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Food Microbiology Based Entrepreneurship

Making Money From Microbes

Springer

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Foreword

The scientific expertise and knowledge of Microbiology in the twenty-first century is a goldmine that has no limitation. The expertise and ingenuity of microbiological scientists can be acquired, cultivated, and transformed into products that generate lucrative fortunes. These assets look infinite as long as people continue to volunteer to be trained and, as a result, develop new science or improve on current knowledge. These goldmines can be established in their backyard. This book describes scholarly works that facilitate job creation and economic growth.

With growing concerns of a high unemployment rate among graduates around the world, it is imperative to translate research outputs into money-making ventures that the unemployed can tap into. This book promotes a paradigm shift in research-related studies to the technology transfer and commercialization of microbial products. The book also facilitates strengthening the knowledge and skills needed for interested entrepreneurs to the commercialization of products for general use.

This book is written for anyone who has a nascent interest in creating a fortune for themselves through microbiological research products. The book provides impetus for developing entrepreneurial skills in academics, graduate students, and unemployed members of the public in building a source of income for themselves using microbiological products. They can leverage the studies in this book to build a fortune for themselves.

Chapters 1–5 and 19 discuss both small- and large-scale production of mushroom and the cost benefits. The chapters looked into various types of mushroom and mushroom products, their production, raw materials needed for production, cost benefits, marketing, and the expected outputs. The chapters describe the practical steps involved in the production and marketing of various products and individuals can tap into their potential.

Chapter 6 describes the production of *Ganoderma* sp., cost analysis, and marketing of the products. It evaluates various products from *Ganoderma* sp. and how those products can be harnessed to generate income.

Chapters 7–10 report the use of microbes as a single cell protein in combating hunger for sustainable development, cost benefits, and production. The chapters focus on the use of *Spirulina*, various yeasts, and bacterial species as single cell protein in combating malnutrition and their production.

Chapters 10–13 highlight the importance, production, cost analysis, and marketing of different fermented foods, such as bread, cheese, and yogurts, while Chaps. 14 and 17 discuss the production, cost analysis, and marketing of probiotics from poultry and livestock production. Chapter 21 highlights the production, cost analysis, and marketing of *Streptomyces* probiotics.

Chapters 14 and 15 give an overview of the production, business plan, and market value of various pigments (phycocyanin and astaxanthin) produced by cyanobacteria and *Haematococcus pluvialis* while Chap. 20 focuses on the production and business plan of red pigments from *Monascus* sp.

Chapter 17 describes the potential benefits, production cost analysis, and marketing of fermented fish.

The last chapter highlights the importance of microbial-based nanotechnology for food packaging.

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Ayansina S. Ayangbenro

Preface

Bio-entrepreneurship is a broad concept and is trending nowadays and has set a very high standard for research and innovation in the country. This book *Food Microbiology-Based Entrepreneurship: Making Money from Microbes* provides a comprehensive overview of the different dimensions in the extraction of the economic value of microorganisms. Entrepreneurship is the ability and readiness to develop, organize, and run a business enterprise using microorganisms such as bacteria, fungi, mushrooms, and algae. Entrepreneurship is about innovation, seeing problems as opportunities, and about changing the world with the commercial production, marketing of microbes, and microbial-derived products, including astaxanthin production from the green alga *Haematococcus pluvialis*, phycocyanin from cyanobacteria, and single-cell proteins. It also includes the small/large-scale production, cost-benefit analysis, and marketing of *Spirulina* single-cell protein, mass multiplication, production cost analysis, and marketing of *Ganoderma* sp.

The chapters included in this book mainly cover the small, large-scale production, cost-benefit analysis, and marketing of button, oyster, milky, shitake, and psilocybe mushrooms. An overview of the red pigments from *Monascus*, cost analysis and marketing of fermented fish, livestock and poultry probiotics are also included. Still, several microbe-derived fermented food products, business plans, commercial production, and marketing do not reach the scientific community, businesspeople, and innovative entrepreneurs, and recent scientific reports suggest that the precise cost-benefit ratio of microbial product production is yet to be available to an entrepreneur to start a new business. This book is a unique volume that covers the small, large-scale production, cost-benefit analysis, and marketing of bread, cheese, yogurt, bacterial, and yeast single-cell protein.

In this book, collectively 21 chapters are allotted for food products including mushrooms, probiotics, fermented milk products, and single-cell proteins. This book is used as a source of information in different fields such as microbiology, biotechnology, food technology, food science, food engineering, business management, small businesses, scalable startups, large companies, innovative entrepreneurship, international entrepreneurs, and imitative entrepreneurs. We strongly believe that in future, more research should be carried out to make money from microbes from the perspective of commercial production and marketing of microbes.

We offer special thanks to Aakanksha and Jayesh, Production Editor, Springer Nature, for their constant support and encouragement and help in bringing out the volume in the current structure of bio-entrepreneurship book. We are also indebted to Springer, Uka Tarsadia University, Gujarat, Bharathidasan University, Tiruchirappalli, India, North-West University, South Africa, for their concerns, and support in the task of publishing the volume as a resource for entrepreneurs.

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Dhanasekaran Dharumadurai is working as an Associate Professor, Department of Microbiology, Bharathidasan University, Tiruchirappalli, India. He was awarded UGC-Raman Post-Doctoral Fellowship and worked in the University of New Hampshire, USA. He was awarded Fellow of Linnean Society, London, UK, and INSA Visiting Scientist Fellowship by the National Science Academy. He has graduated 12 PhDs and 76 MScs with over 106 research publications, 49 book chapters, and an H-index of 30 and citations of 2850. He filed two Indian Patents, and organized 8 national level seminar, conference, and workshop programs. He has edited 11 books and authored 4 laboratory manuals. His research focus is

microbiome profiling of actinorhizal root nodules, lichen, poultry gut, and cattle's reproductive system.

Olubukola Oluranti Babalola the Vice President of the Organization for Women in Science, is an NRF-rated scientist with over 20 years of expertise in rhizosphere metagenomics and an MBA. She has experience from the International Institute of Tropical Agriculture, Weizmann Institute of Science, University of the Western Cape, University of California, University of Mauritius, North-West University, and Bradford University. Olubukola is a member of the editorial board and an international advisor to many journals. She has graduated 27 PhDs and 24 MScs with over 300 publications, an H-index of 47, and >10,200 citations. Olubukola is #1 in Africa for Soil Science and Plant Nutrition. Her international experience spans the Americas, Asia, Europe, and Oceania.

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Small/Large-Scale Production, Cost-Benefit Analysis, and Marketing of Button Mushroom

Dhruti Amin and Jawahar Ganapathy

Abstract

People, in modern times, are becoming more health-conscious. In the present scenario, mushrooms fit themselves well to meet the best low-calorie food's dietary requirements and its delicious taste and flavour. The species grown on most farms is the white button mushroom. *Agaricus bisporus,* belonging to the class Basidiomycetes and Family Agaricaceae, is the most popular mushroom variety grown and consumed worldwide. It is rich in protein and known as a good source of fat, phosphorus, iron, and vitamins, including thiamine, riboflavin, ascorbic acid, ergosterine, and niacin. They are low in calories, carbohydrates, and calcium. The potential benefits of button mushroom resources as human food for centuries are utilized worldwide due to their delicate taste, flavour, nutritional values, functional properties, and medicinal properties. Earlier, their production was possible only in the winter season, which was not fulfilling the market demand in India. However, with technology development, these are produced almost throughout the year in small, medium, and large farms, implementing different levels of technology. The present book chapter deals with general information, cultivation steps involved in small/large-scale production, marketing, and cost-benefit of *A. bisporus* mushrooms. This would help the growers and consumers increase their domestic market utilization.

Keywords

Agaricus bisporus · Start-up · Popularization · Business plan

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1 Introduction

Mushrooms have long ago drawn the attention of human beings for nutritional and medicinal values, and nowadays, it is considered as the leading food. Mushrooms grow in fields, woods, forests, water channels, manure heaps, bunds, and grassy grounds (Kües & Liu, 2000). Mushrooms are the fruiting bodies of macrofungi. It includes edible, medicinal, and poisonous species. However, originally, the word "mushroom" was used for the edible members of macrofungi and "toadstools" for poisonous ones of the "gill" macrofungi. Edible mushrooms were once called the "Food of the Gods" and are still treated as a garnish or delicacy that can be taken regularly as part of the human diet or treated as healthy food or functional food (Zhang et al., 2002).

Mushrooms are proteinaceous and a relatively good source of the following nutrients: fat, phosphorus, iron, and vitamins, including thiamine, riboflavin, ascorbic acid, ergosterine, and niacin. They are low in calories, carbohydrates, and calcium (Werner & Beelman, 2002). For centuries, the medicinal properties of edible mushrooms have been exploited for treating various health conditions, including cancer (Wasser, 2002). The World Health Organization (WHO) has estimated that approximately 80% of the world's inhabitants still depend on traditional medicines for primary health purposes. The most significant medicinal effect of mushrooms and their metabolites that attracted the public's attention is their anti-tumour property (Cragg & Newman, 2001). Having nutritional content such as high fibre, proteins, microelements, and lower caloric content, mushrooms are ideal for a nutrition program to prevent hypercholesterolemia and cardiovascular disease. Mushroom-derived polysaccharides act as anticancer compounds, and their clinical trials are in progress and act as adjuncts to existing chemotherapeutic compounds.

Several medicinal mushrooms like *Agaricus bisporus*, *Pleurotus ostreatus*, *Pleurotus florida*, *Pleurotus rimasus*, *Calocybe indica,* and *Ganoderma lucidum* have recently been reported to possess significant antioxidant activity. The role of free radicals has been implicated in many diseases (Ekonem & Ubengama, 2002). The antioxidant activity of the mushroom is of significant importance in exploiting its therapeutic potential. Mushrooms represent a major and largely untapped source of powerful new pharmaceutical products. The most significant medicinal effect of mushrooms and their metabolites that attracted the public's attention in recent years is their anti-tumour property. Many pharmaceutical substances with potent and unique properties were isolated from mushrooms and distributed worldwide. Many of them are not strictly pharmaceutical products, but represent the novel class of dietary supplements of approximately 10,000 known species of mushrooms, 2000 are safe, and 300 have significant pharmacological properties (Vamanu, 2012). More than 50 mushroom species have yielded potential immunoceuticals, exhibiting significant anticancer activity in experimental animal systems (Kidd, 2000). The protein-bound polysaccharides isolated from the mushrooms have been used as an immune therapy agent to treat cancer in Asia for over 30 years. Many mushroom-derived compounds, cellular components, and secondary metabolites positively impact the immune system. The therapeutic properties and its biomedical application

in human health care and treatment of chronic inflammations and cancers were reported (Dhamodharan & Mirunalini, 2010, 2012, 2013).

India is an agriculture-based country due to the varied agro-climate abundance of agricultural waste and human resources, making it most suitable for cultivating temperate, subtropical, and tropical mushrooms. Mushroom cultivation mainly uses agricultural wastes, so there will be no or minor disposal and atmospheric pollution; these may be ideal tools for recycling agricultural waste. It requires less land than other crops and is an indoor activity. It can be profitable for landless farmers, unemployed youths, and other entrepreneurs—the first culturing of button mushrooms was initiated in the sixteenth century. However, in the seventeenth century, Europe initiated commercial-scale cultivation of button mushrooms. After that were established several farms for the production of button mushrooms, and still, this variety dominates the world's production and consumption. *Agaricus bisporus* is one of the most widely cultivated, highest yielding, and most consumed edible fungi globally (Siwulski et al. 2020). With its diverse agro-climatic conditions and abundance of agricultural wastes, India has been producing mushrooms for more than four decades, mainly for the domestic market. Commercial production increased in the 90s and developed several hi-tech export-oriented farms with foreign technology collaborations. However, a significant share of mushroom production is still on small farms.

The main goal of this chapter is to present a small/large-scale viable bankable model production unit through the adoption of appropriate technology, utilization of resources, and suitable market strategy. In India, about three-fourths of the total mushroom production, mainly of *Agaricus bisporus*, are edible and most popular among other mushroom species. Earlier, the mushroom was being cultivated seasonally during winters, particularly in hilly regions of the country and plains of North India. However, commercial units have spread throughout the years in all parts of the country. Therefore, mushroom cultivation is of economic importance and has a vital role in the integrated rural development programme by increasing income and self-employment opportunities for village youths, womenfolk, and homemakers to make them financially independent.

2 Taxonomical Classification and Botanical Description of Button Mushroom

Agaricus bisporus has several names based on their various developmental stages. When collected from the market or eaten in young, unopened form, it is called "button mushroom", while an immature portobello is known as "crimini mushroom" or "baby bella"; when it is large brown mature mushroom, it is called "Portobello mushroom". In France, mushroom is known as "champignon de Paris"; champignon means "fungus". Mushroom is a member of the large genus *Agaricus*, which has several members that are edible, tasty, and collected worldwide. The next best-known is the commonly collected wild mushroom *A. campestris*, known in North

Fig. 1 Image of *Agaricus bisporus*

America as the meadow mushroom or field mushroom in England and Australia. This can be found mainly in the United States and Europe.

The name *bisporus* comes from *bi*, meaning two, and *sporus*, meaning spored. Most basidiomycetes have four spores per basidia, but this species has only two spores per basidia. This is a very distinctive microscopic characteristic. Commonly found in fields and grassy areas, rain from late spring through to autumn worldwide is especially associated with manure. It is widely collected and eaten, even by those who would not usually experiment with cultivation (Carluccio, 2003).

Scientific Classification

Kingdom:	Fungi
Phylum:	Basidiomycota
Class:	Homobasidiomycetes
Order:	Agaricales
Family:	Agaricaceae
Genus:	*Agaricus*
Binomial Name:	*Agaricus bisporus* (Button Mushroom)

Agaricus bisporus is considered a secondary decomposer. It is found in different colours (white, off-white, brown), sizes (ranging from small mushrooms with a 15 mm cap diameter to large mushrooms with a cap diameter of 50 mm and even more significant), and levels of maturation (fully closed caps up to fully opened caps) (Fig. 1). Mostly used mushroom strains for cultivation in India are S-11, TM-79, and Horst H3. In 2002, scientists of the Horticulture Research Station of the Tamil Nadu Agricultural University at Vijayanagaram, Ooty, released two strains of button mushrooms, such as Ooty 1 and Ooty (BM) 2, for commercial mushroom cultivation.

3 Small/Large-Scale Cultivation

Regarding mushroom production, *Agaricus bisporus* species come first in the world based on their nutrition and market value, therefore mainly selected for cultivation. Mushroom production is a difficult task at the best of times. Successful mushroom growing involves overcoming difficulties such as temperature and humidity control, pest control, and compost preparation. This chapter intended to provide valuable tips to increase the success rate of growing mushrooms.

3.1 The Mushroom Cultivation Process Can Be Divided into Six Following Steps (Source: http://nhb.gov.in/report_files/button_mushroom/button%20mushroom.htm)

Spawn Production

The first step is to collect potential button mushroom strains that may be produced in the lab or obtained from other reputed sources. In India, rather than Indian strains, imported fruiting culture is used, which is reported to produce a high yield. Then spawn is produced from fruiting culture/stocks of selected strains of mushrooms under sterile conditions. The spawn should be of good quality in terms of flavour, texture, and size, apart from having the potential for high yield and longer shelf life.

Compost Preparation

Mushrooms are heterotrophic and saprophytic organisms and thus possess no chlorophylls, but decompose organic materials to feed off (Chang & Miles, 2004). For compost preparation, generally, use the mixture of plant wastes containing lignocellulosic compound (cereal straw/sugarcane bagasse, etc.), salts (urea, super-phosphate/gypsum, etc.), and supplements (rice bran/wheat bran) and water.

Small-scale mushroom production, i.e., 1 kg, required 220 g of dry substrate materials. It is recommended that each ton of compost should contain 6.6 kg of nitrogen, 2.0 kg of phosphate, and 5.0 kg of potassium (N:P:K—33:10:25) and would get converted into 1.98% N, 0.62% P, and 1.5% K on a dry weight basis. The ratio of C:N in a suitable substrate should be 25–30:1 at the time of staking and 16–17:1 in the case of the final compost. Various compositions of compost of *Agaricus bisporus* informed around the world refer to Owaid et al. (2017).

Short Method of Composting

During the first phase of compost preparation, paddy straw is placed in layers, and sufficient water, fertilizers, wheat bran, molasses, etc. are added to the stack. The whole thing is mixed thoroughly with the straw and made into a stack (almost 5 ft high, 5 ft wide and of any length can be made with the help of wooden boards). The stack is turned and again watered on the second day. The stack is again turned for the second time on the fourth day by adding gypsum and water. The third and final turning is given on the 12th day when the colour of the compost changes into dark brown, and it starts emitting a strong smell of ammonia.

The second phase is the pasteurization phase. The compost prepared as a result of the microbe-mediated fermentation process needs to be pasteurized to kill undesirable microbes and competitors and convert ammonia into microbial protein. The whole process is carried out inside a steaming room where an air temperature of 60 °C is maintained for 4 h. The compost finally obtained should be granular in structure with 70% moisture content and pH 7.5. It should have a dark brown colour, sweet unobnoxious smell, and be free from ammonia, insects, and nematodes. Then the final step is to cool down substrate to 25 °C.

Long Method of Composting

The long composting method is usually practiced in areas where facilities for steam pasteurization are not available. In this method, eight times turnings are given for substrate composting. The number of times turnings to be done after substrate addition is as follows:

Turning for composting	Days after addition of substrate for composting
First turning	6th days
Second turning	10th days
Third turning and addition of gypsum	13th days
Fourth turning	16th days
Fifth turning	19th days
Sixth turning	22nd days
Seventh turning and addition of 10% BHC (125 g)	25th days
Eighth turning	28th day after which it is checked whether there is any smell of ammonia present in the compost

The compost is ready for spawning only if it doesn't have any smell of ammonia; otherwise, a few more turnings are given at an interval of 3 days till there is no smell of ammonia.

Spawning

The process of mixing spawn with compost is called spawning. The different methods followed for spawning are given below:

1. *Spot Spawning:* Lumps of spawn are planted in 5 cm deep holes made in the compost at 20–25 cm distance. The holes are later covered with compost.
2. *Surface Spawning:* The spawn is evenly spread in the top layer of the compost and then mixed to a depth of 3–5 cm. The top portion is covered with a thin layer of compost.
3. *Layer Spawning:* Preparation of about 3–4 layers of spawn remixed with compost covered with a thin layer of compost like in surface spawning.
   The spawn is mixed through the whole compost mass at 7.5 ml/kg compost or 500–750 g/100 kg compost (0.5–0.75%).

Spawn Running

After the spawning process is over, the compost is filled in polythene bags (90 × 90 cm, 150 gauge thick, having a capacity of 20–25 kg per bag)/trays (mostly wooden trays 1 × 1/2 m accommodating 20–30 kg compost)/shelves which are either covered with a newspaper sheet or polythene. Temperature is be maintained at around 23 ± 2 °C in the cropping room. Higher temperature is detrimental to the growth of the spawn, and any temperature below that specified for the purpose would result in a slower spawn run. The relative humidity should be around 90%, and a higher than usual CO_2 concentration would be beneficial. The fungal bodies grow from the spawn and take about two weeks (12–14 days) to colonize.

Casing

After a complete spawn run, the compost beds should be covered with a layer of soil (casing) about 3–4 cm thick to induce fruiting. The casing material should have high porosity, water holding capacity, and the pH should range between 7 and 7.5. Peat moss is considered to be the best casing material, but due to its unavailability in India, mixtures such as garden loam soil and sand (4:1), decomposed cowdung and loam soil (1:1) and spent compost (2–3 years old), sand and lime are most commonly used instead of peat moss.

The casing material must be sterilized at least 15 days before use. The casing soil before application should be either pasteurized (at 66–700 C for 7–8 h), treated with formaldehyde (2%), formaldehyde (2%), and bavistin (75 ppm.) or steam-sterilized. After the casing process, again the room temperature should be maintained at 23–28 °C and relative humidity of 85–90% for another 8–10 days. Low CO_2 concentration is favourable for reproductive growth at this stage.

Fruiting

The fruit body initials in pinheads start growing and gradually develop into a button stage under favourable environmental conditions such as.

Temperature	Initially, 23 ± 2 °C for about a week and then 16 ± 2 °C
Moisture	2–3 little water sprays per day for moistening the casing layer
Humidity	Above 85%
CO_2 concentration	0.08–0.15%
Proper ventilation	

3.2 Pest and Diseases

Nematodes, mites, and springtails are the main causative agents for diseases causing in mushrooms. The crop is suspected of several diseases caused by various bacteria, fungi, and viruses like Dry Bubble (brown spot), Wet Bubble (White Mould), Cobweb, Green Mould, False truffle (Truffle disease), Olive green mould, Brown plaster mould, and Bacterial blotch. The entrepreneur will seek professional help and

extension advice to adopt appropriate and timely control measures against pests and diseases.

3.3 Harvesting and Yield

Harvesting is done at the button stage, and caps measure 2.5–4 cm across and closed. The first crop appears about 3 weeks after casing. Mushrooms need to be harvested by light twisting without disturbing the casing soil. Once the harvesting is complete, the gaps in the beds should be filled with fresh, sterilized casing material and then watered.

About 10–14 kg of fresh mushrooms per 100 kg of fresh compost can be produced in 2 months. The short method used to prepare compost under natural conditions gives more yield, i.e., 15–20 kg per 100 kg of compost.

3.4 Post-harvest Management

Packing and Storage

Short-Term Storage

Button mushrooms are highly perishable. Harvested mushrooms are cut at the soil line and washed in a solution containing 5 g KMS in 10 L of water to remove the soil particles and induce whiteness. After removing excess water, around 250–500 g of mushrooms is packed in perforated unlabelled simple polythene or polypropylene bags for retail sale. It can be stored for 3–4 days in polythene bags at 4–5 °C. Bulk packaging does not exist. Modified atmosphere packaging (MAP) and controlled atmosphere packaging (CAP) trend in developed countries.

Long-Term Storage

White button mushrooms are not usually dried by standard procedures as used in the case of oyster, paddy, and shitake mushrooms. Canning is the most popular method of preserving the white button mushrooms. A sizeable quantity of canned produce is exported to international markets. Besides that, freeze-drying, IQF, and pickling are also practised by some units.

3.5 Global and Indian Scenario in Button Mushroom Production

The most significant developments in mushroom cultivation came from France when *Agaricus bisporus* was cultivated for the first time in 1600 A.C. on agricultural media specially prepared for the purpose (Chang & Miles, 2004). Commercial mushroom production was first achieved by a Frenchman in 1780, who cultivated *A. bisporu* underground in quarries near Paris (Beyer, 2003). Commercial production in USA and Canada has been in place for over 100 years (Al-Bahadli &

Al-Zahron, 1991). The first specialized commission for the development of mushroom cultivation was founded in Pennsylvania in 1894, referred to as the mushroom capital of the world (Beyer, 2003). For large-scale production of *A. bisporus*, the main centres are Europe, North America (USA, Canada), and South East Asia (China, Korea, Indonesia, Taiwan, and India). *A. bisporus* mushroom is considered the most popular commercial mushroom grown in the United States and Canada (Halpern, 2006). It has a seasonal growth pattern in China and represents about 70% of Chinese production (Chen et al., 2003). There are currently about 346 growers of *Agaricus* sp. and speciality mushrooms in the United States, and the volume of sales of the mushroom crop in that year in the United States totalled about $450x10^6$ kg (USDA, 2016).

From 2014, India's annual production of mushrooms increased by 30 times till 2019. According to a national survey, this growth will possibly be 50 times greater by 2023. The major producing states in India are Himachal Pradesh, Uttar Pradesh, Punjab, Haryana, Maharashtra, Andhra Pradesh, Tamil Nadu, and Karnataka. Currently, Punjab holds the crown of maximum production of mushrooms which is almost 48% of total mushroom production in India. Almost 85–90% of mushrooms in India are produced in Himachal Pradesh, Haryana, and Punjab. Himachal produces more than 15% of total mushrooms in India. The second largest producer of mushrooms is Haryana which produces 25% of the total production of mushrooms in India. Indian central government provides a 40% subsidy on mushroom cultivation which is a good initiative for the near future. Mushroom farming in India attracts farmers because of its business structure. It is easy, and people can start it as a side business which does not require larger lands, investment, and human resources. It is a profitable business in India that provides 1.5 times the investment return if everything goes well. The marketing of mushrooms is not a big concern in 2021 because it is in heavy demand. There is a vast scope of mushrooms in India because it just started to increase exponentially. People have a chance to get into this business with full awareness and information about the mushroom farming process.

3.6 Constraints of Button Mushroom Production

The production and yield of mushrooms are not satisfactory due to the poor management, low input applications, agro-climatic factors, and lack of marketing knowledge. Poor production and less profit are also due to the poor quality of spawn and casing, lack of spawn units in the locality, lack of compost units in the State, pest contamination and disease occurrence, high transportation charges, and lack of storage facility (Rawat et al., 2020; Dhungana, 2022).

4 Cost-Benefit Analysis

The demand for button mushrooms is fast increasing in international markets, and a significant gap exists between supply and demand. There is a need to take advantage of this situation by encouraging its production. Mushroom cultivation involves investment depending upon the size of the unit/production targets. Before starting this venture, one should have thorough knowledge in this field and survey the market to sell the produce. Expenditure on a mushroom farm can be divided into fixed assets and recurring expenditures. The items permanent in nature and last longer than the duration of one crop include land, building, boiler, blowers, compost handling equipment, computers, air-conditioning equipment, shelves, etc. and are considered fixed assets. However, variable cost/recurring expenditure includes raw materials like compost ingredients, spawn, casing soil, energy cost, pesticides, labour charges, a salary of the employees, etc. Mushroom cultivation is a labour-intensive job if done on a large scale. Skilled labour is employed for composting, spawning, casing, and spraying the beds. Therefore, their wages would be higher and ordinary labour would be hired for harvesting for economizing.

The minimum viable production unit will require a land site of 1.5 acres. Overview of expenditure inclusive of contingencies—the project cost works out to Rs.107 lakhs as below (http://nhb.gov.in/report_files/button_mushroom/button%20mushroom.htm).

Project cost	Amount (in lakhs)	Remarks/justification
Land and site development	5.15	The land would have to be acquired in areas well-connected to urban markets. On average, the land cost might be put at Rs. 3 lakhs per acre The cost of levelling the site (including fencing, etc.) would be Rs. 0.15 lakhs, and putting up guard rooms would be Rs. 50 thousand
Building	44.96	The estimated cost of this component works out to around Rs. 45 lakhs, the major item being the growing room at Rs. 25.92 lakhs
Plant and machinery	47.00	The cost of equipping the production unit works out to Rs. 30 lakhs, that of the compost and casing unit to Rs. 7 lakhs, and installing canning facilities, spawn Lab, and other equipment to Rs. 10 lakhs.
Miscellaneous fixed assets	0.75	This is the estimated cost of building up a communication system and furnishing
Contingency	4.88	These include professional charges, administrative expenses, and other start-up expenses
Pre-operative cost	4.25	
Total	106.99	Three sources of financing the project: Farmer's share (53.50 lakhs), Capital subsidy (21.40 lakhs), Term loan (32.10 lakhs)

Note: Cost, expenditure, and profit vary depending upon the area and scale of button mushroom production

Various subsidies and loan availabilities for mushroom growers in India (terms and conditions apply) are as follows:

1. National bank for Agriculture and Rural Development (NABARD/NHB)
2. National Horticulture Board
3. State government
4. Rashtriya Krishi Vikas Yojna
5. Department of Agriculture and Co-operation under the scheme of National Horticulture Mission.

5 Marketing Strategies

There are two best ways to promote yield, quality, and usage of a button mushroom. The first is online promotion, and the second is banners in local areas. One can also develop a website for mushroom selling. Money required for online marketing depends on the reach of the particular platform. Generally, in India, one can spend 25,000 INR which is enough for one to two times in online marketing. An investment of 15,000 INR is usually needed for posters and banners to print and paste on holdings. One can easily find an individual customer for a whole harvested crop by a proper reach. If quality is maintained, then a single customer becomes a permanent buyer. However, one has to be aware of marketing. It is necessary to start marketing just before 10–15 days of harvesting mushrooms. A better market will be helpful to get a better price and benefit. A critical analysis of mushroom marketing channels and consumer behaviour refers to review article by Shirur and Shivalingegowda (2015).

6 Concluding Remarks

The food processing industry is one of the largest industries in India and ranked fifth in terms of production, consumption, export, and growth prospectus. India, the second largest food producer globally, lags far behind in food processing which is essential for increasing the shelf life of the products and providing more benefit to the growers. India produces more than 0.13 million tons of mushrooms. In India, button, oyster, milky, and paddy straw mushrooms are commonly grown, but button mushroom contributes the highest share of production. Mushrooms could be an important sector for our future agriculture and forestry. The R&D, government schemes, policymakers, and entrepreneurs contribute to the initiation and growth of the mushroom industry. In the current scenario, the best crop and high yield production these days are of propelling mushroom farming as they lead to high profit margins that can be helpful to both farmers and the country's economy. Effective benefit and marketing techniques may reduce post-harvest losses and result in excellent remuneration to the producers and consumers. Mushroom cultivation is an effective bioconversion technology for transforming wastes to wealth; into

potentially valuable resources. Thus, the book chapter explains this edible mushroom: small/large-scale production, precautions, and utilization.

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Large-Scale Production and Cost-Benefit Analysis of Mushroom Spawn

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Abstract

Mushroom is a modified form of fungi. In spawn production process, a mushroom culture is placed on a pre-sterilized grain and it grows on the grain. The mixture of mycelium and grain is called a spawn. Mushrooms are of high nutritive value. There are more than 2000 edible mushroom species; however, few species come under large-scale production. This chapter provides a detailed information about the various phases of spawn production, especially the cultivation of oyster mushroom spawn cultivation, provided an overview about spawn cultivation laboratory and cost-benefit analysis of large-scale production includes the details about infrastructure, equipment, chemicals, and labour charge of mushroom spawn production.

Keywords

Ascomycotina · Mushroom spawn · Nutritional value · Cost-benefits

1 Introduction

Mushrooms neither belong to the plant kingdom nor the animal kingdom. Mushrooms are classified under the category fungi. These are defined as the structures that possess visible fruiting bodies that bear spores. According to taxonomy, mushrooms come under both Ascomycotina and Basidiomycotina (Reddy, 2015). Mushrooms are used as food because of their high nutritional value and they are also used in the field of medicine. The studies show that there are about 1.5

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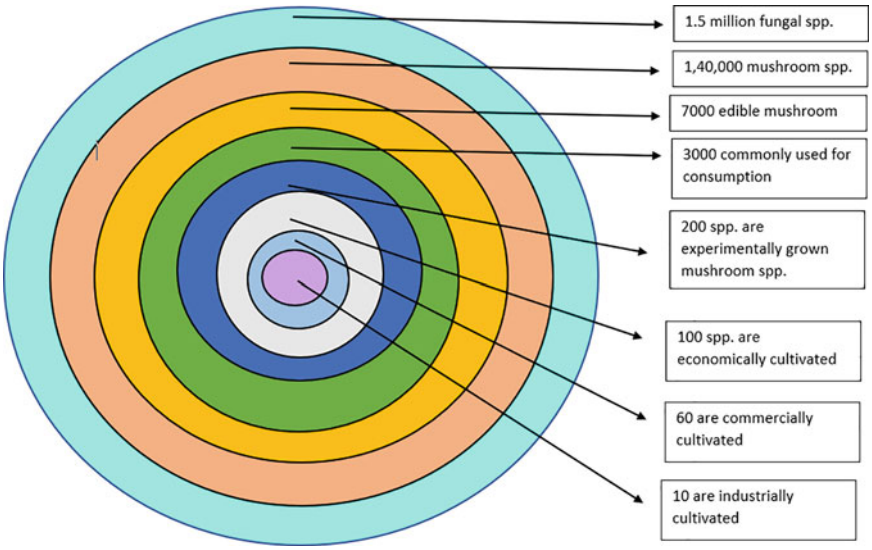

Fig. 1 Diversity of fungal species emphasizing cultivated mushrooms

million species of fungi, among this 140,000 spp. are considered mushrooms. Out of this 140,000 spp., 14,000 are considered as mushroom spp.; 7000 (50%) of these mushrooms are edible, whereas others are considered poisonous mushrooms. Further 3000 spp. out of 7000 are most commonly used for consumption. To date, 200 spp. of mushrooms are grown experimentally, 100 species are cultivated economically, 60 spp. are cultivated commercially, and 10 spp. are cultivated at industrial production (Swayam-NIOS, n.d.) (Fig. 1).

2 Why Mushrooms?

According to FAO, mushrooms are categorized under vegetable due to their high nutritional values. Mushrooms are also considered a functional food, having both nutritional and medicinal values (Chang & Miles, 2004). According to the recent survey, it has been found that mushrooms have high protein content and it is very much helpful for vegetarians or people who like to increase the protein in their body. Sánchez (2010) stated that mushrooms' dietary benefits are contrasted with eggs, meat, and milk and results showed that mushrooms are rich in nutrients and plenty of fundamental amino acids (Sánchez, 2010). Malnutrition is one of the biggest problems that the world is facing right now. The mushroom acts as a very effective source to evade malnutrition as they are rich in many nutrients and also have high medicinal values (Pathmashini, 2008). Mushrooms contain almost 90% of water content and only 10% is its dry weight (Sánchez, 2010). The data regarding the nutritional content of mushrooms per 100 g of fresh mushroom are shown in Table 1.

Table 1 Nutritional values of common mushroom spp. cultivated in India (fresh weight basis)

S. no.	Mushroom spp.	Protein g/100 g	Ash g/100 g	Carbohydrates	Energy Kcal	Fat	Fibre	reference
1.	*Agaricus bisporus*	2.72	0.84	3.35	32.3[a]	0.36	2.39	Solano-Aguilar (2018)
2.	*Pleurotus ostreatus*	3.4	1.18	5.1	46.92[a]	0.68	3.4	Alam (2008)
3.	*Calocybe indica*	2.75	1.28	6.8	56.51[a]	0.65	1.63	Alam (2008)
4.	*Volvariella volvacea*	5.05	0.58	4.43	46.51[a]	0.68	1.24	Lee (1975)

[a]Indicates the manually calculated values using the web link. Weblink: https://www.dairyscience.info/energy/label1.asp

3 Commonly Cultivated Mushrooms across India

There are a few common mushroom spp. that are cultivated across India. Some of these spp. include button mushroom (*Agaricus bisporus*) commonly grown in northern states, oyster mushroom (*Pleurotus ostreatus*) commonly grown in southern states, milky mushroom (*Calocybe indica*), and paddy straw mushroom (*Volvariella volvacea*) in Orissa and its close-by states (Borah, 2020) (Arora, 2018). More information regarding these species is mentioned in Table 2.

4 Spawn

To cultivate mushrooms at the industrial or economical level, the pioneering material used is spawn. Spawn is defined as any substance that is inoculated with mycelium. There are a few steps for the cultivation of any mushroom that need to be followed which are mentioned in Fig. 2.

In this chapter, we discussed about the *Pleurotus ostreatus* (Oyster mushroom). This is one of the species of mushroom that is very widely grown in southern parts of India. The cultivation of oyster mushrooms started back in Germany in the year 1917 by Flack. He started growing them on wooden logs and tree stubs. Further procedure technology on large scale was given by scientists from USA, namely Block, Hau, and Tsao. In India, cultivation of a variety of oyster mushrooms started in the 1960s, whereas production at the commercial level started in the mid-seventies (NHB-National Horticultural Board, n.d.).

5 Spawn Production

For many mushroom cultivators, the main constrain is spawn availability. Most entrepreneurs cannot produce their spawn as it requires the experts and maintenance of high sterility. If sterility is failed to be maintained in the spawn lab, then the owner will be at a great loss. For the mushroom cultivators, it is very important to fetch the spawn from authentic labs and relayed trusted sources. Many government organizations, like, Indian Council of Agricultural research and Indian institute of Horticulture (ICAR-IIHR) and All India Coordinated Research Project on Agro meterology (AICRPM) centres, provide spawn across India. How a farmer chooses the best seed for the crop, in the same way the best spawn should be chosen for mushroom growth (Mbogoh, 2011). Obtaining a good quality mushroom spawn helps in getting a good and high yield of mushrooms at the end. To a great extent, it also helps in avoiding contamination (Borah, 2020). At a commercial level, there are various types of spawn production. The types of spawn production are shown in Fig. 3.

The *Pleurotus ostreatus* is most commonly grown in the southern part of India (Arora, 2018). To grow the spawn of this spp., grain spawn method is most commonly used. Depending on the availability and cost, wheat and jowar are the

Table 2 Some features of commonly cultivated mushrooms across India

Features	Button mushroom	Oyster mushroom	Milky mushroom	Paddy straw mushroom
Botanical name	*Agaricus bisporus*	*Pleurotus ostreatus*	*Calocybe indica*	Volvariella volvacea
Commonly used substrate	Paddy straw (compost + casing)	Paddy straw	Paddy straw	Paddy straw
Spawn running temperature (°C)	14–18	22–26	30–35	30–35
Fructification (°C)	15–20	24–28	35–38	28–32
Relative humidity (%)	85–90	70–85	85–90	80–85
Total life cycle (in days)	90	35–45	45–50	90
Yield	800–900 g/bed (4 kg compost)	635 g (500 g paddy straw)	720 g (500 g paddy straw)	800–900 g/bed (4 kg compost)
Dry weight basis g/100 g				
Fibre	20.90	57.60	64.26	54.80
Fat	3.10	2.20	4.10	2.60
Energy K cal	499	265	305	391
Ash	5.70	9.80	7.43	1.10
Shelf life (days)				
Normal condition	2	2	3	1
Refrigerated	3	3	5–7	2
Production cost/kg[a] (Rs)	90–120[a]	60–75[a]	60–80[a]	45–55[a]
Reference	1. Borah (2020) 2. Manikandan (2011)	1. NHB-National Horticultural Board (n.d.) 2. Borah (2020) 3. Manikandan, (2011)	1. ICAR-Indian Institute of Horticultural Research, (n.d.) 2. Borah (2020) 3. Manikandan (2011)	1. Arora (2018) 2. Borah (2020) 3. Manikandan (2011)

[a]Indicates the change in cost according to the place

most commonly used grains. The procedure for spawn production was first developed by the Pennsylvania State University in 1932. The process of grain spawn cultivation was later on perfected in the year 1962 by Stoller (ICAR-Indian Agricultural Statistics Research Institute, 2020).

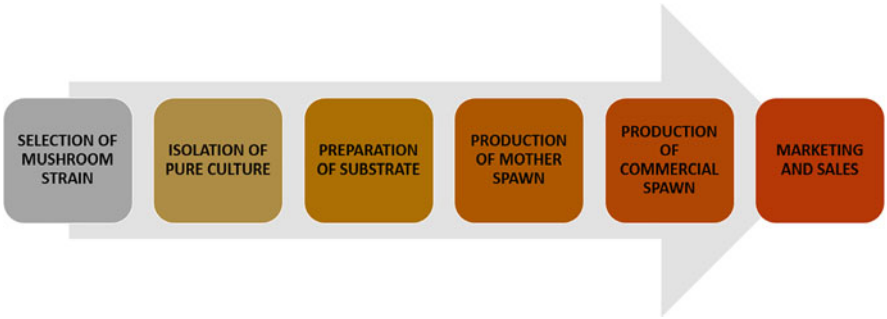

Fig. 2 Mushroom spawn cultivation process steps

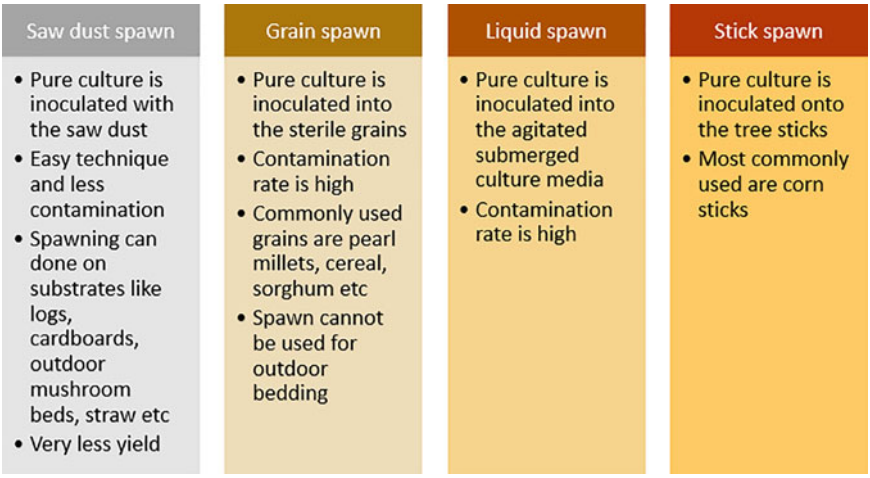

Fig. 3 Different types of spawn (Liu, 2018) and (Mushroom appreciation, n.d.)

Further in this chapter, discussion about the materials required and procedure for the cultivation of grain spawn for oyster mushroom cultivation are included.

6 Oyster Mushroom Grain Spawn Cultivation

6.1 Materials Required

Equipment and Miscellaneous Items in Spawn Lab

Grain cleaner, grain boiler (6 W electric power), autoclave, laminar airflow (LAF), incubator, kettle, sieve, fridge, freezer, hot air oven, grain mixer, bag filling machines, trolleys, stands, pH meter, weighing balance, microwave oven, temperature and humidity meters, fire safety equipment, first aid kit, air coolers, UV tubes, bag filler, racks, weighing balance, stirrer, Bunsen burner, non-absorbent cotton, etc.

Glassware

Petri plates, conical flasks, measuring cylinder, glass rod, inoculation loop, beakers, test tubes, etc.

Chemicals

Ethanol, agar powder, glucose or sucrose, and malt extract, calcium carbonate, calcium sulphate, sodium hydroxide, HCl, Yeast extract, etc.

6.2 Procedure

Basic Steps of Mushroom Spawn Production

There are six basic steps to cultivate the mushroom spawn (Fig. 2). The steps are as follows:

1. Selection of the healthy *Pleurotus ostreatus* species
2. Preparation of potato dextrose agar/malt extract agar media
3. Pure culture of selected mushroom should be obtained
4. Preparation of substrate for spawn culture
5. Mother spawn preparation
6. Multiplication of spawn at commercial level

Selection of Healthy *Pleurotus ostreatus*

The first step is to select the good variety of mushroom species, as the product of our production completely depends on the species selected. It is advisable to purchase oyster mushroom from the market so that we will be sure enough that it is edible and the right spp. is selected.

Preparation of Potato Dextrose Agar/Malt Extract Agar Media: (Hsu, 2018)

PDA Preparation (200 mL)
40 g of thin potato slices
4 g of Sugar (glucose/sucrose)
3 g of Agar powder

Cook the thin potato slices in 100 mL of water. The potato should be chopped into thin slices so that it gets cooked fast. Cook them till it becomes soft. Strain this solution several times and collect the broth. In another flask, take 100 ml of water and to that add 4 g and 3 g of sugar and agar powder, respectively. Now add the strained potato broth to this flask and make up the volume to 200 ml. After making up the volume, place the flask and boil it till the agar dissolves. Keep stirring while boiling so that the nutrient medium won't get charred. The next step is to sterilize the prepared medium by subjecting them to autoclaving. Autoclaving is done at 122 °C and 15 psi for 30 min. Allow the media to cool down approximately to 50 °C and

then pour the media into the sterile petri plates under sterile conditions. So that contamination can be avoided to a large extent.

Malt Extract Agar Media (200 mL)

For 200 ml malt extract agar media preparation, the proportions of the components are given below:

Malt extract -5 g
Peptone-1 g
Agar-4 g

Weigh and add the contents to 200 ml of distilled water. The pH of the solution should be between 7.0 and 7.5. After checking the pH, boil the contents and dissolve the agar. Autoclave the prepared medium at 15 psi for 15–20 min. When the media becomes warm, pour it into the sterile petri plates in sterile conditions.

Pure Culture of Selected Mushroom Should Be Obtained

Selected mushroom strains can be cultured either by multi-spore technique or tissue culture technique to obtain the pure culture.

Multi-Spore Culture

Selected mushroom should be surface-sterilized to avoid contamination. Multi-spore culture is produced using spore print that can be acquired by dangling a fresh surface-sterilized mushroom on a circle of wire over a petri plate or sterilized paper. The collected spore print is then transferred to test tubes with slant media. Media can be either Potato dextrose agar (PDA) or Malt extract agar (MEA). These tubes are further incubated for 2 weeks at 25 ± 2 °C to get pure cultures.

Tissue Culture

Tissue culture is the most commonly used technique in most spawn labs. Surface-sterilize the selected mushroom strain. Prepare the PDA medium and sterilize it using an autoclave. Sterilized media is then poured into the clean petriplates. PDA agar medium should be prepared and poured into the petri plates. This is to avoid contamination. These petri plates are then incubated at room temperature under sterile conditions. In Laminar air flow (LAF), cut the mushroom into 2 halves using a sterilized knife/scalpel. Select a small portion of inner tissue from the junction (where cap and stalk meet) portion. These portions are then inoculated into the petri plates containing medium and incubated at 32 ± 2 °C for 4–5 days in a BOD incubator. Observe for the mycelium growth in the petri plates.

To get pure culture, a small portion of mycelium having a very little amount of media can be transferred into another test tube containing fresh media slants. Incubate these plates at the same temperature and for the same duration of time as mentioned in the above step, i.e. 32 ± 2 °C for 4–5 days in a BOD incubator. After the growth of mycelium on these slants, these can be directly used on spawn substrate and this is also called first-generation (T_1).

Preparation of Substrate for Spawn Culture

There is a wide range of substrates that are used for spawn culture. A few of these include sorghum, wheat, rye grains, cotton waste, tea leaves, etc. The most commonly used substrates are millets (Hsu, 2018). Grain spawn preparation will be discussed further in this chapter.

Grain Substrate Preparation Procedure

Tamil Nadu Agricultural University (TNAU) Agritech Portal suggests that the Jowar seeds are suggested as the best for spawn cultivation (TNAU Agritech Portal, 2016). Grains are washed thoroughly in sufficient water three to four times to remove debris. The damaged and cracked grains are removed by sieving. This can also be done either manually or using the machines. The cleaned seeds are then soaked in water for 20–30 min and not allowed to ferment by soaking for a long time. Water can be drained off by spreading on the sieves. These seeds are then collected and cooked in a vessel for 20–30 min to make the seeds soften. While cooking, it should be ensured that grains should not burst. The overcooked seeds may lead to clumping and the surface area for the mycelial growth may reduce. After boiling, the excess water can be drained by spreading the seeds on a sieve. Leave the seeds on a sieve for some time till the moisture level reaches 50%. The well-drained seeds are then mixed with calcium carbonate (chalk powder) and calcium sulphate (gypsum). For every 1 kg of seeds, 5 g of chalk powder and 20 g of gypsum are added. This is to maintain the pH of the grains at 7 to 7.8 and also to avoid clumps. At the commercial level, rotating drums can be used for uniform mixing. These seeds are filled up to the three-fourths height of saline bottles/Mason jars/commercial spawn bags. They are kept closed until use. These seed bottles/bags are further sterilized in an autoclave under 20-lbs pressure for 2 h. Allow the bottles/bags to cool down and place them in the aseptic room. This finishes the grain substrate preparation for mother spawn culture.

Mother Spawn Preparation

Grain spawn substrate prepared in step iv is inoculated with pure culture obtained in step iii. Before inoculation, some aseptic measures should be taken care of so that the contamination can be minimized; such as sterilization of the grain substrate bags by keeping them under UV for 20 min, turning on the air curtains, and cleaning the working area.

Then open the bags/ bottles and a small amount of the pure culture from the petri dish should be transferred into the grain bag in a sterilized environment. These bags are closed and kept in the incubation room at the temperature of 25 °C for 10 days. After inoculation, they should be labelled providing the details of the date of inoculation and strain used. For incubation, they should be kept in dark conditions or bottles can be covered with aluminium foil. Bottles are examined for contamination and gently shaken to evenly distribute the inoculums periodically. The bottles/ bags will be fully covered with white coloured mycelium by the end of the tenth day. This spawn is referred to as the mother spawn (T2) as it is produced directly from the pure culture of the selected strain. Mother spawn is later used to produce many commercial spawn bags (Borah, 2020). Mother spawn cultures can also be stored for

future use at 4 °C for many months. But, if any contamination or drying of the spawn is observed, then they should be autoclaved and discarded.

Multiplication of Spawn at the Commercial Level

For commercial spawn production, the inoculum is mother spawn and this spawn is called planting spawn as it is directly used for cultivating the mushroom by the growers. At this level, spawn is prepared in heat-resistant polypropylene bags by filling the grains of 500 g to 1 kg. Grain substrate preparation for spawning is the same as mentioned in step 4. For 500 g, 35 × 17.5 cm, and for 1 kg, 40 × 20 cm double-sealed plastic bags can be used. Fill the grains in these bags and then close the bags by using a PP ring and plugging sterile cotton. The filled bags are subjected to sterilization at a pressure of 22 psi for 1.5 to 2 h. After sterilization, the bags are placed under UV light for 20–30 min and then followed by inoculating with a small amount of mother spawn under aseptic conditions. Multiplication from spawn to spawn is not recommended beyond two generations. Post inoculation, the bags should be shaken so that inoculum will get mixed up well. These bags are then labelled providing the details of the date of inoculation, strain chosen, and generation. After labelling, place these bags in the incubation room at a temperature of 25 °C. Within 15–20 days the complete spawn growth takes place; in between, the spawn growth the bags should be shaken and also should be checked for contamination. If any contamination is observed, those bags should be discarded after autoclaving; so that the spreading of the contamination can be avoided to a greater extent. These spawn bags can be stored in the refrigerator at 4 °C for 2–3 months. These bags can be transported in reefer vans where temperature can be controlled; otherwise, they should be packed very neatly in cardboard and made sure that these should get exposed to neither heat nor dust. (Figs. 4, 5, 6, and 7).

7 Layout of Mushroom Spawn Laboratory

Spawn laboratory layout consists of rooms for incubation, inoculation, instrument lab, change room for staff, cold storage room, office and salesroom, parking area and compound wall which can be constructed according to the area of the land. The layout of the spawn lab is shown in Fig. 8.

To maintain the quality of a spawn, some good measures need to be taken. Some of them are following:

1. For spawn preparation, choose a strain that is high yielding, early producing, and has excellent superior sporophore quality.
2. For spawn production, choose unbroken and high-quality grain.
3. Grain boiling should be done according to the instructions to keep the moisture content of the grains between 48% and 50%.
4. By combining an adequate amount of calcium carbonate and gypsum, the pH of boiling grains should be corrected to 6.5–7.5.

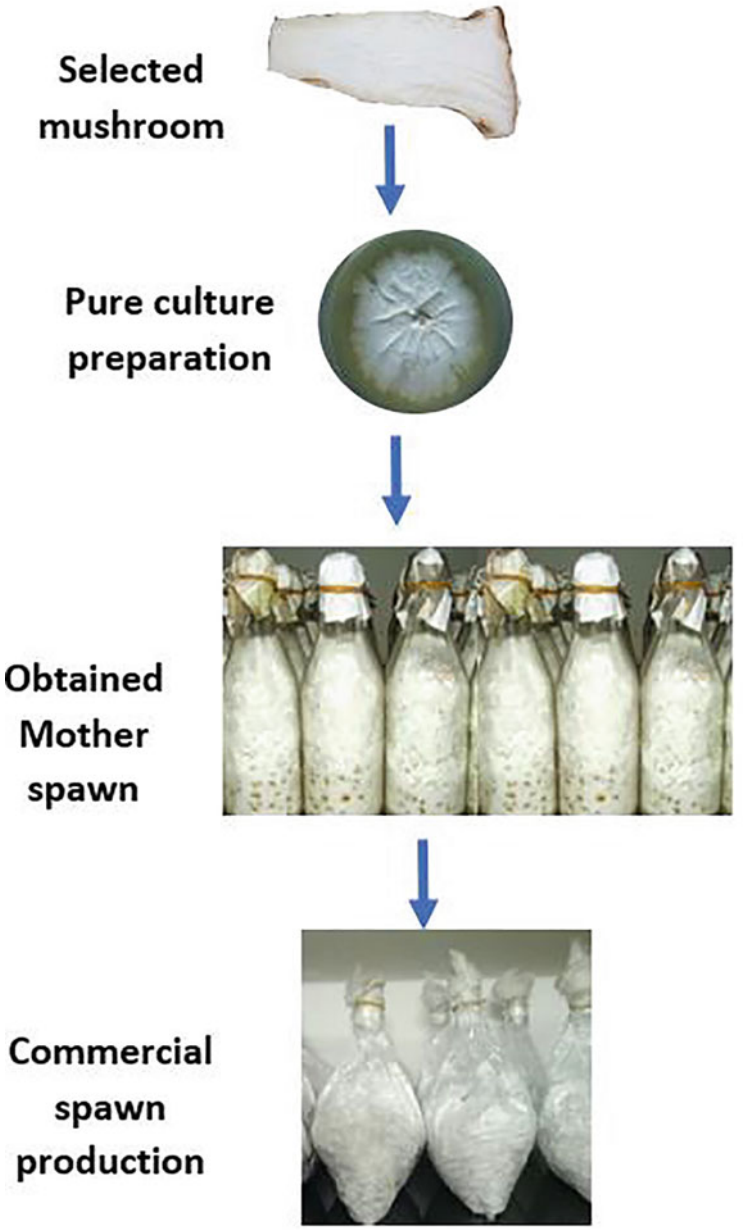

Fig. 4 Steps involved in the preparation of commercial spawn from selected mushroom strain

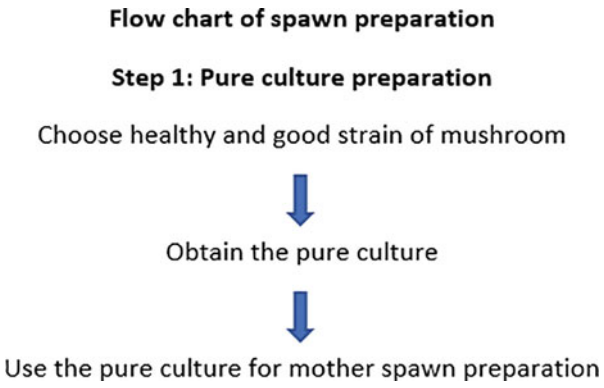

Fig. 5 Flowchart of spawn preparation

5. Only use mother culture to make spawn. Using spawn to spawn do not culture more than two generations.
6. The inoculation and incubation of all the cultures should take place in an aseptic environment.
7. Discard the contaminated cultures after autoclaving.

8 Cost-Benefit Analysis

The cost-benefit analysis calculations were done taking the reference from "Rough estimates for spawn lab" (ICAR-Directorate of mushroom research, 2020). Tables 3, 4, 5, 6, 7, 8, and 9 show the cost-benefit analysis of mushroom.

Net profit can be increased by cutting down the cost of raw materials by purchasing them in bulk. Constructing the lab in high altitude area helps in reducing the electricity charges for cooling purposes.

Usage of windmill power reduces the electricity bills to a great extent. It is advisable for the entrepreneur to have their mushroom farm nearby the spawn lab, so that the charges of spawn procurement and transportation can be eliminated. This may increase profit and also survival capacity of the spawn can be tested.

Decrease in contamination also helps in increasing production and further increase net profit. Good laboratory practices and maintenance of sterility in the spawn lab reduce contamination and chaos in the lab.

9 Summary

Mushroom spawn production is a suitable business for entrepreneurs. It is a profitable risk-free business with low cost of investment. Anyone can take the business opportunity of spawn production and lead their livelihood. This chapter provides a compressive account about spawn production, materials required for spawn production, design of spawn lab, and cost-benefit analysis.

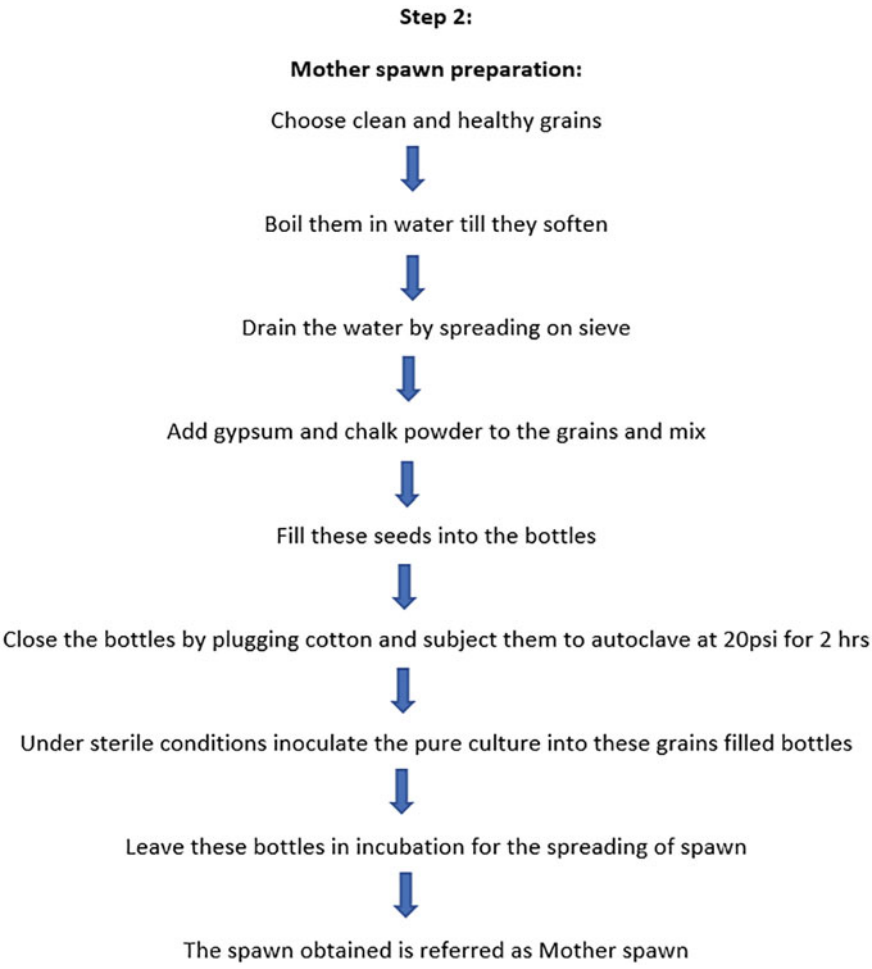

Fig. 6 Steps in preparation of mother spawn

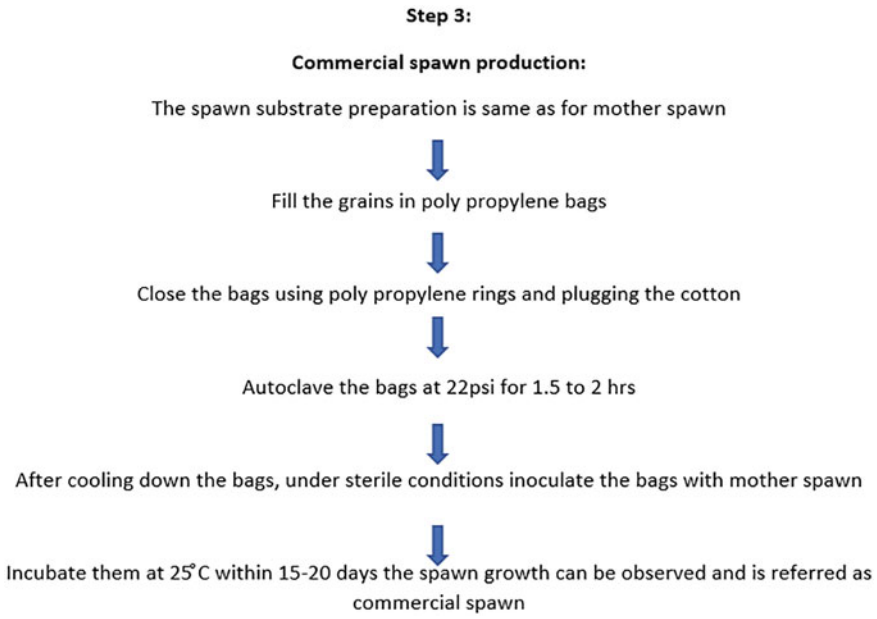

Fig. 7 Steps in commercial spawn production

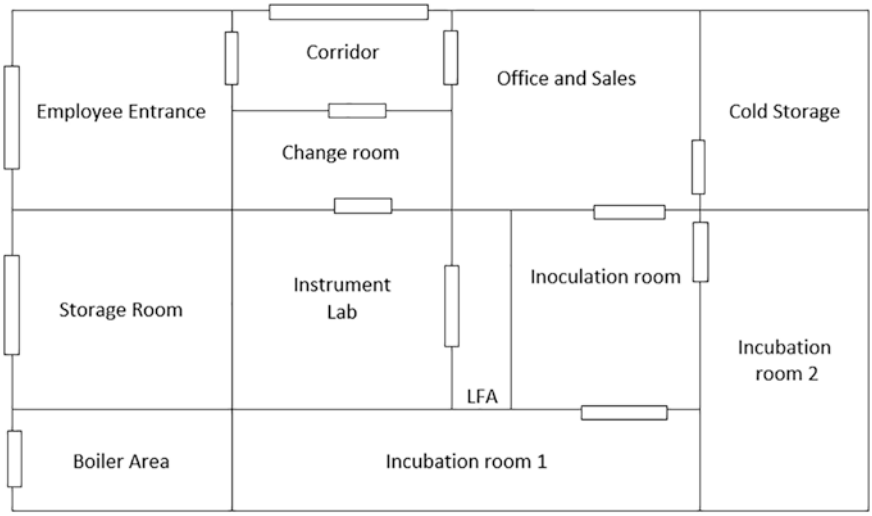

Fig. 8 Layout of mushroom spawn lab.*Laminar flow area (LFA)

Table 3 Target spawn production per annum

Target spawn production per annum	
Target of spawn produced/day	280 kg
No. of lab working days/year	340 days
Incubation of spawn	20 days
Cost of spawn/kg	110 Rs
Required mother spawn for mentioned target	8 kg
Buffer for 5% contamination	14 kg
The total amount of spawn to be produced / day	302 kg
Anticipated spawn output/year	96,640 kg

Table 4 Fixed cost on infrastructure and construction

Fixed cost-infrastructure and construction			
Item	Required area (sq ft)	Cost (Rs/sq ft)	Total cost (Rs)
Spawn substrate and miscellaneous storage area	750	1500	1,125,000
Working area	600	1500	900,000
Boiler area	150	1300	195,000
Change room area	100	1300	130,000
Lab area	600	1500	900,000
Inoculation room	100	1300	130,000
Incubation room(s)	550	3000	1,650,000
Corridor	100	1300	130,000
Cold storage	180	3000	540,000
Office and salesroom	210	1300	273,000
Parking area	300	1000	300,000
Subtotal of lab area	**3640**		**6,273,000**
Compound wall and layout area (app. 50% of total area)	1500	1000	1,500,000
Total area and cost of infrastructure	**5140**		**7,773,000**
Cost of the land/sq. ft	740		
Total cost of the land	3,803,600		

Table 5 Fixed cost on various equipment

Fixed cost equipment				
Item	Capacity	Quantity	Rate (Rs)	Cost (Rs)
Boiler	300 kg	1	300,000	300,000
Grain sieves	Aperture size 2.36 mm: (model no: ASTM E11-No.8)	2	2500	5000
Grain mixer	50Kg	1	48,550	48,550
Bag filling machine	Filling capcity-1 kg holding capacity-50 kg	1	70,000	70,000
Trolleys	300 kg	3	4040	12,120
Autoclaves horizontal	500 L	1	190,000	190,000
Autoclave (vertical)	50 L	1	37,000	37,000
Laminar air flow	4′*2′*2′	1	80,000	80,000
Stands in incubation and cooling room	48*18*72	50	5500	275,000
Air conditioners	5–10 tons	3	80,000	240,000
Incubator	150 L	2	43,000	86,000
Fridge with glass door	320 L	1	32,000	32,000
pH meter	–	1	4000	4000
Temperature and humidity meters		15	1000	15,000
Ozone generator with ULPA (ultra-low particulate air) filter	2 g/hr	1	36,000	36,000
Air curtains	2 feet length	4	7200	28,800
Hot air oven	18*18*18	1	18,000	18,000
Grain cleaning machine		1	10,000	10,000
Reefer van		1	250,000	250,000
Miscellaneous items				100,000
Total cost				1,837,470

Table 6 Chemicals and other miscellaneous items required cost analysis

Chemicals and raw materials required			
Chemicals required	Quantity (kg/month)	Unit cost (Rs)	Total cost (Rs)
Calcium carbonate power	50	2	100
Calcium sulphate (kg)	200	25	5000
Spirit/sanitizer/alcohol	21	100	2100
Agar-agar	1	1750	1750
Glucose	1	500	500
Malt extract	1	3000	3000
Potato	10	20	200
Sodium hydroxide	1	500	500
Hydrochloric acid	1	200	200
Hypochlorite	10	40	400
Yeast extract (kg)	1	1500	1500
Misc chemicals	1	5000	5000
Grains	300	60	18,000
		Total cost per month	38,250
		Total cost per annum	459,000

Table 7 Cost analysis of other requirements in spawn lab

Other requirements (per annum)		
Item	Cost (Rs)/month	Cost/year
Various types of glassware	25,000	25,000
Plastic wares	20,000	20,000
Consumable plastic wares	20,000	20,000
Stationery	10,000	120,000
Accessory items in the lab	20,000	240,000
Misc items	10,000	120,000
	105,000	545,000

Table 8 Cost analysis of labour charges in the maintenance of spawn lab

Labour required and cost				
	No. of person	**Salary/ month**	**Total salary**	**Salary/ annum**
Number of unskilled labour	7	10,000	70,000	840,000
Skilled labour	2	20,000	**40,000**	480,000
				1,320,000
Electricity consumption costs				
	Unit/month	**Cost/unit**	**Cost/month**	**Cost/year**
Electricity cost	7000	7	**49,000**	588,000

Table 9 Profit analysis of spawn lab

Profit analysis	
Land (available)*	3,803,600
Construction and infrastructure (I)	1,837,470
Equipment (II)	7,773,000
Total (I + II)	**9,610,470**
Others	
Chemicals (III)	459,000
Other ingredients (IV)	545,000
Labour (V)	1,320,000
Electricity charges (VI)	588,000
Total recurring charges	**2,912,000**
Project cost	
Interest + depreciation on infrastructure	312,370
Interest + depreciation on equipment	1,554,600
Ingredients	**2,912,000**
Total cost per annum (Rs)	**4,778,970**
Earnings from sale of spawn (Rs) (output*cost)	10,630,400
Net profit (Rs)	**5,851,430**

*Space needed for a moderate spawn farm

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Production, Cost Benefit Analysis and Marketing of Oyster Mushroom

G. Gayathri, S. Gomathi, V. Ambikapathy, A. Panneerselvam, and S. Babu

Abstract

Oyster mushroom is one of the edible mushrooms. It comes under the class Basidiomycetes. Mushrooms are defined as a fleshy fruiting body of macroscopic filamentous fungi. Now environmental condition has produced lot of waste materials by agricultural production; that kind of agro-waste materials are recycled by mushroom cultivation process. In this chapter, we have discussed about oyster mushroom cultivation business plan opportunity, morphology, and its species list; the industrial level small and large scale production of oyster mushroom step by step methodology; and finally, oyster mushroom marketing, uses, and its cultivation profits.

Keywords

Oyster mushroom · Production · Marketing · and Benefits

1 Introduction

The current food and agriculture system is considered environmentally unsustainable due to its substantial emissions, pollution, and resource consumption. Alternative food systems that ensure the well-being of people and the environment have been put forward (Campbell et al., 2017; Kloppenburg et al., 1996), which call for improvements in the environmental sustainability compared to the mainstream systems. These can come from extensive and small scale farming, local food production, short supply chains, and circular economy (Forssell & Lankoski, 2015; Kiss et al., 2019; Erica et al., 2020). One of the most popular businesses

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nowadays is food business. Food business, especially horticulture, is currently aimed to strengthening food self-sufficiency, increasing public incomes, and improving nutrition through food diversification. Consumption of horticultural products, such as mushrooms, has become attractive business in agricultural sector due to its simplicity and flexibility of cultivation and profitability. Having investigated various possible agricultural products, it was found that the growing and marketing of health and gourmet food, in particular gourmet mushrooms, is most promising.

Mushroom continues to increase in line with increasing population, incomes, and public knowledge about nutrition and health. This is the reason that right time for horticulture farming needs serious attention, especially in the aspects of production and development of marketing system. Mushrooms are defined as a fleshy fruiting body of macroscopic filamentous fungi. Mushrooms have been used as a nutraceutical, pharmaceutical, and food source all over the world for decades. Mushrooms are classified into four categories: edible, medicinal, poisonous mushrooms, and others for those mushrooms whose properties remain non-well-established. They are part of the phylum of fungi called Basidiomycota, which is a phylogenetic sister group of Ascomycota, in which truffles are included. These two phyla are part of a wide category called "higher fungi." The high concentration of vitamins, minerals, and proteins in mushrooms makes them ideal as nutritional foods (Sandargo et al., 2019; Ahlborn et al., 2019; Bakratsas et al., 2021).

Mushroom is an exotic and nutritious source of vegetarian food. It is a major horticulture product all over the world and is also becoming popular in India. Fresh mushrooms have very limited shelf life, but dried and packed mushrooms have considerable shelf life. During the COVID-19 situation, the FSMS should be reviewed by all the food business owners or food packaging-related industries where the implementation of different policies should be documented and the effectiveness to be monitored on a regular basis (Chowdhury & Nand, 2021; Montes et al., 2021).

Of all food waste, that produced from the household is the most damaging in terms of environmental and economic impacts. Many efforts have been made to quantify and analyze the reasons for and problems associated with household food waste generation which has led to the development of both technical solutions and behavioral interventions (including education and awareness) to try and reduce its generation (Woolley et al., 2021). Cultivation of edible mushroom is a biotechnological process for waste recycling. The industry of mushroom cultivation uses a wide variety of lignocellulosic waste and is considered a very efficient way to recycle agro-residues and to produce food. It might be the only current process that combines the production of protein-rich food with the reduction of environmental pollution. Cultivation of oyster mushroom has increased tremendously throughout the world because of their abilities to grow at a wide range of temperature and utilizing various agro-based residues.

Oyster mushroom (*Pleurotus ostreatus*) is the second largest commercially produced and important edible mushroom in the world market. Growing oyster mushrooms converts a high percentage of the lignocellulosic substrate to fruiting bodies increasing profitability. It requires a short growth time in comparison to other

edible mushrooms (Pérez-Chávez et al., 2019; Chitra et al., 2018). *Pleurotus* spp., accounting for more than 16% of the mushrooms produced globally, represents one of the most common edible mushroom species cultivated industrially. In Malaysia, they are the most cultivated mushroom species, accounting for more than 90% of the mushrooms produced, and their production increased to 65,000 tons in 2020 (Phan & Sabaratnam, 2012; Amin et al., 2014; Mahari et al., 2020).

2 Business Plan Development of Oyster Mushroom

Mushrooms do not require land or soil in acres for them to grow. This is one of the major reasons why people choose to invest money in mushroom farming. A very small place which is not open to direct sunlight is more than enough to carry out this business. Humidity and a place free from heavy heat conditions will be suitable. A place near kitchen garden or an extra room which has proper ventilation can be productively used.

People always try to use the most unique product of the market and this is irrespective of what kind of product it is. Having this, oyster mushrooms are now gaining more importance leaving back the usual button mushrooms. The demand thus makes it favorable for new business starters. And also, growing them would not require much of science and technology.

Oyster Mushroom Farming is a small scale production process which required (1) Substrate, (2) Spawn, (3) Containers (or) Buckets, and (4) Spray bottle. Industrial level large scale production of oyster mushroom needs lot of requirements and processes.

2.1 Business Opportunity

The mushrooms produced are not restricted to one target place or a country. Since it includes a number of varieties in it, countries which do not have specific variety will import it from the other. Here, there is no statement which says mushroom cannot be grown wherever cultivated. Yes it can be grown, but the cost will be high if there is no natural environment supporting it. This gives chances to export mushroom to countries that do not have the varieties. We can also sell in local market. Apart from selling and exporting mushrooms, you can also go for spawn production and substrate formation.

Spawn Production

Spawn in mushroom farming is like a seed used to initiate a plant's growth. Some small scale industries will not waste their time on production of spawn. This is to save their time and the labor count. They use such opportunities to generate income by supplying spawn for their production.

Substrate Formation

Substrate is the medium on which the mushrooms grow. Opportunities created by small scale industries here in mushroom farming are the outsourcing for assistance in substrate formation. We, as a service provider, can start this by producing perfect substrates for each type of mushroom and thus this service will earn profits.

$$\text{Cost of cultivation} = \text{Total Fixed Cost} + \text{Total Variable Cost}$$

$$\text{Net income} = \text{Total revenue} - \text{Total expenditure for one crop}$$

3 Oyster Mushroom

Oyster mushrooms (*Pleurotus* spp.), which are also commonly known as white rot fungi, abalone, or tree oyster mushroom, are typical saprophytes. The fruiting body of the oyster mushroom (*Pleurotus ostreatus*) can be found on trees or old stumps and form in clusters of shelflike mushrooms. Oyster mushrooms have broad, smooth caps that range in color from tan or brown to white. They have prominent gills on the underside of the cap. Like other mushrooms, oyster mushrooms reproduce via spores released in the air. The spores of the oyster mushroom form in the gills at the tips of structures are called basidia. These clublike structures support the spores until they are ready for release. While they are technically called basidiospores, the term spore is commonly used. Spores are a mushroom's version of seeds and are necessary for reproduction. These microscopic spores are carried by the wind and deposited on the soil, old stumps, and trees. If conditions are favorable for growth, the spore begins to grow until it encounters another spore suitable for mating. The oyster mushroom produces four mating types. When two spores of differing mating types meet, they form a multicellular organism that begins to grow. The oyster mushroom spends the majority of its life cycle in this stage of development. Oyster mushrooms can be grown in the home or garden using a mushroom kit. Indoor kits are self-contained and consist of mushroom mycelium and a growing medium (OECD, 2006).

3.1 Morphology

Fruit bodies of *Pleurotus* are characterized by an eccentric stalk, which may be small or long or even absent; annulus and volva are lacking. The fruit bodies appear like petals of a flower, in clusters or individually. They open up like an oyster shell with the widest margin away from the stalk; therefore, as mentioned earlier *Pleurotus* species are aptly designated as oyster mushrooms. Size varies from species to species and within the same species when cultivated under different climatic and nutritional conditions. Generally, the fruit bodies measure a few to several centimeters in width; the minimum size is about 2 to 3 cm and the maximum around 15 to 20 cm. The margin may be smooth, broken, or slightly serrated or dentated, depending on the species (Rajarathnam et al., 1987) (Fig. 1).

Fig. 1 *Pleurotus* (Oyster mushroom)

Systematic position of oyster mushroom	
Class	Basidiomycetes
Order	Agaricales
Family	Pleurotaceae
Genus	*Pleurotus*

3.2 Species in Oyster Mushroom

Oyster mushroom is regarded as one of the commercially important edible mushrooms throughout the world. It consists of a number of different species including *Pleurotus ostreatus*, *P. sajor-caju*, *P. cystidiosus*, *P. cornucopiae*, *P. pulmonarius*, *P. tuber-regium*, *P. citrinopileatus*, *P. flabellatus*, *P. florida*, *P. eryngii, P. djamor*, and *P. calyptratus*. They thrive on most of all hardwoods, wood by-products such as sawdust, paper, pulp sludge, all the cereal straws, corn and corn cobs, coffee residues such as coffee grounds, hulls, stalks, and leaves, banana fronds, and waste cotton often enclosed by plastic bags and bottles (Fig. 2; Table 1).

4 Oyster Mushroom Cultivation in Small Scale Production

In small scale level, oyster mushroom has been an easy process. It has been done by following steps:

- Select the area to place the containers with substrate and spawn. Cover the room or place with tent or any other sheet to make the place suitable for growth.

Fig. 2 Species list of *Pleurotus*

- Now, mix the substrate with spawn in a correct proportion. Prepare the container to load the mixture in it. Once mixing is done, fill the container with the mix.
- After loading it, spray little amount of water. Monitor the setup as and when we have time to check if the procedures done are working or not.
- The process comes to an end when the farm starts flushing in 3 weeks. Harvest them and use it for the desired purpose.
- Repeat the process by the same steps. We will find a change on the form of increase in production.

Table 1 Species list in oyster mushroom

Species	Strain	Origin of source
Pleurotus sajor-caju	CS-32	Northwest mycological consultants, United States
	H-1	University of Wageningen, Netherlands
	H-2	University of Wageningen, Netherlands
P. pulmonarius	06–1	Zvenigorod Biological Station, Moscow State University
P. ostreatus	38d	Moscow Oblast
	H-8	University of Wageningen, Netherlands
P. djamor	Z1	Russia
P. eryngii	3–1	Korea
	3–2	Russia
	H-6	University of Wageningen, Holland
P. cornucopiae	H-14	Russia
P. calyptratus	C-1	Moscow

- Sell them in the local market or neighbors since it is purely small scale. Don't invest time on marketing and promotion as they may increase the initial capital investment. Make it available to the market by spending moderate amounts for packing and transport.

5 Oyster Mushroom Production in Large Scale

For production in large scale, we will require a lot of elements apart from the basics. Make sure that your capital investment will be sufficient enough to meet all the needs of the firm. Like huge investment, production will also be huge.

5.1 Land

Acres or land will be required to carry out the business for higher quantities of production. Select the land and prepare it for the process. A tent covering an area of land can be used, but remember that this will not allow the use of high tech machines or technologically sound techniques of farming. But, using a building will be suitable for growing it with more new techniques.

5.2 Spawn Preparation (Hsu, 2018)

Spawn production requires a microbiological laboratory style work space, characterized by the following:

- Petri plates or small glass jars, such as baby food jars
- Agar medium such as potato dextrose agar (PDA) medium or malt extract agar (MEA) medium

- Tweezers, needles, and spatulas
- Sterilization flame of alcohol lamp or propane burner
- Parafilm or tape
- Ethanol (200 proof alcohol) for burning and preparation of 70% alcohol as disinfectant
- Plant seeds, such as millet, wheat, sorghum, and rye

Prior to attempting mother culture isolation or transfer, one needs to have prepared media plates for pure culturing. Petri plates or small glass jars such as baby food jars can be used as the media containers.

Potato Dextrose Agar (PDA)

- 200 g of thin slices of potato
- 20 g glucose or 20 g sucrose (table sugar)
- 15 g of agar powder

Boil the potato slices in 500 ml of water until the potato tissue is completely cooked and soft. Strain the 500 ml of potato broth through several layers of cheesecloth.

Add the sugar plus the agar into 500 ml of water. Boil for about 5 min to completely dissolve agar and sugar and keep stirring to avoid burning the material (fungi don't like burned food).

Combine the 500 ml potato broth plus the 500 ml of dissolved agar and sugar to get 1000 ml of PDA (bring up to a 1000 ml volume with additional water if need be), which is ready for sterilization using an autoclave. Alternatively, a pressure cooker may be used to sterilize the medium. In general, 30 minutes at 15 psi is suggested for the sterilization. Be certain to follow the pressure cooker manufacturer guidelines for safety. Let the autoclave or the pressure cooker cool down according to equipment instructions before opening. Do not open when there is positive pressure inside; otherwise, the media may boil over. Let the sterilized media cool down to about 50 °C (122 °F) before pouring into plates or suitable containers, Petri plates, or previously sterilized small glass jars or containers. Alternatively, when using small jars, the media could be placed inside the jars before autoclaving. Hence, the user will avoid the pouring phase, as the containers with media will be ready for use after sterilization. The pouring step should be performed under aseptic conditions and cooled down and solidified under the same conditions. Once inoculated, agar plates used for fungal growth should be sealed by parafilm or tape to prevent possible contamination or getting accidentally opened. If jars are used, they should have metal caps that tightly close their opening. Observe the mother culture plate for cleanness and absence of contamination with bacteria and/or other unwanted fungi. Maintain clean cultures by discarding contaminated plates.

Tissue Isolated for Mother Culture Production

Pure cultures of oyster mushroom can be obtained by tissue culture from desired mushroom fruiting bodies, the basidiocarps. The inside tissue of a fruiting body is exposed by pulling apart the mushroom cap to expose the inside uncontaminated tissues. Then, pluck tiny pieces of the inside tissue using sterile fine-tip tweezers and

transfer onto the agar or gelatin media. Ideally, this is done inside a still-air transfer chamber or a laminar flow hood to avoid airborne contamination. Observe for growth of new hyphae, and when it becomes evident growing out of the transferred mushroom pieces without any contamination, then the new isolate is subcultured onto new growth medium under aseptic conditions. New cultures are assigned unique numbers or codes and maintained as mother cultures (pure cultures). Store the obtained mother cultures at 4 °C (39 °F) and subculture every 6–12 months.

Mother Spawn Substrate Preparation

Millet seed is an excellent substrate for producing oyster mushroom spawn. Seeds should be soaked for about 12 h. Longer soaking causes seed fermentation, lowering seed quality and its suitability for spawn preparation. After draining, transfer the soaked seeds into containers such as the Mason jars or the commercial or homemade spawn bags. Sterilize the seeds inside those containers by heat and vapor pressure sterilization using a pressure cooker. In general, 30 minutes at 15 psi is suggested for the sterilization. Optionally, though it may not sufficiently reduce microbes on the seeds, it can also be prepared by boiling with a small amount of gypsum added during the boiling process. In this case, boil the seeds for at least 60 min. All kinds of glass containers can be used, such as narrow neck bottles and wide mouths jars. In all cases, provision must be made to allow gas exchange for the growing mycelium inside the container and be secure against contamination. Widemouth glass jars with a hole driven through their metal lid and plugged with cotton for gas exchange are a good choice for spawn preparation.

The sterilized seeds, which are the substrate for the oyster mushroom culture to grow on to produce spawn, should be inoculated with mycelium from a pure mother culture under aseptic conditions to prevent contamination and produce clean, healthy spawn. The aseptic, germ-free condition can be created by working under a laminar flow hood or inside an airtight transfer room with strict measures of keeping out all sources of contamination. For the home-based working environment where obtaining these conditions is difficult, having a clean work environment is the key to success.

Mother Tissue Inoculation to Spawn Substrate

The metal transfer tool needs to be sterilized immediately before each transfer with their tip heated red-hot in a flame from an alcohol lamp or a burner. It is highly recommended that operators should wear gloves and manipulate the culture transfer and inoculation deep inside a transfer hood.

Use clean, healthy growing cultures that have just filled the surface of a small Petri plate (60 mm diameter) or ¼ of a large plate (100 mm diameter) to inoculate 400–450 g substrate.

The inoculum (mother culture) in this case is a healthy, non-contaminated, actively growing culture of the desired strain growing on agar or gelatin. Use a wide blade surgical scalpel or equivalent tool to cut the agar culture plate. Heat, sterilize, and then cool down the blade before cutting the agar culture plate. Cut the fungal mycelium and the agar into small cubes. Aseptically transfer those cubes on

to the previously sterilized seeds inside the spawn container. Shake the inoculated spawn container to get the culture pieces distributed inside the substrate, establish growth centers inside, and promote rapid colonization of the substrate. Incubate the inoculated spawn containers at 23 °C (73 °F) in darkness. A clean cardboard box can be used as an incubation container at a room temperature of 23–25 °C. The spawn containers can also be protected from light by wrapping with aluminum foil. The incubation period lasts until complete colonization of the substrate has occurred (around 10 to 20 days). The mature spawn should then be stored in darkness in a refrigerator at 4 °C (39 °F) until used; however, cultures of tropical strains of *Pleurotus* should not be refrigerated. The stored spawn can last for many months unless it dries out or gets contaminated.

5.3 Bed Preparation

Substrate Preparation

The mycelium of *Pleurotus* is saprophytic in nature and it does not require selective substrate for its growth. A large number of agricultural, forest, and agro-industrial by-products are useful for growing oyster mushroom. These by-products or wastes are rich in cellulose, lignin, and hemicellulose. However, yield of oyster mushroom largely depends upon the nutrition and nature of the substrate. The substrate should be fresh, dry, free from mold infestation, and properly stored. The substrates exposed to rain and harvested immature with green chlorophyll patches inhibit the growth of *Pleurotus* mycelium due to the presence of competitor molds. Oyster mushroom can utilize a number of agro-wastes including straws of wheat, paddy and ragi, stalks and leaves of maize, jowar, bajra and cotton, sugarcane bagasse, jute and cotton waste, dehulled corncobs, pea nut shells, dried grasses, sunflower stalks, used tea leaf waste, discarded waste paper, and synthetic compost of button mushroom.

There are various methods to kill undesirable microorganisms present in the straw to favor the growth of *Pleurotus* mycelium.

Steam Pasteurization

In this method, prewetted straw is packed in wooden trays or boxes and then kept in a pasteurization room at 58–62 °C for 4 h. Temperature of the pasteurization room is manipulated with the help of steam through a boiler. Substrate after cooling at room temperature is seeded with spawn. The entire process takes around 3–5 days. This method is adopted on a commercial scale by Zadrazil and Schneidereit in Germany. There are various minor variations of this method adopted in Europe.

Hot Water Treatment

The substrate (wheat straw) after chopping (5–10 cm) is soaked in hot water (65–70 °C) for 1 h or 60 to 120 min at 80 °C or in case of paddy straw at 85 °C for 30–45 min. After draining excess water and cooling, spawn is added. Hot water treatment makes the hard substrate like maize cobs, stems, etc. soft so that the growth of mycelia takes place easily. This method is not suitable for large scale commercial cultivation.

Chemical Sterilization Technique

Various species of *Trichoderma*, *Gliocladium*, *Penicillium*, *Aspergillus*, and *Doratomycs* spp. are the common competitor fungi on the straw during oyster mushroom cultivation. If present on the straw during spawn run, they do not allow the growth of mushroom mycelium resulting in yield loss or complete crop failure. When wheat straw or paddy straw is treated by steeping in a chemical solution of carbendazim 50% WP (37.5 ppm) and formaldehyde (500 ppm) for a period of 16–18 h, most of the competitor molds are either killed or their growth is suppressed for 25–40 days after spawning. The technique, which was standardized at DMR, Solan by Vijay and Sohi in 1987, is as follows: Ninety liters of water is taken in a rust proof drum (preferably of galvanized sheet) or G.I. tub of 200 liters capacity. Ten to twelve kg of wheat straw are slowly steeped in water. In another plastic bucket, Bavistin 7.5 g and 125 ml formaldehyde (37–40%) are dissolved and slowly poured on the already soaked wheat straw. Straw is pressed and covered with a polythene sheet. After 15 to 18 h, the straw is taken out and excess water drained. One can use a larger container or cemented tank of 1000–2000 L for soaking more straw. The chemicals to be added can be calculated as above. The chemical containing water can be reused once again for pasteurization of the straw.

5.4 Cultivation

Spawning

Freshly prepared (20–30 days old) grain spawn is best for spawning. The spawning should be done in a pre-fumigated room (48 h with 2% formaldehyde). The spawn should be mixed @ 2 to 3% of the wet wt. of the substrate. One bottle spawn of 300 g is sufficient for 10–12 kg of wet substrate or 2.8 to 3 kg of dry substrate. Spawn can be mixed thoroughly or mixed in layers. Spawned substrate is filled in polythene bags (30 × 60 cm) of 125–150 gauze thickness. Ten to 15 small holes (0.5–1.0 cm dia) should be made on all sides especially in the bottom for leaching of excess water. Perforated bags give higher and early crop (4–6 days) than non-perforated bags because of accumulation of high CO_2, which inhibits fruiting. One can also use empty fruit packing cartons or wooden boxes for filling substrate. Polythene sheets of 200–300 gauze thickness are spread in rectangular wooden or metal box, spawned substrate is filled, and the polythene sheet is folded from all the four sides to make a compact rectangular box. It is tightly pressed and tied with a nylon rope. The block is incubated as such, and after mycelium, growth polythene sheet is removed. The spawned bags or blocks are kept in incubation room for mycelial growth.

Incubation

Spawned bags can be kept on a raised platform or shelves or can be hanged in cropping room for mycelial colonization of the substrate. Although mycelium can grow between 10 and 30 °C, but the optimum temperature lies between 22–26 °C. Higher temperature (more than 30 °C) in the cropping room will inhibit the growth and kill the mycelium. Daily maximum and minimum temperature of cropping

rooms and beds should be recorded. The bed temperature is generally 2–4 °C higher than the room temperature. Mycelium can tolerate very high CO_2 concentration of 15–20%. During mycelial growth, the bags are not to be opened and no ventilation is needed. Moreover, there is no need for any high relative humidity, so no water should be sprayed.

- Mycelial growth of all the *Pleurotus* spp. can take place between 20 and 30 °C. However, for fruiting different species have different temperature requirement.
- All the *Pleurotus* species require high relative humidity (75–85%) during fruiting.
- Unlike green plants, mushrooms do not require light for the synthesis of food. They grow on dead organic plant material. Light is required to initiate fruit body initiation.
- The optimum pH during mycelial colonization should be between 6.0 and 7.0, while the pH of the water for spraying should be neither too acidic nor alkaline.

Pests and Diseases

Although the mushroom itself is a fungus, it can in turn be affected by a range of fungal pathogens, bacterial diseases, viral diseases, and insect pests.

5.5 Harvesting

Fruit Body Induction

Once the mycelium has fully colonized the substrate, it forms a thick mycelial mat and is ready for fruiting. Contaminated bags with mold infestation should be discarded, while bags with patchy mycelial growth may be left for few more days to complete the spawn run. In no case, bags should be opened before 16–18 days except in case of *P. membranaceus* and *P. djamor* var. *roseus*, which forms fruit bodies within 10 days even in closed bags from small holes. Casing is not required in oyster mushroom cultivation. All the bundles, cubes, or blocks are arranged on iron/wooden platforms or shelves with a minimum distance of 15–20 cm between each bag in the tier. They can also be hanged.

5.6 Packing and Storage

Mushrooms should always be harvested before water spray. The right stage for picking can be judged by the shape and size of fruit body. In young mushrooms, the edge of the cap is thick and cap margin is enrolled, while the cap of mature mushroom is flat and inward curling starts. It is advisable to harvest all the mushrooms at one time from a bag so that the next crop of mushrooms starts early.

Another step is precooling where the product is kept in a plastic bag and stored in cooling unit. Vacuum cooling is another cooling system where water existing in cell walls and inter hyphal spaces of produce is evaporated under pressure which lowers the temperature. But it is cost-oriented system and involves inevitable loss of fresh weight.

After harvesting, lower portion of the stalk with adhering debris should be cut using a knife. Stipe is kept short or almost nonexistent, as it is hard and not liked by many consumers. Packing is essential to protect the mushroom during marketing. It is generally packed in polythene bags of 250–400 gms for local markets. They can also be sun-dried by spreading on a cotton cloth in bright sunlight or diffused light. The dried product with 2–4% moisture can be stored for 3 to 4 months after proper sealing. For long distance transport, pulp board punnets wrapped with PVC films should be used instead of polythene bags. Sometimes, precooled mushrooms are packed in insulated containers having ice in it, so that mushrooms remain fresh, healthy during long transport. (Singh et al., 2011).

Preservation Methods

Due to highly perishable nature, the preservation of mushrooms is necessary to minimize the post-harvest losses. For this, the processing techniques such as Canning, Individual Quick Freezing (I.Q.F.), Vacuum Freeze Drying (VFD), Drying, Vacuum Drying, Pickling, Steeping Preservation, Radiation Preservation, etc. have been developed. These are used on the basis of their market demand and end use.

Canning

Canning is an established process of preserving mushroom pieces in brine, butter, oil, vinegar, etc. It involves six basic operations like cleaning, blanching, can filling, sterilization, cooling, and labeling. Through all these operations, mushrooms are graded, cleaned, blanched (precooked), filled in brine solution of cans, and ultimately sterilized with heat and cooled through water spray and labelled for storage. Canned mushrooms form major share of world trade.

Individual Quick Freezing

Individual quick freezing is another popular processing method followed in large industrial units. In this process, raw materials are washed at processing units after receipt from farms, and then the mushrooms are inspected, sliced, and graded according to quality. After that, blanched and water cooled mushrooms are subjected to tunnel freezing stage.

Vacuum Freeze Drying

Vacuum freeze drying (V.F.D.) is a further development in mushroom processing technology. In this process, the original shape, quality, color size, texture, and freshness properties of thermal-sensitive produce are retained. This processing technique involves the cooling of mushroom much below the freezing point, i.e., –40 °C where moisture present in mushroom is converted to tiny ice molecules which further directly sublime into vapor when subjected to vacuum with a slight rise in temperature and results in a dried end product being obtained.

Drying

Drying is the age old practice of preserving mushrooms at ambient temperatures. With the advancement of technology, different kinds of dehydration processes have

been developed, e.g., Sun drying, mechanical drying, air drying, microwave oven drying, etc. Among these, the microwave oven drying is the best method.

Vacuum Drying

In this process, the produce is subjected to a vacuum drier in which steam is present at about 1.0 to 1.5 bar. A vacuum pump is used to reduce pressure inside the product. The end product is obtained after completion of vacuum drying process.

Pickling

Pickling of mushroom is also a popular method of preserving. It is a more economically viable way during the surplus periods. By this process, mushrooms and different spices/condiments according to preference are fried separately. After that these are allowed to cool and filled in bottles/containers.

Steeping

In case of steeping preservation, mushrooms are preserved by steeping in a solution having 10–12% salt, whereas the preservation of mushrooms is done by radiation with Gamma rays at the dose of 100–150 Krad to stop the post-harvest growth and discoloration/deterioration of mushrooms.

5.7 Marketing

Grading of mushrooms is important for marketing. Generally, the grading is done by segregation of mushrooms into various grade standards as per market demand. For example, button mushrooms are graded into Grade A, B, and C. The Directorate of Marketing & Inspection (D.M.I.) had formulated the grade standards for dried edible mushrooms as Mushrooms Grading and Marking Rules, 1972 under the Agricultural Produce (Grading and Marking) Act, 1937.

Marketing is the most important consideration of all. If you can't sell your mushrooms at a price that ensures a reasonable profit margin, you don't want to invest in this enterprise. Spend some time and even some money educating yourself about marketing your potential product. Market research and evaluation is perhaps the most challenging part of developing a new enterprise.

Large, well-established companies produce virtually all *Agaricus* mushrooms; most are located in Pennsylvania and California. Their production houses are full of mushrooms in every stage of development. Mushrooms raised in these systems can be sold profitably on the wholesale market. It is very difficult for a beginning grower to compete with these companies at wholesale price (Rawat et al., 2020; Beetz & Kustudia, 2004).

Explore as many marketing strategies as appeal to some possibilities. Market the fresh or dried product directly to your customers (at farmers' markets, to gourmet chefs, over the Internet, through mail-order offerings). Add value to the mushroom by creating processed products.

In International level, 20 million Chinese farmers are producing the bulk of all globally grown gourmet mushrooms at very low costs. As an Agri-product, gourmet mushrooms are not older than 30 years. On an industrial scale, gourmet mushrooms are only now emerging as an industry. Every larger producer is developing his own technique and the know-how is guarded. Larger producers are being established in our target market as well as in China. In the West, the Nederland is leading the way. A descriptive method and t-test were employed to analyze the differences in the average cost, income, profit, and benefit-cost of farming (Nur Rahmawati & Marbudi, 2021).

Normally, there are two feasible ways, given below:

1. Buy back agreement
2. Self-marketing

Buy Back Agreement

A Buy back agreement is an agreement done on stamp paper (recommended), by private institutes, where a mushroom grower and institution sign an agreement where the institution promises to buy total quantity of mushroom from the supplier or grower, with a promise of getting the entire quantity sold, after the sale, return the profit to the grower after deducting the previously decided profit margin by the institution. Generally, buy back agreement is done by those institutions, where the grower undergoes training of mushroom cultivation, or purchases spawns, etc. Therefore, it is practically advisable that one should go for buy back agreements from such institutions only. Beginners may find it a challenge in growing without training since a minor mistake in the overall process can negatively impact mushroom growth, hence undergoing training from the relevant institutions is highly recommended.

Self-Marketing

Self-marketing involves entire process of taking out mushroom from the cultivation point to taking them to market place and complete the sale process. For selling, one should ensure that the marketplace or Mandi is near to the production area, otherwise it will lead to rise in cost incurred. In order to increase chances of selling the entire quantity produced in short duration, say a month, it is advisable that one should select 5–10 packets (each packet can be 200–250 g) and give to nearest vegetable vendors who are having good number of customers and who are selling mushrooms as well. Here it is worth noting that better don't take any money initially from the retailer. Instead, that money can be given once the packets are sold. Under this situation, the vendor shall hand over the sale amount to the grower, after subtracting his profit margin.

Always remember that we need to create a market for ourself first. This act will not only enhance trust and goodwill in the mind of the retailer, but increase the interest to accept the packets willingly since we do not have to pay any amount initially. Also, in case there is more than one retail/vegetable shop near your house, then prefer distributing the packets in different shops so as to increase the chances of

sale on the same day. In case few packets are not sold on a particular day, then take it back, dry it and sell as vegetable or make pickle from it and sell it again. This eliminates the chances of mushroom packets getting wasted. So the risk for incurring losses for the unsold packets is almost eliminated and still one is able to get some profit when sold as dried mushroom or as mushroom pickle.

5.8 Post-harvest Management (Fig. 3)

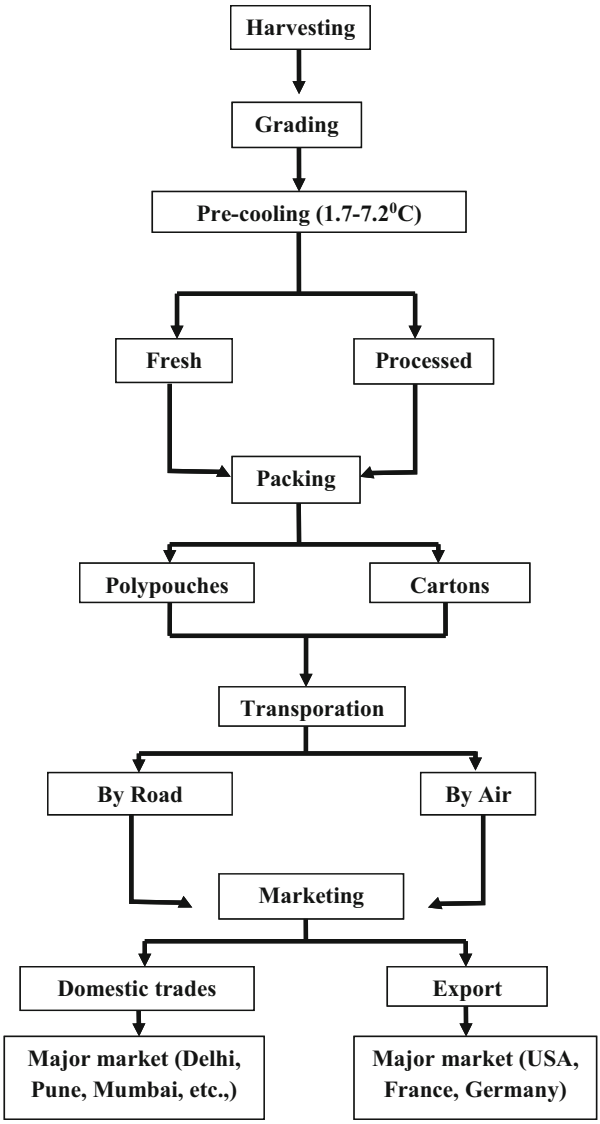

Fig. 3 Post-harvest process of mushroom

6 Uses of Oyster Mushroom

Oyster mushrooms are 100% vegetarian and the nutritive value of oyster mushroom is as good as other edible mushrooms like white button mushroom (*A. bisporus*), shiitake (*Lentinula edodes*), or paddy straw mushroom (*Volvariella* spp.).

They are rich in vitamin C and B complex. Protein content varies between 1.6 and 2.5% on fresh weight basis. It has most of the mineral salts required by the human body such as potassium, sodium, phosphorus, iron, and calcium.

The niacin content is about ten times higher than any other vegetables. A polycyclic aromatic compound pleurotin has been isolated from *P. griseus*, which possesses antibiotic properties.

Using of vegetables waste like faba bean is potential for achieving complete circular use of agro-waste and has implications for development of production systems well-suited in the bio-based society (Ivarsson et al., 2021; Rosdiana et al., 2018).

The beta-glucans in oyster mushrooms make them one of the best foods on the planet for protecting our immune system against short- and long-term illnesses.

Unlike some foods that either stimulate or repress the immune system, mushrooms balance it.

Plus, oyster mushrooms are loaded with other antioxidants to help prevent free radical damage and oxidative stress so that our immune system can defend itself against aging.

Since mushrooms like oysters have a savory flavor and no cholesterol, they're a great substitute for meat in many sautéed dishes.

One study also found that consuming oyster mushrooms reduced triglycerides and cholesterol levels in diabetic patients (as well as high blood sugar levels).

One study found that dietary mushrooms like oysters reduced blood pressure in rats with spontaneous or unexplained high blood pressure.

Oyster mushrooms have plenty of important nutrients for building strong bones; specifically, vitamin D and magnesium.

While most people focus on calcium, your body also needs vitamin D and magnesium to process calcium and store it in your bones.

The beta-glucans and antioxidants in oyster mushrooms make it a great food for lowering inflammation.

Some research shows that beyond beta-glucans, some of the oyster's anti-inflammatory properties come from a unique and somewhat unknown amino acid called ergothioneine.

According to the research, ergothioneine lowers "systemic" inflammation across the entire body which often contributes to diseases like dementia and diabetes.

The beta-glucans in mushrooms like oysters function as potent antioxidants that may protect the body against cancer.

One study found that oyster mushrooms have potential to be effective for certain types of cancer cells.

Ergothioneine is also an excellent amino acid for protecting the heart because it prevents the build-up of plaque. Perhaps this is also why oyster mushrooms are able to lower cholesterol levels.

Finally, the amino acid ergothioneine is a cytoprotectant. In other words, it protects cells against oxidative stress and free radical damage.

That's why mushrooms like oysters have potential for defending against neurodegenerative diseases like Alzheimer's, Parkinson's, and dementia.

Oyster mushrooms are considered safe to be consumed and are very easily cultivated. It is full of various kinds of therapeutic effects (Titas et al., 2019).

7 Profits in Oyster Mushroom Cultivation

- Adopt methods which will increase production. Search for new methods in your locality so that you don't have to invest more to install them. Clearly study about the method to avoid wrong procedure being done.
- Mushrooms require a particular temperature to grow. Thus, fix them accordingly to avoid mismatch between the needs of the mushrooms and your setup conditions. Oyster mushroom requires an optimum temperature of 22–26 °C.
- Utilize the space at its maximum. This will lead to enormous amounts of production since every single unit of the place is productively used. Stack them in a vertical setup to save space and to reduce maintenance costs.
- Use manure to speed up the growth process if needed. Fast growth will reduce the break in between production.
- Sell them at a reasonable price to capture the market. Once we are fixed as a qualified producer, an increase in our price will not matter to our customers.
- Have your production house at a location where markets are easily accessible. This is to reduce your transport costs.
- Avoid middlemen in your business to enjoy more profits. Giving it at a lower price to the middlemen will reduce your margin. Involve the direct supply of mushrooms to your customers.
- Install all the required amenities in your place itself. Do not outsource things in case of a large scale business. Have your own production methods and preparation techniques.
- Choose a right packaging method, but not an expensive one. Since these are perishable items, use and throw packs are more than enough. Don't spend more on fancy items which will increase your cost of production (Table 2).

8 Conclusion

On considering all the above, oyster mushroom farming stays as one among the businesses which will be a life saver for people who are waiting to use their money in a profitable way. Production of mushroom every 3 weeks shows the early income generation feature of mushroom farming. Small scale or large scale care management values the most to predict the results of the farm.

Table 2 Cost of production of oyster mushroom (Chitra et al., 2018)

Particulars	Price (₹)
A	**Nonrecurring expenditure**
Building cost	20,000
Sprayer pump (1 no.)	1500
Galvanized tubs (2 no.)	600
Jute Bags 25no's @ Rs.10 per bag + Nylon ropes	500
Total	22,600
B	**Recurring expenditure**
Paddy straw 5 bales @ Rs.300.00	1500
Polythene bags 150 gauge thick of 30 × 60 cm @ Rs.100/kg (2 kg)	200
Cost of spawn Rs.30/packet (20 no's)	600
Formaldehyde 5 L (commercial grade) @ Rs. 40.00/L	200
Miscellaneous	1000
Total	3500
Depreciation (nonrecurring expenditure) @ 10%	2260
Total for 1 year	2260
Total expenditure	5760
C	**Revenue**
From mushroom 95 kg @ Rs. 200 per kilogram	19,000
By-product	1000
Total revenue	20,000
Net profit	**14,240**

India produces about 600 million tons of agricultural waste per annum and a major part of it is left out to decompose naturally or burnt in situ. This can effectively be utilized to produce highly nutritive food such as mushrooms and spent mushroom substrate can be converted into organic manure/vermi-compost. By just diverting 1% of agro-wastes towards mushroom production, India can produce three million tons of mushroom and about 15 million tons of compost. It is to be concluded that this small initiative by the student generation will serve as a role model for others and create awareness among fellow student community.

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Mass Multiplication, Production Cost Analysis, and Marketing of Psilocybe Mushroom

Boomika Nallaiyan, Priyanka Jayam Rajendran, and Dhanasekaran Dharumadurai

Abstract

Psilocybe mushroom belonging to genus of Hymenogastraceae family found all over the world is a gilled mushroom. Psilocybin, psilocin, and baeocystin are hallucinogenic chemicals found in all species. It is also called shrooms and magic mushroom because of hallugenatic effect. The genus *Psilocybe* has hundreds of species. It can be produced from small scale in Laboratory and large scale in both indoor and outdoor production. Psilocybe mushrooms are widely used for medicinal purposes and for food. They are consumed both fresh and dried material. In this chapter, we explain the mass production, marketing, and cost analysis for the production of psilocybe mushroom. They are produced from spore printing and the spores are transferred into both liquid and agar medium. The fruiting substrate in outdoor production is developed from primary and secondary spawn. In indoor production, the beds are grown under artificial temperature and balanced pH. Also, we discussed about marketing and compared recurring expenditure and non-recurring expenditure of all scale production.

Keywords

Psilocybe · Psilocybin · Mushroom · Spawn · Mass production

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1 Introduction

Psilocybe is a hallucinogenic mushroom found in Europe, South America, Mexico, and the United States. Magic mushrooms are mushrooms that contain psilocybin. Popular variety of psilocybin mushrooms is *Psilocybe semilanceata,* which evolves in northern temperate areas. *Psilocybe azurescens*, a highly energy plant ended to Washington and Oregon in the United States, but prominent in outdoor cultivation. Hence, improving its capacity, nicknamed azies. *Psilocybe cyanescens,* fruiting normally occurs between October and February. It is well-known in the United States and is primarily found in the Pacific Northwest, south to the Bay Area of San Francisco. This chapter is going to explain about psilocybin cubensis mass multiplication, production, and marketing. *Psilocybe cubensis* is the most extensively farmed and consumed psilocybe mushroom, due to its ease of growth and huge fruit bodies. Franklin Sumner Earle, an American mycologist, initially described the species as *Stropharia cubensis* in Cuba in 1906. The surface of the cap is smooth and sticky, with white universal veil remnants clinging occasionally. With age, the crown fades to a golden-brown or yellowish colour, becoming paler to virtually white towards the margin. It is also commonly gathered across the tropics and subtropics, earning it the nickname commercial *Psilocybe cubensis, which* grows on cow dung, rarely horse dung, sugar cane mulch, or rich pasture soil with mushrooms emerging in the southern hemisphere from November to April and in the northern hemisphere from February to December. The top cap is 1.6–8 cm (Musshoff et al., 2000). when fresh; the cap is conic to convex with a central papilla, then extends to plane with the generation with a little umbo often ringed by a ring-shaped depression hollow white stipe that is 4–15 cm (2–6 in) tall by 0.4–1.4 cm (0.2–0.6 in) thick, and it becomes yellowish with age (Stamets, 1996) (Fig. 1).

2 Life Cycle of Psilocybe Mushroom

The basidium spores are produced in sexual reproduction. The cap of mushroom contains basidiospores. In the basidium, the nuclei of two different mating strains unite (karyogamy), forming a diploid zygote that subsequently proceeds through meiosis. Basidiospores are formed by the haploid nuclei, which germinate and create monokaryotic hyphae. A major mycelium is the outcome result of this process. Different mating strains' mycelia can combine to generate a secondary mycelium with haploid nuclei from both mating strains. The major stage of the basidiomycete's lifecycle is the dikaryotic stage (Oss & Oeric, 1992).

Sexual reproduction plays an important role in pathogenic development in many fungal species, increasing genetic variety, adaptability to changing environments, and survival in long term (Nicholas & Ogamé, 2006). (Fig. 2).

a) b)

Fig. 1 *Psilocybe* Mushroom in Grass land, Palani Hills, Tamil Nadu, India. (**a**) *Psilocybe cubensis*. (**b**) *Psilocybe* sp.

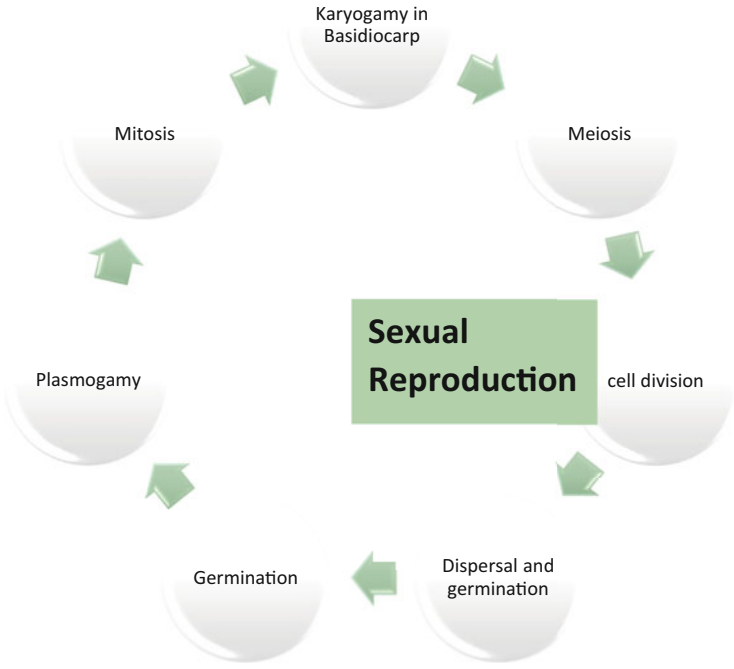


Fig. 2 Life cycle of *Psilocybe* mushroom

3 Isolation and Spore Print Production

Spores are like a seed for fungus; this chapter explains in detail how to make the spore print and how to use spore print in spawn production. Reproductive unit of the fungi is spores.

Spore Print Making Process
The method of developing a spore print is quite straightforward. The vital aspect is to print spore without contamination. Freshest cap mushroom is the first choice. These should be mushroom with recently opened caps and a cap margin that isn't entirely stretched. Spore print can be made on a number of surfaces, such as black and white paper, glass slide, and so on. Lightweight and cheap aluminium foil is vastly used. It is important to maintain humidity (Stamets, 1996).

Step Involved in Spore Print Formation
Using ethanol, clean the work station and materials. Cut a piece of clean aluminium foil to size and lay it flat. Sterilise the blade and cut the cap of mushroom carefully as quickly as probable (Ghouled, 2007). Before using the blade, use the fire lighter to flame the blade's edge. Spot the cap of the mushroom on the foil. Lift the mushroom top from the bowl with care. For a good dark print, let it set for 6 to 12 h. Slowly raise the mushroom cap from the cup/bowl. Enclose the print with the cup/bowl and allow it dry for another 6 to 12 h. Use the foil envelope method for folding and storing. Create an envelope out of the second sheet of aluminium foil by folding it in half and then double folding the edges. While storage, this envelope will maintain the foil with the print clean (Stamets, 1996).

4 Spawn Production

4.1 Spore Print Is Transferred to Agar Medium

Spore print is transferred to agar medium. Mushroom cultivators use agar to evaluate the growth of mycelium. Water, nutrients, and a dry material are mixed together to make agar medium. For cultivation of the mycelia of high fungi, Standard solid nutritional media such as Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) are recommended (Table 1).

Table 1 Media preparation

Potato dextrose agar (PDA)	Malt extract agar
Peeled potato—200 g	Malt extract—20 g
Agar—20 g	Agar—20 g
Dextrose—20 g	Dextrose—20 g
Distilled water—1 L	Distilled water—1 L

Procedure
The media is prepared and placed in a petri dish to solidify. Mushroom culture can commence once a spore print has been obtained. Hold an inoculating loop over a lamp flame to sterilise it. Insert the tip into sterile media in a petri dish to cool it down, then scrape few spores off the print. The spores are transferred by streaking the tip of the transfer tool across the agar surface. Use parafilm to seal the new plate's edge. After few days, the mycelium growth is observed.

4.2 Cardboard Disc Spore Germination

Mycelium germinated in cardboard disc.

Procedure
Sterile cardboard is used as inoculation disc and malt-yeast solution in tubes. Malt yeast inoculated cardboard disc tube for extraction. Inoculate the spore, after some days a spore cluster is formed on the disc's edge. On the surface, spores began to germinate followed by a complete colonisation. After transferring a colonised spore disc to an agar plate with peroxide, spore colonised on the plate (Nicholas & Ogamé, 2006).

4.3 Tissue Sample Is Transferred to Agar

Tissue sample from the fresh mushroom is transferred to agar plate in order to grow the primary inoculum for spawn production.

Procedure
Use ethanol to sterilise the mushroom's outer surfaces. Cut and split the mushroom to expose the sterile mycelium beneath the surface. Take a piece of mycelium from the stipe's inside. Place the mycelium portion on an agar plate. Mycelium starts populating an agar plate from a tissue piece. After that, grown mycelium is transferred to grain for producing spawn.

4.4 Spore Print Is Transferred to Syringe

Spore syringes are now widely available, and while they do not preserve spores as effectively as spore prints, they are useful for beginners learning about mushroom growing.

Procedure
The first step is to sterilise the water in a tiny bottle and close the bottle with tinfoil. Place the bottle in the cooking pot for 15 to 20 min or until it reaches 15 psi. Heat the scalpel and use a set of tweezers with the gas burner to sterilise them until they tune

brilliant red colour. Remove them from the fire and allow them for 20 or 30 s to cool completely. Carefully separate the spore print with the tweezers, then use water to remove the tinfoil from the flash. Scrape some of the spores from the print using a scalpel so that they fall into the water. The spore containing water is filled into the syringe. The syringe cannot be used right away after being filled. In order for the spores to hydrate in the water, they need time. Incubate at room temperature at least for 24 h, but ideally 2–3 days. At 2–4 °C for 2–6 month, the spore syringe must be stored in the refrigerator. Place them in an airtight zip baggie to keep them fresh.

Preparation of Spawn
The most common substrate is wheat grain. The steps to prepare mother spawn are as follows: soak the wheat overnight, and slightly boil it. After boiling, place the grain on a sieve to dry. 20 g of calcium carbonate is added to 1 kg of boiling wheat grain. Fill the jar or polypropylene bags with the substrate (300 g) and make two holes. One hole for inoculating liquid mycelium and another hole for aeration. The hole is closed with a non-absorbent cotton plug. These filled bags or jars are autoclave-sterilised for 90–120 min at 121 °C. The bags are placed in a laminar airflow chamber to cool after sterilisation.

Preparation of Spawn from Agar Medium
Inoculate spawn bag with agar; first make sure to spray and wipe down the bag with ethanol. Open the top of the bag and give it a quick mix; next, with a sterile blade cut a few chunks of agar and then drop them into the spawn bag, then try to mix the agar chunks down into the bag, finally seal the bag then wait for bag to fully colonise, and after 2- to 3-week, mycelium colonised on the spawn bag. Now spawn is ready to produce entire psilocybe mushroom.

4.5 Preparation of Spawn from Syringe

Mycelium has been grown on liquid medium in syringe which is injected in the jar. After inoculation, for 2–3 weeks, these bags or bottles are maintained at room temperature. White mycelium from the mushroom colonised the wheat grain. This is the mother spawn, which is utilised to produce more spawn. (Nicholas & Ogamé, 2006).

5 Mass Production of *Psilocybin Cubensis*

5.1 Lab Scale Production of *Psilocybe Cubensis*

Mono Tub and Substrate Preparation
It is time to create mono tube in 66 quarts after the spawn has been produced. Using a sterilised tub with a clear lid, drill ten 1-in. holes around the tub, 4 in. from the bottom on the long side and 2 in. from the top on the short side. After that, carefully

drill the holes in the mono tub and create a liner. It's critical to use a liner to avoid side pinning. To get the substrate ready, 500 g of vermiculite, 500 g of coco coir, and 100 g of gypsum were used. After adding all three components, pour boiling water over the substrate in the bucket and seal it. Bulk substrate should be cooled and readied to be used after 24 h. It's now time to colonise the mono tube with spawn.

Inoculation of Spawn

Isopropyl spray and clean down the mono tub, liner, and red plugs. For the colonisation stage, apply tape to close all the holes with red plugs. Then, at the bottom, place the liner. Put the bulk spawn substrate in a mono tub and then add the substrate. Combine bulk spawn and substrate in a mono tub. Plug all of the holes in the mono tub and close it. Allow for a period of incubation.

Incubation

Keep an eye on the tub on a daily basis to monitor how quickly the mycelium colonises. Remove the red plugs after 80% colonisation and replace with a foam filter or micro-post filter. Begin fanning; this is a delicate operation that aims to reduce humidity and temperature so that the mycelium knows it's time to begin fruiting by introducing fresh air. This is the initial signal to the mycelium that it should begin pinning and fan tubing for 30 s twice a day. Then, over the next 5–10 days, the mushrooms will begin to fruit, and should fan them twice a day. When the mushroom's veil begins to break, it's time to harvest (Stamets, 1996).

Harvesting and Storing

Carefully twist or cut the mushroom away from the substrate, then insert it immediately into the dehydrator. Dehydrate the dehydrator tray for 8 to 10 h at 125 °F. Keep the 66% dried mushroom in jar in a cool, dark place. The foundation in now complete. A second flush has been constructed. (Nicholas & Ogamé, 2006).

5.2 Small-Scale Production in Outdoor

Outdoor mushroom production has several significant benefits over indoor mushroom cultivation. Once grown, an outdoor production bed will bear fruit annually for several years, or until the substrate is depleted. The life of a bed may be nearly indefinitely extended by occasionally adding fresh wood chips to the dampness, or by building a new bed nearby and using part of the previous substrate as inoculum. (Nicholas & Ogamé, 2006) It requires almost no maintenance other than keeping them damp throughout the drier, hotter months of summer. Outdoor cultivation is economical since wood chips are inexpensive and can even be obtained for free at times. Because wood-based substrates are less prone to bacterial and mould colonisation, they may be handled freely without worry of contamination.

Though the substrate colonisation and fruiting phases of outdoor cultivation require little care, the early stages are more or less maintenance-free. P. cubensis cultivation need the same conditions. The cardboard disc technique is used to

germinate spores, and the resultant mycelium is cultivated on petri plates on agar, and then transferred to sterile grain. When grain spawn has been fully colonised, it is utilised to inoculate tiny amounts of substrate made of sterilised wood. This wood-based spawn is used to inoculate a massive wood chip.

Fruiting begins when ambient temperatures fall into the 40 °F range, which occurs from mid-October to early winter in northern North America. Flushes develop every 2 weeks or so, as long as the temperatures remain stable.

Wood Substrate

The "wood-loving" species' mycelium will quickly grow on just about anything made from trees, as long as it is created from hardwoods or softwoods stripped of their aromatic elements, as most paper products. Wood is composed of cellulose and lignin. Lignin molecules are long cross-linked chains of phenolic organic compound. The lignin which gives longevity and wood hardness is chemically stable. Although it is stable, some fungal creatures can break down the lignin and consume it. The caramel-cap Psilocybes belong to a group of fungus known as "lignicolous" or "wood-inhabiting" fungi, which have the essential enzymes to do so. Wood substrates are much easier to work with than grain substrates because of their lignicolous fungus selectivity. Wood-based substrates are inherently resistant to mould and germs, so they do not require sterilisation prior to use.

Germination of Spore

There are many ways to germinate the spore, but (Nicholas & Ogamé, 2006) choose the cardboard disc technique or streaking on non-peroxidised agar should be used to germinate the spores. The cultures should then be moved to peroxide-containing agar for continued usage after germination.

Wood-Based Primary Spawn

Instead of greater quantities of grain, only a sufficient number of grain jars have been manufactured; they should be used to inoculate bags or jars of wood. Because wood-based substrates have a partial selectivity, the sooner you switch from grain to wood, the less likely your cultures will become contaminated.

There are three types of wood-based substrates for the good growth:

Primary spawn: The wood spawn which is properly sterilised is inoculated in small amounts from grain cultures.

Secondary spawn: It is produced from primary spawn. So, it does not need to be sterilised.

Fruiting substances: The final generation of material before fruiting is known as the fruiting substrate, and it is generally identical in composition to secondary spawn.

Wood Chips

Small to moderate branches or saplings are perfect, and they're best when picked in the late winter or early spring; the wood chips are best when the foliage emerges and the sugar concentration is at its peak. Wood obtained during other seasons of the year

can be used, but to avoid rotting, all leafy sections must be carefully removed before chipping. When it comes to tree species, Nicholas and Ogamé (2006) discovered both alder and oak perform well. Because alder is a soft hardwood, the fungus colonises it considerably more quickly, making it ideal for immediately establishing a new bed. Denser woods, such as oak, take longer time to colonise, therefore beds created of them will last considerably longer before needing to be replaced. You can make your bed out of a mix of light and heavy woods, or add the harder wood after the softer wood has colonised entirely.

Maple, eucalyptus, birch, cottonwood, Balsam poplar, elm, walnut, beech, hickory, dogwood, aspen, yew, and ash are all appropriate broadleaf species. Other hardwoods will work as well, but, advised to try small amounts first before utilising unproven species to make huge beds. If the fungus appears to develop on the wood, it is fair to conclude that it will fruit there as well. Freshly cut wood chips should be sufficiently wet for usage, and only a quick soaking under the hose is required to prepare them. To wet dried wood chips, immerse them in room temperature water for 1 to 2 days. Once moistened, the chips will settle in the bottom of the container.

Chips can also be sintered in a saucepan of water on the stove for approximately an hour, or until they sink, if you just need a little amount. Before usage, drain well the chips.

Dowels

We can use spiral grooved dowels to make main spawn. The ideal ones are 1 to 2 in. long with a 1/4-in. or 5-in. diameter. They're often constructed of birch and will be labelled as such. The spiral groove around the peg's exterior gives the mycelium with the most surface area from which to leap onto following substrates. Birch dowels are quickly colonised by Psilocybes which are lignicolous and serve as a good source of major spawn material. Small and medium quantities of primary spawn are generated. They are developed in sealed containers. So, it can be developed entirely by Dowels and wood chips alone. Maximum contaminated resistance is getting by producing main spawn from bigger and tougher material. (Nicholas & Ogamé, 2006).

Sawdust

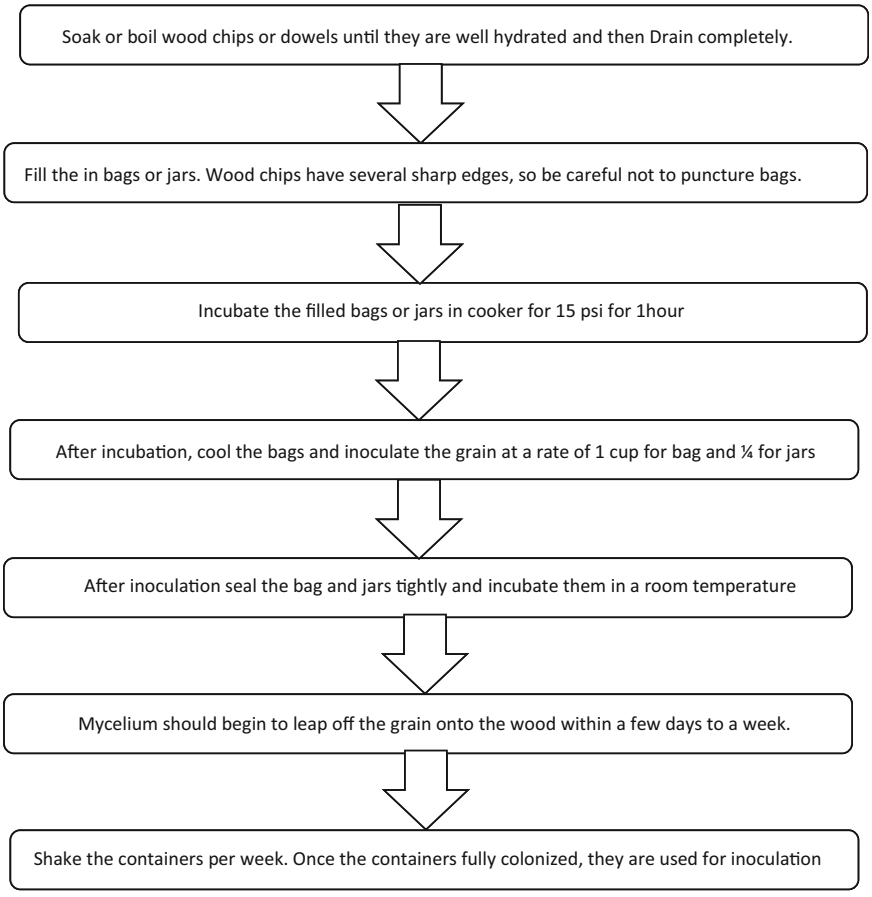
The substrate composition should be composed of many particle sizes to ensure air exchange, rapid colonisation, and good drainage, especially when working with larger amount of material. Substrates constructed entirely of fine materials colonise fast but can get waterlogged or too tied up, whereas those made entirely of huge wood chips colonise slowly and dry out quickly. When creating secondary spawn and the final fruiting substrate, finer materials like sawdust can be added to the substrate mixture. The precise amount of water required to wet your sawdust varies among different species of tree and the brand of pellets used, so check the requirements of water needed for different species. When pressed into a ball, properly hydrated sawdust should yield a few droplets of water and keep its form when released.

Ratio of Spawn

Spawn rate is defined as fresh substrate ratio to spawn. If we use more ratio of spawn, the colonisation will be more successful and faster. Because you're working with unsterile substrates and possible pollutants everywhere while growing outdoors, employing high spawn rates is critical to success. This is why, while cultivating the wood loving Psilocybin species, we recommend aiming for a rate of 20% or higher.

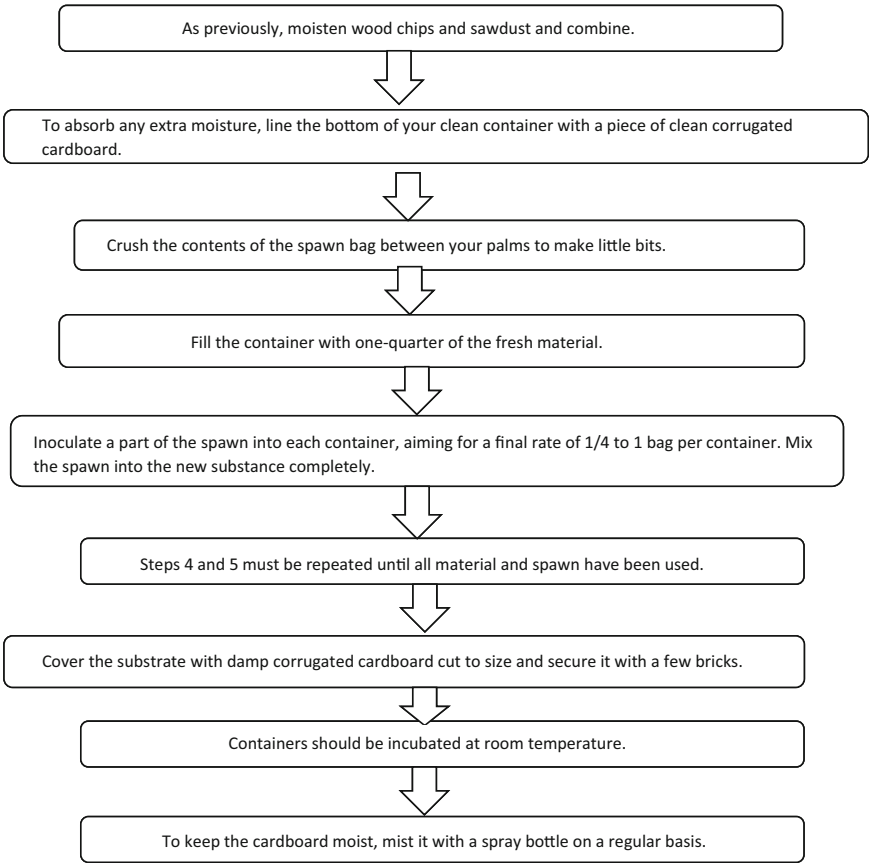
Preparation of Primary Spawn

It is always a good idea to sterilise the spawn before inoculating it. Despite the fact that wood is naturally resistant to infection, sterilising it guarantees that the fungus has a strong foundation on the substrate before it is used again. After colonising the main spawn, it can be freely utilised to inoculate non-sterile wood items.



Preparation of Secondary Spawn

The ultimate colonisation will occur quickly and the health of the bed will be maximised by first doubling the number of pure spawn five- or tenfold, then employing a 25% spawn rate. Large plastic tubs or storage bins are suitable containers for this time. Before usage, wipe them clean with alcohol or ethanol. Cover the infected substrate with a piece of moist corrugated cardboard and a brick or other heavy item. The cardboard aids in maintaining high moisture levels during colonisation, while the weight compresses the substrate, allowing for faster colonisation. In unsterilised secondary spawn, it is preferable to utilise sawdust generated from tougher woods like oak rather than softer species such as alder; when crushed into smaller particles they are prone to contamination.

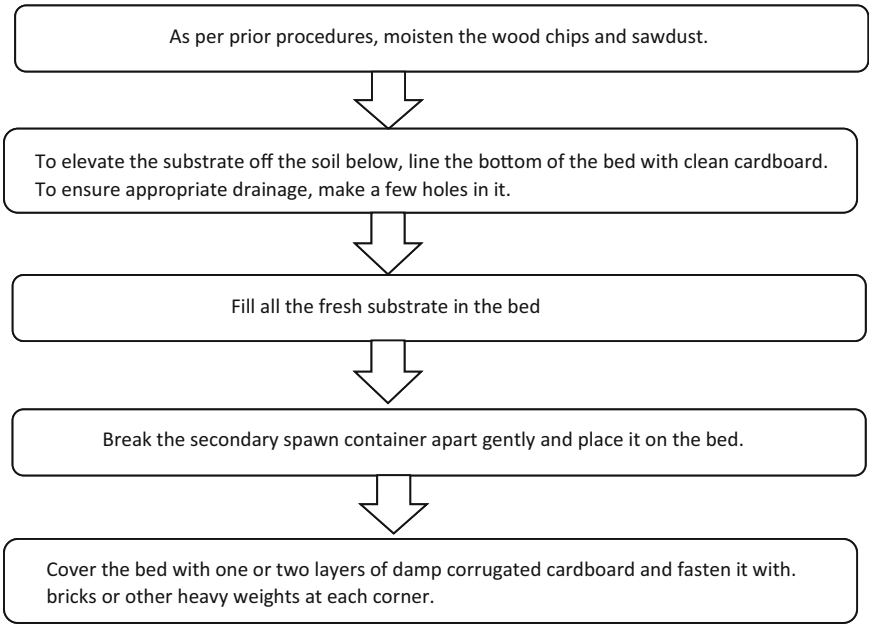


Remove the cardboard after a few weeks, when the container is about one-third colonised, and thoroughly mix the contents by hand while wearing clean gloves. Replace the cover and continue the incubation. Within 1–2 months, the substrate should be fully colonised.

Preparation of Fruiting Substrate

The final fruiting substrate is prepared in the same way as secondary spawn, with dry or fresh wood chips and sawdust added to each 20-pound container of secondary spawn. About 40 pounds of substrate is required for one bed. If the initial number of spawns is restricted, it is advisable to make the bed smaller in order to maintain a suitably high spawn rate and ensure quick colonisation.

The only difference between this process and the one used to make secondary spawn is that the spawn is deposited in a single contiguous layer atop the new material rather than being dispersed throughout. Because this stage occurs in the broad outdoors, where there are many different fungi that may devour the new wood in the bed, "capping" protects the spawn from such attacks and is the new material with a complete covering (Nicholas & Ogamé, 2006).



Ideally, you should place the bed beneath a large plant, such as an ornamental tree, to keep it moist and protect it from direct sunlight. Furthermore, because the fruiting substrate is made of wood chips, such beds are perfectly camouflaged, looking like any other mulched garden area. However, while they are fruiting, they become noticeably more visible, therefore it is always preferable to place the bed in a private, out-of-the-way location.

Incubation

As needed, water the bed. As long as the cardboard covering remains well-hydrated, the bed beneath should be OK. Water carefully with a hose sprayer to avoid

disrupting the growing mycelial mat. Keep an eye on water levels during the months of the summer because beds are most likely to be dried out. Remove the cardboard covering completely and attach the casing layer as directed below when the ambient temperature falls below 65 degrees Fahrenheit in late September or early October.

Fruiting

Fruiting should begin when temperatures drop to continuously 3–4 days in a row around 45 °F and will last until frost sets in. Flushing should take place every 2 weeks or so as long as the environmental conditions are favourable. Apart from picking the mushrooms as they grow, all you have to do at this stage is keep the bed properly hydrated (Nicholas & Ogamé, 2006).

Harvesting

The mushroom stops growing and its cap opens within 30–35 days after preparation of bed, exposing its gills to the environment when it achieves a sufficient size for effective spore dissemination (usually between 3 and 6 in. in height). The optimal time to harvest your mushrooms is immediately before this moment, when the veil is stretched but not torn, because the mushrooms will no longer grow any actual weight beyond this point. Furthermore, you don't want spores landing on your casing soil or containers. Given the enormous number of spores generated by a single mushroom, this may create quite a commotion; also, the gases created by germinating spores may hinder subsequent fruiting.

For determining when a mushroom is going to open, look for the partial vein, a thin protective membrane which covers the gills. Initially, the hat is entirely in rolled, concealing the partial veil. As the cap expands, the veil appears as a circular, light-coloured ring around the bottom hemisphere of the cauldron.

It's as simple as grasping the mushrooms at the strips and slowly twisting them up and far from the casing. Any portion of the mushroom that leaves in the covering will decay, so remove it completely, right down to the stipe, using clean forceps if required. Take extra precautions to avoid injuring nearby less-developed mushrooms or primordia and avoid touching the case layer directly with your hands.

Cleaning After Harvesting

Cleaning the mushroom stems when they are fresh is much easier and more pleasant in the long run than after they have dried. Scrape any remaining casing soil from the base of harvested mushrooms using a knife in a downward motion.

Mushroom Preservation

Fresh mushrooms may be kept in the fridge for up to a week without decaying or losing their vitality. They must be kept in a ventilated container with a slightly ajar cover, such as a paper bag not plastic covers or a plastic container with a sealable facility lined with a paper towel.

Drying is the most basic method of preservation. Dried psilocybin mushrooms potency will last for months, even years, if kept away from light, heat, and moisture.

Fresh mushrooms must be dried gently over low heat (1 10 °F or less) until "cracker" hard and no longer squishy. Then place the mushrooms in a closed bag, such as a zippered freezer bag or, better yet, a heat-sealed food container bag. Before closing the bag, the possible air should be removed from the bag (Nicholas & Ogamé, 2006).

5.3 Large-Scale Production of Psilocybe Cubenises

We will learn the detailed view large-scale production of Psilocybe cubanises.

Raw Material Collection and Mixing

Psilocybe mushrooms require a nutrient-rich growth substrate made of wood. As a result, you must select coco coir from coconut tree (45%). The vermiculite or wood chips by a wood crushing machine (45%), gypsum of good grade (9%), and calcium carbonate (1%) are also utilised for nutrition. Coco coir soaks for 16–18 h, while vermiculite brand soaks for 3–4 h. The coco coir and vermiculite are conveyed into the combination, and the moisture level should be kept at 65% by adding purified water. Keep the pH between 5.5 and 6.6.

Bed Formation and Packing

All of these raw materials are combined and delivered to a bag filling machine automatically. These automatic filling machines fill the polypropylene bags that are packed with the raw material up to 1.5–2 kg in weight. After packing, an iron ring or plastic ring is fitted in the bag's mouth and plugged with non-absorbent cotton.

Cooling and Sterilisation

The trolley is sterilised using an autoclave. Bags are sterilised in an autoclave at 121 °C for 80–120 min at 22 psi pressure. For inoculation, cool the log to 24–26 °C using central air conditioning.

Inoculation and Incubation of Spawn

For inoculating 3% grain spawn, an automatic inoculating machine is employed. The punching and inoculation processes are now complete, and the logs are automatically sent out. The working space is sterilised using ethanol and UV radiation. The inoculating machine is rinsed with distilled water.

Formation of Mycelium

Fresh water or ethanol is used to clean the culture house's floors. The cultural log is moved into the culture house. For pest control, hang a sticky catching card. The importance of humidity, ventilation, and air conditioning cannot be overstated. Spawn will take 10–15 days to complete, including early spawning, mycelial coat creation, blister formation, and browning. After the bumps have formed, oxygen is required. When the substrate turns brown, the polypropylene bags are removed. It was then moved to the cropping room.

Fruiting and Maturity of Spawn

For the correct development, the culture house's floors are cleaned with either fresh water or ethanol. The cultural log has been relocated to the culture house. Hang a sticky capturing card for pest control. The importance of humidity, ventilation, and air conditioning cannot be overstated. Early spawning, mycelial coat formation, blister formation, and browning will all take 10–14 days to complete. CO_2 levels are below 1200 parts per million. Oxygen is required after the bumps have developed. The polypropylene bags are removed when the substrate gets brown. Then it was taken to the cropping room. Spray water into the spawn run substrate for 10–20 min to give it a cold treatment. Small primordia emerged after the substrate was treated with cold water for 3–4 days. The Psilocybe mushroom is ripe and ready to pick after 30–35 days from the bed formation.

Mushroom Harvesting and Drying

When the mushroom cap edge is rolled, harvesting time begins. Holding the mushroom stalk and slowly twisting it from the substrate is one of the most efficient ways to harvest psilocybe mushrooms. Fresh psilocybe mushrooms have an earthy flavour and are also edible. It is kept in a hot air oven (40–50 °C) for 24 h after each complete growth. In the past, psilocybe mushrooms were dried over a charcoal or wood fire for the finest flavour.

Packing

For packaging, you can use either dried or fresh psilocybe mushrooms. The mushroom is packed using an automatic packing machine.

6 Marketing

As Drug

Psilocybin is a hallucinogenic chemical found in over 200 different types of mushrooms across the world. These chemicals, as psilocin prodrugs, behave similarly to and are linked to the natural neurotransmitter serotonin. Psilocybin, popularly known as "shrooms" or "magic mushrooms," was initially synthesised in the late 1950s and was utilised for compulsion therapy and therapeutic purposes. Dried mushrooms are often a reddish rust brown colour with isolated off-white areas. Psilocybin and psilocin levels in dried mushrooms are typically modest, present from 0.2% to 0.4%. The dose of psilocybin varies, with lasting 6 h and peak with 1 to 2 h (Studerus et al., 2011).

As Food

Mushrooms are consumed orally and can be prepared into tea or combined with other meals. The mushrooms can be eaten fresh or dried. Psilocybin has an unpleasant, bitter taste. Any amount of psilocybin can cause a "bad trip," or an unpleasant or even scary experience (Yockey & King, 2021).

As Medicine

Researchers have looked at whether psychologists may use psilocybin and other hallucinogens (Johnson et al., 2008) to treat depression. (Passie et al., 2002) One research looked at psilocybin's capacity to alleviate depressive symptoms without dulling feelings. The other research looked at the link between psilocybin-induced hallucinations and beneficial therapy results (Borowiak et al., 1998).

Psilocybe mushrooms are used in many ways. So, it has a high demand in market. Also, it was consumed by many region people.

7 Cost Analysis

Psilocybe mushroom is grown on both indoor and outdoor. The indoor production was done in lab scale and industry. Outdoor production was done in small scale. Indoor production gives high yield when comes to outdoor production. Six cycles of mushroom cultivation have been both indoor and outdoor cultivation. The total establishment cost is ₹172,250 and income per cycle is ₹200,000. The annual profit percentage of lab scale production of psilocybe mushroom was 78% (Tables 2 and 3). Establishment cost of small-scale production is ₹15,30,400 and income per cycle is ₹3000,00. The annual profit of small-scale production is 80% (Tables 4 and 5). The total establishment cost was ₹15,774,750 and income per cycle was ₹32,000,000. The annual profit of large-scale production was 84% (Tables 6 and 7). The marketing value of the fresh *Psilocybe* mushroom was ₹2000 and dries *Psilocybe* mushroom ₹4450. The annual income of the *Psilocybe* mushroom was ₹1,150,000–₹1,270,000 in lab scale, ₹20,000,000–₹24,000,000 in small scale and ₹220,000,000–₹260,000,000 in large scale. The annual profit is expected to be ₹1,000,000 in lab scale, ₹1,80,00,000 in small scale, and ₹200,000,000 in large

Table 2 Non-recurring expenditure for *Psilocybe* mushroom

Lab scale production				
S. no.	Requirement	Cost of each unit	Total volume	Total cost
1	Air conditioner	₹10,000	1	₹10,000
2	Liner	₹20	100	₹2000
3	Mono tub	₹200	100	₹20,000
4	Hot air oven	₹15,000	1	₹15,000
5	Packing machine	₹2000	1	₹2000
7	Cooker	₹2000	1	₹2000
8	Laminor air flow chamber	₹20,000	1	₹20,000
9	Ventrilator fan	₹3000	1	₹3000
10	Knife	₹25	2	₹50
11	Scissors	₹50	2	₹100
12	Steel or wooden rack	₹4000	10	₹40,000
13	Fridge	₹10,000	1	₹10,000
Total				₹124,150

Table 3 Recurring expenditure of lab scale production of *Psilocybe* mushroom

Lab scale production				
S. no.	Requirements	Cost of each unit	Total volume	Total cost
1	Coco coir	₹20	80 kg	₹1600
2	Vermiculite	₹150	80 kg	₹12,000
3	Calcium carbonate	₹150	10 kg	₹1500
4	Non-absorbent cotton	₹250	1 roll	₹250
5	Tape	₹50	10	₹500
6	Gypsum	₹75	30 kg	₹2250
7	Electricity per month	–	–	₹2000
8	Spawn bottle	₹400	20 bag	₹8000
9	Ethanol	₹400	–	–
10	Labour salary per month	–	1	₹20,000
Total				₹48,100

Table 4 Non-recurring expenditure for *Psilocybe* mushroom

Small-scale production				
S. no.	Requirement	Cost of each unit	Total volume	Total cost
1	Land	30,000	5 cents	₹1,50,000
2	Pressure cooker	5000	2 nos	₹10,000
3	Tubs	200	2000 nos	₹4,00,000
4	Forceps	200	20 nos	₹4000
5	Spray bottle	100	5 nos	₹500
6	Hose, sprinkler	1500	1 set	₹1500
7	Water tank	5000	2 unit	₹10,000
8	Shade cloths	1000	1 Nos	₹1000
9	Refrigerator	15,000	1 unit	₹15,000
10	Baskets	May vary	5	₹1000
Total				₹5,93,000

Table 5 Recurring expenditure for *Psilocybe* mushroom

S. no.	Requirements	Cost of each unit	Total volume	Cost
1	Wood chips	350	2000 kg	₹6,00,000
2	Ethanol	40	1000 ml	₹400
3	Spores	100	2000 nos	₹2,00,000
4	Cardboard	500	10 kg	₹5000
5	Packing boxes and cover	100/kg	–	₹1000
6	Transportation	–	–	₹1000
7	Advertising	10/sheet	500 sheets/ posters	₹5000
8	Utilities (electricity, water, etc.)	–	–	₹25,000
9	Labour	20,000	5	1,00,000
Total				₹9,37,400

Table 6 Non-recurring expenditure for *Psilocybe* mushroom

Large-scale production				
S. no.	Requirement	Cost for each unit	Total volume	Total cost
1	Land expenditure	₹63,000	30 cent	₹18,90,000
2	Building construction	–	1	₹20,00,000
3	Wood crushing machine	₹60,000	2	₹1,20,000
4	Mixing machine	₹1,00,00	1	₹1,00,000
5	Bag filling with cap and ring machine	₹1,50,000	1	₹1,50,000
6	Plastic tray	₹20	2500	₹45,000
7	Autoclave	₹3,50,000	1	₹3,50,000
8	Steel or wooden rack	₹6000	150	₹9,00,000
9	Automatic inoculatic machine	₹3,00,000	1	₹3,00,000
10	Conveyor	₹40,000	2	₹80,000
11	Punching machine	₹10,000	1	₹10,000
12	Motor	₹10,000	2	₹20,000
13	Water connecting motor and pipe	₹1,00,000	2	₹2,00,000
14	Air conditioner	₹1,50,000	6	₹ 9,00,000
15	CO_2 control machine	₹7000	4	₹28,000
16	Hot air oven	₹2,00,000	2	₹4,00,000
17	Knife	₹25	20	₹500
18	Scissors	₹50	20	₹1000
19	Packing machine	₹10,000	2	₹20,000
20	Light	₹250	25	₹6250
21	Computer	₹50,000	2	₹1,00,000
22	Ventrilator fan	₹7000	3	₹21,000
23	Temperature and humidity controller	₹6000	3	₹24,000
24	Sprinkler irrigation pipe	–	–	₹1,00,000
25	Korklift truck	₹10,00,000	1	₹10,00,000
26	Wiring cost	₹3,00,000	1	₹3,00,000
27	Refrigerator	₹20,000	10	₹2,00,000
Total				₹92,65,750

scale (Table 8). The proper maintenance *Psilocybe* mushroom gives a high profit and cultivation cost is low when compared to another mushroom.

8 Conclusions

Cultivation of *Psilocybe* is a very complex process because it needs the specialised technical ability to strengthen and increase the mushroom productivity. The proper maintenance of *Psilocybe* mushroom will provide a good earning result for the cultivators. Mushroom cultivation boosts employment through the financial and

Table 7 Recurring expenditure for *Psilocybe* mushroom

Large-scale production				
S. No.	Requirement	Cost of each unit	Total volume	Total cost
1	Coco coir	₹20	11,000 kg	₹2,20,000
2	Vermiculite	₹150	11,000 kg	₹16,50,000
3	Polyprolylene bag	₹20	22,500	₹4,50,000
4	Non-absorbent cotton	₹250	10 kg	₹2500
5	Plastic or iron ring	₹3	22,500	₹67,500
6	Gypsum	₹75	500 kg	₹37,500
7	Electricity per month	–	–	₹30,000
8	Spawn bottle	₹450	1000 bag	₹4,50,000
9	Ethanol	₹400	2 L	₹800
10	Labour salary per month	–	10	₹3,00,000
11	Trapping card for insects	₹2800	4 pack	₹11,200
Total				₹32,19,000

Table 8 Comparison of lab-scale, small-scale, and large-scale production

	Lab-scale production	Small-scale production	Large-scale production
Price for per kg based on the production	₹2000	₹2000	₹2000
Total yield on one log per fruiting cycle	800 g–1 kg	700–800 g	650–850 g
Total fruiting cycle per log/tub	3	3–4	2–3
Total establishment cost	₹1,72,250	₹15,30,400	₹1,57,74,750
Total income per fruiting cycle	₹2,00,000	₹30,00,000	₹3,20,00,000
Total fruiting cycle per year	5–6	6	5–6
Annual establishment cost	₹2,20,350	₹21,23,400	₹1,57,74,750
Annual income	₹11,50,000–₹ 12,70,000	₹2,00,00,000–₹ 2,40,00,000	₹22,00,00,000–₹ 26,00,00,000
Annual profit	₹10,00,000	₹1,80,00,000	₹20,00,00,000
Annual profit at present	78%	80%	84%

medicinal offerings. People with small land and limited investment can be able to receive good earning by cultivating *Psilocybe* mushrooms in small scale. Cultivators can produce a *Psilocybe* mushroom six cycles in a year. After harvesting, the substrate is used in agriculture land for nutrient. Due to their high profit percentage, *Psilocybe* mushrooms have been developed in many regions. The overall view of cultivating *Psilocybe* mushroom will definitely provide profit in lab scale, small-scale, and large-scale production.

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Small/Large Scale Production, Cost Benefits Analysis, and Marketing of Milky Mushroom

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Abstract

Mushrooms, called 'white vegetables' or 'boneless vegetarian meat', contain high amounts of proteins, vitamins, and fibre. Mushrooms are now getting significant importance due to their nutritional and medicinal value and their cultivation is being conducted in approximately 100 countries. In this chapter, the cultivation of milky mushrooms, its history, spawn preparation, bed preparation, casing, harvesting, and marketing of milky mushrooms are discussed. In addition, the chapter focused on commercial production and different types of storage methods for the mushroom.

Keywords

Milky mushroom · Cultivation · Commercial production

1 Introduction

In India, mushrooms, commonly known as "Khumbi," "Chhatra," "Kukurmutta," "Dhengri," "Dharti ka phool," etc., belongs to ascomycotina or basidiomycotina. Unlike green plant, they lack chlorophyll and thus they cannot utilize the solar energy to manufacture their food as of green plants; having various shape, size, and colours, they are very important food crop and can be produced in varied situations. These could be either epigeal or hypogeal (Chang & Miles, 1991). Mushrooms are very good source of protein, vitamins, and minerals with attracting flavours and are

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cholesterol-free foods with certain important medicinal properties including the antiviral effect. Milky mushroom is also an excellent source of thiamine, riboflavin, nicotinic acid, pyridoxine, biotin, and ascorbic acid (Breene, 1990). In addition to this, the majority of the mineral salts required by human body are potassium, sodium, phosphorus, iron, and calcium. Due to its alkaline and higher fibre content, it is highly suitable for people with hyperacidity and constipation (Doshi & Munot, 1988).

Mushroom cultivation offers an excellent means for recycling agro wastes presently available in India (Sohi et al., 1988). Though we have virtually witnessed a revolution in mushroom cultivation, in order to bring our country on the mushroom map of the world serious efforts are needed to perfect the cultivation technologies of newer edible mushrooms including *Calocybe indica*. This species can be cultivated on a wide variety of lignocellulosic substrates like wheat, rice, corn, and sugar cane bagasse, at a high-temperature range of 30–38 °C (Amin et al., 2010), and has an elongated shelf life of 5–7 days as compared to other commercially grown mushroom species in India (Subbiah & Balan, 2015) (Chadha, 1994).

2 History

Calocybe indica P & C, known as milky mushroom or dudh chatta or summer mushroom, is a big-sized delicious mushroom reported in India by Purkayastha and Chandra (1976). It is an attractive mushroom with large, milky white sporophores, belonging to the family Tricholomataceae of the order Agaricales. It grows naturally on humus soil under roadside trees in the forest. It is sold in city and village markets collected from forests in West Bengal and liked because of its attractive robust white sporocarps, long shelf life, and taste. It grows at a temperature range of 25–35 °C (Sharma et al., 2008). Natural occurrence of milky mushroom in plains of Tamil Nadu and Rajasthan has also been reported (Doshi et al., 1989; Krishnamoorthy, 1995). In spite of sincere efforts by different workers, only limited success was achieved on the cultivation of this mushroom until 1998. The best temperature optima for cultivating this mushroom is 30–35 °C. Some workers have reported that 32 °C was ideal for spawn run and fruit body initiation was more at 30 °C and 90% RH. Yield reduction was reported at temperature below 25 °C. Complete darkness less favoured fruit body formation and diffused light helped in the elongation of stipe. Strandy and vigourous mycelial growth of fungus was observed when it was incubated at 600–800 lux light intensity. Maximum yield was obtained when the cased beds were incubated at 1600 lux light. At higher light intensity, stipe length was very much reduced, whereas pileus breadth increased substantially. During a survey conducted during 2009–2010 in south west monsoon season in Koliyoor area of Thiruvanthapuram district, Kerala, a new species of *Calocybe*, *C. gambosa* (Fr) Donk, was collected and pure culture isolated. The sporophores have bigger sized pileus and club shaped stout and elongated stipe. Cultivation technology was standardized by polybag method on paddy straw and 1:1:1 sand-soil-vermicompost mixture as casing material. Average fruit body weight ranged from 250 to 620 g.

Farm trials conducted in ten locations of six districts of Kerala indicated the highest biological efficiency of 137.4% compared to 90.06% for *C. indica*. First flush appeared early (32 day) in *C. gambosa*, while it took 39.5 days in *C. indica*. A benefit cost ratio of about 3:1 was achieved owing to low cost substrates. Milky mushroom was cultivated on four different agro-residues, viz., paddy straw, coirpith, wood shaving, and banana trash. The energy value of substrates worked out based on cellulose, hemicellulose, and lignin content revealed highest energy value of 466 K Cal per 100 g substrate in coirpith followed by banana trash (435 K Cal per 100 g), paddy straw (391 K Cal per 100 g), and wood shavings (380 K Cal per 10 g values) (Usha, 2007).

3 Morphology and Molecular Characterization

Calocybe is a small genus of about 40 species of mushroom (Kirk et al., 2008), which is edible and is cultivated in India. The name is derived from the Ancient Greek terms kalos "pretty," and cubos "head" (Nilson & Persson, 1977). Around nine species of *Calocybe* are found in neotropical regions. Sixty accessions of the specialty mushroom germplasm maintained in the ICAR-DMR, Solan repository, were characterized using DNA fingerprinting techniques. Phylogenetic analyses based on Random Amplified Polymorphic DNA profiles and direct sequencing of 5.8S rRNA gene region revealed intergeneric, inter and intra specific variations in *Volvariella, Lentinula*, *Ganoderma*, and *Calocybe* groups of the accessions. Multiple sequence alignment of all the accessions within species exhibited polymorphism in ITS-1 and ITS-II, but not in the conserved 5.8S rRNA gene regions. In all four types of *V. volvacea*, two types each of *Lentinula*, *Ganoderma*, *Calocybe*, and one type of *Trametes versicolour* sequences were obtained (Singh et al., 2003). Shekhar and Singh (2014) established phylogenetic relationship among the eleven commercially cultivated edible mushrooms, namely, *A. bisporus* (Portobello), *P. eryngii*, *L. edodes*, *H. tessellates* (Brown shimeji), *H. tessellates* (white shimeji), *F. velutipes*, *P. ostreatus*, *P. djamor*, *C. indica*, and *P. florida* using RAPD markers.

4 Cultivation Technology

4.1 Spawn Preparation (Joshi & Sagar, 2016)

Spawn for milky mushroom can be prepared using standard technology which is discussed in detail in Fig. 1.

4.2 Storage

Temperature of 25 ± 2 °C or at room temperature spawn can be stored up to 30 days, from the date of inoculation. Under refrigerator conditions, spawn can be stored for

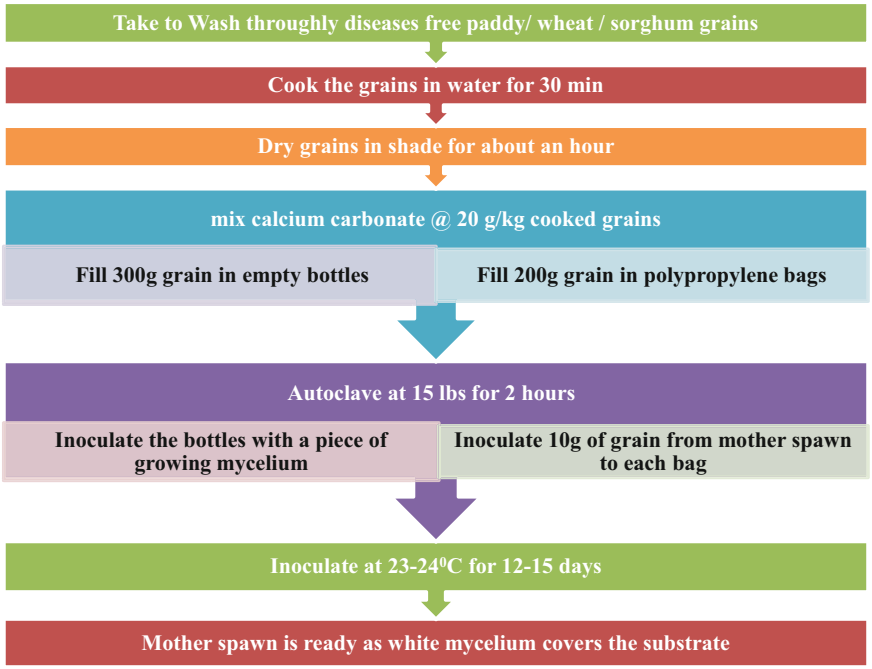

Fig. 1 Preparation of mother spawn

another 3 months. However, there will be some decrease in yield of mushroom with increased storage time above 2 months.

4.3 Contamination or Spoilage

Growth of the mycelia gets restricted in the substrate when bacteria or molds overgrow the mycelia and the spawn gets contaminated or spoilt. Bacterial contamination in spawn packets is generally found as patches of slimy fluid on the substrate where the mycelium growth ceases. Spoilage caused by the molds *Aspergillus* sp., *Pencillium* sp., *Rhizopus stolonifer*, and *Trichoderma* sp. can be seen when the substrate has patches and at times much of it colonized with different coloured mycelia or spores other than white. The factors like excess moisture in the grains, bad quality grains, improper sterilization, and high temperature during storage contribute to spoilage of spawn.

4.4 Pasteurization/Sterilization

Pasteurization/sterilization can be achieved by any of the following ways.

Hot Water Treatment

Water is boiled and chopped wet straw filled in gunny bag is submerged in hot water for 40 min to achieve pasteurization. This is very popular method particularly with small growers.

Steam Pasteurization

Wet straw is filled inside insulated room either in perforated shelves or in wooden trays. Steam is released from a boiler and temperature inside substrate is raised to 65 °C and maintained for 5–6 h. Air inside the room is circulated to achieve uniform temperature in the substrate.

Autoclaving

Substrate is filled in polypropylene bags (35 × 45 cm, holding 2–3 kg wet substrate) and sterilized at 15 psi for 1 h. Once pasteurization/sterilization is over, straw is shifted to spawning room for cooling and spawning.

Chemical Sterilization Technique

Technique defined for oyster mushroom (straw soaked in solution having 75 ppm bavistin and 500 ppm formalin) can also be used. In South India, many farmers are using this technique. However, in 5–10% of bags, spawn run may not be complete and *Coprinus* appears in such cases.

4.5 Bed Preparation (Kavitha et al., 2020)

The cultivation of milky mushroom was done in transparent polythene covers of size 60 × 30 cm, with a thickness of 80 gauge. Polythene cover was tied in the bottom end with a thread and turned inwards. The dried straw was mixed thoroughly to get a uniform moisture level in all areas. Bed spawn, squeeze thoroughly, and divide into two halves. (Two beds are prepared from the single spawn bag.) The straw was filled to a height of 3″ in the bottom of polythene bag, and handful of spawn was sprinkled over the straw layer. The second layer of the straw was filled to a height of 5″ and spawn was sprinkled as above. The process was repeated to get five straw layers with spawns. The beds were gently pressed and tied with thread. Ten ventilation holes were made randomly for ventilation as well as to remove excess moisture present inside the bed. The beds were arranged inside the thatched shed for spawn running by hanging system. The temperature of 22–25 ° C and relative humidity of 85–90% inside the shed were maintained inside the spawn running room. The fully grown spawn run beds were shifted to cropping room after 20 days for casing for initiation of buttons.

5 Casing

Casing means covering the top surface of fully colonized bags, with pasteurized casing material. After complete spawn run, the bags are cut open from the top and are cased. The pond soil and sand, or coir pith, FYM, and other materials can be used for casing. Casing thickness is 3–4 cm. The pH of casing material is adjusted to 7.8–7.9 with chalk powder. The casing is sterilized by autoclaving or using chemicals. It is either sterilized in autoclave at 15 psi for 1 h or chemically treated with formaldehyde solution (2%) about a week in advance of casing. Temperature of 30–35 °C and RH 80–90% are maintained thereafter for entire cropping cycle. When long bags are used, these are cut into two at the time of casing.

6 Cropping

It takes about 10 days for mycelium to reach to top of the casing layer, thereafter fresh air is introduced and minimum 3–4 air changes per hour are required. The bags are watered regularly as the good moisture and humidity are important. Similarly, diffused light is also important for the initiation and growth of the fruit bodies. Light should be provided for maximum duration during entire cropping period. It is believed that blue light is more useful for induction of pinheads. These changes in environment result in the initiation of fruiting bodies within 3–5 days. The mushrooms appear on the top of bag just like that in button mushroom within 2 weeks. Mushrooms are harvested by twisting, cleaned, and packed in perforated polythene/polypropylene bags for marketing. The mushrooms keep on growing, but it is advised to harvest these when these are about 10 cm long. The bigger mushrooms become fibrous. The mushroom has white colour as indicated by its name and has good keeping quality. The fruit bodies can be easily kept at room temperature for 3–4 days. The mushrooms can be used for making pickles or cooked just like other mushrooms. Due to strong aroma, it is advisable to boil these fruit bodies in water for 10–15 min and discard the water before their use in different recipes.

7 Water Management

Water management is very important for a good and healthy crop. During spawning, the water content should be low (around 60–62%) as there are problems in spawn run if water content is high. Also, during rainy season controlled watering is required and watering once in a day may be enough. During winter, watering twice may be sufficient. However, during summer as water loss is high, it becomes very difficult to maintain required RH and moisture of the substrate. During such period, one should spread sand on floor and use mist sprayer 3–4 times and frequently check the

moisture of the casing by touch so as to maintain RH of at least 80–85% inside cropping room. The mushroom is cultivated in huts made of coconut leave or other materials. To maintain high humidity, the bags are kept below ground for homogeneous temperature and humidity. The cost of production depends upon the cost of raw material, yield/unit, production level, and the wholesale price.

8 Diseases and Pests

Pandey et al. (2003) investigated the occurrence of competitor molds and pathogens during the cultivation of milky mushroom. *Trichoderma harzianum* was the most problematic weed mold observed during spawn running. Cobweb disease caused by *Dactylium dendroides* caused complete loss of crop. Carbendazim at 0.01% could effectively control the weed mold and the pathogen without inhibiting the mushroom mycelium. Prochloraz Mn complex controlled the pathogen, but inhibited *C. indica* mycelium by 46.69%.

Studies on sources of nematodes inoculum showed that the most potential sources of nematode dissemination in *Agaricus bisporus* were wheat straw, chicken manure, and spent compost often used as casing material. Since chicken manure is not used in *Calocybe indica*, nematodes were mainly disseminated in this mushroom through other two means. Besides, FYM, loam, and platform soil also acted as source of nematode inoculum. Dipteran flies hovering in the farms also disseminated nematodes from one bed/room to other in both the cases. Interestingly, sporophores of *Calocybe indica* were found to harbour more nematodes as compared to that of *Agaricus bisporus* (Khanna et al., 2006).

9 Shelf Life and Dehydration of Milky Mushroom

Dehydration studies were conducted on milky mushroom under room temperature, in oven (55 °C) and in sun. Polypropylene cover (80–100 gauze) was best suited for the storage of sporophores at room temperature (4–5 days in 0% vent area) and under refrigerated conditions. The sporophores dehydrated at room temperature had better colour and morphology. The rehydration of oven-dried samples was better and browning of sporophores was absent (Pandey et al., 2002). Kasthuri et al. (2007) assessed the physico-chemical qualities and shelf life of mushroom canned with three different concentrations of sodium chloride, viz., 1%, 2%, and 3%, and tomato pulp made from both hybrid and local varieties as canning medium. Canned mushroom samples were analyzed periodically at monthly intervals for physico-chemical parameters such as protein, vitamin C, moisture content, and drained weight. Analytical results of biochemical qualities of canned mushrooms in different caning media showed a gradual decrease during storage period of 1 year. Same trend was observed with physical parameters such as moisture content and drained weight.

Among the treatments, mushroom canned with tomato pulp from hybrid tomatoes performed better at all the quality aspects. Amuthan et al. conducted the studies on osmo air drying of milky mushroom. Samples of milky mushroom were osmosed at different concentrations of common salt, viz., 10%, 15%, 20%, and 25%, and dehydrated in a fluidized bed drier at 55 °C and 60% RH. The moisture removal was higher by osmosis at 25% concentration of salt in 6 h duration. The osmosed samples took about 170 min to dry the samples compared with 195 min for control (air drying) and the complete rehydration of the mushroom osmosed with 25% salt concentration was obtained in a shorter period (50 min). The colour was bright for the samples dried after osmosis.

10 Marketing of Mushroom

The production cannot be said to be complete unless steps are taken to transfer the production to the hands of the consumers. It is a consumer who ultimately pays for everything. Thus, it is very important to provide place utility to the final product and this is where the role of marketing becomes crucial. The aim should be to make available the product to consumer in fresh form and at the least possible price, providing sustainable incentives to all engaged in production and marketing. The ideal marketing system should also take into account the changing tastes and preferences, future demand and supply, processing, stabilization of demand and prices, etc. (Kumar et al., 2017).

11 Marketing Channels

The various marketing channels being used by the sampled mushroom cultivators: Producer–Retailer–Consumer, Producer–Consumer, Producer–Co-operative–Retailer–Consumer, Producer–NGO–Retailer–Consumers, and Producer–Outside collector–Wholesaler–Retailer–Consumer.

All the production of mushroom is disposed of through the above listed marketing channels. The percentage of produce finding its way through different channels varied in each state.

12 Post-Harvest Management (Borah et al., 2019)

Mushrooms have become valuable component of diet owing to their attractive taste, aroma, and nutritional values. Fresh mushrooms are perishable, having a very short shelf life with high moisture content of more than 90%. Preservation of mushrooms in fresh condition for a longer period is a difficult and costly task. Fruit bodies become soggy as many metabolic activities continue after harvest. High water content of mushrooms also becomes conducive for multiplication of various

microbes which cause rot of harvested fresh mushrooms. However, it can be stored for certain time period by adopting the preservation procedures mentioned below.

13 Types and Methods of Preservation

The methods of preservation developed for mushrooms can be grouped into two types as follows:

1. *Short term preservation (for maximum period of 10–15 days only)*
   (a) *Room temperature:* Keeping in room temperature of around 30–33 °C, the mushrooms remain fresh for 8–12 h only, whereas it is possible for 24–36 h during winter at lower temperatures.
   (b) *Refrigeration:* Fresh mushrooms can be stored for 7–15 days in a refrigerator depending on the type of package and storage temperature.
   (c) *Brine solution preservation:* In a solution of common salt (in water) in high concentration (10–15%), fresh mushrooms can be kept safe for 6–7 days.
   (d) *Other methods:* Lactic acid fermentation and gamma irradiation. These are tedious, costly, and sometimes risky also.
2. *Long term preservation*
   (a) *Sun drying:* Fresh mushrooms after sorting and selection (trim off the hard stalk portions) are thinly spread on a sieve and sun-dried for 3–5 days or till when 10 kg mushroom weighs 1 kg. To avoid browning of the fruit bodies, a shade may be provided to the mushrooms by spreading a black cloth at about 1 ft above the sieve. This type of dry mushrooms can be kept in air tight containers up to 5–6 months.
   (b) *Machine drying:* In machine drying method, fresh mushrooms are dried in electrically operated drier within 6–8 h. It is a costly method.
   (c) *Blanching:* Sorted out mushrooms are steeped in warm water of 80–85 °C for 1–2 min. Only and then sun-dried. Sometimes sodium chloride @ 400 ppm and citric acid @ 0.1–0.2% may be added to the water before boiling to retain or improve the natural colour.
   (d) *Other methods:* Freeze drying, canning, pickling, etc (Table 1).

14 Conclusion

Mushroom offers prospects for converting lignocellulosic residues from agricultural fields and forests into protein-rich biomass. Such processing of agro waste not only reduces environmental pollution, but the by-product of mushroom cultivation is also a good source of manure, animal feed, and soil conditioner.

Table 1 Commercial production of milky mushroom approximate budget

S. No	Particulars	Item	Quantity	Approximate cost
1.	Fixed cost	Lab and store room	1	400,000.00
		Electricity		15,000.00
		Water supply		15,000.00
		AC		50,000.00
		Autoclave	1	75,000.00
		Laminar	1	100,000.00
		Refrigerator	1	25,000.00
		BOD incubator	1	50,000.00
		Gas	1	3000.00
		Weight balance machine	1	50,000.00
		Hot air oven	1	50,000.00
		Rack for spawn storage		20,000.00
		Glassware and other materials		40,000.00
Total (A)				868,000.00
2.	Recurring cost	Grains	80 q	120,000.00
		Calcium carbonate	1.5 q	7500.00
		Cotton	1.5 q	10,000.00
		Polythene bags, rubber bands	1.5 q; 30 pockets	15,000.00
		Media	1 kg	30,000.00
		Refilling of LPG		50,000.00
		Miscellaneous		10,000.00
		Contradual		216,000.00
Total (B)				458,500.00
3.	Interest and depreciation	On fixed cost		178,400.00
Total (C)				178,400.00
4.	Cost of production		(B + C)	636,900.00
5.	Gross income (10 tonnes spawn) @ Rs.100 per/kg			1,000,000.00
6.	Net income per annum			352,600

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Mass Multiplication, Economic Analysis, and Marketing of *Ganoderma* sp. (Reishi Mushroom)

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Abstract

Ganoderma species are a large and diverse genus of wood-rotting fungi that are found worldwide. It is made up of species that cause white rot on a wide variety of tree species. It has a long history of use as a medicinal mushroom in Asia and contains a variety of pharmacological properties associated with immunomodulatory action. It grows on decaying wood, and different components of the mushroom, including mycelia, spores, and the basidiocarps, are taken and marketed in a variety of forms, including powder, capsules, and beverages. Recent years have seen the rapid expansion of the *Ganoderma* enterprise, which has been aided by several efforts by academia and business. Numerous commercial products have used the bioactive components and their pharmacological activities. Thus, demand for *Ganoderma* mushroom as a therapeutic

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material is projected to increase in the near future. This is due to consumers who use dietary supplements and nutraceuticals to maintain their health and immunity. This chapter summarizes the significance, current perspectives, methodology for mushroom growth and production, and products in order to ascertain the current market status of *Ganoderma* mushroom products. These include strain characteristics, culture, and product processing, among others. It will serve as a significant information source for both research and commercial production.

Keywords

Entrepreneurship · Lingzhi · Medicinal mushroom · Mushroom cultivation · Reishi

1 Introduction

Many civilizations across the world have long recognized mushrooms as a therapeutic and nutritive food. Mushroom usage has been documented as far back as 18,700 years ago (Spain), 6000 years ago (China), and 4600 years ago (Egypt) (Li et al., 2021). Researchers still don't know how early humans discovered safe and edible mushroom species, but they can assume it happened through iterations, just like native plants or other lifeforms were harvested or acquired for food. Then taste, appearance, and anaphylaxis determine which mushrooms are safe to eat. A comprehensive review of the literature and trustworthy sources with metabarcoding data revealed that 160,000 of the 6.2 million fungal species as macromycete mushrooms produce sporocarps (Niazi & Ghafoor, 2021; Baldrian et al., 2021). There are around 3283 species of mushrooms that have been identified as edible mushrooms, accounting for approximately 20% of all mushroom species recorded in existing data, and approximately 700 are regarded as useful medicinal mushrooms, safe species, and ten of these have reached the level of industrial production in several nations (Barshteyn & Krupodorova, 2016; Siwulski et al., 2015). The global mushroom industry includes edible, medicinal, and wild mushrooms. The world's edible mushroom production has surged 30-fold in the previous 60 years. According to the FAO's Statistical Database, China produces the most mushrooms, followed by the USA and the Netherlands. The upward trend in mushroom cultivation is predicted to rise. On the one hand, the area of medicinal mushrooms has made tremendous strides in the last 40 years. It has been determined that a truly successful new branch of science has emerged (Solomon, 2010). *Ganoderma* sp., one of many medicinal mushrooms, has been used for over two millennia in traditional East Asian medicine to sustain vitality and lifespan. It has three important properties for disease prevention and treatment in traditional medicine. First and foremost, it has no toxicity or side effects; second, it is not restricted to any one organ; and third, its use improves the effects on regulating organ activities (Zhou et al., 2012). It is the most widely cultivated medicinal mushroom throughout the world, especially in China, Europe, Finland, Ghana, India, Japan, Kenya, Korea, South East Asia,

Taiwan, Tanzania, and the USA. It is known for its unique pharmacological characteristics and perceived lack of negative effects; it is revered as the most potent natural remedy in Asia and several African countries. Several experiments have been done on the metabolic and therapeutic activities of *Ganoderma* (reishi). *Ganoderma* produced an incredibly extensive armament of nearly 400 bioactive chemicals that were identified. Its bioactivities rely on β-glucan, ganoderic acids, ganodermanontriol, ganoderiol, germanium, nucleotides, polysaccharides, nucleosides, and triterpenoids from fruiting bodies, gills, spores, and mycelia. The variations are due to the fact that triterpenes such as ganoderic acid and its derivatives have a wide concentration range between species. These bioactive compounds in *Ganoderma* spp. are recognized as antiallergic (antihyperlipidemic), antiasthmic, antiaging, anti-androgenic, anti-atherosclerotic, anticancer, anticonvulsant, antidepressant, antidiabetic, antigenotoxicity, anti-inflammatory, antihypertensive, antimicrobial, antimutagenic, anti-nociceptive, antioxidant, anti-radiation, antiparasitic, cytotoxic, hepatoprotective (anticancer and antioxidant properties), cholesterol inhibition, gut health stimulant, hepatoprotective properties, hypoglycemia, immunomodulatory, inhibition of lipid peroxidation/oxidative DNA damage, neuroprotection, sleep elevation, and prebiotic properties (Ahmad, 2018; Hapuarachchi et al., 2018; Sharma et al., 2019; Venturella et al., 2021), especially the immunomodulation against HIV/AIDS and dengue virus, improving the lives of those affected populations in recent years (Fernandes et al., 2020). Unless *Ganoderma* species are a dietary supplement or medicinal "drug," it is debatable. *G. lucidum* has evolved into the most powerful drug known to mankind. The current states of Ganoderma cultivation, products, and industry are summarized in this chapter, along with suggestions for further research and commercialization.

2 History and Present Status

Medicinal and edible mushrooms have a global impact on human health, and their use is seen as a milestone towards a quasi-revolution. The genus *Ganoderma* is referred to as Lingzhi, Black Reishi (divine mushroom), Shenzhi, Ruizhi, Xiancao, or Rui-Cao (means auspicious herbs) mushroom in China; Red Reishi, Sachitake, Mannendake, Munnertake (10,000-year-old mushroom) in Japan; and "Youngzhi" in the Korean plateau. It is largely deliberated as the "celestial herb" or "herb of spiritual potency," or "mushroom of immortality," or "artist's conk" or "sprint plant" or "super-natural mushroom" or "auspicious symbol for worship" or "miraculous Zhi" and symbolizes happiness, sanctity, prosperity, goodness (Siwulski et al., 2015), and longevity for over two millennia (Hapuarachchi et al., 2018). Several mushroom species have been emphasized in ancient Oriental traditions, including *Ganoderma lucidum* (W. Curt.: Fr.) P. Karst., which played an important role in handling assorted sicknesses and extending life in Chinese and Japanese culture for at least 2000 years (Hapuarachchi et al., 2016). The *G. lucidum* complex, known in Sanskrit as Soma, a Vedic shrub "Soma-Haoma" (Pegler, 2002), Chinese as Lingzhi, was mentioned as a longevity-promoting tonic herb in *Shen Nong's Materia Medica*

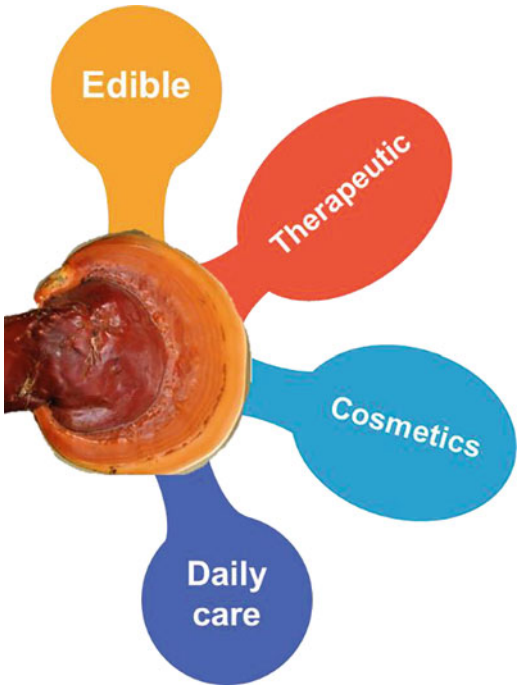

Fig. 1 Major utilities of *Ganoderma* species

(206 BC-8 AD) written by Hong Ching Tao in the Han Dynasty, followed by *Ben Cao Gang Mu* (1590 AD) by Li Shi-Zhen in the Ming Dynasty, as well as listed in the American Herbal Pharmacopoeia, Chinese Pharmacopoeia, and Therapeutic Compendium (Zhou et al., 2012; Karuppiah & Ji, 2020; Wang et al., 2012; Cao et al., 2012; Liu et al., 2021). *Ganoderma*, often known as "red bracket," has the ability to protect humans against a variety of diseases. The scientific effort expended to comprehend mushroom marketing and its use is disproportionate to its significance. *G. lucidum* and *G. sinense* are generally considered to be approved medicinal mushrooms. Additionally, *G. tsugae* is included in the People's Republic of China's list of fungal species that may be utilized in healthy food (Hapuarachchi et al., 2016; Wang et al., 2012; Loyd et al., 2018). The *Ganoderma* mushroom and its secondary metabolites are widely used to treat many diseases, including alcoholism, arthritis, asthma, cancer, chronic hepatitis, diabetes, epilepsy, low blood pressure, cardiac problems, hepatitis A, B, and C, high blood pressure, infertility, mumps, paralysis, psoriasis, ulcers, and tiredness. Because of its long history as a medicinal fungus, *Ganoderma* has been elevated to the rank of a divinity (Bishop et al., 2015). More than ten different species are widely available in eastern markets, but they are identified by different names and are believed to perform distinct functions (Fig. 1). Nonetheless, *Ganoderma* species are still widely used in traditional Asian medicine and are spreading globally.

2.1 Taxonomy

Historically, basidiomycetes were classified by their basidiocarp morphology. The absence of basidiocarps, morphological plasticity, and cryptic species can make identification difficult. Despite extensive taxonomic research, the relationships and categorization of *Ganoderma* species remain a mystery. The genus name *Ganoderma* is derivative of the Greek term "ganos," which means "brightness, sheen," and "derma" which means "skin." The Latin term lucidus implies "brilliant" or "shiny" and refers to its varnished exterior. Petter Adolf Karsten established the genus *Ganoderma* in 1881, with a type species *G. lucidum* in 1881, previously known as *Boletus lucidus* Curtis (1781) and *Polyporus lucidus* (Curtis) Fr. (1821). Also, he split the genus *Ganoderma* into two subgenera in 1889: *Ganoderma* and *Elfvingia* (Hapuarachchi et al., 2016; Loyd et al., 2018). Because of the significance of the species, Hawksworth suggested that the name *G. lucidum* be continued with an Asian type and a new name be introduced for the European species. They are occasionally referred to as polypore mushrooms, shelf fungi, or bracket fungi. *G. lucidum* is still used to refer to *Ganoderma* isolates used in pharmacological, biochemical, or molecular studies, as well as commercially grown strains. Ganodermataceae have generally been regarded as difficult to define due to a lack of precise morphological traits, an abundance of synonyms, and widespread name misinterpretation. *Ganoderma* species identification has been difficult due to genetic variation caused by ethnic origin and intergenerational outcrossing (Midot et al., 2019). There are about 466 records with the name of *Ganoderma* displayed at the Index Fungorum database (http://www.indexfungorum.org/), and most of them are strewn throughout the tropical regions. It is generally accepted that *Ganoderma* species are classified as chaotically, and the binomial nomenclature epithet *G. lucidum* has been applied to the majority of laccate (shiny) and stipitate white-rot *Ganoderma* species. In recent years, it has become clear that *G. lucidum* sensu stricto seems to have a restricted natural distribution in Europe and portions of China. The types and levels of medicinally important compounds in *Ganoderma* species are likely to vary. It has been used for centuries in Asia to treat several diseases, especially *G. lucidum*, *G. tsugae*, and *G. applanatum* (Basnet et al., 2017). During this molecular era, the advancement of genomic phylogenies allowed for the testing of species concept suppositions, which were then used to determine the linkage between the complex and subtle morphological heterogeneities of the laccate *Ganoderma* species. Taxonomic characterization of *Ganoderma* species has used biochemical, genetic, and molecular approaches. There are also a number of studies on the use of nondestructive near-infrared (NIR) methods combined with chemometrics, high-performance liquid chromatography (HPLC), and nuclear magnetic resonance (NMR)-based metabolomics (Liu et al., 2021; Midot et al., 2019; Ferreira et al., 2015; Zhibin Lin, 2019). The first whole-genome sequence of *G. lucidum* using next-generation sequencing technologies was published (Chen et al., 2012). The use of nucleotide sequence data, such as -tubulin gene, nrDNA-ITS, nrDNA-LSU, nrDNA-SSU, mitDNA-SSU, RNA pol II subunit and IGS, RNA pol I subunit, and TEF1- in phylogenetic studies has attempted to comprehend our

understanding of the previously unclear connections among *Ganoderma* species (Wang et al., 2012; Midot et al., 2019; Zhibin Lin, 2019; Chen et al., 2017). These genes were used to taxonomize *Ganoderma*, resulting in tremendous advancement and a plethora of knowledge for future research. Several recent phylogenetic analyses have been published on this genus.

2.2 Diversity and Ecology

A search for *Ganoderma* P. Karst., 1881 in the Global Biodiversity Information Facility (https://www.gbif.org/species/2519220; accessed January 2022) revealed a total of 19,254 described occurrences. Among them, 55,355 occurrences have georeferenced records, mostly confined to Asia, Europe, and North America. These ectomycorrhizal fungi are known for producing lignin-degrading enzymes that preferentially delignify rotting stumps and roots of coniferous or deciduous or mixed forests to mobilize nutrients and for growing as facultative parasites capable of living as saprobes (Siwulski et al., 2015; Hapuarachchi et al., 2018; Rashad et al., 2019; Thiribhuvanamala & Krishnamoorthy, 2021). *Ganoderma* species rot timber yields globally, including acacia, betel nut, coconut, eucalyptus, oil palm, ornamental palms, rubber, and tea. Some species, such as *G. philippii* and *G. boninense* (syn *G. orbiforme*), are causing worrisome diseases such as basal stem rot, butt rot, and root rot (Hushiarian et al., 2013). The fungus causes root rot in cocoa, coffee, and tea and is a major threat to oil palm and coconut in Southeast Asia, incurring annual losses of up to USD 0.5 billion due to upper stem rot and basal stem rot diseases that diminish productivity and increase replant frequency (Midot et al., 2019). In Western countries, *G. lucidum* is frequently found growing on trees, particularly oak trees, causing the wood to decay (Loyd et al., 2018). In India, it has been reported that 62 species of *Ganoderma* attack over 150 distinct hosts, with *G. lucidum* and *G. applanatum* being the most prevalent pathogens. *G. lucidum* appears to be the weak saprophyte responsible for the most common basal rots (Bhosle et al., 2010). *G. applanatum* is the next most prevalent species, having been found on 39 different host trees, the majority of which are forest vegetation. *Ganoderma* spreads via windborne basidiospores or through contact with infected material. There has been no extensive study of *Ganoderma* populations in Indian forest regions and farms, and it is possible that many new species are yet to be unearthed.

2.3 Morphology

The basidiocarp morphology and culture properties like chlamydospore development, growth, and thermophilicity were used to identify *Ganoderma* species. The identification of *Ganoderma* species was based on morphological characteristics of the basidiocarp, as well as cultural traits such as chlamydospore formation, growth, and thermophilicity (Ritu et al., 2020). Moreover, according to the Bencao Gangmu, a sixteenth-century Chinese herbal compendium, a range of *Ganoderma*-like

Fig. 2 Basidiocarp of *G. lucidum*

mushrooms were categorized according to their colour and utilized for a variety of reasons. Researchers have determined that the species *G. lingzhi* is most likely to be the red reishi found in Chinese herbal stores nowadays, despite the fact that no specific modern species can be linked to these ancient *Ganoderma* species (Cao et al., 2012). Mostly, depending on the fruiting body, it is classified into a laccate pileus with a shiny surface or a non-laccate pileus with a dull surface. Laccate forms were traditionally assigned to the *G. lucidum* complex, subgenus *Ganoderma*; types that are not laccate in the *G. applanatum* complex, subgenus *Elfvingia*. Its distinctive fan-like (flabeliform) appearance is due to its reddish-brown, varnished, kidney-shaped sexual basidiocarp (fruiting body), azonate to zonate abhymenial surface, poroid hymenial surface, laterally stipitate or eccentric with bands, and tangentially inserted with laccate, brittle stipe (up to 10 cm) (Fig. 2). Pilear crust as hymeniodermis. Its underside is devoid of gills, with an irregular thin, brown margin, and instead disperses its spores through cream-turning ochraceous yellow minuscule pores (80-120 μm). They are typically characterized by their ovoid to ellipsoid, brownish, inamyloid, acyanophilous, chamois, rugose, bi-walled, and truncate basidiospores (9–12 × 6–9 μm). It germinates as a trimitic hyphal system and produces thin-walled, clamped reproductive hyphae, coenocytic, randomly oriented, thick-walled, rarely branched skeleto-binding hyphae. Unfortunately, morphological traits can change due to culture, development, and functional genomic evolution (e.g. polymorphism, recombination). As a result, *Ganoderma* has multiple names and a muddled taxonomy. Consequently, the number of all these phenotypic species has remained rather steady, accounting for around one third of the genome-sequenced species. Because of the tremendous phenotypic plasticity among *Ganoderma* spp., some taxonomists believe macro-morphological traits are useless in identifying species.

3 Commercial Cultivation

Mushroom farming is second only to yeast culture in terms of economic viability. Using new technologies, global mushroom output climbed from 6.90 to 10.24 MMT in the last decade (Ho et al., 2020). The cultivation of medicinal mushrooms has recently changed dramatically. Incorporating this unconventional crop into current waste-recycling systems as a form of regenerative agriculture could help low-income farmers. Mushrooms, long recognized for their gourmet and nutritional worth, are now increasingly prized for their therapeutic capabilities, being utilized in nutritional supplements, nutraceuticals, and mycotherapy. Artificial cultivation of *G. lucidum* is a major industry in China and other East Asian countries, and it is increasingly spreading globally. *G. lucidum* is an ecologically scarce fungus with inadequate wild specimens to support exploitation. A Chinese researcher produced *Ganoderma* using the "spore separation culture method" in 1970. Since then, *Ganoderma* cultivation has been possible in China. *Ganoderma* is now being produced commercially all over the world, especially in Asia, to meet the mushroom's growing demand as traditional medicine. *G. lucidum* is the name given to both medicinal isolates and commercially cultivated mushroom strains (Wang et al., 2017). Numerous potential strains have been released with crucial features such as Gl-7 and Gl-4 (Hoq et al., 2016), Hunong-1, Lingzhi-2, Xinazhi-2, and Liaolingzhi-2 (Zhibin Lin, 2019). *Ganoderma* (as Lingzhi) was first successfully cultivated in China in 1969, and the annual sales of *G. lucidum* products in Asia, including China, Japan, and South Korea, are projected to be over USD 2.5 billion. In 2015, the worldwide total turnover of the *Ganoderma* business was projected to be USD 5.0 billion, with fruiting body and spore yields of 160,000 tonnes, with China accounting for 30% of total output and 75% of yields. Furthermore, its production exceeds 200,000 tonnes per year and a half comes from China, culminating in around 50,000 tonnes of leftovers being produced annually. However, its quality varies according to the strain, growing habitat, environmental conditions, substrates, cultivation methods, and harvesting period.

3.1 Mycelial Fermentation

Ganoderma cultivation is time-consuming, and the quality of the product is not always constant. Regarding economic uses, large-scale production of *Ganoderma* mycelia in liquid media using a fermenting chamber provides a more inexpensive, productive, and regulated process. After about a week, mycelia and other metabolites can be isolated and processed into various products. *Ganoderma* mycelia fermentation is an attractive alternative due to its shorter production cycle, higher product yield, consistent quality, and reduced cost. Polysaccharides and ganoderic acid are the two most significant active compounds (triterpenoids). Carbon and nitrogen supply, minerals, micronutrients, temperature, pH, duration, and oxygen all play critical roles in the yield of these compounds during fermentation.

3.2 Spawn Preparation

There are two forms of *G. lucidum* spawn formulations: liquid and solid. To prepare the pure culture liquid mycelial spawn, potato-dextrose broth or other nutritional liquid broths are used. The solid spawn can be prepared with dowels, grooved woody plugged spawn, grain spawn, and sawdust-bran spawn. To produce solid spawn, cereal grains such as wheat, barley, sorghum, pearl millet, and maize are commonly used. The grains were rinsed, boiled, and placed in poly bags of the appropriate size. The top bags are covered with heat-resistant plastic tubes. After inserting the cotton into the mouth, it is wrapped and tied in sterile brown paper or aluminium foil. Following sterilization, these packets are transferred to a clean aseptic room and stored for 24 h. With the cotton plug removed, the poly bags are inoculated with a young, equal-sized growing culture of *G. lucidum* and incubated for 8–10 days at 25–28 °C before being inoculated onto the fruiting substrates.

3.3 Nutritional Requirements for Cultivation

Ganoderma is a worldwide significant genus of white rot fungi that contains enzymes that delignify wood selectively. *Ganoderma* growth requires a variety of nutrients, including carbon and nitrogen, inorganic salts, and growth stimulants. The carbon:nitrogen (C:N) ratio in the substrate is claimed to be between 15 and 45:1 for maximum mycelial growth (Zhou et al., 2012) and 70–80:1 for a perceived higher yield (Kumla et al., 2020). *In artificial culture*, *G. lucidum grew optimally on carbon sources such as sorbose*, *dextrin*, *glucose*, *and sucrose (*Jayasinghe et al., 2008*)*. Chemical additives such as ammonium acetate, arginine, calcium nitrate, and glycine are frequently employed to provide nitrogen for the mycelial growth of this fungus. Apart from carbon and nitrogen, *Ganoderma* growth requires inorganic salts and other inorganic elements such as calcium, kalium, magnesium, natrium, phosphorus, sulphur, and zinc, among others. Phosphorus, potassium, and magnesium are the three essential nutrients. The mushroom prefers glucose above other carbon molecules because glucose is easily digested by the mushrooms to provide energy for the cells. *Ganoderma* mycelia may utilize a variety of low molecular weight molecules, including amino acids, urea, and nitrogen.

3.4 Substrates Used for Cultivation

A suitable environment for primary and secondary ingredients is crucial for high yield and quality bracket development. *Ganoderma* grows on organic carbon sources such as sugars, starches, cellulose, hemicelluloses, and lignin. In the local area, carbon is sourced from cottonseed husk, and nitrogen from rice or wheat bran. Several researchers evaluated the applicability of rice bran, rice husks, coconut fibre, corn, peanut hulls, sorghum, and sugarcane bagasse as substrate combinations (Zhou et al., 2012). *G. lucidum* mycelial growth and yield were superior on different

substrate formulations using maize cobs (73–88%) in varied combinations with gypsum, maize flour, sucrose, soybean meal, urea, and wheat bran (Peksen & Yakupoglu, 2009). It is usually formed on grain or lignocellulosic materials like sawdust or wood. An inexpensive substrate such as agricultural residue can be converted into a concentrated product through solid state fermentation. As demand for *G. lucidum* expands, researchers are exploring the commercialization of bag culture using sawdust and agricultural waste. A review of the literature revealed that sawdust from a variety of tree species, including the alder tree, gogon sirish, Indian rosewood, karoi tree, mango, palmyrah, river red gum, sal tree, silk cotton, and their mixed sawdust (Roy, 2015; Subedi et al., 2021), alone and in combination with banana leaves, coconut fibre (coirpith), millet powder, peanut hulls, rice bran, rice husks, sugarcane bagasse, and wheat bran, was evaluated for use in the cultivation of *G. lucidum* (Veena & Pandey, 2011). When it comes to formulated substrates for the growing of *G. lucidum*, sawdust is the most commonly utilized base material, and sawdust mixed with bran substrate containing calcium and sucrose is a popular combination for *Ganoderma* cultivation. For the production of *G. lucidum*, Mishra and Singh reported that among the substrates used, wheat straw modified with rice bran was the most successful. Sawdust is the major element in substrate combinations (Renu & Brij, 2015). Edible mushrooms are grown on cereal straw, which is more readily available than sawdust. Since rural areas lack labor, we require production packages based on appropriate accessible substrates (Veena & Pandey, 2011). Although some of the constituents were present in the growth substrates, they have been added with $CaSO_4$, KH_2PO_4, and $MgSO_4$, particularly the quantity of $CaSO_4$ is up to 1% of the total substrate mass. This is why calcium sulphate can be employed to adjust pH, permeability, air flow, nitrogen fixation, and calcium and sulphur concentrations. Vitamin B1, B6, and biotin are all growth factors involved in Ganoderma metabolism (Zhou et al., 2012). Biological performance is determined as the fresh weight of harvested mushrooms divided by the dry weight of the growing substrate. The biological efficiency of different substrates used for cultivation of *Ganoderma* species is listed in Table 1. A high biological efficiency value ensures strong substrate utilization. Regarding high-profit mushroom farming to be profitable, the biological efficiency has to be above 50%. It provides a sizable net yield from each substrate used for mushroom cultivation.

3.5 Factors Governing Cultivation

Ganoderma evident morphological traits, growth, and reproduction are strongly influenced by environmental factors. The environmental factors are abridged primarily to include several features: carbon dioxide, temperature, humidity, air, water, and light. *Ganoderma* sp. are habitually hygrophilous, aerobic, acidophilic, heterotrophic, and photophobic fungus. The mycelium of *Ganoderma* species grows optimally between 15–35 °C in naturalistic environments (Jayasinghe et al., 2008). Specifically, the optimal temperature for mycelial growth is 25–30 °C, while the optimal temperature for basidiospore germination is 24–26 °C. The relative humidity

Table 1 Biological efficiency of different substrates used for cultivation of *Ganoderma* species

S. No.	Substrate	Biological efficiency (%)	Reference
1.	Paddy straw	6.0	Magday Jr. (2014)
2.	Sunflower seed hull (95%) + wheat bran (5%)	10.0	Gonzáles-Matute et al. (2002)
3.	Sawdust (22.5%) + paddy straw (67.5%) + rice bran (10%)	29.9	Veena and Pandey (2011)
4.	Sawdust (45%) + paddy straw (45%) + rice bran (10%)	27.3	Veena and Pandey (2011)
5.	Elephant-grass (39%) + mango sawdust (39%) + wheat bran (10%) + bagasse (10%) + agro-plaster 2%	72	Rolim et al. (2014)
6.	Oat straw (80%) + wheat bran (20%)	2.0–2.5	Kumla et al. (2020)
7.	Beech sawdust	61.2	Kumla et al. (2020)
8.	Beech sawdust (50%) + olive pruning residues (50%)	20.5	Kumla et al. (2020)
9.	Poplar sawdust (80%) + wheat bran (19%) + molasses (1%)	20.37	Erkel (2009)
10.	Poplar sawdust (75%) + malt extract (5%) + wheat bran (10%)	18.68	Majid et al. (2012)
11.	Rubber sawdust	5.1	Jeewanthi et al. (2017)
12.	Mango sawdust	5.4–12.6	Jeewanthi et al. (2017)
13.	Jack sawdust	2.7	Jeewanthi et al. (2017)
14.	*Lunumidella* sawdust	3.6	Jeewanthi et al. (2017)
15.	Rubber (50%) + mango (50%) sawdust	5.3	Jeewanthi et al. (2017)
16.	Rubber (50%) + jack (50%) sawdust	2.5	Jeewanthi et al. (2017)
17.	Rubber (50%) + *Lunumidella* (50%) sawdust	5.7	Jeewanthi et al. (2017)
18.	Mango sawdust (90%) + wheat bran (10%)	25.2	Renu and Brij (2015)
19.	Mango sawdust (80%) + wheat bran (20%)	21.7–58.57	Renu and Brij (2015), Mehta et al. (2014)
20.	*Jacaranda* sawdust	14.2	Renu and Brij (2015)
21.	*Jacaranda* (90%) + wheat bran (10%)	24.9	Renu and Brij (2015)
22.	*Jacaranda* sawdust (80%) + wheat bran (20%)	27.9	Renu and Brij (2015)
23.	Indian rosewood sawdust	8.3	Renu and Brij (2015)
24.	Indian rosewood sawdust (90%) + wheat bran (10%)	13.1	Renu and Brij (2015)
25.	Indian rosewood sawdust (80%) + wheat bran (20%)	7.1	Renu and Brij (2015)
26.	Paddy straw (90%) + rice bran (10%)	25.7	Veena and Pandey (2011)
27.	Paddy straw (90%) + wheat bran (10%)	27.5	Jandaik et al. (2013)
28.	Sawdust (mixture of *Ficus* spp., *Eucalyptus* spp., *Acacia* spp., *Azadirachta indica*, and *Melia dubia*) (22.5%) + paddy straw (67.5%) + rice bran (10%)	29.9	Veena and Pandey (2011)

(continued)

Table 1 (continued)

S. No.	Substrate	Biological efficiency (%)	Reference
29.	Mahogany sawdust (90%) + rice bran (8%) + $CaCo_2$ (2%)	4.3	Roy (2015)
30.	Mahogany sawdust (90%) + wheat bran (8%) + $CaCo_2$ (2%)	7.6	Roy (2015)
31.	Gurjan sawdust (90%) + rice bran (8%) + $CaCo_2$ (2%)	3.6	Roy (2015)
32.	Gurjan sawdust (90%) + wheat bran (8%) + $CaCo_2$ (2%)	6.8	Roy (2015)
34.	Hornbeam sawdust (80%) + tea waste (20%)	34.90	Peksen and Yakupoglu (2009)
35.	Combination rubber sawdust and palm oil sludge (5%) + $Ca(OH)_2$ (2%) + $Mg(SO_4)$ (0.2%)	22.01	Seephueak et al. (2019)
36.	Rubber sawdust with empty fruit bunch fiber	27	Sudheer et al. (2018)
37.	Maize stem (44.4%) + bean pod (22.2%) + coffee pulp (22.2%) + wheat bran (11.1%) + $Ca(OH)_2$ (0.031%) + $CaSO_4$ (0.084%)	8.2	Bernabé-gonzález and Cayetano-Catarino (2015)
38.	Cotton stalk + paddy straw + bagasse + wheat bran + biochar	19.58	Rashad et al. (2019)
39.	Coconut wood log saw dust (78%) + wheat bran (20%) + $Ca(OH)_2$ (1%) + $CaSO_4$ (1%)	44.3	Thiribhuvanamala and Krishnamoorthy (2021)
40.	Sawdust's (mango + wattle + poplar + Indian rosewood) + wheat bran	54.5	Ralte et al. (2020)
41.	Commercial substrate (sawdust: Rice bran: $CaCO_3$ at 100:10:1) + baby diaper (2%)	36.01	Khoo et al. (2022)
42.	Mango sawdust + wheat bran	58.57	Mehta et al. (2014)

in the atmosphere should be regulated at around 65–70% during mycelial growth and running over the substrate or wood log, and a concentration of ideal oxygen as well as increased carbon dioxide (0.1–10%) in the atmosphere will typically enhance mycelial growth threefold compared to normal conditions. Under artificially dark conditions, acidic substrates with a pH of 4.5–5.5 encourage greater mycelial development (Zhou et al., 2012; Subedi et al., 2021). Inorganic ions like calcium and magnesium become more soluble in alkaline environments, inhibiting enzyme activity, vitamin production, and regular metabolic functions of mycelium. Light,

relative humidity, and temperature are the triadic factors that influence the shape of the fruit body as it develops during its life cycle. The morphology of the fruit body during development is affected by water and air quality, although only to a smaller extent than in the rest of the mushroom. The primordial initiation from the mycelium takes place at 15–30 °C. Basidiocarp production can be differentiated between 24 and 28 °C and normally develops between 25 and 30 °C (Jayasinghe et al., 2008). The relative humidity of the surroundings should be kept between 85–90% throughout basidiocarp genesis and growth. Additionally, clean potable water is being used for large-scale *Ganoderma* cultivation. Wood-log cultivation requires a moisture content of 37–40%. In substrate cultivation, 60–65% of the water content is suitable for growth and development. The substrate relative humidity for primordial initiation and basidiocarp expansion is 85–95%. To avoid stipe deformation, it is critical that the light surrounding the mushroom be as homogenous as possible during cultivation. The basidiocarp requires blue light (400–500 nm). When the intensity of light is above 1500 lux, the emerging bud expands quickly and forms a pileus. Phototaxis occurs in the stipe, and intense light in one direction can cause the stipe to grow excessively long. Air ventilation with a moderate carbon dioxide content influences basidiocarp growth. The needed atmospheric CO_2 concentration for basidiocarp differentiation and growth is 0.03–0.1%. If the carbon dioxide content is too high, the stipe may not form a basidiocarp. Higher carbon dioxide levels induce stipes to lengthen. Others include projections without a cap, which may also be related to carbon dioxide levels, as well as deer-antlers shape with a cap. Moreover, the culture bags and wood logs should not be moved regularly during the growth and fruiting phase.

3.6 Methods of Cultivation

Traditionally, one-meter-long natural logs were used for *Ganoderma* basidiocarp production, which were then entombed in a shallow trough. Yields were lower due to the longer cultivation duration of approximately 6–24 months to generate fruiting bodies, but cropping could continue for 5 years as compared to sawdust-based artificial log cultivation. Several different ways of cultivating *Ganoderma* have been tested, including basswood cultivation, sawdust cultivation, and substituted cultivation. Artificial cultivation methods under controlled conditions consist of five stages: (1) strain selection; (2) medium preparation; (3) inoculation; (4) cultivation in flasks, inoculating chambers, and fermentors; and (5) basidiocarp harvest. Even so, natural log cultivation delivers superior-quality *Ganoderma* mushrooms that fetch the highest prices in Southeast Asian markets (Chen, 2002). However, the yield may be less than that of sawdust artificial log cultivation, and the production period may be extended to that of sawdust artificial log cultivation. In general, commercial production sites employ block wood and substrate cultivation methods. To commence, block cultivation utilizes wood logs (natural or sterile) to cultivate stumpage by inoculation with mycelium or spawn materials. It is widely grown on maple trees in China. While maple trees are scarce in Japan, Japanese oaks and ume plum trees

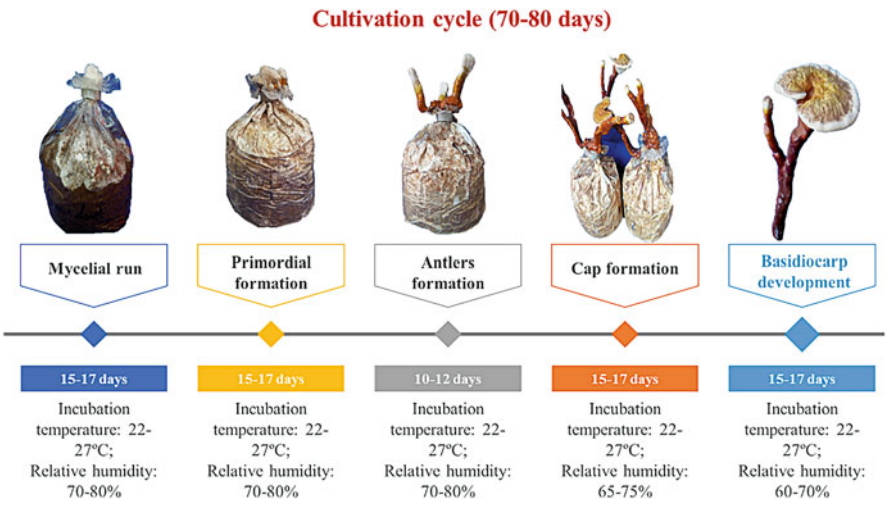

Fig. 3 Cultivation cycle of *G. lucidum* under substitute cultivation

are employed to cultivate *Ganoderma* in Japan (Okamoto & Mizuno, 1997). Second, sawdust or agro-waste resources with minimal additions have been identified as a unique way of inoculating mycelium or spawn materials as growth substrates for basidiocarps. The procedures of substitute cultivation are the following substrate selection (mixture of wet sawdust and rice bran), burdening (65% moisture), bagging (polypropylene bags, plug with non-absorbent cotton), sterilization (121 °C, 15 lbs. pressure for 60–90 min in autoclave), cooling, spawn inoculation, incubation, mycelial running in dark conditions, open the bags and shift to cropping room (blue light, cross ventilation), primordial initiation, basidiocarp development (kidney-shaped, reddish to brown in color), harvest and drying, grading and packaging in various forms of dry mushroom, mushroom powder, and products (Fig. 3). The following method is widely exploited for substitute cultivation (Subedi et al., 2021). Using sawdust, wheat bran and rice bran substrates (200 g) were placed in polypropylene bags (12 × 12 cm^2), autoclaved, and kept at 65% moisture. Then they cultured the synthetic logs for 60 days at 28 °C in diffused light with a relative humidity of 90–95%, sprinkling sterile water with calcium 0.1% twice daily. To allow for air circulation, 1 mm holes were punched in the aerial portions of the bag.

The growth, quality, and yield of artificially cultivated *Ganoderma* species vary in their genetics, physiological, and nutritional requirements (Roy, 2015; Magday Jr., 2014). Nowadays, *G. lucidum* is not the only species in this genus that is cultivated commercially, but also *G. applanatum*, *G. capense*, *G. neojaponicum, G. sinense*, *G. tsugae* (Chen et al., 2017; Ritu et al., 2020). The majority of broad-leaf hardwoods are suitable for cultivation of *Ganoderma* species, and logs are harvested from living trees. Since the late 1980s, innovative technologies utilizing short logs (15 cm or less) have been developed. Numerous aesthetic growth forms, including antler-like formations, can be achieved by modifying the growing

environment, most significantly the temperature and carbon dioxide concentration (Pegler, 2002). Nowadays, the majority of *Ganoderma* natural-log farmers in China, Japan, and Korea use this method (Bhosle et al., 2010; Chen, 2002). The length of time required to develop the fruiting body, mycelia-, and culture broth-based products has been examined as ways to ensure quality control and year-round manufacturing. It is currently produced commercially using a variety of methods, including wood logs, short basswood pieces, tree stumps, paddy straw, sawdust packs, and bottle methods (Veena & Pandey, 2011; Renu & Brij, 2015; Erkel, 2009; Girmay et al., 2016). Cultivation techniques have advanced significantly over the course of more than 40 years of experimentation. Currently, a variety of approaches are commonly employed in economic production (Hapuarachchi et al., 2018). Recently, the billet method of log cultivation was devised. The logs were debarked and chopped to a length of 10–15 cm and a thickness of 4–5 cm. Overnight, these billets were steeped in a 1% malt solution. After tying the billets, they were autoclaved in polypropylene bags. The bags were subsequently inoculated with the spawn of *G. lucidum* (20 gm/bag). These billets were incubated for 15 days at a temperature of 26 °C and a relative humidity of 60–70%. Mycelium growth was aided by a well-ventilated and clean environment. Solarization of the soil was accomplished by combining well-drained sandy soil with 1% lime. These billets were then vertically planted in the sand, one end level with the ground, and covered with soil. The billets were left in the dark until pinheads appeared, at which point they were adequately illuminated. After maturation and spore shedding, fruiting bodies were picked using a sharp knife (Singh et al., 2014; Bijalwan et al., 2021). Since temperature is a crucial determinant in the growth of *Ganoderma*, the billet method may be a successful cultivation method.

4 Industrial Processing

The processes of breaking down *Ganoderma* spore walls, extracting spore oil, and isolating chitosan from basidiocarp residues that succeed in oil or polysaccharide recovery are all critical for industrial development. Mechanical spore wall breakage between 15 and 0 °C, in general, better protects bioactive compounds from oxidative degradation and high temperatures. When the processing parameters are optimal, the breakdown percentage of the spore walls reaches 95–99%. Spore oil is extracted using CO_2 solvent extraction. Following the extraction of water-soluble compounds, fruiting body residues include chitosan. This can be extracted very easily isolated by protein extraction (deproteination), elimination of inorganic impurities (demineralization), which is an unnecessary process in the extraction of chitin from *Ganoderma*, discoloration of obtained chitin, and chitin deacetylation. These processing processes optimize resource utilization and are employed in a number of companies around China (Li et al., 2016).

5 Production Cost Analysis

Details of production cost analysis of the *Ganoderma* commercial cultivation are shown in Table 2, and marketing cost of processed products are depicted in Table 3.

An entrepreneur interested in the production of *Ganoderma* can earn (Rs. 8,795,000), during the first year, and the price goes up from the second year onwards. The bottleneck in *Ganoderma* production is marketing. It is believed that over the next few years, public awareness of the medicinal importance of *Ganoderma* would be created and entrepreneurs would have more benefit from cultivating *Ganoderma*.

Table 2 Economic analysis of commercial production of *Ganoderma*

Items	Budget for 3.2 tonnes/year (INR)	
	1st year	2nd year
Recurring		
1. Manpower (Rs. 10,000/month)	120,000	120,000
2. Consumables	50,000	50,000
3. Other maintenance costs (electricity, plumbing, water, etc.)	25,000	25,000
4. Packing and transportation	10,000	10,000
Sub-total	205,000	205,000
Capital		
1. Shed (20 × 10 ft)	100,000	5000
2. Permanent equipment	500,000	0.00
Sub-total	600,000	5000
Grand Total	805,000	215,000
Yield (800 kg/cycle × 4 cycles/year)	3200	3200
Price/kg is Rs. 3000 (fresh bracket)	9,600,000	9,600,000
Net income (Production cost – Sales cost)	8,795,000	9,385,000
Benefit Cost Ratio = Present value of expected benefits/Present value of expected costs	11.92	44.65

Note: Days/cycle: 70–90 days in commercial cultivation; Yield/cycle: 200–600 (400) g/25 kg substrate bag (200 bags × 400 g = 80,000 g/cycle = 800 kg)

Table 3 Processed products from *Ganoderma* and their price

Products	Approx. price (INR)
Fresh brackets	3000
Dried brackets	5000
Ganoderma dried slices	6000
Ganoderma extract powder	14,000
Capsule powder (450 mg)	4

6 Commercial Uses

Ganoderma species were traditionally grown in the wild and consumed mostly by the nobility, but they are now widely available and frequently used as an alternative or addition to conventional medicine (Sharma et al., 2019). The fruiting body, spores, and mycelium of this fungus are all marketed and used all over the world in the production of a wide range of culinary, medicinal, and cosmetic items, among other things. Specifically, *Ganoderma* species were found to contain economically practicable alkaloids, amino acids, beta-glucan, cytokines, fatty acids, flavonoids, ganomycins, glycoproteins, inorganic elements, lignin, lectins, nucleosides, nucleotides, peptides, phenols, steroids, and vitamins (Hapuarachchi et al., 2018; Ritu et al., 2020). Because of this economic potential, *Ganoderma* cultivation has become a rich business in China and other East Asian countries, and it is now spreading to other parts of the world. To effectively advertise mushrooms or propose an appropriate kind, it is critical to understand client expectations and perceptions. *G. lucidum* products are sold in the form of beverages, functional food supplements, medicines, cosmetics, daily-care products, etc. The polysaccharides found in *G. lucidum* have a broader range of therapeutic effects, including anti-ageing, antioxidant, anti-tumor, and other therapeutic properties; they also enhance the immune structure and lower blood sugar and fat levels, with β-glucan being the most effective. *G. lucidum* strains produce 12.4–19.0 g of β-glucan for every 100 g of dry weight (Hwang et al., 2018). The β-glucan extracted from *G. lucidum*, in particular, has been shown to suppress cancer cell development by triggering the immune response in normal cells (Zhibin Lin, 2019). Recently, it was discovered to have radioprotective properties (González et al., 2020). Tyrosinase (EC 1.14.18.1) is a crucial molecule in melanogenesis in human skin, and the extracts from *G. formosanum* and *G. lucidum,* suppress its activity (Wang et al., 2017). Many skin care companies use *Ganoderma* derived molecules as masking, whitening, moisturizing, toning, and sun-protectant products (Kim et al., 2016). It has been widely commercialized as a food and medicine supplement that supports the immune system and metabolic activities of the body. The extracts of *G. lucidum* also showed an anti-bacterial activity against *Micrococcus luteus*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Hleba et al., 2014). Ganodermin from *G. lucidum* has also been shown to have antifungal mechanisms against *Botrytis cinerea*, *Fusarium oxysporum, Physalospora piricola,* and *Saccharomyces cerevisiae* (Hleba et al., 2014; Wang & Ng, 2006). Similarly, extracted compounds such as ganodermanontriol, lucidumol A, ganoderic acid C2, ganosporeric acid A, ganoderiol F, ganoderic acid, and triterpenoids have been shown to have anti-viral activity against a variety of viruses such as enterovirus (Zhang et al., 2014), human immunodeficiency virus (HIV-1) (Flórez-Sampedro et al., 2016), dengue virus, herpes simplex virus types 1 (HSV-1 and HSV-2), influenza A virus (Flu A), hepatitis B, and vesicular stomatitis virus (VSV) (Bharadwaj et al., 2019). The biomaterial sacchachitin and chitosan from the *G. tsugae* fruiting body can be utilized to treat ocular damage, bacterial acne, endodontic treatment, and dialysis membranes (Huang et al., 2019). The mycelium of *Ganoderma* is progressively

being employed in bioengineering technologies. It can be used to make mycelium blocks, furniture, and fabric items, among a variety of other things. *Ganoderma* extracts were exploited as a raw material to enhance the functional characteristics of beer, yakju, and wine (Leskosek-Cukalovic et al., 2010). *Ganoderma* was used to make bonsai ornaments for lawns, decorations, and a variety of other artistic works. The residual substrate from *G. lucidum* cultivation on sunflower seed hull, rice straw, and rice husk agro-residues was recycled to produce ganocetas as biodegradable containers (Postemsky et al., 2016) and soil amendments (Rashad et al., 2019) for seedlings. To mitigate harmful emissions and a scarcity of paper manufacturing materials, refined *G. lucidum* wastes are employed as raw materials and as a sizing ingredient in the paper industry (Khoo et al., 2020).

7 Market Potential

The *Ganoderma* market has grown tremendously during the last two decades in Asia, now offering thousands of products, notably nutraceuticals, that are eaten as dietary supplements. *Ganoderma* basidiocarp, slices, and spore powders are the three most popular forms in the domestic economy among buyers looking to improve their health or treat and prevent diseases. Based on these forms, there are many different types of *Ganoderma* products available, such as Brain Gano, Heart Gano, Kimshen Gano, Liver Gano, Peacock Gano, and Ruyi Gano, with a variety of names and labels (Karuppiah & Ji, 2020). Despite the enormous market, the industry faces challenges in developing a global market. The multifaceted activity of fungi in the genus *Ganoderma* paves the way for their widespread application in a variety of economic categories. It is a prominent Asian therapeutic agent with a wide range of uses. It is widely utilized in food supplements worldwide, with an increasing number of patents and products utilizing *G. lucidum* as an active ingredient. The extracted and purified compounds are sold globally as capsules, lotions, hair tonics, and syrups. The global *Ganoderma* mushroom market was valued at USD 3096.9 million in 2019 and is predicted to reach USD 5059.4 million by 2027, growing at an 8.1% compound annual growth rate between 2021 and 2027. Demand for *Ganoderma* is also rising due to growing consumer health awareness, spending, and product understanding and knowledge. Additionally, consumers are becoming more receptive to sustainably produced foods and beverages, such as those with simplified labels, no additives or preservatives, and a non-GMO and easily identifiable ingredient list. This is a significant factor contributing to the growth of the *Ganoderma* mushroom market. According to market trend on *Ganoderma* products, the market is segmented by type, end use, origin, and geographic area. Food and beverages, pharmacological, nutritional, and dietary ingredients, and beauty products and personal care are the market segments classified by end usages. The market is segmented geographically into Asia-Pacific, Europe, North America, and Latin America. *Ganoderma* mushroom powder is also a popular cosmetic and personal care product. Powdered *Ganoderma* products dominated the fresh, dried, and sliced *Ganoderma* markets. In both its natural state and usage, powdered

Ganoderma is highly customizable. It contains the same necessary nutrients as the fresh version, but with fewer calories. It is easily incorporated into a variety of food products. This is due to its excellent antioxidant, anti-aging, anti-wrinkle, skin-brightening, and moisturizing qualities. As a result, more global brands are creating and launching personal care products containing the *Ganoderma* powder or extracts. However, *Ganoderma* mushroom products are more expensive than equivalent items, which may limit market expansion. Rather, customers continue to favour foods that promote a healthy lifestyle. Demand for organically grown foods with high nutritional value is rising due to rising consumer health consciousness. This surge in demand for organic *Ganoderma* products creates growth and diversification potential for *Ganoderma* manufacturers.

The liquid *Ganoderma* segment, on the other hand, is expected to expand at a faster CAGR during the forecast period. The liquid extract of the mushroom has fewer impurities, which is important if a consumer wants a specific effect. Mushroom liquids also provide more nutrients than powdered mushrooms. A key consumer factor is that it is easily included in portable flavours. Additionally, China is the world's largest supplier and buyer of *Ganoderma* products. North America, on the other hand, is expected to experience a significant CAGR. India's trade exceeded USD 1 billion annually, owing to imports from China and Malaysia. Numerous Ganoderma-based products have been launched in Indian e-commerce as customers' attitudes toward health-conscious products such as functional foods and beverages have shifted as a result of lifestyle changes (Table 4). As a result, the popularity of mushroom-based nutritional supplements is increasing worldwide.

Strategy and Flow Chart for the Commercialization Process

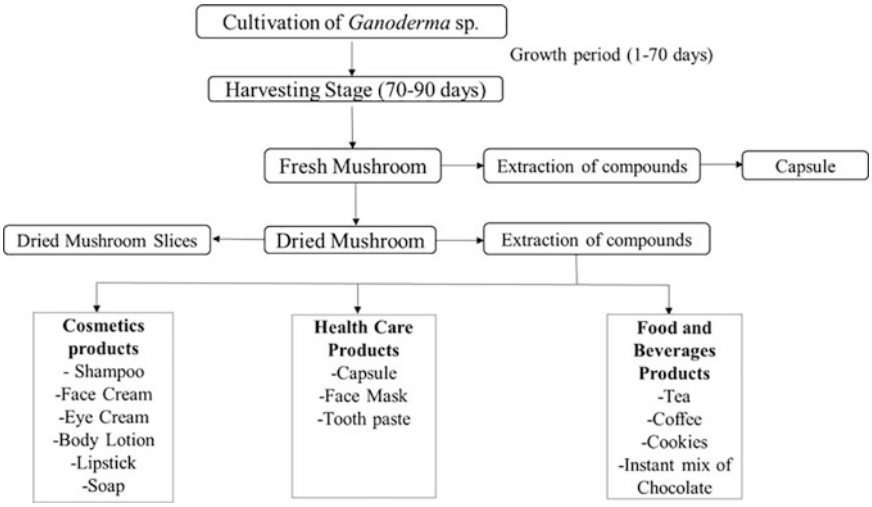

Table 4 Products available in Indian markets

Name of the product	Content	Cost (Rs.)
Vitawin *Ganoderma* capsules, ultimate health and nutrition supplements for boost immunity, detox rejuvenation and anti-oxidant support	60 capsules	499
Nature sure *Ganoderma*	60 capsules	749
Trexgenics® *Ganoderma* 500 mg Reishi Mushroom Extract	60 capsules	899
Viridio reishimax *Ganoderma* extract red reishi mushroom	60 capsules	699
Reishimax Reishi Mushroom *Ganoderma lucidum* extract for immunity booster	60 capsules	600
Cordy gold Reishi Mushroom capsules	60 capsules	600
Amalth Reishi Mushroom extract powder for better sleep, Relaxation	90 capsules	599
Vringra Ultra-Pure *Ganoderma*	60 capsules	949
Vringra Ultra-Pure Sea Buckthorn	60 capsules	320
Zindagi *Ganoderma* Herbal Capsules	60 capsules	461
Solorary- Dietary Supplement	100 capsules	951
Zindagi	60 capsules	409
Woohoo Natural Reishi King Triple Mushroom	60 capsules	1084
Harry Organo Premium Grade Lingzhi Mushroom	50 g	650
Hawaiian herbal *Ganoderma* Complex Drops	60 mL	749
Mahogany Reishi Mushroom Extract Powder	50 g	648
Vestige	90 capsules	750
Reishi Gano Tea	150 g	390
Keva *Ganoderma* Coffee	250 g	600
Teeccino, Mushroom Herbal Coffee, Reishi Eleuthero	284 g	1042
Mushroom Hot Chocolate Blend	8 g	1216
Dxn Ganozhi Toothpaste with *Ganoderma*	150 g	986
DXN Ganozhi *Ganoderma* Palm Oil Based Soap	150 g	224
DXN Ganozhi shampoo	250 mL	370
Shroomness Reishi Mushroom Spawn Liquid Culture	10 mL	660

According to the chart, *Ganoderma* sp. cultivation and fresh mushroom harvesting take place between 70–90 days. Chemical compounds are extracted from fresh mushrooms and converted into capsules. It is used as a supplement to boost human immunity. Fresh mushrooms can be dried in a variety of ways. Dried

mushrooms are used for a variety of purposes by extracting the compounds of *Ganoderma* sp. and developing them as cosmetics, health care, and food and beverage products. The importance of *Ganoderma* products has been realized in western countries as well as south east Asian countries, except in the Indian subcontinent. However, consumer preference for *Ganoderma* is slowly picking up in India. People recognize the medicinal importance of *Ganoderma* and are slowly inculcating it into their diets.

8 Concluding Remarks

Ganoderma has a long and rich history of tradition, cultivation, and human-related applications. Over the last 60 years, substantial research has been conducted on commercial cultivation, use, product development, and marketing in order to meet the global demand. The *Ganoderma* industry has progressed from basic resources and growing knowledge to artificial cultivation, large-scale production, bioactive component isolation, product commercialization, and industry development. Numerous obstacles must be overcome in order to realize this great economic potential. As a result, a comprehensive study of taxonomy is still required. In some cases, the scientific name for *Ganoderma* species may be incorrect. The market dictates that known *Ganoderma* cultivation methodologies be utilized. Training may result in the manufacture of *Ganoderma* products through standard techniques, hence increasing the quality of *Ganoderma* commodities on the market. Furthermore, fermentation methods must be tuned in order to increase the concentrations of specific compounds. Finally, despite its enormous market size, the *Ganoderma* company continues to face obstacles such as product homogeneity, a lack of high-value-added products, substandard quality, and expensive costs. To develop innovative products, it is necessary to identify and characterize bioactive components. Meanwhile, a market order that is viable in nations like India must be established.

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Small/Large-Scale Production, Cost Benefit Analysis, and Marketing of *Spirulina* Single Cell Protein

R. Thangaraj, S. Mahendran, C. Nizhanthini, D. Dhanasekaran, and N. Thajuddin

Abstract

The multi-cellular and filamentous cyanobacterium *Spirulina* sp. has gained substantial predominance in the health sector, food industry, and aquaculture in contemporary times. It has a very high content of macro and micronutrients, essential amino acids, proteins, lipids, vitamins, minerals, and anti-oxidants. In recent years, *Spirulina* sp. has garnered enormous attention from research fraternity as well as industries as a flourishing source of nutraceutical and pharmaceuticals. It is considered a complete food supplement to combat against malnutrition deficiencies. In developing countries like India, malnutrition is a renowned social challenge that can be defeated by the supplement of *Spirulina* sp. products in the diet. The commercial cultivation of *Spirulina* sp. that can be converted into consumable forms (tablets/granules) can be an economic enterprise in India. Such agribusiness has been commenced by an agripreneur under Agri-Clinics and Agri-Business Centers (ACABC) scheme. The agripreneur was interviewed and informed that this agribusiness had a good turnover with low capital investment, and also providing employment opportunities to others.

Keywords

Cyanobacterium agri-business · Super food · *Spirulina* · Agri-business · Food industry

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1 Introduction

Spirulina sp. are the multicellular and filamentous blue green microalgae belonging to two separate genera, *Spirulina* sp. and *Arthrospira* sp., and consist of about 15 species. Of these, *Arthrospira platensis* is the most common and widely available *Spirulina* sp. and most of published research and published health decision refers to this particular species. They have characteristics of the oldest fossils of about more than 3.5 billion years ago (Sigler et al., 2003). They also have an evolutionary significance because they are responsible for present day oxygenic environment. Classifications of cyanobacteria proposed in 1985, in which four orders of the bacteria have been identified as *Chroococcales, Nostocales, Oscillatoriales,* and *Stigonematales* and their phyla are *Chroococcales*, *Gloeobacterales,* and *Pleurocapsales.* Cyanobacteria are immensely important in determining the path of evolution and ecological changes all over the earth's history. In the late proterozoic or the early Cambrian period, cyanobacteria began to take up residence within certain eukaryote cells, which is called endosymbiosis, for the origin of the eukaryotes (Garia Pichel, 2010).

Spirulina contains several nutrients, including B-vitamins, β-carotene, and vitamin-E, and also comprises antioxidants, minerals, chlorophyll, and phycocyanobilin. It was first discovered by Spanish scientist Hernando Cortez and Conquistadorsin in 1519. Now, modern lifestyle personalities endorse *Spirulina* as a **secret, potent 'super-food or a miracle from the sea'** (Fathima & Salma, 2001; Dillon, 2014). The United Nations world at food conference declared ***Spirulina* as the best food for the future** and it is gaining popularity nowadays among the wide population. The *Spirulina* has also multiple applications for clinical practices such as allergy, rhinitis, immunomodulation, antiviral applications, cholesterol-lowering effects, effects on diabetes, anticancer effects, chronic arsenic poisoning, and antioxidant effects (Karkos et al., 2008). Therefore, the demand for this supplementary diet is picking up in many countries to suffice the nutritional requirements of its population. As a result, many healthcare industries are involved in the production of *Spirulina* products since *Spirulina* is considered to be a high nutritive value best food for futur.

Microphotograph of *Spirulina* sp.

2 *Spirulina* as a Functional Food

Spirulina is like most cyanobacteria an obligate photoautotroph; it cannot grow in the dark on media containing organic carbon compounds. It reduces carbon dioxide in the light and assimilates mainly nitrates. The main assimilation product of *Spirulina* photosynthesis is glycogen. *Spirulina* shows an optimum growth between 30–35 °C under laboratory conditions. Outdoors, it seems that an increase in temperature up to 39 °C for a few hours does not harm the blue-green algae or its photosynthetic ability. Thermophilic or thermotolerant isolates of *Spirulina* can be cultivated at 38 °C. Such a property has the advantage of eliminating microbial mesophilic contaminants. The minimum temperature at which growth of *Spirulina* takes place is around 20 °C during the day. At night, *Spirulina* can tolerate relatively low temperatures. The resistance of *Spirulina* to ultraviolet rays seems to be rather high.

Spirulina is nutritionally complete with a balanced amount of all beneficial nutrients. It contains high protein content which range between 50% and 70% of its dry weight (Falquet, 1997), essential amino acids, fatty acids, vitamins, and dietary minerals (Belay, 2008; Sharoba, 2014; Gutiérrez-Salmeán et al., 2015). Furthermore, the biomass is very rich in antioxidants such as phenolics, flavonoids, vitamin E, and various light absorbing pigments (e.g. phycocyanin, chlorophylls, and carotenoids), which are also essential in preventing the body against free radicals (Kumar et al., 2005; El-Baky et al., 2008; Chu et al., 2010; Michael et al., 2018). Due to the exceptional nutritive profile, *Spirulina* has received much attention and is cultivated massively in health-food industries to serve as food for human, animals, feed additive, and pharmaceutical products (Kumar et al., 2005; Habib et al., 2008; Chu et al., 2010; Chen, 2011).

2.1 Mass Cultivation of *Spirulina*

Spirulina is one among various algal species discovered growing in natural fresh waters. These are observed in natural habitats such as soil marshes, seawater, and brackish waters where alkaline waters subsist. It can withstand low temperatures of 20 °C during nights and 38 °C for a few hours in the daytime also.

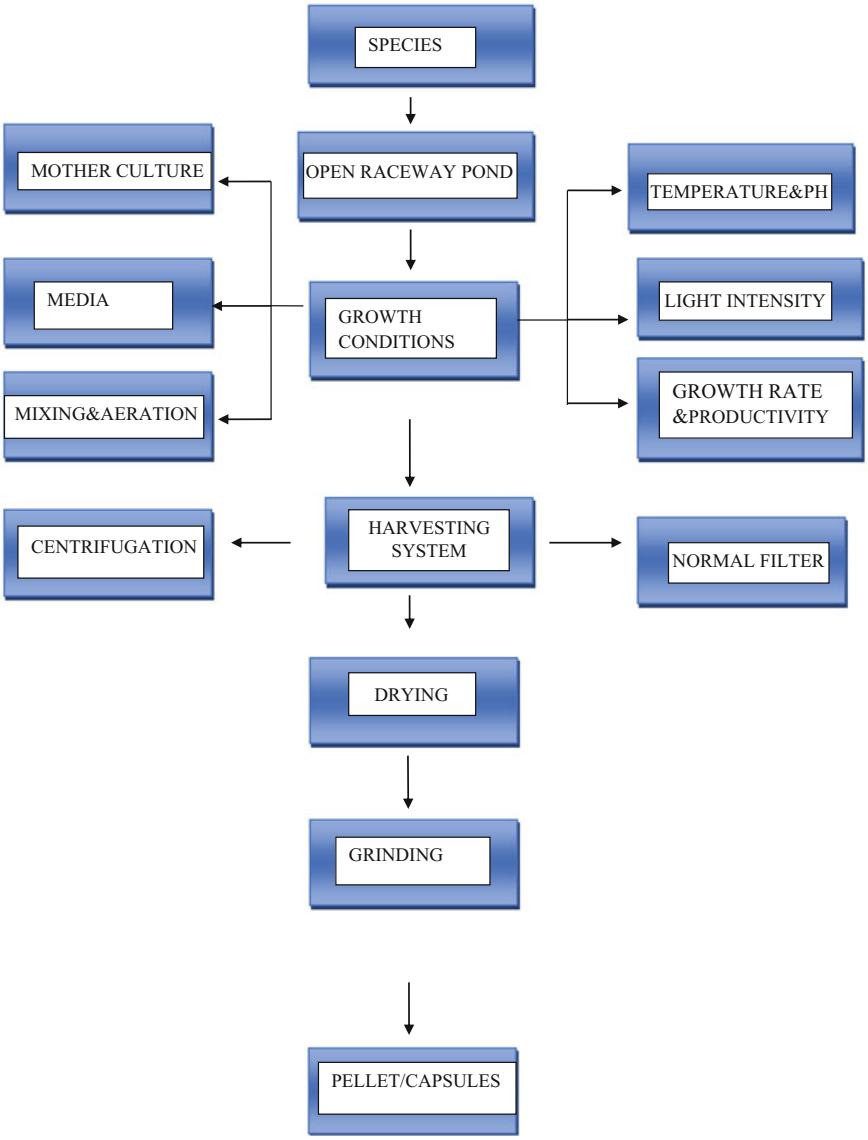
Outdoor cultivation of *Spirulina* sp.

In natural habitats, their growth cycles depend on the limited supply of nutrients. In Japan, large scale cultivation of microalgae of chlorella (early 1960s) followed by *Spirulina* commenced in the early s (Table 1).

Table 1 Nutritional profile of *Spirulina* powder (composition by 100 g)

Macronutrients	Quantity	Vitamins	Quantity
Calories	373 Kcal	Vitamin A (as β-carotene)	352.0 IU
Total fat	4.3 g	Vitamin K	1090 mcg
Total carbohydrate	17.8 g	Thiamine HCL (Vitamin B1)	0.5 mg
Essential amino acids	21.9 g	Rivoflavin (Vitamin B2)	4.53 mg
Non-essential amino acids	35.4 g	Niacin (Vitamin B3)	14.9 mg
Minerals	Quantity	Phyto-nutrients	Quantity
Calcium	468 mg	Phycocyanin	17.2%
Iron	87.4 mg		
Phosphorus	961 mg	Chlorophyll	1.2%
Magnesium	319 mg	β-Carotene	211 mg
Zinc	1.45 mg	Superoxide dismutase (SOD)	531,000 IU
Selenium	25.5 mcg	Zeaxanthin	101 mg
Copper	0.47 mg	Gamma linoleic acid	1080 mg
Manganese	3.26 mg		
Chromium	<400 mcg	Total carotenoids	504 mg
Potassium	1660 mg		
Sodium	641 mg		
Iodine	142 mcg		

Note: *IU* International unit


SPECIES
OPEN RACEWAY POND
MOTHER CULTURE
MEDIA
MIXING&AERATION
GROWTH CONDITIONS
TEMPERATURE&PH
LIGHT INTENSITY
GROWTH RATE &PRODUCTIVITY
HARVESTING SYSTEM
CENTRIFUGATION
NORMAL FILTER
DRYING
GRINDING
PELLET/CAPSULES

2.2 Requirements for Spirulina Cultivation

Water requirements are the most important step to growing spirulina, and although spirulina can grow in a variety of types of high alkaline waters, the following ingredients of Zarrouk medium are required:

Name of the chemical	Quantity (g/L)
$NaHCO_3$	18
$MgSO_4.7H_2O$	0.2
$NaNO_3$	2.5
K_2SO_4	1
$CaCl_2.2H_2O$	0.04
$FeSO_4{\cdot}7H_2O$	0.01
K_2HPO_4	0.5
NaCl	1
NaEDTA	0.08
Distilled water	1 L
Micronutrients	1 mL

Micronutrient composition (g/l): H_3BO_3, 2.86; $MnCl_2{\cdot}4H_2O$, 1.81; $ZnSO_4{\cdot}4H_2O$, 0.222; Na_2MoO_4, 0.0177; $CuSO_4{\cdot}5H_2O$, 0.08.

Other facilities required are:

Harvesting basins, drying rooms or racks, a room to analyse the *spirulina* flakes and transform them, technical requirements, land with a high boundary wall (fence) to avoid intruders, lighting, PV solar panel, raceway pond with paddle, culture pond – where the actual culture is tested for growth, harvesting station and dryer room, infrastructure – office, guard house, admin building, etc. packing and grinding unit, laboratory for testing.

2.3 Species Selection

Eight major environmental factors influence the productivity of *Spirulina*: luminosity (photo-period 16 h/day), temperature (30 °C), inoculation size, stirring speed, dissolved solids (10–60 g/L), pH (8.5–10.5), water quality, and macro and micronutrient presence (C, N, P, K, S, Mg, Na, Cl, Ca and Fe, Zn, Cu, Ni, Co, and Se) (Ciferri, 1983; Ayala, 1998). Today, there are more than 22 countries that cultivate *Spirulina* commercially on a large scale (Ravi et al., 2010). The distinct stages for commercial cultivation of *Spirulina* as practiced by the agripreneur are as follows.

It is a very crucial stage in *Spirulina* cultivation. The two species which are most commonly utilized are *Spirulina platensis* and *Spirulina maxima* (De Smet, 1997) due to their valuable components, positive effects, and being supplements which are nontoxic for human well-being.

2.4 Open Raceway Pond

A raceway pond is a shallow artificial pond utilized for the cultivation of algae. The cultivation of *Spirulina* can be done in open systems like ponds, lakes or lagoons, or a closed system (Singh & Sharma, 2012). The open ponds are utilized commercially to produce high value *Spirulina* products, which may be shallow big ponds, circular ponds, tanks, and raceway ponds.

Open Raceway pond cultivation in Department of Microbiology field facility, Bharathidasan University, Tiruchirappalli

The cultivation is usually carried out in two ways: (a) concrete ponds (b) pits lined with PVC or other plastic sheets. The agripreneur had constructed concrete ponds for its cultivation. The installation of single or multiple ponds can be arranged with ideal pond size of 50 m long, 2–3 m wide, and with 20 to 30 cm depth, but the length of the ponds can be of any measure depending on the land availability (Karthikeyan et al., 2016). With such infrastructures, *Spirulina* biomass yield of 35 tonnes/hectare/year has been reported in a commercial open mass cultivation pond at Siam Algae, Bangkok (Habib et al., 2008).

2.5 Growth Conditions

Spirulina growth conditions are similar to terrestrial plants, but they utilize resources very efficiently to increase biomass productivity with comparatively less water use (Sudhakar et al., 2014) (Lucie et al., 2016). *Spirulina* cultivation for commercial and large-scale production has to be executed in given regions with suitable climatic conditions. The tropical and subtropical regions are well-suited places for its cultivation. It requires sunshine throughout the year. The growth rate and production of *Spirulina* depend on numerous factors such as wind, rain, temperature fluctuation, and solar radiations.

2.6 Mother Culture

The fully developed concentrated *Spirulina* culture is required for inoculums preparation and culture maintenance. The preferred *Spirulina* strain must have a high proportion of coiled filaments (<25% straight filaments or none) and at least 1% of gammalinolenicacid (GLA) based on the dry weight. The colour of the culture should be explicitly green. The growth rate is about 30% per day when the temperature and other climatic conditions are satisfactory (Pal et al., 2011). The growth is proportional to the area of the culture exposed to light. Therefore, the agripreneur had maximized this area at all time.

2.7 Media

The culture of *Spirulina* is practised in different media, especially inorganic and decomposed organic nutrients. Different types of *Spirulina* were cultured to evaluate growth and biochemistry under similar controlled conditions (Bhattacharya & Shivaprakash, 2005). They cultured three species of *Spirulina,* viz., *Spirulina platensis*, *S. laxissima,* and *S. lonar*. Of the three species, *S. platensis* showed highest growth rate, biomass, pigment concentration, and low intracellular phenolics. The results indicate that *S. platensis* reached highest growth in shortest doubling time and the importance of strain selection for large-scale cultivation (Sanchez-Luna et al., 2004). It was found that the intermittent addition of urea in the autotrophic culture of *Spirulina platensis* yielded similar results to those obtained by the continuous feeding.

Spirulina flourishes in alkaline brackish water. The culture medium should provide all essentials to nurture *Spirulina* in a suitable environment. It should compose of sodium carbonate and other suitable medium as source of nitrogen, phosphorus, iron, and trace metals (Raoof et al., 2006). The makeup media should also consist of urea. It can grow on either nitrate or urea alone, but using both at the same time is more advantageous. The water should be clean or filtered to avoid the growth of other algae during cultivation. The media preparations should be arranged in such a way that it meets the local growing conditions for *Spirulina*. The most commonly used is Zarrouk's medium (Zarrouk, 1966; Pragya et al., 2013). The cost of nutrients accounts for about 15–25% of the total production cost (Selvendran, 2015).

2.8 Mixing and Aeration

The agitation of the mother culture in the media is an inevitable step to homogenize and assure a good distribution of light among all the filaments of *Spirulina* during its growth. The mixing of the mother culture plays an important function in increasing the productivity (Chisti, 2016). Similarly, the aeration is also very crucial for obtaining good quality and better yield of cultures of *Spirulina* species. It can be

achieved by rotators, which maintain the cells in suspension by gentle agitation of growing cells. The agripreneur had installed motorized-rotators in its pond for constant mixing as well as stir. It also assists to circulate carbon dioxide concentration uniformly and eliminates inhibitory substances such as oxygen (Richmond & Vonshak, 1978). When aeration is not adequate, the efficiency of energy utilization and biomass production will be low.

2.9 Temperature and pH

Spirulina can develop at 20 °C -37 °C, but the ideal temperature for *Spirulina* for high growth with high protein content is between 30-35 °C. The variation in atmospheric temperature is the main factor affecting the biomass production rates in *Spirulina* cultivation. The bleaching of cultures may take place when temperatures are above 35 °C, while it cannot withstand in temperatures less than 20 °C). The agripreneur has installed exhaust fans to regulate the temperature within the production unit. The pH maintenance of the media over 9.0 is obligatory in *Spirulina* cultures in order to avoid contamination by other algae. The pH adjustment is made by increasing the carbon dioxide level by the addition of carbonate salts into the culture (Selvendran, 2015). When pH is between 9 and 11, it indicates a healthy culture. The effect of pH on the algal growth, pigment production, and protein content of *Spirulina* species has the direct effect on the antioxidant system (Vonshak & Guy, 1987) (Ogbonda et al., 2007). The agripreneur is regularly testing the pH while it is growing. It is controlled by taking necessary measures, accordingly.

2.10 Light Intensity

All photoautotrophic organisms including photosynthetic bacteria, cyanobacteria, and higher plants transform light energy into chemical energy through photosynthesis. In open-air cultivation system, natural light or solar radiation is the sole source of light for the autotrophs. The light has a direct effect on *Spirulina* production for its protein content, growth rate, and pigment synthesis. The optical density of the culture is directly proportional to the light intensity. Higher the optical density, higher is the requirement of light and lower is the optical density, lower is the requirement of light. The agripreneur has covered the production unit by net shades which assist to regulate the light intensity for the cultivation.

2.11 Growth Rate and Productivity

In commercial *Spirulina* farming, it is needed to recreate the culture medium where water is the main source for *Spirulina* to grow naturally. It should have all the essential and required resources of nutrition for the healthy growth of *Spirulina*

(Bharathiraja et al., 2015). The water level in tanks should be controlled which is important for the photosynthesis process to take place in *Spirulina*. A minimum shallow level of 20 cm is an ideal water level height. The deeper the water level, sunlight penetration will be reduced, which will affect algae growth.

2.12 Harvesting System

The concentration of algae in the production unit (pond) will be the determining factor for harvesting. In general, the *Spirulina* will be ready for harvest after 5 days once the seeding process is done. The most suitable time for harvesting is early morning because the percentage of proteins in the *Spirulina* is highest during the morning. Besides, the cool temperature makes the work easier and more sunshine hours (during day-time) will be available to dry the product.

2.13 Centrifugation

Centrifugation is a method to separate *Spirulina* from the media. It is generally carried out by a centrifuge, driven by a motor that puts an object in rotation around a fixed axis, applying a force perpendicular to the axis. This method is reasonably efficient, but sensitive algal cells may be damaged by pelleting against the rotor wall.

2.14 Filtration

During commercial production processes, filtration devices are used for harvesting. These are of two types, inclined and vibrating screens. The inclined screens are 380–500 mesh with a filtration area of 2–4 m^2 per unit and are capable of harvesting nearly about 10–18 m^3 of *Spirulina* culture (Ogbonna et al., 1999). It is considered as most suitable method for harvesting *Spirulina.* The vibrating screens filter the same volume per unit time as the inclined screens, but require one third of the area. The next step is the washing of excess salts from the biomass. The washed cake is frequently homogenized before being dried.

2.15 Drying

Spirulina can be consumed fresh, but it has to be used after slight drying. *Spirulina* is relatively quickly digestible in its fresh form (Richmond & Vonshak, 1978). *Spirulina* should be consumed within 6 h of its harvest, although it can be stored for later consumption for a period of up to one or more year by sun-drying or in a solar drier. After drying, it will last for several months and also the nutritional content can be preserved. There are different drying methods which include sun-drying, freeze-

drying, spray-drying, drum-drying, and cooking. *Spirulina* has a thin and fragile cell wall; hence, sun-drying is sufficient to sterilize the algae and make it consumable.

Sun-drying is the most popular drying method, but requires a few precautions. Direct sun-drying must be very quick; otherwise, the chlorophyll will be destroyed and the dry product will appear blue. The agripreneur was drying the *Spirulina* after its harvest either through sun-drying or by drying machine.

2.16 Grinding/Powdering

Spirulina is consumed as a whole food/dietary supplement which is prepared in tablet, flake, and powder form. The dry chips or rods of *Spirulina* are usually converted to powder by grinding to enhance their apparent density. Grinding is continued for about 6–10 h, till the average powder size reaches 200–800 nm. The two most common forms of commercially available *Spirulina* are powder and tablets. It is also a component in some protein and energy-boosting powder mixes.

2.17 Small-Scale Commercial Production of *Spirulina*

Spirulina cultivation has a number of advantages over traditional agriculture:

2.18 High Yield

With around 60% protein content, rapid growth of *Spirulina* means it yields 20 times more protein per unit area than soybeans, 40 times more than corn, and over 200 times more than beef.

2.19 Soil Requirements

Spirulina culture does not require fertile land and can actually benefit from saline conditions.

2.20 Efficient Water Use

Spirulina uses less water L/kg of protein (approximately 2100 L/kg protein) than other crops. Water can be recycled and the only significant water loss is through evaporation. *Spirulina* culture uses 25% of the water of soy, 17% of corn, and 2% the water required for beef protein. As mentioned above, brackish or saline water can be utilized.

2.21 Efficient Energy Use

Spirulina requires less energy input compared to soy, corn, or beef, including solar and generated energy and energy efficiency of *Spirulina* (food energy output/kg/energy input/kg) is 5 times higher than soy, two times higher than corn, and over 100 times higher than grain-fed beef.

The small-scale production of *Spirulina* is considered as a potential income-generating activity for households or village collectives. *Spirulina* might be also dried and processed for local consumption, especially where poor dietary regimes need to be supplemented. In addition, the extensive or semi-intensive production of *Spirulina* for animal or aquatic feeds might be conducted for small-scale farming and aquaculture. As early in 1949, Spoehr and Milner suggested that the mass culture of algae would help to overcome global protein shortages. Ironically, in spite of the lamentably low per capita protein supplies in many parts of the world, mass cultivation of algae has received only casual interest. The United Nations Environmental Programme (UNEP) is emphasizing nitrogen fixation and nutrient recycling through a programme that will establish microbiological centers (MIRCENS), and it is hoped that this will stimulate interest in microalgae technology as a component of an integrated recycling system for rural communities. *Spirulina* indeed lends itself to simple technology: cultivation may be carried out in unlined ditches through which flow is low (e.g. 10 cm/second). Stirring may be provided by a simple device driven by wind energy or harnessed to humans. Harvesting may be readily performed using some suitable cloth, and the biomass dehydrates in the sun. The quality of the *Spirulina* product obtained in this fashion would not be as high as what is attained in “clean cultures”, but product could serve well as animal feed.

3 Food Source as a Benefit for Humans

Food source: when the algal cells or filaments of *Spirulina* are transformed into powder, it can provide the basis for a variety of food products such as soups, sauces, pasta, snack foods, instant drinks, and other recipes. Attempts have been made by

Proteus, a marketing company mainly associated with Earthrise Farms in the United States of America, to incorporate *Spirulina* into a variety of food products such as granola bars and various kinds of pasta (Vonshak, 1990). *Spirulina* powder is also an ingredient of an orange-flavoured chewable wafer and other types of candy, of protein flours (10% *Spirulina* added to soybean or to milk-egg powders) and of Pastalina, a green soy-whole wheat noodle. The preparation of fermented foods such as cheese, yogurt, and tofu offered many new possibilities to the use of *Spirulina.* Furthermore, extraction methods could provide a decoloured *Spirulina* powder (yellow-white) which is odourless and tasteless, and thus suitable for widespread use. *Spirulina* is also used to prepare food with other ingredients. For example, instant noodles, stylish noodles, nutritious blocks, beverages, and cookies. The first three food items are recommended as luncheon food for middle-grade students. Diets in which *Spirulina* provides up to 100% of the protein produced growth rates comparable with those obtained with standard diets in several animal species. As little as 10 g a day of *Spirulina* brings rapid recovery from malnutrition, especially in infants. This was achieved in Mexico for undernourished children and adults.

3.1 Food Safety Aspects Related to Human Consumption of *Spirulina*

Since many of the existing blue-green algae species are known to produce toxin (microcystins, in particular MCYST-LR), it is very important to clarify the specific species used for human consumption as in all likelihood there is a danger of species substitution and/or contamination of *Spirulina* with other cyanobacteria. It is particularly important in countries where no such regulation exists on this type of products. The studies and the risk management decisions about blue-green algae products are relatively recent. Although there has been no conclusive evidence on the presence or absence of microcystins in *Spirulina*, only products from *Arthrospira platensis* have so far been cleared for consumption (United States of America, Australia, Canada, and probably EC) under specific conditions, by public health authorities. A Canadian study found that no microcystin was detected in blue-green algal products containing only *Spirulina*, while a study conducted for the Oregon Department of Agriculture (ODA), published in 2000, found MCYST-LR in all the 15 *Spirulina* samples (dietary supplements) analysed (Gilroy et al., 2000). Nevertheless, MCYST-LR content in *Spirulina* samples was below the regulatory level established by the ODA for microcystins in blue-green algae products (1 μg/g). *Spirulina* has been recognized as GRAS (generally recognized as safe) under the "indented conditions of use" implying that it is "for use as an ingredient in foods, at levels ranging from 0.5 to 3.0 grams per serving." This means in relatively small amounts. Nevertheless, considering that safety level and possible hazards for consumption of *Spirulina* and *Spirulina*-related products have not been established beyond doubt, special precautionary measures would be necessary on the consumption of *Spirulina* products to some segments of the population at risk to include pregnant women, nursing mothers, and people in dialysis and immune-compromised.

3.2 Costs and Profit in *Spirulina* Farming

The pond should be constructed of size 10′ × 20′ and required about 20 such ponds. Each pond will generate on an average about 2 kg wet culture per day. It should be noted that one-kg wet culture will give 100 grams of dry powder only. Based on this, on an average, a 20 tank *Spirulina* farming business will generate 4–5 kg of dry *Spirulina* powder on a daily basis. The production of *Spirulina* in a month will be around 100 to 130 kg per month. Dry *Spirulina* powder in the market will fetch about Rs. 600 per kg. A farmer can earn about Rs.40000–45,000 per month. A farmer can make more profits by increasing tanks made with low-cost, durable materials apart from concrete ponds by utilizing maximum space available in the land, which will reduce labour and investment with more profit returns.

3.3 Production Economics of *Spirulina* Farming

Capital investment			Economics of farming	
S. No	Particulars	Cost (Rs.)	Total capital investment	12,50,000
1	Pond construction	10,00,000	Operational cost	25,000
2	Plant machinery		Total cost (Rs.)	12,75,000
3	Laboratory equipment 5000.00	5000		
4	Water treatment plant	1,50,000	Sale of spirulina powder (@ Rs. 600 per kg)	72,000
5	Piping work	25,000		
6	Electrical works	15,000		
7	Drying screens	10,000	Income per month (Rs.) (Sale—Operational cost)	47,000
8	Harvesting screens	5000		
9	Packing materials	2500		
10	Chemicals (per month)	2000		
11	Labor (monthly basis)	18,000		
12	Miscellaneous	2500		
	Total capital investment	12,50,000		

Total expected output	10 g/sq.mt/day
500 sq.mt	5 kg per day
Wholesale price per kg	Rs. 50
Total expected production × wholesale price/day	−Rs. 250
Direct cost – water/fertilizer/day	Rs. 50
Gross profit/day	Rs. 200
GP per month (average 25 days)	Rs. 5000.00
Less monthly expense	Rs. 1370.00
Total expected net profit per month	Rs. 3630.00

4 Recent Development and Future Outlook

4.1 Plant Growth Regulators

Plant growth regulators may be used to enhance *Spirulina* growth. Among the plant growth regulators, the combination of 6-BA (6-benzyladenine) and NAA is suitable for the growth of *Spirulina platensis*A9 compared to other growth regulators and/or combination of other regulators. There are some other growth regulators such as GA (Gibberellic acid), IBA, 2,4 –D, and IAA used to enhance the growth of microplant like *Spirulina* and other microalgae, but not effective like the combination of 6-BA and NAA.

4.2 Strain Development and Improvement

The species diversity of *Spirulina* is limited, with not more than 15 species (Tompkins et al., 1995; Phang & Chu, 1999; Bhattacharya & Shivaprakash, 2005). New strains of *Spirulina platensis* with improved nutritional quality and high essential micronutrient levels can be developed through replicable and stable gene transfer system (Torzillo et al., 1985). This transfer system has not been successfully established since 1997. A recipient system for genetic transformation in *Spirulina* with electroporation, isolated single cells by lysozyme-free method, was found to be regenerated with a rate of 28.6% (Gaoge et al., 2004). Ultrastructural observations for electroporated cells indicated that electroporated cells recovered in 7 days. Electroporation is a valid transforming method for transgenic manipulation of foreign genes into *S. platensis*. Thus, strain development and improvement of *S. platensis* through transformation of foreign gene could be conducted using this method.

4.3 Expanding Production to New Countries

At present, *Spirulina* production is restricted to either countries with a high demand for high value and processed products that allow commercially viable intensive production (i.e. the United States of America and China) or to a few countries that have specifically focused on small-scale production to supplement human diets or to integrate animal and fish production. It is suggested that there could be a considerable expansion of the latter category, especially in the following areas:

High Altitude Alkaline Ponds or Lakes

Spirulina can be grown in ponds or lakes at high altitudes if the water is sufficiently alkaline. If *Spirulina* is not found in these habitats, then it can be imported from strains developed for similar environments. A note of caution should be made, in that a detailed investigation should be conducted before any new organism is introduced into a watershed where it is not naturally present in order to ensure that it will not

have any adverse ecological impacts in the probable circumstance of its loss into external watercourses.

Coastal Area with High Temperature

Tropical coastal belt areas with a high ambient water temperature may be suitable for *Spirulina* production.

Saline-Alkaline Water

When the groundwater is rich in calcium and other minerals, conventional crop production can be poor, especially in areas with high summer irradiation levels; the culture of low-temperature-resistant *Spirulina* may be an option, especially for small-scale rural development.

5 Conclusion

Spirulina has considerable potential for development, especially as a small-scale crop for nutritional enhancement, livelihood development, and environmental mitigation. *Spirulina* has the potential for being a 'wonder food supplement' and various leading organizations have recommended its salubrious effects on human well-being. In developing countries like India, it can be very suitably utilized as a diet supplement to overcome the challenge of social malnutrition. Unlike a company set up for *Spirulina* production in other countries, the commercial cultivation of *Spirulina* can be an agribusiness in India to supply *Spirulina* products to a large population of the country. It has good turnover with low capital investment, also providing employment opportunities to others.

In conclusion, in this study the methodology of cultivation of *Spirulina* was provided. *Spirulina* sp. in open raceway ponds on large scale would be an efficient way for CO2 fixation to mitigate greenhouse effects. Overall, *Spirulina* cultivation is an emerging agribusiness providing lot of opportunities for the people. Similar agribusiness under ACABC scheme has a good scope and turnover with low capital investment.

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Conflict of Interest Statement We declare that we have no conflict of interest.

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Production, Economics, and Marketing of Yeast Single Cell Protein

Urjita Sheth and Swati Patel

Abstract

Increase in the world population living below the poverty line actuates the scientific community to find economic alternative to conventional expensive protein sources. Single Cell Protein (SCP), microbial biomass products produced by fermentation for human or animal consumption, is the good protein alternative. SCP is the term used to designate microbial protein cultivated on organic wastes to be utilized as a human food or animal feed. The SCP from microorganisms is convenient over other protein sources as it offers more advantages such as shorter doubling time, i.e., rapid growth on cheap substrates (raw materials), have high protein content, have less reliance upon environmental factors such as soil, water, and climate and small land requirements. Yeasts have been particularly important since it has been consumed by humans since ancient times in fermented foods. This chapter includes discussion on types of yeasts that can be used as SCPs, sources of organic sources for the cultivation of yeasts, fermentation methodology, protein harvesting, marketing, and business plan.

Keywords

Yeast · SCP · Agriculture waste · Fermentation · Marketing

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1 Introduction

SCP is a generic term used for protein extracted from pure or mixed culture of unicellular or multicellular microorganisms such as algae, yeast, fungi, or bacteria, having more than 40% of crude protein on dry-weight bases, cultivated on agricultural or organic wastes, to be utilized as a food for humans or feed for animals (Cooney et al., 1980). In developing countries, one of the main problems faced by the modern world is a large food shortage which affects the growth and health of children; therefore, the researchers turned their attention to trying to find inexpensive food sources. Although alteration in food consumption patterns leads to increase in demand of protein, only dependance on agriculture, animal, and dairy production for rising demand of protein would be non-justifiable, inefficient, or uneconomic as a major source of food (Ritala et al., 2017). Therefore, other protein sources should be exposed which can meet increasing protein demand for humans and animals (Zha et al., 2021). Also, it has been estimated that 10% supplement to the world's supply would be provided by a fermenter of an area equivalent to one-half square mile of the earth's surface (Humphrey, 1968). SCP production from bacteria, fungi, yeasts, and algae has been started 20 years ago utilizing the advantage of their rapid growth which leads to the production of large quantities of SCP in a short time compared to the traditional methods. Also, their capability to carry out a wide range of metabolic reactions makes them utilize cheap raw materials and easy adaptability to different environmental conditions (Nangul & Bhatia, 2021). Thus, this type of protein is cheap. Also, the processes used to produce SCP have merely few issues of waste disposal than other food processes, as almost entire product can be consumed as food. Waste is mostly in the form of heat and the spent growth medium which can be recycled after harvesting of the cells. Besides, it strongly competes with other protein sources and can also provide good nutritional value. In addition to the high protein content, which ranges between 60–82%, SCP contains fats, carbohydrates, vitamins, and minerals (Jamal et al., 2008). It is also rich in essential amino acids such as lysine and methionine which most plants and animal feed lack (Mondal et al., 2012). Since ancient times, the evidences of using SCP as human food are available. For instance, any fermented food contains substantial amount of cellular masses of either bacteria or yeasts or fungi. Thus, to use such organisms as a basic protein source is just an exploitation of our previous experience. It is also reported that various types of SCP can be useful as additional protein and vitamin sources in animal feed (Tannenbaum & Wang, 1975).

2 Yeast for SCP Production

Yeast is a good source of SCP production and has been used for a long time, mainly due to its superior nutritional quality (Bratosin et al., 2021). Advantages of yeasts to be used for SCP production are short doubling time, the larger size of cells which facilitates easier harvesting of the product, have high lysine and malic acid contents, low methionine and nucleic acid contents, capability to grow at acidic pH, and

resemblance of amino acid profile to soya protein and other traditional protein sources (Hamdy, 2013; Kieliszek et al., 2017; Ugalde & Castrillo, 2002; Yousufi, 2012). Yeast extracts are an important source of group-B vitamins too (Ritala et al., 2017). Therefore, this has proved to be a solution of food for developing countries suffering from food shortages and malnutrition (Bacha et al., 2011). But the main limiting factors are low growth rate, lower protein content than bacteria, and low cell wall digestibility (Bennett & Keller, 1997; Nasseri et al., 2011; Raziq et al., 2020).

Most widely used yeasts for SCP production are S*accharomyces cerevisiae*, *S. fibuligera*, *Kluyeramyces marmianus*, *K. lactis*, *K. fragilis*, *K. bulgaricus*, *Candida utilis*, *C. tropicalis*, *C. intermedia*, *C. kefyr*, *C. pseudotropicalis*, *Pichia pastoris*, *Yarrowia lipolytica*, *Hansenula jadinii* (Khan et al., 2010), *Cryptococcus* (Sengupta et al., 2006), *Lipomyces* (Sengupta et al., 2006), *Pitchia* (Khan et al., 2010), *Rhodosporidium* (Sengupta et al., 2006), *Rhodotorula* (Sengupta et al., 2006), *Torulpsis* sp. (Khan et al., 2010), *Trichosporon* (Sengupta et al., 2006), *Yarrowia* (Sengupta et al., 2006), *etc.* (Bozakouk, 2002; Obaeda, 2021). Out of these, yeasts, *S. cerevisiae*, *K. marxianus*, and *C. utilis*, documented as generally safe (GRAS) for human consumption by the US Food and Drugs Administration, have been used widely for production of protein for human consumption (García-Garibay et al., 2014). *C. utilis* has proven to be capable of growing on several carbon sources to produce biomass and can be used to support animal feed (Nigam, 2000). And above-mentioned advantages of yeasts made *S. cerevisiae* a most vital for the production of SCP (Bacha et al., 2011).

3 Nutritional Value of SCP from Yeast

The main nutritional contribution of SCP either for human food or animal feed is its high protein content. Some of the micro and macronutrients provided by SCP, other than protein, are found to be, lipids, carbohydrates, β-carotene, vitamin A precursor, biotin, folic acid, niacin, pantothenic acid, pyridoxine, riboflavin, thiamine, vitamin B12 (cyanocobalamin), vitamin C, and vitamin E (Finnigan et al., 2017). The nutritional value of SCP depends on its chemical composition (amino acids, nucleic acids minerals, enzymes, and vitamins) (Bogdahn, 2015).

Yeasts have a protein concentration ranging from 45 to 70%. Protein quality is also quite acceptable, as compared with vegetable proteins. Some parameters of protein quality are given in Table 1. SCP is also an important source of vitamins; actually, brewer's yeast has long been used as a vitamin supplement. Vitamin contents are also listed in Table 1. As shown in Table 1, comparison of the amino acids profile of different yeast species and the profile of egg or soya cake shows that there is a deficit of sulfur amino acids (especially methionine) and a good balance of the other essential amino acids (lysine, tryptophan). The overall protein content is comparable to that of numerous products used in animal feeds. Yeasts in animal or human diet not only form a protein complement, but can also provide interesting complementary vitamins, lipids, and sterols (Boze et al., 1992).

Table 1 Nutritional values of SCP from various yeasts and comparison of amino acid profiles of yeast SCPs and other protein sources (Bogdahn, 2015; Boze et al., 1992)

	Kluyveromyces marxianus	*Saccharomyces cerevisiae*	*Candida utilis*	Soya bean meal	Fish meal	Egg
Protein (g/100 g dry weight)						
Crude protein True protein	43–58 40–42	48 36	42–57 47	45.5	66.3	–
Essential amino acids (g/16 g of N)						
Isoleucine	4.0–5.1	4.6–5.5	4.3–5.3	2.2	3.2	6.7
Leucine	7.0–8.1	7.0–8.1	7.0	3.5	5.0	8.9
Phenylalanine	3.4–5.1	4.1–4.5	3.7–4.3	2.2	2.9	5.8
Tyrosine	2.5–4.6	4.9	3.3	1.9	3.0	5.1
Threonine	4.1–5.8	4.8–5.2	4.7–5.5	0.6	0.9	1.6
Tryptophan	0.9–1.7	1.0–1.2	1.2	2.3	3.7	7.3
Valine	5.4–5.9	5.3–6.7	5.3–6.3	2.8	4.9	6.5
Arginine	4.8–7.4	5.0–5.3	5.4–7.2	0.7	0.7	2.4
Histidine	1.9–4.0	3.1–4.0	1.9–2.1	0.6	1.9	5.1
Lysine	6.9–11.1	7.7–8.4	6.7–7.2			
Cysteine	1.7–1.9	1.6	0.6–0.7			
Methionine	1.3–1.6	1.6–2.5	1.0–1.2			
Protein efficiency ratio Net protein utilization	1.8 67	2.0	1.7			
Vitamins (μg/g)						
Thiamin	24–26	104–250	8–9.5			
Riboflavin	36–51	25–80	44–45			
Pyridoxine	14	23–40	79–83			
Nicotinic acid	136–280	300–627	450–550			
Folic acid	6	19–30	4–21			
Pantothenic acid	67	72–86	94–189			
Biotin	2	1	0.4–0.8			
Vitamin B_{12}	0.015–0.05		0.0001			

4 Production Process for SCP from Yeasts

Main process for the production of SCP is fermentation which follows certain basic steps as shown in Fig. 1 (García-Garibay et al., 2014).

Fermentation, either submerged or solid state, is used to cultivate selected microorganisms to build biomass utilizing available wastes as growth medium under optimum conditions (Kadim et al., 2015; Queiroz et al., 2007; Sharif et al., 2021). Upon completion of fermentation process, biomass is harvested and processed further for purification, cell disruption, washing, and protein extraction (John et al., 2011; John Rojan et al., 2011; Zepka et al., 2008). In order to make SCP

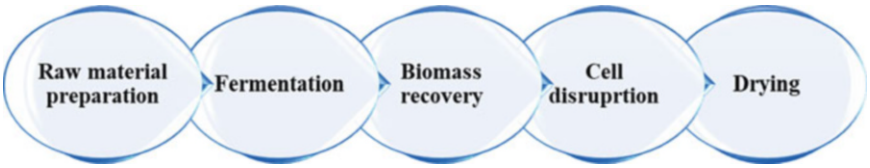

Fig. 1 Process stages of SCP production

with comparable price, it is necessary that all the production/fermentation stages, from substrates selection to downstream processing, are efficient. This section focuses on all these aspects of SCP production from yeast.

4.1 Requirements for SCP Production

The SCP production technology needs fulfillment of some basic requirements as follows (Obaeda, 2021; Srividya et al., 2014):

1. Availability of feasible carbon source with prior physical and chemical treatment facilities.
2. Addition of some nutrients like phosphorous and nitrogen in order to create optimum growth conditions.
3. A large-scale biomass fermenter.
4. Sterilization equipment to maintain sterile conditions to prevent health hazards.
5. Pure culture of selected microbial strain.
6. Adequate ventilation capacity.
7. Biomass collection, extraction/processing, and purification, i.e., downstream processing equipment.

4.2 Raw Material Preparation

Raw material preparation begins with the selection of appropriate substrate which will obviously be justified by the nutritional requirements of yeast.

Nutritional Requirements of Yeasts (Boze et al., 1992)

The qualitative and quantitative nutritional requirements depend on the yeast strain used. Yeast growth depends upon the occurrence of substances essential for the synthesis of cell constituents and the energy produced which can be utilized for biosynthetic process. All the essential growth substances in the proportions similar to that of the cells should be present in the culture media as shown in Tables 1 and 2 (Boze et al., 1992; Fiechter, 1984). Natural media with varying complements are generally used in industry.

Requirements and role of each substances in yeast metabolism have been published as follows (Boze et al., 1992; Jones & Greenfield, 1984):

Table 2 Atomic composition of yeasts (Boze et al., 1992; Fiechter, 1984)

	Saccharomyces cerevisiae	*Candida utilis*		*Saccharomyces cerevisiae*	*Candida utilis*		*Saccharomyces cerevisiae*	*Candida utilis*
	Major elements (%)			Major minerals (%)			Trace elements (%)	
C	47.0	45.94	Ca	0.113	0.57	Co	0.2	A
H	6.0	6.72	Fe	0.01	0.01	Cu	33.0	13.4
N	8.5	7.31	Mg	0.23	0.13	Mn	5.7	38.7
O	32.5	32.08	P	1.43	1.68	Zn	38.7	99.2
			K	1.72	1.88			
			Na	0.07	0.01			

1. Carbon (Boze et al., 1992): Carbon is the main cell constituent, forming approximately 50% (w/w) of the dry weight. Carbohydrates such as glucose, fructose, lactose, sucrose, and cellulose hydrolysate, and other organic compounds such as methanol, and hydrocarbons (n-alkanes) are used as energy and carbon sources. Carbohydrates are the main compounds in numerous industrial by-products, including whey, molasses, and sulfite waste liquor. Hydrocarbons are supplied mainly by the oil industry.
2. Nitrogen (Boze et al., 1992): Nitrogen is quantitatively the second most important element, accounting for some 10% of the cell composition. It is taken up as inorganic nitrogen (ammonium salts) or urea, amino acids, peptides, purines, and pyrimidines. It is essential in protein synthesis and as a component of cell wall polymers. Ammonium is most commonly used because of its price and ease of use. Urea is sometimes used for its buffer capacity.
3. Phosphorus (Boze et al., 1992): This is taken up only as dehydrogeno-phosphate ions (orthophosphate), $H_2PO_4^-$. Phosphorus is found in phosphate sugars, nucleic acids, and di- and tri-phosphate nucleotides and forms about 1.5% of cell dry weight.
4. Other Macroelements: Potassium, Magnesium, and Sulfur (Boze et al., 1992): These are required at different levels as coenzymes (K), enzyme activators (Mg), components of amino acids (S), coenzymes (S), membranes (Mg), and RNA (K).
5. Trace Elements (Boze et al., 1992): Extremely small quantities of elements such as Fe, Mn, Mo, Zn, Co, Ni, B, C1, and Na are required (mM or pJ4 scale). They play an essential role as effectors and constituents of enzymes and coenzymes. The composition of natural media may vary. Excesses of certain trace elements can occur; these may be toxic to varying degrees.
6. Vitamins (Boze et al., 1992): These growth factors are inorganic compounds required sometimes for growth and for obtaining optimum cell yield. Most are precursors or constituents of enzymes or coenzymes. The most important vitamins are thiamine, riboflavin, pantothenic acid, pyridoxine, nicotinic acid, biotin, p-aminobenzoic acid, and folic acid. They are found in adequate quantities in numerous natural substances.
7. Interactions between the various elements (Boze et al., 1992): The definition of growth medium composition must meet two objectives:
   (a) A qualitative aspect: all the substances required for growth must be identified.
   (b) A quantitative aspect: the concentrations of the substances required and sufficient for growth and that do not cause inhibition or modification of the metabolism must be defined.

The compositions of complex growth media used in industry are not always known. Numerous difficulties may be experienced as a result of (Boze et al., 1992):

1. Interaction between the substances
2. An inhibitory effect of certain substances at high concentration (Zn, Cu, etc.)

3. The presence of a chelating agent (Berry & Brown, 1987; Jones & Greenfield, 1984)

Substrate for SCP Production Using Yeast

One of the major advantages of SCP is the flexibility in choosing not only the organism to be produced, but also the substrate to serve as the primary carbon source. Substrates with high carbohydrates as carbon and energy source are extensively used for SCP production (Ugalde & Castrillo, 2002). SCP production process is designed according to the plenty and proximity of substrate to the production plant, their price, accessibility, oxygen demand during the fermentation, rate of heat production and cooling capacity of the fermenter, and even the cost of downstream processing (Raziq et al., 2020; Suman et al., 2015). SCP can also be achieved as coproduct of biorefinery processes which also has added advantage of lowering downstream processing cost and waste disposal costs (Ritala et al., 2017).

Carbon source alone can account for the 60% of the total production cost. Therefore, it is necessary to select inexpensive, easily accessible, and assimilated carbon source with high yielding capacity. The above-mentioned criteria explicit the use of industrial wastes such as molasses, whey, or industrial residues, depending upon the local availability.

Various sources used to grow microorganisms are categorized into three main types as follows (Raziq et al., 2020; Suman et al., 2015):

1. High-energy sources or their derivatives: gas oil, natural gas, ethanol, methanol, n-alkanes, and acetic acid.
2. Industrial wastes like whey from cheese, sulfur from paper pulp, urban sewage, and CO_2.
3. Materials from plant sources such as starch, sugar, cellulose, molasses, and other agricultural wastes.

SCP production is mainly based upon the local sources which met various desirable factors such as availability, purity, cost, and lack of toxicity. Therefore, natural gas or methane would be of great interest in areas where it is available in abundance, or where it may even be discarded (flare gas), whereas carbohydrates may be more suitable for the sun-drenched tropical regions (Cooney et al., 1980). Second type of wastes receives much attention now-a-days because of little or no cost and also the environmental concern of disposing these wastes in the environment. Agricultural wastes have also proven to be a good substrate for the production of good quality, protein-rich SCP with economical value which can be used as an animal feed and human food upon further processing (Yunus et al., 2015). Cellulose, hemicellulose from different plants, nail, hair, feather, and different nitrogenous compounds from animals can also be utilized for SCP production after appropriate physical, chemical, or enzymatic hydrolyzation (Ashok et al., 2000). High yielding capacity, i.e., 1 g of dry biomass per gram of hydrocarbon substrate, makes it advantageous (García-Garibay et al., 2014). Enhancement of carbon assimilation can be achieved using balanced nutrition, i.e., in addition to carbon source, nitrogen

source, minor elements such as P, K, S, Mg, trace elements, and vitamins should be added as per the composition of carbon source and strain to be used. Cost can even be reduced using simple nitrogen sources such as urea, ammonia, and nitrate. Phosphoric acid or soluble phosphate salts can be used as source of phosphorous.

Molasses, a by-product of sugar industry (leftover sugar syrup after crystallization of sugar from sugarcane or sugar beet juice), can be considered as one of the most important substrates for SCP production. Molasses have approx. 50% sucrose eliminated in it. 3.5–4.5 kg of molasses can be obtained from 100 kg of plants (Oura, 1983). It can be obtained over a wide range of geographical locations from tropical to temperate (Ugalde & Castrillo, 2002). In addition to high sugar, molasses also contain other valuable nutrients for fermentation such as minerals, organic compounds, and vitamins (Olbrich, 1973). Yet, yeast biomass cultivation using molasses demands for the addition of suitable nitrogen and phosphorous source. Actually, it has been estimated that about 9% of the dry matter of yeast grown on molasses is originated from substances other than sucrose. In fact, baker's yeast was the first organism to be cultivated in aerobic stirred fermentation on molasses (Chen & Chiger, 1985; Ugalde & Castrillo, 2002; White, 1954). Still, this yeast is rarely intended as food but used for baking purposes.

Another, inexpensive, more yielding or acceptable carbohydrate substrate for SCP production is starch, obtained from bulb plants of tropical and temperate regions or from rice, maize, and cereals. Cassava has been proposed as a good source of starch in tropical countries for SCP processes (Forage & Righelato, 1979). The process named Symba, developed in Sweden, grown two yeasts in sequential manner on starchy waste, one amylase producing *Endomycopsis fibuligira* and the fast-growing *Candida utilis*. The overall process is divided into three parts, first, sterilization of starch waste from potato tubers in heat exchangers, followed by hydrolysis of starch using amylase producing yeast in first bioreactor, and then this hydrolyzed substrate is fed for cultivation of *C. utilis* in second reactor (Jarl, 2013; Oura, 1983).

Another promising substrate for SCP production is Whey, a residual liquid gained after removal of protein or fat from milk during cheese production. Per every kg of cheese production, roughly 9 kg of whey can be generated (Muller, 1969). Whey has 4–6% (w/v) Lactose as the principal component along with significant amount of other nutrients such as 0.3–1.0% (w/v) proteins, 0.6–0.9% (w/v) mineral salts, 0.2–0.3% (w/v) lactic acid, and 92.6–93.5% water (Moebus & Teuber, 1983). Added advantage with whey is its large-scale production in almost every country. Also, it can easily be used for human consumption as it is derived from the milk. Various limitations associated with use of whey as substrate hindering its application are low concentration of lactose makes its transport economically nonviable, it crystallizes at higher concentrations, it has relatively low sweetening power, and also it creates digestion complications in adults as lactose assimilation capacity of adults of Asian and African people weakens with age (Ugalde & Castrillo, 2002). Whey was demonstrated as an appropriate substrate for SCP production. Cultivation of Lactose consuming *Kluyveromyces marxianus* (formerly *K. fragilis*) on whey was pioneered in 1956 by the French dairy company Fromageries Bel and expanded to

the capacity of 8000 tons per year by 1983 (Moulin et al., 1983). The yeast produced was mostly used as animal feed.

Many yeasts such as *Candida*, *Hansenula*, *Pichia*, *Rhodotorula*, *Saccharomyces*, *Torulopsis*, and *Trichocporon* are capable of utilizing n-alkanes (paraffins) and *Candida*, *Debaryomyces*, *Hansenula*, and *Rhodotorula* are capable of utilizing l-alkanes (olefins) in liquid culture (Riviere, 1977). However, hydrocarbons present many complications while used for SCP production like low solubility of substrate, requirement of high degree of aeration for metabolic reactions so elevated cost of aeration, and exothermic nature of the oxidation process which adds high cooling costs (Einsele, 1983). However, suspicions related to safety of SCP produced using alkanes leads to discontinuation of the alkanes as a substrate nowadays (Ugalde & Castrillo, 2002).

By-product of petrochemical industry, methanol has been used as a substrate for a number of SCP production systems. Many benefits of methanol are there for SCP production over other petrochemical by products; the main one in easy removal of substrate from product during downstream processing is the volatile nature of methanol which leads to easy loss of substrate during drying process. *Hansenula*, *Pichia*, *Candida*, and *Torulopsis* are capable of assimilating and tolerating up to 6 g/l of methanol (Ugalde & Castrillo, 2002). A number of methylotrophic yeasts can synthesize enormous number of proteins using methanol. This feature has been exploited for the heterologous expression of proteins at the pilot and industrial scales (Faust & Präve, 1991). A number of industrial examples of SCP production systems using methylotrophic yeasts were implemented in the 70s (Faust & Präve, 1991). However, all have been discontinued.

Usage of Spent sulfide liquor for fermentation has been started in Sweden since 1909, which is then started to be used in many parts of the world. First, *Saccharomyces cerevisiae* was used for the SCP production using spent sulfide liquor, but inability to metabolize pentoses replaces it with other organisms, *Candida tropicalis* and *Candida utilis*, better suited for the assimilation of all the sugar monomers that were selected. Sulfites should be removed prior to fermentation as microorganisms are susceptible to sulfite (Webb, 1964). Yeast produced from sulfite liquor has been used for feeding at war periods, but lost favor in peace time, being destined for fodder in most instances. However, experiences of baker´s yeast produced from sulfite liquor exist in Finland (Oura, 1983).

Composition of Media Used for SCP Production at Industrial Scale

Neither of the raw materials containing carbohydrates such as molasses, sulfite waste liquor, and whey constitutes complete culture media and hence the medium must therefore be modified and complemented (Boze et al., 1992). Also, the compositions of the raw materials are not fully known and constant; therefore, various steps must be taken before using the carbohydrate-based substances as follows (Boze et al., 1992):

1. Inhibitor should be eliminated. E.g., sulfur dioxide from sulphate waste liquor and furfural from hydrolysate of cellulose materials.

2. The components should be purified. E.g., germs should be removed from sugarcane molasses.
3. The components should be sterilized.
4. If concentrated, the media should be diluted properly to adjust chemical composition.
5. Complementary nutrients such as nitrogen, phosphorous, trace elements, etc. should be added.

4.3 Fermentation Process

Fermentation is the main method to cultivate the cells on commercial/industrial scale. Three types of fermentations can be used to cultivate cells for SCP production.

1. Submerged fermentation
2. Semisolid fermentation

In submerged fermentation, the substrate used for fermentation is in liquid state. The substrate is held in fermenter that is operated continuously and simultaneously biomass product is continuously harvested. The oxygen will be sparged and other temperature and pH conditions are maintained same as in regular fermentation processes. The biomass is harvested by techniques such as filtration and centrifugation (Kargi et al., 1980) and then dried. This process has higher operating cost. In semisolid fermentation, substrate used generally remains in solid state (e.g., Cassava waste).

For commercial production, large-scale fermenters along with high oxygen transfer rates are required which can aid in high respiration rates, high metabolism, and high yield. This high metabolic rate is in turn associated with high energy, and therefore, efficient cooling system is also required (Dobariya, 2013). Thus, various engineering operations, like stirring, mixing of multiphase system, heat transfer from liquid phase to surroundings, and transport of oxygen, are involved in the process of SCP production. Important among them is aeration. Heat produced during the cultivation process should be removed subsequently using cooling devices or it may affect the survival of organisms inside the fermenter. Harvesting the produced microbial biomass involves major considerations. Single cell organisms like yeasts are usually recovered by the process of centrifugation. Major portion of the water content from the media should be removed in early stages as much as possible. The final drying of the product must be carried out only under clean and hygienic conditions. In order to maximize fermentation productivity, it is essential to operate continuous fermentation processes.

4.4 Processing of SCP

Depending upon the substrate and intended use of the SCP, i.e., food or feed, various processing steps are required prior to formulation of the final SCP products.

Biomass Recovery

SCP is usually produced using waste materials. The problems that lie in the extraction of SCP from these wastes are that the product remained diluted and that the recovery costs are higher. SCP is found in low concentrations, usually less than 5%. Therefore, various techniques, such as centrifugation, flotation, precipitation, coagulation, and filtration, can be implemented/tested to increase the concentrations of SCP (Dobariya, 2013). Centrifugation is the principal technique for the recovery of the biomass.

Cell Disruption

Methods used to disrupt cell wall use either mechanical force (crushing, crumbling, grinding, pressure homogenization, or ultra-sonication) or hydrolytic enzymes (endogenous or exogenous) or chemical disruption (with detergents) or combinations of any of these methods (Nasseri et al., 2011). Cell disruption may affect the quality and quantity of protein and other components in the SCP. Products such as MarmiteR and VegemiteR are cell extracts, generated by heating the cells to 45–50 °C long enough for intracellular enzymes to partially hydrolyze the cell wall; the proteins are also reduced to smaller peptides (Trevelyan, 1976; Ugalde & Castrillo, 2002).

Drying

Dehydration of SCP to approx. 10% of the moisture content and acidification of the product are required to aid in storage and to prevent spoilage during storage. Drying or concentration of cells can be achieved by dewatering processes like centrifugation, filtration, flocculation, and evaporation (Dobariya, 2013).

5 Existing Methods for Cultivation of Yeast SCP on Commercial Level

Production of Baker's Yeast (*S. cerevisiae*)

For cultivation of Baker's yeast at large scale, first the pure culture of *S. cerevisiae* is grown in the laboratory test tube or flask followed by gradual transfer to larger volume lab scale fermenters. Care is taken to prevent contamination. The media used for cultivation contain molasses and corn steep liquor as C, N, and mineral salts. The reaction conditions for cultivation are pH 4.4–4.6 and incubation temperature 25–26 °C with continuous aeration. During the cultivation, yeast cells oxidize sugar under aerobic conditions and liberate energy, which will then be utilized for the synthesis of cell protoplasm. High growth rate yeasts exhaust sugar within 10 h. After completion of fermentation, yeast cells are recovered by centrifugation followed by

washing, mixing with starch or corn meal, and pressing into cake. The cake is preserved at low temperature to prevent spoilage or the yeast cells are dried. The dry yeast powder can be kept for several months in live condition (Dobariya, 2013). Inactive dried brewer's or baker's yeast is also used as a dietary source of vitamins and trace minerals in specific medical conditions and as flavoring agent.

Candida Cultivation on N-Paraffin (BP Process)

For cultivation of *Candida* using n-paraffin as substrate, mechanically agitated fully baffled fermenters with turbine mixers are used. The reactor is fed continuously with paraffin, gaseous ammonia, and other salts. This process demands for high oxygen, i.e., 2.2 kg O_2 per kg biomass produced by aerobic microorganisms grown on n-hexadecane is 2.5 times higher than that required for growth of organisms on glucose. Heat production rate is 25000 kJ/kg biomass. Fermenters require substantial agitation because of insoluble nature of alkanes. Due to continuous agitation, alkane drops remain in suspension with drop size of 1–100 mm in diameter. Cells are recovered by centrifugation, producing 15% dry solids, evaporation to 25% dry solids, and spray drying (Dobariya, 2013).

Cultivation of _Methylophilus methylotrophus_ on Methanol (ICI Process)

For the said process, pressure recycle fermenter, i.e., a combination of an airlift and loop reactor consisting of an airlift column, a downflow tube with heat removal, and a gas-release space, is used. Here, ammonia gas is used as the nitrogen source and pH is maintained between 6 and 7. Cell specific growth rate is approximately 0.5 h^{-1} and cell yield 0.5 g/g. Cells oxidize methanol via dehydrogenation to formaldehyde which can either be assimilated for conversion to cell mass or further oxidized to CO_2 with concomitant energy production. Cells are recovered by agglomeration followed by centrifugation, flash dried, and ground (Dobariya, 2013).

Cultivation of _Kluyveromyces marxianus_ Using Whey (Bel Fromageries Process)

Whey containing about 5% lactose, 0.8% protein, and 0.2–0.6% lactic acid is used as a substrate. Organism is cultivated in an aerobic fermentation, whereas aeration is minimal for ethanol production. For feed grade biomass, the entire fermentation contents, containing yeast, residual whey proteins, minerals, and lactic acid, may be recovered. For preparation of food grade material, cells are harvested by centrifugation, washed, and dried. Cell yield is 0.45–0.55 g/g based on lactose consumed (Dobariya, 2013).

Other Processes

- Spent brewer's yeast (*S. cerevisiae*) has been sold by Unilever and Sanitarium Health Food for more than a century as MarmiteR (produced from starch-driven glucose), by Bega Cheese Ltd as VegemiteR, by Gustav Gerig AG as CenovisR, and by VITAM Hefe-Produkt GmbH as Vitam-RR (Ritala et al., 2017).
- *Torula* (*C. utilis*, renamed as *P. jadinii*) is high in protein and widely used as flavoring agent, commercialized in combination with *Pichia* and *Kluyveromyces*

yeast, by Provesta Corporation as ProvesteenR T in the 1980s (Hitzman, 1986; Ritala et al., 2017). *Torula*, glutamic acid rich yeast, has been used to replace the flavor enhancer monosodium glutamate (MSG) (Ritala et al., 2017).

- Phillips Petroleum Company carried Industrial scale production of SCP using Methylotrophic yeasts, for example *Komagataella pastoris* (previously *Pichia pastoris*), from methanol (Rashad et al., 1990).

Some of the other active yeast SCP producers are mentioned in Table 3.

Table 3 Active yeast SCP producers

Company, Country	Strain	Substrate	Reference
Amoco (BP), USA	*Candida utilis*	Ethanol	Rudravaram et al. (2009)
Bega Cheese Ltd	*Saccharomyces*	Wheat	http://www.smh.com.au/business/begasnaps-up-vegemite-as-part-of-460mdeal-20170118-gtu7wk.html
Bellyyeast, FRc	*Kluveromyces*	Whey	Rudravaram et al. (2009)
Cangzhou Tianyu Feed Additive Co., Ltd	*Yeast powder*		www.cztymy.com
Flint Hills Resourcesb	*S. cerevisiae*	Corn	https://www.fhr.com/newsroom
Imperial Chemical Industries, UK	*Methylotrophus*	Methanol	Rudravaram et al. (2009)
IFP, FRc	*Candida tropicalis*	n-Alkanes	Rudravaram et al. (2009)
Lallemand Inc, Montreal, Canada	*S. cerevisiae* and *Torula*		www.bio-lallemand.com
LeSaffre	*S. cerevisiae*		http://www.lesaffre.com/
Liquichemica, ITb	*Candida maltosa*	n-Alkanes	Rudravaram et al. (2009)
Mondelez Int.b	*Yeast*	Brewer's spent grain	http://www.mondelezinternational.com/
Nucelisb	*Yarrowia lypolitica*		https://www.nucelis.com/
Phillips Petroleum Company	*Pichia* sp. *Torula* sp.	Sugar feed stock	Rudravaram et al. (2009)
Skotan S.A., PLb	*Yarrowia lipolytica*		http://www.skotansa.pl/
Tangshan Top Bio-Technology Co., Ltd.	*Saccharomyces* sp.		www.tuopobio.com
Unileverb	*Yeast*	Brewer's spent grain	www.unilever.com

6 Economics of Yeast SCP Production

The costs of the SCP depend upon the end use of SCP (food or feed), quality of SCP to be used, and competing protein products with pricing (Litchfield, 1977). As a commodity, SCP must be competitive with commercial animal and plant proteins, in terms of price and nutritional value and must conform with human and animal food safety requirements. SCP production can be a profitable business if appropriate tactics are used. Productivity, yield, and selling price are the major factors affecting the economics of SCP production (Dobariya, 2013). Economic viability of SCP production process depends upon cost of raw material, operation costs, manufacturing costs and capital costs, and profit obtained from the product. Overall cost of the SCP is determined by the strain (growth rate, yield from a given substrate, stability in continuous culture, and size of cell), cost of raw material and chemicals or enzymes needed for pretreatment of substrates (such as lignocellulosic waste), cost of maintenance of process conditions and recovery requirements, the size of plant, and amount of capital investment of facilities (Junaid et al., 2020; Litchfield, 1977; Ritala et al., 2017; Tesfaw & Assefa, 2014). For continuous process, genetic stability of the organism over an extended period of time is vital in order to achieve higher productivity (Litchfield, 1977). To achieve profitable business, improvement in microbial strain, fermentation method, and advanced downstream processing are needed (Bratosin et al., 2021).

Raw Material Costs (Litchfield, 1977) The costs of feedstocks such as hydrocarbons, methanol, and ethanol and nutrients such as ammonia and phosphoric acid directly affect manufacturing costs of SCP products. Though the gas oil (a source of n-alkanes) is having low cost, its use is hampered by the complex and costly purification processes to be followed to meet the requirements of regulatory agencies in various countries. Even one can use purified substrates to minimize the product purification costs, but that in turn increases the cost of substrate. For instance, 98–99% purity of the n-alkanes can be achieved by molecular sieve separation process to meet the U.S. Food and Drug Administration standards for purity for white mineral oil (Code of Federal Regulations, 1976), which increase the cost of substrate considerably over that of gas oil. Nevertheless, the cost of product purification remains less as compared to substrate purification. Preferred nitrogen source for SCP production is ammonia due to its use in pH control, ease of handling, and cost. The price of ammonia is directly related to the cost of energy in the form of natural gas used for its production. For a source of phosphorus, feed grade phosphoric acid is preferred over fertilizer grade because of the presence of undesirable impurities, such as iron, arsenic, and fluoride in the latter. Other mineral nutrients required as sources of K, Mg, Mn, Zn, and Fe should be supplied as hydroxides or sulfates rather than chlorides to minimize corrosion of stainless-steel equipment.

Process Factors Affecting Costs Various process factors such as pH, temperature, aeration and agitation systems, and recycle of nutrients affect cost of operation. For instance, a low pH in the range of 2.5–4.0 minimizes contamination problems in

nonsterile systems and high temperatures in the range of 35–45 °C minimize cooling requirements. Likewise, the aeration system influences productivities and yields and energy consumption and thus directs manufacturing costs. It is important to note that it may not be economical to operate at maximum productivity as there are other factors which can hinder achieving maximum productivity and reduce the productivity. Recycle of nutrients in continuous processes is vital for maximum consumption of raw materials. The costs of cell separation from the growth medium depend mainly on the physical properties of the cells. The cost of recovery of the SCP product is dictated by the concentration of biomass in the growth medium, cell size, density, viscosity, amount of substrate in the cell, purity of the product required (removal of substrate residues, toxins, etc.), and heat susceptibility during drying of the flavor, aroma, color, and nutrient contents of cells. Also, recovery process should be such that it yields final product free of contamination and should be nonpathogenic to domestic livestock or human. It is required to remove as much water as possible from the product by any drying process and the drying is the costly process. Other than centrifugation, filtration, flocculation, and evaporation can also be used to dewater cells. The energy cost of cell separation by evaporation using a multiple effect system may still exceed those of centrifugation. These methods require equipment that is expensive and not always suitable for small-scale operations. It is economically sensible to feed the product locally and shortly after it is produced (Dobariya, 2013). Substantial amount of heat is released during the growth of microorganisms on hydrocarbons, methanol, and ethanol, therefore, to maintain the optimum temperature, cooling system adds the total cost of the product. However, the requirements for refrigeration can be lowered by using organisms that grow in the range 35–45 °C. Water is the primary requirement of all the process steps from media preparation to cleaning and sterilization, etc.; it has been estimated that 100,000 metric tons of SCP per year requires 18.2 million liters of water if substrate is n-alkanes. The cost of the water can be minimized by recycling of water in all possible aspects.

Manufacturing Costs Manufacturing costs depend on carbon and energy sources, mineral nutrients and other chemicals, utilities, labor, maintenance, insurance and taxes, materials and suppliers, and overhead charges/depreciation charges. Therefore, one must be careful in comparing such cost breakdowns.

Capital Cost Along with increase in energy costs, plant construction and equipment costs increase considerably.

7 Marketing Strategies for Yeast SCP Production

Worldwide, the segmentation of SCP market is shown in Fig. 2.

Applications of SCP in food are as source of protein, vitamin, and to improve the nutritive value of several foods including baked products, soups, ready-to-serve-meals, in diet recipes and others and in animal nutrition as fattening calves, poultry,

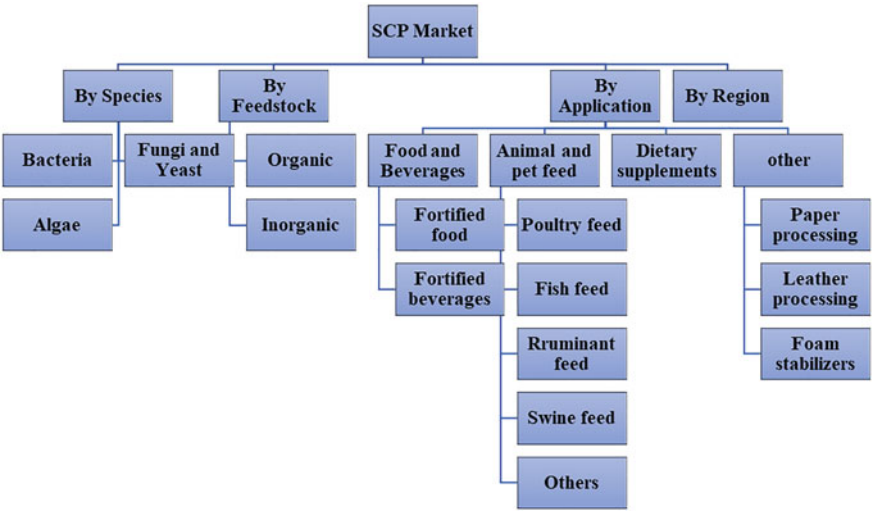

Fig. 2 Segmentation of Global SCP market

pigs, and fish breeding. Growing demand for such additives enhances revenue of foods and beverages segments over the forecast period. On the basis of a region, the global SCP market is segmented into North America, Latin America, Western Europe, Eastern Europe, Asia Pacific excluding Japan, Japan and the Middle East, and Africa. North America and Western Europe are anticipated to have significant revenue shares in the global SCP market over the forecast period. The driving forces behind this are changing life styles and growing demand for value-added food products and growing demands for protein-rich foods. SCP is used as the health food to control obesity and to lower the sugar content in diabetic patient and others. Asia Pacific and Middle East countries are probable to generate significant development in the overall SCP market over the forecast period. SCP Market Drivers are increasing number of malnutrition cases across the globe. As per UNICEF, half of the deaths in children's under age five are due to malnutrition or under nutrition. This creates increasing demand for protein-rich food across the globe and contributes to increasing revenue share in the global SCP market (OpenPR, 2020).

8 Conclusion and Future Prospects

Increasing demand for nonconventional, environment-independent, protein-rich, nutritive supplement, or food makes the SCP market most earning business. Besides the nutritive values, it also helps in utilizing wastes in good ways. Though the nutritional quality of the yeast SCP has been clearly demonstrated, the production costs are much in order to obtain comparable protein source for plants proteins to be used as animal feed/ food and hence development of yeast production is yet inadequate now-a-days. Therefore, though various strains and production processes have

been developed and the product is marketed, there is need of improving the strain utilizing biotechnological techniques for more protein content with required amino acids and vitamins, with favorable physicochemical properties to aid easy separation and purification, operation under conveniently maintained culture conditions, utilizing easy, locally available, cheap raw material, to engineer more efficient fermenters and downstream processing equipment, and to find out more and more easy accessible wastes for high yielding strains. Also, there is need for generation of awareness among the common people regarding the benefits of the SCP.

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Bacterial Single Cell Protein: Applications, Productions, and Commercialization: Opportunities and Challenges

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Abstract

During the evolution of civilization, humans generated the food from farming. Since then, his food habits including nutritional necessities have changed. Today, consumptive manners are highly diversified and cause several diseases and spoil the natural immune characteristics. Out of this background, this chapter delivers about the 'bacterial single cell proteins their applications, productions and commercialization'. Naturally, single cell proteins are a refined or purified form of protein derived from microorganisms used by substrates under artificial conditions. They are the richest sources of proteins, vitamins, and minerals. There is numerous bacterial spp., such as *Acinetobacter calcoaceticus*,

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Achromobacter delvaevate, *Aeromonas hydrophila*, *Bacillus subtilis*, *B. megaterium*, *Brevibacterium spp.*, *Cellulomonas* spp., *Flavobacterium* spp., *Lactobacillus* spp., *Methanotrophic* spp., *Pseudomonas fluorescens*, and *Rhodopseudomonas capsulate*. Bacterial SCPs are produced through semi-solid fermentation with continuous process of cell disruption, removal of nucleic acids, washing, purification, and drying. In addition to bacteria, fungi, algae, and yeasts nutritional supplements like Quorn™ (*Fusarium venenatum*) and Pekilo (*Paecilomyces variotii*) are used by global corporations. It is a growing science-based industry. Furthermore, growth of SCPs depends upon food habits and demand, and in future, it will play a dominant role in human food and health care.

Keywords

Acinetobacter spp. · *Candida utilis* · *E. gracilis* · *F. venenatum* · *Spirulina* spp

1 Introduction

By 2050, the world population may reach 9.3 billion (Pihlajaniemi et al., 2020). Globally, this growth rate causes a high consumption rate of food and provides a large demand for food production, distribution, and breaking a supply chain and a lack of availability of healthy supplies (Ritala et al., 2017). On the other hand, 8.9% (690 million) people are estimated to be affected by undernourished diseases and disorders, viz., anaemia, malnutrition, underweight child, growth retardation, blindness, and Alzheimer's disease; among them most were children and adolescent women. By 2030, this rate will increase and exceed 840 million people (Food and agricultural organization of the United Nations, 2021). Mostly, African, West Asian, Latin American, and Caribbean regions are affected. It indirectly reflects changes in the food habits, destruction of native healthy foods and crop species, and genesis of new epidemics (Bratosin et al., 2021). Naturally, protein stuffed foods play an essential role in all living organisms because of their constituents of nitrogen sources and amino acids such as, arginine, cysteine, glutamine, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tryptophan, tyrosine, and valine (cannot be self-synthesized) for energy synthesis and metabolic functions (Wu, 2009). Worldwide, protein consumption was attained at a rate of 1250 million tons/year by animal- and plant-derived proteins. Under these conditions, plant-derived proteins are inefficient compared with animal proteins and 1 kg of animal protein requires an amount of ~6 kg biomass of plants. In spite of this, the available sources are not enough for global people and their healthy, sustainable, and routine livelihoods (Goldberg, 2013). Under these situations, global food production pushes to the new or alternative strategies such as single cell protein (SCP), their production, distribution, consumption, and commercialization with affordable conditions. Today, single cell protein (SCP) acts as an unavoidable role player in proteinaceous

and high calories constituent food sources (Zamani et al., 2020). Their nature, applications, historical background, sources, types of single cell proteins, production technologies, economics, commercialization, opportunities, challenges, and future perspectives are discussed below.

1.1 Single Cell Protein (SCP)

"Single cell protein" (SCP) is a dried or crude refined protein/bulk of cells (biomass) which is obtained from different microorganisms like algae, bacteria, fungi, and yeasts. It is otherwise termed as biomass, bioprotein, mycoprotein, and microbial protein (Nasseri et al., 2011). These are used as high-nutrition-derived food supplements (proteinaceous) for humans and animals. Mostly, the production cost of a single cell protein is expensive and marketing with more price. These issues can be avoided by identifying the easily available affordable sources that are highly contributed in single cell protein production (Boland et al., 2013).

1.2 Applications

It is a better alternative source for plant-derived proteins (soya meal) and fish meal; especially for the limited quantity of water being required compared to green proteins, microbes derived contain about 45–55% protein on average; additionally, some bacterial species yield more than 80% protein supplements (Ghasemi et al., 2011). These proteins' final products' results in essential nutrients and vitamins viz., Ca, P, Se, folic acid, vit-B (riboflavin, niacin, tocopherol, thiamine, biotin, pantothenic acid, choline, pyridoxine, glutathione, β-aminobenzoic acid, and streptogenin) (Turnbull et al., 1992). Not only for mono-use, it has a wide range of purposes like, aroma carriers, emulsifying acids, food additives, and feed (fish, poultry, pigs, and cattle) (Zhou et al., 2019) and also used in paper and pharmaceutical industries (Nagare et al., 2015).

1.3 Historical Background

Since ancient times, humans have used food as many species of living organisms including microbes. Because these are easily available and consist of all nutrients and minerals, compared to plant-derived sources, they have been rich in all aspects and are compatible with human physiological nature (Srividya et al., 2013). Several years ago, *Spirulina* spp. was grown and used as food to compensate for people's protein shortfalls in Africa (Junaid et al., 2020). After world war I, Germans utilized certain species of *Candida* as food in forms such as sausages and soups. After popularization, most of the people widely used microbes and algal cultures as food sources for protein supplements. In 1968, the term 'single cell protein' was

introduced by scientists (Ali et al., 2017). Later, science developed SCPs which are obtained from numerous microorganisms and algal species at domestic to commercial levels (Voltolina et al., 2005).

1.4 Sources and Substrates

The common sources for single cell proteins are obtained from various sources such as, raw materials or food wastes viz., fruit wastes (apple pomace, orange peels, citrus pulp, pineapple peel, papaya pulp), vegetable wastes (potato starch-peels, yam peels, and cauliflower leaves), sugarcane molasses-bagasse, bran (cereals), husk (millets), etc. (Suman et al., 2015); combustible wastes or by-products (oil products, ethanol, and methanol) (Yunus et al., 2015). There are several types of organic wastes from agriculture and forestry wastes and different organic carbon constituted substrates (natural or artificial media) used for production of single cell protein from a vast diversified group of microorganisms (Godfray et al., 2010). In recent days, these single cell proteins were obtained from living, viz., microbes (bacteria, fungi, yeasts) and algal spp., and non-living sources of organic wastes from domestic, agricultural, and industrial substrates (Gervasi et al., 2018).

Bacterial Sources and Substrates

Naturally, bacteria contain >50–80% protein (dry weight) and they are rich in nucleic acid content (8-12%) and are of low density, cell size is smaller and rapid growth (20–120/min) (Peden & Bamberg, 2000) on wide range of substrates like, sugars, starches (soya meal - *Methylobacterium extorquens*) (Hardy et al., 2018), raw waste materials (i.e. organic wastes, methanol, and ethanol), and agricultural wastes (cereals brans or husk, pulses hull (soybean—*B. subtilis*) (Wongputtisin et al., 2014), and cellulosic nature of stubbles) (Vibha & Sinha, 2005). Commonly, these substrates were used at high levels because of their easy availability and bacterial species requiring nutrient sources are predominantly present at maximized levels. Methane has been utilized by several species, viz., *Methylococcus capsulatus*, *Methylocapsa acidiphila*, *Methylomonas methanica*, and *Methylovibrio soehngenii* for SCP production (Overland et al., 2010). An example of Pruteen (Imperial chemical industries) is produced from methanol as a substrate by the bacterium *Methylophilus methylotrophus* and this was used as pig feed containing high protein levels up to 70% (Jhonson, 2013). Species of *Rhodopseudomonas palustris* (nonsulfur bacteria (Getha et al., 1998; Kornochalert et al., 2014)), *Rhodopseudomonas acidophila* (Pfennig, 1969), *E. coli* (Kurbanoglu & Algur, 2002), and *Haloarcula* sp. (Le Page, 2016) exploited higher efficiency in the SCP production compared to others (Kornochalert et al., 2014). At various substrates, methane is an interesting one majorly from cattle by-product of methanotrophic bacteria such as, *Methylococcus capsulatus* (group I) (Le Page, 2016), *Methylosinus trichosporium* (Stein et al., 2010), and *Methylocystis parvus* (group II) (Gesicka et al., 2021). A large-scale biomass of protein (highest level) and polyhydroxyalkanoate (PHA) has been produced in the artificial medium of

biodegradable polyester by gram negative soil bacterium *Cupriavidus necator*; this protein biomass was fed to rats and was unsafe due to presence with substantial amounts of PHA (Kunasundari, 2011; Kunasundari et al., 2013). The substrate, ram horn hydrolysate, yielded high levels of protein substances by three different species like, *E. coli* (66%), *B. cereus* (68%), and *B. subtilis* (71%), respectively (Kurbanoglu & Algur, 2002). Bacterial cultures such as *Methylococcus capsulatus*, *Brevibacillus agri*, and *Aneurinibacillus* sp. are produced in SCPs with moderate levels of protein content 67–73% in 'C' containing sources of natural gas (Raziq et al., 2020). Numerous and highly diversified group of bacterial species were used for large scale of production for single cell protein, viz., *Acinetobacter calcoaceticus*, *Achromobacter delvaevate*, *Aeromonas hydrophila*, *B. subtilis*, *B. megaterium*, *Brevibacterium*, *Cellulomonas* spp., *Flavobacterium* spp., *Lactobacillus* spp., *Methanotrophic* spp., *P. fluorescens*, and *Rhodopseudomonas capsulate* by using various substrates (Ashok et al., 2000). Commonly used bacterial species and their growing substrates are listed in Table 1.

Fungal Sources and Substrates

Several species of fungi are involved in production of single cell proteins, because of the presence of carbon sources (cellulose, hemicellulose, etc.) and are rich in nucleic acids (10%) compared to algae (6%) (Ardestani & Alishahi, 2015). They consist of the highest level of lysine and threonine but lack the S-containing amino acids, cysteine and methionine. SCPs obtained from fungi can provide vitamin B complexes (riboflavin, niacin, thiamine, biotin, pantothenic acid, choline, pyridoxine, glutathione, amino benzoic acid, folic acid, and streptogramin) during nutrition to humans (Ahmadi et al., 2010). 'Pekilo' is a term used in Finland for obtaining single cell protein from fungi (*Paecilomyces variotii*) to feed the animals. A wide group of filamentous fungi was utilized for a huge level of production in single cell proteins by using various substrates (Nangul & Bhatia, 2013). Using fungal sources and their substrates are listed in Table 2.

Algal Sources and Substrates

Algae are autotrophic organisms which have numerous species and a wide range of genetic diversity. Naturally, they are growing in a broad spectrum of substrates such as water, wood, trees, soil, walls, rocks, waste water, and sludges (Lee, 2008). Their growth and constituents of inorganic compounds (N and P) are mostly decided by environmental factors, viz., light, CO_2 conc. pH, available C/N sources, and water. Commonly, these organisms are photosynthetic in nature and produce carbon sources for their nutrition and growth (Chapman, 2013). Both groups or categories are available based on their genetic nature, macro-algae (multicellular) and micro-algae (unicellular), otherwise known as seaweeds and phytoplankton, respectively. In both categories, macro-algae consist of four classes like, Cyanophyta (blue green algae)—*Spirulina*, *Anabaena*, *Nostoc*; Chlorophyta (*Green algae*, *Marimo*, *Chlamydomonas* spp., *Sea lettuce*, *Dunaliella salina*, *H. pluvialis*, *Ulva intestinalis*, *Volvox globator*, *V. aureus*, *Scenedesmus dimorphus*, etc.); Haptophyta (*Brown algae*, *Fucus* spp., *Laminaria* spp., *Ascophyllum nodosum*, *Macrocystis*); and

Table 1 Bacterial sources and substrates used for single cell protein production under commercial level

Substrates	Bacteria
Ram horn Tuna condensate	*Bacillus subtilis*, *B. cereus*, *E. coli* *Rhodocyclus gelatinosus R7*
Wastes Potato starch waste Pineapple waste Cassava waste Rubber waste Soybean hull Wheat bran Pig farm and animal waste (manure) Agro-wastes	*B. licheniformis*, *B. pumilus* *Rhodobacter sphaeroides P47* *Rhodocyclus gelatinosus* *Rhodopseudomonas palustris* *Bacillus subtilis* *Rhodocyclus gelatinosus* *Rhodocyclus gelatinosus*, *P. fluorescens* *Cellulomonas* spp.
Carbon sources	*Corynebacterium ammoniagenes* (G+F) *Corynebacterium glutamicum*, *Rhodopseudomonas capsulate* (G); *Lactobacillus* sp. (G, A, M) *B. subtilis*, *Cellulomonas* spp., *Flavobacterium* sp., *Thermomonospora fusca* (H, C), *Methylococcaceae* family (C-1), *Brevibacterium* spp. (C1 to C4)
Waste water Petrochemical Brewery Rubber latex Poultry house Soybean Noodles waste water Sugar refinery Seafood processing Municipal Stimulated waste water	*Haloarcula* sp. *IRU1* Rhizospheric diazotrophs *Rhodopseudomonas blastica* *Rhodocyclus gelatinosus* *Rhodobacter sphaeroides Z08* *Rhodopseudomonas palustris* *Rhodopseudomonas*, *R. fulvum* *Rhodocyclus gelatinosus* *Rhodopseudomonas* sp. *CSK01* *Rhodopseudomonas palustris*
Natural gases Methane, biogas, natural gas Solvents Methanol Ethanol	*Methylococcus capsulatus*. *Ralstonia sp*., *Brevibacillus agri*, *Aneurinibacillus sp*., *Methylomonas* spp., *Methylocapsa acidiphila*, *Methylophilus* spp., *Methanomonas methanica* *Methylophilus methylotrophus*, *Streptomyces* spp. *Acinetobacter calcoaceticus*
Media Synthetic growth media Miso-like effluent media	*Cupriavidus necator*, *Rhodopseudomonas* sp., *Bacillus* spp., *Pseudomonas* spp., *Streptomyces* spp., *Rhodopseudomonas* *acidophila*, *R. gelatinosus*. *Rhodocyclus gelatinosus*
Sludges Oil and gas Photosynthetic sludge	*Methylomonas* and *Methylophilus* spp., *Methylococcus capsulatus* *Rhodopseudomonas palustris*

G glucose, *F* fructose, *A* amylose, *M* Maltose, *C* cellulose, *H* hemicellulose (Kadim et al., 2015)

Rhodophyta (*Red algae*). Another category of micro-algae mostly occurring in the marine ecosystem, it is considered with 5 classes and additionally Dinophyta (*Crypthecodinium cohnii*) compared to macro-algae (Guiry Michael, 2012; Hallegraeff et al., 2021).

Table 2 Fungal sources and substrates used for single cell protein production under commercial level

Substrates	Fungi	Trade name
Wastes Rice bran Banana waste, wheat straw, Apple pomace Poultry litter, *Capsicum* waste powder Glycerol waste, crude oil Waste liquor Cheese whey filtrate Potato starch waste	*Cladosporium cladosporioides*, *P. citrinum*, *A. flavus*, *A. niger*, *A. ochraceus*, *A. oryzae (deoiled)*, *Monascus ruber and Fusarium semitectum* *A. niger*, *Pleurotus florida*, *A. niger*, *T. virideae*, *Candida utilis* *Yarrowia lipolytica* *A. niger* *T. harzianum* *A. niger*	Quorn™* Pekilo*
Edibles Citrus pulp Potato pulp, orange pulp (edible) Molasses; brewer's spent grain	*A. niger*, *T. virideae* *Kluyveromyces marxianus* *Kluyveromyces marxianus*	
Carbon sources Lignin Glucose Maltose Hemicellulose Lactose, Galactose Cellulose, Pentose	*Chrysonilia sitophilia* *Fusarium venenatum**, *A. fumigatus*, *P. cyclopium*, *Rhizopus chinensis* *A. fumigatus*, *Rhizopus chinensis* *Cephalosporium eichhorniae*, *Chaetomium cellulolyticum* *Penicillium cyclopium* *Scytalidium acidophilum*, *T. virideae*, *T. alba*, *Paecilomyces varioti**	

Ugalde and Castrillo (2002), Valentino et al. (2016), and Wiebe (2002)

Classically, algae are taken as food in human civilization. Especially, certain groups of microalgae are gathered or cultivated for consumption. Due to the protein content up to 70%, other healthy sources of fats (ω^{-3} fatty acids), carotenoids, minerals, vitamins A, B, C, and E present in least percentage (Wells et al., 2017). Mostly, these supplements from algae are obtained in various forms such as tablets, capsules, syrup (liquid), and ready to eat, pastas, snacks, and healthy drinks (Gouveia et al., 2008). Commercially, species such as *Arthrospira platensis*, *A. maxima*, *Chlorella*, *Scenedesmus*, *D. salina*, and *Aphanizomenon flos-aquae* are widely cultivated and used as food. Globally, there are several algal species that are cultivated artificially, obtaining the SCPs from a huge amount (Barka & Blecker, 2016). These species are described in Table 3 with their substrates.

Yeasts Sources and Substrates

Yeasts are unicellular organisms that have evolved from multicellular organisms belonging to *Ascomycota* with more than 1500 species recognized. Yeast is used as a model organism (*Saccharomyces cerevisiae*) for their potential use in food

Table 3 Algal sources and substrates used for single cell proteins production

Substrates	Algae
Natural habit CO_2 + Sunlight CO_2	*Gracilaria domingensis*, *G. birdiae*, *G. verrucose*, *Laurencia filiformis*, *L. intricate*, *Palmaria palmate*, *Porphyra umbilicalis* and *Chondrus crispus* *Sargassum*, *Dunaliella*, *Laminaria*, *Diatoms*, *Chlorella* spp., *Porphyrium* sp., *Spirulina*, *Chondrus crispus*, *Scenedesmus* sp. and *Spirulina maxima* *Spirulina* spp., *Chlorella* spp. and *Caulerpa racemosa*
Wastes Soda ash effluent Tofu, Cheese waste Saline sewage effluents Water Waste water Salinated and Desalinated	*Ulva fasciata*, *Chaetomorpha antennina* *Chlorella* sp. *Chlorella salina* *Chlorella sorokiniana*, *Scenedesmus obliquus* *Spirulina* spp.

Fleurence (1999), Garcia-Garibay et al. (2014), and Sharif et al. (2021)

microbiology, viz., baking, wine preparation, and studies on cell biology from ancient to current (Angiolo et al., 2020). During world war I, some yeasts spp. (*Candida utilis* and *S. cerevisiae*) are used for production of SCPs by Professor Carol Winston. Nowadays, it is frequently used as feed for animals, fish, and vegetarian foods. Compared to bacteria, some typical characteristics are highly enhanced in the usage of yeasts in SCPs production; characters like cells are larger than bacterial cells, low methionine and high lysine and malic acid contents, able to grow in low pH conditions, and abundant intake of 'C' sources from various different substrates (Broach, 2012). However, some limiting factors are there such as low cell wall digestibility and few species, *viz.*, *Saccharomyces lipolytica*, *C. oleophila*, and *C. tropicalis* using substrate alkanes for single cell protein production (Kieliszek et al., 2017). Several toxic or carcinogenic substances like aflatoxins B1, B2, G1 and G2, citrinin, trichothecenes, and zearalenone are generated during the process (Bennett & Klich, 2003). Table 4 reported using species of yeasts for single cell proteins production.

1.5 Industrial Production

Microbes utilize the available sources such as waste, water, and natural sources (light, CO_2) for their growth, development, and reproduction in morphological and genetic. Mostly, SCP production involves the biological process of fermentation either submerged or solid (Hedenskog & Morgen, 1973). Following completion of fermentation and available biomass further processing techniques, viz., cell wall disruption, removing nucleic acids, washing, purification, and followed by protein extraction (Jhon et al., 2011). These are further discussed with progresses like selection criteria of microorganisms, selection and nature of substrates, fermentation processes, and production technologies (Fig. 1).

Table 4 Commonly used yeasts spp. and substrates using for single cell proteins production

Substrates	Yeasts spp.
Edibles Orange pulp, molasses Cheese whey	*S. cerevisiae*, *Kefir* sp., *Kluyveromyces marxianus* *Candida krusei*, *Kefir* sp., *Kluyveromyces marxianus*
Agricultural/food/poultry wastes Brewer's spent grain Bagasse Potato pulp Spoiled date palm fruit Waste *Capsicum* powder and potato starch industry waste Poultry litter Inulin (plant waste)	*S. cerevisiae*, *Debaryomyces hansenii*, *Kluyveromyces marxianus* *Candida tropicalis* *Kefir* sp., *Kluyveromyces marxianus* *Hanseniaspora uvarum*, *Zygosaccharomyces rouxii* *Candida utilis* *C. utilis* *Yarrowia lipolytica*
Industrial waste water/effluent Potato waste water Crude oil effluent and glycerol waste	*Candida utilis* *Yarrowia lipolytica*
Carbon sources Ethanol Glucose Maltose Lactose Pentose	*Amoco torula* *C. tropicalis*, *C. utilis* *C. tropicalis*, *S. cerevisiae* *Candida intermedia*, *S. cerevisiae* *S. cerevisiae*

Ashy and Abou-Zeid (1982), Dharmadurai et al. (2011), and Wu et al. (2014)

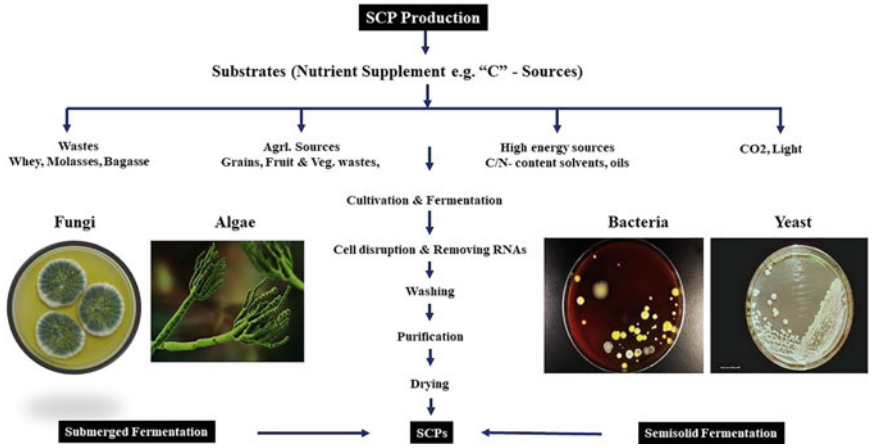

Fig. 1 Commercial production of single cell proteins from various beneficial microorganisms

Selection Criteria of Microorganisms

Selecting fast-growing microorganisms with better outcomes (biomass) is one of the most important criteria in single cell production (Reihani & Khosravi-Darani, 2019). Sometimes, heavy biomass produced by microorganisms is unfavoured due to high levels of RNA in the cells. It acts as an anti-nutritional factor in the final product.

Therefore, optimization of biomass and selection of organisms are better for SCP production initially (Waldron & Lacroute, 1975). Additionally, fast-growing microorganisms require a large amount of substrate and availability is essential one. During production, more consumption of substrates and low biomass produce a negative result (Leuenberger, 1972). Potential heat shock-tolerant organisms or species play a vital role in SCP production, because it is another way for identification and synthesis of new heat shock proteins from various microorganisms. A mild temperature of 37 °C for 30 min induce oxidative stress aerobic and anaerobic cells. Choosing the wider temperature-tolerant organisms is suited for SCP production due to reducing the temperature regimes during fermentation (Steels et al., 1994). The pH of media influences the growth, development, sporulation, and biochemical compounds in microorganisms and especially changes the fermentation time and progress during production. Fungi require lower pH than bacteria and it depends upon selection of favourable substrates chemical nature of C/N ratio (Serrano et al., 2006).

Selection and Nature of Substrates

In SCP production, selection of substrates plays a crucial role for better yield in quantity and quality. Abundance of substrates, proximity of production area, and nature of compounds (CHO-derived sources) give the highest energy value for microorganisms and yield maximized biomass (Ukaegbu-Obi, 2016). Especially, some microorganisms want additional nutrition supplements like minerals (N′), sterols, and salts (Na^{2+}) for the fulfilment of growth (e.g. bacteria and algae). Ratio of C:N should be maintained given the better results in biomass; mostly 10: 1 ratio is followed in several organisms, but this is varied among different microorganisms. Increasing the level of this ratio will cause absence of ammonia before sugar consumption for enhancement of growth, biomass, and may result in poor yield (Jay et al., 2005). Using agricultural and industrial wastes reduced the cost of input on substrates at 20–30% and 80% biological oxygen demand during cultivation. Avoid the usage of petrochemical wastes and refinery effluent is better for prevention of the accumulation of harmful or toxic substances in biomass (Royer & Nakas, 1983).

Fermentation Process

It is a vital process involved in the production of single cell proteins at industrial level. Selected specific microorganisms are grown and multiplied in the suitable substrates with available environmental factors such as light, air, CO_2 conc., and pH. After mass multiplied, the biomass was obtained from it, further undergoing screening for removal of toxic substances and getting a pure SCP (Anupama & Ravindra, 2000).

Normally, there are two types of fermentations involved like submerged and semisolid. In submerged conditions, substrate is always used in the form of liquid containing adequate necessary nutrients. The whole process mostly depends on the available CO_2 and O_2; heat is generated during this process in the fermenter and continuously provides cooling at final for better optimized yield of biomass.

Generally, fungi and algae are recommended and involved in this process (Kumar Sadh et al., 2018). At semisolid fermentation (SSF), microorganisms grow on solid support materials or substrates in absence of free-flowing of water (visible like colloidal state). There are two types of substrates used like natural (lignocellulosic wastes, CO_2, polysaccharides and molasses) and inert (plastic foams, n-alkanes, ethanol, methanol, effluents of breweries) (Machado et al., 2013). For the remaining, solid state is carried out by pure solid form of wheat rice and millets brans inoculated with desirable microorganisms with 60–65% moisture. Regularization of favourable temperature, ionic conc. O_2 level, nutrient supplements, and pH were optimized with the desired biomass of microbes and quality and quantity of SCPs (Anupama & Ravindra, 2001).

Cell Wall Disruption

Sometimes, SCPs used as whole cell proteins act as direct food (e.g. Quorn™), while for others the cell wall may be broken to make the proteins. In fungal-derived chitin, glucan contributed as a fibre diet (Bogale, 2020). Numerous methods are handled for disrupting the cell wall, by mechanical (crushing, grinding, crumbling, pressurized homogenization, and ultra-sonication) and chemical - using exogenous or endogenous hydrolytic enzymes (bacteria—glycosyl hydrolases, oxidoreductases, lyases, pectinases, esterase; fungi—cellulase, glucanase, xylanases, proteases, pectinases) which are used commercially. After digestion or removal, the cell wall further goes to the whole cell for nucleic acid removal (Geciova et al., 2002).

Removal of Nucleic Acids

Naturally, removing the nucleic acids is an essential part in the production of SCP because the accumulation of proteins with DNA/RNA is harmful to humans. When ingested as food, it may cause kidney stones and urinary tracts problems in humans (Chee Tan & Chin Yiap, 2009). Different types of methods are employed in removing endogenous nucleic acids from cells. Particularly, using DNA/RNA degrading enzymes (DNA/RNase) continuously treated with hot temperature 60–70 °C diffuses out the nucleic acid components, but some folding of RNase released through utilization of 4M guanidinium thiocyanate, phenol, and SDS. Afterwards, give a sterile wash with water 3-4 times to remove unwanted molecules and contaminants (Hames & Demir, 2015).

Purification and Drying

It's a most important function during SCP production; proteins obtained by some popular methods such as detergent lysis, shearing force, treated with low ionic salts, and rapid changes in pressure. Additionally, these are specific due to any changes occurring in the buffer conditions; pH and temperature that may produce protein inhibitors and provide least yield. Using water and detergents are highly specific for avoiding contamination and yielding end products as good. Final process of drying mostly depends on the air flow or circulation and temperature present in the process (4 °C) (Omar & Sabry, 1991).

Factors Affecting SCP Production

SCP production is a specific one, because it is derived from controlled conditions in a specific manner. Under production part, several exogenous factors (Molnar & Pal, 2020) such as identification and characterization of beneficial microorganisms (life cycle, biomolecules, toxins, and biomass), their availability, sources of substrates and selection, cost of substrates, area of production, selection of fermenters, size, models or types and requirement of time, available skilled persons (man-forces) and indigenous factors (Uckun Kiran et al., 2015) like, C:N ratio (e.g. 1:6 and 1: 8 for Candida spp. and *Rhodotorula* sp.) in substrates, pH (3.5–7), temperature (25–35 °C), CO_2, O_2 levels (derived from RQ value: Vol. of CO_2 released/Vol. of O_2 absorbed—both are direct proportional), and least or unavailable of toxic substances. Finally, under commercialization factors, viz., global demand, affordable price, free movement of legislative structures, and consumption rate also influenced the SCP production (Xie et al., 2015).

Biosafety of SCP

Any food materials consumed by humans or animals must be noted in safety to all organisms. So, before commercialization, it must be screened by biosafety measures and applicable usage levels of components or ingredients are mentioned clearly and absence of harmful toxins (endo/exo) or substances (heavy metals or metabolites) is mandatory. Totally, it is only for human's health and wealth-related one (Bourdichon et al., 2012).

1.6 Economic Aspects

SCP production is always driven by the need of proteins. Nowadays, the agriculture and industrial wastes are converted and used as substrates rather than animal feeds because of their economic viability and environmental safety potential. Additionally, implementing the some tactics like, source available in native, lesser cost, capital load, and it's clear definition of outcomes are studied before it, generate this production as profitable one (Cooney et al., 1980).

Economic Analysis

Before analysis, consider the total capital load inoculated, cost of substrates, time consumption, yield per unit, enzyme cost, miscellaneous charges (power bills, manual, transportation), and these totals are compared with plant proteins available in current markets. An example of 40,000 tons of dry matter (wheat straw) used for 333 days/annum process was designed. Under production, pre-treatment, enzymatic hydrolysis, fermentation, and downstream processing are handled. At all processes, 2% loss is calculated, enzyme cost €10–20/kg other electric wages (20%) and labour wages, and finally, the minimum protein selling price (MPSP) estimated at € 5160–9007/ton, then it was lesser than milk proteins (€10,500/ton). So, providing

a ratio of benefit in cost, viz., it is profitable and affordable than plant and animal proteins (Mateles, 2007).

Commercialization

Currently, several multinational corporations are more involved and are starting new SCP-based industries with government to international tie-ups; because the human's consumptive habits are changed and it makes demand and available (Jones et al., 2020). At global level, there are unaccountable corporate companies that are involved and produce several types of proteins for human and animal nutrition from different sources with huge amounts. Additionally, household industries are developed in villages by support of government and women's empowerment groups (VSHGs - village self-help groups) for production of mushroom and yogurt (Enzing et al., 2014). Following Table 5 mentioned the active global industries in single cell protein productions from various microorganisms.

1.7 Opportunities

Nowadays, science is developing day-to-day in all man-essential sectors. Humans' food habits are changing due to their essential needs and demands. Furthermore, agriculture traditionally followed practices like agricultural wastes as feed for cattle. This approach is converted as feed to production substrates, so this new change is given a positive result in national income by these industries associated with new inventions in biological approaches. Therefore, the application of this science-based industrial take-off will make a big revolution on youngsters who are interested in science-oriented by giving a better opportunity for SCP production from small scale to large (Abdullah et al., 2021).

1.8 Challenges

Single cell proteins are mostly derived from natural substances and organisms; it may be not hazardous to be ingested by humans, animals, and is eco-friendly after consumption also. Despite this, some tragic or unwanted issues are occurring during production to ingestion due to following biosafety concerns and legislative policies. These issues are categorized under two segments, viz., during production and during and after ingestion (Bankra et al., 2009).

During Production

It acts as a basic screening manner by choice of organisms, characterization of organisms (growth, development, toxic studies, heavy optimized cells yield), availability of substrates, well-known beneficial production technologies and skilled persons (scientists), and well-established research and development sectors which all play a crucial and initial role in single cell proteins productions and commercialization (Spalvins et al., 2018).

Table 5 Prime industrial corporations and their multi-products in SCPs at world wide

S. No.	Name of the companies/organisms used	Products
1.	Algaeon (USA)/*Euglena gracilis*	β-Glucan
2.	BlueBioTech Int. (Germany)/microalgae	Food supplements ($11.59 million/year)
3.	Calysta Inc. (USA)/soil microbes	Enzymes (20,000 MT./year—($40 million/year)
4.	Cangzhou Tianyu Feed Additive Co., Ltd (China)/*B. subtilis*, Photosynthetic bacterial spp., *Saccharomyces cerevisiae*	Yeast powder (5000 MT.), Allicin (5000 MT.), Betaine (3000 MT.), Choline Cl_2 (20,000 MT)
5.	CBH Qingdao Co., Ltd (China)/Agrl. wastes; Algae; *X. campestris*	Arachidonic acid, docosahexaenoic acid, inulin, L-cysteine, taurine, vital wheat gluten, Xanthan gum
6.	Cyanotech Corporation (USA)/*Spirulina* sp.	BioAstin®—Natural food additive ($30 million/year)
7.	E.I.D Parry Ltd., Parry Nutraceuticals (India)/*Spirulina* sp.	Health supplements
8.	Euglena Co. Ltd (Japan)/*E. gracilis*	Healthcare and cosmetics ($23.29 million/year)
9.	KnipBio. (USA)/methanotrophic bacteria	Affordable feed for aquaculture
10.	Lallemand Inc. (Canada and France)/*S. cerevisiae* and *Torula*	Human consuming proteins
11.	LeSaffre (France)/*S. cerevisiae*	Lynside® Nutri, Lynside® ProteYn (human consuming proteins)
12.	Marlow Foods Ltd. (UK)/*Fusarium venenatum*	Quorn™ (human consuming proteins)
13.	Nucelis Inc. (USA)/*Yarrowia lipolytica*	Vit-D derivatives, nutritional oils
14.	Nutrinsic. (USA)/waste water	Organic fertilizer, animal feed (ProFloc™)
15.	Tangshan Top Bio-Technology Co., Ltd (China)/Yeast spp.	Brewer yeast powder, hydrolysed yeast, yeast cell wall, yeast extract ($5.00 million/year)
16.	TerraViaHoldings, Inc. (USA)/eucaryotic algae	Algal oils, food ingredients
17.	UniBio A/S (Denmark)/natural gas	UniProtein® (animal feed)
18	Unilever (UK)/Brewer's spent grain	Yeast extract (Marmite®)
19.	Vega Pharma Ltd. (China)/natural substance, agrl. wastes, bacteria, yeasts	Vitamins, veterinary pharmaceuticals, nutritional supplements ($99 million/year)

MT Metric ton (Vigani et al., 2015)

During and After Ingestion

Regular intake of SCPs sometimes produces side effects or health issues to humans and animals. Because of impurified or partially purified form of proteins contaminants (toxins) with nucleic acids (RNase), it causes heavy issues on biological functions of kidney, liver, and digestive systems. So, following health standards in products is mandatory (Spalvins et al., 2018).

1.9 Future Perspectives

In the future, the aim of FAO is to alleviate the hunger index rate and DFH ('die from hunger') with growth and development of every nation. Today, agriculture and allied sectors are highly influenced by new transgenic seeds, evolved fertilizers, and chemical inputs; another side is growing development in organic farming. It has a highly controversial impact on human's health and environmental safety. So, production and distribution of food had all nutrition is the prime goal in future global wide. This may be derived from only possibilities with natural-derived SCPs' source at >50% (Hansen & Cheong, 2019).

2 Conclusion

Single cell proteins exhibit attractive characteristics in nutrient supplements for plants, animals, and humans. Under production, it can be produced at any place and time during the year because of their sources available in nature and not much influenced by any climatic changes. Compared to other organisms, bacteria play a superior role by their rapid growth and production of biomass and yield. Furthermore, it benefits human welfare and socio-economic welfare by their easiest ways of production approaches from small household to corporate level with influencing the national GDP.

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Small/Large Scale Production and Cost Benefit Analysis of Bread

Kohila Durai, Sri Sneha Jeya Kumar, and Dhanasekaran Dharumadurai

Abstract

Bread has been considered to be an easiest processing from wheat flour that suits our modern world. Bakery goods are an integral component of a modern lifestyle. Bread contains all the required sources as much as possible for a diet including calcium, potassium, iron, and magnesium. Bread may be leavened by naturally occurring bacteria, chemicals, yeast developed industrially, or aeration at high pressure. This article presents a detailed note on production of bread at small and large scale level also the economic analysis. The investment and cost for production all comprised under economic analysis.

Keywords

Bread · Small and large scale production · Cost benefit · Revenue generation

1 Introduction

Bread is a well-known dietary item that is prepared by baking in specialized oven. By the intentional trail with water and grain, the first bread was found. People of Egypt are the pioneers who are the discoverer for the bread production across the globe. Russia, Ukraine, and Kazakhstan are considered to be the major wheat producing countries. Approximately, it is estimated that it produces 18% of world's wheat production and 22% of wheat's global exports. Production and exports of the

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bread can be increased immensely by land utilization with a great yield which would be said as "Breadbasket of the world" (Glauben et al., 2014). The substance containing flour proteins is known to be an important component in the bread making quality to be best.

2 Ingredients

The most used ingredient for bread making are water and flour as they form apt texture and crumb. The breads are prepared with the composition of flour that need (Mondal & Datta, 2008) 14–14.5% moisture, 12.8–13% protein, 0.56% ash, pH range 5.6–6.1 which would make a volume of 100%, and water approximately 50%.

Other than this, some ingredients are added on own interest to enrich their quality which is a percent of that amount of ingredient by weight. Other than 100% flour, rest of ingredients may include yeast 1.5–2%, sugar for sweetness 3–4%, salt 1.8–2%, and ghee or margarine 2.9–3% (Gobbetti, 1998). The bread was derived from the process of fermentation that occurs in wheat flour. The sugars have been liberated from the starch by action with natural enzymes in the flour (Bell et al., 2001). *Saccharomyces cerevisiae* is a microorganism that causes fermentation to be taken place (Table 1) (Zanoni & Peri, 1993; Oda & Ouchi, 1990).

Soon after the preparation of dough, the next is baking of bread which results in a product of soft and pleasant aromatic pieces (Brinker & Schmidt, 2008). The fresh bread is of alluring brownish and crunchy crust (Mondal & Datta, 2008). The need for the production for bread has been increased nowadays. Many large scale productions have been initiated to overcome need for exclusive. This results in necessity of emulsifiers and anti-staling agents to produce the expected quality in these controversies. Emulsifiers also improve crumb structure and the capability of splicing characteristic and enhance gas holding capacity.

Table 1 Role of constituents used

S. No	Actions	Uses
1.	Fermentation (leavening process)	Sucrose is improved to CO_2 and moisture
2.	High temperature	Carbon-dioxide (CO_2) and vapor gas expand acting as insulating agents that prevent overheating
3.	Sugar	Initiation of fermentation
4.	Salt	Strengthen gluten and controlled expansion of dough by yeast
5.	Shortening (ghee margarine)	Increases low work machines' efficiency and slicing mechanism
6.	Emulsifiers	Strengthen dough to withstand machine handling and improve rate of hydration

3 Baking Technology

Over past 150 years, the bakery industry has seen a known revolution. From the small artisan bakeries to large bread manufacturing industry, many techniques are developed over these times to readily meet the demand of bread in market (Mondal & Datta, 2008). Many ingredients and new technologies are introduced for the new enriched nutritional, flavored bread at superior quality and lucrative benefits (Hutkins, 2006). Comparing to other products, bakery products have special quality with reduced density with high healthy nutrient content. The engineering of food structures is done such as apt composition of mixture (dough) that fences in fermenter and finally the heat is applied.

4 Bread Waste

The bread wastes are utilized in following ways; the breads are frozen and drilled, then (Rosing & Nielsen, 2004)

1. Replacement of bread waste in place of barley as an animal feed was done.
2. In the place of flour, the bread waste is used as bread crumbs in bakery.
3. After processing the bread waste into bread crumbs, it can be utilized as an animal feed.
4. Future technologies avoid drilling. It is alternatively used by baking the waste around the stick with more air on them and creating new recipes by which the hotdog man creates a hole with spear.

5 Types of Bread

All the types of bread are made with the same composition such as water volume of 600 ml and sourdough quantity of 200 g with same baking procedures (Table 2).

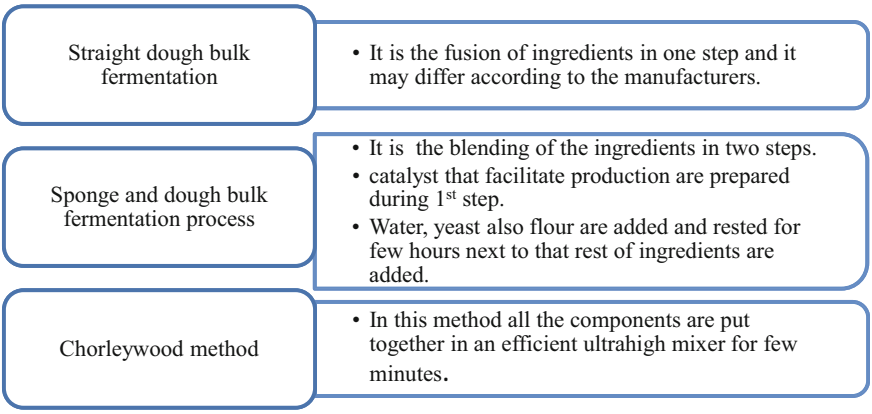
6 Methods

The confectioneries are prepared according to three categories of methods:

Table 2 Varieties of bread (Mondal & Datta, 2008)

S. No	Types of bread	Source
1.	Oat bread	Oat
2.	Rye bread	Rye
3.	Light wholemeal bread	Wheat gluten
4.	Dark wholemeal bread	Dark Bavaria
5.	Multicereal bread	Sesame, sunflower, linseed, carob

- Straight dough bulk fermentation method
- Sponge and dough bulk fermentation method
- Chorleywood bread processes (CBP)



Dehydrated sour dough was utilized for quick preparation from specialized companies.

The preproofed frozen dough is fermented prior to freezing was developed by the improver technology by the end of 1990s (Hino et al., 1987). To have better fulfilling needs according to the marketing concepts, cycle includes freezer to oven, ready to bake but the quality is not compromised. Though it is not satisfied the price for that product has increased. The frozen dough involves the steps as follows:

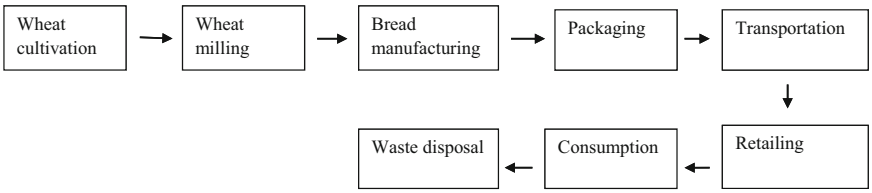
- Dough preparation
- Freezing
- Thawing
- Baking

The effect of freezing and thawing plays a vital role in developing the relationship between temperature and duration holdings.

7 Production

The term RDM refers to redistributed manufacturing as a political interest which is of high economy and accompanied with expeditious technological, regulatory, social, and environmental changes in UK (López-Avilés & Leach, 2016).

The fundamental methods involved in production of bread are;



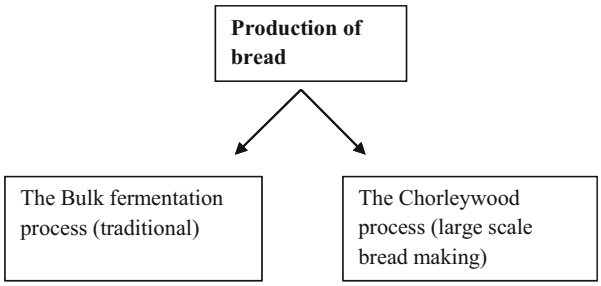
The most energy demanding process in the food sector is bread making when compared to canning (Le-bail et al., 2010).

8 Bread Manufacturing in the UK

The technologies have developed to increase desired yield at short span, and when compared to grading and cleaning, most of the energy is required for the intensive processes in flour milling such as

- Sucking air to remove dust
- Stirred mixture
- Smashing
- Disparate
- Grinding mixture into flour
- Packaging

The next prominent processes included in bread manufacturing are that ingredients are mixed, next to mixing it is fermented and baked.



Most of the breads are baked in large centralized manufacturing facilities that sell to supermarkets and some shops (López-Avilés & Leach, 2016). The variation observed in the production of bread was illustrated.

9 Small Scale Production

In small scale production, the microorganisms such as yeast and bacteria play an important role in leaven, consisting of dough in which the fermentation process is active for making artisanal bread. Sourdough starters contained *Saccharomyces cerevisiae* and *Kazachstania exigua* yeasts. When bread was made, merging this type of yeasts alone and in combinations with LAB (lactic acid bacteria) also sequestered. The sourdough starts with *Lactobacillus plantarum*, *Lactobacillus brevis*, *Lactobacillus rossiae*, and *Lactobacillus casei*, the dough finally comes out like a tang shapes and small pieces, adding of aromatic compounds produces some aromas. The mixture of microorganisms, starch, and sugar in the flour initiates fermentation process to produce CO_2 (Arendt et al., 2007; De Vuyst et al., 2009). In artisanal bread, sourdough is used as a leaven and this sourdough create some numeral of superiority features are improved. Some investigated originate lactic acid bacteria dough containing amplified aromatic combinations (Petel et al., 2017) and yield complete collection of aromatic acids (Hansen & Schieberle 2005).

10 Steps

The significance of bread in the growth of mankind is undeniable. Bread is used up throughout the world in many profiles and customs, be an average spending of 70 kg per year, even though Europeans consume a reduced amount, be an average of only 59 kg (De Boni et al., 2019; Edwards, 2007; Gębski et al., 2019). Refined flour, purified water, and some kind of yeasts and salt are the common ingredients for making bread. The mixing of above mentioned ingredients to prepare a dough flour and make them into knead flour and after finishing of fermentation and baking process, the bread is prepared, while around variety of steps are involved for making bread (Fig. 1). The most vital fact following bread accomplishment is a primary cooking, in addition to the variety of cereals also used for baking. Finally, baking bread is packed into packed grit together with cohesiveness, chewiness, stiffness, and resilience. Then, color texture of the bread crumb was also analyzed by using colorimeter.

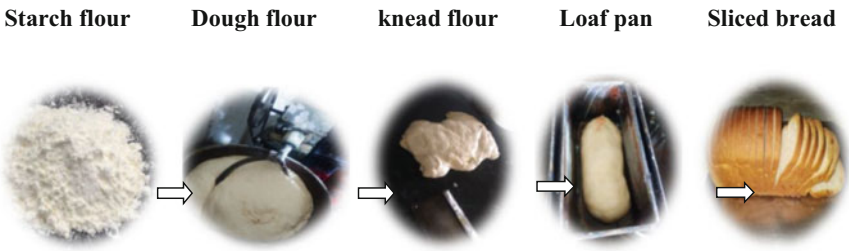


Fig 1 Bread making steps

11 Machine

Sunbeam Bakehouse Compact BM2500

Sunbeam Bakehouse Compact BM2500 is a machine for making bread (Fig. 2). The bakehouse BM2500 will combine all the ingredients such as refined flour, purified water, some kind of yeast, milk, sugar, and salt. And after adding of ingredients, the bakehouse BM2500 mixed together to make a dough. This sunbeam bakehouse compact BM2500 takes the duration for fermentation up to 1 h to 1 1/2 h. After that the dough separated into pieces and put into the loaf tins. The loaf tins are placed into the oven at 30 °C for 2 h. And then cool the loaf tins for some more minutes. And slice the baked bread into small pieces.

12 Techniques

Fermentation is the fundamental process for making the mold foodstuffs. The yeast *Saccharomyces cerevisiae* absorbs the sugar in dough flour during straight dough fermentation process to produce CO_2. In fact, CO_2 creation is too high, the dough becomes risk and may fall down. The amount of fermentation process depends upon the presence of sucrose in the refined dough flour. In dough fermentation process, the most significant fermentable sugar is maltose. The yeast is the common leavening agent present in both straight dough fermentation and sourdough fermentation, but the duration of sourdough fermentation, pH, and microbial ecology may be different from straight dough fermentation. The yeasts described in the sourdough fermentation process are generally nonconventional yeasts, for instance, Candida and Kluyveromyces species.

Fig. 2 Bread slices

13 Chemicals

The processed foods of bakery products are definitely focused in their physical, chemical, and microbiological spoilage. High moisturized products are spoiled by microbes such as bacteria, yeast, and molds. The reason for mold-free shelf life of many bakery products is CO_2-enriched atmospheres and addition of chemical additives.

14 Investment

Bread products are essential part for our balanced diet. In this world, a wide variety of bread products are available in markets. The common bread mixing method (i.e., mixing of cereal grains and water) and the mixing are cooked by using fire. This is our ancestor's method. Our ancestor's method (i.e., primitive cottage industry) developed into industrial scale modern manufacturing industry. This improvement developed an assortment of giving out and wrapping technology, together with custom-made atmosphere wrapping. This kind of technologies is used to increase the availability of wide variety of bakery products and increase the revenue.

15 Large Scale Production of Bread

The mass production can be achieved either by production of very large quantities of same artifact or of essential artifacts by same production means. The large quantities of required products can be produced by large scale production by creating a large sector for production or replicated individual artifacts. The large scale production generally involves certain requirements: division of labor, standardization of sizes and forms. The most important step involved in the bread making is the fermentation which is a small scale operation, and if we go for large scale production the small units are multiplied and so the mass production can be achieved (Figs. 3 and 4; Table 3).

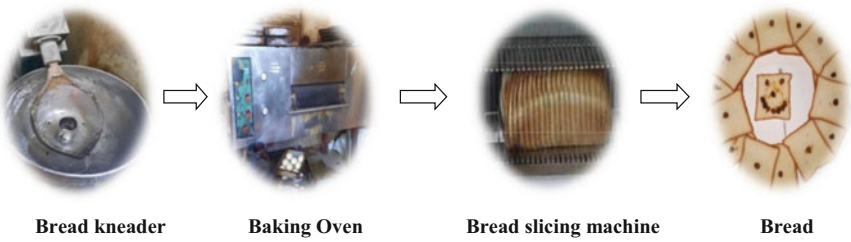


Fig. 3 Steps involved in pilot scale production

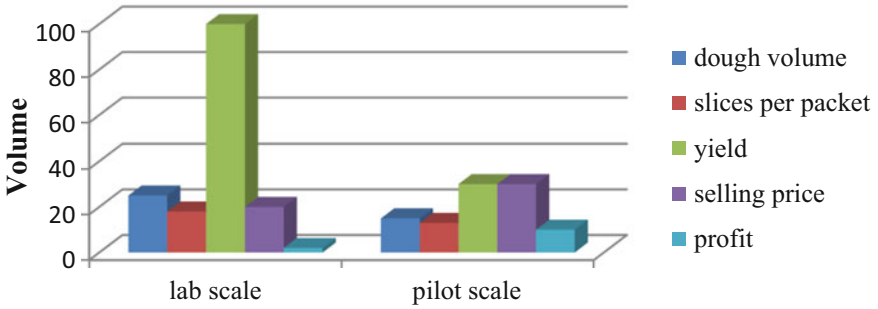


Fig. 4 Volume analysis b/w small and large scale

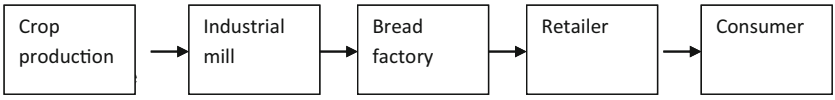
Table 3 Comparative chart of bread production and the brief description

Comparative analysis			
Ingredients		Ingredients	
Lab scale	Volume	Pilot scale	Volume
Maida	25 kg	Maida	7 1/2 kg
Sugar	10 kg	Sugar	4 1/2 kg
Yeast	250 g	Yeast	150 g
Salt	500 g	Vanilla extract	50 g
		Milk powder	50 g
		Milk	1 l
		Oil	1 l
Water	$14_{1/4}$ l	Water	2 l
Total volume	50 kg	Total volume	15 kg
Profit analysis			
Yield	200 packets	Yield	30 packets
No. of slices per packet	20 pieces	No. of slices per packet	13 pieces
Profit	Rs. 2.51	Profit	Rs. 10

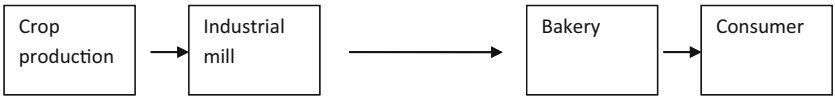
16 Life Cycle Scenarios of Bread Production

The lifecycle scenarios for conventional crop production and organic both include same processes in four stages (Borken et al., 1999; Patyk & Reinhardt, 1997).

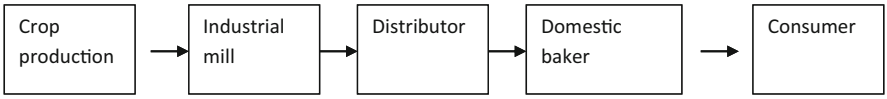
First stage



Second stage



Third stage



Fourth stage

17 Cost Benefits Analysis of Bread Production (Table 4)

Formula

$$\text{Cost benefit ratio} = \text{Total benefits (₹)}/\text{Total cost (₹)}$$

$$= ₹4000/₹2410$$

$$= 1.65$$

This cost benefit analysis is analyzed based on lab scale production of bread and the volume and cost of each ingredients are mentioned in Table 5.

Chemicals, Media (Consumables), Basic Laboratory Equipment, and their Cost for the Production of Bread in Lab Scale Production

Table 4 Cost benefit analysis

Cost benefit analysis	One day investment
Total costs	₹2410
Total benefits	₹4000
Cost benefit ratio	1.65

Table 5 Cost for the production of bread in lab scale production

Ingredients	Cost for each items of ingredients (per gram and per kg)	Total cost for each items of ingredients	Total cost of the production for one day
Yeasts (250 g) Maida (25 kg) Sugar (10 kg) Salt (500 g) Water (14 1/4)	₹2 ₹60 ₹40 2 paisa Required amount	₹500 ₹1500 ₹400 ₹10 Required amount	₹2410

Basic Laboratory Equipment and Tools

- Siever
- Measuring cups
- Digital scale
- Dough mixer
- Rolling pin
- Dough scraper
- Loaf pan
- Baking rack
- Deck oven
- Baking Tester
- Bread slicer

18 Nonrecurring Expenditure, Recurring Expenditure, Revenue Generation, and Net Profit

Nonrecurring Expenditure

In lab scale production, the nonrecurring expenditure is not involved because the lab scale production of bread is the day by day continuous making process.

Recurring Expenditure

Recurring expenditure is the day by day investment in lab scale production of bread industry. Recurring expenditure is shortly defined as daily investment for making bread (Fig. 5).

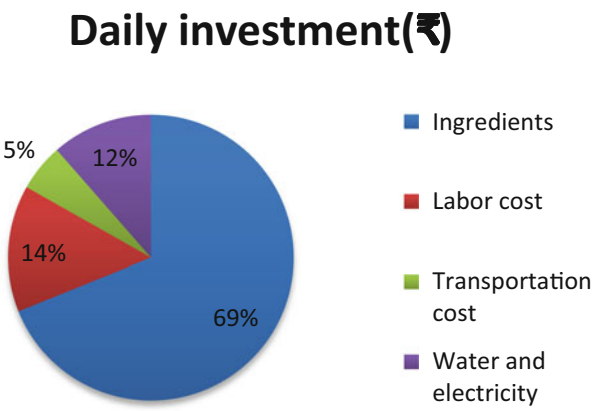


Fig. 5 Recurring expenditure for lab scale production of bread

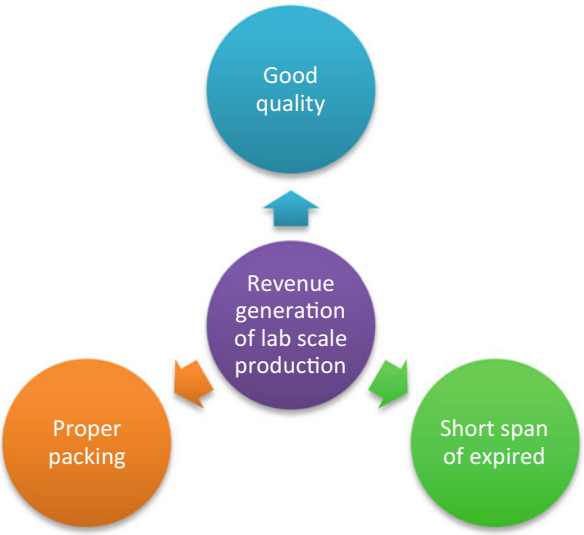


Fig. 6 Revenue generation for lab scale production of bread

19 Revenue Generation

Revenue generation is the process of marketing and selling of products with the aim of increasing income. Based on some changes in bread production such as unique taste and freshness of bread, the revenue income increased (Fig. 6).

20 Net Profit Analysis

Net profit analysis is the calculation of profit from its total revenue.

Formula

Table 6 Net profit analysis for one day

Profit for one day (₹)	
Total investment for one day (recurring expenditure)	₹2410
Yield for one day	200 packets
Cost for one packet of bread	₹20
Profit for one packet of bread	₹2.51
Total profit for one day investment (revenue)	₹4000
Recurring expenditure	₹2410
Salary and maintenance	₹1088
Remaining profit for one day	₹502

Table 7 Net profit analysis for 1 month

Profit for one month (₹)	
Total investment for one month (recurring expenditure)	₹72,300
Yield for one month	6000 packets
Total profit for one month investment (revenue)	₹120,000
Recurring expenditure	₹72300
Salary & maintenance	₹32640
Remaining profit for one month	₹15060

$$\text{Net profit analysis (₹)} = \text{Revenue (₹)}/\text{Revenue} - \text{cost (₹)}$$

$$= ₹4000/₹4000 - ₹2410$$

$$= ₹2.51$$

This net profit is analyzed based on lab scale production of bread, and the volume and cost of each ingredients are mentioned in Tables 6 and 7.

Cost of 1 kg of Bread and the Cost for Total Production in 1 Day

This cost is analyzed based on lab scale production of bread and the volume and cost of each ingredient are mentioned in Table 8.

Table 8 Cost for total production in one day

Total volume of production for one day	Cost of one kg of bread production	Cost for total production in one day
50 kg	₹48.2	₹2410

21 Conclusions

Nowadays, a wide variety of bread products are being sold and marketed. This wide variety is classified based on their different ways of production, e.g., sweet bread, plain bread and flavor bread. In ancient days, breads were prepared in homes, but now the home preparation (cottage industry) is reduced and the handmade preparation of bread level is totally reduced. But in recent days, lot of machines are available for preparation and packaging of bread products. Recurring expenditure, nonrecurring expenditure, revenue generation, and net profit are important factors for increasing revenue. The bread products have short span of expiry. And it is one of the balanced diet food products. The microbes play an important role for making money in bread production.

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Production Cost Analysis and Marketing of Fermented Food: Cheese

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Abstract

Cheese has been produced from both raw and pasteurised milk for almost 7000 years in our world's history. It is currently being introduced as a vital part of specific diet regimens like the ketogenic diet, as it is classified as a leading supplier of proteins and good fats. Curdling, coagulation with proteins like casein and whey, fermentation, ripening, and other processes are all required in the manufacture of cheese. Different types of cheeses, such as Mozzarella, Gouda, Feta, Blue cheese, Cheddar cheese, and others, are created as a result of these processes. Cheese markets are most commonly found in hypermarkets and supermarkets, where cheese is a popular sales item. There is enough demand for cheese all across the world to support new businesses.

Keywords

Cheese · Fermentation · Manufacture · Bioentrepreneurship

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1 Introduction

The roles of probiotic bacteria in dairy fermentations are to aid in: (1) the preservation of milk through the production of lactic acid and possibly antimicrobial compounds; (2) the generation of flavour compounds (e.g. acetaldehyde in yoghurt and cheese) and other metabolites (e.g. extracellular polysaccharides) that will provide a product with the organoleptic properties desired by the consumer; and (3) to boost the nutritional value of food, such as the release of free amino acids or vitamin synthesis; and (4) to provide particular medicinal or preventive qualities, such as cancer prevention (Reddy et al., 1973; Fernandes et al., 1987; Gilliland, 1990; O'Sullivan et al., 1992) and control of serum cholesterol levels (Lin et al., 1989). The development and action of microorganisms during the manufacture of cultured foods may have potential benefits (Shahani & Ayebo, 1980). Prophylaxis against certain forms of intestinal infection is also a therapeutic benefit (Fernandes et al., 1987) Lactose digestion is improved, which helps to prevent lactose maldigestion (Sawada et al., 1990). Metchnikoff (1908) is credited with being the first to suggest that fermented dairy products had health benefits. There has been a resurgence of interest in the nutritional and medicinal elements of these items over the last two decades. Several studies (Donna, 2001; Lee et al., 1999; Taranto et al., 1998) have demonstrated that lactic cultures and their fermented products provide customers with a wide nutritional and therapeutic benefits.

Cheese production consumes around a third of the world's milk production. Cheese is a nutrient-dense food. It's convenient and adaptable, with a wide range of flavours and textures. Cheese accounts for over 30% of total dairy product sales globally, with sales expected to increase by 9.8% between 2003 and 2007. Convenient packaging, growing usage of cheese in the food service sector, and rise in specialty and unusual cheeses are all contributing to the cheese industry's expansion. During the 5-year period from 1998 to 2002, the cheese market in the United States, the United Kingdom, France, and Germany grew by more than 9%. Because milk is perishable, making cheese is a method of milk preservation. Depending on their moisture content, all cheeses, whether rennet or acid set, can be classed as soft, semisoft (semihard), hard, or very hard. Although arbitrary and utilitarian, this classification aids in the methodical grouping of cheeses that share some basic qualities or characteristics (e.g. moisture content), as moisture impacts the body, consistency, and compactness of cheese. As a result, the word "soft cheese" refers to cheese that is soft to the touch or when pressed between fingers.

In contrast, the terms hard cheese (for example, Cheddar) and very hard cheese (for example, Parmesan) refer to cheeses that are firm or very firm and require some type of pressure to break apart. Semisoft, hard, and extremely hard cheeses have moisture content upper and fat content lower limitations, which are commonly stated as fat in dry matter (FDM). Soft cheeses must contain a minimum of 50% FDM, but the US Code of Federal Regulations does not define a maximum moisture level. However, there is a practical moisture limit of roughly 80%, after which the product becomes a liquid rather than a semisolid mass. The process used to coagulate milk

for cheesemaking has an impact on the cheese's overall structure, qualities, and hardness.

Rennet and acid are the two most common procedures for clotting milk for cheese production, resulting in the phrases rennet- and acid-coagulated cheeses, respectively. Acid-coagulated cheeses are often soft, while rennet-set cheeses are stiff. Economic factors, equipment/engineering, consumer needs, and regulatory standards are the primary drivers of cheese technology. Examining typical cheese production stages, using Cheddar as an example and because it pertains to Professor P. F. Fox's contributions to the science and technology of cheesemaking, is the best way to analyse the contributions of these aspects.

Lactic acid production from lactose is perhaps the most critical stage in cheesemaking, and it is accomplished by carefully selecting cultures of various lactic acid bacteria (LAB) that are given to the milk immediately before renneting. Because they initiate (start) the formation of acid, these cultures are referred to as starters. They are sometimes known as lactic cultures since their primary job is to make lactic acid; however, their enzyme systems are also vital in the development of flavour creation during the ripening of cheese and certain starters. Other molecules produced by LAB include acetaldehyde, acetic acid, and diacetyl, which are significant in the perception of flavour in fresh fermented foods such as cottage cheese, Quarg, and yoghurt. Lactic acid generation during cheesemaking lowers the pH of the curd and whey and serves three purposes: it stimulates rennet activity, aids in the evacuation of whey from the curd, lowering the cheese's moisture content, and helps to prevent the growth of harmful bacteria.

The primary cultures used in cheese production are starter LAB. Other microbes, such as *Brevibacteria* and *Corynebacteria* in surface-ripened cheeses, *Propionibacteria* in Emmental, and moulds in Brie and Camembert, are utilised in some cheeses. These secondary cultures are only active throughout the ripening process. Cheese was manufactured without the purposeful inclusion of starters until the end of the nineteenth century. Starters were initially used in the production of butter in Denmark around 1890. Mesophilic starter cultures have an ideal temperature of 30 °C, while thermophilic cultures have an optimum temperature of 42 °C. *Lactococcus lactis* subsp. *cremoris* is the most common strain found in mesophilic cultures; however, minor numbers of *Lc. lactis* subsp. *lactis* and/or *Leuconostoc* sp. can also be found. *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lb. delbrueckii* subsp. *lactis*, or *Lb. helveticus* are used in thermophilic cultures. *Streptococcus gallolyticus* subsp. macedonicus has recently been isolated from the Greek cheese Kasseri, which is thermophilic and related to *Streptococcus equinus* rather than *Sc. thermophilus* (de Vuyst & Tsakalidou, 2008). It's unclear whether or not this species should be regarded a starting culture because the cheese from which it was isolated is created without the organism being intentionally included. Each sort of starter can be further separated into cultures with mixed strains and cultures with defined strains.

Traditionally, cheese has been consumed directly. However, the use of cheese in food service and as an ingredient has skyrocketed in recent years. Due to the increased popularity of pizza, approximately 40% (1528 million kg) of the

approximately 3904 million kg of natural cheese produced in the United States in 2002 was used in the foodservice sector, with mozzarella cheese being the most commonly used variety. Cheese's capacity to be chopped, sliced, diced, or cubed into various forms, as well as grated, ground, and dried, gives it considerable diversity in terms of cuisines and food uses. Cutting, dicing, slicing, cubing, and other techniques are confined to semisoft, hard, and extremely hard cheeses, whereas soft cheeses require pumping and spreadability. For specialised functional purposes, processed cheese products, cheese powders, and enzyme-modified cheeses are becoming increasingly popular. There is also a lot of interest in developing cheese variants for baked, deep-fat fried, and retort culinary applications. Paneer, queso blanco, and other Hispanic-style cheeses have numerous health benefits (Farkye et al., 1990; Van Hekken & Farkye, 2003). Cheese contains a variety of nutrients. One ounce of hard cheese, or roughly the size of a thumb, has 120 calories, 8 g of protein, 6 g of saturated fat, and 180 mg of calcium. A half-cup of soft cheese, such as 4% full-fat cottage cheese, contains 120 calories, 14 g of protein, 3 g of saturated fat, and 80 mg of calcium. Because salt is a critical element in holding moisture and preventing microbial overgrowth, most cheeses have a high sodium content of 300–450 mg/serving. Some cheeses, such as goat, whole-milk mozzarella, and Swiss, are sodium-free, containing only 50–100 mg/serving. Many entrepreneurs are turning away from large business and mass production in favour of a more concentrated and deliberate return to their craft. More importantly, consumers, particularly millennials, are demanding this change. They desire high-quality items with integrity and complete transparency. They are also willing to pay a higher price for them. In this chapter, we see about cheese production and its marketing through the eyes of entrepreneur.

2 Cheese Production

Professionals estimate the global cheese market to be worth between $65 billion and $68 billion. In India, the cheese market is expected to increase rapidly between 2015 and 2020. Because India is currently the world's largest producer of milk, the cheese market is poised for significant expansion. With the rise of western cuisines and rising disposable incomes, buyers are shifting away from paneer and toward cheese, which has growing demand in the country. In addition, manufacturers are launching a variety of flavoured cheese products, such as pepper, garlic, red chilli flakes, and oregano pickle, to appeal to the diverse tastes and objectives of Indian consumers.

Cheese is a good source of fat, calcium, and protein, and its consumption can help an individual gain weight in a healthy way. These factors are expected to contribute to the demand for various types of cheese in the coming year. A growing number of restaurants, cafes, and bars are expected to drive demand for fast food or small bites, which will undoubtedly have a positive impact on the cheese market. The desire of manufacturers to replace chemical weapons, non-organic ingredients with natural variants is gaining enormous attention in the global market as major players in the cheese market focus on venturing into the green market. Growing consumer

awareness for natural products and the desire of manufacturers to replace chemical weapons, non-organic ingredients with natural variants is gaining enormous attention in the global market. Following elaborates on the steps of cheesemaking.

2.1 Selection of Milk

The process of manufacturing cheese begins with the selection of milk. The quality of raw milk used in cheese production has a significant impact on the quality of the cheese produced. Starter growth, rennet coagulation, manufacturing procedures, the formation of taints and other flaws in the body and texture of cheese, and other changes that occur throughout the ripening of cheese are all influenced by milk quality. Sensory, microbiological, and chemical quality criteria are used to evaluate milk. The odour and appearance of milk are assessed as soon as it arrives at the cheese factory's reception dock. It must not have an unpleasant odour and must be devoid of foreign stuff. The milk, or raw material, can come from a variety of animals, including cows, buffaloes, sheep, and goats, or it might be a blend of milks from many species. Because of its distinctive aroma, physical characteristics, and high income, buffalo milk is said to be more suitable for Mozzarella cheese than cow milk (El-Koussy et al., 1995).

Quantitative Factors

Composition of Milk

The production and texture of cheese are mostly affected by the content of milk. The qualities of coagulum are affected by the composition of milk, affecting yield and texture. Milk proteins (especially casein), milk fat, and mineral salts are the most important milk ingredients in cheese manufacturing (particularly calcium). The primary milk protein, casein, is mostly found in micellar form in milk. It accounts for roughly 70–80% of total milk protein, -casein, -casein, -casein, and a few other components make up the casein micelles. Furthermore, genetic variations of these components have been discovered. Due to the influence of species, breed, animal health, lactation stage, climate, time of year, and environmental factors, the composition and concentration of such a combination is not constant and prone to fluctuations. This has an impact on milk cheesemaking features such as clotting time, curd strength, syneresis, cheese yield, cheese proteolysis, and cheese composition. Whey loses serum proteins and lactose throughout the cheesemaking process. As a result, casein-rich milk is preferred.

Milk Fat

The same factors that impact the volume, composition, and type of milk fat are explored in the case of milk proteins. The physical and chemical properties of milk fat play an important role in the production of cheese. As the fat content of milk increases, the syneresis decreases, requiring longer durations to obtain the desired moisture content in cheese. Too little fat in milk is also undesirable since it causes

moisture retention issues, resulting in hard, dry body cheese. The fat content of cheese has an impact on its texture. Cheese made with low fat milk has a leathery feel and lacks mellow and velvetiness, whereas cheese made with high fat milk has a soft, buttery, and greasy texture. Fat is also necessary for the development of cheese taste. Lipolysis of fat occurs during ripening, resulting in the creation of fatty acids, which give cheese its distinct flavour. In the casein matrix, fat is integrated. Fat incorporation is influenced by the size and content of fat globules. Larger globules are more difficult to mix into curd than smaller ones. Higher melting point milk fat is better absorbed into the curd. In terms of fat and casein content, the milk used to make cheese must be of consistent quality. As a result, prior to standardisation to a specific casein/fat ratio, it is always preferable to pool all of the milk supply. Milk is regulated to a casein/fat ratio of 0.67–0.72 while manufacturing Cheddar cheese.

Milk Salts

Normally, milk salts are classified as ash. Ash is high in metallic elements such as potassium, sodium, calcium, zinc, chromium, and nickel, as well as non-metallic elements like sulphur, chlorine, phosphorus, and iodine. Calcium and magnesium salts of phosphoric and citric acid are the salts in milk that are most important in the cheesemaking process. Casein and phosphates form a compound when calcium is present. The calcium level of milk has a big impact on rennet coagulation time, clot strength, and cheese body and texture. The size of casein aggregates is influenced by calcium levels. Casein micelle size increases when calcium level in milk increases. The RCT of milk is directly influenced by variations in calcium, magnesium, phosphates, citrates, and salt concentrations. Slow rennet coagulation of milk has been discovered to be caused by high soluble phosphates, citrates, and salt, as well as low soluble calcium and magnesium and a low fraction of casein bound calcium.

Qualitative Factors

There are three types of chemical characteristics in milk that affect cheese quality:

- Starter growth is inhibited by such quality.
- Coagulation-affecting quality.
- In cheese, the quality that causes taints, gas-holes, and other defects.

The relevance of the starter in cheesemaking is reported to be 90%. The relevance of starting organisms in cheesemaking is highlighted in this remark. As a result, cheesemaking milk must be devoid of physiologically active inhibitors, preservatives, and antibiotics. Antibiotics in milk, even at a concentration of 0.01 IU/ml, can impact the growth of the starter. The time it takes for milk to coagulate with rennet is one of the most critical aspects in cheesemaking. The coagulation of mastitis milk, colostrum, or late lactation milk is harmed when they are mixed. As a result, milk must be free of toxins. Mastitis milk contains a lot of serum albumins, as well as a lot of salt and chloride. It has fewer calories, fat, casein, lactose, phosphate, potassium, and calcium than regular milk. It generates a poor yield and wet cheese because of its low casein content. Biochemically, late lactation milk resembles

subclinical mastitis milk. It has a slightly alkaline pH, is high in albumin and chloride, and is low in casein, lactose, and calcium, and has a similar effect on rennet coagulation of milk as mastitis milk.

Colostrum differs significantly from regular milk. It is high in proteins, vitamin A, and sodium chloride, but low in carbs, lipids, and potassium when compared to regular milk. Lactoferrin and lactoperoxidase (LP), two antibacterial compounds found in it, inhibit starter cultures used in cheesemaking. To avoid coagulation delays, colostrum should not be blended with milk for at least three days, but preferably up to 15 days. The composition of cheese milk, particularly the level of fat, protein, calcium, and pH, has a significant impact on the composition of cheese. Several factors influence the contents of milk, including the producing animal's species, breed, individuality, nutritional status, health, and lactation stage.

Natural Inhibitory Substances and Antibiotic Residues in Milk

Immunoglobulins, lactoferrin, lysozyme, and the LP system are all natural inhibitory mechanisms found in raw milk. The presence of inhibitory mechanisms in milk can affect the qualities of cheesemaking, particularly starter growth and acid development. Aside from these natural inhibitors, some antibiotics may enter milk as a result of animal medical treatment for a variety of disorders. Antibiotics interfere with acid development and may cause starter failure, resulting in faults such as high moisture in cheese, early and late blowing, a weak and pasty body, cracks, an open texture, and sponginess.

2.2 Standardisation of Milk

Milk standardisation allows the producer to manage the composition of the starting milk in order to fulfil the legal definition of the specific variety and improve yields while also meeting the legal definition of the specific variety. The use of standardised milk, on the other hand, prevents the production of excessively fat cheese and reduces fat and casein losses into the whey (Lucey & Kelly, 1994). The addition of skimmed milk powder, liquid skim milk, and the removal of cream are the three basic ways for standardising milk for cheese manufacturing. The quality and temperature history of the skimmed milk powder or liquid are critical in the first two circumstances, especially in the production of high-quality Cheddar cheese. Standardisation resulted in a slight loss of yield from a standard volume of milk when Cheddar cheese was manufactured from milk that showed normal seasonal variations in composition and from milk that had been standardised to a crude protein-to-fat ratio of 0.9 (by the addition of skimmed milk) (averaged over a complete year). This loss was offset by a nearly equivalent increase in fat retention efficiency (Banks et al., 1989).

Different cheese varieties have a distinct fat-in-dry-matter composition, resulting in a specific fat-to-protein ratio, which is codified in several cheese kinds' "Standards of Identity." While the manufacturing technique determines the moisture

content of cheese and hence the level of fat and protein, the fat: protein ratio is primarily regulated by the fat: casein ratio in the cheese milk.

- Eliminating some fat by natural creaming, as in the preparation of Parmigiano Reggiano, or centrifugation
- Adding skim milk
- Adding cream
- Adding micellar casein (made by ultrafiltration)
- Adding milk powder, evaporated milk, or ultrafiltration retentate, depending on the ratio required. As a result of these additions, the total solids content of the milk rises, and the cheese yield rises as well.

Because calcium is important in the rennet coagulation of milk and subsequent processing of the coagulum, it is usual to add $CaCl_2$ (e.g. 0.01%) to cheese milk. Milk's natural pH ranges from 6.6 to 6.7, however it varies (e.g. it increases in late lactation and during mastitic infection). Preacidification of milk by 0.1–0.2 pH units, either through the application of the acidogen gluconic acid—lactone or by limited growth of a lactic acid starter, followed by pasteurisation, is indicated to offset these differences and to reduce the pH as an alternative to ripening.

2.3 Heat Treatment of Milk

All cheese was traditionally prepared from raw milk, a process that persisted until the 1940s. Even today, raw milk is used to make a substantial volume of cheese throughout Europe. The usage of raw milk may be unsuitable because of:

- Public health concerns
- The presence of unwanted bacteria that can create flaws or flavour and texture variability.

Pasteurisation (72 °C for 15 s) is required for cheese milk. Raw milk is still widely used in the production of cheese in various countries. In the United States, raw milk cheese must be kept at a temperature of 1.7 °C for at least 60 days before being consumed. This law restricts the production and sale of unripened soft cheeses made from raw milk. Fox's research group recently completed a series of tests to better understand the distinctions between raw and pasteurised milk cheeses. Non-starter bacteria were found in larger concentrations in raw milk cheeses than in pasteurised or microfiltered milk cheeses. Pasteurisation is one of four methods for lowering the amount of bacteria in milk:

- Activation of the lactoperoxidase-H_2O_2-thiocyanate system through H_2O_2 treatment
- Microfiltration
- Bactofugation

2.4 Pre-treatment Before Cheesemaking

Hydrogen Peroxide/Catalase Treatment of Milk

Good quality, clean-tasting milk with a low somatic cell count, no antibiotics, and a low microbiological count is the beginning material for good grade cheese. The quantity, quality, and functional properties of cheese are controlled by the composition of milk, which is influenced by the breed of cow, season, stage of lactation, disease, and genetics. Raw milk may be treated with hydrogen peroxide depending on the microbiological quality of the raw milk and its source, as well as to ensure consistent cheese quality (H_2O_2). This technique is not currently employed in hygienic milking circumstances in technologically sophisticated countries. Pat Fox's first research project focused on the effects of H_2O_2 treatment on caseins and the consequences for cheesemaking (Fox & Kosikowski, 1967). Pat Fox demonstrated that treating milk with H_2O_2–catalase increased the utilisation of chymosin and boosted proteolysis in cheese, resulting in a soft cheese. It's usually used at a concentration of 0.07–0.1% for a maximum of 40 min at 50–54 °C (Scott et al., 1998). H_2O_2–catalase treatment killed practically all coliforms in milk, according to Kuchroo and Fox (1982). Catalase's job is to break down the toxic effects of leftover H_2O_2 and the quantity of catalase added.

Bactofugation

The development of the bactofuge has aided in the control of milk quality in areas of the world where milk quality is low bacteriologically. The effect of bactofugation on the regulation of microbiological quality of cheese milk was originally demonstrated by Kuchroo and Fox (1982). Bactofugation of milk reduced bacterial counts by 95.3%, according to the researchers. The bactofuge is a high-speed centrifuge that removes germs and bacterial spores from milk at high temperatures. Bacteria and bacterial spores are reduced by at least three logs after a double bactofuge treatment at 73 °C (Walstra, 1999). Bactofugation reduces the initial volume of milk by 2–3% and the milk protein content by 7% (Scott et al., 1998), resulting in a 6% reduction in cheese yield due to the loss of milk solids in the sludge (Scott et al., 1998) (Walstra, 1999). The sludge is treated with ultrahigh temperature (UHT) for a few seconds at 130–140 °C before being reintroduced back to the cheese milk (Walstra, 1999).

Chemical Treatment

In countries that allow the use of nitrates in cheesemaking, a particular level of nitrate (2.5 g/100 kg milk) is useful to limit spore germination even when milk is subjected to bactofugation treatment (Walstra, 1999). In the United States, nitrates are not permitted in the production of cheese. Lysozyme (100–250 mg/100 mL) can also be given to the milk as an alternative. Lysozyme binds to casein micelles and hydrolyses peptidoglycan bonds of primarily Gram-positive bacteria (e.g. *Clostridium tyrobutyricum*) that produce late-gas blowing when present in cheese kinds like Swiss cheese. Although lysozyme can be found in bovine milk at concentrations of 0–2 mg/L, these levels are insufficient to be effective (Walstra, 1999).

Use of Membrane Technologies

The dairy industry is increasingly using ultrafiltration (UF), reverse osmosis (RO), and microfiltration (MF) to concentrate/fractionate milk for the production of high-moisture cheeses in high yields. Commercially, ultrafiltered retentates are used to make precheese for the production of several soft and semisoft cheeses, as well as to prepare a cheese base for processing (Ernstrom et al., 1980). However, using highly concentrated milks (>5 times the concentration factor) to make semihard and hard cheeses remains a difficulty because the resultant cheese's sensory characteristics differ from those created with regular milk using standard procedures. Cheddar and similar cheeses have been successfully produced using ultrafiltration with a low concentration ratio. To remove germs and bacterial spores from milk, microfiltration using membranes with hole sizes ranging from 0.01 to 10 m is utilised (Scott et al., 1998). Membrane technologies are also used to create novel liquid and dry ingredients for use in cheese milk standardisation. St-Gelais et al. (1998) and Rehman, Farkye, Considine, et al. (2003), Rehman, Farkye, and Drake (2003) both gave examples of employing liquid or dry milk protein concentrate in cheese production.

2.5 Conversion of Milk to Cheese Curd

After the milk has been homogenised, pasteurised, or otherwise processed, it is moved to vats (or kettles) that can be hemispherical, rectangular, vertical, or horizontal cylindrical, and range in size from a few hundred litres to 20,000–40,000 l. It is then processed into cheese curd, which entails three main steps: acidity, coagulation, and dehydration.

Acidification

Acidification is commonly accomplished by producing lactic acid in situ by lactic acid bacteria fermenting lactose, the milk sugar. Initially, the native milk bacteria were depended upon to produce acid, but because this microflora is varied, so is the rate and extent of acidification, resulting in cheese of variable quality. Lactic acid bacteria cultures for cheesemaking were first commercially available some 130 years ago and have since been enhanced and refined. Direct acidification with an acid (typically lactic or HCl) or an acidogen (generally gluconic acid—lactone) can be used as an alternative to biological acidification and is widely utilised in the commercial production of Cottage, Quarg, Feta-type cheeses, and Mozzarella made from UF-concentrated milk. Furthermore, because the starter bacteria play a vital role in cheese ripening in addition to acidification, chemical acidification is utilised only for cheese kinds where texture is more important than flavour. The rate of acidification varies according to the variety, ranging from 5 to 6 h for Cheddar and Cottage to 10–12 h for Dutch and Swiss cheeses. The rate of acidification varies according to the variety, ranging from 5 to 6 h for Cheddar and Cottage to 10–12 h for Dutch and Swiss cheeses. The rate of acidification, which is affected by the

amount and type of starter used as well as the curd's temperature, has a significant impact on the texture of cheese, primarily by solubilising colloidal calcium phosphate; regardless of the rate of acidification, the ultimate pH of the curd for most hard cheese varieties is in the range 5.0–5.3, but it is 4.6 for soft, acid-coagulated varieties such as Cottage, Quarg, and Cream, and some rennet-coagulated varieties, e.g. Camembert and Brie.

Starter

For cheesemaking, a variety of starting cultures are utilised. Starters help to acidify cheese milk to the required pH throughout the manufacturing process. Furthermore, starter bacteria are vital in the maturing and flavour development of cheese. Genetically engineered starters, supplementary starters, and fast-acid starters are already marketed commercially as liquid, frozen, or dried starters. Liquid starters are propagated daily from bulk starter vessels in major commercial operations using internal or external pH-controlled starter material before being inoculated into milk. Whey can also be used as a starting medium. Starters are propagated in sterile milk or reconstituted skim milk powder by smaller, less complex plants. Frozen cultures can be inoculated directly into milk and come in concentrated or unconcentrated forms (also called direct vat cultures). Frozen cultures may indeed come in the form of frozen pellets, which are easier to use. Spray-dried or freeze-dried starters are available. Although immobilised cell technology is possible, it has yet to be commercialised (Tamime, 2002). Fox's starter culture technology research concentrated on the role of starters in cheese flavour development (see Farkye et al., 1990; Law et al., 1992; O'Keeffe et al., 1976), as well as the involvement of indigenous microflora and nonstarter bacteria in flavour development (McSweeney et al., 1993, 1994; Lynch et al., 1996).

Coagulation

The coagulation of the casein component of the milk protein system to form a gel that entraps the fat, if present, is a crucial characteristic stage in the manufacturing of all cheese kinds

- Limited proteolysis by chosen proteinases (rennets);
- Acidification to pH 4.6;
- Acidification to a pH value >4.6 (possibly 5.2) in combination with heating to 90 °C

Rennet coagulation produces the majority of cheese kinds and accounts for around 75% of overall production, but acid-coagulated varieties such as Quarg, Cottage, and Cream are important. Acid-heat-coagulated cheeses are of modest importance and are often made from whey or a blend of whey and skim milk. They are thought to have originated as a means of recovering nutritionally significant whey proteins. They have quite different qualities than rennet- or acid-coagulated

cheeses, and they are commonly employed as food additives. Ricotta and related variants are important varieties.

Coagulant

The main milk-clotting enzyme used in cheesemaking is chymosin. Coagulants come from both animal and microbial sources. Calf chymosin, bovine and porcine pepsins, *Mucor meihei* protease, *Mucor pusillus* protease, *Cryophonectria prasitica* protease, and fermentation-derived chymosins are only a few examples. The micelle-stabilising -casein is hydrolysed by these enzymes, which are known as rennets, at or near the Phe 105–Met 106 link. At around 30 °C, the rennet-altered milk coagulates in the presence of Ca^{2+}. Rennet hydrolyses-casein to produce a hydrophobic para-casein (f1–105) that stays with the curd and a hydrophilic (glyco) macropeptide (f106–169) that is lost in the whey. Chymosin and other milk clotting enzymes have a pH-dependent action, with activity increasing as pH drops. Several enzymes, including bovine, porcine, and ovine pepsins, as well as fermentation-produced chymosin, have been examined by Fox's group for their milk-clotting activity (see Fox, 1969; O'Keeffe et al., 1976; O'Sullivan et al., 1992). Fox's research also revealed that less than 10% of the clotting enzyme employed in milk remains active in cheese and plays a function in the ripening process (O'Keeffe et al., 1978). At the pH of cheesemaking, porcine pepsin becomes unstable and loses its activity. Microbial enzymes *M. meihei* and *M. pusillus* have residual activities that are unaffected by the pH of the milk at the time of setting (Farkye & Yim, 2003).

Post-Coagulation Operations

The curd is heated, or scalded, after coagulation and chopping to remove whey through syneresis. Cooking also aids starter development and acid production. Fox and his colleagues investigated the impact of acidification rate (O'Keeffe et al., 1975) and cooking temperature on the production and quality of Cheddar cheese (Mullan et al., 1981; Wilkinson et al., 1995). If kept in a quiet environment, rennet or acid-coagulated milk gels are relatively stable, but if sliced or fractured, they syneresis and discharge whey. Syneresis reduces the lipid and casein content of milk by a factor of 6–12, depending on the type. Concentration in the dairy business is usually accomplished by thermal evaporation of water, or more recently, by eliminating water through semi-permeable membranes. Syneresis of rennet- or acid-coagulated milk gels is thus a relatively unusual process of dehydration, reliant on the caseins' particular properties. Milk composition, particularly Ca^{2+} and casein concentrations, pH of the whey, cooking temperature, rate of stirring of the curd-whey mixture, and, of course, time all influence the rate and amount of syneresis. The extent of syneresis determines the finished cheese's composition, and because this is under the control of the cheesemaker, it is here that the differentiation of individual cheese varieties really begins, though the type and concentration of the milk, the amount and type of starter, and the amount and type of rennet are also significant.

2.6 Removal of Whey, Moulding, and Pressing of the Curd

When the desired degree of syneresis and, in some cases, the desired pH have been achieved, the curds are separated from the whey using a variety-specific method, such as transferring the curds-whey into perforated moulds (common for soft varieties, e.g. Camembert), allowing the curds to settle in the vat and sucking off the supernatant whey (e.g. Gouda and Emmental, Cheddar and Pizza cheese).Many cheeses are created in conventional forms and sizes, such as small fl at cylinders (e.g. Brie and Camembert), taller cylinders (e.g. Cheddar and Parmesan), huge low cylinders (e.g. Emmental), spheres, and so on (Edam). Traditional shapes have been abandoned in some cases; for example, Cheddar and Emmental are now routinely manufactured as rectangular or square blocks.

The size and form of a cheese may be cosmetic and traditional in certain circumstances, but the size of a cheese has significant implications for the ripening of many varieties. Surface-ripened cultivars, such as Camembert, must be small since the surface microflora is important for ripening but only works over a short distance. For cultivars that produce eyes owing to propionic acid fermentation, such as Emmental, which must have a tight texture and be large enough to retain enough CO_2 for eye development, the opposite is required. During the maturing of an 80 kg Emmental cheese, 120 l of CO_2 are produced, 60 l remain dissolved in the cheese body, 40 l diffuse out of the cheese, and 20 l are in the eyes; too much CO_2 is lost from a small or open cheese, and eye formation is poor or absent.

Curds for high-moisture cheeses congeal under their own weight, whereas curds for medium- and particularly low-moisture cheeses must be pressed to form a well-matted body, for example, Cheddar cheese is pressed at 2.7 kPa. Pressing eliminates some whey in addition to consolidating the curd mass; for example, 1.3% of the volume of milk used in Cheddar cheese is in the press whey. Certain varieties of curds or pressed cheese curds are treated to specific treatments in order to achieve a specific texture or physico-chemical quality, or to promote the growth of specific microorganisms. Cheddar, Pasta Filata, washed-curd variants, and blue cheeses are examples of such types.

2.7 Salting

The final step in the production process is salting. Salt is used in cheese to reduce or stop the action of the starting bacteria, as well as to improve the flavour. During cheese ripening, salt content has an impact on enzyme activity. The salt concentration of cheese is best stated as salt-in-moisture (S/M), which determines how long starter activity lasts after it has been salted. S/M levels in Cheddar-type cheeses are typically 4–6%; above this level, most starter bacterium activity is blocked. The method utilised widely in industry is based on Fox's pioneering research report on salt determination in cheese (Fox, 1969). His group has also looked on salt diffusion in dry-salted (such as Cheddar; Morris et al., 1985) and brine-salted cheeses (e.g. Romano; Guinee & Fox, 1986).

2.8 Ripening

Glycolysis, lipolysis, and proteolysis are three biochemical and metabolic processes that contribute to cheese ripening. The relative importance of each process varies according to the type of cheese. Fox has made a significant contribution to our understanding of and progress in the field of cheese ripening. He has studied the role of coagulant, native milk enzymes (such as plasmin), starter, and nonstarter microorganisms in proteolysis during cheese ripening. Proteolysis is the most complicated process, with varying degrees of complexity depending on the variety—from little in mozzarella to considerable in blue mould variants.

The following are the general reaction steps:

- Initial hydrolysis of caseins by residual coagulant and plasmin to large peptides
- Breakdown of large peptides by starter proteinases and peptidases into medium and small peptides
- Further hydrolysis of medium and small peptides by starter peptidases into dipeptides, tripeptides, and free amino acids

While the amount of residual coagulant in cheese is determined by the pH of the milk at the time of setting (Farkye & Yim, 2003), plasmin activity is determined by the cooking temperature used during manufacturing and is higher in high-cook cheeses (such as Swiss) than in low-cook cheeses (e.g. Cheddar; Farkye et al., 1990). The peptides formed by residual coagulant and plasmin action are frequently bland or bitter, and they do not contribute directly to the flavour of cheese. The combination of tiny peptides and amino acids, on the other hand, has a direct impact on the taste and mouthfeel of cheese (Law et al., 1992). The free amino acids can also be catabolised into flavour compounds that are specific to each cheese and are dependent on the enzymes and microorganisms (especially nonstarter lactic acid bacteria, NSLAB) present. The relevance and significance of NSLAB to cheese ripening have been extensively studied (Gobbetti et al., 1999; McSweeney et al., 1993; Rehman, Farkye, Considine, et al., 2003; Rehman, Farkye, & Drake, 2003).

The water-soluble fraction of cheese contains the highest concentration of flavour components. As a result, in cheese research and technology, methods for researching proteolysis, fractionation, and defining water-soluble nitrogen (WSN) are critical. Cheese proteolysis is studied using methods such as electrophoretic examination (Shalabi & Fox, 1987), extraction, separation, and quantification of WSN (Kuchroo & Fox, 1982), and detection of free amino acid concentration using the cadmium–ninhydrin reagent (Folkertsma & Fox, 1992). Some mesophilic *Lactobacillus* spp., especially *Lb. casei* and *Lb. paracasei*, and maybe *Pediococcus* and *Micrococcus*, may cause secondary microflora to emerge from the indigenous microflora of milk that survive pasteurisation or obtain entrance to the milk following pasteurisation. Citrate-positive *Lactococcus* or *Leuconostoc* spp. in Dutch-type cheese, Propionibacterium in Swiss cheese, *Penicillium roqueforti* in Blue varieties, *P. camemberti* in Camembert or Brie, or *Brevibacterium linens* in surface smear-ripened varieties, e.g. Tilsit and Limburger, may also be added as a secondary starter.

The metabolic activity of these secondary bacteria often determines the qualities of the finished cheese. Secondary catabolic changes to the compounds produced in these primary pathways include deamination, decarboxylation, and desulphurisation of amino acids, oxidation of fatty acids, catabolism of lactic acid, and even some synthetic reactions, such as esterification.

Accelerated Ripening of Cheese

Ripening cheese is a costly operation. Fedrick (1987) estimated interest charges for ripening cheese in Australia of around $40/ton/month. As a result, methods that shorten the time and cost of keeping and maturing cheese until it is sold are important to both the dairy industry and cheese consumers. Exogenous enzymes, starter adjuncts, attenuated starters, genetically engineered starters, high pressure processing, and enhanced ripening temperature are just a few of the options for speeding up the ripening of cheese.

Guinee et al. provide an example of Fox (1969) contribution in this area. Accelerated ripening procedures can also be utilised to make enzyme-modified cheeses for use in processed cheese or as ingredients in goods that require a strong cheese flavour.

2.9 Processed Cheese Products

A variable amount of mature cheese is consumed as such, depending on culinary traditions, and is often referred to as "table cheese." Natural cheese is used in a large number of other meals, such as Parmesan or Grana on pasta, Mozzarella on pizza, Quarg in cheesecake, and Ricotta in ravioli. A third important use for cheese is the manufacture of a wide range of processed cheese products, which can be used in a variety of ways, such as spreads, sandwich fillers, and meal additives.

S. No	Cheese type	Characteristics	Fat content	Examples
1.	Fresh cheese	No rind	19–24%	Ricotta Mozzarella Cottage cheese
2.	Aged-fresh cheese	Wrinkled white to grey blue rind	Typically 24%	Ricotta Mozzarella Cottage cheese
3.	Soft white rind cheese	White fuzzy rind	Typically 24–26% Sometimes, 45%	Camembert Brie de Meaux Chaoure Cherre log
4.	Semisoft cheese	Fine to thick grey-brown rind or orange and sticky	26–28%	Edam Reblochon Port Salut Raclette St. Nectaire

(continued)

S. No	Cheese type	Characteristics	Fat content	Examples
5.	Hard cheese	Crusty, grey-often polished, waxed or oiled	28–34%	Cheddar, Pecorino, Beaufort, Manchego, Gruyere, Parmesan
6.	Blue cheese	Gritty, rough, sometimes sticky rind	28–34%	Stilton Gorgonzola Roquefort Picos de Europa
7.	Flavour-added cheese	Hard cheeses like Gouda, White Stilton, Wensleydale or Cheddar to which a variety of flavours like nuts, fruit, or herbs are added	28–34%	Gouda with cumin, Lancashire with chives, pecorino with truffles

2.10 Whey and Whey Products

Only about half of the solids in milk are absorbed into cheese; the rest (90% of lactose, 20% of protein, and 10% of fat) is found in the whey. Until recently, whey was thought to be a largely useless by-product that should be discarded as inexpensively as possible. However, whey processing has become an important feature of the whole cheese industry, not only in the purpose of decreasing environmental pollution, but also because it is now possible to manufacture valuable food products from whey.

Fat, which is normally contained at 0.3% (w/w) in bulk cheese whey, is recovered from clarified whey using a centrifugal separator to a level of 0.07% (w/w). The resulting whey cream (50% fat) is usually used to make whey butter, which is utilised as a food ingredient in processed cheese products, for example. The clarified whey can be used as a foundation for beverages that have a good amino acid profile and are blood isotonic. They're frequently flavoured with fresh or concentrated fruit juices, and they can even be fermented to make whey wine. Such items are available, albeit only on a small scale. Non-hygroscopic whey powders, demineralised whey powder, delactosed, and delactosed/demineralised whey powders are among the processed and dried whey products.

3 Cost Analysis for Cheese Production

Over the last decade, the number of artisan dairy processors has increased dramatically. Since 1999, the number of artisan dairy enterprises in Oregon has increased from three to twenty-six (Fig. 1). The lack of solid economic data is one of the major factors restricting new company growth. The absence of readily available information on start-up and manufacturing expenses is a hurdle to entrepreneurs who start these businesses. This makes it more difficult to establish convincing company strategies and secure financing. The limited studies that are available focus on

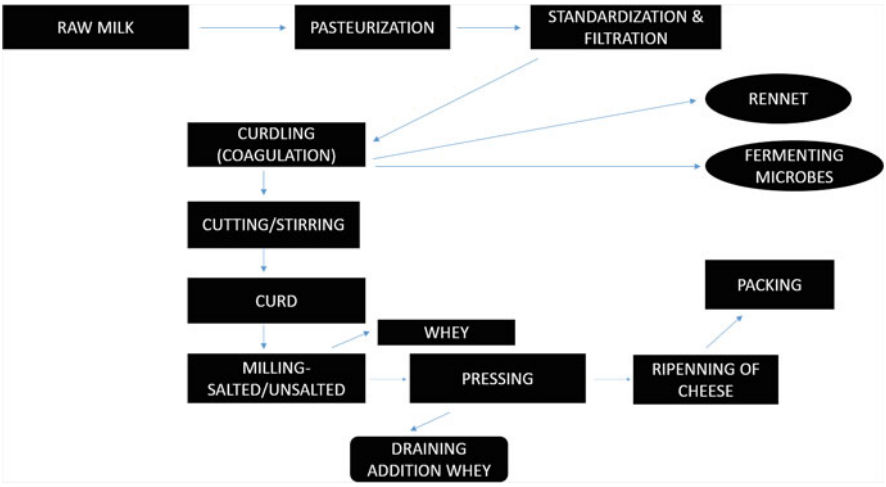

Fig. 1 Cheese making

large-scale commodity milk processing (Erba et al., 1997), economic feasibility of small commodity cheese processing facilities (Becker et al., 2007), and artisan company start-up without mentioning financial factors (Reed et al., 2011).

3.1 Cost of Capital and Discount Rate

All financial analyses were evaluated using a ten percent discount rate. This was found by assessing the risks involved in starting a speciality food business. Debt financing was shown to have the lowest cost of capital, at 6%, with an additional risk of 4% added for conservative calculations.

3.2 Investing in the Beginning

Estimates for initial capital investment were calculated and reported as monthly loan payments. As an output, the model calculates the initial capital required for a down payment on the loan, 20% of the principal, and additional capital required to operate the business during negative cash flow years. The model calculated the size of the facility, essential equipment, and ageing room capacity needed to accommodate the total cheese produced at the end of the research period (15 years).

3.3 Equipment

The survey of artisan cheese producers indicated what equipment was required, and price was based on bids from various equipment manufacturers as well as a dairy

equipment installation specialist (H. Schuller, C. van't Riet Dairy Technology, DuBois, PA; personal communication).

3.4 Processing Facility

Based on the survey results, the required facility size was estimated. The size of the facility (excluding the ageing room and other tasting and sales spaces) is compared to total annual cheese output. The total yearly cheese production data collected ranged from around 1361 kg (3000 lb) to 36,287 kg (80,000 lb). The relationship between the area required for the facility and the intended capacity was nonlinear, as seen by the data points, hence several nonlinear relationships were evaluated. The double (natural) log format, in which the y and x variables are logged before being regressed using ordinary least squares, provided the best fit for these data; thus, log Ap (area of processing facility) is regressed on the log of production capacity.

3.5 Aging Facility

Specialised costs, such as those associated with air manipulation, are associated with ageing rooms (to control humidity, flow, and temperature). The ageing facility's capacity is dependent on cheese styles and production levels, hence it was calculated individually in the model. Due to the longer ripening period and inventory storage requirements, harder cheeses require more ageing space. Fresh cheeses, on the other hand, may simply require short-term cold storage rather than a maturing facility.

3.6 Operating Expenses

The results of the Operating Expenses Survey revealed the most significant costs associated with running an artisan cheese business.

3.7 Labour

Managerial (Lm), retail (Lr), and production labour were all calculated (Lp). Hourly salaries of $20.00 and $10.00/h were used to estimate Lm and Lr, respectively. Production work was a salaried full-time job that paid $45,000 a year. The owner/labour, operator's as well as all other work required for production, was allotted. It was believed that the owner would spend 10 h/week in management positions, 20 h in production, and 10 h in retail sales. Because the business enterprise is considered to be classed as a sole proprietorship, the total owner labour cost was then accounted for and subtracted as an expense after business taxes were determined (Tables 1 and 2).

Table 1 Variables and values used as a model for production cost analysis in cheese production

Variable	Value used
Raw milk price	$0.47/L ($1.78/gal) for cow
Labour	Manager: $20.00/h Cheese maker: $45,000/year Retail worker: $10.00/h
Owner/operator labour per week	Manager duties: 10 h Production: 20 h Retail sales: 10 h
Cost of gasoline	$0.92/L ($3.50/gal)
Creamery location	Suburban
Production during 3 years after scale-up	Scenario 1: 3402 kg/year (7500 lb/year) Scenario 2: 6804 kg/year (15,000 lb/year) Scenario 3: 13,608 kg/year (30,000 lb/year) Scenario 4: 27,216 kg/year (60,000 lb/year)
Cheese production days per year	Scenario 1: 52 Scenario 2: 104 Scenario3: 156 Scenario 4: 156
Business loan parameters	6% APR, 5 years
Average miles driven to farmers' markets	97 km (60 miles)
Average miles driven each week to deliver product for wholesale (e.g. restaurants and local grocers)	320 km (200 miles)
Maximum days at farmers' markets producer would realistically be able to attend per year	156 (3 days/week year round)
Maximum number of retailer outlets available for wholesales	15
Product attributes: Cheddar	Yield: 10% Ageing: 150 days

Table 2 Start-up costs ($) for 1 operating costs ($) for facility producing Cheddar cheese aged for 150 days production in kg

Cost	3402	6804	13,608	27,216
Facility	128,700	168,200	219,900	287,400
Ageing room	30,400	36,700	49,300	74,500
Equipment	108,148	108,148	112,578	261,974
Year 1 production costs	65,245	107,411	324,315	620,094
Total	332,493	420,459	706,093	1,243,968

4 Marketing of Cheese

Cheese has been a staple in many people's diets around the world. According to the United States Department of Agriculture, the country produced 12.7 billion pounds of cheese in 2017, and the average American consumes 30 pounds of cheese each year. Without a doubt, the cheese sector is booming, as its products are used in practically every cuisine on the planet. According to Industry ARC analysts, the worldwide cheese market size in 2018 was predicted to be between $65 billion and $68 billion. The market is expected to develop at a standard CAGR of 2.5–3.5% over the projected period of 2019–2025. Due to the diversity of cheese produced by 28 different countries in the European Union, Europe leads the cheese industry with a regional share of 38–40% as of 2018. Cheese is a staple of European cuisine, and it's used in almost every meal in most homes. The cheese industry was worth $156.9 billion in 2020 and is expected to grow to $199.7 billion by 2030, with a CAGR of 2.3% between 2021 and 2030.

4.1 Growth Factors in the Cheese Industry

The Ascension of Fast Food Corporations

Fast food establishments such as McDonald's and Domino's Pizza have successfully created a global pizza and burger eating culture. Both items are laced with cheese and are sold at subsidised prices, attracting customers to the dishes. The achievement of the Asian market, where cheese was essentially non-existent, is the most significant milestone in the evolution of the cheese market. Since fast food chain restaurants took over the Chinese people's taste buds, for example, China has seen a surge in cheese demand. Chinese producers are now finding it difficult to keep up with the rising demand and are heavily exporting cheese. The Chinese government imported roughly 90,000 tons of cheese in 2017, with New Zealand leading the way with 51 million kg.

Increased Disposable Income as a Result of Urbanisation

As the world moves closer to an exponential expansion in urbanisation, it is clear that households with discretionary money are multiplying. This leads to more frequent trips to the shop and a higher consumption of foreign foods. People are cooking at home using foods like cheese, which are touted as a healthy source of protein and fats, as a result of a recent fitness wave that has already engulfed the first world countries. Cheese is an important part of the ketogenic diet, which has gained in popularity and spreads the benefits of cheese consumption.

Cheese Market Obstacles

Cheese has a short shelf life, therefore it's best to eat it as soon as possible. Cheese wastage is a constant result of rising demand and supply, as well as its short shelf life. Consumers are preferring unique flavours over conventional cheeses as more flavours reach our supermarkets. As a result, conventional cheese sales and

consumption are steadily declining. Around 1.4 billion pounds of American cheese, such as Cheddar, are sitting in warehouses in the United States, waiting to be consumed. Only by inducing innovation in the cheese market and altering the quality of their products in response to consumer needs can this challenge be overcome.

4.2 Key Players in the Cheese Market

Parag Milk Foods, an Indian dairy company, launched Go Cheese as an indigenous brand. Go Cheese owns and operates India's largest cheese production plant, which is located in Manchar, a small district in Maharashtra. Cheese Market, according to the corporation, has expanded its reach by providing not only hotels and restaurants, but also street food sellers. Almarai Co. Ltd., Sargento Foods Incorporated, Friesland Campina, Dzintars, Go Cheese, Parag Milk Foods, GebrderWoerleGes. m.b., Mother Dairy, Old Fashioned Cheese, Vindija DD, and Bletsoe Cheese, Inc. are the top firms in the worldwide cheese market, according to the analyst.

4.3 Market Trends in Cheese

Vegan Cheese

Veganism, which is expected to be the most popular food trend in 2018, is motivating people to create alternative, healthier goods that taste just like the real thing but are less harmful to the environment. Vegan cheese is now widely available around the world, as the cheese market has caught up with the trend. Veganism has spawned an obsession with plant-based diets that can easily include vegan cheese. The handcrafted charm and organic nature of this product are driving demand.

Expanding Tastes

With globalisation at its pinnacle in the twenty-first century, we live in fascinating times where we may easily be exposed to cuisines from around the world. The cheese business has taken advantage of this characteristic, with producers combining cheese with a variety of odd flavours. Anand Milk Union Limited (Amul), an Indian dairy corporation, has coupled its cheese spreads with flavours such as red chilli pepper flakes, tikka dip, oregano pickle, among others. Consumers are drawn to innovative products like this, which help the cheese market thrive.

The cheese market study also examines the market's primary geographical areas as well as the major nations within these regions. The study includes the following areas and countries:

- North America: The U.S., Canada, Mexico
- South America: Brazil, Venezuela, Argentina, Ecuador, Peru, Colombia, Costa Rica
- Europe: The U.K., Germany, Italy, France, The Netherlands, Belgium, Spain, Denmark

- APAC: China, Japan, Australia, South Korea, India, Taiwan, Malaysia, Hong Kong
- Middle East and Africa: Israel, South Africa, Saudi Arabia

4.4 Cheese Sale Promotions

The steps to cheese promotions that work:

1. Plan early: Plan promotions months in advance. Promotions are scheduled at least three months in advance, and more frequently four to five months. Staffers can line up merchandise, make agreements, and plan signage photographs with the extra time. Merchandisers construct a model display, photograph it, and transmit it to all stores two months ahead of time so managers know how to set up their sections.
2. Choose a theme: Keep things basic. Many companies associate promotions with holidays, countries, or regions, which is a simple notion for buyers to comprehend. Choose a symbol to use on signage and banners to visually link featured products, such as the Italian flag or the Super Bowl logo. Posters and photographs can be provided by tourism promotion boards to emphasise a country's concept. To make counter employees feel like they're a part of the promotion, give them distinctive aprons or pins to wear on their uniforms.
3. Select featured items strategically: Whole Foods aims for a mix of familiar, popular cheeses that customers will be happy to find at a discount, as well as more obscure options that excite the staff, in its monthly promotions.
4. Make signs that are both appealing and educational: Use signage to tell customers something about the cheese they didn't know. Make a suggestion for a use, a recipe, or a cross-merchandising opportunity. Colour photos can suggest how a product should be served or what it might be paired with. Use iconography and signage to link specific wines or sauces to featured cheeses in other sections, such as wine.
5. Think big: Clients respond positively to plenty because it implies that the retailer believes in and has invested in the goods. Whole Foods runs its promos for a month, according to Bair, so the retailer can buy huge supplies for stunning displays.
6. Cheeses were thoughtfully featured by price: A promotion indicates that the things on display are discounted, however businesses claim that discounts aren't always required.
7. Offer active sampling with knowledgeable staff: Early on, involve the counter personnel. Explain the promotion, introduce the cheeses, and provide talking points, literature, usage suggestions, and tales so they understand what makes each cheese unique and can engage customers in conversation. Invite vendors to speak with your team.

8. Get excited: Enthusiasm is contagious. During the campaign, hold a sales contest to motivate your counter personnel. If you have more than one store, give the one with the finest display a prize.
9. Cross-merchandise: Make use of your theme to create sales for related products.
10. Above all, specialist cheesemakers provide a high-quality, one-of-a-kind product; your customers will recognise this and be willing to pay a premium for it. However, it is critical that your cheese represents good value for money, so think about what your competitors charge for their products and what supermarkets price for any specialty cheeses they provide.

5 Conclusion

Because of the expanded variety of cheese and intense flavours, the cheese business has seen great development in sales and consumption. Cheese is also a healthful and versatile food that may be eaten as a snack or used as a component in other dishes or cuisines. Pat Fox's work has aided in a better understanding of the science and technology of cheesemaking, as well as the biochemistry and microbiology of ripening, in order to ensure high-quality, consistent cheese. Thus the above chapter shows the global demand for cheese and its business strategies.

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Production Cost Analysis and Marketing of Fermented Foods: Yoghurt

Ami Naik

Abstract

Yogurt, often known as yoghurt, is one of the most popular fermented dairy products in the world, with a wide range of health advantages in addition to basic nutrition. Consumer demand for yogurt and yogurt-related products has surged as a result of these well-known health benefits, and it has become the fastest-growing dairy category in the world. Yogurts are currently available in a variety of styles and variations, each with its own fat content, flavor profile, and texture, making them suited for a variety of meal settings and plates as a snack, dessert, sweet, or savory dish. This chapter describes various constituents and their composition for use in the production of yoghurt. The quality of milk and their characteristics for yoghurt manufacture are briefly discussed. Several steps required for formulation and processing of popular styles of yogurt are described. A brief description of various types of yogurt is also given. The primary objective of this chapter is to give insight knowledge about the production of yogurt; to discuss the main ingredients, main unit operation, and equipment's used in yogurt production. This will facilitate the entrepreneurs in understanding the importance of setting up unit of yoghurt plant. This content will serve as guidance to the entrepreneurs on starting up such a new project and basic technical knowledge for setting up such a facility.

Keywords

Yoghurt · Dairy product · Milk · Production · Entrepreneurs

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1 Introduction

Yoghurt is one of the most popular fermented dairy products for its wide variety of flavors and fruits that are added to it. Different forms of yoghurt are available in the market like stirred, set, frozen, and liquid yoghurt. Yoghurt is a low fat, calcium-rich food with "active cultures" (Lee & Lucey, 2010). Yoghurt can pump up your immunity, regulate your digestive health, nourish your memory, lower your cholesterol, maintain your muscles, boost your fiber intake, strengthen your bones, help you lose weight, and make you happy (Meydani & Ha, 2000). Yoghurt has become prune juice, bran, and chicken soup, all rolled into one.

Yoghurt is a fermented milk product that contains the characteristic bacterial cultures *Lactobacillus bulgaricus and Streptococcus thermophilus*. All yoghurts must contain at least 8.25% solids not fat. Full fat yoghurt must contain not less than 3.25% milk fat, low fat yoghurt not more than 2% milk fat, and nonfat yoghurt less than 0.5% milk (Tamime & Deeth, 1980).

Yoghurt has its origins in Eastern Europe, but is now consumed throughout the world. The French called it the milk of eternity, as it was believed to have therapeutic powers and give long life to those who consumed it. France is the leading consumer of yoghurt in Europe, followed by Ireland, with the average person consuming 21.3 kg and 13.2 kg/annum, respectively. The yoghurt market has been growing steadily in recent years, benefitting from yoghurt's image as both a healthy alternative for snacking or meals, and also as a treat or dessert. Health and wellness trends have driven the growth of naturally lower-sugar varieties such as Greek yoghurt, fat-free yoghurts, high-protein products, as well as increased offerings in cholesterol-reducing and added-nutritional value yoghurts. The demand for dairy-free and plant-based products is also growing, and this demand extends beyond traditional vegetarian/vegan consumers. High-value growth has been observed in sour milk products, largely due to growing demand for kefir (a fermented milk drink), a trend noted in markets other than Ireland as well (Kroger, 1976).

2 Yoghurt as Functional Food

Current dairy product intake recommendations vary from region to region (Lourens-Hattingh & Viljoen, 2001). Some countries provide general recommendations to consume milk and other dairy products daily, but most countries have quantitative recommendations that usually range from 2 to 3 servings of milk, yoghurt, or cheese (Larsson et al., 2008). It has been reported that people who avoid dairy products have difficulty achieving the recommended intakes of some nutrients, and consuming the recommended portions substantially improves the population's average intake of certain vitamins and minerals that are otherwise underconsumed (Quann et al., 2015).

Fermented foods and beverages such as yoghurt have been a part of the human diet for thousands of years. With supplementation of probiotic microbes, in some cases, they offer nutritional and health attributes that suggest they should be

recommended for regular consumption. Despite the importance of fermented foods and dairy products for both general and gastrointestinal well-being and disease risk reduction, nutritionally based recommendations on their consumption and their many benefits have not been included in food-based dietary guidelines (Gómez-Gallego et al., 2018). Yoghurt has long been consumed in many regions and cultures, but the pattern of consumption varies greatly from country to country.

3 Varieties of Yoghurt (Chandan & O'Rell, 2006)

3.1 "Traditional" Yoghurt

Yoghurt is created and thickened through adding cultures to milk, creating that familiar tangy, sour flavor. Milk is heated to prevent curds from forming, cooled, and then has the bacterial culture mixed in. Most yoghurts have live and active cultures that promote gut health. The traditional yoghurt Americans are used to has a smooth, creamy texture (Baspinar & Güldaş, 2021). It is unstrained, so it's not as thick as other yoghurt options, but it's still too thick to drink.

3.2 Greek Yoghurt

Once the yoghurt-base is created, Greek yoghurt is strained to remove the liquid and whey, creating a thicker consistency than traditional yoghurt (Malik et al., 2021). Greek yoghurt has a strong tangy flavor, which makes it a popular substitution for sauces used in savory dishes.

3.3 Australian Yoghurt

Like traditional yoghurt, Australian yoghurt is unstrained (Chandan & O'Rell, 2006). However, it's cooked slower and longer than regular yoghurt, creating a creamier texture that is somewhere between traditional and Greek yoghurt in thickness.

3.4 French Yoghurt

Instead of being made in a large vat and then divided into containers, French-style yoghurt is cultured in the individual-sized containers it is sold in (Chandan & O'Rell, 2006). It is unstrained like traditional yoghurt, but it has a smooth, creamy texture and is not as sweet as other yoghurts.

3.5 Skyr/Icelandic Yoghurt

Icelandic yoghurt or Skyr is mildly tangy and noticeably thicker than Greek yoghurt (Chandan & O'Rell, 2006). There is a debate on whether Skyr should be considered a variety of yoghurt. Because the process allows curds to form, it can be argued that it's a cheese. Whatever Skyr technically is, it is consumed like a yoghurt, and so it is marketed as Icelandic yoghurt to Americans. Skyr is strained 4 times, creating one of the thickest consistencies of yoghurt available.

3.6 Lactose-Free Yoghurt

Though yoghurt with live, active cultures is already a good choice for those with a lactose sensitivity, there is yoghurt available with no lactose (Skryplonek et al., 2017). Lactose-free yoghurt has been treated with a special enzyme that breaks down the lactose found in milk. Without this lactose, your body doesn't experience the discomfort of lactose intolerance. It tastes like traditional yoghurt because it is; the lactose has just been broken down before it enters your body.

3.7 Drinkable Yoghurt

Drinkable yoghurts come in a variety of options, and we're not only talking about flavors. They have a consistency for everyone, ranging from a thinner consistency like skim milk to thicker consistencies closer to traditional yoghurt. You can also choose if you want to sip on a yoghurt that is tart or sweet (Allgeyer et al., 2010).

3.8 Kefir

Kefir is a similar fermented drink to drinkable yoghurt, but with a longer fermentation process (Ahmed et al., 2013). What makes kefir unique is the kefir grains blended into the milk. The taste is slightly tart and you'll notice an almost bubbly quality to it.

4 Ingredients

The main ingredient in yoghurt is milk. The type of milk used depends on the type of yoghurt—whole milk for full fat yoghurt, low-fat milk for low-fat yoghurt, and skim milk for nonfat yoghurt (Chandan & O'Rell, 2006). Other dairy ingredients are allowed in yoghurt to adjust the composition, such as cream to adjust the fat content and nonfat dry milk to adjust the solids content. The solids content of yoghurt is often adjusted above the 8.25% minimum to provide a better body and texture to the

finished yoghurt. The CFR contains a list of the permissible dairy ingredients for yoghurt (Lagrange et al., 2015).

Stabilizers may also be used in yoghurt to improve the body and texture by increasing firmness, preventing separation of the whey (syneresis), and helping to keep the fruit uniformly mixed in the yoghurt. Stabilizers used in yoghurt are alginates (carrageenan), gelatins, gums (locust bean, guar), pectins, and starch (Chandan, 2017).

Sweeteners, flavors, and fruit preparations are used in yoghurt to provide variety to the consumer (McGregor & White, 1986). A list of permissible sweeteners for yoghurt is found in the CFR (code of federal regulations) (Kilara, 2017).

5 Bacterial Cultures

The main (starter) cultures in yoghurt are *Lactobacillus bulgaricus* and *Streptococcus thermophiles* (Nagaoka, 2019). The function of the starter cultures is to ferment lactose (milk sugar) to produce lactic acid. The increase in lactic acid decreases pH and causes the milk to clot, or form the soft gel that is characteristic of yoghurt. The fermentation of lactose also produces the flavor compounds that are characteristic of yoghurt. *Lactobacillus bulgaricus* and *Streptococcus thermophilus* are the only 2 cultures required by law (CFR) to be present in yoghurt (Adolfsson et al., 2004).

Other bacterial cultures, such as *Lactobacillus acidophilus*, *Lactobacillus subsp. casei*, and *Bifido*-bacteria, may be added to yoghurt as probiotic cultures. Probiotic cultures benefit human health by improving lactose digestion, gastrointestinal function, and stimulating the immune system (Mazahreh & Ershidat, 2009).

The starter cultures used for most yoghurt production are a mixture of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* (Wasilewska et al., 2019). Although they can grow independently, they are symbiotic and the growth rate and acid production are better if they are grown together. These bacteria are ultimately responsible for the flavor and aroma of the final product. To achieve this, they produce a number of products, such as: n lactic acid; n acetic acid; n diacetyl; n acetoin; and, penetration in the Indian market. As a part of their strategy, makers have chosen to either slash n acetaldehyde.

6 Market Opportunities

Milk is one of the essential items of daily life in our country and it is more so as the majority of Indians are vegetarians and thus milk products are an indispensable necessity. Frozen yoghurt players have up their ante to increase price points, offer smaller affordable portions, or value meals for families, while global players have come up with flavors that entice Indian taste bud. Indian brand Cocoberry, one of the earlier entrants in the frozen yoghurt space in the country back in 2009, has given an advantage compared to the newer entrants; it has managed to generate significant volumes already. Yoghurt market in India is still at a nascent stage, and as more and

more companies enter the space, the market will expand exponentially in the coming years (Sharma, 2005).

7 General Manufacturing Procedure

The following flow chart and discussion provide a general outline of the steps required for making yoghurt. For a more detailed explanation, see the literature references (Temesgen & Yetneberk, 2015).

The production of yoghurt is a relatively simple procedure (Soukoulis et al., 2007). Milk, which can be fortified with milk powder to give a thicker product, is pasteurized, cooled, and inoculated with starter bacteria, which ferment the milk, causing it to clot or gel. The differentiation in yoghurt formats is due to changes in the production method.

For example, set yoghurt is packed immediately after inoculation and is incubated in the package. Stirred yoghurt is produced by adding fruit and other condiments after setting, followed by a gentle stirring motion. Drinking yoghurt is produced by adding fruit juice and other flavoring compounds to a thick liquid using high speed mixers. Frozen yoghurt is set yoghurt, combined with sugar and stabilizers before being pasteurized and frozen (Inoue et al., 1998).

7.1 General Yoghurt Processing Steps

Adjust Milk Composition and Blend Ingredients

Milk is required as the major raw material for the manufacturing of curd. Average raw material (cost per Liter): Rs. 50–55. Skimmed milk powder is usually added to improve solids content and the texture of the final product. Milk composition may be adjusted to achieve the desired fat and solids content. Often dry milk is added to increase the amount of whey protein to provide a desirable texture. Ingredients such as stabilizers are added at this time.

Modifying Milk Composition

When the milk arrives at the plant, its composition is modified before it is used to make yoghurt. This standardization process typically involves reducing the fat content and increasing the total solids. The fat content is reduced by using a standardizing clarifier and a separator (a device that relies upon centrifugation to separate fat from milk). From the clarifier, the milk is placed in a storage tank and tested for fat and solids content. For yoghurt manufacture, the solids content of the milk is increased to 16% with 1–5% being fat and 11–14% being solids-not-fat (SNF). This is accomplished either by evaporating off some of the water, or adding concentrated milk or milk powder. Increasing the solids content improves the nutritional value of the yoghurt, makes it easier to produce a firmer yoghurt, and improves the stability of the milk substance that is fermented until it becomes yoghurt. Fruits and flavorings are added to the yoghurt before packaging.

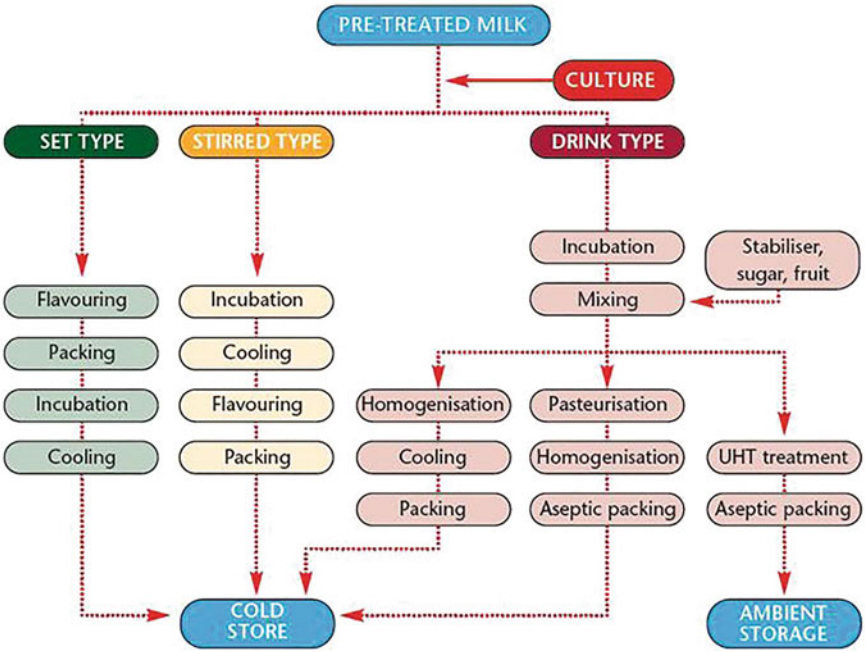

Fig. 1 General yoghurt processing steps

The milk substance is fermented until it becomes yoghurt. Fruits and flavorings are added to the yoghurt before packaging (Fig. 1).

Pasteurize Milk

Milk is heated to 90 °C for five minutes, which denatures the protein in it. This helps to increase viscosity and improve mouth feel (texture). The milk mixture is pasteurized at 185 °F (85 °C) for 30 min or at 203 °F (95 °C) for 10 min. A high heat treatment is used to denature the whey (serum) proteins. This allows the proteins to form a more stable gel, which prevents separation of the water during storage. The high heat treatment also further reduces the number of spoilage organisms in the milk to provide a better environment for the starter cultures to grow. Yoghurt is pasteurized before the starter cultures are added to ensure that the cultures remain active in the yoghurt after fermentation to act as probiotics; if the yoghurt is pasteurized after fermentation, the cultures will be inactivated.

After the solids composition is adjusted, stabilizers are added and the milk is pasteurized. This step has many benefits. First, it will destroy all the microorganisms in the milk that may interfere with the controlled fermentation process. Second, it will denature the whey proteins in the milk which will give the final yoghurt product better body and texture. Third, it will not greatly alter the flavor of the milk. Finally, it helps release the compounds in milk that will stimulate the growth of the starter culture. Pasteurization can be a continuous-or batch-process. Both of these processes

involve heating the milk to a relatively high temperature and holding it there for a set amount of time. One specific method for batch process pasteurization is to heat a large, stainless steel vat of milk to 185 °F (85 °C) and hold it there for at least 30 min.

Homogenize

This step disperses any fat present and helps to dissolve milk powders, while improving mouth feel. The blend is homogenized (2000–2500 psi) to mix all ingredients thoroughly and improve yoghurt consistency. While the milk is being heat-treated, it is also homogenized. Homogenization is a process in which the fat globules in milk are broken up into smaller, more consistently dispersed particles. This produces a much smoother and creamier end product. In commercial yoghurt making, homogenization has the benefits of giving a uniform product, which will not separate. Homogenization is accomplished using a homogenizer or viscolizer. In this machine, the milk is forced through small openings at a high pressure and fat globules are broken up due to shearing forces.

Fermentation

Then pasteurization and homogenization are complete, the milk is cooled to between 109.4–114.8 °F (43–46 °C) and the fermentation culture is added in a concentration of about 2%. It is held at this temperature for about 3–4 h while the incubation process takes place. During this time, the bacteria metabolize certain compounds in the milk producing the characteristic yoghurt flavor. An important by-product of this process is lactic acid.

Depending on the type of yoghurt, the incubation process is done either in a large tank of several hundred gallons or in the final individual containers. Stirred yoghurt is fermented in bulk and then poured into the final selling containers. Set yoghurt, also known as French style, is allowed to ferment right in the container it is sold in. In both instances, the lactic acid level is used to determine when the yoghurt is ready. The acid level is found by taking a sample of the product and titrating it with sodium hydroxide. A value of at least 0.9% acidity and a pH of about 4.4 are the current minimum standards for yoghurt manufacture in the United States. When the yoghurt reaches the desired acid level, it is cooled, modified as necessary, and dispensed into containers (if applicable).

Cool Milk

Milk is then cooled to 38–42 °C, the optimum temperature range for the growth of bacteria. The milk is cooled to 108 °F (42 °C) to bring the yoghurt to the ideal growth temperature for the starter culture.

Inoculate with Starter Cultures

Use a blend of *Streptococcus thermophilus* and *Lactobacillus bulgaricus.* They consume the milk sugar (lactose) and convert it to lactic acid and distinctive yoghurt flavor compounds. The starter cultures are mixed into the cooled milk.

Hold

For optimum growth of bacteria, the temperature should be held uniformly for four to six hours to ensure a nice, thick flavor for some product. The milk is held at 108 °F (42 °C) until a pH 4.5 is reached. This allows the fermentation to progress to form a soft gel and the characteristic flavor of yoghurt. This process can take several hours.

Cool

Once a pH of 4.5 is reached, the yoghurt coagulum needs to be cooled to less than 30 °C to stop excess acid production. The yoghurt is cooled to 7 °C to stop the fermentation process.

Add Fruit and Flavors

After mixing these into the yoghurt, it is important the mixture is chilled without disturbing it to give maximum texture and flavor. Fruit and flavors are added at different steps depending on the type of yoghurt. For set style yoghurt, the fruit is added in the bottom of the cup and then the inoculated yoghurt is poured on top and the yoghurt is fermented in the cup. For Swiss style yoghurt, the fruit is blended with the fermented, cooled yoghurt prior to packaging.

Fruits, flavors, and other additives can be added to the yoghurt at various points in manufacturing process. This is typically dependent on the type of yoghurt being produced. Flavor in non-fruit yoghurts is added to the process milk before being dispensed into cartons. Fruits and flavors can also be added to the containers first, creating a bottom layer. The inoculated milk is then added on top and the carton is sealed and incubated. If the fruit is pasteurized, it can be added as a puree to the bulk yoghurt, which is then dispensed into containers. Finally, the fruit can be put into a special package, which is mixed with plain yoghurt upon consumption. The finished yoghurt containers are placed in cardboard cases, stacked on pallets, and delivered to stores via refrigerated trucks.

The yoghurt is pumped from the fermentation vat and packaged as desired. The majority of yoghurts are packed in plastic tubs, but some luxurious and high-quality products are packed in glass jars.

Storage

Pack the yoghurt, in whatever form, should be stored and transported at less than 10 °C, but preferably below 5 °C. This slows down the biological and biochemical reactions and hinders the growth of potential contaminants, such as yeasts and mold.

7.2 Quality Control

Milk products such as yoghurt are subject to a variety of safety testing (Ares et al., 2006). Some of these include tests for microbial quality, degree of pasteurization, and various forms of contaminants. The microbial quality of the incoming milk is determined by using a dye reaction test. This method shows the number of

organisms present in the incoming milk. If the microbial count is too high at this point, the milk may not be used for manufacture (Aryana & McGrew, 2007). Since complete pasteurization inactivates most organisms in milk, the degree of pasteurization is determined by measuring the level of an enzyme in the milk called phosphatase (Chandan et al., 2008). Governmental regulations require that this test be run to ensure that pasteurization is done properly. Beyond microbial contamination, raw milk is subject to other kinds of contaminants such as antibiotics, pesticides, or even radioactivity. These can all be found through safety testing and the milk is treated accordingly.

In addition to safety tests, the final yoghurt product is also evaluated to ensure that it meets the specifications set by the manufacturer for characteristics such as pH, rheology, taste, color, and odor (Izadi et al., 2015). These factors are tested using different laboratory equipment such as pH meters, viscometers, and human panelists.

8 Project Components

8.1 Capacity of the Project

- The total capacity of the unit is to be produced 48 MT Yoghurt per year.

8.2 Production Targets (Per Annum)

- The scheme is worked out per shift (8 h) basis and 300 working days per annum.
- Assume there'll be 70% production in first year.
- Quantity: 33.60 MT Yoghurt per year or 2.80 MT per month.

8.3 Land

- Land required 300 m^2 approx.
- Approximate rent for the same is Rs. 25,000–30,000 per month.

8.4 Machinery and Equipment

- Mixing or blending vat
- High-speed mixer
- Pasteurizer and homogenizer
- Fermentation vats
- Fruit feeder/mixer
- Pot filler
- Chilled storage area
- Packaging

- Storage
  Note: cost of the machinery is approx. Rs. 800,000 excluding GST and other transportation cost.

8.5 Misc. Assets

No.	Item description	Rate
1	Electricity connection	50,000
2	Equipment and fixtures	50,000

8.6 Power Requirement

The borrower shall require power load of 20 HP which shall be applied with Power Corporation. However, for standby power arrangement, the borrower shall also purchase DG Set.

8.7 Manpower Requirement

- 7–8 manpower are required for the curd manufacturing unit.
- Includes
  - 2 Skilled labors
  - 2 Unskilled labors
  - 2–3 helpers
  - 1 Accountant

8.8 Assumptions

- Production Capacity of Curd is 600 kg/day.
- Working shift of 8 h/day can be considered.
- Raw Material stock is for 2–3 days and finished goods Closing Stock can be taken for 3 days.
- Take the salary and wages rates as per the current market scenario.
- Power consumption can be taken at 15 kW.
- Selling prices and raw material costing has been increased by 5% and 5%, respectively, in the subsequent years.

9 Financial Aspects

9.1 Application of Funds

Particular	Amount (in Lacs)
Land and building	Rented/owned
Plant and machinery	8.00
miscellaneous assets	1.00
Working capital	2.78
Total	11.78

9.2 Source of Fund

Particular	Amount (in Lacs)
Own contribution (min 10%)	1.18
Subsidy at 35% (Max. Rs 10 Lac)	3.15
Term loan at 55%	4.95
Working capital (bank finance)	2.50
Total	11.78

9.3 Projected Profitability

Projected profitability statement (in Lacs)					
Particulars	1st year	2nd year	3rd year	4th year	5th year
Capacity utilization (%)	60	65	70	75	80
Sales (gross sale)					
Total sale of yoghurt	64.15	73.65	83.10	93.09	103.62
Cost of sales					
Raw material consumed	43.20	49.14	55.44	62.10	69.12
Electricity expenses	2.16	2.48	2.86	3.29	3.61
Depreciation	1.30	1.11	0.95	0.81	0.69
Wages and labor	5.04	5.54	6.10	6.71	7.38
Repair and maintenance	1.12	1.29	1.45	1.16	1.04
Consumables	1.28	1.47	1.66	1.86	1.87
Cost of production	54.11	61.04	68.46	75.93	83.71
Add: opening stock/WIP	–	0.54	0.61	0.68	0.76
Less: closing stock/WIP	0.54	0.61	0.68	0.76	0.84
Cost of sales	53.56	60.97	68.39	75.85	83.63
Gross profit	10.59	12.68	14.72	17.23	19.99
Salary to staff	3.24	3.56	3.92	4.31	4.74
Interest on term loan	0.49	0.43	0.31	0.19	0.07

(continued)

Projected profitability statement (in Lacs)					
Interest on working capital	0.28	0.28	0.28	0.28	0.28
Rent	3.00	3.30	3.63	3.99	4.39
Selling and administrative expenses	2.57	2.95	3.32	3.72	4.14
Total	9.57	10.51	11.46	12.49	13.62
Net profit	1.02	2.17	3.26	4.74	6.37
Taxation					0.14
Profit (after tax)	1.02	2.17	3.26	4.74	6.22

9.4 Production and Yield

Computation of Production of curd Items to be manufactured: yoghurt		
Machine capacity per day	600	kg
Total working hours	8	
Working days in a month	25	Days
Working days per annum	300	
Machine capacity per annum	180,000	kg
Production of yoghurt		
Production	Capacity	kg
1st year	60%	108,000
2nd year	65%	117,000
3rd year	70%	126,000
4th year	75%	135,000
5^{th} year	80%	144,000

9.5 Power, Salary, and Wages Calculation

Utility charges (per month)		
Particulars	Value	Description
Power connection required	15	kWh
Consumption per day	120	Units
Consumption per month	3000	Units
Rate per unit	10	Rs.
Power bill per month	3000	Rs.

Break up of labor charges			
Particulars	Wages (Rs. per Month)	No of employees	Total salary
Skilled (in thousand rupees)	13,000	2	26,000
Unskilled (in thousand rupees)	8000	2	16,000
Total salary per month			42,000
Total annual labor charges	(in lacs)		5.04
Break up of staff salary charges			
Helper	6000	2	12,000
Accountant	15,000	1	15,000
Total salary per month			27,000
Total annual labor charges	(in lacs)		3.24

10 License and Approvals

- Obtain the GST registration
- Additionally, obtain the Udyog Aadhar registration Number
- Fire/pollution license as required
- FSSAI license
- Choice of a brand name of the product and secure the name with Trademark if required

11 Recent Development and Future Outlook

11.1 Yoghurt Market Segmentation

Yoghurt market is segmented on the basis of product type which includes regular yoghurt and fat-free yoghurt. Among both of these segments, regular yoghurt is expected to occupy the largest position on the pie in terms of revenue contribution. However, rising awareness among the consumer regarding the consumption of healthy and fat-free products is expected to support the demand of fat-free yoghurt product segment in the near future (Nyanzi et al., 2021).

Yoghurt market is further segmented on the basis of form which includes set yoghurt, frozen yoghurt, Greek yoghurt, and yoghurt drinks (Chandan, 2017). Among all these set, yoghurt occupied the major position on the pie in terms of market share. High usage of set yoghurt in various cooking recipe and consumption as a dessert is contributing towards the growth of set yoghurt product segment. However, frozen yoghurt and yoghurt drinks are expected to show a healthy growth during the forecast period. Frozen yoghurt is further subsegmented as regular and flavored.

Among both of these sub segments, flavored yoghurt is expected to show a fastest growth during the forecast period. Also, yoghurt drinks are subsegmented as regular and flavored yoghurt. Increasing availability of various fruit flavored yoghurt with health benefits is expected to support the demand of flavored frozen yoghurt and flavored yoghurt drinks in the near future.

Yoghurt market is also segmented on the basis of distribution channel which comprises supermarket/hypermarket, grocery stores, convenience stores, online, and others. Among all these segments, supermarket/hypermarket is expected to occupy the major share on the pie in terms of revenue contribution. Increased penetration of retail industry, especially in developing region, is expected to support the growth (Afridi et al., 2021). Moreover, purchasing of yoghurt through online is expected to show a substantial growth during the forecast period. Increasing internet penetration and urge of consumers towards convenience purchasing of product is predicted to be the driving factor for online purchasing in yoghurt market in the near future.

11.2 India Packaged (Retail) Yoghurt Market Outlook 2023

According to the report 'India Packaged Yoghurt Market Outlook, 2023', India packaged/retail yoghurt market has grown with 23% of Compound Annual Growth Rate (CAGR) approximately from the year 2011–2012 to 2016–2017.

It is segmented into Spoonful yoghurt and drinkable yoghurt. Spoonful yoghurt market is further divided into Plain/Natural yoghurt, Frozen yoghurt, and others (Greek yoghurt, shrikhand, probiotic yoghurt, mishti doi), whereas Drinkable yoghurt are divided into lassi, buttermilk, and others (raita). Gujarat Co-operative Milk Marketing Federation Ltd. (GCMMF) is the market leader in both the segments. Region-wise, North and South have constituted more than 65% in the year 2016–2017. In the spoonful yoghurt, plain/natural yoghurt has dominance over frozen yoghurt and other yoghurt. In the drinkable yoghurt, other type of drinkable yoghurt is the least contributor at the end of review period.

Indian consumer, especially the affluent urban consumer, is consuming more value-added products like yoghurt, which bring in bigger profits for dairy companies than raw milk. However, the Indian cooperatives dairies have largely stuck to basic milk, butter, processed cheese slices, and ice cream for many decades and had left a gap in the market which was filled by new players with new product offerings.

Danone was among the first to introduce a series of frozen yoghurts (Caetano, 2013), but its innovations were quickly copied by his rivals, including Amul (Sharma et al., 2013). Nestle has launched Greek yoghurt, Nestle-a+ GREKYO, which is a super concentrated yoghurt, is a fledgling category in India, and is stocked by premium retailers (Pathan & Pol, 2017). As cold food supply chain is an obstacle in India, companies have innovated and created products with greater shelf lives. They have innovated products, such as smoothies, buttermilk, and lassi, which are packaged in ultra-high temperature (UHT) packs. Thus, all the value-added products including yoghurt will grow at colossal rate in India in future.

11.3 Market Trends and Developments

- Yoghurt has become daily habit.
- New products are the key factors contributing the market.
- Product development is taking place continuously.
- Greek and Probiotic Yoghurt are newcomers.
- Shifting focus from raw milk to Value-Added products.

11.4 Yoghurt Market Drivers

Rising disposable income coupled with shifting consumer eating habits is expected to drive the demand of yoghurt market in the near future. Moreover, increasing availability of flavored yoghurt, especially in developing region, is predicted to be major factor supporting the growth of ice cream and frozen dessert market during the forecast period (Kumar et al., 2021). In addition, rising awareness among the people regarding the benefits of yoghurt, such as it has good bacteria or probiotics that boost the immune system, provide calcium, and help combat constipation and diarrhea and others, is also expected to drive the growth of yoghurt market in the near future.

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Production, Cost Analysis, and Marketing of Livestock and Poultry Probiotic

Nikita Patel, Hemant Borase, M. A. Belewu, and R. Krishnamurthy

Abstract

The gastrointestinal (GI) tract of livestock and poultry animal is a complex ecosystem of microbes which interacts with feed nutrients and cells of the recipient animal. Poultry and meat animal plays an integral role in maintaining the economic stability in developing countries. Because of several unwanted impacts on animal growth and animal originated food products, the utilization of antibiotics as growth promoter has been restricted in animal and poultry farming in many countries across the globe. Along with the extensive farming needs, breeders are concerned and interested with non-toxic and cheaper feed supplement as functional food to accelerate the maturation and development of highly nutritious animal products. Probiotics/live microorganisms have emerged as non-invasive tool for increasing performance in poultry and livestock animals. In the view of this, the present book chapter deals with types of feed additives, cost effectiveness, production, and market potential of probiotics used in animal farming.

Keywords

Probiotics · Microorganisms · Gut health · Market analysis · Cost-effectiveness

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1 Introduction

Probiotics are the live microorganisms (bacteria and yeast) which confer the health benefit to humans and animals by maintaining their gastrointestinal flora and performing multiple host beneficial activities. Optimum gastrointestinal function can be maintained by proper absorption and digestion of nutrients. This can be achieved by providing healthy food or feed material with essential nutrients which leads to production of quality poultry and livestock products (Yeoman et al., 2012). Livestock and Poultry probiotic has become a major economic activity in many parts of developed and emerging nations. Much of emphasis is made on large scale production of poultry and livestock products, leading to stressful and disease condition in animals, resulting in economic loss (Khan & Naz, 2013). In the view of increasing population which is predicted to rise up to 10 billion by 2060, the need for animal-based food also arises by several billion. This can be maintained by proper and sustainable animal nutrition in the form of non-toxic probiotics as direct feed. Owing to the ban imposed by European union on usage of antibiotic free feed in 2006, poultry and animal nutritionist are in search of antibiotic-free alternative for animal nutrition (Lillehoj et al., 2018; Van Boeckel et al., 2019).

Generally, livestock and poultry animals harbour diverse microbial communities under their internal body organs, fluids, and skin which directs various biochemical function sustaining their health and body. The interrelationship between gut microbial community and feedstock has suggested high efficacy of animal performance and their ability to produce high yielding animal-based products in terms of sheep, pigs, lamb, cattle, and hen. Several reports have suggested that modifying the gut microbiota with the help of Direct feed Microbials (DFM) or probiotics could benefit the host in terms of high yielding products (Guan et al., 2008; Perea et al., 2017; Walter et al., 2018). Many hypotheses and definitions were coined for probiotics during 1965–1989; however, after twentieth century, the actual meaning and importance of probiotic were hypothesized by Russian scientist Elie Metchnikoff. According to him, consumption of *Lactobacillus* or fermented milk product could eliminate the pathogenic bacteria and enhance the beneficial microflora in gut (Fesseha, 2019; Maurya et al., 2014).

After the ban on use of chemical-based growth enhancer in animal feed by European union, several countries have partially (and completely) banned the antibiotic containing additive in feed material. Moreover, more than 30 probiotic products are registered by European union with most commonly used strains including *Lactobacillus* sp., *Lactococcus* sp., *Bifidobacterium* sp., and *Candida* sp. Generally, probiotics can be administered through oral gavage as vaccine or as spray, in form of capsules, powder, or tablets. Each form of probiotic has common strategy to follow that is to eliminate pathogens and maintenance of overall health. However, large scale production of probiotics varies from species to species of microorganisms (Jiang et al., 2017; Park et al., 2016). Hence, the present book chapter throws light on important aspects of production, cost-effectiveness, and marketing of poultry and livestock probiotic with an aim to provide recent advances and beneficial properties of probiotics in animal health.

2 Probiotics in Animal Feed (Feed Additives)

Large scale production of animal feed requires balanced production system and nutritional requirements in terms of providing sufficient nutrition in concentrated form so that they can produce bulk amount of their products. In case of animals, the digestion process takes place in rumen and sometimes this process becomes suboptimal for certain microbes to digest fibrous material. However, the efficacy of utilization of feed material is dependent on nutritional, antinutritional, enzymatic inhibitor, palatability, and digestibility of feed additives (Beauchemin et al., 2006; Encinas et al., 2018). Apart from above, the age, sex, and health of animal are also important factors for efficient digestion and absorption of various metabolites present in feed. Feedlot cattle technique is mostly followed in Russia, Australia, and Mexico for getting highly nutritious meat. Various strategies were implemented to enhance the efficiency of feedlot cattle. However, increasing antibiotic resistance has enabled use of live microbes or direct fed microbes as feed additives in feedlot production (Encinas et al., 2018; Thompson et al., 2016). Studies were carried out in past to isolate and identify microbial strains as feed additives for feedlot cattle which resulted in identifying *Lactobacillus* strain as potent probiotic strain for farm animals (Maldonado et al., 2018). Generally, feed additives are classified into nutritional additives, sensory additives, technological additives and zootechnical additives, and coccidiostats or histomonostats (Hameed, 2021) (Fig. 1). Below are the important points about feed additives.

- Nutritional additives are those that are required in specific amount by livestock or poultry animals such as vitamins, minerals, protein and essential amino acids, fat, etc.
- Sensory additives are those that add palatability and taste to the feed by making it more appealing to stimulate the diet like they add flavour, colour, and taste to the feed.

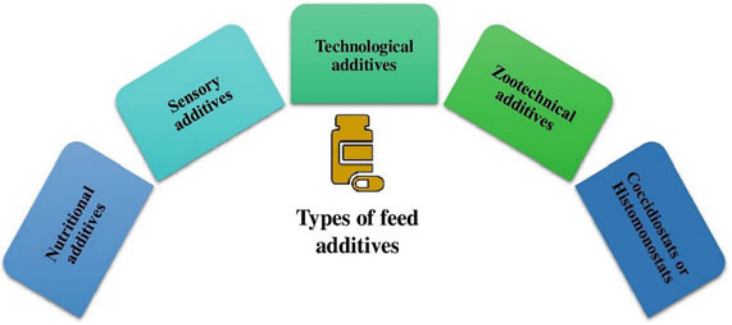

Fig. 1 Types of feed additives

- Technological additives are those that maintain the shelf life and stability of food products and they are often in form of acidity regulators, preservatives, or emulsifiers.
- Zootechnical additives are those that help in proper digestion of food by enabling efficient use of nutrients ingested in the food. They are normally the gut flora enhancers or digestibility enhancers.
- Coccidiostats and histomonostats are those that inhibit pathogenic and parasitic organisms by maintaining the gastro intestinal health of poultry or livestock animal

Over the past years, it has been cleared that the modern rearing method of animals causes stress in animals that may change the gastrointestinal microflora of animals which may compromise their ability to cope up with infectious bacteria. Thus, the prebiotics and probiotics aim to repair these deficiencies and restore their health.

2.1 Microorganisms Used as Probiotics in Animal Nutrition

Majority of probiotic products comprises one or even more type of bacteria or their consortia. The most frequent strains used in animal nutrition belong to *Bifidobacterium*, *Lactobacillus*, *Pediococcus*, *Bacillus*, and *Streptococcus*, including some strains of fungi and yeast (Fig. 2). However, there are certain criteria that need to be fulfilled or satisfied for selecting microbes to be used as probiotics: they could be isolated from single host generally referred as autochthonous; or isolated from different sources generally referred as allochthonous or may be in form of consortia known as multi-strain probiotics (Nandi et al., 2017; Seghouani et al., 2017).

The criteria to be addressed for selecting potent microbial strain include:

- The microbial culture must be pure and strain-specific.
- Must be acid- or bile-resistant.
- Microbial culture must be safer for the host.
- Microbial strain used must be able to reduce parasitic or pathogenic microbes from the host.
- Microbial culture must have antimutagenic and antagonistic activity.
- They must be normal habitant of the (GI) gastrointestinal tract.
- Microbial strain must not be carcinogenic.
- Microbial strains must be able to treat the selected target site for better efficiency.
- The strain should yield high quantity of the end product.
- The strain must be amenable to be produced at industrial level.
- Strain must be grown on relatively cheaper substrates (food industry waste/whey).
- Strain must perform appropriate fermentation to reduce the cost of production.

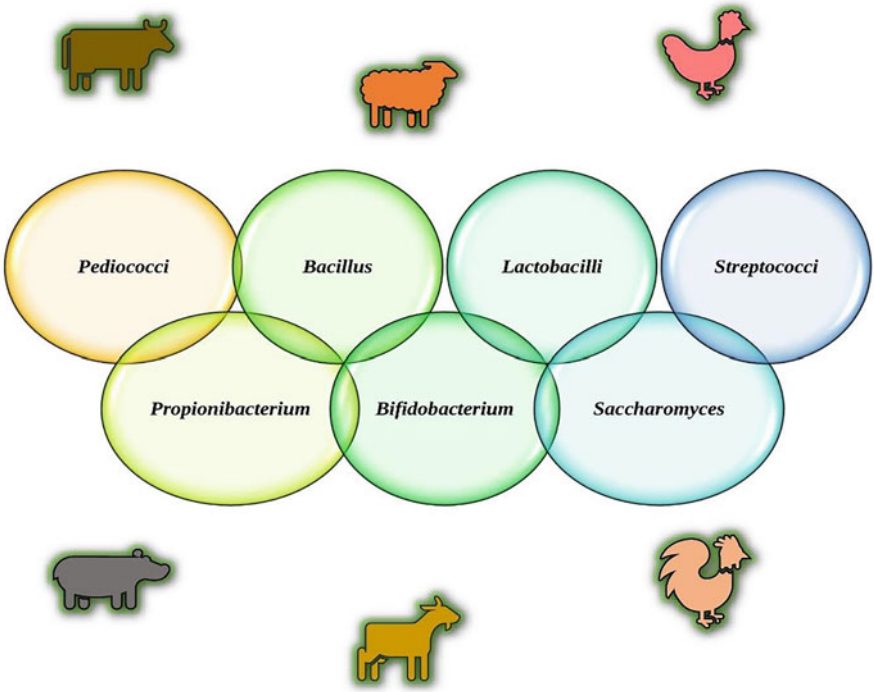

Fig. 2 Microbial strains commonly used in livestock and poultry probiotic

The success rate of any microbial stain as probiotics depends on the selection criteria, and on contrary to that, the microbial strain mostly colonizes within colonic epithelium, enterocyte caecal, and rumen. Among the common strains of microbes, *Saccharomyces* strains generally work for ruminants and *Lactobacillus*, *Bacillus*, and *Enterococcus* species mainly work for pigs/poultry animals. The most common microbial species used in probiotics are depicted in Table 1 (Kechagia et al., 2013; Seo et al., 2010). Apart from the common microbes used in probiotics, there are certain protozoa and archaea present in the rumen of animal that aid in fermentation process. The rumen protozoa are mostly composed of ciliates or flagellates which help in cellulolytic activity of rumen. Protozoa help in digestion of carbohydrates, fats, and proteins by engulfing feed particles and bacteria (Nagaraja, 2016; Puniya & Singh, 2015). On the other hand, Archaea are the methanogens that produce methane basically relying on production hydrogen gas and reducing their inhibitory effects on fermentation (Puniya & Singh, 2015). Subsequently, these groups of microbes are associated with ruminal fermentation process by breaking complex molecules into digestible form and then into acetate by methanogens (Millen et al., 2016).

The microorganisms that are inhabited in rumen have interdependent relationship in breaking down the complex matter through specific enzymes. However, most of the feed ingested remains undigested by the rumen microbiota because of the lack of

Table 1 Common microbial strains used in probiotics

Sr. no	Microbial genera	Strain
1	*Aspergillus*	*Aspergillus niger*, *Aspergillus oryzae*
2	*Bifidobacteria*	*Bifidobacterium pseudolongum*, *Bifidobacterium longum*, *Bifidobacterium animalis*, *Bifidobacterium infantis*, *Bifidobacterium bifidum*
3	*Bacillus*	*Bacillus coagulans*, *Bacillus bicheniformis*, *Bacillus lentus*, *Bacillus pumilus*
4	*Enterococci*	*Enterococci faecium*, *Enterococci faecalis*
5	*Lactobacilli*	*L. casei*, *L. crispatus*, *L. acidophilus*, *L. gasseri*, *L. fermentum*, *L. johnsonii*, *L. paracasei*, *L. plantarum*, *L. reuteri*, *L. rhamnosus*, *L. bulgaricus*
6	*Pediococcus*	*P. cerevisiae*, *P. pentosaceus*
7	*Saccharomyces*	*S. boulardii*, *S. cerevisiae*

enzymes. Hence, probiotic microbes provide optimum and favourable conditions to rumen microorganisms in order to enhance the digestibility and nutrient uptake of the livestock animal (Myer et al., 2015).

2.2 Effect of Probiotics on Animal Health and Performance

Probiotics generally exert an optimistic effect on animal production by improving digestion of fibres, cellulolysis, and synthesizing microbial proteins. The potency of probiotic microbes in livestock and poultry health and nutrition is best achieved when the dynamics of multiple factors work collectively in a well-unified fashion. Generally, *Saccharomyces cerevisiae* is the main strain used as probiotic for cows and buffaloes along with lactic acid sustaining bacteria in feedlot animals (Nocek & Kautz, 2006). There are many reports on utilization of yeast as probiotic as it is found to improve body weight, milk productivity, and food intake; however, exact mechanism of improving fermentation is yet to be targeted (Corcionivoschi et al., 2010). It was observed that yeast cells help in maintaining ruminal pH by providing appropriate nutrients, vitamins, minerals, and free radicals, thereby creating anoxic environment for anaerobic microbes (Dehority, 2003; McDonald, 2010). Supplementing animal feed with probiotics has enhanced fat quality, milk yield, and protein content of milk. The haematological analysis has shown an improved total lipid concentration and serum cholesterol level of livestock animals (Kritas et al., 2006; Yunus, 2017). A milk-based product commonly known as cheese was optimized by using *Bifidobacterium* and *Lactobacillus* sp., whereas combination of *Aspergillus* and yeast has enhanced the solid not fat levels of bovines, respectively (Yu et al., 1997).

Nowadays, the consumption of forage and non-toxic feed supplement has increased meat production to meet the demand of nutritious and healthy meat products. Jukna and his co-workers have observed increase in water retaining capacity and carcass of animal with a net decrease in meat hardness and processing

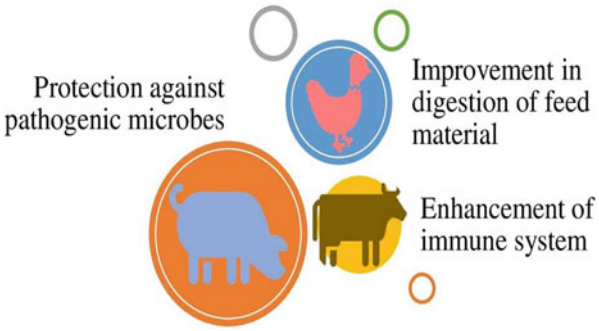

Fig. 3 Role of probiotics in terms of animal performance and application

loss (Jukna & Šimkus, 2005). To surpass the pathogenic infections, *Lactobacilli* strains were prominently used as probiotics in meat industry (Trabelsi et al., 2019). The primary role of animal probiotics regarding performance and application includes: (a) Protection against pathogenic bacteria or fungi; (b) Improvement in digestion of feed material; and (c) Enhancement of immune response (Fig. 3).

There are several factors that affect the health and performance of an animal; that is, age, sex, genetics, and diet also play a crucial role in the animal development. The beneficial flora obtained from probiotics tends to remove pathogens by colonization or competition or by stimulating the immune response. An experiment on mice model and pigs reported that they could be protected from *Salmonella/Listeria* infection and diarrhoea, respectively, with the help of probiotics with an increase in weight and food intake (Corr et al., 2007; Shu et al., 2000). Apart from this, probiotics are known to inhibit *Campylobacter*, *Clostridium*, Enteropathogenic *E. coli*, Enterohemorrhagic *E. coli*, and *Helicobacter pylori* (Johnson-Henry et al., 2005; Ogawa et al., 2001; Stern et al., 2001).

Probiotics are reported to boost immunity in multiple ways. They stimulate the production of IgA in piglets, whereas they also tend to variate the levels of IgG and IgM. The IgA secretion aids in mucosal defence which benefits weaning piglets (Cetin et al., 2005; Scharek et al., 2007). Combined effect of yeast and lactobacillus noted higher yield of CD8+, CD3+, and CD4+ T-lymphocytes in chickens (Bai et al., 2013). Both the cell and humoral immune response are influenced by probiotics by providing shield to the recipient organism, for example, certain *Lactobacillus* species regulate the inflammation by rebuilding the physiological balance of animals through secretion of Interleukin 6, 8 and 10 (Hardy et al., 2013; Luongo et al., 2013; Perez-Cano et al., 2010). Probiotics helps in the managing and treating acute diarrhoea, prophylaxis of antibiotic-associated diarrhoea, and the enhancement of lactose-metabolism. Probiotics in ruminant regimens improve ruminal digestive efficiency (feed digestibility, degradability, and rumen microbiota), rumen pH and lactate levels, fibre digestion, and rumen methane production which have an impact on production performance. Probiotics can assist animals to absorb more nutrients, improve their gastrointestinal health, and minimize diarrhoea, to enhance their performance. Rumen acidosis and enteric infection were

reported to be reduced when probiotics are delivered directly to the rumen (Gomes et al., 1998; Zamojska et al., 2021).

2.3 Probiotics in Animal Breeding

Regardless of species, livestock or poultry animals are subjected to number of stress in form of diet, handling, or rearing methods. These variables may cause discomfort and disturbance to their healthy lifestyle affecting the production system of animal. The utilization of probiotics in livestock farming/animal breeding is usually associated with alteration of gut microbiota with healthy microbes which may impact their body weight, health, and breeding period. In case of weaning pigs, the transition in their diet from lactation to vegetables poses systemic disturbance to their productivity resulting in reduced size of their offspring. It was reported that addition of *Enterococcus*-based probiotics in normal diet of pigs resulted in improved consumption and offspring size of pigs (Böhmer et al., 2006). Favourable results were observed in a study conducted with chickens by using *Lactobacillus* strain as feed supplement. There was prominent increase in egg production and quality of eggs with reduced levels of cholesterol in yolk (Haddadin et al., 1996; Kurtoglu et al., 2004).

2.4 Probiotics in Poultry Feed

The world poultry production has increased to tenfold since past 40–50 years and the GLEAM (Global Livestock Environmental Assessment Model) has estimated that around 80 million tons eggs and more than 100 million tons of meat are required worldwide (Gleam, 2018). To meet this requirement, probiotics exert a massive support without any side effects. Many researchers have observed that feeding probiotics to poultry animals increases growth, productivity, and performance of broilers by preventing gastrointestinal disorders including *Campylobacteriosis* and *Salmonellosis* (Abd El-Hack et al., 2020; El-Sharkawy et al., 2020; Fazelnia et al., 2021; Mookiah et al., 2014). Some of the probiotic strains are interconnected through enzyme production such as amylase, protease, and mannase which led them to the advancement in enzyme industry (Hmidet et al., 2009). A study on broilers in thermoneutral and heat stress condition indicated that there is an increase in enzymatic activity in thermoneutral broilers treated with consortia of probiotics (Sohail et al., 2010).

As the intestinal mucosal framework of broiler is important for both digestion and absorption of nutrients affecting the overall growth, certain *Bacillus* strains were identified to maintain villus height and crypt ratio along with few strains of *Lactobacillus*, *Enterococcus*, and *Pediococcus* (Abdel-Rahman et al., 2013; Afsharmanesh & Sadaghi, 2014; Biloni et al., 2013). Apart from Salmonellosis, Campylobacteriosis is also a major concern for poultry animals and to cope up with this certain strains of *Pediococcus*, *Lactobacillus* and *Enterococcus* were given to

the broilers in an *invitro* study. This resulted into reduction of Campylobacteriosis among them (Ghareeb et al., 2012; Morishita et al., 1997).

Supplementing probiotics to poultry feed improves the integrity of flesh meat in broiler chicken by converting the unfavourable fat into favourable form and thus increasing the tenderness of meat. The overall tenderness, juiciness, and texture were improved by the use of *Clostridium* and *Lactobacillus* (Yang et al., 2010). Mohammed et al. (2021) reported that *Bacillus* can upgrade the chicken meat, while Abou-Kassem et al. (2021) noted that Bifidobacterium bifidum and *Bacillus toyonensis* can enhance growth rate and meat quality of quail.

3 Livestock and Poultry Probiotic: Associated Risk Factors

In last few decades, the utility of probiotics in livestock and poultry production has expanded at an unprecedented rate where India ranks 5th in the world in terms of their products including eggs, meat, beef, etc. However, there are some risk factors associated with them which implies to both folks and animals. One of the factors associated with them is zoonoses. Zoonoses include Campylobacteriosis and Salmonellosis. The infection of Campylobacteriosis is higher than Salmonellosis. *Campylobacter*, a thermotolerant bacteria, is originated from broiler chickens. There are only few methods to eliminate or treat them. Mostly, they are treated through vaccination, bacteriophage, probiotics, and prebiotics. But due to antigenic variability and high cost production of bacteriophage-mediated system, they are less preferred (Meunier et al., 2016; Saint-cyr et al., 2016). There are other factors also, which include extensive immune stimulation in susceptible individual, systemic infection, and gene transfer (Marteau & Boutron-Ruault, 2002).

Majority of microorganisms used as probiotics/feed supplement in animals are innocuous, though some have issues including enterococci, which may carry antibiotic resistance determinants that are transmissible, and the *Bacillus cereus* group, which is known to produce enterotoxins and an emetic toxin cereulide (Anadón et al., 2006; Chaves et al., 2017). Among the poultry and livestock animals, there are other factors that impose deleterious effects via transovarial transmission of microbes. Reason behind this transmission includes contaminated feed, accommodating high density of livestock and poultry animals in as smaller space and intensive fattening of the animals (Dankowiakowska et al., 2013). The necrotic enteritis by *Clostridium perfringens* and aflatoxins produced by several strains of fungi leads to mortality and morbidity in farm and poultry animals most specifically due to the sporadic nature of aflatoxin produced by *Aspergillus* (Otim et al., 2005; Sokale et al., 2019; Tarus et al., 2019). Although less information is available on adverse effect of probiotics on animal and human health, the immune response and status of both humans and animals play a key role (Hempel et al., 2011).

4 Production and Marketing of Livestock and Poultry Probiotics

Breeding and development of healthy animal are the important task of animal industry. There is also a need to produce beneficial non-toxic strain with good viability. To ensure this batch, fermentation is mostly carried out. Although the number of cells that are required is not known, the desired therapeutic activity can be obtained by utilizing the density of 10^6 cells/ml or per gram of feed product. Industrial production process mostly relies on suspended cells under conventional batch fermentation process. The survivability and viability of any probiotic strain under this process is mostly dependent on their capability to adapt adverse or new environment (Yousef & Juneja, 2002). Ali et al. (2021) worked on production of *Bacillus* (KT427377) probiotic by shake flask culture method with 4.9×10^{10} CFU/ml, making it a good source of probiotics with cheap raw material in form of molasses.

Recently, continuous two staged fermentation process for heat/environmental stressed probiotics has become a new tool for bulk production. However, very less information is available on merits and demerits of this process (Lacroix & Yildirim, 2007). The consumer interest has increased in demanding safer and healthier new products around the world. The major factor driving the market potential of probiotics includes health consciousness and functional food diet in form of probiotics. With respect to this, the European market boasted around 48–50% increase in total turnover with easily available probiotic feed supplement. The cost and sale of raw materials, supplements in preparation of this probiotics, amounted to be more than 21 billion US dollars in 2011, which increased to 61.6 billion US dollars by 2021. According to the report/statement generated by Market Research, "Probiotics Market: Global Industry Analysis, Market Size, Share, Trends, Analysis, Growth and Forecast", the possibility of probiotic market is projected to rise by 91.1 US dollars by 2026 with 8.3% annual rate as the consumption has expanded because of the novel covid-19 pandemic. The sudden upsurge of Covid-19 has led to panic in global population and hence probiotics are known to be immune boosters for both animals and humans.

Marketing could be defined as activity of promoting and advertising products or services which include market research with a purpose of generating revenue for the company. According to the market report of 2020, the livestock or poultry feed is categorized into vitamins, binders, amino acids, acidifiers, enzymes, and probiotics considering them to be a growth promoting industry in near future. This report also suggested the economic expansion of probiotics, enzymes, and feed supplement at the rate of 6.1% compound annual growth rate by 2027 (Fior Markets, 2020). Although the marketing of probiotics as feed supplement is quite segmented, the data of individual country including Australia is predicted to rise at the rate of 4.3% compound annual rate with 20% share by revenue of eubiotic supplements (Mordor Intelligence, 2020). The marketing and production of any probiotic strain are generally performed by international companies. However, small scale laboratory also helps in development of probiotic strains before coming into commercialization

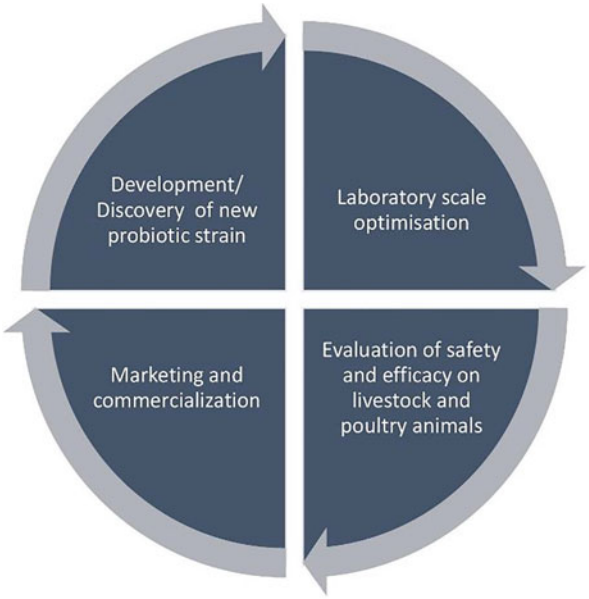

Fig. 4 Strategy for production of probiotics

and hence new products can be identified (Fig. 4). Due to the large market size and capacity of poultry and livestock animals, global companies have started commercializing several probiotics products for animals that could work against necrotic enteritis caused by *Clostridium* sp. The major companies that produce this kind of probiotics are: AB Vista, Novozymes, Danisco animal nutrition, Evonik, Gallipro, CLOSTAT, and Bioplus. They have long history of working with feed supplements with established facilities including development, production, registration, quality assurance, marketing, and distribution (Jayaraman et al., 2013). After the development of potential probiotic strain, it is necessary for the strain to pass through processing criteria as there is a larger difference between production of efficient product in shake/flask culture rather than big fermenters. Once it has been developed at small scale, it is transferred to large scale fermenters to manufacture bulk volume for poultry and livestock evaluation. This sort of production is most widely accepted by the well-developed companies as they have knowledge and experience with large scale production with advanced setup when compared to new or emerging companies. It is always important to maintain the pH and temperature of growth media as the growth rate of species and strains of probiotic microorganisms varies according to them. Recently, submerged fermentation has also gained importance in production of probiotics along with enzyme producing strains of bacteria to get optimized growth of that particular strain. In case of probiotics after the optimum growth of microbial strain, the pellet is dried and used as product directly (Fasim et al., 2021; Patel et al., 2017).

Manufacturing/ producing large amount of probiotics is also dependent on the raw materials that are used as there could be larger difference in the substrate that is

used for microbial strain. Suppliers, nowadays, are sourcing for cheaper materials to overcome the cost cuttings which may ultimately change the overall quality and quantity of the by-product that is to be obtained at the end (Tanguler & Erten, 2008). With the successful production of probiotic strain, the next step to the process is drying/freeze drying/fluid drying where they are initially freezed by using liquid nitrogen followed by reducing moisture under high vacuum for long-term preservation (Muller et al., 2009).

5 Market Strategies for Probiotics

Market strategies could be defined as the overall business plan for reaching prospective consumers and turning them into customers of the products. The market strategies include the four Ps (products, price, place and promotion, packaging, positioning, and people) (Fig. 5) (El-Ansary, 2006; Morgan et al., 2019).

Product: What you are selling, e.g., probiotics (is it physical good, services, consulting, etc.)

Price: How much do you charge and how does that influence your customers about your brand?

Place: Where do you promote your product or service? Such as Newspaper, internet, television, Facebook, Twitter, and many more. Where do your ideal customers find information about your products?

Promotion: How do customers find out about you? What are your strategies and are they effective?

All the "four P" must come into play before having effective and efficient marketing.

Fig. 5 Market strategies for probiotic

1. It was noted that players in probiotics production are leveraging on both organic and inorganic growth strategies
2. Players are focusing on clinical trial program with the state-of-the-art technologies with bacterial strains that are targeting swine, cattle silage, and poultry
3. Strengthening of research and development for the development of New Innovative products that provide feed ratio and be able to quantified mycotoxin risk are the target of major players in the production of probiotics (Foligné et al., 2013).

6 Cost Effectiveness of Livestock and Poultry Probiotics

The rational and practical use of beneficial microbes as probiotics for farm and poultry animal has gained much importance over the past few years due to its health benefits and highly nutritious products. The main challenge faced by this industry lies in providing high quality animal protein enriched with vitamins to large group of population. By approaching sustainable means of production, this sector works on controlling inherent pollution output and guarantees efficient production of animal products including animal welfare (Barba-Vidal et al., 2019). As the usage of probiotics for gut welfare has gained momentum, it is necessary to analyse their cost effective ratio. This ratio is defined as total cost of probiotics per estimated health benefits presented in euro as DALY- disability adjusted life year (Van Wagenberg & Van Horne, 2016). As feed constitutes the 80% share of production cost, any sort of reduction in feed production leads to the reduction in total production cost.

As per the report generated by IMARC (International market research and consulting), the animal feed market was around 817 billion INR in India between 2017–2018, which was projected to rise by 1680 billion INR by 2024. This is owing to an upsurge in demand for dairy products leading to farming livestock in India. Owing to the competition in procuring raw materials for probiotic preparation in humans and animals, local animal farmers are utilizing cheap and low productive materials for animals affecting their health (Mallick et al., 2020). Cam-Con model for controlling the campylobacter infection was applied (Van Wagenberg & Van Horne, 2016) to determine the cost effectiveness of poultry probiotics among broilers in six European countries. It was estimated that building an anteroom, fly screen, and hygiene barrier has low cost on replacing old house with new and varied results were obtained in this six EU countries depending upon the availability of technical inputs. The poultry and livestock integrators have limited resources in their production system and hence probiotics can help them to achieve desired product at a cost effective rate (Thirumalaisamy et al., 2016). In a study conducted by Ray and his co-workers, it was observed that *Bacillus* species, most specifically *B. coagulans* and *B. subulans*, could impose great cost economical production (Ray et al., 2019).

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Small-scale Production and Business Plan for Phycocyanin from Cyanobacteria

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Abstract

Phycobiliproteins are water-soluble proteins present in cyanobacteria and certain algae. They capture light energy, which is then passed on to chlorophylls during photosynthesis. The major phycobiliproteins are phycocyanin, phycoerythrin, and allophycocyanin. The C-phycocyanin (C-PC) is a blue coloured pigment in cyanobacteria, which is considered as a healthy ingredient in cyanobacterial-based foods products while its colouring, fluorescent, or antioxidant properties are utilized only to a minor extent. However, recent research and developments in C-PC synthesis and functionality have expanded the potential applications of C-PC in biotechnology, diagnostics, foods, and medicine. The productivity of C-PC has been increased in heterotrophic, high cell density cultures that are grown under well-controlled and axenic conditions. C-PC purification protocols based on various chromatographic principles or novel two-phase aqueous

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extraction methods have expanded in numbers and improved in performance. The biggest constrain on pigment bioprocessing comes from the installation and operation costs; thus, fundamental and applied research are still needed to overcome such constrains and give the cyanobacteria industry an opportunity in the world market. Several factors can affect the extraction of pigments, including the target pigment, organism, market trends, available technology, and costs. In this, the main extraction methodologies were discussed, taking into account the advantages and disadvantages for C-phycocyanin pigment, type of organism, cost, and final market.

Keywords

Phycobiliproteins · Cyanobacteria · C-phycocyanin · Applications · Productivity · Purification

1 Introduction

Cyanobacterial morphology ranges from simple unicellular, colonial and multicellular filamentous forms. The vegetative cell wall is of Gram-negative type but in some species, the peptidoglycan layer is considerably thicker than in other bacteria. Minute pores are present in regular or scattered order in the wall of all cyanobacteria, but the arrangement varies greatly. Many unicellular and filamentous cyanobacteria possess an "envelope" outside the lipopolysaccharide (LPS) "outer membrane," which is called sheath, glycocalyx, or capsule.

The photosynthetic apparatus of cyanobacteria contains photosystem I and photosystem II as found in higher plants with Chlorophyll *a* and specific accessory pigments, including allophycocyanin, phycocyanin, and phycoerythrin. Cyanobacteria possess the ability to use low light intensities effectively, since they are able to produce the accessory pigments needed to adsorb light most efficiently in the habitat in which they are present, providing them a great advantage for the colonization of their wide range of ecological niches. Phycobiliprotein synthesis is particularly susceptible to environmental influences, especially light quality. The chromatic adaptation is largely attributable to a change in the ratio between phycocyanin and phycoerythrin in the phycobilisomes. The photosynthetic pigments are located in thylakoids that are free in the cytoplasm near the cell periphery. Cell colours vary from blue green to violet red due to the chlorophyll *a* masking by the carotenoids and accessory pigments. The pigments are involved in phycobilisomes, which are found in rows on the outer surface of the thylakoids.

Cyanobacteria get their name from the bluish pigment phycocyanin, which they use to capture light for photosynthesis. In some cyanobacteria, the colour of light influences the composition of phycobilisomes. In green light, the cells accumulate more phycoerythrin, whereas in red light, they produce more phycocyanin. Thus, the bacteria appear green in red light and red in green light. This process is known as complementary chromatic adaptation.

2 Cyanobacterial Pigments-Phycobiliproteins

Phycobiliproteins (PBPs) are a family of accessory light-harvesting macromolecules organized in supramolecular complexes, called phycobilisomes (PBSs) that function as components of the photosynthetic apparatus in cyanobacteria and some eukaryotic algae. Light energy captured by PBS complexes is transferred to the chlorophylls of the inner chlorophyll antenna, CP43 and CP47 (containing chlorophyll A and carotenoids) and finally to the reaction centre II. PBPs are nitrogen storage compounds and may constitute up to 60% of the soluble proteins of the cell. PBSs may undergo changes in the ratio of phycocyanin to phycoerythrin in rods to improve light harvesting in changing habitats. PBSs complexes are highly ordered, having supramolecular assemblies that carry covalently linked bilins (open-chain tetrapyrrole chromophores) and linker polypeptides (Richa et al., 2011).

Phycocyanin contains natural blue pigment and is present in almost every phycobiliproteins containing organisms predominately in cyanobacteria. The different species of cyanobacteria, which are reported to produce phycocyanin, are *Arthrospira (Spirulina) platensis*, *Arthrospira (Spirulina) maxima*, *Porphyridium* sp., *Synechocystis* sp., etc (Manirafasha et al., 2016). Phycocyanin has application in a different domain, such as food additives, health food, cosmetic, pharmaceutical, and medicine. It has pharmaceutical application, as it is known to have anti-inflammatory, anticancerous, and antioxidant activities (Liu et al., 2013; Manirafasha et al., 2016). As well as, it is used in diagnostics due to its fluorescent properties in a particular wavelength. Phycocyanin is a rich source of protein supplements (Kent et al., 2015).

Phycobiliproteins have wide usage and great economic potential. Nevertheless, the widespread use of C-PC and other phycobiliproteins has been somewhat limited by the high cost of these purified macromolecules, ranging from $10 to 50 per mg (Prozyme, ABD Bioquest Inc.). For uses as colourant, this price reduces to $1–5/g because lower purity is requested (Sigma). Conventional methods for phycobiliprotein purification involve the pretreatment of the sample to liberate the intracellular material, making a crude extract ready for isolation step in which the phycobiliproteins are separated using conventional chromatography processes. For C-PC purification, several chromatographic methods are described in the literature involving adsorption, hydrophobic interaction, gel filtration, or ion exchange chromatography. These methods involve a large number of steps, resulting in complex and difficult to scale up, are time-consuming, and have a low product yield. Modern research and development in the synthesis and function of PBSs have expanded the potential applications of PBPs in biotechnology, diagnostic, food, and medicine. They are extensively commercialized for fluorescent application in clinical and immunological analysis.

3 Structure of Phycobilisomes (PBSs) and Phycobiliproteins (PBPs)

PBSs are multimolecular configuration made up of several polypeptide classes. PBPs, mainly composed of α and β polypeptides (some phycoerythrin have γ subunits), are bright coloured systematic assembly of disc-shaped proteins bearing covalently attached open-chain tetrapyrrole known as phycobilins and are the main components of PBSs α and β subunits associated into heterodimers and subsequently aggregate into trimers and hexamers. On the basis of colour and spectral properties, PSBs are categorized into four groups: Phycocyanin (PC; λmax = 610–620 nm), phycoerythrin (PE; λmax = 490–570 nm), allophycocyanin (PE; λmax = 650–660 nm), and phycoerythrocyanin (PEC; λmax = 560–600 nm). A number of colourless linker polypeptides maintain the overall structure of the phycobilisome complex as well as direct its assembly. Linker polypeptides not only serve as structural elements involved in the biosynthesis and stabilization of PBS, but also facilitate efficient flow of excitation energy to the photosynthetic reactions centres (MacColl et al., 1971). The membrane-phycobilisome association is mediated by a large chromoprotein present within the phycobilisome core, which also has linker polypeptide features; it is referred to as the anchor protein or core-membrane linker polypeptide.

4 Extraction Methods Used for Isolation of (C-PC) C-phycocyanin

Several different physical and chemical extraction methods were combined for the disruption of cell walls for the extraction of the water-soluble phycobiliproteins. One of the common methods used for extraction is to homogenize the cell suspension in dilute phosphate buffer, which results in osmotic shock that results in the breakage of cell walls (Sun et al., 2009). The overview of phycocyanin extraction and purification process was shown in Fig. 1. The phycocyanin was extracted from dried *Arthrospira platensis* biomass, by re-suspending the biomass in 0.1M phosphate buffer, at pH 7 (Adams et al., 2002). Phycocyanin has been successfully extracted from wet biomass, when exposed to repeated freezing and thawing cycle. The highest phycocyanin concentration was obtained when the *Synechococcus* sp. biomass was subjected to cycles of freezing at −21 °C and thawing at ±4 °C (Abalde et al., 1998). Some protocols require other mechanical disruption by grinding, sonication, or by use of a French press (Furuki et al., 2003). Sonication is a commonly used method for the extraction from *Synechococcus* sp. promoting cell breakage, and the use of silica beads further aids the disruption process (Abalde et al., 1998). Cell disruption by a French press depends on the blunt force used to treat the samples as they are squeezed through a small orifice by the press, which breaks the cells (Stewart & Farmer, 1984). Nitrogen cavitation is a gentler method of cell disruption that has not been used as much as the other techniques for the extraction of phycobiliproteins (Viskari & Colyer, 2003). A combination of EDTA and lysozyme was used for phycobiliproteins extraction by many researchers

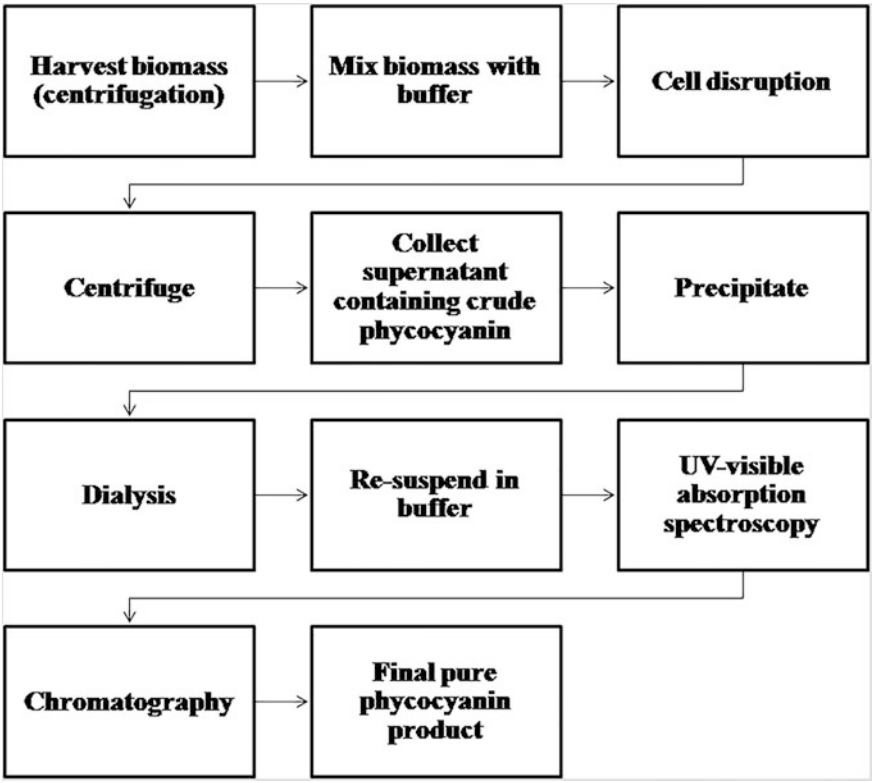

Fig. 1 Schematic diagram showing the overview of phycocyanin extraction and purification process

(Hemlata et al., 2011). Phycobiliproteins from *Arthrospira platensis* have previously been extracted using lysozyme treatment which was more effective than mechanical methods, since a higher yield of phycobiliproteins was obtained. Furthermore, this method was easier to handle (Santiago-Santos et al., 2004).

5 Physical and Chemical Parameters of C-PC Extraction

C-PC extraction is mainly influenced by the following physical and chemical parameters: temperature, pH, solvent type, biomass/solvent ratio, and biomass form (dried or fresh).

5.1 Temperature

It is well known that temperature influences the extraction of intracellular compounds due to modifications on cell membrane structure, increasing mass

transfer rates of internal compounds to the extraction medium. In the case of C-PC extraction, the yield increases with temperature up to a maximum, from that degradation and denaturation are favored; it was reported an increase of the extraction yield with temperature from 30 to 50 °C and a further decrease of C-PC concentration at higher temperatures (Su et al., 2014). Above 50 °C, denaturation of C-PC proteins structure occurs, leading to modifications in the chromophore stability and colour changes (Jespersen et al., 2005; Su et al., 2014). These degradation reactions are probably related to modifications on secondary, tertiary, and quaternary structures promoted by high temperatures (Faieta et al., 2020).

Therefore, high temperatures may be avoided, being preferable extraction methods performed at relatively low temperatures. Most of the mechanical disruption methods, as bead milling, ultrasound, and homogenization, have been performed at 25–30 °C (Ilter et al., 2018; Pan-utai & Iamtham, 2019; Tavanandi & Raghavarao, 2020). In addition, temperature has a strong influence on the purity of the crude extract; an increase on temperature enables the extraction of cytoplasmatic proteins and other interference compounds, decreasing C-PC purity (Silveira et al., 2007).

5.2 pH and Solvent Type

pH directly affects C-PC solubility due to solvent ionic strength influence on proteins structure. Best pH conditions were reported to be between 6 and 7 (Su et al., 2014), as C-PC was unstable at pHs below 5 and above 8 (Patil & Raghavarao, 2007). The isoelectric point of C-PC varies between 4.1 and 6.4 depending on the cyanobacteria species (Fernandez-Rojas et al., 2014). Near the isoelectric point proteins interact less with water, making the extraction harder (Silveira et al., 2007). In addition, the purity of the extracts was reported to be lower at low pH values (below 5) (Patil & Raghavarao, 2007).

To control the pH of the extraction medium, aqueous buffer solution is generally used as solvent within the pigment stability pH range. The most common buffer solution is sodium phosphate (pH 6–8). Besides buffer solutions, distilled water and other aqueous solutions, as $CaCl_2$ (1.5%, w/v) and NaCl (0.15M), were also used as solvent. Some studies were conducted to optimize pH and solvent type for C-PC extraction from *Spirulina platensis* (Ilter et al., 2018; Silveira et al., 2007), and the results are contradictory. Some researchers found out that aqueous 1.5% $CaCl_2$ solution led to higher extraction yield when compared to distilled water and sodium-phosphate buffer (pH 7.0). Other researchers obtained the same extraction yield when sodium phosphate buffer (pH 7.0), distilled water, NaCl solution (0.15M), and $CaCl_2$ solution (10 g L^{-1}) were used; on the other hand, lower yields were observed using acetate buffer (pH 5.0) (Silveira et al., 2007).

5.3 Biomass/Solvent Ratio

Generally, the higher the biomass/solvent ratio, the higher the extraction yields. However, high biomass/solvent ratio leads to the reduction of extracts purity. Few researchers have evaluated biomass/solvent ratio for C-PC extraction from *Spirulina*, and the results are ambiguous. Sodium phosphate buffer (pH 6.8) with ratios of 1:6, 1:8, and 1:10 (w:v) is evaluated, and the ratio 1:10 resulted in the highest extraction yield. However, the ratio 1:6 led to the highest extract purity; the increase on solvent availability may extract more compounds, mainly interference proteins (Tavanandi et al., 2018). In a subsequent study (Tavanandi & Raghavarao, 2020), the researchers evaluated the biomass/solvent ratio under other extraction conditions (solvent, surfactants, and enzymes combination). Similarly to the previous study, the ratio 1:6 resulted in higher purity extracts; however, with this ratio, the extraction yield was comparable to the other evaluated ratios. Other researchers have evaluated 1:50, 1:25, and 3:50 extraction ratios using sodium phosphate buffer (pH 7.0) and Ultra-Turrax as cell disruption method. The highest extraction yield and purity were obtained using 1:50 biomass/solvent ratio (Pan-utai & Iamtham, 2019).

Most part of the extraction studies have not detailed biomass/solvent ratio choice, but commonly used ratios are: 1:25 (Silveira et al., 2007), 1:20 (Ferraro et al., 2020; Su et al., 2014), and 2:25 (Silveira et al., 2007). Besides the extraction yield and the purity of the extracts, it is also important to take into account the processing costs, once an increase on the biomass/solvent ratio may increase the solvent costs.

5.4 Biomass for C-PC Extraction

Dry biomass is generally used for C-PC extraction, and few studies were performed with fresh biomass (Jaeschke et al., 2019). It is still unclear whether the use of freeze-dried or fresh biomass impacts the extraction yield. However, powder rehydration (for 2 h) in the extraction buffer resulted in higher extraction yields, if compared to the results obtained with dry biomass (Tavanandi & Raghavarao, 2020). Conventional drying is not recommended once the longer exposure at high temperature conditions (up to 60 °C) may impact C-PC content (Ilter et al., 2018).

6 Purification of C-Phycocyanin

Phycocyanin is the major phycobiliproteins in cyanobacteria. Phycocyanin comprises a protein and chromophore, and the protein moiety consists of *cpcA* and *cpcB* genes encoded alpha and beta proteins of molecular weights in the range of 18 and 20 kDa, respectively. Phycocyanin is obtained from the microalgae cellular biomass by a freeze thawing process or by using a French pressure cell and is purified by successive steps of ammonium sulphate precipitation and further DEAE-cellulose chromatography. Phycocyanin is considered pure when the absorption

ratio of visible maximum to 280 was greater than 4. Another criterion of purity was that allophycocyanin was not observed at 650 nm (MacColl et al., 1971).

Purification of phycocyanin is a multistep procedure involving a combination of various methods. The first step after extraction of the phycocyanin pigment is precipitation using ammonium sulphate, centrifugation, and dialysis to obtain a crude extract (Ramos et al., 2010). Ammonium sulphate is used as a purifying agent since it readily precipitates phycobiliproteins whilst reducing the quantity of sample to be handled. Proteins precipitation occurs due to the salting out effect, as a result of the competition between protein and saline ions for water molecules, leading to the removal of water from protein. A greater protein-protein interaction occurs, which becomes stronger than protein-water interaction, resulting in the aggregation of protein molecule followed by their precipitation (Silva et al., 2009).

Following precipitation of the phycobiliproteins, chromatographic methods which include ion exchange chromatography and gel filtration, chromatography on hydroxyapatite, and expanded bed adsorption chromatography can be performed (Bermejo et al., 2003). Besides this common process, attempt was made to optimize phycocyanin purification by including the use of rivanol (Minkova et al., 2007), chitosan, charcoal (Patil & Raghavarao, 2007) and hydrophobic interaction chromatography (Soni et al., 2008).

These techniques have been employed to obtain phycocyanin of food, reactive and analytical grades. The purity of phycocyanin extracts is evaluated based on the ratio between absorbencies from PUB at 620 nm and aromatic amino acids in all proteins at 280 nm (Niels, 2008). Phycocyanin with A620/A280 greater than 0.7 was considered food grade, while A620/A280 of 3.9 was considered reactive grade, and A620/A280 greater than 4.0 analytical grade (Kamble et al., 2012). Polyacrylamide gel electrophoresis (PAGE) and sodium dodecyl sulphate—polyacrylamide gel electrophoresis (SDS–PAGE) are also employed to complement or enhance the purity evaluation. Electrophoresis is also used as a simple, less-expensive, and reliable method for separation and identification of the phycobiliproteins. Under the given electrophoretic conditions, the separation of a complex phycobiliproteins depends partially on the different iso-electric points (IEP) of the phycobiliproteins (Morisset & Kremer, 1984).

The purification of phycobiliproteins from *Nostoc* sp. was done using ultrafiltration 30kDa cut off, ammonium sulfate $(NH_4)_2SO_4$ precipitation, gel filtration chromatography, and ion-exchange chromatography. The result showed that 66% protein losses was due to damage by thermal and the hollow fiber membrane and permeate losses from ultrafiltration method (Reis et al., 1998).

The purification of phycocyanin by 40% ammonium sulfate $(NH_4)_2SO_4$ precipitation, gel filtration and 70% $(NH_4)_2SO_4$ precipitation showed that the last step of purification yield of phycocyanin was 45.7% from crude extract and determine molecular masses 19.5 and 21.5 kDa, corresponding to the *cpcA* and *cpcB* genes products, respectively (Minkova et al., 2003). By the purification of phycocyanin from *Spirulina fusiformis* by hydroxyapatite column chromatography and gel filtration chromatography, the result showed molecular masses of the *cpcA* and *cpcB* genes products of 16 and 17 kDa, respectively. The homology of the alpha and beta

proteins when compared with other cyanobacteria phycobiliproteins, *Spirulina fusiformis*, shared the highest degree of homology with *Spirulina platensis* (93% for *cpcA* and 95% for *cpcB*) followed by *Mastigocladus nidulans* (57% for *cpcA* and 64% for *cpcB*) (Madhyastha et al., 2006).

A lot of advantage in phycocyanin was studied by many researchers but the scale-up of these methods is difficult and expensive, so some of the researchers tried to study in the field of molecular biology to clone and express phycocyanin for more convenience and it is inexpensive to scale up protein desire.

7 Production of C-Phycocyanin

7.1 Photoautotrophic Production of C-Phycocyanin

C-Phycocyanin is produced commercially in outdoor, photoautotrophic cultures of the cyanobacterium *A. platensis* (syn. *Spirulina platensis*) grown in open ponds and raceways predominantly at tropical and subtropical locations around the Pacific Ocean (Spolaore et al., 2006). Production of *A. platensis* has also been investigated as far North such as Italy (Carlozzi, 2003) and Spain (Jiménez et al., 2003). *A. platensis* tolerates alkaline conditions and is grown at pH values up to pH 10.5 (Richmond & Grobbelaar, 1986). It is among the few photoautotrophic microorganisms that can be grown in open ponds without being out-competed by contaminating organisms, although contaminants do appear in open *A. platensis* cultures (Richmond et al., 1990). *A. platensis* has been chosen as host for C-PC production merely because of its availability rather than due to particular qualities of its C-PC. The worldwide production of *A. platensis* has been increasing since 1980 (Pulz & Gross, 2004). The majority of more than 3000 tons dry weight of *A. platensis* that are annually produced worldwide is used for health food products and animal feed additives.

The productivity of C-PC in cyanobacterial cultures is determined by the biomass productivity and the specific C-PC content in the biomass. In outdoor open raceways, the areal productivities of dry biomass and C-PC in cultures of *A. platensis* and *Anabaena* sp. have reached values of 14–23.5 and 0.82–1.32 g m^{-2} day^{-1}, respectively (Jiménez et al., 2003). These values correspond to volumetric productivities of 0.05–0.32 g biomass L^{-1} day^{-1} and 3–24 mg C-PC L^{-1} day^{-1}, respectively.

The biomass productivity is determined by the supply of light and the efficiency by which light energy is used to make biomass. Open raceways are operated with liquid depths no less than 10–30 cm so the liquid phase can be circulated and mixed by paddle wheels. Light penetrates only the top centimeters of the culture, and each cell spends a large proportion of its time in darkness where the productivity is zero or even negative. Cells close to the culture surface experience high light intensities, overloaded reaction centers, and low photosynthetic efficiencies, and they suffer

from photodamage and light inhibition (Grobbelaar, 2007). Improved productivities of photoautotrophic *A. platensis* cultures have been obtained in various enclosed photobioreactors, in which culture depth is decreased and cell density increased (Carlozzi, 2003). In dense cultures of *A. platensis*, light is depleted within the first millimetres below the culture surface (Gittelson et al., 1996), and rapid liquid circulations between light and dark zones create what the cells experience as flashing light regimens. When the exposure times to the high surface light intensities are short, the photosynthetic efficiency is increased and photoinhibition decreased. Volumetric biomass productivities above 1.3 g L^{-1} day^{-1} have been reported from enclosed photobioreactors. This is 5 to 20 times above what is obtained in open raceways, but the differences in a real productivities are much smaller. The volumetric productivity of C-PC is also increased in *A. platensis* cultures grown in enclosed photobioreactors. Enclosed photobioreactors also offer better temperature control, which improves culture productivities of *A. platensis* in temperate climates, and very importantly, cultures can be maintained axenic. However, it has so far not been possible to scale-up and operate large-scale *A. platensis* cultures in cost-competitive enclosed photobioreactors.

7.2 Mixotrophic Production of C-PC

A. platensis also grows mixotrophically, the specific growth rate of mixotrophic cultures grown on glucose corresponded to the sum of the photoautotrophic and heterotrophic specific growth rates (Marquez et al., 1993). Although this simple model was questioned by Chojnacka & Noworyta, 2004, mixotrophy does result in faster growth and increased maximal biomass concentrations compared to photoautotrophic cultures (Chojnacka & Noworyta, 2004; Marquez et al., 1993). Feeding of glucose to fed-batch cultures of *A. platensis* allowed to increase final biomass concentrations above 10 g L^{-1} (Chen & Zhang, 1997). Because glucose apparently has no or only minor effects on the specific C-PC concentration in *A. platensis* (Chen et al., 1996; Marquez et al., 1993), high biomass productivities also result in high C-PC productivities. The C-PC contents of mixotrophic *A. platensis* cultures grown indoors have been higher than in outdoor photoautotrophic cultures, but this has been a result of lower incident light intensities in the indoor cultures rather than an effect of glucose. Mixotrophic cultivations have to be carried out in enclosed reactors since the relatively slow growing *A. platensis* would be unable to compete with contaminating bacteria for organic carbon.

Most *Arthrospira* strains can grow heterotrophically on glucose and fructose in darkness (Mühling et al., 2005), but heterotrophic production of C-PC in *A. platensis* seems not a viable option. The specific growth rates and pigment contents in heterotrophic *Arthrospira* strains are simply too low (Chojnacka & Noworyta, 2004; Marquez et al., 1993).

7.3 Recombinant Production of C-Phycocyanin

Recombinant protein production is also an option for heterotrophic synthesis of C-PC. Production of this multichain holo-protein phycobiliproteins is more challenging than production of other recombinant proteins. Complete synthesis of recombinant phycobiliproteins depends on co-expression of the α- and β-chains as well as parallel synthesis and insertion of the correct phycobilin chromophores. Recombinant C-PC, in which cpcA and cpcB genes were fused to His_6 tags for affinity chromatographic purification, has been produced in photoautotrophic *Anabaena* sp., which naturally synthesize and insert phycocyanobilin into C-PC (Cai et al., 2001). Also oligomerization domains coding for tri- or tetra-meric coil-forming peptide domains were fused to His_6-tagged cpcA and cpcB. These fusion proteins were expressed as stable C-PC complexes. Coding sequences for different biospecific recognition domains were also fused to the stabilized C-PC fusion constructs, and the expressed multidomain fusion proteins were used as biospecific fluorescent probes.

In heterotrophic hosts, recombinant holo-C-PC α-subunit has been expressed in *Escherichia coli* (Guan et al., 2007). The cpcA gene encoding the CPC α-subunit was co-expressed with genes encoding heme oxygenase, 3Z-phycocyanobilin: ferredoxin oxidoreductase and phycocyanobilin lyase. The yields of recombinant CPC α-subunit were less than 0.4 mg g^{-1} wet weight and suffered from inclusion body formation (Tooley et al., 2001). However, engineering of genes has resulted in recombinant C-PC subunits with novel functionalities. His_6 tags have allowed purification by affinity chromatography, and domains with affinities for specific biological structures have been incorporated (Guan et al., 2007; Tooley et al., 2001). Recombinant apo-C-PC α- and β-subunits, apo-APC, and holo-APC α-subunit have also been expressed in *E. coli* (Yang et al., 2008) and in the chloroplasts of the green alga, *Chlamydomonas reinhardtii* (Su et al., 2006). When co-expression and insertion of chromophores are not needed, high product yields are obtained and final yields in *E. coli* have reached 0.9 g L^{-1} for recombinant apo-C-PC β-subunit (Wang et al., 2007) and 2.75 g L^{-1} for recombinant apo-APC (Ge et al., 2005). Recently, Yang et al. (2008) obtained up to 1 g L^{-1} or 44 mg g^{-1} of holo-APC α-subunit in an *E. coli* construct similar to the constructs used for expression of holo-C-PC α-subunit.

8 C-Phycocyanin Stability

C-PC structure consists of an open-chain tetrapyrrole chromophore (phycobilin) covalently linked to protein molecules. Changes on the chromophore structure favor colour fading and antioxidant activity losses. Therefore, compounds that react with the tetrapyrrole structure, such as oxygen, free radicals, and acids, promote C-PC degradation. These reactions occur faster at high temperatures and under light exposure (Wu et al., 2016).

Protein structure is highly responsible for C-PC stability, promoting a protective effect on the chromophore. Hence, any factor that affects protein stability and structure may prevent or accelerate C-PC degradation. The hexameric form has a higher protective effect if compared to trimers and monomers. However, in aqueous solution, protein partially dissociates in discrete aggregates, such as trimers and monomers, exposing phycobilin to reactant species. Phycobilin linear conformation maintenance is also important to prevent C-PC degradation (Selig et al., 2018); when protein denaturation occurs, the reduction in hydrogen bonding network leads to the rearrangement of phycobilin molecules from linear to cyclic conformation, resulting in losses of colour (Berns & MacColl, 1989). Consequently, the main parameters that affect C-PC stability are pH, temperature, and light. C-PC is more stable at pH varying from 5 to 7.5 once the hexameric form predominates at this pH range. Close to the isoelectric point of the proteins, aggregation and precipitation occur, favoring turbidity, colour fading, and losses of C-PC content (Sarada et al., 1999).

Temperature is also a critical factor for C-PC stability. C-PC degradation rates are increased at temperatures above 50 °C. Chaiklahan et al. (2012) demonstrated that C-PC degradation rates were lower at 26–43 °C (pH 5–7) and increased when the pigments were heated up from 47 to 64 °C. The colour fading at higher temperatures was attributed to protein precipitation. At lower temperatures, C-PC is relatively stable. However, at ambient temperature, losses of 20% were reported after 10 days of incubation.

Regarding light exposure, C-PC is more sensitive to photochemical degradation at pH 7 than 5. Under intense light exposure (3×10^5 lux) for 24 h, losses of around 80% were observed at pH 7 and around 65% at pH 5. In both conditions, a precipitate was observed, indicating protein denaturation (Jespersen et al., 2005). Even a short exposure to light led to losses of colour: yellow, white, UV-A, and UV-B light exposure for 5 h induced up to 13% of C-PC losses (Munawaroh et al., 2020).

In the light of the above discussion, C-PC application in food formulations is limited due to its sensitivity to acids, heat, and light. To overcome these problems, stabilizing agents have been evaluated to protect C-PC molecule. Among them, small saccharides have shown a positive contribution, maintaining protein native structure and C-PC colour. The preferential exclusion theory has been proposed to explain sugar effects on protein structure: sugars act as cosolute, promoting modifications of protein-solvent interactions, increasing the free energy of the system, and favoring protein folding state (Faieta et al., 2020). The type of sugar and concentration influence C-PC stability. The addition of sucrose (20%, w/w) and glucose (20%, w/w) was considered suitable for prolonging stability of C-PC extracts (Stanic-Vucinic et al., 2018). Sucrose (20%, w/w) had a more positive effect than trehalose in the same concentration. When comparing different concentrations of sucrose, 70% promoted a better protective effect than 20% (Faieta et al., 2020).

Besides sugars, preservatives, such as sodium azide and dithiothreitol, have been used as C-PC stabilizing agents for analytical purposes. However, these compounds are toxic and not suitable for food or pharmaceutical applications (Mishra et al., 2008). In addition, protein crosslinking agents have been used for C-PC stability maintenance, as formaldehyde and glutaraldehyde. The crosslinkers may prevent

protein dissociation in subunits by creating networks between polymer chains and proteins. Besides presenting some positive results (Sun et al., 2006), most of these compounds are toxic and may not be used in food applications. Recently, natural polymers have been evaluated for C-PC protection. Some studies showed that the addition of polysaccharides contributes to increasing the viscosity of the medium, being unsuitable in high concentrations depending on the application. Among the studied compounds, whey protein seems to contribute to blue colour stability at low pH values (3–7), high temperature (80 °C), and under high pressure conditions (Zhang et al., 2020). However, more studies are needed to understand the effect of these compounds on C-PC structure under different processing conditions.

9 Commercialization of Phycobiliproteins

Several multinational companies exploit these phycobiliproteins as a commercial commodity (Table 1). They are developing micro-algae-based technologies and producing micro-alga-derived products. They also produce phycobiliproteins, which are sold to the medical and biotechnology research industry. Their special properties make them useful as tags or markers in many kinds of biological assay such as flow cytometry, fluorescence immunoassay, and fluorescent microcopy.

Cyanotech produces a line of four phycobiliprotein products with various spectral properties. R-Phycoerythrin (R-PE) is a red pigment used primarily in flow cytometry, allophycocyanin (APC) is a blue pigment also used in flow cytometry, but typically in combination with R-PE to form a fluorescent tandem phycobiliprotein conjugate which improves sensitivity. Crosslinked (XL-APC) is a stabilized form of APC which can be used in much diluted solution without the problem of degradation. C-phycocyanin is also a blue pigment, although not used externally in cytometry, has potential application in food and cosmetic colouring. The company's phycobiliproteins currently range in price from US$5000–$33,000/g.

Prozyme produces R-phycocyanin, C-phycocyanin, GT5 allophycocyanin, and crosslinked allophycocyanin and has recently added a new cyanobacterial phycobiliprotein, to which they have assigned the designation Y-phycoerythrin (Y-PE) in recognition of the shift of its fluorescence emission toward the yellow, relative to R- and B-phycoerythrins. Its absorbance and excitation maxima are located at ~495 nm, making it particularly suitable for excitation with a 488-nm laser. Its shorter emission wavelength (~563 nm), relative to other phycoerythrins (575 nm), makes it a good candidate for multicolour fluorescence applications, where separation from higher wavelength emission is desired. In addition, preliminary results suggest that Y-PE may be a more efficient donor in fluorescence resonance energy transfer (FRET) applications. The shift in its spectral characteristics, relative to other phycoerythrins, reflects a high content of the phycourobilin chromophore. Indeed, their properties (high molar absorbance coefficients, high fluorescence quantum yield, large Stokes shift, high oligomer

Table 1 Products marketed using phycobiliproteins

Phycobiliproteins products	Company
Allophycocyanin (APC) B-Phycoerythrin (B-PE) C-Phycocyanin (C-PC) R-Phycoerythrin (R-PE), and crosslinked allophycocyanin	Cyanotech Corporation (Hawaii and United States)
Phycolink®—allophycocyanin (APC) conjugation kit Phycolink®—R-phycoerythrin (R-PE) conjugation kit Phycolink®—B-phycoerythrin (B-PE) conjugation kit C-Phycocyanin (C-PC) GT5 allophycocyanin Crosslinked allophycocyanin R-Phycocyanin (R-PC) Y-Phycoerythrin (Y-PE)	PROzyme Inc. (United States)
R-Phycoerythrin (R-PE)	Pierce Biotechnology Inc. (United States)
Phycoerythrin Avidin D, phycoerythrin streptavidin	Vectors laboratories (USA)
B-Phycoerythrin (B-PE)—labeling kit—NH2 B-Phycoerythrin (B-PE)—labeling kit—SH R-Phycoerythrin (R-PE)—labeling kit—NH2 Phycoerythrin (R-PE)—labeling kit—SH Allophycocyanin (APC)—labeling kit—NH2 Allophycocyanin (APC)—labeling kit—SH	Dojindo Molecular Technologies (United States)
Allophycocyanin (APC) B-Phycoerythrin (B-PE) R-Phycoerythrin (R-PE) Crosslinked allophycocyanin (CL-APC)	Flogen® (Canada)
Allophycocyanin (APC) AnaTag™ APC protein labeling kit AnaTag™ B-phycoerythrin (B-PE) protein labeling kit AnaTag™ R-phycoerythrin (R-PE) protein labeling kit B-Phycoerythrin (B-PE) R-Phycoerythrin (R-PE), crosslinked allophycocyanin (CL-APC)	ANAspec Inc. (Fremont, CA, USA)
R-Phycoerythrin (R-PE) B-Phycoerythrin (B-PE) SureLight® allophycocyanin (APC) Sensilight™ dyes (stabilized phycobilisomes) PBXL-1 PBXL-3 P3L Phycoerythrin conjugates SensiLight™ P3L conjugates PBXL-1 conjugates PBXL-3 conjugates APC conjugates	Martek Bioscience Corporation (United States)
Phycoerythrin (R-PE) B-Phycoerythrin (B-PE), allophycocyanin (APC), crosslinked allophycocyanin (APXL) R-Phycoerythrin (R-PE)-streptavidin conjugates	Invitrogen-Molecular probes (Eugene, OR, USA)

(continued)

Table 1 (continued)

Phycobiliproteins products	Company
B-Phycoerythrin (B-PE)-streptavidin conjugates, allophycocyanin (APC) streptavidin conjugates	
PhycoPro™ C-Phycocyanin, PhycoPro™ Crosslinked Allophycocyanin (CL-APC) PhycoPro™ B-Phycoerythrin (B-PE) PhycoPro™ R-Phycoerythrin (R-PE) Phycolink®-FLAG® R-Phycoerythrin (R-PE) conjugate, Streptavidin—R-Phycoerythrin (R-PE)	Europa Bioproducts Ltd (United Kingdom)
R-Phycoerythrin (R-PE), allophycocyanin (APC)	Chromaprobe Inc. (United States)
C-Phycocyanin	Dainippon Ink and Chemicals (Sakura, Japan)
C-Phycocyanin	Sigma Aldrich (St. Louis, MO, USA)
C-Phycocyanin	Fisher Scientific (Waltham, MA, USA)
Phycolink® C-phycocyanin	Europa Bioproducts Ltd (United Kingdom)
C-Phycocyanin (spirulina pigment)	Japan Algae Co., Ltd. (Okinawa/Japan)
Phycocyanin colour	Ozone™ Naturals (Haryana/India)
Natural phycocyanin	Norland Biotech (Tianjin/China)
C-Phycocyanin	Hash Biotech Labs (Punjab/India)

stability, and high photo stability) make them very powerful and highly sensitive fluorescent reagents.

Dojindo offers two types of R-PE, B-PE, and allophycocyanin labeling kits. For example, in R-PE, one is NH_2- reactive labeling kit (NH_2 type) and the other is SH-reactive labeling kit (SH type). NH_2 type reacts with a primary or secondary amine group of target molecules, and SH type reacts with thiol groups. They are useful in immunoblotting and Immunostaining. Flogen® phycobiliproteins have applications in high sensitivity direct fluorescence detection in flow cytometry, fluorescence in situ hybridization, fluorescence activated cell sorting (FACS), receptor binding in fluorescence resonance energy transfer (FRET), fluorescence immunoassays, fluorescence microscopy, multicolour immunofluorescence, and other imaging techniques. The properties such as excellent stability, high water solubility, extremely high quantum efficiencies, easy to link to antibodies, and other proteins, large Stokes shifts-excitation and emission bands at visible wavelengths, intense long wavelength excitation and emission (relatively free of interference from other biological materials), stability of the chromophore after

binding to their ligands and target make phycobiliproteins a valuable commercial commodity.

Martek Biosciences Corporation produces a variety of fluorescent pigments from algae, includes R-Phycocyanin (R-PC) and B-Phycoerythrin from *Porphyridium cruentum*, a red alga, and R-Phycoerythrin (R-PE) from *Porphyra yezoensis*, C-Phycocyanin (C-PC) and allophycocyanin (APC) from *Spirulina platensis*. They have also developed stabilized phycobilisomes, a line of proprietary pigments from the photosystem II antennae complex of red algae and cyanobacteria which includes PBXL-1, PBXL-3, and P3L named SensiLight™ dyes. The PBXL™ pigments are water soluble and have been successfully applied to different immunodiagnostic formats (e.g., sandwich, competitive displacement assays, microtiter, flow cytometry, microscopy, and paramagnetic beads). In addition to their proprietary PBXL dye conjugates, they developed phycoerythrin conjugates. B-Phycoerythrin and R-Phycocyanin streptavidin conjugates have been favorably evaluated for use on microarray imaging and immunohistochemistry.

For more than 150 years, phycobiliproteins and phycobilisomes were extensively studied and revealed a lot of their mystery hidden behind their glowing colours. Their remarkable physio-chemical properties, and their wonderful structure, so precisely fitted to harvest and transfer light energy to the photosynthetic reaction centres, still fascinate researchers and will certainly continue for a long time to hold captivating interest for mankind. The number of cyanobacterial strains are currently amenable to phycobiliproteins productions is miniscule; none of the characterized strains are significant producers of the products. Basic research is needed to expand the range of organisms represented in culture collections beyond the fast-growing strains that currently make up the bulk of available cultures. Screening of additional cyanobacteria for the presence of biliproteins with desirable characteristics will result in additional discoveries of this nature. It is very clear that existence of patents in this field is huge and the applied research is also gaining upper hand, the problem lies in the commercialization. The future depends on the expansion of the range of products to be manufactured as well as improvements in bioprocess engineering.

Phycocyanin is already being produced commercially. It was introduced in Japan as a natural colouring agent for feed and cosmetics and is produced at a rate of 600 kg/month. The main use of phycocyanin is as a food pigment. However, small quantities are used as a biochemical tracer in immunoassays, microscopy, and cytometry owing to the fluorescent properties of the pigment. Phycocyanin could be utilized as a natural pigment for the food, drug, and cosmetics industries to replace the currently used synthetic pigments that are suspected of being carcinogens. Indeed, phycocyanin from *Spirulina* has already been commercialized by Dainippon Ink & Chemicals of Japan under the name of Lina blue. The product is an odorless non-toxic blue powder with a slight sweetness. When dissolved in water, it is brilliant with a faint reddish fluorescence. Its colour (abs. max. 618 nm) is between those of blue colours No. 1 (brilliant blue) and No. 2 (indigo carmine). It is stable from pH 4.5 to 8.0, and thermostable up to 60 °C, but exhibits poor light stability. Its uses include colouring of candy, ice cream, dairy products, and soft

drinks (Dainippon Ink and Chemicals Inc.). In another patent, Dainippon Ink describe buffer extraction of *Spirulina* and treatment of the extract with an organic solvent, which denatures and precipitates the phycocyanin. The obtained blue pigment is used in eye shadow, eye liner, and lipstick. Since it is not water soluble, it does not run when it is wet by water or sweat. It does not irritate the skin.

Different cultivation methods have specific features that make them more adequate for the production of certain species and commercial applications. Factors like land requirements, construction and operational costs, technological development, maintenance, and control of environmental parameters vary according to the cultivation method used. Cultivation in open ponds involves lower investment, operational and energy costs, and has the potential to produce higher biomass volumes (Narala et al., 2016). This production method is thus more commonly used in the production of biomass for low-value applications, although some technical issues still need to be solved to unfold its potential in the upscaling of cyanobacterial biomass production. Some infrastructures and facilities required for the production of phycocyanin pigment are given in Table 2. The market value for biomass varies depending on several factors such as the production system, production costs (energy and work force), geographical origin, certification schemes, and steps of the economic evaluation is shown in Tables 3 and 4.

9.1 Infrastructure and Facilities Required for Lab Scale Production

Facilities Required

Autoclave, mortar and pestle, cooling centrifuge, refrigerator, −20 °C freezer, UV-Vis spectrophotometer, lyophilizer, and aerator pump.

Economic Evaluation

Revenue

Dried *Spirulina* per month = 85 kg

Phycocyanin pigment extracted = 80 g/kg

Total phycocyanin pigment = (85 kg × 80 g) = 6800 g (6.8 kg)

Total profit per month (approx.) = 6.8 kg × Rs. 35,000 = Rs. 238,000

(the selling price of phycocyanin estimates of approximately Rs. 35,000/kg for food grade C-phycocyanin but the cost varies according to the purity of the pigment)

Total profit per year (approx.) [Rs. 238,000 × 12] = Rs. 2,856,000

Instrument cost (one-time investment) = Rs. 1,086,000

Laboratory land cost = Rs. 120,000/year

Recurring expenditure = Rs. 732,000

Table 2 Media composition

BG11 medium (fresh water cyanobacteria)	
BG-11	Quantity (g/25 L)
$NaNO_3$	37.5
K_2HPO_4	1.15
$MgSO_4{\cdot}7H_2O$	1.875
$CaCl_2{\cdot}2H_2O$	0.9
Citric acid	0.15
Ferric ammonium citrate	0.15
$NaCO_3$	0.5
Trace metal mix A5	25 mL
EDTA (disodium salt)	0.025 g
Distilled water	25 L
Noble agar (if needed)	250.0 g
pH 7.1	
Trace metal mix A5	g/25 L
H_3BO_3	71.5
$MnCl_2{\cdot}4H_2O$	45.25
$ZnSO_4{\cdot}7H_2O$	5.55
$NaMoO_4{\cdot}2H_2O$	9.75
$CuSO_4{\cdot}5H_2O$	1.975
$Co(NO_3)_2{\cdot}6H_2O$	1.23
Distilled water	25 L
MN medium (marine water cyanobacteria)	
MN	Quantity (per 25 L)
$NaNO_3$	18.75 g
$K_2HPO_4{\cdot}3H_2O$	0.5 g
$MgSO_4{\cdot}7H_2O$	0.04 g
$CaCl_2{\cdot}2H_2O$	1.0 g
Citric acid	75 mg
Ferric ammonium citrate	75 mg
EDTA (disodium potassium salt)	12.5 mg
$NaCO_3$	0.5 g
Trace metal A5	25.0 mL
Sea water	18,750 mL
Distilled water	6250 mL
Noble agar (if needed)	10.0 g
pH 8.0	
Trace metal mix A5	g/25 L
H_3BO_3	71.5
$MnCl_2{\cdot}4H_2O$	45.25
$ZnSO_4{\cdot}7H_2O$	5.55
$NaMoO_4{\cdot}2H_2O$	9.75
$CuSO_4{\cdot}5H_2O$	1.975

(continued)

Table 2 (continued)

BG11 medium (fresh water cyanobacteria)	
BG-11	Quantity (g/25 L)
$Co(NO_3)_2 \cdot 6H_2O$	1.23
Distilled water	25 L
Ingredients standard Zarrouk's media (g/25 L)	
NaCl	25.0 g
$CaCl_2 \cdot 2H_2O$	1 g
$NaNO_3$	62.5 g
$FeSO_4 \cdot 7H_2O$	0.25 g
EDTA (Na)	2 g
K_2SO_4	25.0 g
$MgSO_4 \cdot 7H_2O$	5 g
$NaHCO_3$	420 g
K_2HPO_4	12.5 g
A5 micronutrient (H_3BO_3, $MnCl_2 \cdot 4H_2O$, $ZnSO_4 \cdot 4H_2O$, Na_2MoO_4, $CuSO_4 \cdot 5H_2O$)	25.0 mL

Table 3 Non-recurring expenditure-lab scale production

S. No	Head	Approx. cost (in Rs)
1.	Laboratory for production unit on rental basis	120,000 per year
	Equipment (one-time investment)	
2.	Autoclave	250,000
3.	Mortar and pestle	1000
4.	Cooling centrifuge	300,000
5.	Refrigerator	20,000
6.	−20 °C freezer	35,000
7.	UV-Vis spectrophotometer	250,000
8.	Lyophilizer	200,000
9.	Aerator pump	30,000
	Total	1,206,000

Table 4 Recurring expenditure

S. No	Head	Cost (in Rs) per year (approx)
1.	Manpower (four persons—two persons per shift) Rs. 8000	384,000
2.	Consumables	
(a)	Chemicals (culture medium expenses)	78,000
(b)	Glasswares and other miscellaneous expenses	50,000
(c)	Maintenance	35,000
(d)	Transport	25,000
(e)	Electricity (approximately 450 units/month at Rs. 12)	64,000
	Total expenses	732,000

Cumulative Revenue

Total profit = Rs. 2,856,000

Total expenditure = Rs. 1,938,000

Net profit = Rs. 918,000

After initial investment, by reducing the instrument cost, the net profit per year will be Rs. 2,004,000 (approx).

10 Main Challenges and Future Prospects

Main challenges regarding C-PC extraction are related to develop methods that allow high extraction yields and a selective extraction. Most part of the studies performed so far are focused on obtaining high extraction yields. However, purity aspects of the extracts (purity ratio, chlorophyll concentration in the extracts) are not fully explored and may be better discussed in the future. In addition, since C-PC is very temperature sensitive, methods without temperature control may be avoided. Regarding C-PC stability, main challenges are related to find a stabilizing agent that could be applied in food formulation without affecting sensorial and technological properties. Future works may focus on applying natural compounds, such as polymers, in the extracts to avoid C-PC degradation. The other important aspect is the stabilizing agent concentration that should be as low as possible to avoid changes in food properties.

Phycobiliproteins have potential and diverse applications, used for colouring purposes in foods was well exploited in confectioneries, dairy products and ice creams, soft drinks, beverages, and cake icing. In food application, there is an increasing interest in the production of light fluorescent products. The scope for the use of phycobiliproteins in textile and printing dyes is in the offing. Furthermore, it is likely to have applications as colourant in pharmaceutical products like pills and syrups. However, use of phycobiliproteins for food purposes has to be further supported with toxicity testing. Only in the case of C-phycocyanin, categorical reports on the safety for use in food are available (Dufosse et al., 2005). In relation to other pigments, toxicological evaluation has to be performed. There is a need to improve thermo stability, aqueous stability, pH stability, alcohol resistant, light stability, and shelf life of the pigments. In addition, smaller size seems to be advantageous, and hence, lot of scope exists with cryptomonads which also harbor novel phycobiliproteins and phycobilins. Moreover, suitability of separated phycobilins for application purposes was not looked into. Furthermore, exploration of phycoerythrocyanin, finding new and novel phycobilins and phycobiliproteins as well as making colour combination using phycobiliproteins should also be investigated.

Phycocyanin is extensively used as a colourant rather than phycoerythrin. However, phycoerythrin is very much used in fluorescent applications. Patent analysis revealed that most of the US patents are related to fluorescent application while most of the Japanese patents are related to the production, purification, and application for therapeutic and diagnostic purposes. It also covers the novel use of phycobiliprotein

as lipase activity inhibitor, serum lipid reducing agent, skin function activation factor, and reagent to obstruct the environmental pollutant deposition in the body, fabric dye, ingredient in tissue culture media, plant growth regulator, and molecular weight maker.

A comparison of patents, applied research, and commercial activities indicates the common use of phycobiliprotein for fluorescent purposes. Furthermore, biomedical properties such as anti-inflammatory, anti-oxidant, liver protection, anti-tumor, lipase activity inhibitor, and serum lipid reducing agent are documented both in patents and applied research that are not commercially ventured. Similarly, a number of other novel properties are reported only in patents. Some of these properties documented in patents and applied research can be looked for commercialization.

11 Conclusions

In relation to the nature of organisms and yield potential, wide variations are encountered. Phycocyanin is commonly produced using cyanobacteria, and its yield is reported as high as 46%. The success in the production of phycobiliproteins depends on the nature of organisms, its growth characteristics, availability of mass cultivation technology, the extent of accumulation of pigments, etc. It also depends on the efficacy of downstream processing. Further research is needed to develop fast growing organisms adapted to suit conditions in mass cultivation like resistant to photobleaching, contaminants, and variation in temperature are required. Furthermore, technologies for the high accumulation of phycobiliproteins using organismal and genetic modification are required. Efforts should also be made to isolate high pigment producers too. Consequently, further investigation may focus on evaluating new molecules, such as other natural polymers, to better understand C-PC behavior under different processing conditions and design strategies to increase C-PC application in various fields.

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Commercial Astaxanthin Production from Green Alga *Haematococcus pluvialis*

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Abstract

Astaxanthin is a secondary carotenoid that is widely used as a dietary supplement and feed in aquaculture industries. Among other commercially significant microalgae, *Haematococcus pluvialis* is considered as the best non-genetically modified microalgae for accumulation of natural astaxanthin. Natural astaxanthin extracted from *H. pluvialis* has a significantly better antioxidant potential than artificially manufactured astaxanthin. Astaxanthin promotes the health conditions of humans by reducing oxidative stress and free radicals. Natural astaxanthin is recognized as one of the high-value-added products of the future, with noticeable advantages and a great growth in demand. The present chapter describes the mass cultivation of *H. pluvialis* for high-end production of natural astaxanthin. Cultivation of *H. pluvialis* in photobioreactors and open raceway ponds via two-stage and one-stage methodologies are summarized in detail. Production processes including harvesting, cell rupturing, downstream processing, and biorefinery models were reviewed. Total cost and profit involved in the mass production of *H. pluvialis* were elaborated by lab-scale production, small-scale production, and large-scale production in detail. Thus, the chapter can serve as a baseline for entrepreneurship opportunities in the commercial production of astaxanthin from *Haematococcus pluvialis*.

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Keywords

Astaxanthin · *Haematococcus pluvialis* · Photobioreactors · Open raceway ponds · Mass production

1 Introduction

Haematococcus pluvialis is a single-celled freshwater microalga acknowledged for its preeminent organism of natural astaxanthin (Lorenz, 1999). The bright red-colored secondary carotenoid of astaxanthin (3,3′-dihydroxy-carotene-4,4′-dione) has distinctive biochemical characteristics. Due to the presence of carbonyl and hydroxyl functional groups, carotenoid exhibits remarkable anti-inflammatory and antioxidant properties (Hussein et al., 2006). Dietary supplements, cosmetics, aquaculture, and pharmaceutical sectors are the most dominant applications utilizing astaxanthin. It fulfils several biological functions in aquatic animals, including prevention of oxidative stress, increasing immune response and pigmentation, and protection from ultraviolet radiation (Zuanon et al., 2011). The free radical scavenging efficiency of astaxanthin is 10 times more powerful than canthaxanthin, zeaxanthin, lutein, and β-carotene, 54 times stronger than β-carotene, 65 times more dominant than vitamin C, and 100 times more efficacious than α-tocopherol (Borowitzka, 2013; Koller et al., 2014; Pérez-López et al., 2014). Astaxanthin slows down the growth and regulates immune responses against tumor cells (Nagendraprabhu & Sudhandiran, 2011). This carotenoid acts against various human health conditions like diabetes, cardiovascular diseases, liver diseases, and obesity (Ambati et al., 2014). It also improves the brain, skin, and eye health (Nakagawa et al., 2011; Yamashita, 2005), promotes the reduction of body fat percentage, and enhances physical performance (Aoi et al., 2008).

Nowadays, the natural astaxanthin extracted from *Haematococcus pluvialis* present in market is only around <1%, while over 95% is synthetically manufactured (Koller et al., 2014). Synthetic astaxanthin has 20-folds lesser antioxidant capacity than natural astaxanthin, and it is still not approved for human consumption (Koller et al., 2014; Lorenz & Cysewski, 2000). Synthetic astaxanthin is produced from Wittig reaction with C_{10}-dialdehyde and asta-C_{15}-triarylphosphonium salt, and there is safety concern for human consumption due to its different stereochemistry. It is only allowed to be implemented in aquaculture. These factors increase the demand for natural astaxanthin in the global markets. *H. pluvialis* extracted natural astaxanthin has ANVISA (Brazilian Agency of Health Surveillance) status in Brazil, FDA (US Food and Drug Administration) granted GRAS (generally recognized as safe), and FSA (UK Food Standards Agency) granted "novel food" status (Capelli & Cysewski, 2013; Grewe & Griehl, 2012).

This chapter provides the prior budgetary estimation for profitable production of natural astaxanthin from *Haematococcus pluvialis*. By correlating the production expenditure of natural astaxanthin towards lab-scale, small-scale, and large-scale production scenarios, it be less expensive than synthetically produced astaxanthin. It

also summarizes the mass multiplication process, cultivation methods, harvesting, and downstream processing of biomass. Current global market and biorefinery strategies are also discussed.

2 Morphology and Life Cycle of *Haematococcus pluvialis*

Haematococcus pluvialis is a freshwater single-celled freshwater microalga which is an efficacious producer of astaxanthin in massive amounts (Fig. 1). It is also called as *Sphaerella lacustris* or *Haematococcus lacustris*. Typically present in freshwater bodies such as artificial pools, birdbaths, man-made ponds etc., and it is distributed worldwide (Shah et al., 2016). The cell is oval to spherical with a diameter of 30 μm (Oslan et al., 2021). At the start, *Haematococcus pluvialis* begins as a free-swimming, biflagellate greenish microalgae with a singular pyrenoid-carrying chloroplast, then transforms into a non-motile palmella by losing its flagella, and finally into the thicker-walled aplanospore via eliminating its flagella (Niizawa et al., 2018).

H. pluvialis does have a four-staged life cycle that is segmented into macrozooids, a flagellated cell stage which is also called as early growth phase, microzooids, palmella stage (immature cyst) followed by maturation, and aplanospore (mature

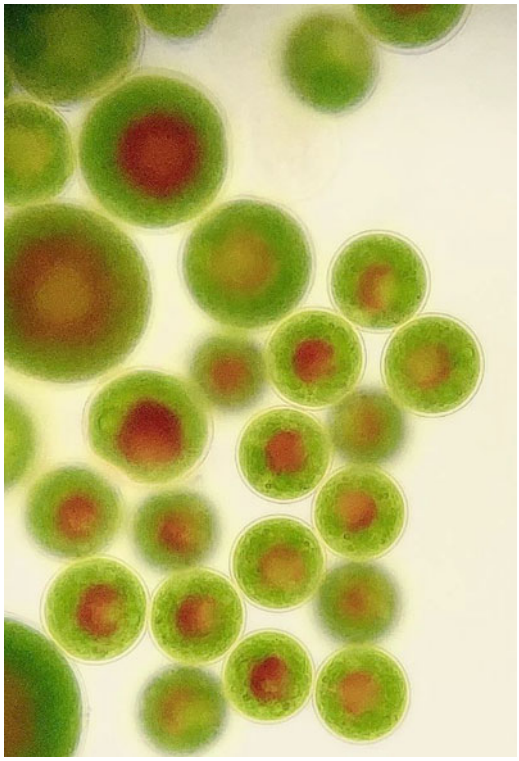

Fig. 1 Light microscopic image of *Haematococcus pluvialis*

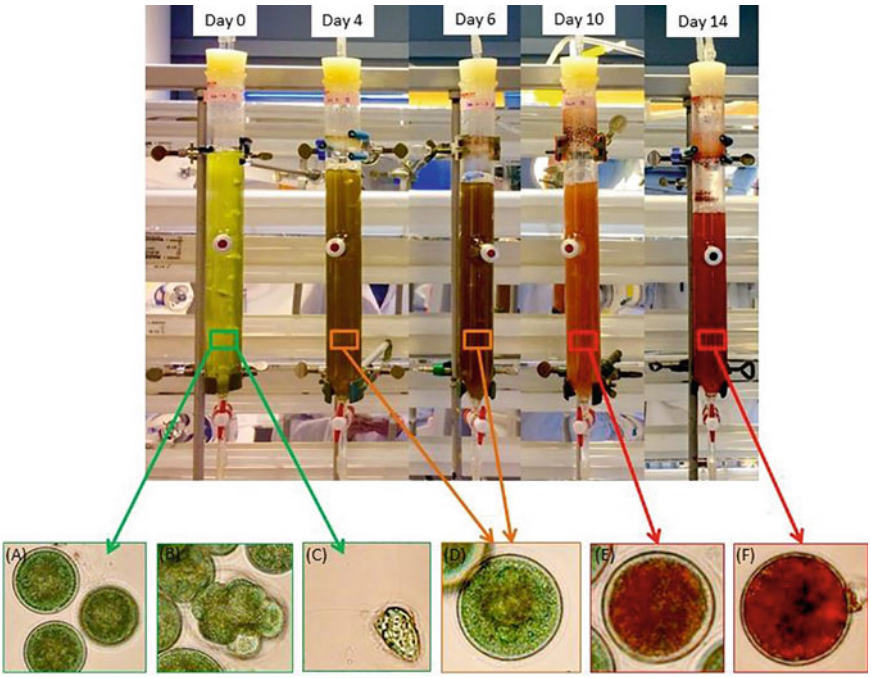

Fig. 2 Life cycle of *Haematococcus pluvialis*. (**a**) Palmelloids, (**b**) dividing cells, (**c**) zooids, (**d**) palmelloids, (**e**) intermediates, and (**f**) mature cysts

cyst). It consists of two phases: a vegetative phase and a red non-motile encysted phase. The green vegetative phase involves the palmella stage and microzooids, whereas the reddish non-motile encysted phase includes aplanospores or hematocysts (Shah et al., 2016). Aplanospore develops from the vegetative cells due to morphological changes occurs during unfavorable growth condition and other stress factors (Mota et al., 2021). Due to the formation of aplanospore, flagellum degenerates with increasing cell size and the astaxanthin content reaches about 81.2% (Fig. 2).

3 Facilities Needed

H. pluvialis growth can take place in open tanks or closed photobioreactors. For the large-scale biomass production, open raceway ponds are the most popular as well as feasible cultivation system. While photobioreactors provide higher biomass yield, level of contamination is comparatively lower than in raceway ponds (Mota et al., 2021). Current production process is schematically represented in Fig. 5.

3.1 Photobioreactor

Photobioreactors are generally designed as transparent tanks, in which environmental conditions like temperature, light illumination, pH, and carbon dioxide levels can be controlled. PBR increases the production of biomass by supplying the best growth condition for certain strains of microorganisms. Light and temperature are essential for biomass production, and they should be optimized as it occurs in nature (Metsoviti et al., 2019). By limiting the delivery of optimum exposure of light to other cells, the exterior layer of the culture accepts most of the light source (de Mooij et al., 2016). Light-emitting diodes (LEDs) are widely used light source due to their effective as well as simultaneously low of cost.

When compared to open systems, PBR necessitates less area, minimizes contamination, and provides unique culturing growth parameters independent from environmental conditions (Pawar, 2016). The operation of control modules and sensors in PBR systems facilitates the automation of biomass cultivation. In the aspects of disadvantages, the construction and operation of these systems are more expensive. However, it requires enormous supply of electricity to provide light illumination and complexity in designing as well as building the PBRs. Moreover, the formation of biofilm in the course of cultivation reduces light entry and causes difficulties with tank cleaning (Dębowski et al., 2013). In any case, the increased productivity and homogeneity of green algal biomass mitigate for its higher expenditure of these technologies (Blanken et al., 2013). Due to their production of high value-added products, including biopharmaceuticals, nutraceutical, components of healthy food product, and high-grade cosmetics, these PBR systems on large scale is developing to be more and more popular (Hubo et al., 2017).

Closed photobioreactors are effective for the growth of *H. pluvialis*, as it is sensitive towards environmental changes (Fig. 3). The selection of suitable bioreactors is a crucial step at an early stage. Tubular systems are distinguished by a huge illuminating space along heavy workload, as well as significantly greater maintenance and operation expenditure (Fazal et al., 2018). Use of the plastic bag PBRs is a cheaper alternative, but it faces some obstacles including lower light illumination, culture mixing, and mechanical damage (Huang et al., 2017). Flat panel and column airlift PBRs are known for its high capital investment, longer lifespan, high light illumination, efficient mixing, and mass transfer. The rectangular form of airlift PBRs promotes the sedimentation of microbes causing complications while cleaning (Ting et al., 2017).

Wan et al. (2015) carried out a comparative study between raceway tanks and column photobioreactors and reported that during the photoinduction phase of *H. pluvialis*, the biomass and astaxanthin concentration in column photobioreactor were 0.9 g L^{-1} and 2.8%, respectively. Raceway tanks showed 0.6 g L^{-1} and 2.2%, respectively. The contamination and parametric control capabilities of flat-panel and closed tubular bioreactors are excellent; however, the CO_2 restriction and limited illumination efficiencies have an impact on biomass buildup (Olivieri et al., 2014). It is essential to optimize the mixing effect in the bioreactors to avoid the accumulation of CO_2 and O_2, during growth phase. To overcome this issue, Yoo et al. (2012) used

Fig. 3 Closed photobioreactor—capacity: 5 L; light module: LED lamp; max. lux: 20,000 lux; sensor control and recording: pH, DO, temp., foam, and pressure; sparger and gas mode: micro sparger, 2 gas and 4 gas mixers; agitation range: 10–1500 rpm; feeding control system: peristaltic pump control acid, base, foam, and feed; power voltage: 110–220 V, 20.60 Hz, 10 A

6 L indoor bag-type PBR with adequate hydrodynamic mixing of *H. pluvialis* and obtained a yield of 2.62 g/L biomass concentration and 78.37 mg/L astaxanthin content. Productive features of *H. pluvialis* cultured in various photobioreactors and culture mediums are enumerated in Table 1.

3.2 Open Raceway Pond

Open raceway ponds are well known since the 1950s (Borowitzka, 1999), and it is more economical than photobioreactors for microalgal cultivation (Huntley et al., 2015). They are closed-loop recirculation channel which includes paddlewheel and baffles (Fig. 4). The fixed depth of raceway pond is 30 cm, so that all the cells utilize ideal light irradiation. Paddlewheel promotes the proper mixing and circulation of the stream. Nutrients are supplemented near the paddlewheel for optimal mixing all over the pond. While baffle brings the uniformity of stream right through the curved bend plus limits the dead zone formation. The dead zones negatively impact on mixing, unwanted energy loses and enables solids to settle (Bompolakis et al., 2019). It is challenging to regulate the temperature, lighting, and evaporation rate in open raceway ponds and thereby affecting the cooling process (Chisti, 2007). Open ponds contribute a low yield of biomass, and it is prone to contamination (Terry & Raymond, 1985).

The integration of photobioreactor along with open raceway pond is known as hybrid two-stage cultivation. At first, photobioreactor controls the optimum growth condition, and after that, raceway pond exposes the cell to nutrient deprivation. As a result, the desired product's synthesis is enhanced (Huntley & Redalje, 2007; Rodolfi et al., 2009). Productive features of *H. pluvialis* cultured in several open raceway ponds are enumerated in Table 2.

Table 1 Productive characteristics of *Haematococccus pluvialis* in different photobioreactors and its biomass and astaxanthin yield

Refs.	Li et al. (2020)	Azizi et al. (2020)	Sun et al. (2016)	Wang et al. (2019)	del Río et al. (2005)	Aflalo et al. (2007)	Harker et al. (1996)	Onorato and Rösch (2020)	Onorato and Rösch (2020)	Onorato and Rösch (2020)	Choi et al. (2018)
Astaxanthin productivity (mg L^{-1} day^{-1})	11.4	8.0	9.2	18.1		11.5	0.44	750	70	210	
Astaxanthin content (mg L^{-1})	38.02	49.71	92	13.51	8	40	26.67	3750	880	1160	170.1
Biomass productivity (g L^{-1} day^{-1})	0.66	0.25	0.43	1.34		0.3		1	0.32	0.39	
Biomass yield (g L^{-1})	2.0	1.75	1.8	20.1	1.3	2.8	1.5	5	2.13	4.83	1.43
Culture medium	BG-11	BG-11	MCM	BBM modified	Inorganic basal	BG-11	BBM modified	–	–	–	NIES-C
Cultivation system	Bubble column photobioreactor	Photobioreactor	Tubular PBR	Column PBR	Bubble column tubular PBR	Tubular PBR	Air lift PBR	Flat panel airlift PBR	Unilayer horizontal tubular PBR	Green wall panel PBR	Polymeric thin film PBR

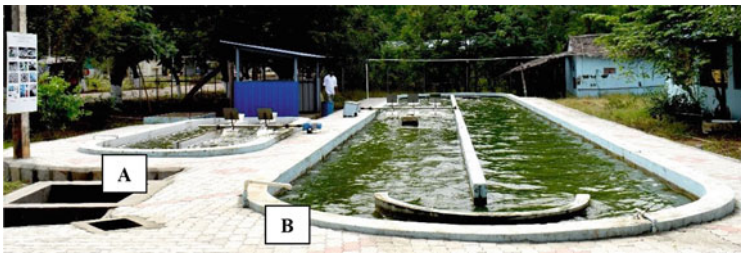

Fig. 4 Open raceway pond—capacity. (**a**) 5000 L and (**b**) 35,000 L

Table 2 Productive characteristics of *Haematococccus pluvialis* in different capacity of raceway ponds

Refs.	Olaizola (2000)	Aflalo et al. (2007)	Zhang et al. (2009)	García-Malea et al. (2009)
Astaxanthin productivity (mg L^{-1} day^{-1})	–	10.1	51.06	8
Astaxanthin content (%, DW)	2.8–3.0	3.8	2.79	1
Biomass productivity (g L^{-1} day^{-1})	0.036–0.052	0.37	–	0.7
Biomass yield (g dry wt. L^{-1})	–	–	1.83	
Culture medium	BBM modified	BG-11	BG-11 modified	Inorganic medium
Capacity (L^{-1})	25,000	200	20,000	1.8

4 Mass Production Processes

4.1 Selection of Microalgal Strain

Haematococcus pluvialis is a widespread global species. Annually, a great percentage of strains were isolated and characterized all over the universe. Existing biotic biodiversity would allow higher functioning variants to be preferred for astaxanthin synthesis without any need for genetic modification (Li et al., 2020). Variant selection is still an important phase in commercial microalgal cultivation. Cost for large-scale production can be diminished by analyzing many potent species and attributes like inflation of biomass and carotenoid productivity. Use of native microalgal species is best suited for commercial production as they can adapt effectively to the local environmental and weather condition (Fig. 5).

Kiperstok et al. (2017) studied the total astaxanthin and biomass production in 25 strains of *H. pluvialis* in twin layer photo bioreactor. Among the 25 strains, strain CCAC 0125 is selected as best strain for mass cultivation while whole biomass obtained and astaxanthin productivity of 91.2 g/m^2 and 1.4 g/m^2 and astaxanthin

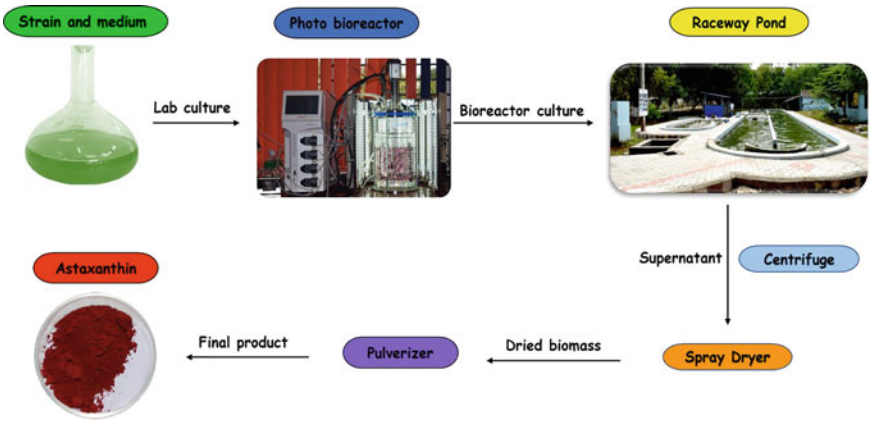

Fig. 5 Current production processes for high-end astaxanthin yield

content of 1.5% dry weight. Zhang et al. (2009) selected *H. pluvialis* 26 and *H. pluvialis* WZ that are suitable superior variants for its biomass yield when compared to other two strains (*H. pluvialis 30* and *H. pluvialis 34*). *H. pluvialis* 26 showed astaxanthin and biomass productivity of 51.06 mg L^{-1} and 1.83 g dry wt. L^{-1} with 2.79 g $100g^{-1}$ dry wt. of astaxanthin content. Therefore, the selection of optimal strain enhances the production process and cost effective.

4.2 Microalgae Cultivation

To attain high biomass and astaxanthin productivity, optimization of light, temperature, pH, growth media composition, etc. is essential. Carotenogenesis initiation requires intense exposure to stress condition to accumulate higher levels of astaxanthin. The source of stress can be induced either via combination of multiple stress factors, or from high levels of one stress factor (Shah et al., 2016). It is also interesting to notice that when cells are subjected to excessive stress, their proliferation completely stops and they die within a short period of time (Su et al., 2014). When culture is exposed to nutrient deprivation, it triggers the astaxanthin accumulation inside the cells (Saha et al., 2013).

Most prevalently used medium for growth are BG-11 (Rippka et al., 1979), OHM (Fábregas et al., 2000), BBM (Bischoff, 1963), KM1 (Kobayashi et al., 1993), and their alterations. It is significant to supply nutrients during the day time with maximal sun irradiation, when photosynthesis occurs. The best source of inorganic nitrogen was found to be sodium nitrate (Sarada, Bhattacharya, & Ravishankar, 2002), and urea can be used as an alternative source. Nitrogen starvation leads to the production of approximately double the astaxanthin ratio than the phosphorus deficiency. It might be caused due to lack of nitrogen resulting in higher cellular damage, which displays a remarkable degradation of chlorophyll (Boussiba et al., 1999). Addition of

NaCl (0.25–0.5% w/v) to the growth media induces astaxanthin production. Also, astaxanthin accumulation can be enhanced by combination of NaCl and 2.2 mM sodium acetate (Sarada, Tripathi, & Ravishankar, 2002).

The ideal temperature for *H. pluvialis* proliferation and astaxanthin generation is between 20–28 °C (Fan et al., 1994; Kang et al., 2010; Wan et al., 2014). It is preferred to gradually adjust the temperature, which enables better adjustment to the new circumstances (Hata et al., 2001). The optimal pH for *H. pluvialis* is within 7–7.8 pH (Hata et al., 2001; Sarada, Bhattacharya, & Ravishankar, 2002). Irregular shift in pH can have negative results on the biomass cultivation.

Standard irradiance for culture improvement ranges within 40–50 μmol photons m^{-2} s^{-1} (Hata et al., 2001; Park et al., 2014). Optimal irradiance tends to be higher to reach high growth rates for about 70 (Zhang et al., 2014) to 177 μmol photons m^{-2} s^{-1} (Domınguez-Bocanegra et al., 2004). The use of white or blue LED light at the ratio of 3:1 at 7000 lx induces carotenogenesis. Park et al. (2014) recorded continuous escalation of light intensity results the cells with progressive conversion to cysts and causes better accumulation of astaxanthin. It is due to the potential of cells to manage increasing stress levels.

Two-step Strategy

In this technique, the biomass is initially generated under ideal growth circumstances (green stage), and then, the growth is subjected to harsh ecological parameters to trigger astaxanthin accumulation (Fig. 6). In this two-stage procedure, astaxanthin can be generated effectively (Aflalo et al., 2007; Orosa et al., 2005).

In commercial setups, astaxanthin accumulation is usually induced by increasing solar irradiance or temperature and combination of nutrient deprivation especially

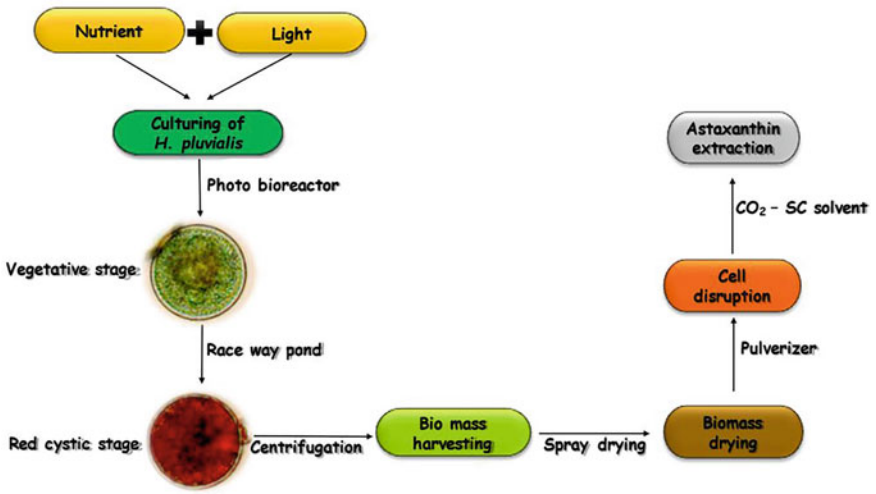

Fig. 6 Schematic flow chart of two-step cultivation for obtaining astaxanthin from *Haematococcus pluvialis*

phosphate and nitrate (del Río et al., 2005; del Río et al., 2008; García-Malea et al., 2009). Green and red phase of biomass production ranges from 0.1 to 0.5 and 0.1 to 4.8 g L^{-1} day^{-1}, respectively. The astaxanthin content and its productivity ranged from 0.8 to 4.8% of DW and 0.44–21 mg L^{-1} day^{-1}, respectively (Tables 1 and 2).

One-step Strategy

Simple method for efficient production of astaxanthin is by *H. pluvialis*. It incorporates the employment of nitrate starving in combination with a constant standard irradiance with in growth media (del Río et al., 2005; del Río et al., 2008; García-Malea et al., 2009). Thus, the one-step technique was used to induce synchronous cellular proliferation and astaxanthin accumulating at a considerable level in a laboratory environment under continuous light illumination, yielding a median astaxanthin yield of 20.8 mg L^{-1} day^{-1} (García-Malea et al., 2009). On a pilot scale, researchers investigated the practicability of this approach in an outdoor tubular bioreactor. The productivity of biomass and astaxanthin was calculated to be 0.7 g L^{-1} day^{-1} and 8 mg L^{-1} day^{-1}, respectively.

Two-step Vs One-step Strategy

However, the one-step procedure is less complicated than the two-step technique and astaxanthin manufacture occurs in a continuous fashion as preferred. But it drops two significant drawbacks. First, when compared to two-step method, the actual astaxanthin production is remarkably lower. Second, it requires light illumination during night as it is unsuitable for outdoor cultivation. Thus, it is too expensive (Aflalo et al., 2007).

4.3 Harvesting

This is the most difficult and restricting component of *H. pluvialis* industrial biomass production. Harvesting of biomass is depended on the morphological features of the cell, which includes size, shape, specific weight, and cells concentration (Lam & Lee, 2012). Centrifugation is the most usual technique for harvesting and combined with other processes. The hematocysts are split up by means of a passive settlement, due to its high density and eventually robust after centrifugation (Lorenz & Cysewski, 2000; Olaizola, 2000; Pérez-López et al., 2014). Around 13.5% of total suspended solid in the form of algal cake is acquired via this procedure (Li et al., 2011). Disk-stack centrifugation and flotation efficiently recovered biomass of more than 95%, and both are alternatives for *H. pluvialis* harvesting technique (Panis & Carreon, 2016).

4.4 Rupture of Cells

Cell disruption step can be carried out either before or after drying the biomass. It attempts to enhance the recovery efficiency of the intracellular compounds.

Accumulation of astaxanthin induces the thickness of the cells, turning firm and rigid. The cell wall of hematocysts possesses three layers, one sporopollenin, and two cellulose with mannose (Hagen et al., 2002; Sun et al., 2016). Hence, it is mandatory to apply pretreatment methods capable of weakening this resistance.

Mechanical process is involved widely in commercial scale and more particularly bead milling along with high pressure homogenizer (Razon & Tan, 2011; Shah et al., 2016). Bead milling technique follows by colliding minute spheres by revolving at intense speed (Onumaegbu et al., 2018). When the biomass concentration after accumulation is between 100 and 200 g/l, it is considered the most effective method (Greenwell et al., 2010). On the other hand, either one two displacement pumps compress the cells at elevated pressure to disrupt the thick cell wall in a high pressure homogenizer (Lee et al., 2012). It is simple and minimizes contamination. Nearly 75% of recovery efficiency can be reached in a single step. After cell disruption, it is suggested to keep cells from exposure to light and immediately execute the astaxanthin extraction (Khoo et al., 2019). Cheap and best alternative for cell disruption is the use of pulverizer. It is widely used in food industries, and the mechanism is similar to bead milling.

4.5 Drying

Drying preserves the quality of the pigment, longer shelf life, and it must occur immediately after harvesting to avoid spoilage. Most suitable methods are spray-drying and freeze-drying techniques (Li et al., 2011). Freeze-drying deals with freezing of algal cake. It inflicts less damage and more expensive on industrial scale when compared to spray-drying. Spray-drying is rooted on a forced course of hot air inside a drying compartment. It suddenly vaporizes the droplets when they get exposed to the air, and it is regarded as the most convenient process (Li et al., 2011; Panis & Carreon, 2016). Spray-drying delivers a recovery efficiency of about 95% of dry biomass in powder (Leach et al., 1998). Main setbacks of this method are certain risks in deterioration of pigments and high operational costs (Grima et al., 2003).

4.6 Astaxanthin Extraction

The recovery of specific product is feasible whenever the cell wall is shattered, and the biomass is perfectly dry. Astaxanthin is soluble in solvents and oils, as it is lipophilic in nature. Solvents used for extraction includes alcohols (ethanol, methanol, etc.), acetone, ether, concentrated acids or bases (potassium hydroxide, dimethyl sulfoxide, etc.), aliphatic hydrocarbons (hexane), edible oils, and supercritical carbon dioxide (CO_2-SC) (Khoo et al., 2019; Shah et al., 2016). Between these, CO_2-SC and conventional solvents are regarded as the most effective, well suited, and sustainable to be assigned in *H. pluvialis* (Shah et al., 2016). In commercial scale, CO_2-SC is widely employed due to its quicker extraction period, low toxicity, low

degradation, cost effective, and high fineness of astaxanthin when compared to conventional solvents. While in the case of solvents, it is significant to assign the type of solvent used, since many possess higher toxicity leading to serious health issues (Khoo et al., 2019).

5 Establishment of Mass Production System

For establishment of the production setup, a sum of ₹555,900 was required for lab-scale production, ₹2,716,350 for small-scale production, and ₹17,159,400 for large-scale production. The main sections of production units are a bioreactor, raceway pond, centrifuge, pulverizer, spray dryer, and storage containers. Non-recurring expenditure and laboratory equipment's essential for astaxanthin production is denoted in Tables 3 and 7. A standard two-step approach was practiced for the production of astaxanthin. The vegetative stage of *Haematococcus pluvialis* was cultivated in a 5 L photo bioreactor; here, we have accounted a total of 3 and 10 bioreactor in small-scale and large-scale production, respectively. These photo bioreactors are operated in a semi-continuous mode which supplies inoculum (cyst stage) for the pond. In small-scale production, a single pond is considered and a total of five ponds have been constructed for large-scale production. While in the case of lab-scale production, open raceway ponds are absent and single 5 L photo bioreactor is utilized for cultivation of both vegetative and cyst stage.

The temperature of bioreactors is maintained between 10 and 25 °C using cooler. Amount of CO_2 is also controlled in both pond as well as bioreactor to control the pH. The aeration is provided through the sparger with high pressure pumps, and bioreactor consists of both air and media filter which are involved in sterilization. A motor-powered paddlewheel is used to keep the suspension of cells by turbulence. The cells are harvested and stored in cell storage containers, which form a cell slurry. These cell slurries are dehydrated using a centrifuge, and resultant algae paste was powdered using a spray dryer. Then, dried cells were further powdered using pulverizer. Recurring expenditure for each batch of biomass production is represented in Table 4.

5.1 Production Parameter

The production parameter was considered as per (Li et al., 2011). The temperature is maintained below 25°C using coolers. The inoculum of size 5–9 $\times$ 10^4 cells/ml are required for initial cell concentration. The working cell standard and final concentration are 4.2–6.0 $\times$ 10^5 and 5–8 $\times$ 10^5 cells/ml respectively. Pond depth must be around 13–15 cm, and water flow rate is adjusted to 25–30 cm/s. The initial cell concentration for inoculation is 5–7 $\times$ 10^4 cells/ml. It takes up to 8 – 10 days for reddening of inoculum. The above-mentioned values are prescribed in Table 5.

For the downstream processing of high-quality astaxanthin, the final dry weight is required to be 0.25–0.59 g/L. Cell slurry acquired after sedimentation needed to be

Table 3 Non-recurring expenditure for astaxanthin production

S. No	Requirements	Lab-scale production		Small-scale production		Large-scale production	
		Volume	Total cost (in ₹)	Volume	Total cost (in ₹)	Volume	Total cost (in ₹)
1	Land expenditure	–	–	0.5 acres	6.5 lakhs	2 acres	5,227,000 lakhs
2	Building construction	–	–	200 sq. ft	2 lakhs	600 sq. ft	7.5 lakhs
3	Raceway ponds (10,000 L)	–	–	1 unit	3 lakhs	5 units	15 lakhs
4	Photobioreactor (5 L)	1 unit	100,000	3 unit	3 lakhs	10 units	30 lakhs
5	Centrifuge	1 unit	15,000	1 unit	50,000	5 units	25 lakhs
6	Pulverizer	1 unit	20,000	1 unit	20,000	2 unit	3 lakhs
7	Spray dryer	1 unit	1 lakh	1 unit	1 lakh	2 unit	7 lakhs
8	Storage container for harvested cell broth	1 unit	10,000	1 unit	50,000	4 unit	2 lakhs
9	CO_2 storage container	–	–	1 unit	1 lakh	1 unit	5 lakhs
10	Tubing along with air compressor	–	–	1 unit	50,000	1 unit	2 lakhs
11	Piping, pumps, valves, and controls	–	–	1 unit	1 lakh	1 unit	5 lakhs
12	Electrical and engineering cost	–	–	1 unit	1 lakh	1 unit	4 lakhs
Total cost		₹245,000		₹2,020,000		₹15,777,000	

Table 4 Recurring expenditure for astaxanthin production

S. No	Process	Lab-scale production		Small-scale production		Large-scale production	
		Consumption	Total cost (in ₹)	Consumption	Total cost (in ₹)	Consumption	Total cost (in ₹)
1	Temperature maintenance and cooling of culture in bioreactor	10 kWh	80	60 kWh	480	200 kWh	1600
2	Sparger or aeration power consumption	15 kWh	120	50 kWh	400	150 kWh	1200
3	Paddlewheel—power consumption	–	–	100 kWh	800	1000 kWh	8000
4	Total CO_2 consumption	2 kg	800	15 kg	6000	50 kg	20,000
5	Centrifuge—power consumption	2 kWh	16	5 kWh	40	30 kWh	240
6	Spray-drying—oil consumption	0.2 kg	200	1 kg	700	8 kg	5600
7	Pulverization—power consumption	2 kWh	16	3 kWh	25	18 kWh	150
8	Medium filters and air filters	1 unit	500	3 unit	2000	10 unit	5000
Media composition (BG-11)		5 L		15 L		50 L	
Sodium nitrate		7.5 g	7	22.5 g	23	75 g	63
di-potassium hydrogen phosphate		0.2 g	0.3	0.6 g	0.9	2 g	4
Magnesium sulphate		0.37 g	0.19	1.2 g	0.6	3.7 g	2
Calcium chloride		0.18 g	0.5	0.55 g	1.6	1.8 g	5
Sodium carbonate		0.1 g	0.06	0.3 g	0.2	1 g	0.7
Citric acid		0.03 g	0.03	0.09 g	0.09	0.3 g	0.3
Ferric ammonium citrate		0.03 g	0.02	0.09 g	0.06	0.3 g	0.2
EDTA		0.005 g	0.003	0.015 g	0.009	0.05 g	0.03
Total cost		₹1750		₹10,500		₹42,000	

Table 5 Production parameters of photobioreactor and open raceway pond

Photo bioreactor	
Parameter	
Temperature	Below 25 °C
Cell concentration for inoculation	5–9 × 10^4 cells/ml
Working cell concentration	4.2–6.0 × 10^5 cells/ml
Final cell concentration	5–8 × 10^5 cells/ml
Renewal rate of photo bioreactor	0.28/day
Raceway pond	
Parameter	
Pond culture depth	13–15 cm
Water flow rate	25–30 cm/s
Cell concentration for inoculation	5–7 × 10^4 cells/ml
Reddening of inoculation	8–10 days
Final cell concentration	6–7 × 10^5 cells/ml

Table 6 Downstream processing of biomass

Downstream processing	
Final concentration of biomass in dry weight	0.25–0.59 g/L
Percentage of dry biomass in cell slurry	1.1–1.6%
Percentage of dry biomass in cell pastes	10–16.2%
Astaxanthin content in dry biomass	2–3.7%

around 1.1–1.6% of dry biomass. In case of cell paste, the dry biomass percentage ranges in between 10% and 16.2%. By estimating all the above values, the astaxanthin content produced will be around 2–3.7% (Table 6).

6 Cost Benefit Analysis

Considering the time taken for the inoculum to accumulate astaxanthin in the pond, we have assessed the overall expense per each batch which is demonstrated in Tables 7 and 8. It includes temperature maintenance, cooling of culture, aeration consumption rate, power consumption of centrifuge, paddle wheels and pulverizer, rate of CO_2 utilized, oil consumption of spray dryer, air filters, and media composition cost.

The direct production cost of biomass and astaxanthin per batch is around ₹1750 and ₹250 (lab-scale), ₹10,500 and ₹2000 (small-scale), and ₹42,000 and ₹9000 (large-scale), respectively. Annual production cost of biomass and astaxanthin is estimated to be ₹52,500, ₹315,000, and ₹ 1,260,000, respectively. The astaxanthin production per batch is expected around 5 kg per batch in large-scale production, eventually producing 650–800 kg each year. Astaxanthin yield may vary due to certain factors such as pH, sunlight, temperature, etc., While in small-scale production, astaxanthin production is around 0.5–1 kg per batch and produces 30 kg/year approximately. Although the astaxanthin production is comparatively lesser in small-scale production, it provides a sustainable profit. Astaxanthin production per

Table 7 Laboratory equipment for astaxanthin production

S. No	Laboratory instruments	Lab-scale production		Small-scale production		Large-scale production	
		Unit	Total cost (in ₹)	Unit	Total cost (in ₹)	Unit	Total cost (in ₹)
1	Light microscope	2	26,000	5	65,000	10	130,000
2	Measuring cylinder						
	100 ml	2	500	5	1250	10	2500
	250 ml	2	1000	5	2500	10	5000
	500 ml	2	1250	5	3000	10	6200
	1000 ml	2	1500	5	3700	10	7000
3	Beaker						
	500 ml	2	600	5	1500	10	3000
	1000 ml	2	1000	5	2500	10	5000
4	Erlenmeyer flask						
	100 ml	5	1250	10	2500	20	5000
	250 ml	5	2000	10	4000	20	8000
	500 ml	5	3000	10	6000	20	9000
5	Test tube	20	800	40	1400	60	2000
6	Weighing balance	2	2500	5	7500	10	15,000
7	Mortar and pestle	2	3500	5	9000	10	15,000
8	Autoclave	2	40,000	5	100,000	10	200,000
9	Pressure cooker (3 L)	2	3000	5	7000	10	10,000
10	Tissue culture storage rack	1	5000	5	25,000	10	50,000
11	Biosafety chamber	1	200,000	2	400,000	4	800,000
12	LED light	20	6000	40	12,000	60	24,000
13	Tissue culture flask	40	10,000	150	37,500	300	75,000
14	Polypropylene beaker (5 L)	2	2000	5	5000	10	10,000
	Total cost	₹310,900		₹696,350		₹1,381,700	

year in lab-scale is estimated to be 1000 g. The current market value of astaxanthin varies between ₹166,534 and ₹ 501,118 per kg and around ₹300,000,000 per ton. The biomass will be harvested 30 times a year. In large-scale production, 750 kg of astaxanthin is expected to be harvested which provides a capital of ₹225,000,000 per year through the sales of astaxanthin. In small production, as mentioned earlier, 30 kg will be produced per year generating a total of ₹9,000,000 per year. Laboratory scale generates a capital of ₹ 300,000 per year. The cost may vary based on the nature and purity of the pigment accumulated. With deducting expenses, including production cost, labor cost, and some additional expenses. The net profit is estimated to be ₹197,500 (lab-scale production), ₹6,885,000 (small-scale production), and ₹216,740,000 (large-scale production) (Table 8).

Table 8 Revenue generation and net profit analysis

Cost analysis	Lab-scale production	Small-scale production	Large-scale production
Total set-up cost	₹555,900	₹2,716,350	₹17,159,400
Direct production cost of biomass per batch	₹1750	₹10,500	₹42,000
Direct production cost of astaxanthin per batch	₹250	₹2000	₹9000
Total cost of production per year	₹52,500	₹315,000	₹1,260,000
Estimated amount of astaxanthin production per batch in each pond	–	1 kg	5 kg
Total production of astaxanthin per year in each pond	–	30 kg	150 kg
Total production of astaxanthin per year considering all ponds	1000 g	30 kg	750 kg
Current net worth of astaxanthin per kg (1 ton—300,000,000)	₹166,534–501,118	₹166,534–501,118	₹166,534–501,118
Total cost produced by sale of astaxanthin per year	₹300,000	₹9,000,000	₹225,000,000
Labor cost per year	–	₹1,500,000	₹6,000,000
Total production cost per year + labor cost per year + additional expenses—total cost produced by sale of astaxanthin per year	₹52,500 + ₹50,000 − ₹300,000 = ₹197,500	₹315,000 + ₹1,500,000 + ₹300,000 − ₹9,000,000 = ₹6,885,000	₹1,260,000 + ₹6,000,000 + ₹1,000,000 − ₹225,000,000 = ₹216,740,000
Net profit	₹197,500	₹6,885,000	₹216,740,000

7 Current Global Market for Astaxanthin

With the current trend of using natural components in cosmetics, food, and medical applications, there is a considerable concern about synthetic ingredients entering the human food chain. Increasing knowledge of the health benefits of natural astaxanthin, the global demand for astaxanthin extracted from *H. pluvialis* has been rapidly increasing. In 2021, Astaxanthin's global market is predicted to be worth USD 647 million. During the forecast period, it is predicted to reach around USD 1206.52 million by 2026 and recording a CAGR of 7.7% (Market Research Future, 2021). Depending upon the purity levels, the market value of astaxanthin tends to vary between USD 2000–15,000/kg (Koller et al., 2014). In the aquaculture field, yeast-derived astaxanthin and synthetic astaxanthin are predominantly consumed. While for human consumption, the *H. pluvialis*-derived astaxanthin is recommended for cosmetics, dietary supplements, and food (Nguyen, 2013).

When compared to synthetic astaxanthin, in medicinal and nutraceutical uses, natural astaxanthin is 3–4 times more effective and valuable than artificial astaxanthin (Han et al., 2013). Because there is an increasing interest in natural astaxanthin, mass growth of *H. pluvialis* on a commercially has a lot of promise and could be a lucrative company with huge opportunities in trading enterprises. Synthetic astaxanthin can be toxic and carcinogenic, due to its presence of stereoisomers (Khoo et al., 2019).

Astaxanthin business has been gaining interest due to the following reasons:

1. Bioactive profile which involves antioxidant and free radical scavenging activity.
2. Growing consumer needs on personal care, cosmetic, and nutraceutical products (Global Market Insights, 2018).
3. Huge potential for industrial applications.
4. Gaining popularity in several health spa service providers and cosmetic producers (Global Market Insights, 2018).
5. Rise in health consciousness and demand for natural food additives (Grand View Research, 2017).
6. Natural astaxanthin promotes better pigmentation in some species of fishes.
7. U.S. FDA approved astaxanthin as safe (GRAS) for dietary supplements and authorization from several European food organizations (Shah et al., 2016).

As a commodity of extraordinary commercial value, astaxanthin alone explains the high price of *H. pluvialis* cultivation and its processing setups, making it an economically practical job (Shah et al., 2016). Application of biorefinery models for the astaxanthin production from *H. pluvialis* turns into more sustainable and feasible. In this method, incorporation of bioprocesses leads to production of desired products and co-products of more superior value with slightest waste (Fig. 7). Biorefinery of microalgae enables the processing of various biomass products, which includes carbohydrates, proteins, lipids, and bioenergy utilizing a single raw material. As follows, revenues are maximized (Chew et al., 2017). Simultaneous production of astaxanthin, triglycerides, and polyhydroxybutyrate (PHB) can be obtained and becomes a possible source of biodiesel and biopolymers, respectively. The residual microalgal biomass can be utilized in biogas plants for its production processes (Prieto et al., 2017). The residual biomass performs vital role in producing methane to generate thermal energy for industrial facilities, protein rich feed for animal consumption, and carbon source for fermentation industries (Chew et al., 2017; Oliveira et al., 2020).

8 Conclusion

Haematococcus pluvialis holds the highest capacity in accumulation of astaxanthin, and it is the source for obtaining the carotenoid with tremendous market value. Even if it comes with high production costs, these can be reduced with the addition of effective methods of cultivation and processing of biomass. Biorefinery modelling

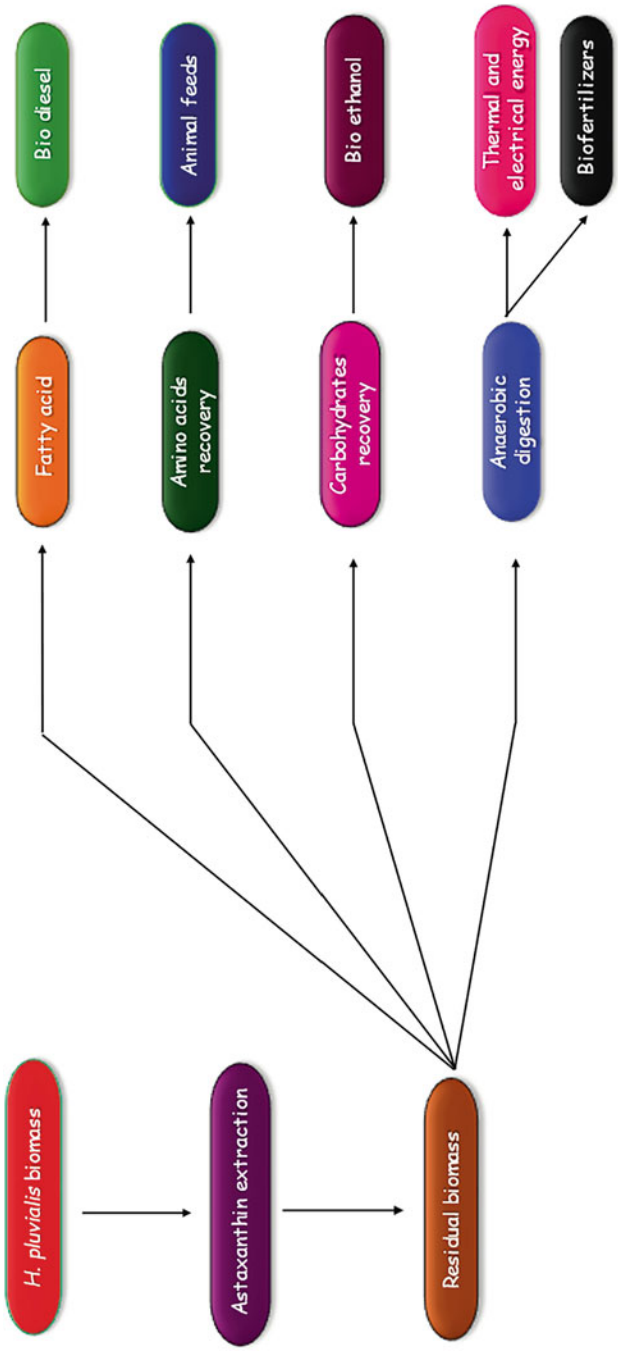

Fig. 7 Application of biorefinery approach to *H. pluvialis* and astaxanthin as desired product of interest

makes it feasible to attain high-value added products and co-products. Organic astaxanthin has a higher demand than artificial astaxanthin, leading to increased rate of production. Also, the animal feed industry has exhibited prominent growth and demand for astaxanthin. As a result, this bioproduct has the potential to reach the top of the market.

Commercial synthesis of astaxanthin using *Haematococcus pluvialis* is indeed a potential using current modern technologies. The total manufacturing expenditure of astaxanthin per batch in large-scale production is estimated to be ₹51,000. While in lab-scale and small-scale production, the production cost is evaluated to be ₹2000 and ₹12,500, respectively. When compared to current industrial processes, the production cost is considerably inexpensive. Lab-scale production of astaxanthin can be preferred for optimization of production processes, due to its low yield. The large-scale production is more efficient due to better quality and quantity of photobioreactors, raceway ponds, and other instrumentation used in the cultivation process. Small-scale production is budget friendly and best option in the case of low investment scenarios.

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Production, Cost Analysis, and Marketing of Probiotics

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Abstract

A significant focus has been given to understanding the probiotic manufacturing process at a commercial scale with different production settings, and a thorough evaluation of probiotic-product performance as well as a benefit-cost analysis is needed for the coveted future marketing. This notion is gaining the acceptance among the customers as food-makers become more commending about designing foods that create consumers' awareness in association with health, nutrition, and diet, as well as add value to food via inclusion of desirable ingredients. Probiotic products are the important topics in the functional foods all over the world, and researchers are striving hard to improve dairy products that include beneficial probiotic organisms. Such probiotic products may alter composition of microorganism in the gut, enhanced gut health. People can tolerate the milk proteins (casein) and regulate their cholesterol intake exponentially by using these probiotics. A large quantity of living microorganisms are likely to be required in the food product, which should be ingested on a regular basis to gain the benefits. All over the world, the probiotic business, especially, the dairy products yogurt and fermented milks have escalated. Many factors are responsible to fulfill consumers' expectation such as sound and scientifically proven health-promoting outcomes, accurate product information, and effective marketing strategies. Since the probiotics have demonstrated long-term success as health promoters both in human and animal health. This is to be applauded for the decision to formulate an

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effective probiotic product as the high numbers of viable organisms will be required at the time of intake. Despite the several health benefits of probiotics, research is underway by which the culture utilizes their effects is still at the earlier stage. Therefore, continuing scientific efforts have targeted to understand health-promoting effects of probiotic cultures in the cellular level as a critical obligation for ensuring probiotics' future as a functional dietary supplement. In the upcoming days, the global market for purposeful foods is anticipated to be increased considerably. Therefore, the aims of this exploration were to delve the production-processing considerations and alternate cost variables for probiotic production including all-inclusive benefit-cost analysis in global perspectives. Additionally, these insights have focused on how to gain money from microbes and probiotic marketing status as well as ensure a food microbiology-based entrepreneurship in probiotic domain.

Keywords

Probiotic · Manufacturing · Food makers · Ingredients · Global market · Microorganisms · Probiotic products · Entrepreneurship

1 Introduction

Based on the type of products, probiotics can be classified into dietary supplements, probiotics drinks, and drugs, including animal/livestock foods/feeds. Probiotic food is again sub-classified into breakfast/bakery, yogurt, infant formula, and baby food cereals including other probiotic foods. Moreover, the probiotic drinks are sub-segmented into dairy-based probiotic drinks and fruit-based probiotic drinks (Modor Intelligence, 2022).

In every step of probiotic production, process is dependent on the one before it; thus, this is challenging to determine the species and/strains which are dependent to sensitivity. Because of the cut-down method for producing cells at experiment, development work is intrinsically more closely regulated and each phase in the process has shorter pause durations, and thus, scaling up can be complicated over the times. Commercial centrifugation of cells from the spent media could require hours of time due to the large volume of cells versus minutes at lab-scale as the smaller volumes involved. These results reflect at different stresses in generally heat and absolute stress condition than those experienced at upright lab-scale centrifuge (Crittenden, 2009). In addition, there are a number of stages relating to the commercial production that are not usually involved in bench-scale production. Also, throughout commercial manufacturing, cells are likely to be exposed to a variety of pH and temperature conditions that are difficult to replicate at the lab-scale. As a result, this is critical to scale up to an intermediate level in trial to evaluate and optimize the typical production environments, and to mitigate the challenges before moving to commercial operations. The scaling up from trial to commercial basis can be difficult for the same grounds as scaling up from lab scale to pilot scale which needs further trials for commercial applications (Kurt et al., 2019). In livestock

production, cost benefit analysis would be streamlined to substantiate the usage of probiotics and the profitability at the production level to end of experimental trails. All cost items which included chicks, feed, vaccine, electricity, and casual labor were taken into consideration when estimating the total cost of production. Besides, the expense of trial ingredients was also included to the treated groups. The base cost (cost/bird) and also cost/kg live weight basis were used to calculate the cost benefit estimation (Ray et al., 2019).

In this moment, assurance of a probiotic impact, including the development of foodstuffs which contained large numbers of living organisms, needs to be explored and involved with the formation of effective probiotic items. Notwithstanding the fact that probiotics are connected with a variety of health benefits, research onto the progressions by which this culture work is still in its initial stages. As a result, persistent scientific efforts targeted to the health-promoting effects of probiotic cultures at the cellular level should be investigated as an impotent necessity to confirm probiotics' future as functional food additives (Catherine et al., 2001).

Food manufacturers will be continuing to develop new diets, resulting in more food ingredients being linked to health claims. In the near future, sport-related items, fortified foods and drinks, and dairy products, for example, yogurts, cheese, ice cream, and milks containing prebiotics and probiotics are all likely to be incorporated. Although the market for the functional ingredients is favorable, scientific validation is essential for future expansion as well profitability issues. In present time, since most of antimicrobials are found to be ineffective globally, thus, antimicrobial resistance (AMR) is escalating at an alarming rate as Neu (1994), which have serious impact on health and overall development throughout the world. Therefore, multisectoral actions are to be needed to obtain the Sustainable Development Goals (SDGs) (WHO, 2021). Given the situation, very high concern about antibiotic uses (AMU) use and antimicrobial resistance (AMR), therefore, alternative options for pathogen suppression, such as probiotics and prebiotic including organic acids, herbal product, are becoming more demanding. The World Health Organization (WHO) advocates to increasing efforts towards prevention of disease by enhancing immunization with vaccination including global initiatives to lessen the use of antibiotics in food animal production (Kabir, 2009; Kamruzzaman et al., 2005) including human health perspectives.

Probiotics have also been recommended as an alternative to antibiotics in livestock (Canganella et al., 1996; Fuller, 1986; Gardiner et al., 1999; Underdahl et al., 1982) and poultry productions (Arif et al., 2021; Edens et al., 1997; Gardiner et al., 1999; Islam et al., 2014).

2 Historical Aspects

Historically, usually the fermented foods are connected with the exploration and understanding of probiotics or useful microbial agents, and it is thought that we are using the probiotics since the first edible stuff experienced from the fermentation process. The International Scientific Association for Probiotics and Prebiotics

(ISAPP) gave the definition of a fermented food as "foods made through desired microbial growth and enzymatic conversions of food components" (Marco et al., 2021). The fermented food items like beer, kefir, cheese, wine, and bread have a long the past, and their usage can be carried back to hundreds of years, even earlier to Egyptian and Middle-Eastern civilization.

Numerous preliminary approaches of treatment, such as bacterial interference, serum therapy, and the activation of macrophages to kill germs, may be worth to reconsidering (Bengmark, 1998). In this regard, several probiotic strains have been demonstrated to reduce the growth of enteropathogens such as *Salmonella enteritidis*, enterotoxigenic *Escherichia coli*, and *Serratia marcescens* (Gonzalez et al., 1993; Drago et al., 1997) and may have therapeutic potential in vitro. The recent data indicate that *Lactobacillus* GG has antagonistic activity against *Salmonella typhimurium* C5 infection both in vitro and in vivo (Hudault et al., 1997), which makes the groundwork for the clinical application of probiotics through pathogen suppression. Also, probiotics' immunostimulatory characteristics (Marteau & Rambaud, 1993) have likely to increase the host's resistance to disease, potentially reducing the need for antibiotics. In addition to their role in human health maintenance, new evidence suggests that some probiotic strains, particularly *Lactobacillus* strains, may protect the urogenital tract from microbial infections, and that probiotic microorganisms can reduce the risk of infections associated with the use of medical devices (Reid et al., 1994, 1995, 1998).

Table 1 represents the time-line summary on the discovery of probiotics which gives a primary understanding of the idea of probiotics, prebiotics, paraprobiotics, postbiotics, and synbiotics that evolved in 1905 and continuing its advancement as of date (Cuevas-González et al., 2020; Hill et al., 2014; Lee & O'Sullivan, 2010; Lilly & Stillwell, 1965; Mackowiak, 2013). However, robust efforts are ongoing for development of new probiotics considering benefits both in human and animal health.

In most recent years, the probiotics have been documented as multifaceted benefits as functional ingredients, such as anticancerous (Górska et al., 2019; He et al., 2019; Mendoza, 2019), prophylactic and antigenotoxic, (Chandel et al., 2019), antioxidative (Mishra et al., 2015), anti-inflammatory (Plaza-Díaz et al., 2017; Posocco et al., 2018), antiosteoporotic (Lee & Kim, 2020), antihypertensive (Rai et al., 2017), and antidiabetic prospects (Wang, Bai, et al., 2020; Wang, Shang, et al., 2020). There is unanimous consensus on the positive impact of probiotics on psychological state of the well-being via alteration of sleep, cognitive reactions, and mental condition (Marotta et al., 2019).

3 Production and Processing

3.1 Detection of Strains

Strain identification is the first pivotal step in the probiotics production process. The strain selection exclusively be influenced by the objectives for producing a particular probiotics supplement which have significant health claims. When you intend to

Table 1 Time line for successful development of probiotics, prebiotics, synbiotics, postprobiotics, and postbiotics (adapted from Gao et al., 2021)

Year	Discovery	Reference
1905	Detection of lactobacillus in yogurt and its connection with the longevity and gut performance	Mackowiak (2013)
1906	Detection of Bifidobactin in feces of infants and their role in protection against organisms in the intestine and prevention of childhood diarrhea	Lee and O'Sullivan (2010)
1960	The term probiotic was first introduced as "substances produced by the one microorganisms which stimulates the growth of another microorganisms"	Lilly and Stillwell (1965)
1974	The definition of the term "probiotic" was corrected as growth promoting animal feed supplements	Parker
1989	The definition of the term "probiotic" improved as "microbial feed supplement which beneficially affect the host animal by harmonizing its microbial balance"	Mackowiak (2013)
1989	The most updated definition of probiotic is "live microorganism that when administered in adequate amounts, confer a health benefits to the host" introduced	WHO (2021)
2013	A probiotic recently defined as a substance selectively utilized by the host microorganisms conferring health benefit in symbiotic condition.	International Scientific Association for Probiotics and Prebiotics (ISAPP)
	The definition was further revised and grammatically corrected as "live microorganisms which when administered adequate amount confer a health benefit to the host"	
2015	Non-viable microbial cells or supernatants (paraprobiotics or interactive probiotics), microbial metabolites, and growth promoting elements (postbiotics) have also emerged	Mackowiak (2013)

make a supplement to favor digestion, to boost health of your immune mechanism, prove a healthy response to infrequent stress, or further (Nutra Science, 2022).

Since each strain has particular features, they confirm specific benefits. A few strains help healthy immunity, whereas others assist lactose digestion. This is unnecessary to depict that for manufacturing highest quality supplements, raw materials contained excellent-grade probiotics are needed. The identified strains should live within the gut to validate the effectiveness of the probiotics. The commonly applied probiotics are generated under the genus *Lactobacillus*, is a group of lactic acid producing beneficial bacteria that consist of around 400 probiotic species in the human body (Healthline, 2022). Other genus like *Bifidobacterium*,

Bacillus, and *Streptococcus* are commonly used for probiotic production (Nutra Science, 2022).

3.2 Nutritional Prerequisites

The dietary requirements for growth and performance, the nutritional capacity of the environment in which the microbe was adapted and from which probiotics was sourced and is typically connected with the complexity of these auxotrophies, and nutrient demand (Hebert et al., 2004). Understanding the nutritional requirements of the microbe and establishing a particular fermentation medium that promotes growth including improving the cells' ability to survive, and respond to the stresses imposed by the manufacturing process is vital to producing a high-performance final product. Identifying strain-dependent dietary requirement necessitates a multidisciplinary, empirical knowledge-based approach. For probiotic preparation, LAB and *bifidobacteria* are demanding microbes in these regards. Around 20 amino acids are auxotrophic in LAB and *bifidobacteria*, and they have food requirements that must be met from the outside environment in order to grow. The ability to assess the genome (genomics), gene expression (transcriptomics), protein expression (proteomics), and metabolism (metabolomics) of strains provides important knowledge which is useful for the assessment of strain-dependent nutritional needs and capabilities, and the final performance of the manufactured product (Sieuwerts et al., 2010; Siragusa et al., 2014; Smokvina et al., 2013).

Using these methods to understand the composition of intricate basic ingredients allows researchers to match critical fermentation media ingredients for the nutritional needs of the species and/or strain development, allowing them to squeeze the media and process to improve species and strain performance while also lowering manufacturing costs and increasing efficiency. Thus, there is a wealth of empirical data that can be collected with the proper skills and innovative ideology. When combined, these approaches were found to be extremely powerful for understanding species and strain dependencies, species and strain sensitivities, nutritional needs, and nutritional limitations, and therefore, high-performance strains to be successfully developed and manufactured that meet customer expectations.

3.3 Raw Materials with Special Requirements for Growth Media

The raw ingredients used to make probiotics and other dairy starter cultures must be carefully selected and monitored. However, there may be need for the media coverage from the client and/consumer in addition to the organisms' development requirements (Kurt et al., 2019).

3.4 Fermentation and Processing of Raw Materials

The raw material for producing probiotics can be obtained from around the world. Probiotics are specially manufactured to conserve wholesomeness and excellence. Culture of bacteria could take up to 6 weeks of duration. This could not be shortened the cultivation time when the cultures are developed. Every time, detailed strain ID records are specific in the material received from supplier. Therefore, this is needed to be dependent on a specific supplier as others could not afford you with the desired strain with IDs. Likewise, other resources may not be instantly obtainable in the essential quantity with the agreement manufacturer, which may further delay from submission of a request to supply of products.

All the equipment and nutrients should be decontaminated to remove any undesirable contamination. The strain to be included to the media is in a big tank. The strain reproduces in the nutritious and warm component bath till it confirms the desired count colony-forming units (CFU). The metabolites are also generated during the process as a byproduct of the bacteria's metabolism (Nutra Science, 2022).

Fecal microbiota and fermented products with indefinite microbiota cannot be considered as probiotic (Hill et al., 2014). If there is no cell count level of a probiotic strain in a food product, this could not be assured the health benefits. However, this is to be needed a minimal CFU of 10^6–10^8 CFU/g is recognized as an adequate number which can ensure probiotic benefits (Champagne et al., 2011).

The fermentation medium is so important in the production of probiotics; however, several modifications in the raw materials can have a significant impact on growth and performance. The supplier's modifications to raw materials could be the result of cost reductions from the process improvements, which result in a change in ingredient sources or variation in the production process. Such changes to complex ingredients can sometimes go undetected, with some probiotic strains have apparently consistent performance; however, the performance of other strains is more obviously affected in a positive or negative way, depending on the nutritional requirements and sensitivities of the probiotic strains being manufactured (Tanguler & Erten, 2008).

3.5 Centrifugation

When the cultures are available, the metabolites are required to segregate from the probiotic strains. A close attention is needed during probiotics production process for confirming the stability of probiotics. Probiotic products start to misplace their freshness during the moment of packaging. Various maneuverers are employed to keep stability of supplements including potency for a long duration storage. There are several processes which are important and affect the viability of probiotic strains' and appropriateness of the application, are refrigeration: probiotic bacteria are taken to severe low temperatures; avoiding hot condition: this step allows the bacteria remove from humidity (a few drying methods); freeze-drying—a more longer,

however gentler process and spray-drying: a shorter process with higher temperatures, however, not too high for the bacteria to live (Nutra Science, 2022).

3.6 Probiotics in Dietary Supplements

Probiotics in the form of freeze-dried powder are commonly used in dietary supplements. For human beings, the most prevalent formats on local stores are capsules, tablets, and powder in stick packaging or sachets, which are typically maintained at room temperature. Beside these, we observed that liquid probiotic formats are available in poultry production. However, dietary supplement items should confirm the probiotic count and clearly mentioned on the label regarding product's shelf life. This ensures that the consumer gets the right amount of probiotics to effect the health claim's targeted structural function or otherwise indicated health benefits. This is crucial to describe each strain's stability, so that the appropriate amount of overage can be added during the preparation of a food additive format to corroborate that each individual strain in a multi-strain formulation has the lowest possible count.

Single strain product quality in a multi-strain formulation is extremely difficult especially when numerous strains from the same species are used. Though theoretical molecular-based strategies have been reviewed, there are currently no widely applicable and effective procedures available (Lai et al., 2017).

3.7 Bottling

The shelf life of dietary supplement forms is often measured in years; therefore, significant precautions should be taken to ensure that the proper probiotic cell count is provided. As the probiotics are living microorganisms, even when freeze-dried, they need proper care and attention than other dietary supplements and food ingredients (Broeckx et al., 2016; Forssten et al., 2011); however, the availability of technical facilities, expertise, and cost of production should be ensured in the format of probiotic supplements. Considering probiotic viability, not all plastic bottles are appropriate, since PET bottles should never be utilized since their structure permits for too much moisture absorption than the high-density polyethylene bottles (Muller et al., 2014).

3.8 The Probiotic and Nutritional Food Markets in Previous Decades

In general, the market demonstrated substantial increase for health-based probiotic products especially among the younger generations. Therefore, probiotics are found to be a part of beverages and functional foods, presented for enhancing gut function, including other benefits such as boosting immunity (Modor Intelligence, 2022).

During the last decade, the public awareness was created due to potentiality and efficaciousness of probiotics, and thus, consumers' trust was developed for something have health benefits, and the people were willing to pay extra money was the primary reasons for the success of probiotics fortified foods in these markets. Until 2011, Japan, United States, and Western Europe were the biggest markets for probiotics in terms of consumption and production. Besides, after 2011, Eastern Europe, Asia Pacific, and Brazil have demonstrated enormous growth in probiotics and are anticipated to remain this production pace over the next 5 years (Lim et al., 2015).

At present time, probiotics documented a very big business, where global functional food market has been approximated yearly USD 50 billion share (Pineiro & Stanton, 2007), including world probiotic market is calculated to USD 15 billion. Nowadays, this market is soaring at a direction of 5% to 30% considering type of product and geographical locations (Bhadoria & Mahapatra, 2011). The marketing company like Frost and Sullivan thinks that very likely to use salutistic marking on the label of the probiotic contained products in accordance to the CE 1924/2006 rules which may further boost the consumers' trust. Sufficient communication in combination with appropriate marketing plan will demonstrate to be competent to this goal. The acceptance among the consumers varied widely across Europe, including Scandinavian countries where the probiotic products were found to be consumed a long traditional way (Bhadoria & Mahapatra, 2011). However, the prevailing consumers' confusion on the different probiotic strains including suspicion about their efficacy cannot be resisted by the salutistic propaganda of the media (Caselli et al., 2013).

In 2011, the global probiotics revenue was earned USD 27.9 billion which was expected to grow with 6.8% per annum. Escalating trend on consumption of fermented dairy products and high concerns on gastrointestinal health are the main contributing factors, and these forces have triggered into high research and development expenses for development of new products over the last three years (Lim et al., 2015).

Probiotic dairy products, particularly probiotic yogurts and milks, have been found the most active segment of the European functional foods market (Catherine et al., 2001). In 1997, these products covered 65% of the European functional foods market with a calculated revenue of USD 889 million which was escalated from 23% of the market with a revenue of USD 320 million at the beginning. However, the market for the functional foods in the United Kingdom, France, Germany, Spain, Belgium, the Netherlands, Denmark, Finland, and Sweden was examined in a recent study conducted by Leatherhead Food RA. According to the findings, the probiotic yogurt market in these nine nations totaled more than 250 million kg in 1997 with France noticed the highest market, with sales of 90 million kg with value of USD 219 million. On the contrary, in Germany, the probiotic yogurt is growing quickly; for instance, during 1996–1997, it increased by 150%, whereas, in the United Kingdom, the market grew by a slow pace of 26% during the same period.

Averagely, the probiotic yogurts accounted for 10% of all yogurts sold in the nine countries studied, with Denmark having the highest consumption (20%) followed by Germany and the United Kingdom (both at 13%), and then France (11%). However, the Netherlands and Belgium (both at 6%) and then Finland and Sweden (both at 5%) (Hilliam, 1998) were documented lower sales.

The market for functional foods in Europe could ultimately account for 5% of total food expenditure in Europe, which is based on current prices and equivalent to USD 30 billion (Young, 1996). Additionally, diversified soft drinks, the original functional foods are still dominating the Japanese market; dietary fiber and probiotics are the significant functional ingredients in many of these products. The Bikkle which is considered to be the typical functional drink, was introduced in 1993 by Suntory (Osaka, Japan) and contains bifidobacterial cultures, whey minerals, xylooligosaccharides, and dietary fiber. Overwhelmingly, this product achieved sales of 11 billion yen in its first year. However, the fermented milk drink Yakult (Yakult, Japan), which is classified as a functional food in Europe, was not observed as such in Japan as the presence of probiotics in isolation from other functional ingredients does not carry functional food status in Japan (Young, 1996). In addition to functional drinks, functional milk products and products for children are also important, with innovations in a variety of foods and drinks such as ice creams, confectionery, biscuits, snack foods, and calcium-fortified drinks.

There have been several developments in the dairy products category including the development of yogurts supplemented with oligosaccharides and calcium (Young, 1996). It is expected that prebiotics and probiotics will continue to be important for the future growth and expansion of the market for probiotics and functional foods in the United States. Leatherhead Food RA's (1996) report assessed the global market for functional foods at USD 6.6 billion in 1994, with Japan accounting for just under one-half of that among the major functional food ingredients for the projected future in Japan. Estimating the size of the functional foods market in Japan is difficult as there is a lack of a clear boundary between health foods and functional foods; however, the current estimates are in the range of USD 3 to 3.5 billion.

The US functional foods market is comparatively undersized by European standards, with fortified dairy products, particularly, those containing active cultures, obtaining popularity in recent time. In contrast with the situation in Europe, there is lack of notable development of prebiotics in the United States. Vitamin- and mineral-enriched products continue to be incorporated among the more successful functional foods in the United States. Market development has been held back by criticism leveled at companies that have introduced products bearing raucous health claims. However, this is predicted that the US market for functional foods will experience the rapid growth rates compared with other countries in the coming future (Young, 1996).

4 Cost–Benefit Analysis

4.1 Animal Health

Overall production costs may decrease if commercial agriculture's growth performance and feed effectiveness improves. According to Torres-Rodriguez et al. (2007), a cost-effective analysis of the use of probiotic in diet in turkeys revealed that the cost for yielding per kilogram live body weight was lower, even after, including the cost of probiotics, when compared to the same for the control group, and that a small decline in FCR associated with the addition of probiotics would assist in lower production costs (Akhter et al., 2018; Islam et al., 2014).

In case of poultry production paradigm, the main variables of cost items include day old chick cost (DOC), litter cost, vaccination and biosecurity related cost, schedule medication cost, electricity bill cost, cost for purchased probiotic/antibiotic, transportation cost, and other operational cost which are considered to be the primary factors of cost components in chicken production.

In present market, a number of commercial probiotics and prebiotics for poultry production, all of which are typically administered in small doses. However, the cost of probiotics or prebiotics varies depending on the manufacturer and the active components in the final probiotic product (Young, 2008). Besides, in their research, Gutierrez-Fuentes et al. (2013) established the economic effectiveness of employing the probiotic. Their cost-benefit study revealed that body weight gained 100 gm for every dollar for probiotics spend, which was then converted to a cost-benefit ratio of 1: 22.57.

The benefits of all treatment groups overshadowed those of the control groups, and they depicted that using probiotics of any kind is cost-effective and profitable, as well as agreeing with other research statements. Thus, it was evident that that the newly designed probiotics are effective (Ferdous, 2021; Khatun et al., 2017).

4.2 Human Health

Among the side effects of antimicrobial use, the antibiotic-associated diarrhea (AAD) is one of the greatest common. Several meta-analyses confirm that probiotics have pivotal role on the risk, length, and acuteness of AAD (Hempel et al., 2012; Ouwehand et al., 2014). A number of research have made efforts to interpret the economic effect of probiotic use is on AAD-related costs.

The study confirmed in particular hospital, setting an extra annual cost of EUR 77,800 for isolation of the patients with AAD, however, the probiotic use would lessen these specific costs at EUR 63,400. Taken into consideration the extra cost for the probiotic (EUR 4600/annum), the net savings could be EUR 58,800/annum (Dietrich et al., 2014).

The use of oral probiotics as a preventive measures for *Clostridium difficile*–associated diarrhea (CDAD) in adults admitted in hospitals who were having a course of antimicrobials to lessen the CDAD risk and proceeded in a cost-savings

of USD 518/person/treatment. Inferring to a population size >380,000 hospitalized/year, the healthcare system would likely to spend USD 2.2 million for oral probiotics usage; however, this would likely to lessen total cost-savings of USD $44 million (Leal et al., 2016). Since the CDAD is a sub-class of AAD, *C. difficile* is documented to be accountable for nearly 25% of total AAD cases, and this could be terminated to be acute cases of AAD (Larcombe et al., 2016).

A separate study in human health evaluated the cost-benefit of probiotics usage (*Bifidobacterium bifidum* and *Lactobacillus acidophilus*) in the treatment of hospitalized >100 children with severe diarrhea via both a double-blind randomized and placebo trials. The study confirmed pointedly shorter hospital stay in the probiotics treated group than in the controlled group; however, the median length of diarrhea and direct medical expenses were not significantly changed. Thus, parental income loss, a non-significant low-cost, was seen in the probiotics group. A wider cost-benefit with the probiotic treatment is plausible, however, statistically found to be non-significant due to small sample size. The probiotics application reduced the length of hospitalization in children with diarrhea ailments but the total expenses were not different (Phavichitr et al., 2013).

5 Recent and Future Probiotic Marketing

Compound annual growth rate (CAGR) is a business and investing specific term for the geometric progression ratio that gives a constant rate of return over the time. The CAGR is the yearly average rate of revenue growth over a 2-year period, assuming growth is compounded exponentially. The mathematical progression ratio that gives a constant rate of return over time is referred to as compound annual growth rate in business and investing as per formula given by Fernando (2021).

$$\mathrm{CAGR} = \left(V_{\mathrm{final}}/V_{\mathrm{begin}}\right)^{1/t} - 1 \tag{1}$$

where, CAGR = compound annual growth rate, V_{begin} = beginning value, V_{final} = final value, t = time in years.

However, the probiotics market witnessed significant growth due to incessant demand for health-based products among the younger generations. Additionally, probiotics are vital part of functional foods and beverages, they are known for improved intestinal function and other health benefits, such as boosting immunity. Of the total retail probiotic food products sales, 71% of market shares is occupied by the probiotic yogurt segment, as stated by the International Probiotics Association (Modor Intelligence, 2022).

The global probiotic markets were estimated to be USD 34.1 billion in 2020 and expected to grow at USD 73.9 billion by 2030, with an 8.6% CAGR. Probiotics are bacteria and yeast-like microorganisms that assist humans and animals maintain a healthy intestinal microbial balance. The probiotics element promotes the body's natural digestive juices and enzymes, ensuring conducive environments for digestion. They can be taken as supplemental or administered orally. Probiotics also

protect healthy microorganisms from potentially dangerous pathogens. In case of human being, probiotics are being used tremendously to diagnose and treat mental illness, as well as digestive and neurological ailments. They also help to strengthen the immune system, protect proteins and lipids from oxidative damage, and reduce pathogen levels in the body. However, a major aspect to influencing the market expansion is due to boost in consumer preference on natural products about preventative healthcare and the efficacy of probiotic microorganisms on health benefits. Demand for probiotics has been risen as a result of growth in the use of functional foods, which, in addition to providing basic nourishment, have the potential to improve health. Thus, market actors have created probiotics products to aid in the treatment of disorders of living hosts. The influence of the rise in health concerns on the probiotics market is mild. Many people, however, are ignorant of the advantages of probiotics. In developing nations such as India and China, the supplement business is rapidly expanding due to health-related concerns. To address the needs of the industry and remain competitive in the market, the companies today have their own research and development facilities. Huge investments in research and development institutes are projected to increase probiotic product quality in the future. The probiotics market is likely to be driven by growth in the food and beverage industry as a result of higher consumer expenditure and positive government backing. Probiotics market growth is likely to be powered by an increase in demand for nutritional supplements among health-conscious consumers. Market expansion is expected to be hampered by a lack of proper regulations in the industry. Furthermore, because probiotics are highly sensitive organisms that can be readily weakened by a range of environmental factors, it requires safe storage facilities.

The growing recognition of the health benefits of probiotics, along with the aging population, is predicted to present a major growth opportunity. The market is predicted to rise as the number of workers has increased, as well as consumer expenditure on healthy food products. The market has experienced an increase in demand for goods that boost immunological health as the impact of COVID-19, which is expected to boom the probiotics market. Therefore, the major products formulation are taking place in order to meet the increased demand of both in animals and humans. Human consumers prefer probiotic dairy products such as yoghurt, ice cream, and cheese to absorb beneficial bacteria in addition to existing medical health supplements.

5.1 Probiotics Market by Ingredients

The market can be classified by ingredient, function, application, end user, and geography, according to probiotics market analysis. Bacteria and yeast are the two types of ingredients that are being used in probiotics. Considering these ingredients of the end product, the yeast segment is anticipated to grow at highest CAGR of 8.9% during the forecast period (2020–2030) (Fig. 1). However, due to an increase in demand for bacteria-derived probiotics food products, the bacteria category had the greatest probiotics market share by ingredient in 2020.

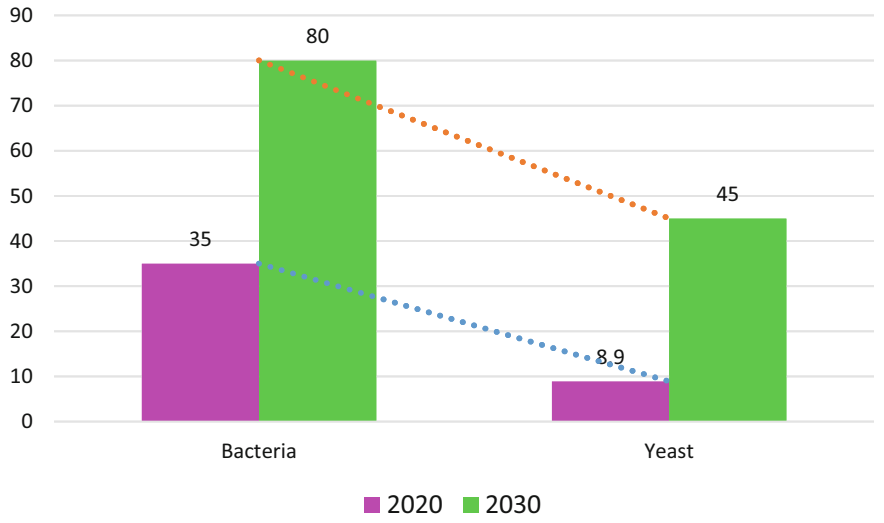

Fig. 1 Probiotic market by ingredients. The yeast segment is anticipated to grow at highest CAGR of 8.9% during the forecast period (adapted from Jaya & Roshan, 2021)

5.2 Probiotics Market by Purpose of Use

The probiotics industry is divided into three categories based on function: daily, preventative healthcare, and therapeutic healthcare. Among the three categories (regular, preventive, and therapeutic healthcare), interestingly, preventative healthcare segment is anticipated to grow at highest CAGR of 9.4% during the forecast period (Fig. 2).

However, the utilization of *Streptococcus* probiotics strains and other new species and/or strains in strengthening animal feeds are the major current market's

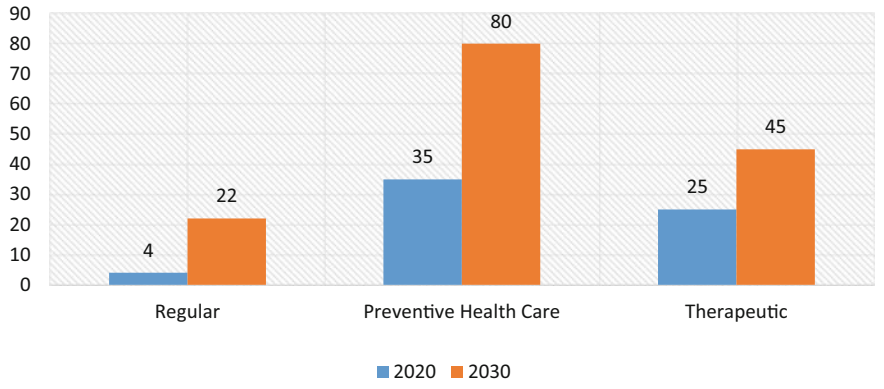

Fig. 2 Probiotic market by functions (adapted from Jaya & Roshan, 2021)

breakthroughs. Also, probiotics are increasingly being introduced in supplement formulations to treat inflammatory skin infections, viz., atopic dermatitis and eczema. All of these reasons are expected to contribute to the probiotics market's development.

According to the probiotics market projection, the preventative healthcare category lead the industry in terms of market share. This can be related to the rise in health concerns, which has prompted market participants to engage in research and development and offer new and innovative probiotics products. The dietary supplements category is expected to take the lead with the greatest market share over the projection period, based on application. This is due to the fact that some dietary supplement manufacturers concentrate on including a specific type of dietary fiber that contains probiotics and prebiotics (synbiotic). Diarrhea, indigestion, dermatitis, yeast infections, and other health complications, all are now attempt to treat with these substances (Jaya & Roshan, 2021).

5.3 Probiotic Market by Application

Food and beverage, dietary supplement, and animal feed are the three categories according to application. The dietary segment is expected to grow the highest CAGR (9.2%) during the anticipated period (Fig. 3).

During the projection period, the dietary supplement segment is predicted to increase 9.2% of compound annual growth rate. In 2020, the human group led the market by end user. This is owing to a rise in health concerns and understanding about the need of keeping the gut healthy, which has increased consumer demand for probiotics. The market is likely to be dominated by Asia-Pacific. Due to an increase in demand for food and health products that contain probiotics, Japan is the biggest consumer for probiotics in Asia-Pacific. Probiotics are live bacteria that, when taken in adequate amounts, give health advantages to humans. Probiotics for humans are

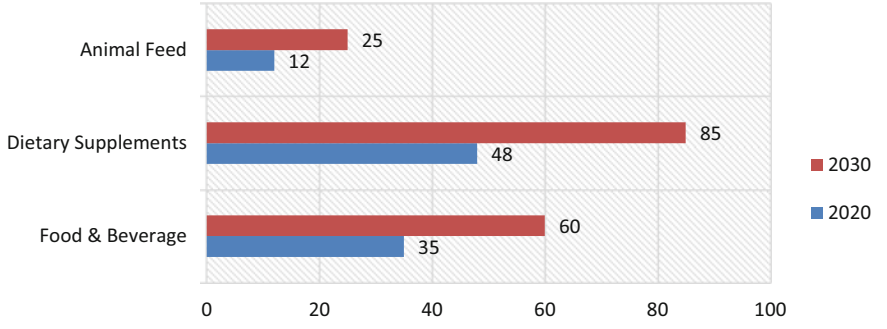

Fig. 3 Probiotic market by applications. The dietary segment expected to grow the highest CAGR (9.2%) during the anticipated period (adapted from Jaya & Roshan, 2021)

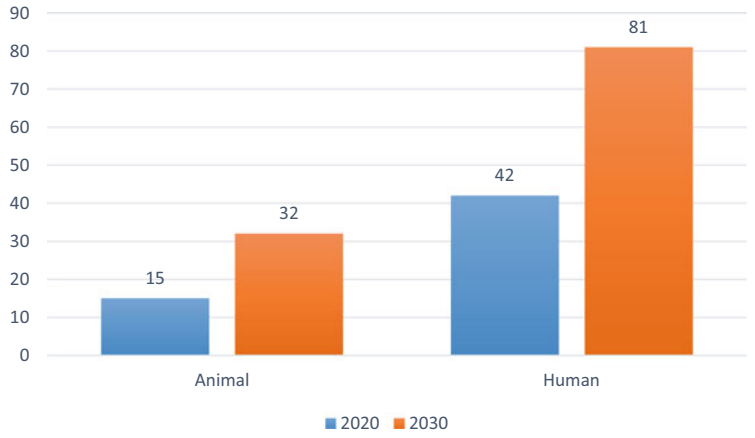

Fig. 4 Probiotic market by end users. The animal segment is expected to grow at highest CAGR (9.1%) during the forecast period (adapted from Jaya & Roshan, 2021)

used to address intestinal issues. Antibiotic-induced diarrhea and spasms are also treated with them.

5.4 Probiotic Market by End User

It is divided into human and animal categories based on the end user. During the projection period, the animal segment is predicted to increase 9.1% of compound annual growth rate (Fig. 4).

Around the world, BioGaia AB, Danone, Chr. Hansen Holding A/S, Yakult Honsha Co. Ltd., Probi AB, Lifeway Foods, Inc., Nestle S.A., Ganeden, Inc., E. I. du Pont de Nemours and Company, and Protexin are leading most probiotics companies. The most common growth strategy employed by market participants is product introduction, which is followed by partnership and agreement, expansion, and acquisition. To broaden their product offers, several companies have developed unique goods. Product launches are used by a variety of companies to broaden their market reach and service international clients (Jaya & Roshan, 2021).

5.5 Probiotics Market by Regions

The probiotics market is divided into four regions: North America, Europe, Asia-Pacific, and Latin America and the Caribbean (LAMEA). Asia-Pacific countries currently leads the market, with a compound annual growth rate (CAGR) of 9.0% predicted over the projection period, followed by North America, Europe, and Latin America (Jaya & Roshan, 2021).

6 Future Trend of Prebiotics and Probiotics

In coming days, prebiotics will expected to be identified from novel sources (Fig. 5) considering sustainability, price, and its judicial use (Mano et al., 2018). Yearly, 1.3 billion tons of food waste produced per year in the food chain demonstrated a good and viable source of natural bioactive substances.

Important prebiotics are produced from fruits, grains, and vegetable processing, for example, pectin from orange peel (Gómez et al., 2014) and arabinoxylans from distillery and brewing waste (Monteagudo-Mera et al., 2018). Application of sonication future prebiotic compounds may also be chemically or structurally altered using high pressure, enzyme, acid, and oxidation treatments for functionality modification. Finally, unique mixtures of prebiotics in improved mixtures could offer the capability to produce novel profiles of benefits (Lam & Cheung, 2019).

Current advancement in microbiome science is permitting new horizons of enquiry for probiotics and prebiotics. New genres, mode of action, and practices presently under review have the importance to adjust the scientific knowledge including healthcare and nutritional applications of these inventions. The extension of related domains of microbiome-targeted findings and an emerging landscape for execution across policy, regulatory, prescriber, and consumer-levels signify an epoch of meaningful change (Cunningham et al., 2021).

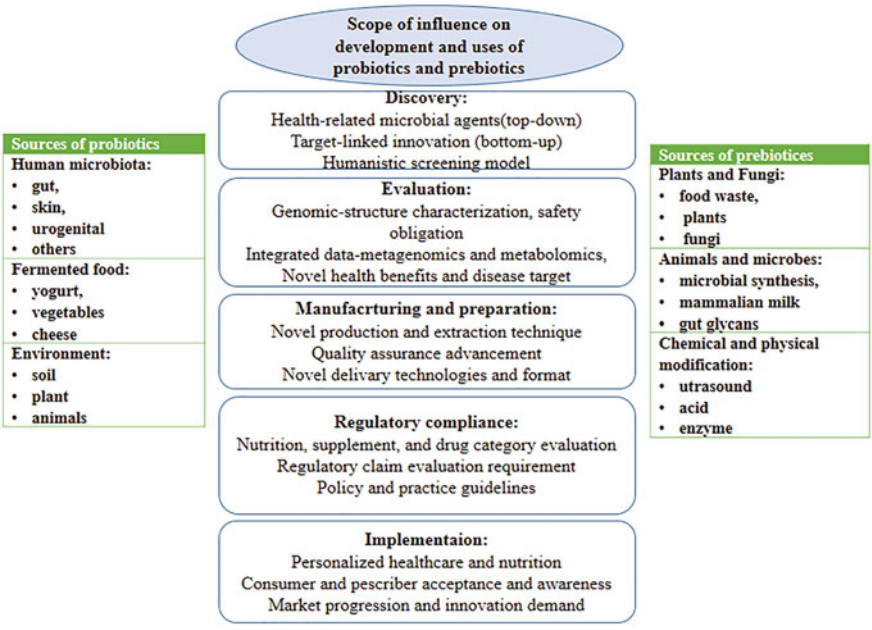

Fig. 5 Development and application of future of probiotics and prebiotics. The snapshot displays present and evolving effects on probiotics and prebiotics along with new sources, novel discovery and assessment techniques, manufacturing and formulation advances, regulatory issues, including effect on execution in nutrition and healthcare (adapted from Cunningham et al., 2021)

7 Conclusion

In view of multifaceted health-promotion effects connected with the use of probiotics in humans and animals, these organisms are currently occupied by many industries in producing probiotic-origin food and animal-feed, pharmaceutical products, dietary supplements including natural therapeutic agents. Successfully conveying probiotic benefits to the consumers and qualitative business with probiotics must be ensured. Precautions to be taken to select the most suitable species and/or strains, cultivation conditions, and product manufacture. The probiotic commercialization will run concurrently with media engagement which vary greatly according to cost and other considerations. The required doses of the species and/or strains towards the end of shelf life, as well as incorporating them in consumer goods, are often ignored challenges in the manufacturing of probiotics. To assure the supply of high-quality prebiotic-probiotic-containing products to customers, legislation and regulation should be developed around the world. Furthermore, manufacturers of probiotic-containing goods should have responsibility for delivering scientifically and legally accurate information to consumers. Inclusion of probiotics into a product is predominately investigated thorough sensory and physicochemical characteristics, however, to assess whole metabolite profile using metabolomics can give deep-insights into many substances of appropriateness to odor and food value. The future probiotic microbes will significantly lead for making money and food microbiology based entrepreneurship will arise dramatically around the earth.

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Production, Cost Analysis, and Marketing of Fermented Fish

Tulsi Kumari Joishy and Mojibur Rohman Khan

Abstract

Fermented fish have long been an important cuisine of the human diet across the world. Fermentation is an important technique for preserving perishable fish products which also aid in nutritional quality. Fermented fish serve as a stable and significant source of proteins, vitamins, minerals, and nutrients. Fish products serve as stable and significant sources of protein, vitamins, minerals, and nutrients. The differences among the fish products mainly occur due to different types of fish species used as substrates and the associated microbes involved in its manufacture. Fermented fish contain a plethora of beneficial microbiota, making them a valuable source of probiotics that may confer nutritional and health benefits. However, production of fermented fish is still confined to small scale or local cottage industries. Many countries of Asia and Africa still follow spontaneous fermentation while in Europe, defined starter cultures are used to maintain the consistency of the products. The introduction of starter culture in fermentation industries has led to greater quality, consistency, and safety of fermented fish, while choice of culture is critical. Fish industry and its mass production have accelerated over the last decades. This chapter cumulates the different methods of fermented fish productions, the variable marketing chains, and important elements of marketing fermented fish products for sustainable development.

Keywords

Fish products · Fermentation · Marketing · Production · Starter culture · Probiotics

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1 Introduction

Fermentation is one of the ancient techniques of preserving perishable food products. Fermentation is defined as a method in which the metabolic activity of various microorganisms transforms and stabilizes food products (Wilburn & Ryan, 2017). The microbial populations in fermented foods are derived either from autochthonous microbes residing within the raw materials, or from a defined starter culture (Tamang et al., 2020; Narzary et al., 2021). During Neolithic time, foods were fermented spontaneously without using any starter culture, known as natural fermentation (Shangpliang et al., 2018; Dewan & Tamang, 2007). However, another method of fermenting food is using loopful from previously fermented batch, also known as the back sloping method (Joishy et al., 2019). During fermentation, microorganisms break down carbohydrates into organic acid, alcohol, carbon dioxide, and bacteriocins (Nout, 2014; Ananou et al., 2007). Microbes transformed volatile and bioactive compounds into anew edible forms with enhanced nutritional properties, flavours, and aromas. Therefore, fermented foods are a stable source of vitamins, calories, proteins, minerals, and other nutrients. Fermentation also enhances the shelf-life, and organoleptic properties of perishable food items (Altay et al., 2013; Hwang et al., 2017). Fermented foods exert several beneficial effects on human health such as reduces high blood pressure, cholesterol levels, osteoporosis, diabetes, obesity, allergies, and also protection against pathogens which might be attributed to the presence of bioactive peptides synthesized from protein by bacteria involved in fermentation (Sekar & Kandavel, 2002).

With the development of civilization, the method of fermentation has also evolved. Fermented fish is one of the most popular fermented foods and is widely consumed in different parts of the world. Fermented fish products were originated in Mekong basins of South East Asia around 200 BCE to 200 CE (Tamang et al., 2020). The first ethnic groups of people to initiate fermentation using freshwater fish are reported to be Thai – Lao, Khmer, and Burmese (Tamang et al., 2020). Fish sauces such as *garum* and *muria* were originated in Greece (Tamang & Samuel, 2010). Fermented fish products are a staple food of many ethnic tribes of Asian countries, including Korea, Japan, China, Thailand, Malaysia, Cambodia, India, Philippines, and Indonesia (Fig. 1). However, these products are typically produced at the family or village level using traditional methods in the regions. Fermented fish products are of three different types: fish fermented with or without salt, paste, and sauce (Fig. 2). Food and Agricultural Organization (FAO) suggests that dietary habits of the populations directly influence the production and marketing growth of food items in a country (FAO, 2020). In the developing countries of Southeast Asia, South Asia, and Africa, there is an increasing demand for fermented fish products due to its enormous health beneficial property. The Scandinavian countries in Europe also prefer fermented fish products, including *surstromming* (fermented herring) and *rakorret* (fermented trout) (Narzary et al., 2021).

Spontaneous fermentation is mainly preferred in Asia, Southeast Asia, and Africa, whereas in Europe, North America, Australia, and New Zealand defined starter culture is widely used to initiate fermentation. Fish fermentation is a

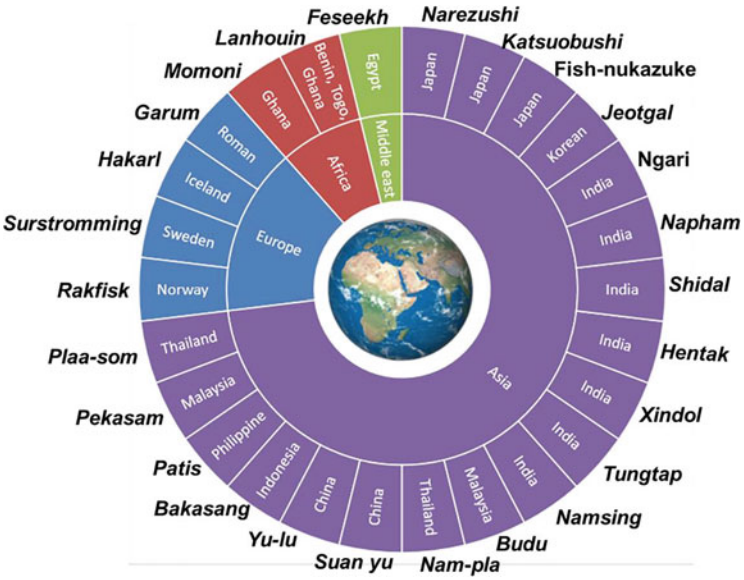

Fig. 1 Varieties of fermented fish consumed across the world

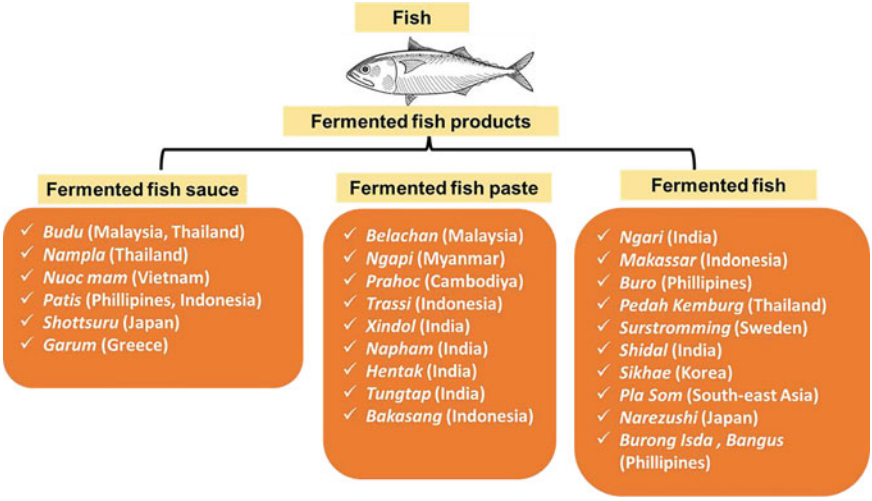

Fig. 2 Different forms of fermented fish produced in different parts of the world

time-consuming method that can be reduced using defined starter cultures which enhances fermentation, reduces time period, and ensures the quality and consistency of the products for commercial purpose (Narzary et al., 2021). Lactic acid bacteria (LAB) are widely used in food fermentation due to enormous health beneficial attributes. LAB enhances lactic acid production, decreases biogenic amines

secretion, and aids in nutritional property of the end product (Hill et al., 2017; Parente & Cogan, 2004; Bintsis, 2018). Additionally, non-lactic acid bacteria such as *Bacillus*, *Staphylococcus* sp., and *Micrococcus* sp. also play a crucial role in fermenting fish (Narzary et al., 2021). Previous studies reported that *Virgibacillus* sp. SK 33 and *Staphylococcus* sp. SK1-1-5 strains increase the desired volatile compounds in the fish sauce by inducing higher production of proteinases (Narzary et al., 2021). *Tetragenococcus halophilus* improves fish sauce's flavour (Dıncer et al., 2010) and reduces histamine accumulation in low-salted fermented fish products (Narzary et al., 2021).

In India, the fish curing industry has high demand but paradoxically lacks commercial strategies (Pillai et al., 1956). The indigenous fermented fish product is an intrinsic part of the dietary habits of an ethnic tribes residing within Himalayan belt (Narzary et al., 2021). Fish is fermented in an artisanal way by different tribes, whose method varies in a considerable way, including salting, drying, and fermenting. The fermented fish product *Ngari* is prepared using dried *Puntius sophore*, while *hentak,* which is a fermented fish paste, is prepared using *Esomus danricus* are intrinsic dietary habits of Manipur. Similarly, *Tungtap* is a fermented fish paste which is prepared using *Puntius sophore* found in Meghalaya. In Sikkim, *Gnuchi*, a smoked fish product, is widely preferred by Lepcha community and is prepared using *Schizothorax richardsonii*, *Labeo dero*, *Acrossocheilus* spp., and *Channa* sp., while *Sidra* is a sun-dried fish product prepared from *Puntius sarana*. *Sukuti* is trendy cuisine among Gorkhalis, which is prepared from *Harpodon nehereus* and is consumed as curry, soup, and pickle. *Shidal* is a fermented fish product that is prepared by keeping semi-dried *Puntius* species in earthen pots for around 4–6 months. *Shidal* is widely consumed in Tripura, Assam, and Arunachal Pradesh and is also known as *Matkashidal*. The traditional knowledge of the ethnic women of this region plays a vital role in manufacturing fermented food, their marketing within and outside area (Tamang et al., 2009). Fish fermentation is a small-scale industry and can provide direct employment to its people (FAO, 2020). The nutritional value of fermented foods can improve public health and provide opportunities for economic development. However, good manufacturing practices (GMP) should be followed by the small-scale producers to have sustainable development goals, paving way for its mass productions and economic upliftment.

2 Mass Multiplication

Fermentation of fish is still practice in home or small-scale level using manual labour in different parts of the world. Recently, the demand of fermented fish has become global due to its health beneficial attributes (Tamang et al., 2020). However, to meet the demands of the same, a large-scale production is required. Fermentation of fish demands longer time period of about 4–6 months which is a major limitation in its mass multiplication. Therefore, implementation of starter cultures or well-characterized strains with sequenced genomes might play an important role in

shortening the fermentation time and mass production of the fermented fish (Tamang et al., 2020).

Fermented fish contain beneficial metabolites such as ascorbic acid, and are antioxidant-rich food products. However, few fermenting bacteria such as *Leuconostoc*, *Lactobacillus*, *Lactococcus*, *Escherichia*, *Enterobacter*, *Enterococcus*, *Salmonella*, *Shigella*, *Pseudomonas*, *Staphylococcus*, and *Bacillus* in fermented fish produces biogenic amines (BA) which might be lethal if consumed in excess quantity (Tamang et al., 2020; Narzary et al., 2021; Suzzi & Gardini, 2003). BA is nitrogenous compound formed through decarboxylation of amino acids (Sivamaruthi et al., 2021). *Lactobacillus* produces histamine, putrescine, and tyramine while *Enterococcus* additionally produces cadaverine (Bover-Cid & Holzapfel, 1999). Although BA are omnipresent but high concentration of the same (1000 mg of total BAs/kg; 8 mg of histamine) might cause a minor allergic reaction to serious health issues in susceptible individuals (Erdag et al., 2018). There are several factors which influence accumulation of BAs in fish products such as raw material quality, moisture, fermentation duration, storage conditions, starter culture, and temperature (Sivamaruthi et al., 2021). Therefore, during strain selection for fermenting fish, isolates which are unable to produce BA should be an important criterion. Recent advancements in omics approach such as metabolomics, next-generation sequencing, and bioprocess technology provide enormous opportunity to address such challenges along with enhancement of flavour, improved shelf-life, defense against bacteriophages, and preservation.

Additionally, implications of starter culture and controlled fermentations provide consistent quality products, reduce spoilage, and assure food safety (Tamang et al., 2020). The demand of such quality products is accelerating within low income countries in Asia, and sub-Saharan Africa (Tamang et al., 2020). Mass multiplications of fermented fish products might facilitate efficient production of similar kind of quality products throughout the year. Mass multiplication methods are based on two principles: (1) specialization and division of labour and (2) automation such as use of tools and machinery for manufacturing standard products. Across the continent, every ethnic tribe has emerged diverse artisanal methods of preparing fermented fish products. Therefore, method validation and proof of concept is very crucial in the process of mass multiplications (Fig. 3). This will heighten the new business forum paving way for global consumption.

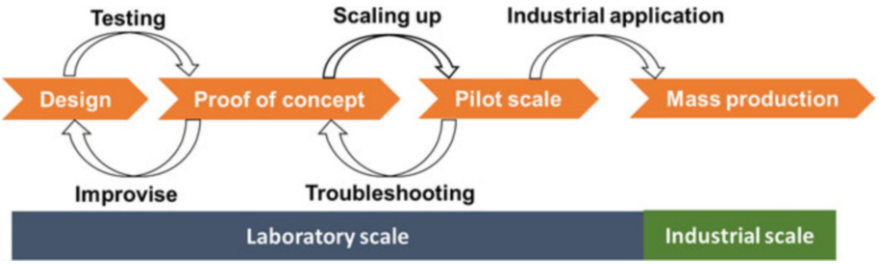

Fig. 3 Strategies for the mass production of fermented fish

3 Advantage of Mass Production

3.1 Economically Efficient

Mass production is a process which judiciously utilizes resources, require fewer labour charges, while decrease total expenditure per unit by saving unnecessary expenditure.

3.2 Fast Production Rate

Mass production employs advanced technology and conveyor methods to streamline and boasts the fastest production rate of the manufacturing procedure.

3.3 Production Accuracy

Mass production allows low margin error with high accuracy based upon auxiliary and repetitive primary operations, which work together to create a final product.

4 Disadvantage of Mass Productions

4.1 Inflexible to Consumer Demand

Consumer demands are an essential parameter in mass production. Therefore, it is difficult to change or redesign if consumer demands decline for the same. Additionally, if the end product is perishable, mass multiplication can be wastage.

4.2 Disengaged Workforce

In mass production, quality control is a crucial factor in maintaining the safety of the fermented fish product. Therefore, motivated staff is necessary, which might be supported by rotating them frequently.

4.3 Difficult to Restructure Production

Mass multiplication is a unison strategy in which machines work together to produce a quality product. Therefore, a change in the production line might have significant financial and logistical consequences.

5 Business Plan Development

Fish and fishery products are the most traded food commodities across the world. However, fermented fish are nominal in the global food map due to its strong odoriferous flavour that repulse western taste (Sally, 2020). Fermented fish products are popular cuisine in Africa, Asia, and Europe (Xu et al., 2021). Each continent has unique fermented fish products prepared in an artisanal way based on differences in environmental conditions, raw materials, and microorganisms (Zang et al., 2020).

Fermented fish products are manufactured on small-scale or local cottage industries harbouring microbiome of respective geographic conditions resulting in variety of product qualities (Xu et al., 2021). The demand of fermented fish products continues to increase globally due to its health beneficial attributes in addition to its unique flavour, and texture (Xu et al., 2021). However, introduction of cold chain and various preservation technologies in Western countries have laid preservation methodology of fermented fish largely outdated for commercial purpose (Xu et al., 2021). The standardization of the final product is crucial which might be obtained by implementing the selected dominant native microorganisms, maintaining fish quality as the microbial enzymes found in fish flesh is important in fermentation (Narzary et al., 2021; Xu et al., 2021). *Prahok*, a national staple fermented food of Cambodia, is produced at industry standards by maintaining the specific characteristics like smell and colour of the product (Narzary et al., 2021). Previous studies reported that finest quality fish products are produced by controlled growth of microorganisms and conversion of enzymes (Xu et al., 2021). To maintain the consistent quality of fish and its availability during dry season, few methods of aquaculture or aquafarming might be implemented. Aquaculture is a method of fish farming which is performed offshore to rear fish in completely artificial facilities. For example, biofloc technology (BFT) is defined as aggregates of bacteria, algae, or protozoa, held together in a matrix with particulate organic matter to improve or treat water used in aquaculture system (Abakari et al., 2021; El-Sayed, 2021). BFT has been adjudged as novel "blue revolution" and an outstanding technology for sustainable aquaculture that could contribute to FAO sustainable development goals (SDGs) related to food security (El-Sayed, 2021). BFT is capable of solving some of the environmental and economic challenges faced by traditional aquaculture production systems (Abakari et al., 2021). BFT is based on the recycling of nutrients and their reuse in the same system, which is designed as a zero-exchange or minimal exchange (water) system (Emerenciano et al., 2017). In aquaculture, BFT prevents from water-borne diseases, improves water quality, feed conversion, and biosecurity. A report suggests that the fish farming is vision to expand from 2018 to 2030 (FAO, 2020). Fish farming is therefore beneficial to meet fish demands and also for using in fermentation technology.

In India, various types of fermented fish products are produced in the Northeastern and coastal region at local cottage industry (Narzary et al., 2021). Asia's largest dried fish market is in Jagiroad, Assam.

However, the fermented fish like *puthi* and *telesh shidal* are imported from Bangladesh (Upadhyay, 2016). The consumption and demand of fermented fish

has shot up in the past few months due to the health beneficial belief (Narzary et al., 2021). *Shidal* is believed to enhance immunity during fever, cough, and cold. Therefore, to meet the consumers demand, a proper business development strategy and certain standards need to be maintained (Uchoi, 2020). It is extremely important to maintain the health and safety issues related to the product (Narzary et al., 2021). Although fermented foods are safe to consume, however, if fermented improperly, then the same product might be lethal to consume (Tamang et al., 2020). Therefore, improved hygienic practices such as education and information to rural or small-scale manufacturers, implementation of starter culture, maintaining good manufacturing practices and hazard analysis critical control point for extremely perishable products like fish, emphasis on workers personal hygiene, selecting or maintaining high-quality raw materials to reduce pathogens and undesirable microbial load, use of heat for proper pretreatment of raw materials, fermentation should be practiced always under optimal environment including aerobic/anaerobic or enclosed and constant temperature to maintain the quality of end product (Tamang et al., 2020). To develop business plan for food products, it is important to have blueprint and executive summary of the same (Uchoi, 2020). Executive summery provides a detailed overview of the plans, table of contents, background, history, goals, objectives, description of the product, a detailed market assessment, competitors in the existing market, marketing, and manufacturing plans (Fig. 3). The vision, mission, and objectives of fermented fish business is to producing the best quality fermented fish products or providing customer satisfaction by returning value for money. Data of complete details of total fish and type of fish used in the business and the varieties of equipment acquired with their billing details are also important. A detailed market analysis will provide the status of the competitors and information about the innovation which will be needed to be maintained in new fish products. The major components required to start business of fermented fish products are a dedicated space/land to carry out different procedures for fermenting fish, regular supply of pots, and skilled manpower, regular water supply to keep fresh fishes.

6 Production and Cost-Benefit Analysis

Globally, fish consumption has rose significantly since 1961 from 9.0 kg per capita to 20.3kg in 2017 (FAO, 2020). This might be due to the increased availability of fish and fish products across different regions contributing to the food security and nutrition of the world populations. In 2018, 35% of global fish production has remained from China which is still major fish producer across the world (FAO, 2020). Additionally, 34% accounted from Asia, 14% from America, 10% from Europe, 7% from Africa, and 1% from Oceania contributed in global fish production (FAO, 2020). However, in last decades, total fish productions in Asia and Africa have doubled in comparison to Europe and Americas where the productions have been gradually decreasing since 1980s (FAO, 2020). In 2017, consumption of fish accounted for 17% of the global population's animal protein intake covering 50% of

several small island developing countries (SIDS), Bangladesh, Gambia, Indonesia, Sri Lanka, Sierra Leone, and Cambodia (FAO, 2020). However, in 2018, 88%, i.e., 156 million tonnes of global fish production was utilized for direct human consumption while remaining 12%, i.e., 22 million tonnes of fishes were used for the production of its meal and oil (FAO, 2020). Fish farming dominates in Asia, followed by Africa while in Europe and Oceania, fish farming is declining. Fish and fishery products are the most traded food commodities across the world.

With the increase in fish and fish products demand, the fish industries wastage is also expanding (Marti-Quijal et al., 2020). These by-products represent approximately 50–70% of total fish weight which can be processed further to obtain high-quality products. Protein hydrolysates obtained after fish by-product processing is widely applicable for animal feed production to obtain increased animal protein intake (Saadaoui et al., 2019). Similarly, fish liver is the main organ for lipid accumulations in addition to skin or head due to which varieties of oils can also be produced using fish by-products (Vázquez et al., 2019; Rustad, 2003). Biogas, biodiesel, and fertilizers can also be produced using fish by-products as they are rich in phosphorus, calcium, and nitrogen (Arvanitoyannis & Tserkezou, 2014; Illera-Vives et al., 2015). Fish by-products are widely used in manufacturing mainly marine proteases enzymes such as trypsin, pepsin, collagenase, elastase, and chymotrypsin. All these enzymes have large-scale industrial application including food technology and detergent (Klomklao, 2008; Ferraro et al., 2013).

In fish industries, acids or bases are mostly used for protein hydrolysis because of simple and inexpensive procedures (Kristinsson & Rasco, 2000). However, implementation of fermentation might provide several benefits in addition to the protein and lipid hydrolyzation. Fish flesh harbour lipases which aid in lipids hydrolyzation, while protein hydrolyzation is achieved by an array of fish gut and bacterial enzymes (Xu et al., 2021). Additionally, fermentation produces amino acids and antioxidant peptides which act together with glutathione to protect against oxidative stress (Ramírez et al., 2013; Rajendran et al., 2018). In various fermentation procedures, enzymes from plant and animal sources such as ficin, papain, bromelain, or trypsin and chymotrypsin might be used to accelerate the rate of hydrolysis (Xu et al., 2021). Previous studies reported that the quality of oil produced by fermentation was high in comparison to the fish treated with formic acid for hydrolysis (Özyurt et al., 2018). Implementation of fermentation technology might be very useful tool to improve and enhance the nutritional quality of fish products (Özyurt et al., 2019).

7 Small-Scale Production of Fermented Fishes by Artisanal Method

7.1 Asia

In a country, the production of fermented fish is solely depended on two factors including food habits of the native people and its national or international market demand (Essuman, 1992). In Asian countries, fish fermentation is a traditional

method and is mostly prepared at home in small scale. For example, in India, various fermented fish has been reported from its Northeastern region which is produced at small or household scale. Each ethnic tribe of Northeast India has a unique artisanal method of fermenting fish. Previous studies reported that oil is common in *Ngari* and *Telesh shidol* production which initiates anaerobic fermentation process inside the earthen pot utilised in both types of fish fermentation (Figs. 2 and 3). However, *xindol* is prepared in bamboo poles in an artisanal way which is different in comparison to other fermented fish (Fig. 4). *Xindol* is prepared by mixing stem of

Fig. 4 Business plan provides a general overview

Fig. 5 Flowchart depicting production strategy of *Telesh shidal*

Colocasia esculenta while *Hentak* is prepared by mixing with fresh petals of *Alocasia gigantean* (Figs. 5, 6, and 7). *Colocasia esculenta* are rich and natural source of bioactive proteins, which is reported to act as immunostimulators in distinct mice strains (Ribeiro Pereira et al., 2020).

7.2 Africa

Similarly, in African countries, the demand of fermented fish is more in comparison to salted or dried fish. Fermented fish is an important condiment and is important source of animal protein. Few major fermented fish product of Africa are *Lanhouin*, *Momone*, and *Gued* produced in Benin, Ghana, and Senegal, respectively (Essuman, 1992; Anihouvi et al., 2012; El Sheikha et al., 2014). *Lanhouin* is produced using 25 different species of fish, few which are *Pseudotolithus senegalensis*, *Galeoides decadactylus*, *Chloroscombrus chrysurus*, *Scomberomorus tritor*, and *Caranx hyppos (*Anihouvi et al., 2012*)*. Fish is firstly dressed by scaling, gutting, and washing thoroughly followed by overnight ripening which is the most crucial step because its influences the texture and the aroma of *Lanhouin* (Anihouvi et al., 2012). After ripening, fishes are washed and salt is applied to the belly cavity, gill, and the whole fish. The quantity of the salt used depends on the 20–35% of the weight of

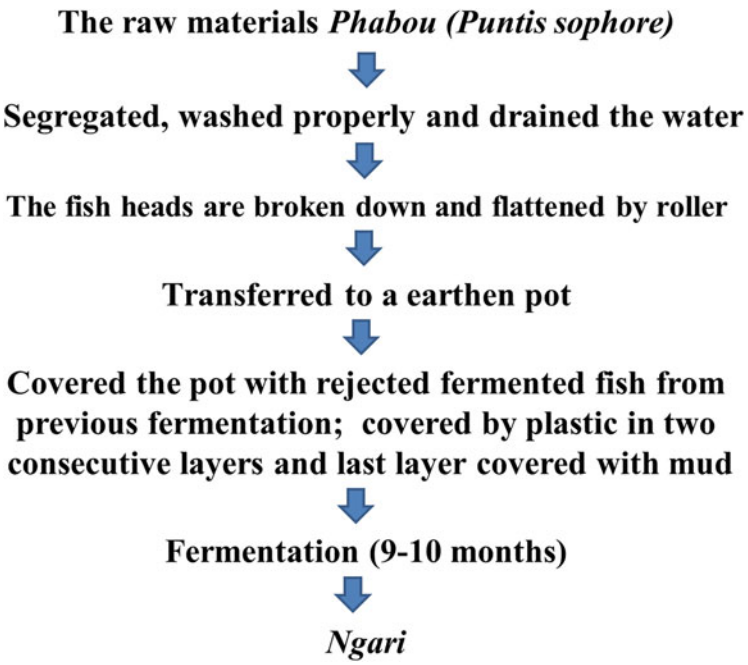

Fig. 6 Flowchart depicting production strategy of *Ngari*

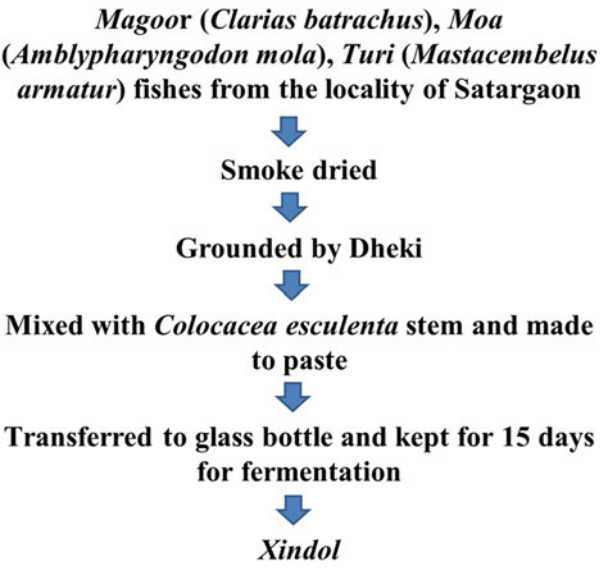

Fig. 7 Flowchart depicting production strategy of *Xindol*

fresh fish (Anihouvi et al., 2012). The salted fish is then arranged in earthen jar which is wrapped with sacks and buried in a 2 meters depth hole for 3 to 8 days fermentation. Later, the fermented product is sun dried to reduce the water content. *Momone* is widely consumed in Ghana and is produced using catfish, barracuda, sea bream, *Galeoides decadactylus*, *Pseudotolithus senegalensis*, *Sphyraena* spp., *Caranx hippos*, *Caranx rhoneus*, and *Scomberomorus tritor* (Anihouvi et al., 2012). The fish is washed thoroughly, salt is applied at a concentration of 15–40% of fish weight and allowed to ferment for 3 to 8 days. After fermentation, fish are dried for 1 to 3 days. *Guedj* is a fermented fish product of Senegalese and Gambiam which is used as flavouring agent by the local populations because of its exceptional flavour and taste (Anihouvi et al., 2012). The raw fish is dressed, washed, and fermented for about 2 to 3 days, followed by the drying step which is done for 3 to 5 days.

8 Cost-Benefits Analysis of *Shidal*

In Northeast region, *shidal* is widely consumed fermented fish product (Majumdar et al., 2016; Gupta et al., 2021). In Tripura, two different forms of *shidal* including *puthi shidal* and *Bashpati shidal* are processed and marketed. *Bashpati shidal* are low-priced value added product in comparison to *puthi shidal*. The total variable cost processing of *puthi shidal* was reported to be Rs. 213,647.5/t in Tripura whereas it was Rs. 285,129.99/t in Manipur (Upadhyay, 2016). The cost processing of *Bashpati shidal* is low approx. Rs. 231,344.43/t which might be due to low price of fishes. Cost of raw material or dry fish utilized for processing accounted for 90% of total variable cost while 10% accounts for transportation charge, labour charge for preparation of paste, loading and unloading the materials, cost of mustard oil which is very important for curing the *matka* (Upadhyay, 2016). In Tripura, the gross return of *puthi shidal* production was Rs. 266,241.3/t in comparison to Manipur where the gross return was reported to be Rs. 362,195.30/t. In Tripura, the gross return of *Bashpati shidal* was reported to be Rs. 231,344/t. The net return in processing of *puthi shidal*, in Manipur is reported to be Rs. 77,065.31/t whereas in Tripura, it is Rs. 52,593.8/t. The net return of *Bashpati shidal* is reported to be 59,616.17/t. The net return for processing of puthi and *Bashpati shidal* in Manipur and Tripura were reported to be 20–26% of the total revenue. Therefore, both types of *shidal* processing is economically viable and also provides direct employment to the women (Upadhyay, 2016).

9 Marketing of Product with the Main Objective of Entrepreneurship

Marketing is the connecting link between the producers/farmer and consumers (Nayeem et al., 2010). The marketing system directs products to reach consumers in an acceptable form. Marketing of fish also involve the buying and selling of fish by an individual who need not be a fish farmer (Nayeem et al., 2010). Marketing

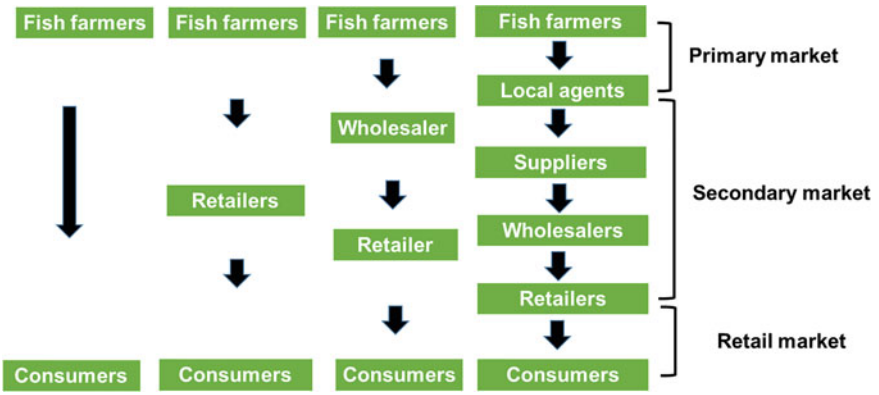

Fig. 8 Different forms of marketing channels

system operates in a chain formation through a set of intercessors that performs necessary commercial functions (Fig. 8). Fish farmers consider fish marketing as major challenges because of poor prices, lack of proper marketing platform, and transport system. Fish marketing provides international relationship and foreign exchange earnings. Additionally, it determines the most preferred forms of fish products, consumer preferences, creates employment opportunities, and encourages entrepreneurs. The marketing system is operated by private sector to improve marketing chain. Previous report suggests that in Bangladesh, fish and fishery products are marketed through different channels because of lack of proper marketing infrastructure (Reza et al., 2005). The major limitations in marketing of fish products are poor transportation and storage facilities (Nayeem et al., 2010).

The major elements of marketing fish and processed fish products include assemblage, storage, sorting, grading, packaging, transportation, purchase commission gent, wholesaler, retailer, and consumers. Assemblage is a process which enables farmers or marketer to measure precisely all the record of total fish products which may or may not be of same species. Storage is a procedure in which processed fish, dry fish products are stored in a warehouses or cool and dry environment. However, fresh fish are stored at freezer or cold room to control the growth of spoilage microbes in the fish. Storage of fish products is important to prevent fish market from over flooding which may hamper its market price. Similarly, sorting is the process of categorizing fish products for easy grading, handling, and marketing. It is done based on size, colour, species, and weight of fish products. Grading is a process of allocating prices to the sorted fish products based on its value and market demand. Market survey is important factor to get assured about the market price and prevent from arbitrary costing that may lead to loss or inability to sale. Packaging is a process of wrapping or loading fish products in containers including cans, bag, and cartoons. Packaging aids in handling during transportation and also plays an important role in attracting consumers. Labelling is equally important as it describes or provides necessary information about the raw materials, form of consumption, price,

and shelf-life of the product. Transportation entails moving fish products from farm, warehouses, to the consumers or middle men. During transportation, temperature should be maintained properly. Transportation is a vital part in the distribution of fish products from farmers to wholesale markets and further to the retailer outlet. For marketing, advertisement of the fish product may provide awareness to the public regarding the availability, and quality of the fish products. It can be done by telephonic contacts, radio, newspaper, online platforms, and television. Selling and buying of fish products initiates with the farmers and ends with consumers while middleman obtains it with extra commission from the retailers. The increase in price of the fish products is due to the involvement of enormous number of commission agents (Ahmed et al., 1993).

10 Marketing of *Shidal*

Matkashidal which is popularly known as *shidal* is widely traded in the Northeast region of India and Bangladesh (Narzary et al., 2021; Zang et al., 2020; Nayeem et al., 2010). *Shidal* and dry fish products are marketed through similar channels due to which both products have similar kind of marketing chains as well as centres in the Northeast India (Nayeem et al., 2010). A survey of *shidal* marketing conducted in Bangladesh reported that there was no fixed marketing chain for fermented fish products, and the length varies depending on the locations as well as season (Nayeem et al., 2010). The fish farmers also functions as traders or wholesalers due to which they are aware of market prices.

In Northeast India and Bangladesh, there is high demand of salted, smoked, and fermented fish products. However, there is no information available on the marketing system of these products especially those produced from small indigenous species such as dried *punti* and semi-fermented products locally known as "*Chepa shutki*" in Bangladesh. Therefore, it is extremely important to know about the existing marketing channel which will help to identify marketing inefficiencies that pose negative impact on all the components of the value chain.

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Mass Multiplication, Production Cost Analysis and Marketing of Shiitake Mushroom

Rajendran Priyanka Jayam, Nallaiyan Boomika, and Dharumadurai Dhanasekaran

Abstract

Shiitake mushroom is one of the edible mushrooms native to East Asian countries, cultivated and consumed in different parts of the world. Currently, it is the second largest edible mushroom in the world. Earlier, the wood and their waste are used for making furniture, fuel, etc. In recent times, wood is used for the production of shiitake mushroom worldwide. These mushrooms are rich in taste and flavour. Shiitake mushroom is considered as a medicinal mushroom which induces the immune system and maintains the cholesterol level in the blood. Shiitake mushroom is rich in vitamin C and antioxidant, which protect the skin, and is also used for making many cosmetic products. Indoor production of shiitake mushroom produces high quantity yield, while outdoor production of shiitake mushroom produces high quality yield. In this chapter, we discuss the production, cultivation and cost analysis of shiitake mushroom in lab scale, small scale and large scale. Spawn is the main product for the production of shiitake mushrooms and is also explained in this chapter.

Keywords

Shiitake mushroom · Medicinal mushroom · Primordia · Spawn production · Lab scale

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1 Introduction

Shiitake, scientifically called *Lentinula edodes*, from the Division Basidiomycota is a cultivable fungus native to East Asia. Shiitake was named by the Japanese term "Shii" and "take" meaning tree mushroom (Przybylowicz & Donoghue, 1988). Shiitake mushroom is an edible fungus. They are naturally produced on dying hardwood trees and eaten like vegetables. Their colour ranges from brown to dark brown and height ranges from 5 to 10 cm. They are grown in Japan at a rate of around 83%. It can grow well in winter season as well as other climates under regulated conditions. The origin was tracked into thousands of years to Japan (Kumar et al., 2019). Shiitakes have abundant nutritional content and have many medicinal properties. They are also grown artificially in countries such as the United States, China, etc. Shiitake mushrooms have been utilized in the Orient for over 2000 years and have been commercially farmed since 1940 (Kumar et al., 2019; Rahman & Choudhury, 2012). They are purchased as fresh mushroom, dried mushroom and also as variety supplements. Shiitake is the second most often grown mushroom (Breene, 1990). Shiitake mushrooms are cultivated in moist sub-woods and on cambium trees. It may be grown both indoor and outdoor in cool areas. Shiitakes are grown in natural log and sawdust. Natural log is preferred mostly for small-scale production, and synthetic log is preferred for large-scale production. Shiitakes are used as an ingredient in many products including shampoo, dietary supplements and also in food industries. The production of shiitakes is fairly a simple process (Gold et al., 2008).

This chapter provides the production of shiitake mushroom in small and commercial levels. Also, the economical and cost analysis of small and large is compared and discussed. It also summarizes the marketing strategy and uses of shiitake mushrooms, as well as solutions to overcome difficulties.

2 Life Cycle

Shiitake's life cycle begins when a manure mushroom discharges basidiospores into the air, which are disturbed by the wind. Basidiospore germinates and starts a new colony when conditions are favourable (Przybylowicz & Donoghue, 1988).

Shiitake mushrooms spend the most of their life cycle as secondary mycelium. Mycelium colonizes and digests logs during this stage, collecting and storing nutrients in preparation for fruiting. When little knots of hyphae called primordia grow below the surface after the fruiting stage, mushroom development begins. This primordium eventually turns into a mushroom (Fig. 1).

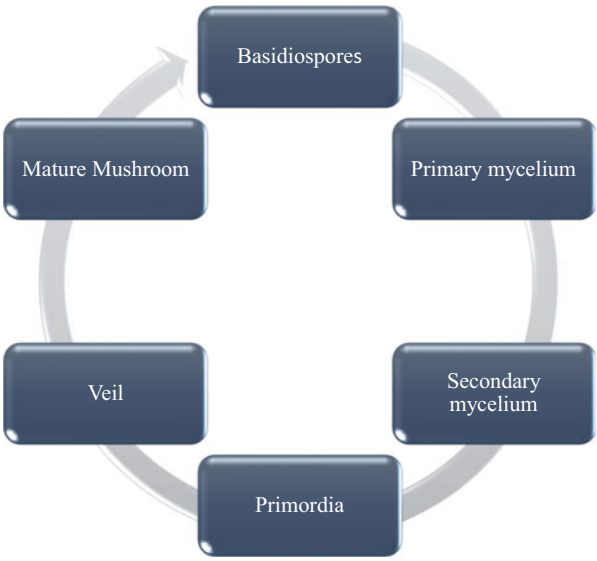

Fig. 1 Life cycle of shiitake mushroom

3 Production of Spawn

Tissue culture technique is used for the production of spawn. A fresh shiitake mushroom inoculum is used for making a shiitake spawn. Fresh mushroom undergoes surface sterilization. The fruit body of the mushroom was cut into small pieces; the pileus and stipe are placed in the Petri plates which contain 2% of sodium hypochlorite and allowed to grow in the potato dextrose agar medium (Table 1) (Sharma et al., n.d.). These are incubated for 7 days at room temperature, and growth of mycelium was observed which is used as mother spawn (Kamalakannan et al., 2020) (Fig. 2).

3.1 Preparation of Mother Spawn

The suitable substrate for spawn production of shiitake mushroom are sawdust, cereal grain, wheat grain or wood plug (Sharma et al., n.d.). Wheat grain is mostly used as a substrate. The procedure for preparation of mother spawn is as follows: soak the wheat for overnight and boil them partially. After boiling, keep the grain for drying in the sieve. About 20 g of calcium carbonate is added to 1 kg of boiled wheat grain. Fill the bottles or polypropylene bag with the substrate (300 g) and make an inoculation hole; the end of the bag is plugged by a non-absorbent cotton plug. These filled bags or bottles are sterilized in autoclave at 121 °C for 90–120 min (Rahman et al., 2019). After sterilization, keep the bag in a laminar air flow chamber and allow them to cool. An inoculating loop is used to inoculate the mycelium in the hole. After

Fig. 2 Mycelium formation

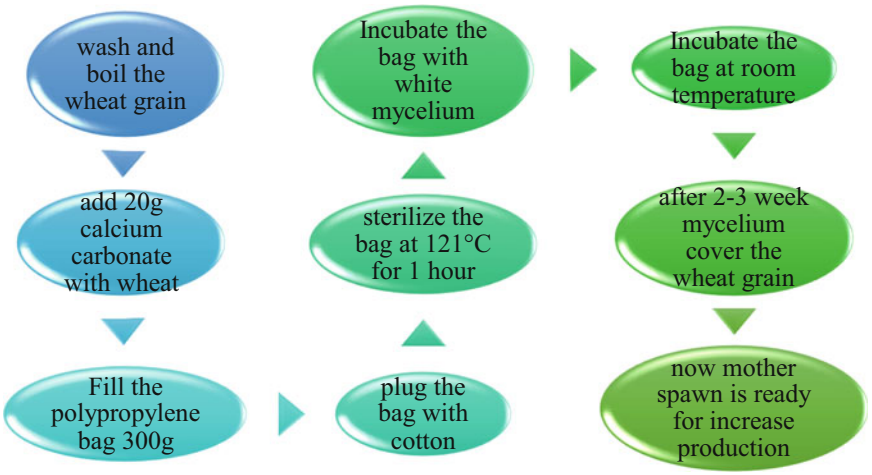

Fig. 3 Production of mother spawn

inoculation, these bags or bottles are maintained at room temperature for 2–3 weeks. The white mycelium of the mushroom is colonized in the wheat grain. This is the mother spawn which is used for further production of spawn (Fig. 3).

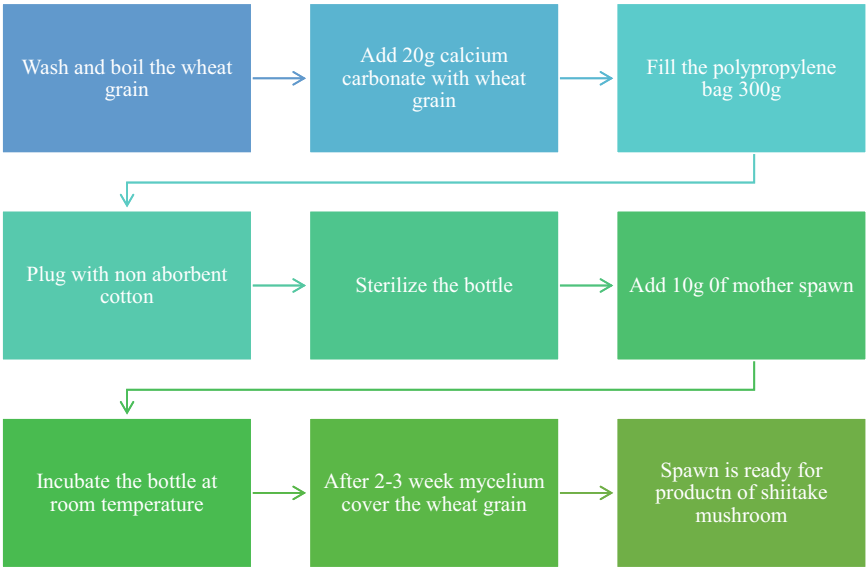

Fig. 4 Development of spawn

3.2 Mother Spawn Used for Proliferation of Spawn

Calcium carbonate mixed to the boiled and dried wheat grains acts as a substrate for spawn production. Fill the bottles or bag with substrate and sterilize them (Sharma et al., n.d.). Inoculate 15–20 g of wheat grain which consists of mycelium (mother spawn) into the bottle and incubate the bottle or bag in room temperature (Przybylowicz & Donoghue, 1990). After 2–3 weeks, white mycelium colonizers are formed in the wheat grain which is present inside the bottle or bags. Now, this spawn is ready for growing entire shiitake mushroom (Kamalakannan et al., 2020) (Fig. 4).

3.3 Preparation of Potato Dextrose Agar Medium

Table 1 Media Composition

Component	Amount
Sliced and peeled potato	200 g
Dextrose	20 g
Agar	15 g
Distilled water	1 L
pH	5.5–6.5

4 Mass Production of Shiitake Mushroom

4.1 Lab Scale Production of Shiitake Mushroom

It is necessary to know the details of cultivation of shiitake mushroom production in lab scale.

Substrate Preparation and Sterilization

Broad-leaf trees like mango, oak and maple are used as a popular sawdust. Sawdust and wheat brans are used as a substrate which are mixed together by sanitized hand. Calcium carbonate is also used about 20 g for per kg of sawdust (Mushroom, n.d.). Calcium carbonate is added to maintain the pH of 5.5–6.0. Immediately fill the polypropylene bag with the substrate and allow for sterilization (Ho & Suzuki, 2019). Sterilization takes place in an autoclave at 121 °C by maintaining pressure of 22 psi for 1–2 h. After sterilization, bags are kept in the laminar airflow chamber to prevent contamination (Przybylowicz & Donoghue, 1990).

Inoculation and Incubation

Spawn is inoculated to the substrate in the laminar air flow chamber, and for the even distribution of spawn, shake the bags. After inoculation, the bag is allowed to incubate. At this process, mycelium, spawn running and bristle formation will be formed. When sawdust substrate turns to brownish colour, remove the bags. Temperature, water, humidity and oxygen are maintained in the cropping room (Ho & Suzuki, 2019). Fruiting body is formed in the substrate after 60–70 days. The substrate with fruiting is maintained by spraying with water for 2–3 days. After 4–5 days, the mushroom gets matured and ready for harvesting (Mushroom, n.d.).

Harvesting

Matured mushroom is harvested either by hand picking, scissors or using knife. One half of the matured fresh mushroom is allowed for packing while the other half is taken to hot air oven for drying the mushroom. Dried mushroom has high aroma and flavour; then, these dried mushrooms are allowed for packing. Waste substrates are used as fertilizer in agriculture land and for fuel utilization (Sekino & Jiang, 2021).

4.2 Small-Scale Production

Shiitake mushroom cultivation in India began around 15 years ago. The average price of shiitake is ₹1000–1200 per kg. As a consequence, many farmers and investors around the country are choosing shiitake to produce them. Shiitake mushroom is the ideal alternative for the person interested in recreational scales or at modest local scales. Shiitake is a kind of wood fungus that grows on sawdust (Dwyer, n.d.). Sawdust manufacturing is a high-volume operation that must be carried out in facilities with tight temperature, light and humidity control. Experience is the most significant compound in shiitake mushroom. Unexperienced persons may

suffer losses as a result of sawdust contamination. On a small scale, outdoor production is done with tree logs, while indoor production is done with a shiitake growing kit at home (Tables 2 and 3).

Table 2 Non-recurring expenditure for spawn production

Spawn production				
S. no	Requirment	Cost of each (in ₹)	Total volume (in sq.ft)	Total cost (in ₹)
1	Land	167	300	50,000
2	Construction	–	1	1,45,000
3	Rack	6500	1	6500
4	Inoculating loop	300	1	300
5	Punsen burner	350	1	350
6	Autoclave	6000	1	6000
7	Laminar air flow chamber	50,000	1	50,000
8	Sprayer	100	1	100
9	Boiling kettle	25,000	1	25,000
10	Test tube	15	10	150
12	Petri dish	20	10	200
11	pH meter	7000	1	7000
12	Gas connection	1000	1	1000
13	Water connecting motor and pipe	15,000	1	15,000
14	Light	150	5	750
Total cost				3,07,350

Table 3 Recurring expenditure for spawn production

S. no	Requirment	Total cost of each unit (in ₹)	Total volume	Total cost (in ₹)
1	Empty bottles	30	350	10,500
2	Wheat grain	40	300 kg	12,000
3	Cotton	400	5	2000
4	Calcium carbonate	450	7 kg	3150
5	Polythene bag	24	350	8400
6	Wood	–	–	500
7	Electricity	–	–	3000
8	Labour charge	–	2	30,000
9	Bucket	200	1	200
10	Plastic or iron rod	3	350	1000
11	Washing material	–	–	200
12	Potato	–	–	50
13	Dextrose	–	100 gm	150
14	Agar agar	–	100 gm	800
15	Ethanol	–	500 ml	200
Total cost				72,150

Selection of Wood

Shiitake mushrooms get their nutrients from sapwood and cambium. The species for Shiitake cultivation is genus *Quercus*, *Liriodendron tulipifera, Carpinus caroliniana, Liquidambar styraciflua, Acer rubrum*, and *Betula papyrifera*. Woods with high density are ideal. Tree species should be cut during the dormant period to avoid harm. We should maintain the water level in the logs until inoculation. Healthy logs with length 3–4 ft and diameter 3–8 in. are highly preferred (Davis, 1993; Dwyer, n.d.; Royse et al., 1985).

Inoculation

It is the process of introducing spawn into the logs. The spawn is the mixture of strain, sawdust, grains and nutrients. At the time of inoculation, the log should be in 35–55% moisture. For inoculation, drill holes in log. Both electric hand drill and high-speed drill are used based on the number of logs we have. Spawn should be inoculated immediately to prevent the contamination. Then, the holes are sealed with hot wax like cheese and paraffin. For less consumption (Davis, 1993), mix the wax (80%) with mineral oil (20%).

Incubation

After inoculation, the logs are kept undisturbed as stacking for the formation of mycelium. The spawn is run into the logs and begins to colonize throughout the wood. The logs should be put in the shaded area with sufficient air circulation. This process can take time up to 6–8 months based on the logs and strain. Temperature of 20–25 °C is best for mycelium growth. After the growth of the mycelium, it looks like white patches in the inoculated area and also in the ends of the logs.

Stacking

During incubation, many stalking methods are used as follows: frame stack, lean to stack, triangle and crib stack. According to Davis (1993) and Dwyer (n.d.), the most common stacking methods are crib stack and lean to stack because these methods allow more logs stacked in a small area and exposure to sun and rain.

Log Moisture

As well, the moisture of the log should be maintained for good production and checked periodically throughout the growth of the mushroom. The moisture content of log ranges between 35% and 55% on average (Frey & Heath, 2020). If the log moisture goes down to 35%, then it was sprinkled with water using overhead irrigation system (Davis, 1993).

Fruiting

After the mycelium formation, if the logs are left alone, then fruiting occurs naturally. But for early production, we have to induce fruiting artificially. To stimulate fruiting, the logs are immersed in water for 24 h in a container or tank. The humidity should be kept between 60% and 85% (Frey & Heath, 2020; Royse et al., 1985). Soaking helps to increase the production. In summer, logs are soaked in

cold water at 50 to 60 °F; in winter, after soaking, logs must be kept between 59° and 68 °F. Fruiting can be done in two ways: outdoor fruiting and indoor fruiting. For indoor fruiting, the logs are transferred after soaking into green house. In outdoor production, the logs are kept closed with shade or agricultural cloths for maintaining moisture and humidity.

Harvesting

Shiitakes should be harvested usually for 5–7 days after mushroom appears. Although shiitake mushrooms are plucked at varying stages of maturation, the top-grade mushrooms retain an in-rolled edges on the caps (Dwyer, n.d.). The cap should open above 50% when picked. It was collected by breaking the stem at its base. After harvesting, it should be placed on a sterilized container. Refrigerate the mushrooms at 41 °C within 1 h after harvesting.

Refrigeration

Shiitake should be refrigerated soon after picking to delay degradation as much as possible. The rate of respiration may be as high. So, mushrooms should be kept as cool quickly as possible. The coolers used should be having adequate cooling capacity, a high airflow rate and high relative humidity. Furthermore, mushrooms should never be frozen because this would ruin the product (Davis, 1993; Dwyer, n. d.; Kumar et al., 2019) (Fig. 5).

Drying

Shiitakes are also sold as dried mushrooms. Drying preserves the quality of the mushroom over a year. The mushrooms with commercial uses are dried for later production. For drying, mushrooms of same size and grade are separated for easy drying (Przybylowicz & Donoghue, 1988; Wang et al., 2014).

Methods of Drying (Fig. 6)

Packing

The market decides the sort of packaging utilized. Mushrooms are frequently offered in bulk or prepared form; they are also available in compact retail containers made up of trays coated in a gas permeable plastic coating.

Gold et al. (2008) differentiated advantages and disadvantages on Growing on log as follows:

Advantages	Disadvantages
Taste as well as nutritional and therapeutic benefits have made it popular	Hard work and serious commitment
Sustainability and quality product	About 1 year will be taken for the investment
Low investment	Production available only in certain period
Use of available resource	Growth depends on weather

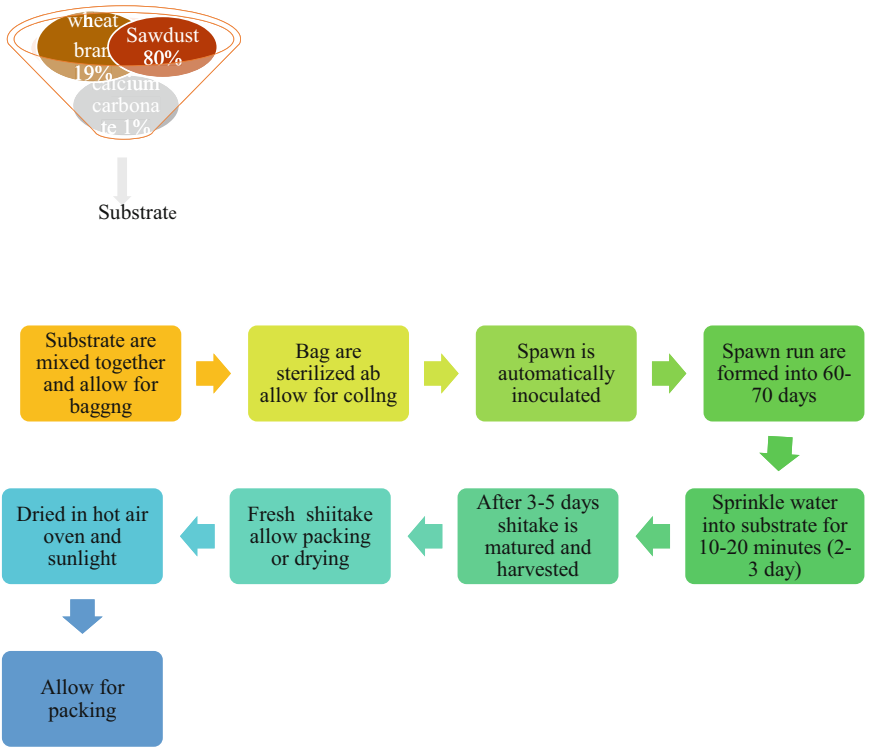

Fig. 5 Large-scale production of shiitake mushroom

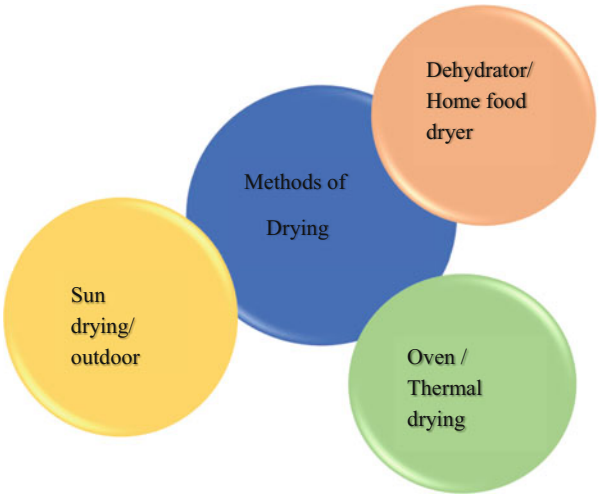

Fig. 6 Method of drying

4.3 Shiitake Mushroom Growing Kit

A mushroom growth kit is simply a pre-colonized fruiting block that has not yet been placed in circumstances that is favourable to mushroom fruiting. The mycelium-covered block is often housed in a mushroom grow bag, which may remain dormant for an extended period of time, especially if kept cold in the fridge.

Growing shiitake differs from growing other mushrooms. They grow well in kits, because shiitake requires much fresh air and can handle low humidity, which is comparable to what is required to grow shiitakes on your kitchen counter. These kits are identical to the fruiting blocks on their farm. For up to 3 months, you should be harvesting many times from this fast-growing shiitake variety. This package includes complete instructions for growing our own shiitake mushrooms. The kit ranges from ₹1000 to ₹2000.

4.4 Large-Scale Production of Shiitake Mushroom

In this method, we are going to know the elaborate view of production of shiitake mushroom in industries and cost analysis for the production (Fig. 5).

Collection and Mixing of Raw Material

Shiitake requires wood-based growing medium to get their nutrient. So, you have to choose sawdust of broad-leaved trees like mango, oak, maple, beech and ironwood, which are popularly used sawdust (80%) (Ranjbar & Olfati, 2017). A wood crushing machine cuts the wood into small pieces or wood chips (Mushroom, n.d.). For nutrient, rice bran or wheat bran in good quality (19%) and calcium carbonate (1%) are also used. Sawdust is soaked for 16–18 h; wheat bran is soaked for 3–4 h. The sawdust and wheat brans are transferred to the mixture by conveyor, and 65% moisture should be maintained by adding purified water. Calcium sulphate dihydrate is used to maintain the pH up to 5.5–6.6 (Mushroom, n.d.).

Packing and Bed Formation

All these raw materials are mixed together and automatically transferred to bag filling machine. These automatic filling machines fill the polypropylene bags which are packed by using that raw material up to the weight of 1.5–2 kg (Ho & Suzuki, 2019). Iron ring or plastic ring is inserted into the mouth of the bag after packing and plugging with non-absorbent cotton.

Sterilization and Cooling

The trolley is autoclave for sterilization. Bags are sterilized at 121 °C by maintaining the pressure at 22 psi for 90–120 min in the autoclave. Cool the log with central air condition to 23–26 °C for inoculation (Przybylowicz & Donoghue, 1990).

Spawn Inoculation and Incubation

An automatic inoculating machine is used for inoculating grain spawn of 3% (wet weight basis). Now the process of punch, inoculation is finished and logs are sent out automatically (Mushroom, n.d.). Ethanol and ultraviolet rays are used to sterilize the working area. Distilled water is used to wash the inoculating machine (Figs. 7 and 8).

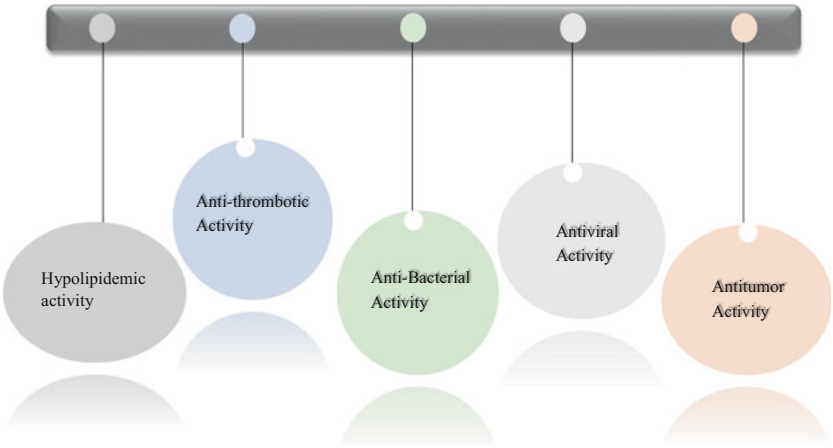

Fig. 7 Medicinal value of shiitake mushroom

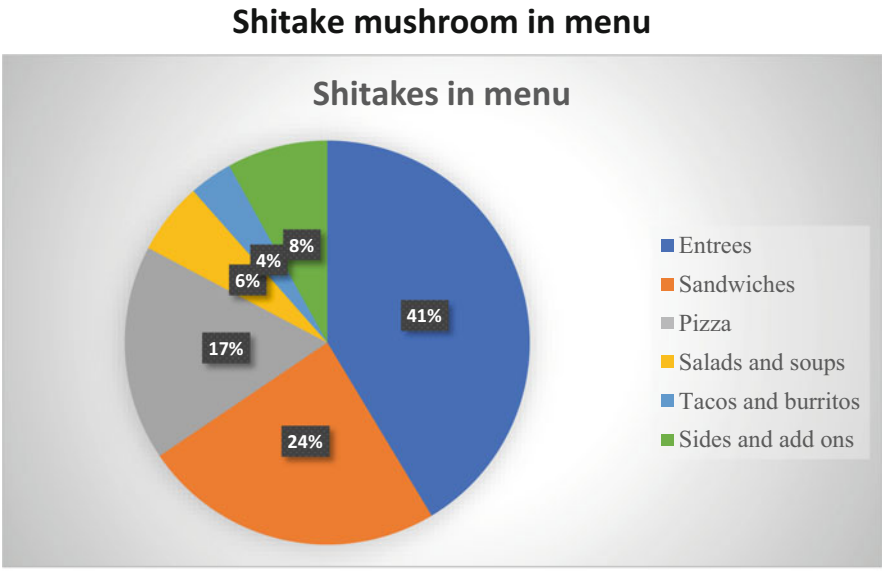

Fig. 8 Fine art of shiitake mushroom

Mycelium Formation

The floors of the culture house are cleaned using fresh water or ethanol. The culture log is transferred to the culture house. Hang sticky trapping card is used for insect prevention. Humidity, ventilation system and air condition are important. Spawn will run around 60–70 days including initial spawn running, mycelial coat, blister formation and browning. CO_2 level is reduced below 1200 ppm. Oxygen is needed after the bumps are formed. Polypropylene bags are removed when the substrate turned brown. After that, it is transferred to the cropping room (Mushroom, n.d.).

Fruiting and Maturation

For the development, proper temperature, ventilation, RH (85–90%), and cold water are required (Przybylowicz & Donoghue, 1990). Cold water treatment by sprinkling water into spawn run substrate for 10–20 min (Mushroom, n.d.). The substrate was treated with cold water for 2–3 days, and small primordia is developed. After 4–5 days, shiitake mushroom is matured and ready for harvesting.

Harvesting and Drying

Harvesting time arises when the mushroom cap edge is rolled. Hand picking is one of the efficient ways for harvesting shiitake mushroom by holding the stalk of mushroom and slowly twisting it from the substrate. Fresh shiitake mushroom having earthy flavour is also an edible one. After successive complete growth, it is maintained in hot air oven (40–50 °C) for 24 h. For best taste and aroma, in olden days, charcoal fire or wood fire is used to dry shiitake (Ho & Suzuki, 2019). After drying at high temperatures, it exposes the flavour and aroma. Furthermore, it is placed in sunlight for complete drying, and it also increases the vitamin D content (Wasser, 2005).

Packing

Dried or fresh shiitake mushroom is allowed for packing. An automatic packing machine packs the shiitake mushroom and packed mushroom.

5 Cost Analysis

Indoor production of shiitake mushroom is cultured on both lab scale and large-scale industries. For both the production, instrument and constructed building or green house are required (Kwon et al. 2018). Outdoor production of the shiitake mushroom is cultured in small-scale production. For this production, construction of building is not required. Amount needed for lab scale production of shiitake mushroom is ₹1,36,000, and income of lab scale production is ₹2,00,000 (Tables 4 and 8). In lab scale production, a total of 5 to 6 cycles can be produced in a year. Total establishment cost for shiitake mushroom production in small scale production is ₹9,83,800 and earning is ₹12,00,000 (Tables 5 and 6). Due to seasonal changes, small-scale production can only be produced three cycles a year. While in high

Table 4 Recurring expenditure of lab scale production of shiitake mushroom

Lab scale production				
S.no	Requirment	Cost of each unit (in ₹)	Total volume	Total cost (in ₹)
1	Air conditioner	10,000	1	10,000
2	CO_2 controll machine	5000	1	5000
3	Wood crushing machine	20,000	1	20,000
4	Hot air oven	15,000	1	15,000
5	Packing machine	2000	1	2000
6	Plastic tray	20	22	440
7	Autoclave	6500	1	6500
8	Laminar air flow chamber	30,000	1	30,000
9	Ventrilator fan	3000	1	3000
10	Knife	25	2	50
11	Scissors	50	2	100
12	Temperature and humidity controller	6000	1	6000
13	Steel or wooden rack	6000	1	6000
Total				1,04,050

Table 5 Non-recurring expenditure for shiitake mushroom

Small-scall production				
S. No	Requirement	Cost of each unit (in ₹)	Total volume	Total cost (in ₹)
1	Land	30,000	18 cents	5,40,000
2	Drilling stand	1500	2 stands	3000
3	Driller	2000	5 nos	10,000
4	Inoculation tool	400	5 tools	2000
5	Brush	100	5 nos	500
6	Hose, sprinkler	1500	4 sets	6000
7	Water tank	5000	3 units	15,000
8	Shade cloths	1000	5 nos	5000
9	Refrigerator	15,000	2 units	15,000
10	Baskets	May vary	5	1000
Total				6,06,500

budget plan, total establishment cost for shiitake production is ₹3,48,65,500 and income for per cycle is ₹4,50,00,000 (Tables 7 and 8). Large-scale indoor production is cultured six times in a year. Expenditure cost, procedure and profit differ for every scale. Indoor production shows high quantity but outdoor production shows higher in quality. The current market value of fresh shiitake mushroom is ₹1000–1200 and dried shiitake mushroom is ₹2000–₹2500 per kg. The shiitake mushroom production per cycle is expected to be 200–230 kg in lab scale, 900–1200 kg in small-scale production and 40,000 kg in large-scale production. Annual profit of shiitake mushroom is expected to be ₹10,00,000–₹12,00,000 in lab scale,

Table 6 Recurring expenditure for shiitake mushroom

S. no	Requirement	Cost of each unit	Total volume	Total cost (in ₹)
1	Logs	350	1000 nos	3,50,000
2	Drill bit	300	5 nos	500
3	Sawdust	500	40 kg	20,000
4	Wax	500	2 kg	1000
5	Packing boxes and cover	100/kg	–	800
6	Transportation	–	–	1000
7	Advertising	10/sheet	100 sheets/poster	1000
8	Utilities (electricity, water, etc.)	–	–	3000
Total				3,77,300

Table 7 Non- recurring expenditure for shitake mushroom

Large-scale production of shiitake mushroom				
S. no	Requirment	Cost for each unit	Total volume	Total cost (in ₹)
1	Land expenditure	₹75,000	50 cents	37,50,000
2	Building construction	–	1	45,00,000
3	Wood crushing machine	₹72,500	2	1,45,000
4	Mixing machine	₹1,00,00	1	1,00,000
5	Bag filling with cap and ring machine	₹1,50,000	3	4,50,000
6	Plastic tray	₹20	4800	96,000
7	Autoclave	₹4,00,000	1	4,00,000
8	Steel or wooden rack	₹6000	200	13,00,000
9	Automatic inoculatic machine	₹3,00,000	1	3,00,000
10	Conveyor	₹40,000	5	2,00,000
11	Punching machine	₹10,000	2	20,000
12	Motor	₹5000	2	10,000
13	Water connecting motor and pipe	₹75,000	2	1,50,000
14	Air conditioner	₹2,00,000	7	14,00,000
15	CO_2 controll machine	₹7000	4	28,000
16	Hot air oven	₹2,50,000	2	5,00,000
17	Knife	₹25	25	500
18	Scissors	₹50	20	1000
19	Packing machine	₹10,000	1	10,000
20	Light	₹250	20	5000
21	Computer	₹50,000	2	1,00,000
22	Ventrilator fan	₹7000	4	28,000
23	Temperature and humidity controller	₹6000	4	24,000
24	Sprinkler irrigation pipe	–	–	1,00,000
25	Korklift truck	₹10,00,000	2	20,00,000
26	Wiring cost	₹3,00,000	1	3,00,000
Total				1,94,17,000

Table 8 Recurring expenditure for shiitake mushroom

S. no	Requirment	Cost of each unit (in ₹)	Lab scale production		Large-scale production	
			Total volume	Total cost (in ₹)	Total volume	Total cost (in ₹)
1	Wheat bran	30	30 kg	900	19,000 kg	3,80,000
2	Wood	18	160 kg	3000	60,000 kg	1,20,00,000
3	Polyprolylene bag	20	200	4000	43,200	10,36,800
4	Non-absorbent cotton	250	1 roll	250	20 kg	12,500
5	Plastic or iron ring	3	200	500	43,200	1,29,600
6	Calcium carbonate	30	10 kg	300	1000 kg	4,50,000
7	Electricity per month	–	–	–	–	50,000
8	Spawn bottle	450	8 bags	3600	1728 bags	7,77,600
9	Ethanol	400	–	–	2 L	800
10	Labour salary per month	–	1	20,000	20	6,00,000
11	Trapping card for insects	2800	–	–	4 packs	11,200
Total				32,550		1,54,48,500

Table 9 Comparison of lab scale, small scale and large scale

	Lab scale production	Small-scale production	Large-scale production
Price for per kg based on the production	₹1000	₹1200–1500	₹1000
Total yield on one log per fruiting cycle	1.5–2 kg	800–900 gm	1.5–2 kg
Total fruiting cycle per log	1–2	3–4	1–2
Total establishment cost	₹1,36,600	₹9,83,800	₹3,48,65,500
Total income per fruiting cycle	₹2,00,000	₹12,00,000	₹4,50,00,000
Total fruiting cycle per year	5–6	3	5–6
Annual profit	₹10,00,000–₹ 12,00,000	₹25,00,000–₹ 30,00,000	₹22,50,00,000–₹ 27,00,00,00

₹25,00,000–₹30,00,000 in small-scale production and ₹22,50,00,000–₹ 27,00,00,000 in largescale production (Table 9).

6 Marketing Plan

6.1 Medicinal Value

Shiitakes have many medicinal values. Ancient Chinese suggested that consumption of shiitake mushroom could acquire long life and good health (Wasser, 2005). Also, it conserves health, enhances stamina and circulation, treats the cold and decreases

the blood cholesterol in the modern terminology. Protein, lipid, carbs, vitamins and minerals are found in shiitake mushroom (Rahman & Choudhury, 2012). Certain activities that are found in the shiitake mushrooms are as follows:

Hypolipidemic Activity
A meal supplemented with the dried powdered sporophore of *L. edodes* reduces average plasma cholesterol levels (Mori, 1974). In both animal and humans, the major and active component called eritadenine decreases all lipid components of serum lipoproteins. The use of dried shiitake mushroom reduces both HDL and VLDL cholesterol levels.

Anti-Thrombotic Activity
Individuals who consume shiitake mushroom oil have been proven to have a lower risk of thrombosis. Also, the quantities of Lanthionine contained in shiitakes reduces platelet aggregation (Breene, 1990; Jasrotia & Prashar, 2012).

Anti-Bacterial Activity
When the host comes into contact with pathogens, shiitake helps to modulate the inflammatory response. It also boosts macrophage activation by activating the complement system (Rahman & Choudhury, 2012).

Antiviral Activity
The antiviral medicines such as proteinase inhibitors are found in shiitakes. Also, anti-HIV properties are found in the mycelial culture and have antiviral properties against influenza and polio viruses as well as various bacteria and parasites (Rahman & Choudhury, 2012).

Antitumor Activity
Ikekawa et al., 1969 discovered that an interperitoneal injection of an aqueous extract of shiitake significantly supresses the tumour development by 81% generated from sarcoma 180 ascites cells transplanted in albino mice.

Shiitake mushrooms have several health advantages that might help with weight loss (Rahman & Choudhury, 2012). According to American Heart Association, eating well-balanced diet that contains nutritious mushrooms is the greatest way to reduce weight.

Also, shiitake mushroom is useful for the production of capsules for nutritional supplements. Shiitake serves a variety of roles in the medical field (Kumar et al., 2019; Senti et al., 2000). “Food is medicine,” as the adage goes more intake of shiitakes leads to longer life. So, demand for shiitakes is higher than marketing shiitakes was quite simpler.

Nutritional Analysis
Shiitake mushroom is nutrient dense, including protein, lipids, carbs and variety of vitamin and minerals (Breene, 1990). When it comes to quality and quantity, shiitake mushrooms outer performs well. The ratio of all nine necessary amino acids is

Table 10 Nutritional analysis

Nutrients in shiitake mushroom		In caps	In strips	Activity
Primary nutrients	Protein	284 g/kg	188 g/kg	Reduces cholesterol level, keeps hydrated, prevents the diseases and maintains healthy
	Lipid	21 g/kg	20 g/kg	
	Fibre	25 g/kg	82 g/kg	
	Water	914 g/kg	872 g/kg	
	Ash	62 g/kg	41 g/kg	
Minerals	Copper	9 g/kg	4 g/kg	For humans, they serve as a key role in cell metabolism, biosynthesis and physiological function
	Zinc	72 g/kg	53 g/kg	
	Iron	36 g/kg	35 g/kg	
	Calcium	174 g/kg	370 g/kg	

comparable to that of optimum protein for human diets. It also contains a lot of leucine and lysine (Li et al., 2018) (Table 10).

6.2 Fine Art of Shiitake Mushroom Cooking

Shiitake is one of the most common foods throughout the food. From 2007 to 2015, the average yearly mushroom intake in the United States was 3 kg per person. It becomes more popular due to their nutritional and culinary qualities. Mushrooms are cooked as soup, curry, biryani, snacks, biscuits, ketchup, masala, etc. in food industry. Dried shiitake mushrooms are used in the production of instant soup. Demand for shiitakes in food industry is also high (Singh et al., 2017).

6.3 Shiitake for Glowing the Beauty

Shiitake mushroom is also used for making the cosmetic product. Shiitake mushroom is rich in vitamin C and antioxidant that protect the skin from ageing, wrinkles and acne. For brightening the skin, shiitake mushroom extract is used which is high in kojic acid content. Shiitake mushroom extract is used for making shampoo and conditioner which have iron content; it strengthens the hair and reduces the hair fall. Age bright clearing serum is produced from the extract of shiitake mushroom which contain antioxidant and anti-inflammatory properties to promote skin tone. Shiitake is also used for making under eye cream. Many cosmetic products like face cream, foundation, moisture cream, face pack, etc. are made by using shiitake mushroom.

6.4 Advertising and Publicity Method

In the starting stage, the main goal of the seller is to create name and fame to their product. For promoting the product, the seller must give advertisement in television, promotion is done in newspaper or in radio about the shiitake mushroom,

having partnership with another company to create and develop market value and creating collaboration with charities for making the product sell more efficient. This is one of the easiest and finest ways to reach the customer. Catalogues, brochures and leaflets can also be distributed in the target area or directly mail to customers.

6.5 Disadvantage and Way to Overcome

Slug and Snail

Slugs, termites and snails are the most common pests on shiitake. They cause serious damage by feeding. Also, crack and other diseases on mushroom lead to loss. By keeping the logs at good environment, we can avoid damage by sprinkling lime water, removing dead leaves and keeping soil dry should be appreciable (Ashrafuzzaman et al., 2009; Przybylowicz & Donoghue, 1988). Birds and deer also feed the mushroom. To protect the shiitake, keeping the dogs and constructing tall fence are best ideas. Every day, check and hand pick the slugs if you find. Chicken or ducks destroy slugs in high level. Wood ash is also used to remove the slugs (Rahman et al., 2019).

Soldier Beetles, Squirrels and Way for Prevention

They eat the mushroom before harvesting and disturb the shiitake grown and destroy the mycelium growth. The way for prevention is to harvest the mushroom quickly. Cover the mushroom with cloth to avoid it (Przybylowicz & Donoghue, 1990).

7 Idea for Reducing Investment

S. no	Requirement used	Alternative idea
1	Packaging and labels	Make own labels on a home computer
2	Log drilling stand	Use vase grips or work with person help
3	Wax melting pot	An old fryer or hot plate
4	Water hose and sprinkler	May already have one in home or farm
5	Shade cloth	Small diameter crooked branches that will keep cover off top logs
6	Soak tanks	Small kid's pool or stock trough that already have on farm
7	Constructed building	Instead of building, greenhouse used for industrial development of shiitake
8	Storage baskets	Any baskets in home with good airflow

8 Financial Support

There are several subsidies and loans available to help with the growth of mushroom farming. In India, the National Horticulture Board (NHB) also assists mushroom producers with credit-linked bank end subsidies. The subsidy is equal to 20% of the overall cost. Subsidies are also offered for spawn making unit and compost making units. Training is also available for peoples who are interested in mushroom farming.

9 Conclusion

The Shiitake mushroom is the most effective way to do business in all countries. Although Shiitake is a good choice for entrepreneurs; experience is the most important factor for growth. Shiitake mushroom growing kits and small-scale growth are the best options for beginners. Shiitake mushroom is in high demand in the pharmaceutical, cosmetic and food sectors. Despite the high manufacturing costs, the value of shiitake is equally considerable. The mass production of shiitake mushrooms is elaborately discussed. The costs were examined, and methods for lowering them were explored. The overall revenue of shiitake production on a small scale every fruiting cycle is ₹12,00,000; whereas on a large scale, it is ₹4,50,00,000. Large-scale manufacturing is recommended for high volume, whereas small-scale production is preferable for high quality.

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Production and Entrepreneurship Plan for Red Pigment from *Monascus* sp.

Mahalakshmi Karthikeyan and Dhanasekaran Dharumadurai

Abstract

The fungus *Monascus purpureus* belongs to the phylum *Eucomycota*. These fungi are recognized for their fermented products and provide a variety of polyketide-type secondary metabolites. The yeast is used to prepare cholesterol-lowering statins in the commercial world. Statins, which are prescription drugs used to treat high cholesterol, have the same active ingredients as red yeast. During growth, *Monascus* spp. break down the starch substrate into a range of metabolites, including secondary metabolites such as colours. *Monascus* pigment can be produced through solid state or submerged fermentation and then recovered, dried, and used. Thus, this chapter illustrates the entrepreneurship of *Monascus* pigment is characterized by the ability and willingness to start, organize, and run a *Monascus* pigment business, including all of its associated risks, in order to profit.

Keywords

Monascus pigment · Red yeast · Solid state fermentation · Submerged fermentation

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1 Introduction

Monascus purpureus is a fungus that belongs to the phylum Eumycota group or phylum, the Ascomycotina subphylum, the class Ascomycetes, the order Eurotiales, and the Monascaceae family. *Monascus pilosus*, *M. purpureus*, *M. ruber*, and *M. floridanus* are among the nine species of Monascus. *M. argentinensis*, *M. eremophilus*, *M. lunisporas*, *M. pallens*, and *M. sanguineus* are the three species listed, which has received critical appreciation on a global scale. These fungi are recognized for their fermented products and are a source of a variety of secondary metabolites of the polyketide type. The majority of higher fungi are heterokaryons, meaning they have genetically distinct nuclei that share the same mycelium. When the colonies of a fungus have genetically identical nuclei, the fungus is homokaryotic. Heterokaryosis has been demonstrated to produce filamentous fungus variety as well as metabolite instability or loss during fermentation operations. *M. purpureus* belongs to the *Monascus* genus, which encompasses a group of fungus that are normally regarded asexual; yet, perfect forms (sexually reproducing forms) have been discovered. Similar to many fungi, *M. purpureus*' taxonomy is based mostly on visual qualities rather than biochemical and physiological properties or genetic makeup, which are commonly used to categorize bacteria. The yeast *Monascus purpureus* is utilized in the commercial manufacture of blood cholesterol-lowering statins. Fungus is the most important because it is used in the creation of fermented foods in China in the form of red yeast rice. In healthy persons, red yeast is used to keep cholesterol levels in check. Red yeast contains the same active component as statins, which are prescription medications used to treat high cholesterol. As a result, red yeast comes with all of the toxicity, drug interactions, and warnings that come with this sort of treatment. Monascus purpureus works by selectively inhibiting the enzyme that produces cholesterol. During the growth of *Monascus* spp., the starch substrate is degraded into a range of metabolites, including colours produced as secondary metabolites. The structure of pigments is influenced by the type of substrate used as well as a variety of other parameters such as pH, temperature, and moisture content during culture. This fungus is significant because it is used to make red yeast rice, which is a fermented dish popular in China (Table 1). The structure of various *Monascus* pigments are given in Fig. 1.

Table 1 Pigment types from *Monascus* spp.

S. no	*Monascus* spp	Substrate used for production	Type of pigment	Reference
1	*Monascus purpureus*	Rice and sorghum	Rubropunctamine	
2	*Monascus ruber*	Rice and grains	Monascorubramine	
3	*Monascus anka*	Rice	Ankaflavin	
4	*Monascus pilosus*	Rice	Ankaflavin	

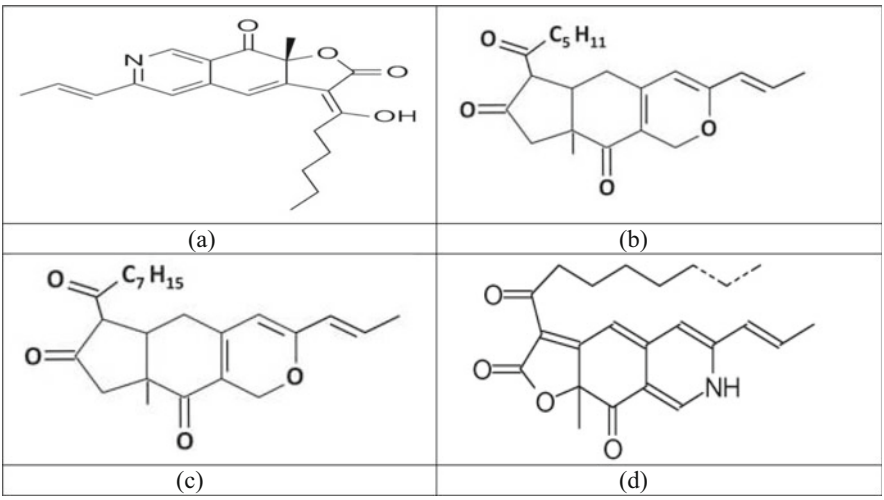


Fig. 1 Chemical structure of pigments from *Monascus* spp. (**a**) Rubropunctamine. (**b**) Monascin. (**c**) Ankaflavin. (**d**) Monascorubramine

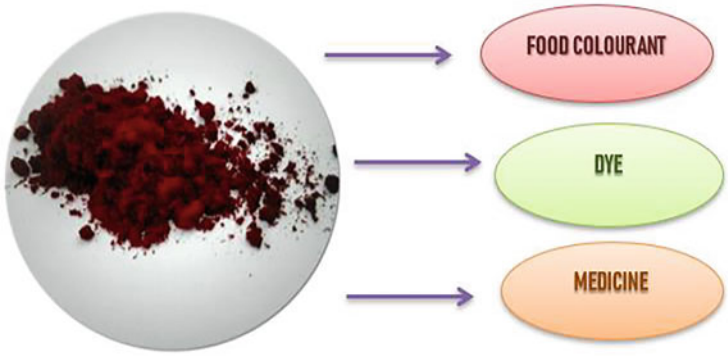


Fig. 2 Uses of red pigment

Three strains of the fungus Monascus purpureus AKI, AKII, and 915 were chosen to produce angkak colours and lovastatin in potato dextrose agar (PDA) media. After that, the best fungal strain AKII has been used in three various rice media (Danuri, 2008). The *Monascus* pigment can be produced from naturally available substrates like rice, corn, whole sorghum grain (WSG), and dehulled sorghums (DSG). The grown fungi were used for the production of the *Monascus* pigment. It was found by the studies conducted by Ignatius Srianta and the colleagues that *Monascus purpureus* grew well in rice grain compared to the other substrates (Srianta et al., 2016) (Fig. 2; Table 2).

This chapter describes the many criteria and standards that must be met in order to commence a bio-business. It is currently a rapidly developing discipline that

Table 2 Uses of *Monascus* pigment

S. no	Pigment name	Uses	Industry	Reference
1.	Rubropunctatin	Food colourants	Food industry	
2.	Ankaflavin	Anti-inflammatory property	Medicine	Hsu and Pan (2014)
3.	Monascin	Anti-tumor property	Medicine	Hsu and Pan (2014)

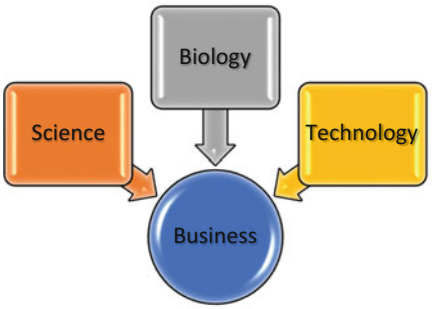


Fig. 3 Integration of science, biology, and technology with business

connects two huge domains: microbiology and business. It emphasizes how science, biology, and technology are all interconnected with business (Fig. 3).

2 Production of *Monascus* Pigment

2.1 Raw Materials

Monascus purpureus grows on different substrates like:

Jackfruit seeds (Babitha et al., 2007).
Grape waste (Daroit et al., 2007).
Wheat straw (Liu et al., 2020).
Broken rice (Johns & Stuart, 1991).
Corn (Mu et al., 2015).
Sorghum (Mu et al., 2015).

The raw materials that are used for the growth of *Monascus* spp. are cheap and easily accessible. Raw materials have to be selected in such a way that they are cheap, so that funds can be saved. Compared to chemically synthesized compounds as raw materials, naturally available compounds could help with saving finance.

Monascus ruber was tested for red pigment synthesis using a complex culture mix that included glucose or dextrose (10 g/L), corn steep liquor (5 or 10 g/L), and monosodium glutamate (0, 5.0, 7.6, 11.4, or 15.2 g/L). The extracellular red colour absorbance (20.7 U) and productivity (0.35 U/h) were best in a medium with 10 g/L sucrose, 5 g/L corn steep liquor, and 7.6 g/L monosodium glutamate. With analytical

grade compounds, this medium also outperformed semi-synthetic media (12.4 U and 0.21 U/h). The cells grew in the same way in both mediums (6.5 g/L), showing that the more sophisticated culture media may produce more red pigments. Furthermore, when compared to the semi-synthetic medium, the complicated culture medium accumulated fewer intracellular red pigments (9.1% and 30% respectively). Because of the cheap cost of the product and the lack of purification, the culture medium is almost certainly a considerable portion of the total cost in the synthesis of red pigment by Monascus sp. For example, raw fermentation medium from rice-based semisolid Monascus sp. cultures is employed directly as a red pigment. The purpose of this research is to create a complex culture medium for Monascus ruber that will allow it to manufacture red pigments, primarily using corn steep liquor, monosodium glutamate (to obtain water-soluble red pigments), and sucrose rather than glucose (to increase the production of certain red pigments and because sucrose is less expensive) (Hamano & Kilikian, 2006).

3 Fermentation

3.1 Solid State Fermentation

Inoculating steamed rice grains spread on huge trays with a strain of Monascus anka and incubation for 20 days in an aerated and temperature-controlled environment is a traditional Chinese process. Moisture content, oxygen, and temperature are all important factors in these cultures carbon dioxide concentrations in the atmosphere, as well as the most significant aspect of cereal medium composition is parameters to regulate. The amount of moisture in the air is a critical factor. In plastic bags holding rice grains, red pigments were created. Pigmentation was only detected at a small scale. The initial moisture level was rather modest (26–32%). The water content of the substrates controlled initial pigmentation. It was observed that when the initial moisture level of the substrate increased, so did glucoamylase activity. As a consequence of the large enzyme activity produced at high moisture content, glucose was promptly released in proportions that inhibited colouring. After that, the sugar was converted into ethanol (Dufossé et al., 2005).

Due to the increased degree of starch hydrolysis, *Monascus* growth was greater on cooked autoclaved rice (4.4 OD/g) than on adequately sterilized rice (3.11 OD/g). High oxygen pressure (3.11 OD/g autoclaved rice) allowed for three times more growth than low oxygen pressure (1.14 OD/g autoclaved rice) in the experiment. Oxygen and carbon dioxide levels have a significant impact on pigment development and, to a lesser extent, growth on solid substrates such as rice. *Monascus* growth and pigment output are influenced by the initial pH of the substrate (Kaur et al., 2009). Monascus cultivated on 50 g/L tapioca starch as a carbon source obtained a biomass dry weight of 8 g/L in a simple batch culture. When cell development slowed to 40 h, the red and yellow polyketide pigments were still generated, with OD units of 31 and 26.5, respectively. After 60 h of batch development, a 200 g/L starch medium was regularly supplied to a Monascus culture, and

the biomass dry weight reached 16 g/L after 140 h. The concentration of red and yellow pigment was 70 and 60 OD units, respectively, at this moment. Mycelia were grown in a two-state (solid-liquid) batch bioreactor, with a solid state containing 400 g/L gelatinized starch substrate and a liquid state containing all culture substrates except starch. The amylolytic enzymatic activity produced by the Monascus culture was used to degrade the starch block gently. The culture expanded at a reasonably consistent rate for the first 170 h, resulting in a cell concentration of 37.5 g/L and pigment concentrations of roughly 145 OD units for both red and yellow pigments.

3.2 Submerged Fermentation

Experiments were conducted to determine the best process parameters for producing colours from potato pomace and its sugar hydrolysate. After inoculating with 10 mL of spore suspension and incubating at 28 °C, 200 rpm for seven days, the volume of media in the flask was 100 mL (Chen et al., 2021).

In 250 mL Erlenmeyer flasks, submerged fermentation was conducted with 50 mL of raw rice straw part of the process fermentation medium. 2.5 g/L yeast extract, 2.5 g/L malt extract, 2.5 g/L peptone, 5 g/L K_2HPO_4, 0.1 g/L $CaCl_2$, 0.58 g/L $MgSO_47H_2O$, 0.02 g/L $FeSO_47H_2O$, 0.02 g/L $ZnSO_47H_2O$, 0.03 g/L $MnSO_47H_2O$, and 0.03 g/L $MnSO_47H_2O$ has to be used in the medium for fermentation. Each experiment is performed in triplicate for 10 days at 30 °C and 150 rpm. Samples were taken on a scale to determine total sugar intake and MPs development. Fermentation kinetics investigations were performed out in a 1000 mL Erlenmeyer flask containing 400 mL fermentation media for 14 days. Experiments on the effects of 50-HMF and Fur on MPs production were conducted out like in a 1000 mL Erlenmeyer flask containing 400 mL of glucose-based fermentation medium (GBFM) containing 0.60 g/L of 50-HMF or 0.36 g/L of Fur and 80 g/L of glucose, to the same other compounds as the raw rice straw hydrolysate-based fermentation medium.

Because MEB broth contains more free amino acids than PDB broth, MEB broths produced greater red pigment (0.176 OD/mL) than PDB broth (0.123 OD/mL). When compared to submerged fermentation, solid-state fermentation increased pigment production in Monascus purpureus MTCC 410. The amount of pigment produced on cooked autoclaved rice (4.4 OD/g) and autoclaved rice (3.11 OD/g) was higher than MEB broth (0.176 OD/mL), and solid-state fermentation produced more biomass than submerged fermentation. Solid-state fermented autoclaved rice yielded 1.09 g wet biomass, while solid-state fermented autoclaved rice yielded 0.44 g wet biomass (Kaur et al., 2009).

M. sanguineus mycelial growth and red pigment optimization in potato dextrose broth fungal medium were explored under adverse conditions. About 50 mL of medium had been prepared in 100 mL conical flasks. After that, it was autoclaved at 121 °C for 20 min to disinfect it. After cooling, the sample was inoculated with 0.5 mL of *M. sanguineus* culture and stored at a temperature of 5.5 to protect it. For a

total of 16 days, the experiment was kept in a static state. The samples were kept at 30 °C for incubation. Glycerol, peptone, and NaCl stress were studied prior to autoclaving by adding glycerol at concentrations of 0.25, 0.5, 0.75, 1, and 1.25 M to the media, as well as peptone and NaCl at concentrations of 0.25, 0.5, 0.75, 1, and 1.25% (w/v) to the media. At 30 °C, 40 °C, 50 °C, 60 °C, and 70 °C, the spore suspension was tested. The effects of temperature stress were assessed 1 min before inoculation. These spores are obtained from fungi. It was also necessary to apply inoculum (Dikshit & Tallapragada, 2014).

4 Recovery of *Monascus* Pigment

Overnight at 55 °C, the fermented materials were dried. The ratios of substrate to solvent differed from one experiment to the next. By using Soxhlet extraction, the pigments were retrieved for 12 h in 95% ethanol under partial vacuum at 65 °C. Except where mentioned, static extractions were performed with 1 g of fermented substrate in 250 mL Erlenmeyer flasks with various organic solvents for 24 h at room temperature. Agitated extractions in 250 mL Erlenmeyer flasks with 95% ethanol or ethanol-water compositions were done at room temperature for 1 h at 110 rpm on a rotary shaker. The extracts were centrifuged for 15 min at 10,000 g in all cases.

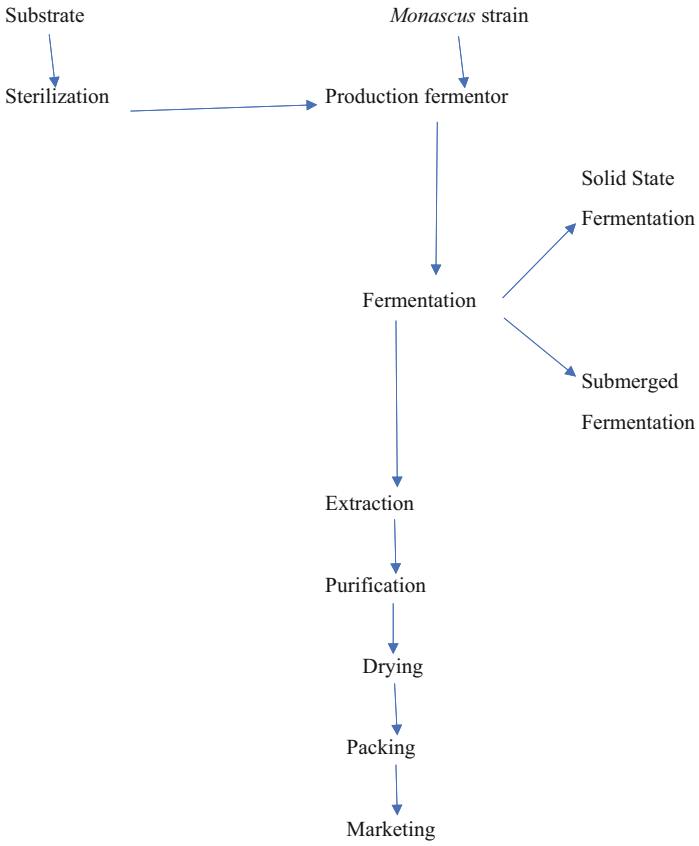
Various ethanol: water ratios of 10:0 were used to extract a properly weighed MFDS from roughly 1 g. About 9:1; 8:2; 7:3; 6:4; and 5:5 were filtered through Whatman no. 1 Paper at 30 °C or 60 °C at 100 rpm for 2 h. The pigment was analyzed using spectrophotometry (Srianta et al., 2020). Monacolins were extracted from solid and liquid samples of fermented food using somewhat different extraction procedures. The solid fermented product was dried and powdered coarsely. The sample (0.5 g) was incubated for 2 h at 60 °C with agitation in 5 mL of ethanol/water solution (75:25), then centrifuged for 10 min at 3000 g. To break the mycelia cells in the liquid fermented product, it was homogenized. The homogenized sample (5 mL) was extracted for 2 h at 60 °C with agitation in 5 mL of 95% ethanol before being centrifuged for 10 min at 3000 g. The supernatant (1 mL) from both experiments was concentrated and dried under vacuum before being resolved in 1 mL ACN. Through a membrane filter, the mixture was filtered (Ajdari et al., 2011). Fermented red "mould" rice (10 g) was placed in a 100 mL flask, dissolved in 20 mL acetone, and shaken for 15 min at 25 °C in an orbital shaker. The top acetone filtrate was filtered using Whatman filter paper and centrifuged for 10 min at 2500 rpm.

Cell-free fermented broth was used to purify the pigments. Scraped off the target band (Rf 0.42), diluted in ethanol, then filtered to remove silica purification of the red pigment generated using preparative TLC was done using semi-preparative HPLC.

5 Purification of *Monascus* Pigment

After extractive fermentation, Monascus pigments were produced from fermentation broth. They went from a nonionic surfactant micelle solution to an ionic liquid phase. According to the findings, Monascus pigments did not interact with primary amines in an aminophilic manner (Zhong et al., 2015).

6 Flowchart of Production of *Monascus*



7 Entrepreneurship Concept in *Monascus* Pigment Production

Entrepreneurship is described as the ability and willingness to create, organize, and run a business in order to profit, with all of the risks that go along with it. The most visible manifestation of entrepreneurship is the establishment of new companies. An entrepreneur is a person who has the capacity and motivation to establish, manage, and succeed in a new business, as well as the risk that comes with it, in order to profit. Starting a new business venture is the best example of entrepreneurship. Entrepreneurs are typically referred to as innovators or providers of new ideas since they bring new ideas to market by replacing old ones with new technologies. The goal of productization is to create a product investment that can be mass-customized. Companies in the high-tech sector must strike a balance between standardization and customization. Product data management techniques can cut down on engineering time and help a product get to market faster. Marketing and sales are two distinct yet intertwined functions. Marketing and sales may be handled by employees from a variety of divisions inside a major organization. Because they lack the financial resources to hire specialized staff for each, startups usually fail to discern between the two (Fig. 4).

Both recurring and non-recurring expenses have an impact on a company's cash flow and profitability of *Monascus* pigment production. Because recurring expenses have a year-over-year influence on profitability, they must be examined, monitored, and controlled to stay within budgeted limits. Non-recurring expenses are rarely anticipated for, but they can have a significant influence on cash flow and profitability in the year they occur. Non-recurring expenses, such as the price of a new location or new equipment are beneficial since they improve business operations. Non-recurring expenses such as substantial consumable like culture media, chemicals, solvents, laboratory glassware or plasticware, legal fees, costs of ceasing operations, and costs associated with labour unrest, among others, can result in business losses, and their causes should be identified and remedied.

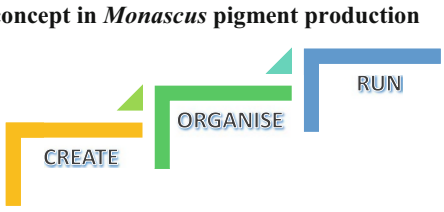


Fig. 4 Entrepreneurship steps

8 Budget Proposal for *Monascus* Pigment Production in Small-Scale Operation

1. Permanent expenses

S. No	Requirements	Amount (in Rupees)
1.	Hot air oven	10,000
2.	Autoclave	15,000
3.	Laminar air flow chamber	30,000
4.	Fermenter (150 gallon, ie.567 L)	59,000
	Total	1,14,000

2. Recurring expenses

S. no	Requirements	Amount	Total expense for a month
1.	Substrate for *Monascus* growth: Rice bran	Rs. 14 (for 1 kg) Rs. 7952 (for 568 kg)	15,904
2.	Culture media and chemicals	10,000	10,000
3.	Laboratory glass wares and plasticwares	10,000	10,000
4.	*Monascus* strain	500 (for 5 g)	1000
5.	Microbiologist (1)	25,000 (per month)	25,000
6.	Labor (3)	10,000 (for 1 per month)	30,000
	Total		91,905

Rice bran is the cheapest source of *Monascus* production, and it is best suited for the growth as it has optimal nutritional requirement for the growth and development of the strain. It is highly suggested to use rice bran in the production of *Monascus* pigment. Rice bran has the optimum amount of carbohydrates, proteins, and other trace minerals like phosphorous that is required for the *Monascus* production.

9 Production Income

Substrate used	Dry weight of Monascus obtained per kg	Dry weight of Monascus obtained from 568 kg
Rice bran	215 g	122.12 kg

Commercial rate of packed *Monascus* pigment = Rs. 560/− per 200 g

	Rate for 200 g (rupees)	1 kg (rupees)	10 kg (rupees)	122.12 kg (rupees)
Monascus pigment powder	560	2800	28,000	3,41,936

Monascus pigment powder can be produced in 15 days. It requires 13 days of fermentation, 1 day of drying and consequently packaging. In 150 gallon fermenter, 122.12 kg of *Monascus* pigment can be produced in 15 days. For a month, 244.24 kg *Monascus* pigment can be produced.

10 Profitability

Total *Monascus* pigment that could be produced in a month with 150 gallon fermenter	244.24 kg
Total marketing price that could be earned in a month	Rs. 6,83,872
Recurring expense estimated for a month	Rs. 91,904
Profitability in a month	Rs. 5,90,000 approximately

11 Conclusions

Monascus pigments have generated global interest due to their potential anti-mutagenic, anti-tumor, anti-obesity, anti-inflammation, anti-diabetes, and cholesterol-lowering properties. *Monascus* pigments can be produced with the starter culture of *Monascus* strain that could be commercially bought and can be made to synthesis the red *Monascus* pigment through fermentation, namely, solid state fermentation and submerged fermentation with the easily accessible raw materials of less cost. After fermentation, they could be dried and powdered and easily packaged and then brought out to sale and sold to various firms that requires *Monascus* pigment. The new entrepreneurs can make out huge profit with commercializing this biopigment as it has got a various attention in various industries. Recurring costs that have to be used are comparatively very less than commercializing other pigments as *Monascus* red pigment grows very well on the substrates like rice bran, jackfruit seeds, corn etc. Out of the aforementioned substrates, rice bran is the main substrate with optimal growth conditions for the *Monascus* strain. Production of *Monascus* pigment and its entrepreneurship plan ideas are illustrated in small-scale production, which could bring out many more entrepreneurs with production of *Monascus* red pigment.

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Mass Production and Cost Analysis of Marine *Streptomyces* as Probiotics

Guhanraj Radhamanalan and Dhanasekaran Dharumadurai

Abstract

In 2021, the aquaculture market in India reached a volume of 11.40 million tonnes. According to IMARC Group, the market would reach 18.40 million tonnes by 2027, with a CAGR of 8.20% from 2022 to 2027. Disease prevention, infection resistance, increased growth performance, and immunomodulation are among the issues facing aquaculture's long-term development. Because of their detrimental environmental effect and the formation of carcinogenic microbe strains, chemicals and antibiotics are no longer advised for disease outbreak management. Alternative feed additives, such as microbial supplements, can help aquaculture-related animals enhance their physiology, growth performance, and immune responses. Therefore, marine and soil, animal, and fecal *Streptomyces* are used as supplementary probiotics in the aquaculture and poultry field.

Keywords

Aquaculture · *Streptomyces* · Probiotics · Feed

1 Introduction

Probiotics, which are beneficial microorganisms, or their products that provide health benefits to the hosts have been used in aquaculture as disease control agents, and supplements to improve growth (Dharmaraj & Dhevendaran, 2010). According

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to data, global aquaculture production is rapidly increasing and showing no indications of slowing down. Meanwhile, global catch fisheries production has been steady at around 90 million tonnes since the mid-1990s. The United Nations Food and Agriculture Organization reported that global aquaculture production reached an all-time high of 90.4 million tonnes in 2012, including 66.6 million tonnes of food fish and 23.8 million tonnes of aquatic algae, in response to rising domestic and international seafood demand. For the world's expected population of 7.3 billion people, food fish is currently said to provide an average of one-fifth of total animal protein intake. In many places of the world, the rapid growth of aquaculture has resulted in increased fish stocking density, overcrowding, and a lack of sanitary control. Global disease losses are projected to reach over a quarter billion dollars per year due to the rapid spread of infections. Antibiotic-resistant infections are becoming more common in aquaculture, demanding the development of an antibiotic-free alternative for illness prevention and treatment, as well as for increasing the quality and sustainability of aquaculture production. According to rigorous studies, probiotics have been proved to be a viable alternative to antibiotics in aquaculture, demonstrating good effects on the host by curing diseases, promoting growth, and activating immune responses to infections. Aquaculture is the most rapidly expanding and potential food-producing agricultural industry, accounting for about half of worldwide fish output. It also creates jobs and contributes significantly to human food security and socio-economic growth in many nations. Disease prevention, infection resistance, increased growth performance, and immunomodulation are among the issues facing aquaculture's long-term development (Shefat, 2018). Feeding a 9.6 billion-strong global population by 2050 is a daunting problem that has drawn the attention of academics, technologists, and politicians all around the world. According to an underestimated but encouraging truth, fish can help satisfy the palates of the world's growing middle class while also meeting the food security needs of others with less money. Fish currently accounts for 16% of all animal protein consumed worldwide, and its part of the global food basket is expected to rise as consumers with growing incomes seek higher-value fish and aquaculture expands to meet rising demand. Aquaculture has grown at a tremendous rate during the previous several decades. The FAO is continuing to work on reports for 2015 and 2016 on the condition of aquaculture across the world. Over the previous 50 years, global aquaculture output has risen considerably, from less than a million tonnes in the early 1950s to more than 167.2 million tonnes in 2014. Aquaculture output increased at a 5.8% annual pace from 44.3 million tonnes in 2005 to 73.8 million tonnes in 2014. In 2014, aquaculture output was valued at USD $160.2 billion, and this figure is likely to rise in the future. In the 1980s, aquaculture increased at a quick pace of 10.8% per year, and 9.5% per year in the 1990s. However, between 2005 and 2014, the pace of growth dropped to an average of 5.8%. Asia is said to have the most aquaculture production when compared to the other continents (quantitative and value). The proportion of fish produced through aquaculture has steadily grown, reaching 44% in 2014. In 2014, Asia produced more

farmed fish than wild fish for the first time since 2008, with aquaculture accounting for 55% of total output. Other continents' aquaculture contributions varied from 17% to 18% in the same year, with the exception of Oceania (13%). The top 10 aquaculture producers in 2014 (excluding aquatic plants and nonfood items) were China (45.5 million tonnes), India (4.9 million tonnes), Indonesia (4.3 million tonnes), Vietnam (3.4 million tonnes), and Bangladesh (two million tonnes), followed by Norway, Chile, Egypt, Myanmar, and Thailand. They produced 89% of the world's production. Many millions of people throughout the world rely on the fisheries and aquaculture sectors for their income and livelihood. In 2014, around 56.6 million people worked in the primary sector of catch fisheries and aquaculture, with 36% working full-time, 23% working part-time, and the remainder working as occasional fishermen or with no status. Total involvement in fisheries and aquaculture has remained constant for the first time since 2005–2010 (Hariharan & Dharmaraj, 2018). Probiotics are beneficial bacteria or bacterium compounds that promote the health of their hosts. In aquaculture, these probiotics have been used as disease control agents, feed additions, growth boosters, and, in certain cases, antibacterial chemical substitutes. Despite the fact that significant research has been done in the field of probiotics in the last 30 years, Metchnikoff may have invented the notion in the early 1900s. Parker developed the word "probiotic," which he defined as "organisms and substances that aid microbial balance in the gut." "Probiotic" is derived from the Greek words "pro" and "bios," which mean "for life." According to Browdy, one of the most important developments that evolved in response to illness management issues is the use of probiotics. Probiotics are live bacteria that may be used to boost the human gut flora's microbial balance and growth ability. Because of the existing over-reliance on antimicrobial drugs, the development of probiotics in aquaculture management will reduce the usage of antimicrobial treatments for prophylaxis, perhaps creating hazards to individuals who consume them. He describes probiotics as "a live microbial feed additive that enhances the host animal's gut microbial balance." Fuller's explanation was a development of the probiotic concept, which characterized protozoans that generated substances that stimulated the growth of other protozoans. Verschuere defines a live microbial adjunct as "a live microbial adjunct that has a beneficial effect on the host by modifying the host-associated or ambient microbial community, ensuring improved use of the feed or enhancing its nutritional value, enhancing the host response to disease, or improving the quality of the host's ambient environment." According to Kesarcodi-Watson et al., it should aid the host nutritionally or by altering its immediate surroundings. According to current probiotic usage and scientific knowledge on mechanisms of action, nonviable microbial components operate favorably, and this effect is not limited to the digestive tract (Hariharan & Dharmaraj, 2018) (Table 1).

Table 1 *Streptomyces* bacterium as a probiotic

Probiotic features	*Streptomyces* probiotic	Results	Reference
Synthesis of antagonistic substances	Strains A03 and A05 of *Streptomyces cinerogriseus*	CAS-agar was used to identify all of the strains that produced siderophores	You et al. (2005)
Siderophores are produced	*Streptomyces griseorubroviolaceus* A26 and A42 *Streptomyces lavendulae* A41 is a *Streptomyces* strain	It has been hypothesized that siderophore-producing *Streptomyces* strains might compete for iron and thereby reduce *Vibrio* infections in the marine environment	You et al. (2007)
Quorum sensing and anti-biofilm activity	*Streptomyces albus A66*	With a 99.3% inhibition rate at 2.5% (v/v), *V. harveyi* biofilm formation was inhibited. At 2.5% (v/v), the mature biofilm of *V. harveyi* was disseminated with a 75.6% disintegration rate *Streptomyces* degradation of the quorum-sensing component N-AHSL is thought to be responsible for A66's anti-biofilm activity (N-acylated homoserine lactone)	Iwatsuki et al. (2008)
Activity against virulence	*Streptomyces*. K01–0509	Guadinomine B was developed as a Gram-negative bacteria type III secretion system inhibitor with an IC50 of 14 nm	
Antiviral properties	*Streptomyces* AJ8	An intramuscular injection of an ethyl acetate extract of the secondary metabolite reduced the white spot syndrome virus load in the *Fenneropenaeus indicus* by 85% after the third day of administration	Jenifer et al. (2015)
Secretion of exoenzymes	*Streptomyces*CLS-28; *Streptomyces*CLS-39; *Streptomyces*CLS-45	All strains had high proteolytic activity, although their	Das et al. (2010)

(continued)

Table 1 (continued)

Probiotic features	*Streptomyces* probiotic	Results	Reference
		amylolytic and lipolytic activities were different When added to the meal, it is suggested to improve the host's feed consumption and digestion, resulting in higher *Penaeus monodon* weight	
Growth-stimulating effects	*Streptomyces fradiae and Streptomyces* sp.	Shrimp in their post-larval stage both *P. monodon* and *Xiphophorus helleri*, an ornamental fish, developed quicker The production of indoleacetic acid, a growth-promoting hormone, aided the development of *X. helleri*	Dharmaraj and Dhevendaran (2010), Aftabuddin et al. (2013)
Bio-functional attributes of an exopolysaccharide	*Streptomyces griseorubens GD5*	Exopolysaccharide (EPS) generated by the actinobacterial probiont *Streptomyces griseorubens* GD5 has biofunctional properties. From the HPLC chromatogram, arabinose, glucose, galactose, mannose, and xylose were found, indicating that GD5EPS is a heteropolysaccharide in nature.	Vinothini et al. (2018)
Cell aggregating temperament and biopotency of cultivable indigenous actinobacteria	*Streptomyces* sp.	Cultivable gut actinobacterial flora derived from chicken feces and evaluation of its probiotic properties	Vinothini et al. (2018)
High throughput of *Streptomyces* sp. biomass production	*Streptomyces* sp.	Response surface methodology (RSM) was used in studies for improved antibiotic, enzyme, and probiotic production utilizing Streptomyces, with the	Latha et al. (2015)

(continued)

Table 1 (continued)

Probiotic features	*Streptomyces* probiotic	Results	Reference
		goal of highlighting the importance of Streptomyces and RSM to the scientific community and industry	
The cell aggregating propensity of probiotic *Streptomyces*	*Streptomyces* sp.	The capacity of a probiotic to auto-aggregate is required for colonization and protection of the gastrointestinal tract, whereas co-aggregation allows for intimate contact with harmful bacteria	Muthu Selvam et al. (2016)
Intestinal enzyme resistance and low pH tolerance	*Streptomyces* sp. JD9	The viability was good at pH 2 Pepsin resistance was measured at 3 mg/mL, bile resistance was 1 mg/mL, and pancreatin resistance was measured at 1 mg/mL to be calculated at % A significant degree of gastrointestinal survival was seen	Latha et al. (2015)
Water quality improvements	*Streptomyces fradiae* *Streptomyces* sp. *StreptomycesCLS-28*	The amount of ammonia in the water has been lowered Increased the overall amount of heterotrophic bacteria in the water, allowing waste items to degrade more quickly	Das et al. (2006, 2010), Aftabuddin et al. (2013)
Single-cell protein SCP	*Streptomyces* sp.	It increased the host's meal conversion rate and efficiency, as well as the host's growth performance, when employed as a protein source	Dharmaraj and Dhevendaran (2010), Suguna (2012), Selvakumar et al. (2013)

2 Aquaculture

Despite the fact that outbreaks of a variety of diseases have resulted in economic losses, aquaculture has been developing for decades. Modern aquaculture operations have placed a strong focus on the use of pharmaceuticals and chemical additives. Chemicals and antibiotics are no longer recommended for disease outbreak management due to their negative environmental impact, as well as the creation of carcinogenic microbial strains and negative effects on fish health. As a result, in recent years, the use of ecologically acceptable feed additives such as microbial supplements to improve the physiology, growth performance, and immunological responses of aquaculture-related species has got a lot of attention (Fig. 1). Natural-occurring microorganisms play a vital role in aquatic habitats because, among other things, they may recycle nutrients, decompose organic debris, and protect fish from diseases. All of these functions led to the use of these microbes in aquaculture and the creation of probiotics. Probiotics are entering a new era in aquaculture, with increasing commercial and scientific interest. Nowadays, probiotics are widely used as therapeutic and preventative supplements. Lactic acid bacteria (LAB) such as *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, and others are extensively used in fish nutrition as probiotics. The use of LAB has been shown to have the most promising advantages on disease resistance, survival, and growth characteristics for a wide range of fish species. Bacteria (both Gram-negative and Gram-positive), yeasts, unicellular algae, and bacteriophages are all examples of probiotics used in aquaculture (Jahangiri & Esteban, 2018). *Lactobacillus*, *Enterococcus*, *Bacillus*,

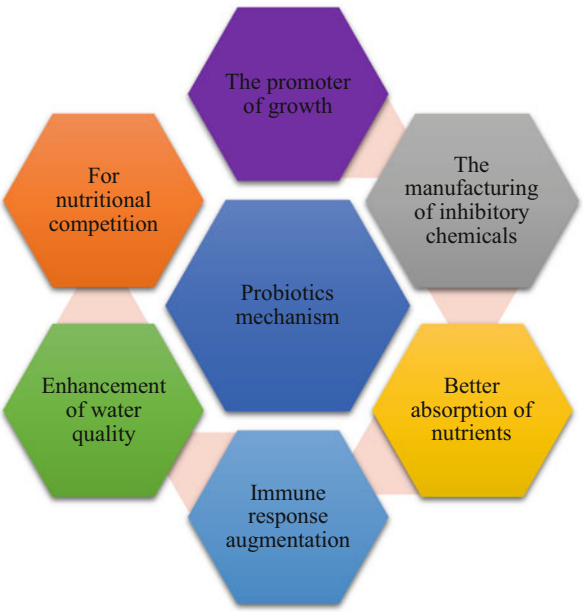

Fig. 1 Probiotics mechanism on host

Aeromonas, *Alteromonas*, *Arthrobacter*, *Bifidobacterium*, *Clostridium*, *Microbacterium*, *Paenibacillus*, *Phaeobacter*, *Pseudoalteromonas*, *Pseudomonas*, *Rhodosporidium*, *Roseobacter*, *Streptomyces*, and *Vibrio* are among the probiotics used in shellfish *Lactobacillus*, *Enterococcus*, *Bacillus*, *Aeromonas*, *Alteromonas*, *Arthrobacter*, *Bifidobacterium*, *Clostridium*, *Microbacterium*, *Paenibacillus*, *Phaeobacter*, *Pseudoalteromonas*, *Pseudomonas*, *Rhodosporidium*, *Roseobacter*, *Streptomyces*, and *Vibrio* are among the probiotics used in shellfish. Streptomycesis the type genus of the Streptomycetaceae family, which belongs to the Actinomycetales order of the Schizomycetes class. This species of bacteria is mostly found in soil, although it is also isolated from manure and other sources on occasion. Though Streptomyces are eubacteria, they develop as filaments or mycelium rather than the typical bacillary or coccoid forms found in bacteria. Conidia, which are created in chains from spore-bearing aerial hyphae, are also formed. Gram-positive reactions are seen in Streptomyces species. They have GC values of 69–78% in their DNA (Sharma, 1999). The most important property of *Streptomyces* is its ability to produce bioactive secondary metabolites such as antifungals, antivirals, anti-tumors, anti-hypertensives, antibiotics, and immunosuppressives. Germinating spores generate hyphae, and multinuclear aerial mycelium forms septa at regular intervals, generating a chain of uninucleate spores (Procópio et al., 2012).

3 Aquaculture Probiotics

However, there is a scarcity of knowledge on the utilization of actinomycetes as probiotics in aquaculture. In shrimp aquaculture, probiotics are frequently used; however, imported formulations are costly. It is necessary to find alternatives that are both effective and cost-efficient. As a consequence, this study looked at the efficacy of *Streptomyces* as a probiotic in a laboratory culture of *P. monodon (Fabricius)* (Das et al., 2006). The use of probiotics in animal feed is gaining acceptance, particularly in poultry and aquaculture. Probiotic products commonly contain organisms such as *Aspergillus oryzae*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus plantarum*, *Bacillus sp.*, *Bifidobacterium bifidum*, *Streptococcus lactis* and *Saccharomyces cerevisiae*. These ingredients can be taken as a drink or added to the diet. Despite being the source of multiple novel antibiotics, marine actinobacteria have received less attention as probiotics in aquaculture and poultry production. There has been little research on the use of marine Actinobacteria, particularly *Streptomyces*, as probiotics in shrimp aquaculture (Dharmaraj & Dhevendaran, 2010). Globally, marine fishery populations are diminishing, offering an incentive for rapid aquaculture growth. As a result, worldwide farm-based fish and shellfish output increased nearly threefold between 1987 and 1997. As new cultivable species and innovative farming techniques become available, the seafood industry is rapidly changing. More than a quarter of all fish consumed by humans comes from aquaculture. In human history, fish and shellfish have long been important suppliers of protein. Certain amino acids are obtained by a portion of the human population from the meat of domesticated animals and fish.

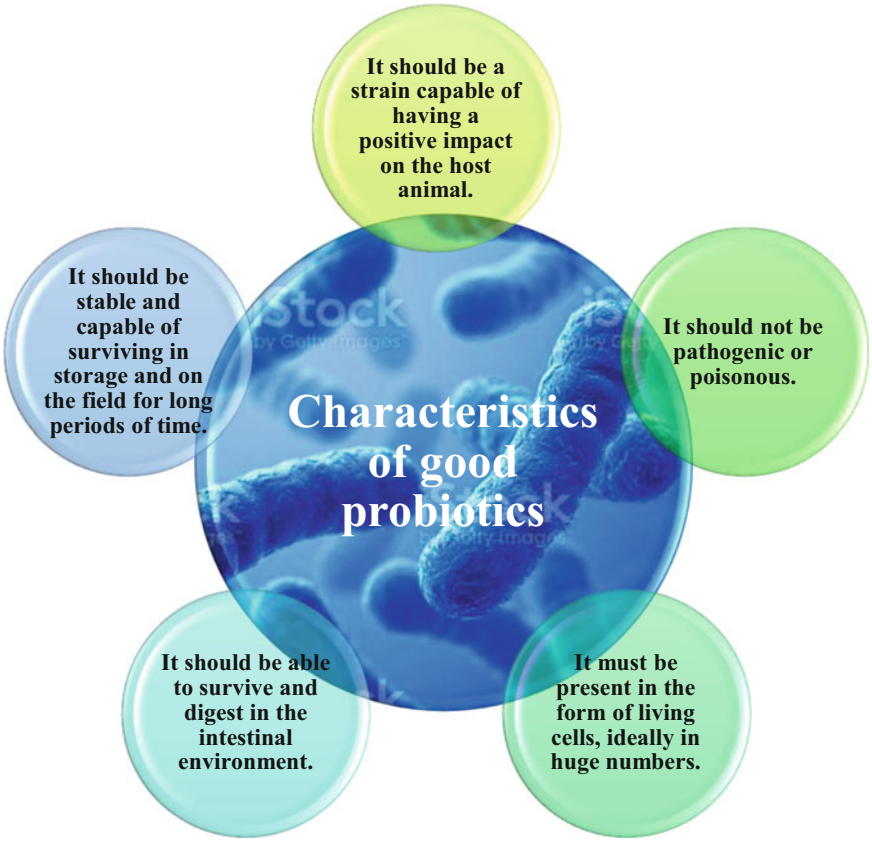

Fig. 2 Characteristics of beneficial probionts

Fish and fisheries products make up around 16% of all animal protein consumed worldwide. According to the Bible, ancient Egyptian monuments illustrate the art of fish cultivation and sale. In India, this age-old industrial practice has just lately acquired traction. Shrimps have been cultivated utilizing a variety of farming techniques due to the pressing requirement for marine food for consumption and export. Aquaculture has been the world's fastest expanding food production sector for the past 30 years, among other things. However, in certain situations, the aquaculture industry's fast growth has outpaced planning and management. As aquaculture has increased in popularity, environmental effect and marketing have become unavoidable overriding issues (Ramachandran, 2017).

Probiotics are ushering in a new age in contemporary aquaculture, with commercial and scientific interest growing. Probiotics are widely utilized as medicinal and preventive supplements nowadays (Fig. 2). Lactic acid bacteria (LAB), such as *Lactobacillus plantarum*, *Lactobacillus acidophilus*, *Lactobacillus thermophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, and others, are commonly employed

as probiotics in fish nutrition. For a wide range of fish species, the usage of LAB has been proven to have the most promising effects on disease resistance, survival, and growth parameters. Probiotics in aquaculture, on the other hand, include a broad variety of bacteria (both Gram-negative and Gram-positive), yeasts, unicellular algae, and bacteriophages. Probiotics have been shown in many studies to be able to create inhibitory chemicals, boost immunity, and prevent pathogen colonization in the gut (Jahangiri & Esteban, 2018).

4 Common Microorganisms Used as Probiotics

These all are the probiotics now employed in the aquaculture business (Hariharan & Dharmaraj, 2018) (Fig. 3; Table 2).

4.1 *Streptomyces* sp. Probiotics in Aquaculture

Streptomyces (phylum: Actinobacteria) are Gram-positive, soil-dwelling bacteria with a distinct branching filamentous shape and a high G + C genome content

Fig. 3 Characteristics of potential probiotics

Table 2 Different probiotic forms of various bacterial strains

Microorganisms	Forms of probiotics
Lactobacillus	Powder/liquid/capsule
Bifidobacterium	Powder/liquid/capsule
Pediococcus	Powder/liquid/capsule
Streptococcus	Powder/liquid/capsule
Corynebacterium spp.	Powder
Bacillus	Powder/liquid
Flavobacterium	Powder
Pseudomonas	Powder
Aeromonas	Powder/liquid/capsule
Enterococcus	Powder
Nitrosomonas	Powder
Nitrobacter	Powder
Vibrio spp.	Powder
Yeast	Powder/liquid/capsule

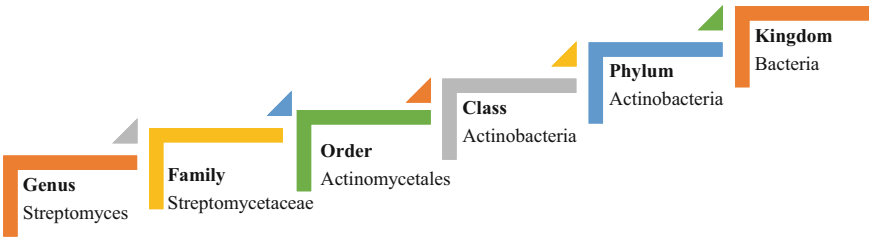

Fig. 4 Taxonomy of genus *Streptomyces*

(70%). *Streptomyces* sp. has long been recognized as an industrially important bacterium due to its capacity to create a wide range of secondary metabolites (Fig. 4). *Streptomyces* has demonstrated the capacity to create a variety of chemical compounds, including antagonistic and antibacterial substances that might be beneficial as probiotics in aquaculture. The ability to create hostile compounds may help probiotics compete for resources and attachment sites with the host. *Streptomyces* not only works well as a probiotic in aquaculture, but also is a cost-effective method because the probiotic bacteria replace 30–40% of the fish meal in the diet (Fig. 3). According to the study, *Streptomyces* might be a cheaper alternative protein source in aquaculture feed.

4.2 Marine Streptomyces as Probiotics

Life Cycle of Marine *Streptomyces*

Streptomyces are abundant in nature and can be dormant for extended periods of time as spores until conditions are ideal for development. The life cycle of a *Streptomyces* species is shown in Fig. 5.

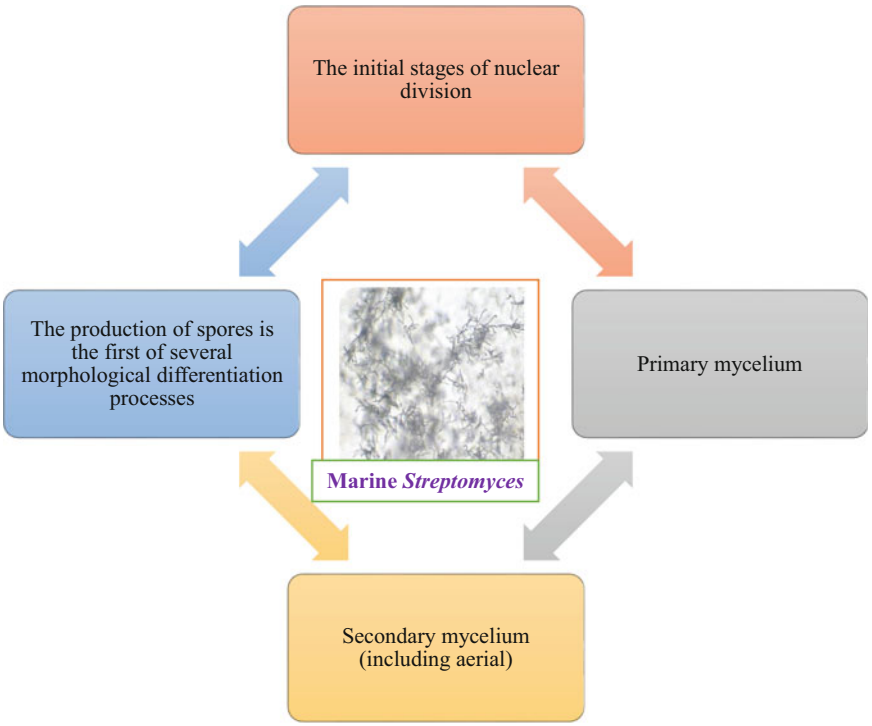

Fig. 5 Mycelial formation of *Streptomyces* species

During vegetative growth, a germ tube emerges from the spore and elongates into long branching filamentous cells, generating a mesh of hyphae known as the substrate or vegetative mycelium that develops deep into the solid growing medium (Hariharan & Dharmaraj, 2018).

Taxonomical Classification of Marine Streptomyces

Indigenous marine actinomycetes indeed exist, according to recent data from culture-independent and culture-dependent investigations, and it has been established that they constitute autochthonous flora in the marine environment. Recent research has found a higher quantity and diversity of actinobacteria, as well as a number of new species. As a result, genuine actinomycetes may be found not just in the oceans, but also in a variety of marine environments. Based on genetic investigations, members from six families have been identified in the maritime environment. *Micromonosporaceae*, *Nocardiaceae*, *Nocardiopsaceae*, *Pseudonocardiaceae*, *Streptomycetaceae*, and *Thermomonosporaceae* are the representative families (Hariharan & Dharmaraj, 2018). *Actinomadura*, *Actinosynnema*, *Amycolatopsis*, *Arthrobacter*, *Blastococcus*, *Brachybacterium*, *Corynebacterium*, *Dietzia*, *Frankia*, *Frigoribacterium*, *Gordonia*, *Kitasatospora*, *Micromonospora*, *Micrococcus*, *Microbacterium*, *Mycobacterium*, *Nocardioides*, *Nocardiopsis*,

Nonomuraea, *Pseudonocardia*, secondary metabolites are abundant in marine actinomycetes, with the great majority of these chemicals coming from the single species Streptomyces. *Streptomyces species* can be found in both marine and terrestrial environments (Hariharan & Dharmaraj, 2018). Marine *Streptomyces* are saprophytic bacteria that live in a variety of environments and have branching hyphal filaments. This unusual growth adaption allows marine *Streptomyces* to colonize solid substrates by attaching and penetrating to acquire access to the host environment's insoluble organic components. Marine *Streptomyces* can produce a variety of hydrolytic enzymes such as amylase, protease, and lipase to break down insoluble organic materials and provide nutrients for the formation of densely packed substrate mycelium, which is then reused to fuel the reproductive phase of aerial growth in the production of spore chains. The release of exoenzymes, which may aid feed consumption and digestion after they colonize the host gut in aquaculture, is thought to make marine *Streptomyces* suitable probiotics (Hariharan & Dharmaraj, 2018). *Streptomyces* are largely saprophytic, thriving in a variety of soil environments and developing branching hyphal filaments in the right conditions. *Streptomyces* uses this unusual growth adaption to colonize solid substrates by attaching to them and piercing them to obtain access to insoluble organic compounds in the soil. Streptomyces' specific physiological adaptations, such as the release of exoenzymes, are thought to make them suitable probiotics, aiding feed consumption and digestion after they invade the host gut in aquaculture. The presence of *Streptomyces* in the feed enhanced the weight of Penaeus monodon shrimp, implying that these *Streptomyces sp*. produced hydrolytic exoenzymes to boost the amylolytic and proteolytic activity in the shrimp digestive tract, allowing the feed to be used more efficiently. The diet supplemented with *Streptomyces fradiae* isolated from mangrove sediment was also demonstrated to help the post-larval *P. monodon* develop faster. After 50 days of feeding trials, all of the feeds supplemented with Streptomyces sp. were demonstrated to increase the growth performance of the ornamental fish, *Xiphophorus helleri* (red swordtail fish), when compared to the control without the *Streptomyces* sp. Furthermore, as established by *Xiphophorus helleri* fed with *Streptomyces* supplemented diets, the generation of the growth-promoting hormone indoleacetic acid by the *Streptomyces* sp. might contribute to the superior growth rate. Because of its high protein content, good amino acid profile, and high digestibility, a fish meal has traditionally been an essential element in commercial aquaculture diets. *Streptomyces* microbial single-cell protein has been used and evaluated for better food conversion efficiency and growth in fish and shrimp. The use of *Streptomyces* shows not only beneficial effects as a probiotic in aquaculture, but also the incorporation of *Streptomyces* in the feed is also a cost-effective approach, as the probiotic bacteria replaced around 30–40% of the fish meal used in the feed. *Streptomyces* was shown to be a less expensive protein source in aquaculture feed, according to the study.

5 Microbial Strains Selection

Probiotic microorganisms are chosen for their capacity to survive in the gastrointestinal environment and their ability to endure low pH and high bile acid concentrations. Furthermore, the selected strain must be able to withstand the production, shipping, storage, and application operations while still retaining viability and desired properties.

5.1 *Streptomyces* Probiotics: Production

- By streaking single colonies of *Streptomyces* strains obtained from seawater fish and shellfish, they were purified.
- In a 1-liter Erlenmeyer flask, the strains were inoculated in 500 mL of starch casein broth and incubated at room temperature for 7 days.
- The *Streptomyces* formed a mat on the broth's surface (non-motile form).
- The cell mass was lyophilized and combined with the prescribed feed components after the mat was harvested (Dharmaraj & Dhevendaran, 2010).

5.2 Probiotic Screening

Isolation and Identification of Probiotic Strains

Survivability tests (resistance to low pH, pepsin, bile, and pancreatin), colonization (auto-aggregation, hydrophobicity, and coaggregation), and safety (antibiotic susceptibility test and nonhemolytic activity) will be used to characterize the strains as potent probiotics.

Isolation of *Streptomyces* from Marine Sources

Samples were taken from marine sources such as seawater, fish, and shellfish. About 0.1 mL samples were serially diluted 10–3, 10–4, and 10–5 to distribute on Starch casein agar plates. Plates were incubated at 30 °C for 7 days. Rifampicin (2.5 mg/mL) and amphotericin B (75 mg/mL) were added to the SCA medium to avoid bacterial and fungal contamination. The detected isolates' SCA medium slant cultures were maintained at (282). The strains are identified and characterized using morphological (mycelial color, soluble pigments, melanoid pigmentation, and spore morphology), biochemical (carbon utilization, amino acid effect, and sodium chloride tolerance), and physiological (pH and temperature), and chemotaxonomic methods.

Mycelia Pigmentation

The aerial and substrate mycelium colors were assessed after the cultures were inoculated on starch casein agar and incubated for 7 days at 30 °C. The cultures were categorized into white, grey, yellow, red, blue, green, or violet series based on mycelial color.

Morphology of Spores

The cultures were grown in a Petri plate with a coverslip inserted at a 45° angle on casein-starch-peptone-yeast extract (CSPY) agar media. The coverslip was removed after 7 days of incubation, air dried, and the spore morphology was studied under a scanning electron microscope (Fig. 6).

6 Fermentation

Fermentation procedures are used to create huge quantities of microbial cells or extracellular microbial compounds (Fig. 8).

6.1 Growth Media

To develop probiotics in a cost-effective manner, microorganism-specific culture mediums, either synthetic or dairy-based, are typically utilized. The cost of media accounts for around 30% of the entire cost of fermentation. Fermentation mediums for the synthesis of probiotics for animal use, on the other hand, are not subject to these limits. Different probiotic strains necessitate various mediums.

6.2 Growth Conditions

Fermentation growth rates are affected by temperature and pH, which are species and strain-dependent.

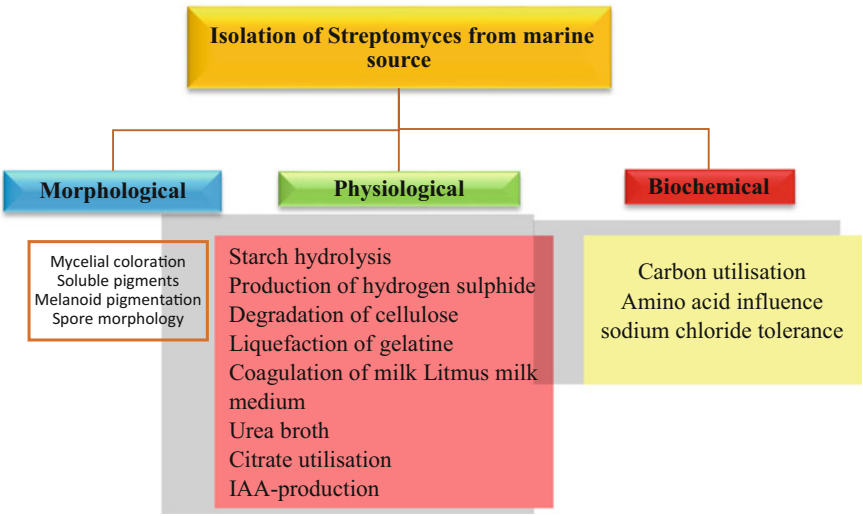

Fig. 6 Isolation of Streptomyces from marine source

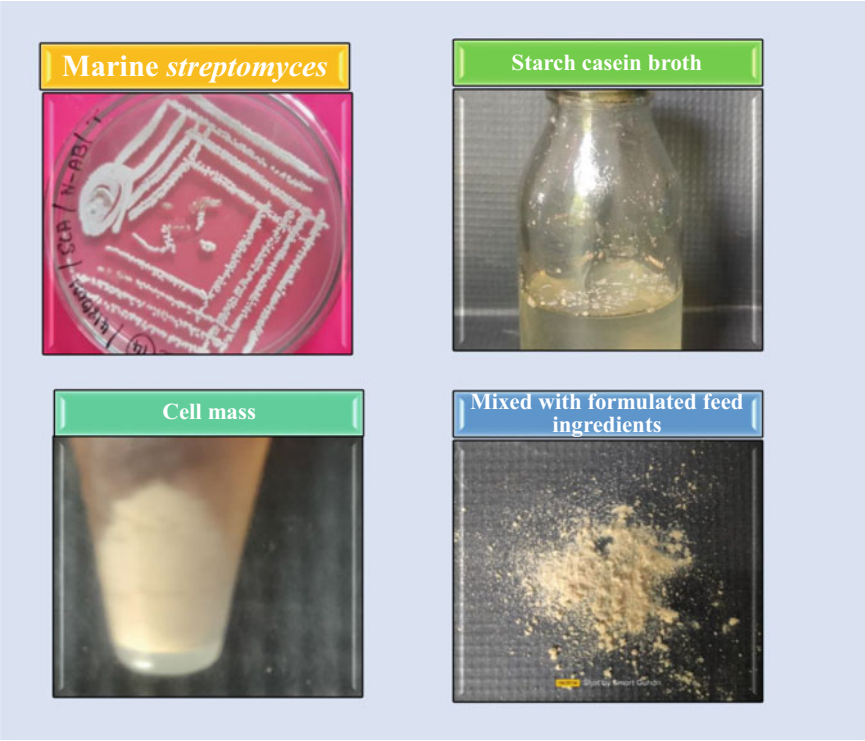

Fig. 7 Isolation and probiotic feed preparation of *Streptomyces*

6.3 Feed Preparation

The probiotic feed and the control feed were two of the eight feeds produced (without probiotics). These diets were provided to the fish for 50 days (Fig. 7 Table 3).

6.4 Control Feed

Fish meal, rice bran, groundnut oil cake, and chickpea flour were utilized in the manufacture of the control feed. Tapioca flour was employed as a binder. The ingredients were finely crushed and properly blended with enough water to make a smooth dough. The dough was then steam baked for 30 min before being allowed to cool. This was pelletized after being extruded. The pellets were dried and kept at 28 °C in dry, airtight containers (Fig. 8).

Table 3 Mass fraction of proteins in the ingredients added to the formulated feeds

Ingredient	Weight (protein)/%
Rice bran	16.5
Chickpea flour	18.5
Groundnut oil cake	23.5
Tapioca flour	16.0
Fish meal	25.5
Streptomyces cell mass	10

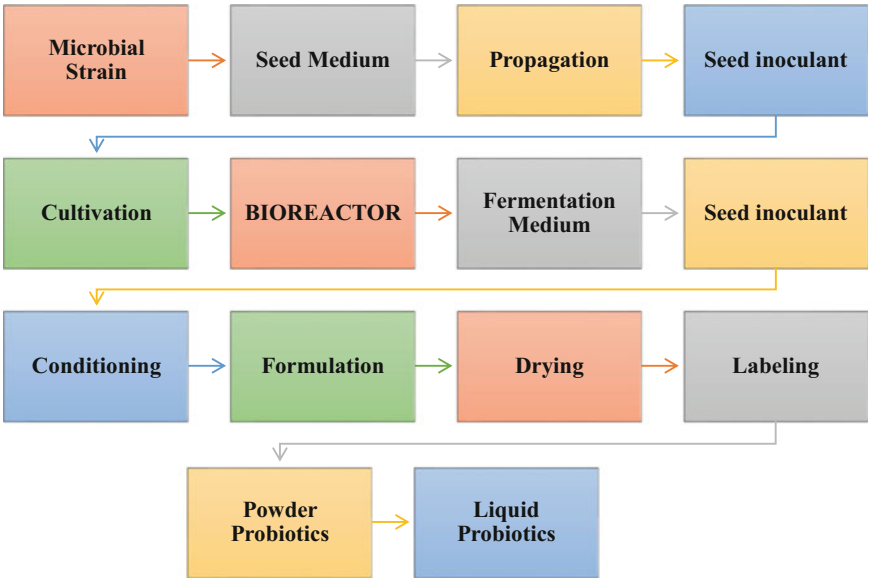

Fig. 8 Schematic illustration of probiotic feed preparation

7 The Range of Probiotic Administration Through Water

Supplementation of raising water is the only form of probiotic delivery in aquaculture that is appropriate for all ages of fish. Due to the undeveloped digestive tracts of fish at that time of development, administration by feeding (dry feed) has obvious disadvantages during the early larval stages. Furthermore, the injection causes a lot of stress in the larvae, which is not the case with adults. Direct addition of probiotics to the rearing water, on the other hand, can begin as early as the first day after hatching in incubators. In larviculture, a combination of probiotic administration via water and enhanced live feed (particularly rotifers) has been strongly advocated as the best strategy to use probiotics. Directly adding enriched rotifers and a commercial probiotic (Remus®, Avecom, Ghent, Belgium) to water containing cod larvae

(*Gadus morhua* L.) increased growth-related proteins while downregulating stress-related proteins (Jahangiri & Esteban, 2018).

7.1 Benefits of Probiotic Administration Through Water

According to some findings, when probiotics were administered through the water compared to other administration protocols, a high level of incorporation of probiotic bacteria into treated aquatic organisms (especially in marine environments) was observed, possibly due to continuous drinking in the aquatic environment (Jahangiri & Esteban, 2018).

Equations for calculation.

$$\text{Absolute growth rate (AGR)} = (\text{final average mass} - \text{initial average mass})/\text{g}$$

$$\text{Specific growth rate (SGR)} = [(\ln \text{ final mass} - \ln \text{ beginning mass})/\text{time of raising}] \times 100$$

$$\text{Relative growth rate(RGR)} = (\text{starting mean mass} - \text{final mean mass})/(\text{final mean mass}) \times 100$$

$$\text{Feed conversion efficiency(FCE)} = (\text{feed given} - \text{unconsumed feed})/(\text{final mass} - \text{initial mass}) \times 100$$

$$\text{Feed conversion ratio(FCR)} = (\text{final mass} - \text{initial mass}) \times [(\text{feed given} - \text{unconsumed feed})]$$

Once a day, the fish were fed and prepared meals at a rate of 5% of their body mass. About 6 h after feeding, the unconsumed feed was drained off. The experiment was done three times and statistically analyzed each time.

8 Probiotic Products (Tables 4 and 5)

Table 4 Aquaculture probiotics in liquid and powder form are produced using the five strains that passed the final screening procedures

S. no	Parameters	Powder probiotics	Liquid probiotics
1.	Live microbial cell count	Trillion cfu/g	1.25 × 1013
2.	Appearance	Free-flowing white powder	Viscous, beige-colored liquid
3.	Odor	Milky sweet	Milky sweet
4.	Moisture	10%	–
5.	Ph	Neutral	4.7

Table 5 Storage period of probiotics

	Months of storage	room	Refrigerator	Freezer
Powder probiotics	3	4.00 × 1016	1.87 × 1016	1.91 × 1016
	6	3.62 × 1017	1.66 × 1016	8.06 × 1015
	9	7.80 × 1014	5.70 × 1013	4.64 × 1014
	12	2.37 × 1015	1.14 × 1015	1.21 × 1015
Liquid probiotics	3	9.00 × 1016	7.40 × 1017	1.12 × 1017
	6	1.48 × 1016	1.94 × 1017	4.57 × 1016

8.1 Role of Probiotics in Aquaculture

- Live microorganisms that have a beneficial effect on the host by modifying the microbial community associated with the host
- Ensure improved use of the feed or enhance its nutritional value
- Enhance the host response to disease
- Improve the quality of its ambient environment

9 Cost Analysis

9.1 Global Aquaculture Production

Probiotics, which use bacterial culture as dietary supplements and medications, are a developing area of the market. According to the market analysis, Europe ranks first in terms of the number of products available as well as the fastest-growing probiotic market, while Japan ranks second. Probiotics are also accessible in Japan as meals and medications. The demand for probiotics in India is largely driven by rising consumer concerns about the high prevalence of a variety of gastrointestinal illnesses. Furthermore, rising awareness of the multiple linked advantages of probiotics in improving gut health and increasing immunity is supporting the country's market expansion (Table 6).

Table 6 Production cost for *Streptomyces* probiotics

Ingredients	Cost
ISP medium no. 5 (500 g)	5255
Rice bran 50 kg	1000
Chickpea flour 10 kg	2000
Groundnut oil cake 10 kg	1000
Tapioca flour 10 kg	2000
Fish meal	1800
Total	13,055

Therefore, to produce 50 kg of probiotic *Streptomyces*, we have to invest around Rs. 13,000–15,000. The market price of 1 kg *Streptomyces* feed is Rs. 500.

The companies marketing the *Streptomyces* feed for Aquaculture are Anfotal Nutrition, Optilite Pro, Probact, Refit, Bioflok, Promax, Prions, LifePro, Probioflok, Virbac.

10 Conclusion

Probiotic bacteria produce macromolecules and vitamins that are beneficial to animal nutrition. There are just a few publications on the use of marine actinomycetes as probiotics in aquaculture, notably *Streptomyces.* This is the first report on the use of *Streptomyces* as a probiotic feed for ornamental fish development, and it was discovered that feeding probiotics to the ornamental fish *Xiphophorus helleri* resulted in a considerable increase in growth and length. As a result, *Streptomyces* as probiotics will play a significant role in aquaculture nutrition in the near future. In future research on probiotics in shellfish aquaculture, the effects of probiotics on growth performance, immunological response, gut microbiota, and disease resistance should be studied.

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Nanoparticles and Its Application in Food Packaging

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Abstract

Nano-equipment is the study as well as application of arrangements within choice of 1–100 nanopatterns in width. The meaning of nanotechnology is be contingent on instigate "nano" that is the Greek term to clue dwarf. In supplementary method languages, the term "nano" precedes 10^{-9} or one billionth of approximately. The term nanotechnology is often used once mentioning to constituents with the extent of 0.1 to 100 nanometers (nm). The innovative characteristics of nanomaterials suggest several novel occasions for nutrition and scientific discipline producing, as an example simpler nutrition coloring, flavoring, wholesome flavors, antiseptic constituents for nutrition packaging, a lot of agricultural

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chemicals, and stimulants. In this book, definition, classifications, and applications of nanotechnology in food packaging and also cost effectivity of nanoparticles are discussed.

Keywords

Nanotechnology · Nano-scale · Packaging · Food processing

1 Introduction

In the era of quickly proceeding evidence knowledge, it is attractively perfect that novel expedients necessity industrialized to grip extraordinary size data for evidence packing and dispensation. Increasing the abilities of these novel expedients has become critical to their expansion. More explicitly, one of the areas of enlargement is spin-tronics, and diluted magnetic semi-conductor (D-M-S) ingredients that supposedly are semi-conductors, but can display ferro-magnetism at room humidity. Nanotechnologies are the examination and solicitation of arrangements within variety of 1–100 nm in length. The definitions of nanotechnologies are contingent on the prefix "nano" that is the Greek word for indication of "dwarf." In supplementary method languages, the term "nano" precedes 10^{-9} or one billionth approximately. The term nanotechnology is often used once mentioning to constituents with the extent of 0.1–100 nm. The innovative characteristics of nanomaterials suggest several novel occasions for nutrition and scientific discipline producing as an example simpler nutrition coloring, flavoring, wholesome flavors, antiseptic constituents for nutrition packaging, a lot of agricultural chemicals, and stimulants.

D-M-S offer a method for assimilating measurable and handling into a solo substantial since D-M-S are intelligent to make use of both the spins and charges of electrons. In addition, the spin degree of self-determination to conservative charges grounded electronics permit many compensations as well as non-volatility, augmented data dispensation speediness, reduced electronic supremacy feasting, and amplified addition thicknesses. The refinement of these resources will be of greatest prominence in reducing the size of compelling fields to permit for spins differentiated current to be transported through spin-tronic expedients to attain both interpretation and scriptabilities. Nanostructured supplies have apprehended the consideration of investigators cross ways the biosphere due to their provisional physical and chemical characteristics (Dong et al., 2022). Metallic oxide nanoparticles perform a serious and leading part in varied parts that are accomplished in creating expanded oxide mixtures approving many geometrical constructions with exclusive belongings because of their size and edge corner sites (Ashfaq et al., 2022).

2 Nano-Packing

Nano-packaging is outlined because the method of interrelating, driving, cooling, and shielding N-component products is insubstantial to create microelectronic and bio-electronics schemes for developed practicality and price. Nano-packaging is already creating a bearing within the development of useful or interactive foods, which answer demand and deliver nutrients expeditiously. In addition, nanoparticles help in the production of bottles and packages with a lot of lightweight and fireplace confrontation, solider powered and thermal performance, and less gas preoccupation (Dong et al., 2022; Ashfaq et al., 2022). These possessions will be significant in the growth of the old-fashioned, expeditiously reserved taste and pigment, and smooth transport and practice. Additionally, nano-structure films can efficiently break the nutrition from the attack of microorganisms and creatures and provide nutritional protection. With entrenched nanosensors within the wrapping, consumers are successful to be able to "read" the nutrition within. Instruments will alarm the U.S. previously that nutrition goes unfortunate or apprise U.S. the precise nutrition standing contained within the contents (Videira-Quintela et al., 2022).

3 Nanomaterials

The advantages of nanomaterials in food wrapping have been previously reported. A solitary example is thermoses created with nanocomposites that reduce the run of carbonic acid gas available in the thermos; this will increase the period of effervescent brews while avoiding the use of denser glass bottles or dearer fires. Another example is a nutriment storing basket with silver nanoparticles entrenched within the malleable. The silver nanoparticles destroy microorganisms from any food and antecedently keeps within the bins, reducing risky microorganism. There exists square measure alternative food packaging product that is presently beneath development. For example, nanosensors in plastic packaging will find gases given off by food once they spoil, and therefore, the packaging itself changes color to provide you with a warning to food being dangerous. Malleable flicks square measure being established that may enable the food to remain underclassman longer. These films square measure jam-choked with salt nanoparticles to scale back the flow of chemical elements into the package and therefore the leaky of wet out of the bundle (de Oliveira & Tavares, 2022). Nanosensor square measures are being industrialized to find microorganisms and alternative contaminates such as enteric on the surface of food at packaging plants. This can provide everyday challenges at a far lower price than that incurred by sending samples to a science laboratory for analysis. This point-of-packaging testing, if conducted properly, has the potential to dramatically scale back the prospect of contaminated food reaching food market drops (Flórez et al., 2022).

Nanostructures can thus be categorized into three assemblies contingent upon the imprisonment of subdivisions in a specific crystallographic way within organization. The three clusters are as follows:

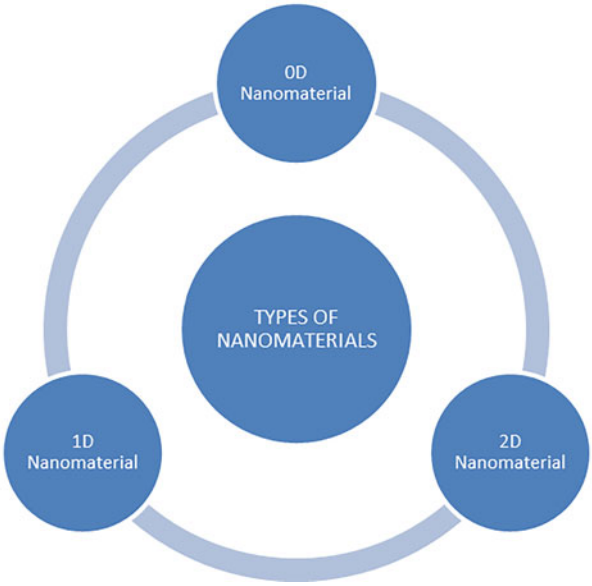

Fig. 1 Category of nanomaterials

1. Zero dimensional (0D) nanostructure: the ingredients that restrain electrons in three magnitudes or the configuration do not license free subdivision motion in any way. Semi-conductor quantum dots (QD), nanoparticles as well as colloidal subdivisions are certain examples to be contained in this cluster.
2. One dimensional (1D) nanostructure: the ingredients that restrain electrons in two magnitudes or the configuration do not permit free particle gesticulation in two dimensions. Some examples include nanobars, nanowires, nanotubes, and nanothreads.
3. Two dimensional (2D) nanostructure: the ingredients exhibit imprisonment of electrons in one length or the configuration does not permit free subdivision motion in one dimension, such as nano discuses or platelets, thin film on a surface, and multifaceted substantial. Figure 1 illustrates the categories of nanomaterials.

4 Nanoparticles

In engineering science, a subdivision is outlined as a slight body that performs as a full component in relation to its transportation and belongings. Particles quadrangular measures any categorized in keeping with size: in footings of width, well particles cowl a spread between 100 and 2500 nm. On the opposite side, ultra-fine particles square measure sized between one and 100 nm. Similar to ultrafine particles, nanoparticles square measure is sized between one and 1 nm. The explanation for this dual name of an alike object is that, throughout the 1970s to 1980s, once the primary thorough basic studies were running with "nanoparticles" within the USA

and Japan (Paidari & Ibrahim, 2021), they were referred to as "ultra-fine particles" (UFP). Nevertheless, throughout the 1990s before the National Engineering Science Initiative was launched within the USA, the innovative name "nanoparticle" had become trendy. Nanoparticles may or may not exhibit size-related properties that differ considerably from those ascertained in fine subdivisions or wholesale materials. Although the dimensions of furthermost molecules would work on top of definition, individual molecule square measure is sometimes not brought up as nanoparticles. Nanoclusters have a minimum of one dimension between one and 10 nm and slim magnitude dissemination. Nanopowder squares measure agglomerates of ultrafine atoms, nanoparticles, or nanoclusters. Nanometer-sized solitary crystals, single-domain ultra-fine subdivisions, and square measures are usually brought up as nanocrystals. Chemical larger Bayer yields a clear film (called Durethan) comprising nanoparticles of earthen. The nanoparticles square measure spread throughout the plastic; and square measure is able to block chemical elements, carbonic acid gas, and wet from attaining contemporary essences or alternative nourishments. The nanoclay additionally makes the plastic brighter, tougher and a lot of heat resilient. Recently, manufacturing's expedition to bundle brews in malleable flasks (for inexpensive transport) was ineffective, attributable to decay and flavor issues. Nowadays, nano or, a subordinate of Amcor Worldwide firm is manufacturing nanocomposites to be used in malleable brew bottles that offer the beverage six-month shelf life. By implanting nanocrystals in malleable materials, investigators have formed a molecular blockade that assists stop the seepage of chemical elements. Nanocor and Southern Clay product square measure currently acts on a malleable flask which will increase layer life to 18 months (Qasim et al., 2021).

5 Nanosyntheses in Food Packing

Nanomaterials has prospective to come up with new-fangled food packing. Nanocomposites will expand automated power, scale back heaviness, increase heat confrontation, and increase blockade in contradiction of chemical elements, carbonic acid gas, ultraviolet illumination, dampness, and volatiles of food bundle resources. The major types of nanoparticles are premeditated for use in nutrition packing organizations. In addition, their properties and uses have been reviewed (Javad et al., 2017). Acceptable nanoparticulate (100 nm or less) square measures assimilated interest in plastics to boost the belongings over those of typical complements. Compound nanocomposites square measure thermo-plastic polymers that have nanoscale additions of 2–8% by weight. A nanoscale addition comprises nanoclays, carbon nanoparticles, nanoscale metals as well as oxides, and compound mastics. Nanocomposite square measures are categorized by a very high surface-to-volume magnitude relation, creating them extremely sensitive compared to their macro-scale complements, and therefore awarding fundamentally totally altered properties. Food and drink wrapping contains fifty-fifth to sixty-fifth of the 130 billion dollar price of wrapping in the US (Anvarinezhad et al., 2020). Furthermore,

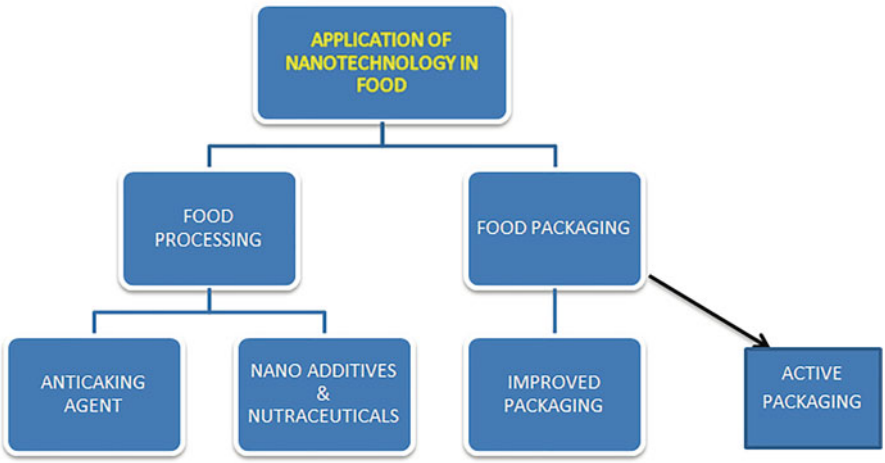

Fig. 2 Application of nanotechnology in food industry

nanocomposites may even be categorized by their associate degree anti-microbial bustle. Packages comprising nanosensors offer data concerning enzymes made within the breakdown of nutrition molecules, creating them dangerous for humanoid ingesting. Figure 2 depicts the application of nanotechnology in the food industry.

6 Nano-wrapping for Chocolates

Previously Nano-wrapping was used in production and farm manufacture square measure nanofilters-screens, thus little they will separate microorganisms, even infections. In science laboratory experiments, the color has been off from beetroot extract, going the flavor; and mauve was snowy. Milk sugar will currently be clean from benefits, and replaced with other sugars, creating all milk appropriate for the lactose prejudiced. This might indicate less use of compounds and temperateness conduct in the nutriment process. Once it involves changes in state secretion, nanoparticles can only be used to transport teeth scrubbing compounds that you simply will not be able to style. Beautiful to the indolent, as are going to be self-cleansing flatware, associate degree improvement created attainable by the manufacturing, at infinitesimal level, of hydrophobic planes that enables substances to interrupt depression and drop off. This can be previously applicable with manufacturing glass products (Khan et al., 2018). Nanofilters can enable you to decide on the number of alkaloids you would like to get rid of from your stumpy (Ajitha et al., 2019).

7 Nano-packaging for Chicken and Spinach

Nanoscale instruments are square measures of growth that can manage toxins and microorganisms in the least phases of nutrient dispensation. This can spot enteric bacteria in cowards or *E. coli* in spinach, extending before the product reaches the outlets. Self-monitoring food wrapping can be established into knowledge similar to nano-patterns. Strengthened interested in your refrigerator, it will find and caution you of a full variety of elements given off by decay nutrition, or the attendance of microorganisms; then sanitary them (Mehmood et al., 2020).

8 Nano-pckaging for Beverages

Nanotechnology, the knowledge of terribly little ingredients, is graceful to possess a giant influence in nutrition and infusion wrapping. The accumulation of certain nanoparticles in formed matter and layers has been shown to reduce them inconsequential, incombustible and solider in expressions of automated and updraft presentation, in addition as less semipermeable to vapors. Innovative wrapping solutions can seek a lot of attention on nutrition protection by dominant microorganism development; postponing oxidization, up interfered discernibility, and suitability. The straightforward classes of engineering science use and usefulness seem to be in growth for food wrapping: improvement of malleable ingredients barricades; combination of active parts which will deliver purposeful characteristics on the far side of those of typical active wrapping; and detecting and gesturing of applicable data (Abutalib & Rajeh, 2021; Lu et al., 2021; Praseptiangga et al., 2021; Hong et al., 2021; Sehar & Khan, 2021).

9 Various Technologies in Nanopackaging

9.1 Chemical Unharness Nanowrapping

Biochemical unharness nanowrapping allows nutrition wrapping to act with food covers. The interchange will progress in each direction. Wrapping will unharness nanoscale anti-microbials, anti-oxidants, flavors, perfumes, or nutraceuticals into the nutriments or liquids to increase their time period or to enhance their style or fragrance (Yuhan et al., 2021). In several occurrences, biochemical unharness wrapping additionally integrates police work components, that is, the discharge of nano chemicals can occur in reaction to a specific activate event (Chawla et al., 2021). On the contrary, nano wrapping mistreatment carbon nanotubes are being industrialized with the plasticity of the flexibility gas or greenhouse gas that would otherwise lead to food or liquid worsening. Nano-wrapping that may absorb unwanted flavors is additionally in development. Nano oxide or nano element dioxide, nano percales, nano copper compounds, nano pigments, and carbon

nanotubes are foreseen for future use in anti-microbial nutrition wrapping (Singh et al., 2021).

9.2 Nano Established Mostly Anti-microbial Wrapping

Nutrition wrapping and vessels are created by integrating anti-microbial nanomaterials, to forestall or hamper the degeneration of nutrition to microorganism action. These merchandise normally use nanoparticles of silver, nano oxide, and nano dioxide. Wrapping ingredient mistreatment percales, oxides and pigments in the nano type, such as carbon nanotubes are being developed to be used in anti-microbial food wrapping. Silver nanoparticles are combined in a very large diversity of commodities, including clothing, electrical products, kitchen utensils, and looped bandages. Nanoparticulate silver releases ions a lot of expeditiously than wholesale metals, and it is the silver ions that have a disinfectant; thanks to the reserve of a good style of organic procedures at intervals of the microorganism. Because the levels of silver ions unconventional square measure are too low to own venomous belongings in humans, it is probably that nanoparticulate silver will be enclosed in more complex ingredients. On the other hand, there is some anxiety terminated the results of enormous quantities of silver ions being quitted into the atmosphere and accruing in ecosystems, as silver ions square measure celebrated to be venomous to marine life. Zinc oxide exhibits medicament activity that will increase with declining particle magnitude (Mahato et al., 2021; Wang et al., 2021; Rukmanikrishnan et al., 2021).

The use of nanomaterials to reinforce bioplastics (plant-based plastics) might change bio plastics to be used rather than vestige fuel based plastics for nutrition packing and transport luggage. When the bioplastic square measure is mixed with nano stone particles, the ensuing nanocomposites exhibit better-quality blockade properties compared with the pure bioplastic, and once their helpful life is composted they come to the soil. Alternative nanomaterials can be used with nanoparticles, nanofibers and nanowhiskers. Several biopolymers such as chitosan, cellulose, collagen and metal (derived from corn) are synthesized as nanofibers from numerous biopolymer mistreatments by the electrospinning technique. In some cases, these have superior properties to the traditionally cast compounds, together with redoubled heat resistance. In addition, the mats of such nanofibers possess an extremely nonporous structure and may be used as support matrixes for added practicality as they are doing all-time low thermal conductance of all solids (Shahvalizadeh et al., 2021).

Application of nanoparticles sustenance growth and developmement, color, flavor, feel and constancy of foodstuffs, redoubled pre-occupation and bio-availability of nutrients and wellbeing additions, new food packing resources with value-added mechanical, barrier and anti-microbial properties, and Nano-sensors for trace-ability and observation the complaint of nourishment throughout transportation and packing (Babaei-Ghazvini et al., 2021).

10 Conclusion

Generally, several republics have recognized prospective of applied science within the nutriment segments as well as genuine measure investigation of a major quantity within it. Equivalent prominence has been prearranged to the community problems related to applied science and to enhance open consciousness. Several interventions have appointed training to investigate the novel and prospective uses of applied science in nutrition, particularly on packing. At equivalent periods extra currency has been paid by the govt branches for analysis as well as growth, which have expansions of applied nutrients, nutrient-delivery-system and techniques for enhancing nutrition look, such as color, flavor and constancy. Regardless of the influence of applied science on the food business and merchandise in the market, the protection of food can stay the major anxiety. This would like to reinforce the acceptance of applied science in detecting solicitation, which can guarantee nutrition care and sanctuary, further as technology that alarms clienteles and retailers once the food is approaching the tip of its time period. It is predictable that applied science may permit a lot of economical and property food manufacture procedure to be established wherever less material square measure disbursed and food with higher nutritionary superiority is achieved.

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