Chapter 10

Production of Antioxidants, Aromas, Colours, Flavours, and Vitamins by Yeasts

CHARLES A. ABBAS

ADM Research, JRRRC, 1001 Brush College Road, Decatur, IL 62521, USA
(e-mail: abbas@admworld.com, Tel.: 217451-4220, Fax: 217-451-4230)

10.1 Introduction

The application of yeast and yeast-derived products by the food industry continues to be a rapidly growing area. Recent focus on improving human health through capturing greater value from yeast products has led to a rise in the recognition of the nutraceutical potential of many of the current products and has renewed emphasis on research that demonstrates the efficacy of new and existing products. Historically, yeast products have consisted of whole yeasts that are provided as yeast slurry or yeast paste, dried active yeasts, yeast autolysates, yeast extracts, separated components, such as protein isolates and amino acids, cell wall glucans and mannoproteins, vitamins, sterols, carotenoids, other lipids, enzymes, nucleic acids, polysaccharides, and chemically, physically, or enzymically modified components. Some examples of derivatives of yeast cell components are the chemically modified proteins (acylated, phosphorylated, encapsulated enzymes, immobilized enzymes, etc.), physically modified proteins that are partly denatured or texturized, enzymatically modified proteins that are re-partially digested by acid or enzymatic treatment or enzymatically modified proteins with covalently attached amino acids, nucleotides, and nucleosides, flavouring products and flavour substances, salt replacers, and substances or immobilized enzymes that are encapsulated inside the yeast for use as flavours and pharmaceuticals (Abbas 2001, 2003, 2004; Benítez et al. 1996; Halasz and Lasztity 1991; Peppler 1967, 1979; Reed 1981).

Yeast and yeast-derived products contribute to food flavour and aroma in a number of ways as added ingredients or as biocatalysts that carry out fermentation or biotransformation of food components thereby yielding a variety of products with desirable features. The use of yeast and yeast-derived products as bioflavouring agents and biocatalysts for edible meats, breads, other bakery products, cheeses, margarine flavours, yogurts, kefirs, other fermented dairy products, animal feeds, alcoholic beverages, fragrances, fruity flavours, soya-derived products, fermented cocoa beans, fermented tea, fermented vanilla beans, fermented syrups, pickles, ciders, vinegars, and a great variety of other fermented foods and beverages is well

The Yeast Handbook
Amparo Querol, Graham H. Fleet (Eds.):
Yeasts in Food and Beverages

documented. Yeasts have also been tapped as sources of colorants, vitamins, antioxidants and as supplements for their nutraceutical or health-promoting attributes.

A number of yeast genera have found uses in the previously mentioned applications. These range from the widely used species and strains belonging to the genus *Saccharomyces*, to other genera such as *Candida*, *Debaryomyces*, *Geotrichum*, *Hansenula*, *Kloeckera*, *Kluyveromyces*, *Pichia*, *Schizosaccharomyces*, *Sporobolomyces*, *Yarrowia* and *Zygosaccharomyces*, to name only a few.

The primary goal of this chapter is to provide a background summary and an update on recent developments in this area with emphasis on the production of aromas, vitamins, antioxidants, colours, and flavours. It should be noted that these products are synthesized by metabolic pathways that parallel the biogenetic relationships of other naturally derived materials from plants and animals as illustrated in Fig. 10.1 (Sanderson 1978).

10.2 Background and Definitions

No serious overview of the topics selected can proceed without delineating its scope by providing the reader with concise definitions that can help assemble the products whenever possible into best-fitting groups. This task is made difficult as there is considerable confusion and overlap in the published literature, particularly when it comes to aromas and flavours produced by yeasts. For example, esters produced by yeasts can contribute to both aroma and flavour in the case of alcoholic and nonalcoholic fermentations. Therefore, in many cases of aromas and favours made by yeasts there is no clear or consistent distinction found in the literature. To a food scientist flavour primarily consists of two elements: (1) taste, which is perceived in the mouth and is mainly due to nonvolatile constituents present in food, and (2) aroma, which is perceived in the nose and is mainly attributed to volatile components

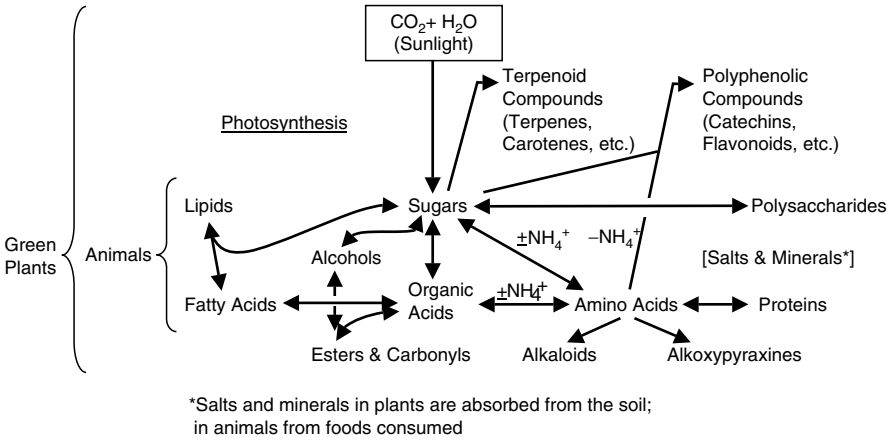

Fig. 10.1. Biogenetic relationship of compounds that comprise living organisms including those that serve as food materials. (Adopted from Sanderson 1978)

(Sanderson 1978). In sensory evaluation, descriptive aroma procedures have been developed which objectively and precisely define aroma as part of flavour (Noble 1978). While the biogeneses of the volatile and nonvolatile components of food are highly interrelated, it is well established that minor variations in a volatile component can lead to a major impact on flavour (Sanderson 1978). For the purpose of this chapter, aromas will be defined as esters, other fusel oil components, and other volatile products that are readily detected by smelling, while flavours will be defined as yeast and yeast-derived products that contribute to organoleptic properties or taste such as yeast extracts, nucleotides, organic acids, and polysaccharides and simple chemical compounds which include polyols such as inositol and glycerol. These products contribute as a whole or in part to the five primary recognized elements of food flavour: sweetness, saltiness, sourness, bitterness, and astringence (Sanderson 1978).

Providing working definitions for yeast-produced colours, vitamins, and antioxidants is a somewhat easier task as long as it is understood that some of the common yeast-derived or yeast-produced pigments may provide in addition to colour enhancement, antioxidant activity as is the case of astaxanthin, a salmon fish feed additive. A similar situation exists for the vitamin B_2, riboflavin, which can be listed as a vitamin as well as a food colorant and as an antioxidant. Keeping the aforementioned in mind, what follows is a summary of the topics under consideration.

10.2.1 Antioxidants

Yeasts and yeast extracts have been recognized as a source of antioxidant compounds for some time (Forbes et al. 1958). In the process outlined by Forbes et al., a 20% suspension of yeast is subjected to a series of organic extractions to yield a white crystalline preparation that can be added to prevent oxidation of food products such as fats, baby foods, whole milk, or milk products. More recently, the increased interest in natural antioxidants has given rise to the screening of microbial sources for compounds to replace the synthetic compounds currently in use as food antioxidants. Natural antioxidants can also be used in nutraceutical applications as supplements (Gazi et al. 2001; Nishino and Ishikawa 1988). Natural oxidants are presumed to be safer for human beings (Gazi et al. 2001). The function of antioxidants and the exact mechanism of their mode of action are still open to conjecture, but it is generally agreed that they act by donating hydrogen protons to substrates, thereby rendering them nonreactive to oxygen-derived free radicals that are referred to as reactive oxygen species or ROS (del Rio et al. 2003; Heath 1981). It is hypothesized that yeast peroxisomes play a similar role to plant peroxisomes. Therefore the response in yeasts to oxygen-derived radicals would involve several enzymes, including catalases, superoxide dismutases (SOD), glutathione (L-γ-glutamyl-L-cysteinylglycine), as well as several NADP-dependent dehydrogenases (del Rio et al. 2003). It is well established that antioxidants are inactivated in the process and that their activity is proportional to the quantity used based on their permitted usage level (Heath 1981).

Yeasts synthesize a number of bioactive compounds which can serve as antioxidants. These have found numerous uses in foods to retard oxidative degeneration of

fatty substances and in nutraceutical supplements to improve health and well-being (Bastin et al. 2002; Doll 2002; Gazi et al. 2001; Ok et al. 2003). They consist of the oxygenated carotenoid torulahodin, both the organic acid and the salt forms of citric acid, coenzyme Q or ubiquinone, glutathione, hydroxymethyl and hydroxylethyl furanone (2*H*), tocotrienol, α-tocopherols (α-TOHs) and other forms of tocopherols, riboflavin (vitamin B_2) and the flavins derived from it, FMN and FAD, and 2,4-hydroxyphenyl ethanol (Cremer et al. 1999; Do et al. 1996; Imai et al. 1989; Kawamukai 2002; Padilla et al. 2004; Penninckx 2002; Sugawara 2001; Suzuki et al. 2003). Other factors that are produced by yeasts and/or that are present in yeast-fermented products or in yeast cell biomass at the end of fermentation that have antioxidant activity or free-radical scavenger activity include several other oxygenated carotenoids, selenium-enriched yeast cells, the wine component resveratrol, octacosanol, yeast-derived cell wall β-glucans, uncharacterized soluble proteins that are produced in yeasts under oxidative stress, sulphur-containing amino acids, cytochrome c, the yeast enzyme Cu,Zn-SOD, and products of SOD genes, CUP1 and SOD1 (An 1996; Archibald 2003; Becker et al. 2003; Combs et al. 2002; Farid and Azar 2001; Forbes et al. 1958; Forman et al. 1983; Imai et al.1989; Lee and Park 1998a, b; Marova et al. 2004; Mast-Gerlach and Stahl 1997; Park 2003; Sakaki et al. 2001, 2002; Shitazawa et al. 2002; Visser et al. 2003).

Glutathione plays an important role as an antioxidant. The depletion of glutathione leads to the accumulation of ROS following the treatment of *Saccharomyces cerevisiae* with the pungent sesquiterpenoid unsaturated dialdehyde, polygodial (Machida et al. 1999). Polygodial exhibited a strong yeasticidal activity against cells of *S. cerevisiae*, in which production of ROS at a significant level could be detected with a fluorescent probe. The production of ROS in polygodial-treated cells was further confirmed by its elimination and the accompanying protection against yeasticidal effects in the presence of antioxidants such as L-ascorbate and α-TOH. Polygodial could accelerate ROS production only in cells of the wild-type strain but not in those of a respiratory-deficient petite mutant (rho0), indicating the role of the mitochondrial electron transport chain in the production of ROS. Unlike antimycin A, which accelerates ROS production by directly targeting the mitochondrial electron flow, polygodial caused depletion of cytoplasmic and mitochondrial glutathione, which functions in eliminating ROS that is generated during aerobic growth. It was hypothesized that the polygodial-mediated depletion of intracellular glutathione was possibly dependent on a direct interaction between its enal moiety and the sulphydryl group of the cysteine in glutathione by a Michael-type reaction. The breeding of high-glutathione-producing strains and their production by fermentation has been described recently (Liu et al. 2003; Sakato 1992; Shimizu et al. 1991; Udeh and Archremowicz 1997; Wei et al. 2003).

Increasing the level of Cu,Zn-SOD in beer was attempted through the genetic engineering of a beer brewing strain of *S. cerevisiae* yeast by the cloning of the Cu,Zn-SOD gene coupled to an α-factor leader (Cremer et al. 1999). The protein product was secreted by the transformed strain (RHS1) and the transformants were active in catalysing the reaction with superoxide. The enzymic activity of SOD was assayed directly and by determining antioxidant activity. The quantity of SOD secreted was insufficient for fermentation processes. Additional expression of the

protein may be achieved by further optimization of the transformation process (e.g. change of the promoter region) and by the use of various recipient cultures.

The antioxidative role of coenzyme Q or ubiquinone in yeasts is well established. Ubiquinone is an essential component of the electron transfer system in both prokaryotes and eukaryotes and is synthesized from chorismate and polyprenyl diphosphate in eight steps. The enzyme *p*-hydroxybenzoate (PHB) polyprenyl diphosphate transferase catalyses the condensation of PHB and polyprenyl diphosphate in ubiquinone biosynthesis. The gene for PHB polyprenyl diphosphate transferase (designated ppt1) was isolated and the gene was disrupted in a strain of the fission yeast *Schizosaccharomyces pombe* (Uchida et al. 2000). This strain could not grow on minimal medium supplemented with glucose. The expression of COQ2 from *S. cerevisiae* in the defective *S. pombe* strain restored growth and enabled the cells to produce ubiquinone-10, indicating that COQ2 and ppt1 are functional homologues. The ppt1-deficient strain required supplementation with antioxidants, such as cysteine, glutathione, and α-TOH to grow on minimal medium. This supports the role of ubiquinone as an antioxidant and the observation that the ppt1-deficient strain is sensitive to H_2O_2 and Cu^{2+}. The ppt1-deficient strain produced a significant amount of H_2S. Thereby the oxidation of sulphide by ubiquinone may be an important pathway for sulphur metabolism in *S. pombe*. Ppt1-green fluorescent protein fusion proteins localized to the mitochondria, indicating that ubiquinone biosynthesis occurs in the mitochondria in *S. pombe*. Thus, analysis of the phenotypes of *S. pombe* strains deficient in ubiquinone production clearly demonstrated that ubiquinone has multiple functions in the cell apart from being an integral component of the electron transfer system.

The formation of several antioxidants can be induced in yeasts grown under stressful conditions or in response to fermentation medium ingredients such as phenolics or additives that are known to be toxic to cells grown aerobically (Cruz et al. 1999; Fung et al. 1985; Larsson et al. 2000; 2001; Millati et al. 2002; Wang et al. 2001). In another variation, synthetic antioxidants are added to fermentation media during the production of fodder yeast to stimulate aerobic growth and to increase cell biomass (Pobedimskii et al. 1998; Larsson et al. 2000). The screening of yeasts for free-radical-scavenging activity is an active area of research (Gazi et al. 2001). The recent publication by Gazi et al. (2001) describes the screening of 25 yeast strains that were cultivated in yeast peptone dextrose broth (YPD) and in yeast malt extract broth (YMB) media under both shaking and stationary conditions. This was followed by measuring the decrease of absorbance at 517 nm of a solution of 1,1-diphenyl-2-picrylhydrazyl after mixing using the supernatant of each cell culture. It was found that all strains tested are capable of producing the activity in at least one condition. Among the tested strains, *Hansenula anomala* (134 units/mL) produced the highest activity during YPD shaking culture. On the other hand, *Rhodotorula glutinis* (199 units/mL) produced the highest activity during YMB stationary culture.

In a recent patent, Nishino and Ishikawa (1998) describe antioxidants that can be used in pharmaceutical, cosmetic, and food applications. These antioxidants comprise whole yeast cells, their cultured products, or their extracts and are selected from a number of genera and yeasts that consist of *Candida gropengiesseri*, *C. parapsilosis*, *C. maltosa*, *C. stellata*, *C. tropicalis*, *Hansenula holstii*, *Hyphopichia*

burtonii, *Pichia membranaefaciens*, *R. glutinis*, *R. minuta*, *R. rubra*, *S. bayanus*, *S. elegans*, *S. unisporus*, *Torulopsis magnoliae*, and *Zygosaccharomyces bisporus*. In this patent, the antioxidant activities of microorganisms were evaluated by adding microorganisms to both Rose Bengal (I) containing medium and in a dye-free medium, culturing yeast under light irradiation, and measuring the viable count or the turbidity of each medium. The antioxidants disclosed inhibit oxidation of low-density-level lipids and are useful as antiarteriosclerotics. A lotion was prepared by combining a 15 mL EtOH extract of *C. parapsilosis* ATCC 6295, 2 g poly(oxyethylene) hydrogenated castor oil, 3 mL 1,3-butylene glycol, 0.2 g perfume, 0.2 g antiseptic, and H_2O to 100 mL.

Another recent publication, by Kakizono et al. (2003), outlines an efficient method for screening antioxidant high-production yeast cells comprising performing a mutation treatment upon a group of cells, reacting the cells with a redox fluorescent indicator capable of generating fluorescence upon being oxidized by ROS, detecting the fluorescence generated from the intracellular fluorescent indicator with an optical detector upon irradiating with excitation light, and isolating the cells possessing the relatively low fluorescence with a fractionation mechanism (Kakizono et al. 2003). This method enables the selection of high-antioxidant-producing cells which appear normally with an extremely low frequency.

A recent patent application describes the isolation and purification of a natural antioxidant compound from natural sources including yeasts [*S. carlsbergenesis*, *S. cerevisiae*, or from a commercially available yeast extract, and saltbush (*Atriplex halimus*)] that can be synthesized chemically, by processes that improve the potency of the product (Mirsky et al. 2001). These antioxidants when used with/without chromium can be formulated for use in animals and humans.

Bio-Catalyzer α-ρ no. 11 (Bio-Normalizer) or BN is a complex natural health food product prepared by yeast fermentation of medicinal plants or unripe papaya fruits which has been reported to possess antioxidant properties (Afanas'ev et al. 2000; Haramaki et al. 1995). The effects of BN have been compared in vivo and in vitro with those of some classical antioxidants to determine their protective properties against free-radical-mediated damage of erythrocytes of thalassemic patients as well as damage to liver, and to peritoneal macrophages of iron-overloaded rats. The principal difference between the protective mechanisms of BN and rutin was observed only in thalassemic cells. Rutin was able neither to remove iron from cells nor to affect haemoglobin oxidation. Thus, rutin's antioxidant effect seems to depend exclusively on its oxygen radical scavenging activity. To better understand the effects of orally administered BN on oxidative damage in the rat heart, the BN-supplemented animals were (1) exposed to ischemia-reperfusion using the Langendorff technique or (2) homogenized and exposed to peroxyl radicals generated from 2,2′-azobis(2,4-dimethylvaleronitrile) (AMVN). During reperfusion following 40 min of ischemia, leakage of lactate dehydrogenase from hearts isolated from BN-supplemented rats was significantly lower than from hearts of control animals. Furthermore, lower levels of AMVN-induced accumulation of thiobarbituric acid reactive substances and of protein carbonyl derivatives were detected in homogenates prepared from hearts isolated from BN-supplemented rats than in samples from control animals. The findings confirm an antioxidant action of BN

and show that it protects the heart against ischemia-reperfusion-induced damage. From these results, it is clear that yeasts generally have radical scavenging activity and are a good source of potent natural antioxidants.

10.2.2 Aromas

During fermentation yeasts synthesize a vast number of aroma and flavour compounds (Fig. 10.2; Berry 1995; Suomalainen and Lehtonen 1979). The numerically and quantitatively largest groups of aroma compounds synthesized by yeasts consist of fusel alcohols, fatty acids, and their esters (Suomalainen and Lehtonen 1978, 1979). These are generally compounds with a molecular weight of less than 300 (Noble 1978). It has been shown that these are primarily due to yeast metabolism since significant differences in their production have been demonstrated by the use of different yeast genera, species, and strains. In addition to the choice of yeast, several factors contribute to aroma production. These include changes in fermentation conditions such as temperature, pH, aeration, agitation, and the nature and concentration of the substrate utilized (Suomalainen and Lehtonen 1979). Yeast-derived aromas or odours are primarily products of the application of yeasts for the production of alcoholic and nonalcoholic beverages such as beer, wine, sherry, sake, brandy, spirits such as rum and whisky, and other fermented beverages. Modern aroma research has revealed that the complex aroma of alcoholic beverages involves over 400 different chemical compounds which include acids, esters, carbonyl compounds,

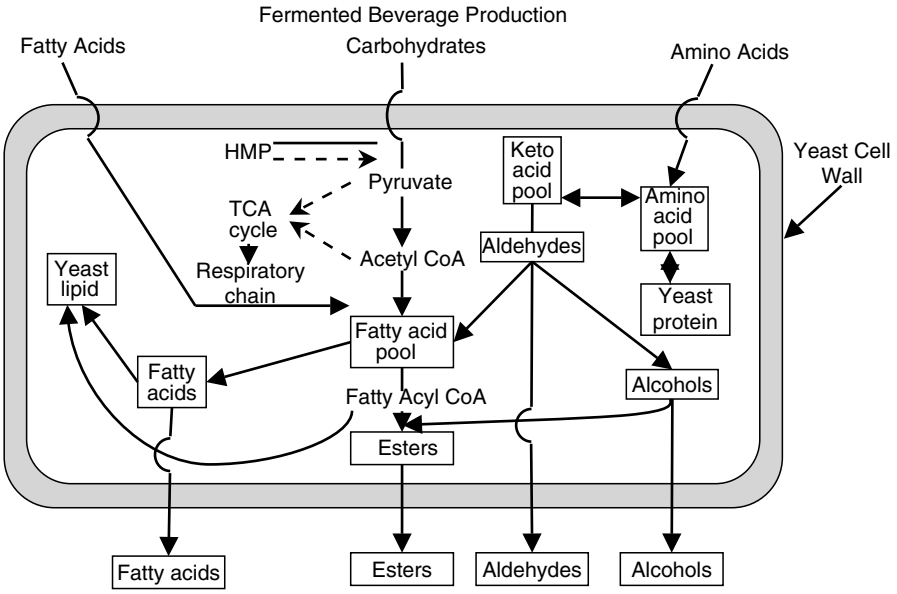

Fig. 10.2. Basic routes by which yeasts form the major flavour groups during fermentation. (Adopted from Ramsay 1982)

acetals, phenols, hydrocarbons, nitrogen compounds, sulphur compounds, lactones, sugars, and a variety of other unclassified compounds that are listed in Table 10.1 in addition to alcohols (Berry 1989, 1995; Dickinson 2003; Garafolo 1992; Suomalainen and Lehtonen 1978, 1979). The unique aromas of the many alcoholic beverages produced by yeasts with some exceptions are frequently the result of a pattern or specific ratios of the previously listed components rather than caused by the presence or absence or a specific concentration of one or a few components (Noble 1978). Yeasts also contribute significantly to the aroma of fermented foods such as breads, cheeses, other dairy products, fermented meat products, vanilla, cocoa, and fermented soy-derived foods. Since many of the aroma compounds that are associated with alcoholic beverages are also present in other fermented foodstuffs, I have chosen to combine all of these into six groups.

10.2.2.1 Fusel Alcohols

Fusel alcohols comprise the largest group of aroma compounds in alcoholic beverages. Their concentration varies considerably in spirits, with rums containing an average of 0.6 g/L, whiskeys about 1.0 g/L and brandies about 1.5 g/L (Dickinson 2003; Suomalainen and Lehtonen 1978, 1979). The main fusel oil synthesized by yeasts is isoamyl alcohol, with *n*-propyl alcohol, isobutyl alcohol, phenethyl alcohol, tryptohol, and optically active amyl alcohol as the other long-chain and complex alcohols (Dickinson 2003; Etschmann et al. 2003; Fabre et al. 1997, 1998; Kunkee et al. 1983; Mo et al. 2003; Pan and Kuo 1993; Suomalainen and Lehtonen 1978, 1979; Ter Schure et al. 1998). Several theories have been proposed to explain the formation of fusel alcohols by yeasts, the oldest of which is Ehrlich's so-called catabolic derivation from exogenous amino acids such as leucine, isoleucine, valine, and threonine (Dickinson 2003; Suomalainen and Lehtonen 1979, 1978; Ter Schure et al. 1998). This theory is

Table 10.1 The number of aroma compounds identified in alcoholic beverages

Compound	Number
Alcohols	38
Acids	80
Esters	118
Carbonyl compounds	41
Acetals	17
Phenols	41
Hydrocarbons	11
Nitrogen compounds	18
Sulphur compounds	11
Lactones	17
Sugars	4
Unclassified compounds	11
Total	407

Adopted from Kahn (1969)

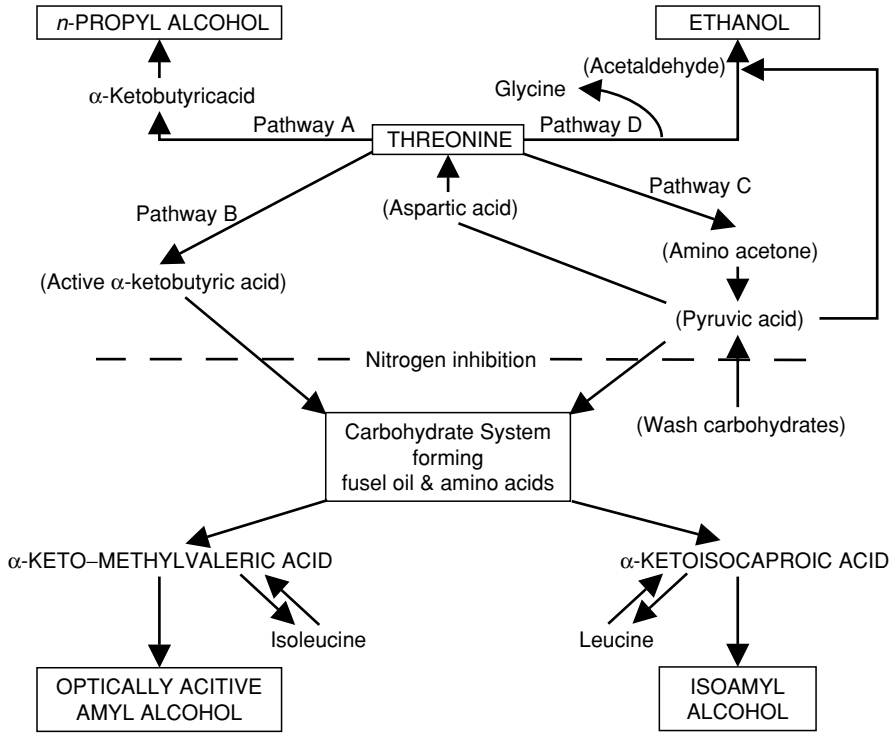

Fig. 10.3. Biochemical relationships of threonine and isoleucine in the formation of fusel alcohols. (Adopted from Reazin et al. 1973)

consistent with more recent work which provides evidence that *S. cerevisiae* grown on isoleucine forms primarily optically active amyl alcohol with significant additional production of *n*-propyl alcohol and isoamyl alcohol when cells are grown on threonine (Suomalainen and Lehtonen 1979). The biochemical pathways describing the production of these alcohols from amino acids is provided in Fig. 10.3 (Suomalainen and Lehtonen 1979). The constitutive expression of the BAP gene which codes for the permease involved in branched amino acid transport has been demonstrated to increase the production of isoamyl alcohol derived from leucine but no comparable increase in isobutyl alcohol derived from valine or amyl alcohol derived from isoleucine was observed (Kodama et al. 2001). The genes for two aminotransferases (ECA39 and ECA40) that actively participate in the transamination of branched amino acids in the mitochondria and cytoplasm have been deleted in order to determine their role in the formation of higher alcohols (Eden et al. 2001). Deletion of the ECA39 and ECA40 genes had little impact on the production of propanol, while the deletion of ECA40 had a drastic impact on the production of isobutyl alcohol and partial impact on the production of active amyl and isoamyl alcohol (Eden et al. 2001). The production of isoamyl acetate in *S. cerevisiae* is regulated at the co-transcriptional level of two genes involved in cytosolic branched-chain amino acid aminotransferase

and L-leucine biosynthesis (Yoshimoto et al. 2002). These findings provide added support to the findings that the catabolic pathway from amino acids fails to explain the formation of fusel alcohols by yeasts grown on carbohydrates with inorganic nitrogen sources or under nitrogen limitation. Therefore, the anabolic formation route from sugars first proposed by Ayräpää provides a plausible alternate explanation (Suomalainen and Lehtonen 1979). Thus, the formation of fusel alcohols in yeasts involves both degradative and synthetic pathways as illustrated in Fig. 10.4 (Suomalainen and Lehtonen 1979). Evidence indicating a high level of specificity of the enzymes involved in the catabolic and anabolic pathways points to the complex operation and regulation of yeast genes involved in the formation of fusel oils as summarized in a recent review by Dickinson (2003). Gaps in the current knowledge of the genetics and biochemical basis for fusel alcohol formation in yeasts will continue to provide the impetus for more research since its delineation will have many practical applications in the production of alcoholic beverages.

10.2.2.2 Fatty Acids

Fatty acids with a carbon chain length ranging from C3 to C16 represent another major group of aroma compounds that are synthesized by yeasts during alcoholic fermentations (Suomalainen and Lehtonen 1979). An important component of alcoholic beverage aroma is provided by the shorter-chain volatile fatty acids such as propionic acid, isobutyric acid, butyric acid, isovaleric acid, valeric acid, caproic

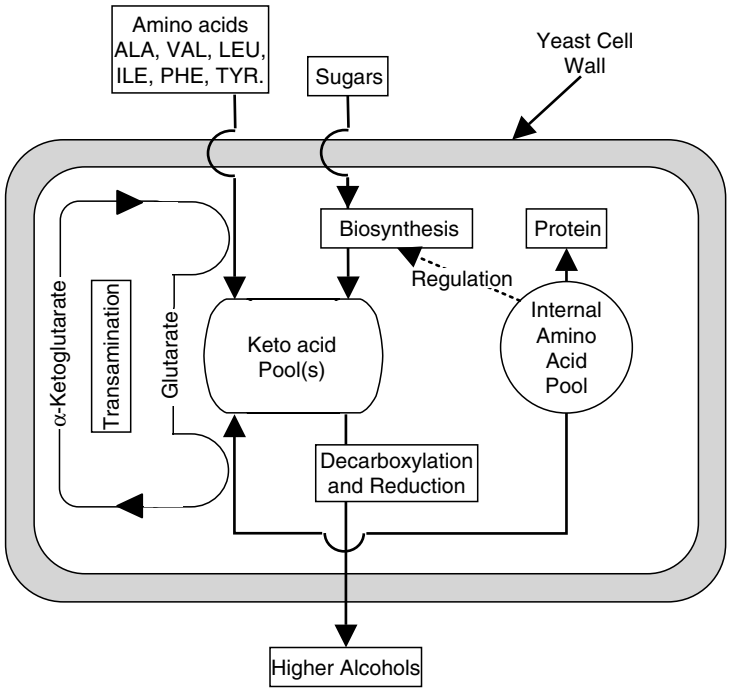

Fig. 10.4. The formation of fusel alcohols. (Adopted from Äyräpää 1973)

or hexanoic acid, caprylic or octanoic acid, and capric acid. These fatty acids are synthesized by yeasts irrespective of the raw material used but with noticeable clear differences in the relative proportions of these acids in the various alcoholic beverages (Edwards et al. 1990; Suomalainen and Lehtonen 1978, 1979). The differences in the relative amounts in the main volatile fatty acids in three spirits are provided in Table 10.2 (Suomalainen and Lehtonen 1979). The acid content of alcoholic beverages varies considerably, with low levels reported for Scotch whisky and cognac, which contain levels in the range 100–200 mg/L, when compared with heavy rum or wines, which tend to have significantly higher levels of volatile acids reported in the range 500–1,000 mg/L (Suomalainen and Lehtonen 1979).

In yeasts, the synthesis of fatty acids begins with acetyl coenzyme A (CoA) formed from the oxidative decarboxylation of pyruvate obtained from glycolysis and involves a multienzyme complex which binds all of the intermediates until the fatty acids are formed (Suomalainen and Lehtonen 1979). This synthesis is illustrated in Fig. 10.5 (Suomalainen and Lehtonen 1979), which provides a scheme for the formation and elongation of fatty acids that leads to even- or odd-numbered chains depending on whether acetyl-CoA or propionyl-CoA is the starting material. The way in which yeasts influence the fatty acid composition of alcoholic beverages is not known. The yeast strain used, the composition of the medium, the temperature, and the aeration employed influence to a great extent the final levels of fatty acids, carbon chain length, and level of saturation (Suomalainen and Lehtonen 1979). At lower temperatures, yeasts synthesize a larger amount of fatty acids when compared with the amounts synthesized at higher temperatures, with more of the unsaturated fatty acids being synthesized under aerobic and semiaerobic conditions. For example, the amount of the fatty acids caproic and caprylic secreted into the fermentation medium at 10°C by *S. cerevisiae* is higher than that secreted at 30°C, whereas

Table 10.2 Relative amounts of the main volatile fatty acids, excluding acetic acid, in the acid fractions of Martinique rum, Scotch whisky and cognac

Acid	Martinique rum (%)	Scotch whisky (%)	Cognac (%)
Propionic	15.7	1.5	2.7
Isobutyric	3.6	4.9	3.6
Butyric	15.3	1.5	3.6
Isovaleric	4.7	5.9	3.3
Valeric	6.5	0.1	Traces
Caproic	5.4	4.2	8.2
Caprylic	14.5	26.7	35.0
Capric	17.5	31.6	30.4
Lauric	6.5	16.2	8.6
Myristic	1.1	2.2	1.6
Palmitic	4.0	1.7	1.1
Palmitoleic	1.0	2.0	0.5
Others	4.2	1.5	1.4

Adopted from Nykanen et al. (1968)

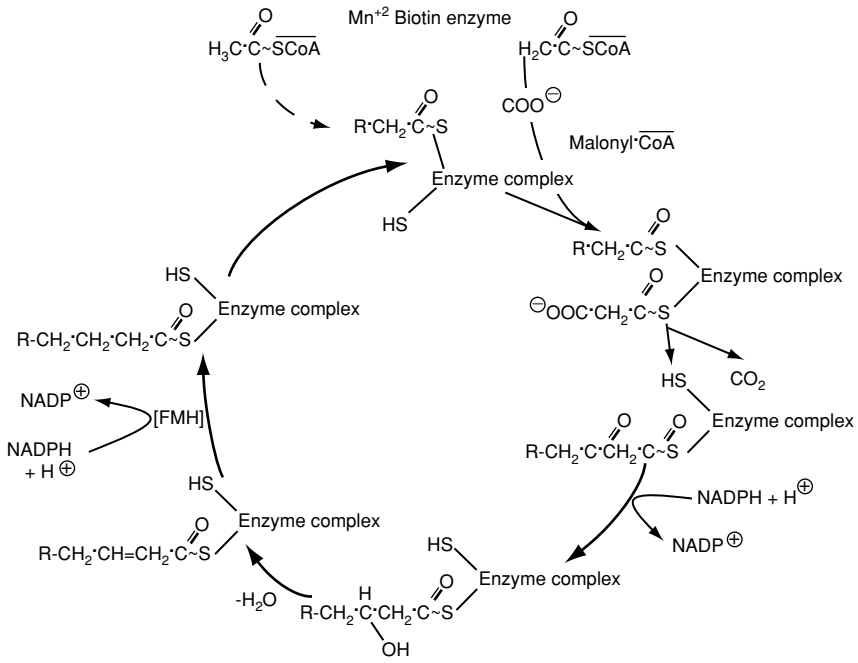

Fig. 10.5. The formation of fatty acids. (Adopted from Lynen 1967)

that of capric and lauric acids is independent of the fermentation temperature (Suomalainen and Lehtonen 1979). When C6–C10 fatty acids are present in beer in levels that exceed 1 mg/L, they contribute to what is known as a "caprylic flavour" characterized by a musty, rancid, or soapy odour (Tressl et al. 1980).

10.2.2.3 Fatty Acid Esters

Fatty acid esters are by far the most prevalent group of aroma compounds produced by yeast fermentation (Buzzini et al. 2003; Cristiani and Monnet 2001; Suomalainen and Lehtonen 1978, 1979; Verstrepen et al. 2003). The ester content of alcoholic beverages is reflected in the perception of their aromas as demonstrated by comparing light rums which have an ester content of 50 mg/L with heavier variety of rums which contain greater than 600 mg/L (Suomalainen and Lehtonen 1979). Fatty acid esters of alcoholic beverages are classified into three major fractions: light, middle, and heavy on the basis of their boiling-point ranges (Suomalainen and Lehtonen 1979). The light fraction consists of fruit esters with pleasant aromas which include all esters that have a boiling point lower than that of isoamyl alcohol such as ethyl, isobutyl and isoamyl esters of short-chain fatty acids (Suomalainen and Lehtonen 1979). The middle fraction comprises ethyl esters that elute during distillation between ethyl caproate and phenethyl alcohol. These include primarily ethyl esters

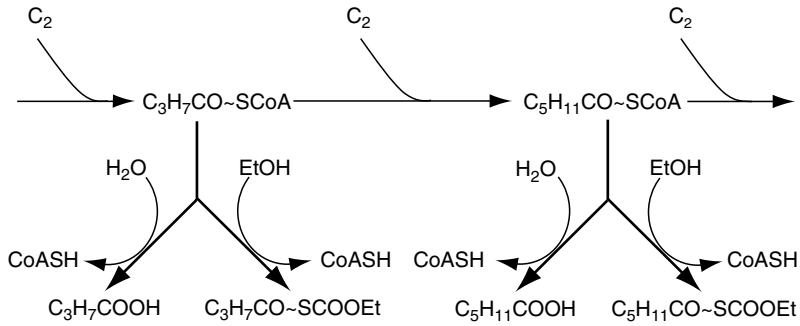

Fig. 10.6. Simplified scheme for the formation of esters and higher fatty acids (Adopted from Nordström 1964)

of caprylic and capric acids. The main components of the heavy distillate fraction are all other esters that elute after phenethyl alcohol (Suomalainen and Lehtonen 1979). The biosynthesis of esters is similar to that of fatty acids and is illustrated in Fig. 10.6 (Suomalainen and Lehtonen 1979). An increase in ethyl esters of caprylic, capric, and palmitoleic acids is noted if the yeast is present during distillation (Suomalainen and Lehtonen 1979; Verstrepen et al. 2003).

During fermentation in yeasts, the formation of esters is carried out by intracellular enzyme-catalysed reactions. The expression level of two genes coding for the yeast alcohol acetyltransferases, ATF1 and ATF2, was shown to influence the production of ethyl acetate and isoamyl acetate (Verstrepen et al. 2003, 2004). Double deletion of the former two genes resulted in partial reduction in the two previously mentioned esters, suggesting the existence of as-yet-unknown other ester synthases (Verstrepen et al. 2003). Ester production by yeasts is dependent to a great extent on the composition of the carbon source and the type of assimiliable nitrogen in the medium used, the fermentation parameters selected, such as pitching rate and top pressure, dissolved oxygen, as well as the genus, species, and strain employed (Verstrepen et al. 2003).

Several fatty acid esters produced by yeasts during alcoholic fermentations have found other applications and an example of this is the use of the short-chain acetate esters of ethyl acetate as a solvent, of isoamyl acetate as a banana food flavour, and of phenyl ethyl as a flowery or rose aroma enhancer in cosmetics and foods (Armstrong 1986; Ashida et al. 1987; Fabre et al.1998; Fukuda et al. 1990a, 1998a, b; Fujii et al. 1996; Furukawa et al. 2003a; Janssens et al. 1987; Quilter et al. 2003; Verstrepen et al. 2003). Screening a selected yeast for 2-phenylethanol production in a molasses-containing medium supplemented with phenylalanine has shown that *Kluyveromyces marxianus* CBS 600 and *K. marxianus* CBS 397 can produce up to 3 g/L at 35°C (Etschmann et al. 2003; Fabre et al. 1997, 1998). The production of the important food and cosmetic aroma compound ethyl oleate can also occur through enzymic catalysis by using immbolized cells of *S. bayanus* to produce ethanol in the presence of oleic acid in a fermentation medium (Kiss et al. 1998). The increased synthesis of ethyl caproate by *S. cerevisiae*, which is an important aroma and flavour compound in sake, has been attributed to inositol limitation

(Arikawa et al. 2000; Furukawa et al. 2003b). Fatty acid ethyl ester synthesis by *S. cerevisiae* is also important in the development of Scotch whisky aroma and flavour (Goss et al. 1999). The release of fatty acids and the production of medium-chain fatty acids and their ethyl esters in the absence of exogenous lipids by yeast strains isolated from musts and wines in the absence of aeration has recently been described (Bardi et al. 1999; Ravaglia and Delfini 1993). Medium-chain fatty acids and their esters are responsible for stuck alcoholic fermentation and their formation is strongly dependent on the yeast strain used and the fermentation medium (Ravaglia and Delfini 1993).

10.2.2.4 Carbonyl, Sulphur, and Phenolic Compounds

Carbonyl, sulphur, and phenolic compounds are other types of yeast fermentation by-products that impact the aroma of alcoholic beverages (Suomalainen and Lehtonen 1979). Owing to their lower sensory thresholds, these compounds are of particular interest as they can contribute to undesirable odours in alcoholic beverages such as beer (Arai 1980; Russell et al. 1983; Suomalainen and Lehtonen 1979; Tressl et al. 1980). The carbonyl compounds of great interest are the aldehydes that are intermediates in the formation of fusel alcohols. Diacetyl and 2,3-pentanedione are formed during fermentation from the decarboxylation of the two α-keto acids, α-acetolactic and α-aceto-α-hydroxybutyric acid, respectively (Suomalainen and Lehtonen 1979). These compounds, illustrated in Fig. 10.7, contribute to a buttery aroma in beer (Tressl et al. 1980). Sulphur compounds derived from the degradation of the amino acids cysteine and methionine lead to the formation of off-flavours in beer, such as the offensive smelling hydrogen sulphide, diethyl sulphide, and dimethyl sulphide (Suomalainen and Lehtonen 1979; Tressl et al. 1980; Van Haecht and Dufour 1995). Sulphites produced by yeasts during alcoholic fermentation can also have a positive effect as they act as antioxidants and flavour stabilizers (Hansen and

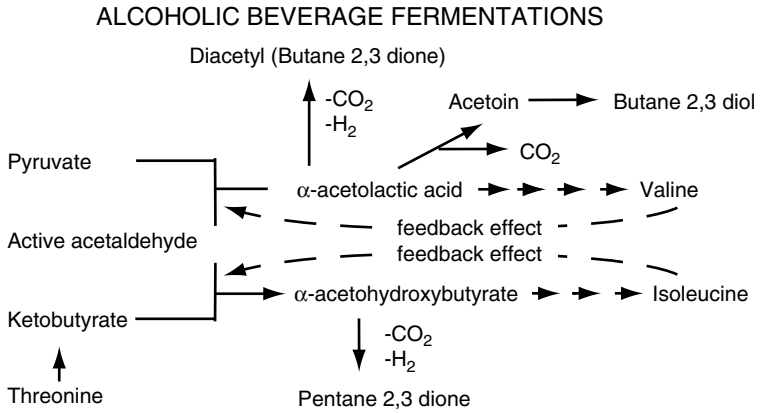

Fig. 10.7. Reduction of the level of diacetyl in wort by reduction of α-acetolactic acid through a feedback mechanism. (Adopted from Berry 1995)

Kielland-Brandt 1996). Inactivation of the MET2 gene which encodes the enzyme homoserine *O*-acetyltransferase increases the level of sulphite in beer (Hansen and Kielland-Brandt 1996). The constitutive expression of MET25 which codes for the enzyme *O*-acetylserine sulphydrylase leads to a reduction in the off-flavour compound hydrogen sulphide (Omura et al. 1995). Phenolic compounds produced during alcoholic yeast fermentations are derived from the catalysis of *p*-coumaric acid, ferulic acid, and vanillin, which are typically of plant origin (Ettayebi et al. 2003; Meaden 1998; Suomalainen and Lehtonen 1979). The products formed by yeast action consist of 4-ethylphenol, 4-ethylguaiacol, and 4-methyl guaiacol as shown in Fig. 10.8 (Dias et al. 2003; Meaden 1998; Suomalainen and Lehtonen 1979). These compounds, while desirable in low levels, can lead to phenolic off-flavours when present in levels in excess of 100 ppb in beer (Tressl et al. 1980). The production of phenolic off-flavours by wine yeast strains of *S. cerevisiae* and other yeast genera such as *Rhodotorula*, *Candida*, *Cryptococcus*, *Pichia*, *Hansenula*, *Dekkera*, and *Brettanomyces* that contribute to phenolic off-flavours in wine products (Coghe et al. 2004; Edlin et al. 1995; Shinohara et al. 2000). Mousy off-flavour production in grape juice and in red and white wines attributed to the yeast *Dekkera* (*D. bruxellensis* and *D. anomala*) and *Brettanomyces* yeasts has been recently described by Grbin and Henschke (2000) and by Dias et al. (2003).

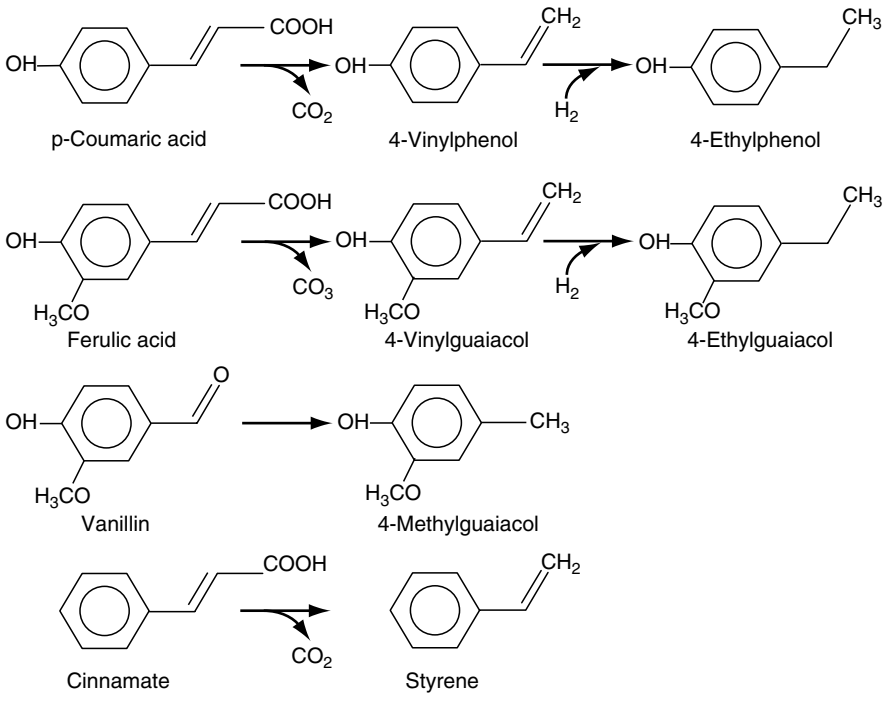

Fig. 10.8. The decarboxlyation of cinnamic acids by *Saccharomyces cerevisiae*

10.2.2.5 Lactones

Lactones are an important group of compounds that are of interest to the food industry as they impart highly pleasant ripened fruit aromas characteristic of peach, apricot, or coconut when added to over 120 foodstuffs (Dufosse et al. 2000, 2002; Endrizzi et al. 1996; Feron 1997; Gatfield et al.1993; Groguenin et al. 2004; Heath 1981; Vandamme 2003; Vanderhaegen et al. 2003; Van Der Schaft et al. 1992; Wache et al. 2003). Lactones are made up of a carbon ring with an oxygen atom and are formed by intramolecular esterification between the hydroxyl and carboxylic group of a hydroxy fatty acid as illustrated in Fig. 10.9. The recognition that the pigmented yeast *Sporobolomyces odorus* can synthesize the lactones responsible for the peach-like odour, γ-decalactone (GC10) and dodecenolactone, has given rise to interest in the de novo microbial production of these compounds from the readily available, natural ricinoleic hydroxy fatty acid (Endrizzi et al. 1996). This hydroxyacid represents over 90% of castor bean oil and thereby provides an economical source as a feedstock which can compete with the chemical synthetic route (Endrizzi et al. 1996). While several yeasts can carry out the aforementioned biotransformation most recent focus has been on using three yeasts *Yarrowia lipolytica*, *Sporidiobolus salmonicolor* (*Sporidiobolus odorus*), and *Sporidiobolus ruinenii*, with reported production of GC10 in the range 0.4–1.2 g/L (Groguenin et al. 2004; Dufosse et al. 2000, 2002; Feron et al. 1997). These levels are below the 5.5 g/L reported earlier for *Y. lipolytica* (Farbood and Willis 1985). A schematic representation for the pathway for the key acyl oxidase enzymes involved in the production of GC10 from ricinoleic acid in *Y. lipolytica* is provided in Fig. 10.10 (Groguenin et al. 2004). The key challenges to the further improvement of industrial processes with the three yeasts are the toxicity, yield, and final concentration and composition of the lactones produced. Several strategies can be employed to overcome these challenges, ranging

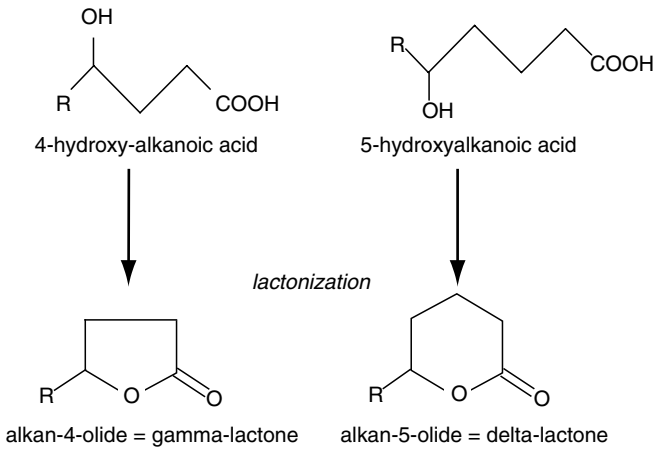

Fig. 10.9. Structure of γ- and δ-lactones and of corresponding hydroxy acids. (Adopted from Groguenin et al. 2004)

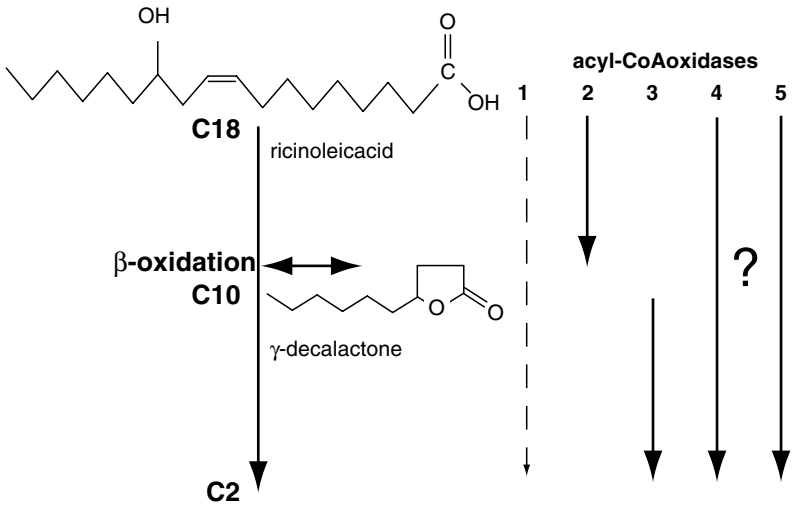

Fig. 10.10. Schematic representation of the pathway from ricinoleic acid to γ-decalactone and activities of the acylcofactor A (*acyl-CoA*) oxidases of *Yarrowia lipolytica*. (Adopted from Endrizzi et al. 1996)

from genetic engineering and/or modification of yeast strains, use of batch vs. fed-batch fermentation, to the continuous extraction of the GC10 decanolide with the use of a membrane based solvent-extraction technique (Akita and Obata 1991).

10.2.2.6 Other Chemical Compounds

Other chemical compounds that contribute to the aroma of yeast-fermented products include breakdown products from sugars such as the furanones, furfural and hydroxymethyl furfuryl, and their alcohol derivatives, production of chiral alcohols from prochiral ketones, volatile organic acids such as acetic acid, which imparts a sour or vinegar smell, pyrazines which are formed from reactions of sugar aldehydes with amino acids which undergo a Strecker degradation reaction followed by self-condensation and oxidation to produce these compounds with burnt or roasted aroma notes, and monoterpene-derived alcohols which are formed from monoterpene hydrocarbons, which are important components of essential oils and convey a fresh fragrance to foodstuffs (Heath 1981; Hecquet et al. 1996; King and Dickinson 2000; Kometani et al. 1995; Maga 1982, 1992; Vandamme 2003).

10.2.3 Colours

Concerns about the use of chemically produced dyes in foodstuffs have resulted in the increased use of naturally produced colorants that are derived from animal, plant, and microbial sources. As a part of this trend, yeasts that are commonly found as part of the food flora and thereby generally recognized as safe (GRAS)

have been tapped as sources of a number of carotenoids for use as food colorants such as astaxanthin, β-carotene and γ-carotene, lycopene, lutein, torulene, torulahordin, and zeaxanthin (An et al. 2001; Bekers et al. 1972; Bhosale and Gadre 2001a–c; Bogdanovskaya 1973; Bogdanovskaya and Zalashko 1982; Bon et al. 1997; Buzzini 2000; Calo et al. 1995; Cang et al. 2002; Cannizzaro et al. 2003; Chen and Liu 1999; Farid and Azar 2001; Fontana et al. 1996a, b, 1997; Frengova et al. 2003, 2004; Guirnovich et al. 1966; Guirnovich and Koroleva 1971; Jacob 1991; Johnson and Schroeder 1995; Johnson 2003; Kilian et al. 1996; Koroleva et al. 1982; Kusdiyantini et al. 1998; Lewis et al. 1990; Liang and Zhang 2003; Martin et al. 1993; Matelli et al. 1990; Misawa 1997, 1999; Misawa et al. 1998; Misawa and Shimada 1998; Miura et al. 1998; Nikolaev et al. 1966; Parajó et al. 1997, 1998a, b; Pshevorskaya and Zabrodskii 1972; Ramirez et al. 2000; Reynders et al. 1996; Sakaki et al.1999, 2001, 2002; Shih and Hang 1996; Shimada et al. 1998; Shitazawa et al. 2002; Simova et al. 2003; Slyusarenko et al. 1976; Squina et al. 2002; Vazquez et al. 1998; Vazquez and Martin 1998; Yang et al. 1993; Zalaskho et al. 1973; Zeile 1972). A number of yeasts are known to produce carotenoids that have been evaluated as food colorants and these include several species of *Rhodotorula* (*R. glutinis*, *R. lactis*, *R. gracilis*, *R. rubra*), *Rhodosporidium*, *Phaffia rhodozyma*, and *Sporobolomyces pararoseus*. Improving the commercial potential for yeast production of the previously mentioned carotenoids has been pursued with improved yeast strains from the aforementioned yeasts and from selected and genetically engineered strains of *S. cerevisiae* (Misawa and Shimada 1998), *C. utilis* (Misawa et al. 1998; Shimada et al. 1998), so-called red yeast, (Fang and Chiou 1996; Yang et al. 1993), and other *Candida* species that have been developed through classical selection and genetic engineering methods (Bekers et al. 1972; Bhosale and Gadre 2001a–c; Bogdanovskaya and Zalashko 1982; Buzzini 2000; Cang et al. 2002; Chen and Liu 1999; Cheng et al. 2004; Frengova et al. 2003, 2004; Girard et al. 1994; Jacob 1991; Koroleva et al. 1982; Martin et al. 1993; Misawa et al. 1998, 1999; Misawa and Shimada 1998; Nikolaev et al. 1966; Pshevorskaya and Zabrodskii 1972; Sakaki et al. 1999; Shih and Hang 1996; Shimada et al.1998; Simova et al. 2003; Slyusarenko et al. 1976; Squina et al. 2002; Zalaskho et al. 1973; Zeile 1972; Yang et al. 1993). The commercial exploitation of one of these yeasts, *Phaffia rhodozyma* or *Xanthophyllomyces dendrorhous*, has resulted in industrial-scale production of the carotenoid astaxanthin (Abbas 2001, 2003, 2004; Jacobson et al. 2000; Palagyi et al. 2001; Kim et al. 2003). Astaxanthin-containing yeasts and astaxanthin extracted from these yeasts has in addition to aquafeed uses as a colorant, other uses as an immunostimulant in poultry, fish, mammalian feed, and food when combined with other antioxidants derived from plant sources such as vitamin E. Other colorants of foods that are produced by yeast fermentation are the vitamin B_2 or riboflavin and caramel colours produced from processing of yeast extracts (Halasz and Lasztity 1991).

10.2.4 Flavours

Flavours can be classified in multiple ways depending on their mode of formation either naturally by biogenetic pathways from known precursors or by processing in which biological, chemical, physical, or a combination of these approaches is

employed (Heath 1981). Natural flavours are mostly metabolites produced in living cells and thereby their formation is strongly dependent on the genetic traits and the environmental conditions under which they are produced (Heath 1981). Flavours produced by processing are generally breakdown products or complex products derived from the interaction of components within a food matrix under the conditions selected (Heath 1981; Chao and Ridgway 1979). Products produced by brewer's yeast that contribute to both aroma and flavour include higher alcohols and their derived esters, carbonyl compounds, and sulphur-containing compounds. Many of these products are derived from amino acid metabolism in yeasts, while others are derived from carbohydrate and lipid metabolism. Yeast-produced flavours can be classified into three very general categories: yeast metabolic products, and this includes products synthesized or derived through yeast biocatalysis, yeast cell mass derived products, which include products prepared through yeast autolysis, and complex products resulting from the interaction of yeast-derived products with other food matrix ingredients. Products representing each category are provided in Table 10.3 and it should be noted that in some cases many of these products have in addition to flavour attributes, aroma, antioxidant, colorant, vitamin, and nutraceutical properties.

10.2.4.1 Yeast Metabolic Products

The metabolic products of yeasts that are used as flavours include a variety of short or long branched or unbranched alcohols, and aromatic alcohols which include in

Table 10.3 Yeast products

Yeast metabolic products	Yeast-derived products	Yeast beverage and food flavour products
Alcohols	Yeast extracts	Baking or bread flavours
Aldehydes	Ribonucleotides	Beer or malt flavours
Amino acids	Cell wall mannoproteins	Cheese and other dairy product flavours
Carbohydrates and other glycosides	Cell wall glucans	Cocoa flavours
Carotenoids	Edible proteins	Distilled spirits flavours
Fatty acids	Choline, glycerol, and inositol	Edible meats flavours
Esters(fragrances)	Mineral-enriched yeast	Roasted flavours
Yeast metabolic products	Yeast-derived products	Yeast beverage and food flavour products
Lactones	Sterols	Soy-derived flavour products
Phenolic compounds flavours	Vitamins	Maple or syrup or caramel
Polyols	Whole cell or single-cell proteins or single-cell oils	Wine, cherry, brandy, and sake flavours
Organic acids	Yeast enzymes	
Terpenes		
Vitamins		

addition to ethyl alcohol, phenyl ethyl alcohol, propyl alcohol, butyl alcohol, octyl alcohol, isoamyl alcohol; amino acids and their breakdown derivatives that are used for their sweet or bitter taste; carbohydrates, glycosides, or polyols such as glycerol, xylitol, and inositol that are used as thickeners, sweeteners, emulsifiers, or for their organoleptic properties; fatty acids and fatty acid esters, and organic acids such as isoamyl acetate, ethyl acetate, ethyl caproate, acetic, formic acid, citric acid, caprylic acid, and lactic acid, which impart a mouth taste of pleasant, sour, or salty notes; and esters, lactones, and aldehydes such as acetaldehyde and GC10 that contribute to a fragrarent, astringent, and sweet taste (Asano et al. 2000; Hecquet et al. 1996; Vandamme 2003; Van den Bremt et al. 2001).

10.2.4.2 Yeast-Derived Products

10.2.4.2.1 Yeast Extracts and Yeast-Hydrolysed Proteins

Yeasts extracts are flavour products that are primarily derived from the soluble fraction of yeasts enriched in amino acids such as glutamic acid in the free acid form or as monosodium glutamate provide a bouillon-like, brothy taste to food (Nagodawithana 1992). These products have found uses by the food industry as lower-cost-flavour protein sources that are competitive with hydrolysed vegetable proteins (HVPs) from soy, wheat, and from other vegetable origins (Cooper and Peppler 1959; Nagodawithana 1992). Yeast extracts are usually derived from yeasts specifically grown under optimized conditions for food application (Chae et al. 2001; Cooper and Peppler 1959; Nagodawithana 1992). In some cases yeast extracts can be derived from spent brewer's yeast, which is readily available from alcohol production facilities in great abundance and thereby represents an inexpensive readily available feedstock (Chae et al. 2001; Nagodawithana 1992). Yeast extracts are provided as powders or pastes and are used at 0.1–0.5% finished dry product basis (Nagodawithana 1992). The manufacturing of yeast extracts employs one of three distinct processes that use autolysis, plasmolysis, or hydrolysis (Chae et al. 2001; Halasz and Lasztity 1991; Lieske and Konrad 1994; Nagodawithana 1992; Peppler 1967, 1970; Reed 1981). The yeast extracts prepared from these processes have different cost structures and product and flavour attributes, and consequently have some tradeoffs. These tradeoffs consist of the cost of using commercial enzymes to autolyse the yeast vs. yeast self-autolysis or the use of high salt, mechanical disruption, or an acid hydrolysis step followed by soluble product recovery (Chae et al. 2001; Lieske and Konrad 1994). Modifying the flavour and colour profile of yeast extract can be accomplished with selected fermentation processes and media as well as by subjecting yeast slurry prior to extraction to O_2 at 60°C for up to 30 min as described in a recent patent (Lieske and Konrad 1994). In this process pasteurized yeast (15% cells by weight) was treated with pure O_2 in a closed reactor at an O_2 partial pressure of 1 atm and pH 6.1, with stirring at 500 rpm. After 15 min, the yeast flavour was modified from very yeasty to mild and the pink colour present initially reduced significantly (Lieske and Konrad 1994). Another mild process for the extraction of yeast was developed that consists of preparing a slurry of a low-moisture active-spray-dried yeast in water at a ratio of 1:5 at 39°C followed by stirring for 30 min and centrifuging the treated material at 2,300 rpm to remove the

soluble fraction which contains trehalose (Cooper and Peppler 1959). The residue after centrifugation was diluted to provide a cream of 16% solids that can be drum-dried. The product derived by this process had a light colour, reduced flavour, and was low in ash but high in protein and nucleic acids when compared with the original composition of the viable yeast. The extract prepared by this process has found many uses in various types of food and feed products. Another yeast extraction process was developed that uses brewer's yeast from a beer factory by combined enzymatic treatments using the enzymes endoprotease, exoprotease, 5′-phosphodiesterase, and adenosine monophosphate (AMP) deaminase (Chae et al. 2001). The effects of enzyme combination, enzyme dosages, and treatment sequence on the recovery of solid and protein, flavour, and compositional characteristics were all investigated (Chae et al. 2001). It was determined that exoprotease dosage strongly affected the recovery of protein and the degree of hydrolysis and sensory characteristics. When the yeast cells were treated using optimal combination of endoprotease and exoprotease (0.6% Protamex and 0.6% Flavourzyme), high solid recovery (48.3–53.1%) and the best flavour profile were obtained. Among various treatment sequences using multiple enzymes, treatment with protease followed by nuclease resulted in the highest 5′-guanosine monophosphate (5′-GMP) content. The optimal concentrations of both 5′-phosphodiesterase and AMP deaminase were found to be 0.03%. After treatments using the optimal combination of enzyme, enzyme dosages, and treatment sequence for four enzymes, a high solid yield of 55.1% and a 5′-nucleotides content of 3.67% was obtained (Chae et al. 2001). A cruder form of yeast extract that is made from both soluble and insoluble fractions by autolysis is employed when a cost-effective product with increased water-binding capability and milder yeast flavour is desired as is the case for meat analogues (Nagodawithana 1992).

Yeast extracts are very powerful savoury flavour ingredients. The sensory quality of yeast extracts is highly influenced by the specific molecular cell composition of the yeast cell. Hydrolysed edible proteins and peptides from yeasts are a good source of the amino acid L-lysine and therefore can be used in food products that need supplementation with this amino acid. The sulphur-containing amino acids cystine, cysteine, and methionine are the limiting amino acids in yeast extracts and therefore attempts have been made to enrich yeast extracts with these amino acids (Reed 1981). Cysteine is a source of sulphur in the generation of meat-like flavours by Maillard chemistry (Hurrell 1982; Stam et al. 2000). Recently, an enriched yeast extract in the amino acid cysteine was produced through the application of genetic engineering to overexpress and overproduce the yeast cysteine-rich protein metallothionein (Stam et al. 2000). This approach can result in the enhancement of the savoury value of yeast extracts prepared by this approach for meat, baked goods, and cheese food applications. Another example of widespread use for yeast proteins and peptides in foodstuffs is as cocoa replacement flavouring components as these can be produced by roasting of yeast extracts in combination with chemical processing (Liggett 1978).

10.2.4.2.2 Nucleotides

The RNA compositions of baker's yeast and of *C. utilis* yeast used to produce single-cell protein have been reported in the range 8–11 and 10–15%, respectively

(Nagodawithana 1992). The ease and cost effectiveness of the methods of production of these two yeasts has resulted in their use to prepare yeast extracts with a high concentration of RNA. The ribonucleotides 5′-inosine monophosphate (5′-IMP) and 5′-GMP are important flavour components of yeast extracts. Ribonucleotide-enriched fractions of yeast extracts are prepared with the nucleotides 5′-GMP and 5′-IMP by enzymic degradation of yeast cellular RNA in hydrolysed yeast extract cell preparations (Chae et al. 2001; Halasz and Lasztity 1991; Nagodawithana 1992; Noordam and Kortes 2004; Patane 2004). A yeast–malt sprout extract was produced by the partial hydrolysis of yeast extract (derived form *S. fragilis* or *C. utilis*) using the sprout portion of malt barley as the source of enzymes, and may be used under the Federal Food, Drug, and Cosmetic Act as a flavour enhancer in food. It contains a maximum of 6% 5′-nucleotides (USA Food and Drug Administration 1973). An improved method for the production of these flavouring agents using liposomes containing 5′-phosphodiesterase that is fused with spheroplasts or protoplast prepared from yeast cells is described in a recent patent application (Patane 2004). The enzymes used are fungal or plant 5′-phosphodiesterases which can release the four free RNA 5′-nucleotides as the final products. Further treatment of the 5′-nucleotide-enriched yeast extract preparations with the enzyme adenyl deaminase from *Aspergillus melleus* converts the 5′-AMP to the flavour-enhancing nucleotide 5′-IMP (Kondo et al. 2001; Nagodawithana 1992; Steensma et al. 2004). The results of the safety evaluation and toxicological data of phosphodiesterases derived from *Penicillium citrinum* and from *Leptographium procerum* are summarized in recent publications (Kondo et al. 2001; Steensma et al. 2004). When used in conjunction with monosodium glutamate or glutamic acid containing peptides in yeast extracts, the aforementioned nucleotides enhance mouthfeel in soups, sauces, marinades, soft drinks, cheese spreads, and seasonings by contributing a meaty flavour with sour, sweet, salty, and/or bitter notes (Noordam and Kortes 2004).

10.2.4.2.3 Yeast Polysaccharides

The major yeast cell wall polymers glucans, mannans, phosphomannans, and mannoproteins have recently been tapped for their potential flavour application as food fibre additives, for their emulsifying properties, and for their nutraceutical potential (Abbas 2003; Halasz and Lasztity 1991; Peppler 1970; Reed 1981; Sucher et al. 1974). These cell wall polymers have been characterized from a number of yeasts which include *S. cerevisiae*, *Pichia holstii*, *Hansensula* sp., a number of *Candida* species, and several *Rhodotorula* sp. Other yeast-produced exopolysaccharides that have gum-like properties that have received recent attention are those produced by *Cryptococcus laurentii* and the yeast-like fungi *Tremella mesenterica* and *T. fuciformis* (De Baets and Vandamme 1999).

Sucher et al. (1974) have described in some detail capturing the greatest value from yeast cell mass from the processing and fractionation of cells to prepare yeast extract proteins, ribonucleotides, and cell wall glucans. The approaches detailed by Sucher et al. in 1974 are still characteristic of current practices employed in the commercial production of yeast-derived products. The process described involved the homogenization of washed yeast cells, followed by alkaline treatment, and centrifugation to remove cell wall constituents. The glucan thus separated was further

washed and dried under vacuum to yield a mild-tasting product which formed a viscous solution upon rehydration. The alkali extract was then acidified to pH 4.5 to produce an isolated yeast protein with a considerable RNA content. The RNA content could be reduced by nuclease treatment and the resultant product used as an amino acid supplement for human consumption. The original RNA contents of 9–14% were typically reduced to less than 3%. After precipitation of the enzyme-treated isolated yeast protein, a yeast extract with a fried-meat flavour is produced with a glutamic acid content of 27–40% of the corresponding remaining protein (Sucher et al. 1974).

10.2.4.2.4 Mineral-Enriched Yeast

Yeasts grown in a medium containing high levels of metallic cations such as chromium, selenium, germanium, or zinc tend to accumulate these metals intracellulary (Halasz and Lasztity 1991; Reed 1981). Owing to their important biological functions as cofactors in enzyme reactions and in other intracellular proteins such as in the case of the chromium-containing glucose tolerance factor (GTF), the use of yeasts cells fortified in these metals as food supplements has gained ground (Halasz and Lasztity 1991; Reed 1981).

10.2.4.2.5 Membrane Lipids and Extracts

Yeast-derived lecithin, inositol, choline, glycerol, and glycolipids can be recovered from the cell membrane lipid extracts or from fermentation broth (Bednarski et al. 2004; Halasz and Lasztity 1991; Harrison 1970). Owing to their physical attributes the use of these components has long been established as flavours in foodstuffs as emulsifiers and surfactants. Owing to their additional health benefits, these components can also be used to fortify foods as food supplements. A commercial process to produce glycerol by fermentation using a strain of *C. glycerinogenes* has been developed and genetically engineered strains of *S. cerevisiae* that can overproduce glycerol or inositol have been reported (Omori et al. 1995; Zhuge et al. 2001).

10.2.4.2.6 Vitamin-Enriched Yeast

Yeast cells are good sources of a number of the B vitamins such as thiamine, pantothenic acid, riboflavin, vitamin B_6, and vitamin B_{12} (Halasz and Lasztity 1991; Harrison 1970; Peppler 1970; Reed 1981). Yeast cells are also good sources of biotin, folic acid, and of ergosterol, which can be converted by UV radiation to vitamin D_2 (Reed 1981). In addition to vitamins, yeasts are also a good source of coenzyme Q.

10.2.4.2.7 Single-Cell Proteins or Oils

A number of other whole-cell yeast products in use consist of use as a single-cell protein or single-cell oil primarily in animal feed applications where yeasts are grown on food processing waste streams from grain, oilseed, candy, brewery, dairy, and wood processing and vegetable oil refinery by-products (Farid and Azar 2001; Ghazal and Azzazy 1996; Halasz and Lasztity 1991; Harrison 1970; Papanikolaou et al. 2002; Peppler 1970, 1979; Reed 1981). Owing to regulatory issues and finished product quality consistency issues, these products have had a limited direct application in foodstuffs.

10.2.4.2.8 Yeast Enzymes

Intracellular yeast enzymes can be prepared from whole yeast cell mass by mechanical disruption and other means. These enzymes have found several food uses (Harrison 1970; Halasz and Lasztity 1991; Mosiashvili et al. 1971; Peppler 1979; Reed 1981). Examples of these are the use of yeast invertase obtained from *S. cerevisiae* and other sucrolytic food yeasts in the confectionary industry to break down sucrose to manufacture liquid-centre candies (Halasz and Lasztity 1991; Reed 1981). Yeast lactases obtained from the GRAS milk sugar fermenting yeast genus of *Kluyveromyces* are used to hydrolyse milk sugar or cheese whey to prepare sweeter sugar slurry. Baker's yeast ribonuclease for is used for RNA digestion during the manufacture of yeast nucleotides. Most recently yeast cytosolic oxidoreductases of brewer's yeast have been used to block Maillard reaction of dicarbonyl intermediates, thereby preventing their decomposition to off-flavour final products (Halasz and Lasztity 1991; Reed 1981; Sanchez et al. 2003).

10.2.4.3 Yeast Beverage and Food Flavour Products

Yeast and yeast metabolic products contribute to the formation of unique distinctive flavours through the formation of complex chemical substances and through other physical interactions with food and beverage matrices in several fermented foods. These reactions involve numerous components that are not fully characterized and contribute to a yeasty character that is frequently used to identify yeast-fermented and/or yeast-containing product.

10.2.4.3.1 Bread and Baked Products

Among these many products are crusty baked breads that are characterized by intense roasted odorant flavours. These flavours are attributed in great part to bread dough composition and preparation methods as nonfermented doughs lack the desired bread taste (Schieberle 1990). It is currently recognized that bread flavour is affected by a great number of compounds produced during fermentation and during baking which include alcohols, diacetyls, esters, organic acids, and carbonyl and other compounds (Annan et al. 2003; Imura et al. 2003; Watanabe et al. 1990). A recent review with 58 references covered many attributes of bread production and included commercial formulations of bakers' yeast, yeast production practices, yeast metabolism and nutrition, application of yeast to bread making, leavening activity, and taste and flavour of yeast-leavened bakery products (El-Dash 1969; Randez-Gil et al. 1999; Van Dam 1986). In this review it is indicated that at least 211 different compounds have been identified in baked breads (Van Dam 1986). Baker's yeast contributes significantly to the formation of these flavour compounds in doughs and breads and these include the alcohols ethanol, propanol, butanol, butan-2-ol, pentanol, pentan-2-ol; the organic acids acetic and lactic; the carbonyl compounds acetaldehyde, propanal, butanal, pentanal, and furfural; and browning reaction products such as melanins and caramels that are concentrated in the bread crust (Van Dam 1986). Several detailed studies have indicated that bakers' yeast is an important source of Maillard-type bread-flavour compounds (Schieberle 1990). The most important odorants present in nonheated

yeast/sucrose and heated homogenates were determined and these consisted in nonheated homogenates, of the odorants 2- and 3-methylbutanoic acid, furaneol, butanoic acid, 2-methylpropanoic acid, 4-vinyl-2-methoxyphenol, and phenylacetaldehyde. After heating the homogenate, 2-acetyl-1-pyrroline (ACPY), methional, GC10, and γ-dodecalactone, followed by 2-acetyltetrahydropyridine (ACTPY), 3-methylbutanol, and 2,5-dimethyl-3-ethylpyrazine became the predominant odorants. A comparison with the primary odorants of wheat bread crust revealed that the yeast is a potent source of the important crust odorants ACPY, methional, ACTPY, and furaneol. Model experiments carried out further to determine the source of these crust odorants showed that ACPY and ACTPY are formed in yeast from the reaction of proline with 2-oxopropanal. The importance of sulphur-containing compounds in enhancing the roasty notes of bakery products is confirmed by the use of the beef broth flavour compound 2-acetyl-2-thiazoline and other sulphur-containing compounds (Bel Rhlid et al. 1999).

The role played by higher alcohols in bread flavour has been demonstrated through the selection of isobutyl and isoamyl alcohols overproducing mutants resistant to 4-aza-DL-leucine from a bakery yeast strain of *S. cerevisiae* (Watanabe et al. 1990). Many mutants that produced more isobutyl alcohol or isoamyl alcohol than the parent strains were obtained. In the evaluation of these mutants, bread containing more isobutyl alcohol was evaluated as giving a favourable characteristic flavour, but bread with more isoamyl alcohol was unfavourable. These mutants were able to ferment dough at similar rates to commercial bakers' yeasts. The mutants overproducing isobutyl alcohol or isoamyl alcohol were released from inhibition of the key enzymes acetohydroxy acid synthase and α-isopropylmalate synthase, respectively, in the pathway of branched-chain amino acid synthesis (Fukuda et al. 1990b; Watanabe et al. 1990).

Baker's yeast has also been demonstrated to contain chemoenzymatic synthetic capability through the synthesis of the aroma active 5,6-dihydro- and tetrahydropyrazines from the aliphatic acyloins that it produces (Kurniadi et al. 2003). The published work described the generation of 25 acyloins by biotransformation of aliphatic aldehydes and 2-ketocarboxylic acids using whole cells of baker's yeast as a catalyst. Six of these acyloins were synthesized and tentatively characterized for the first time. Subsequent chemical reaction with 1,2-propanediamine under mild conditions resulted in the formation of 13 5,6-dihydropyrazines and six tetrahydropyrazines. Their odour qualities were evaluated, and their odour thresholds were established. Among these pyrazine derivatives, 2-ethyl-3,5-dimethyl-5,6-dihydropyrazine (roasted, nutty, 0.002 ng/L air), 2,3-diethyl-5-methyl-5,6-dihydropyrazine (roasted, 0.004 ng/L air), and 2-ethyl-3,5-dimethyltetrahydropyrazine (bread crust-like, 1.9 ng/L air) were the most intensive-smelling aroma-active compounds (Kurniadi et al. 2003). While bread making is one of the oldest food-manufacturing processes, it is only in the past few years that recombinant-DNA technology has led to dramatic changes in formulation, ingredients, or processing conditions. New strains of baker's yeast that produce CO_2 more rapidly, are more resistant to stress, or produce proteins or metabolites that can modify bread flavour, dough rheology or shelf life are now available (Randez-Gil et al. 1999).

10.2.4.3.2 Beer Flavouring

The liquid substances recovered following ethanol removal from beers produced with malts and other grains have found many uses in foods and alcoholic beverages where natural flavours or bioflavours are sought (Vanderhaegen et al. 2003; Ziegler 1972). Recent growth in the use of bioflavouring agents has been fuelled by the increased demand of consumers for natural products that do not pose health or environmental disposal risks (Vanderhaegen et al. 2003). These bioflavouring substances can be produced from beer refermentation using nonconventional yeasts or genetically engineered yeasts that impart unique or additional flavours. In another variation, spent yeast cells from stillage from the production of spirits are reslurried and combined in an aqueous malty fermentation medium that has been treated with enzymes to saccharify the sugars (Vanderhaegen et al. 2003). Where a colourless flavoured malt beverage is desired, enzymes are added first to malt water slurry to convert the nonfermentable sugars in malt (Word et al. 1994). The mash is heated and the liquid extracted and combined with a fermentable carbohydrate to yield a mixture which is boiled and inoculated with yeast. The resulting yeast-fermented product is decolorized to produce a clear and colourless base, which is combined afterwards with a sweetener, tartaric acid, a buffer, and a flavouring agent, followed by carbonation, until the product contains CO_2 in the range 0.48–0.57% by weight. The final product is clear and colourless, has a finished alcohol-to-extract weight percent ratio of 1:0.4–1.5, and is relatively low caloric (8–15 cal/oz). The selected parameters serve to minimize consumer sensations of fullness and excess tartness, sweetness, and astringency, while producing desirable taste characteristics and an attractive appearance.

10.2.4.3.3 Cheeses

When freshly made, most cheeses have little flavour and are often bland (Heath 1981; Moskowitz 1980). It is during ripening that distinctive flavours develop in cheese from the degradation of carbohydrates, proteins, and fats (Heath 1981). These reactions proceed until equilibrium is reached under the conditions of aging employed (Heath 1981). Cheese flavour compounds include a number of hydrocarbons, alcohols, carbonyls, acids, esters, lactones, sulphur-containing compounds, amines, and other miscellaneous flavour components. Yeast flora of dairy products, yeasts included in starter cultures with bacteria and/or moulds, and yeast-derived extracts all contribute to the flavour of a variety and types of cheeses and other fermented dairy products. The yeast flora of dairy products is diverse and includes *Debaryomyces hansenii*, *Geotrichum candidum*, *Y. lipolytica*, *K. lactis*, *C. zeylanoides*, *C. lipolytica*, *C. mycoderma*, *D. kloeckeri*, and *C. lambica* (Anderson and Day 1966; Arfi et al. 2003; Chang et al. 1972; Fadda et al. 2004; Hosono and Tokita 1970; Kalle and Deshpande 1977; Martin et al. 2001; Petersen et al. 2002). The yeast floras of artisanal Fiore Sardo cheese, of a cheese curd, a processed cheese, cheeses produced primarily with yeast, of Limburger cheese, and blue and Roquefort cheese provide examples of the contribution of yeasts to cheese flavour development during ripening (Anderson and Day 1966; Chang et al. 1972; Fadda et al. 2004; Hosono and Tokita 1970; Kalle and Deshpande 1977; Martin et al. 2001). *K. lactis* produced large amounts of alcohols, aldehydes, esters, and terpenes when cultured alone or in association with the mould *G. candidum* and especially *G. candidum* strain G3, and

generated the largest amount of sulphides when cultured alone or in association in cheese curd (Martin et al. 2001). Some of the yeasts tested were shown to metabolize the milk sugar lactose, are tolerant to 3–7% NaCl, grow on lactic acid, a product of bacteria used in starter cultures, and are frequently proteolytic and/or lipolytic (Anderson and Day 1966; Chang et al. 1972; Fadda et al. 2004; Hosono and Tokita 1970; Kalle and Deshpande 1977; Martin et al. 2001). Cheese ripened mainly with *K. fragilis* contained upon analysis four aldehydes, four methyl ketones, seven alcohols, five esters and dimethyl sulphide after 3 weeks (Chang et al. 1972). The amounts of carbonyl compounds were rather small when compared with those for various cheeses of other types but the production of methyl ketones, alcohols, and esters was enhanced by the use of this yeast. The concentrations of ethanol, isoamyl and active amyl alcohol, and ethyl acetate were especially high and these compounds were considered to be responsible for the characteristic flavour of yeast-ripened cheese. *C. mycoderma* and *D. kloeckeri* grew in the presence of 3 and 7% NaCl, respectively, in Limburger cheese and produced relatively high proteolytic activities (Hosono and Tokita 1970). H_2S, volatile fatty acids, and volatile carbonyl compounds were produced by both *C. mycoderma* and *D. kloeckeri*, although the amounts of these volatiles differed. In comparison with the volatiles produced by *Brevibacterium linens*, the kinds and amounts of the volatiles produced by these yeasts were generally limited. These yeasts appear to play an important role in the development of flavour in Limburger cheese (Hosono and Tokita 1970). Yeasts associated with Blue cheese are capable of reducing methyl ketones to secondary alcohols and may play a role in flavour development by producing ethanol, other alcohols, and certain esters. Methyl and ethyl esters and 2-phenylethanol produced by yeasts are important in Blue cheese flavour. Quantitative data on the C3, C5, C7, C9, and C11 methyl ketones in Blue and Roquefort cheese showed considerable variation among samples, but no consistent differences between Blue and Roquefort cheese. This is in contrast to the selective conversion of the C8:0 fatty acids and, to a lesser extent, the 6:0 and 10:0 fatty acids to methyl ketones during cheese curing. The C5, C7, and C9 secondary alcohols were measured in Blue cheese by gas chromatography using the methyl ketones as internal standards. The alcohols were present in lower concentrations than the ketones. Ribonucleotides have been shown to contribute to the taste and/or the aroma and/or the mouthfeel of a low fat spread cheese that resembles the taste and/or the aroma and/or the mouthfeel of the full fat spread cheese (Noordam and Kortes 2004).

10.2.4.3.4 Cocoa Flavours

The yeast flora associated with the fermentation of cocoa bean pods in the fields of the countries of origin contributes to the early development of cocoa flavours (Schwan and Wheals 2004). The first stage of chocolate production consists of a natural, 7-day microbial fermentation of the pectinaceous pulp surrounding beans of the tree *Theobroma cacao*. There is a microbial succession of a wide range of yeasts, lactic acid bacteria, and acetic acid bacteria that carry out a fermentation during which high temperatures of up to 50°C are reached. The physiological roles of the predominant microorganisms are now reasonably well understood and the crucial importance of a well-ordered microbial succession in cocoa aroma has been

established (Schwan and Wheals 2004). During the fermentation process in which the pulp is broken down to release the cocoa beans, the microbial flora produces a number of enzymes which include oxidases, a number of glycohydrolyases, peptidases and proteases, lipases and other esterases, and compounds such as alcohols, the volatile organic acid acetic acid, lactic acid, ketones, amino acids, fatty acids and fatty acid esters, and hydrocarbons that alter the colour of the beans to brownish red and contribute to the elimination of the bitter taste associated with the tannins (Heath 1981). Most of these compounds are removed in the drying step which follows which inactivates all microbial and cocoa bean enzymes and removes all gumminess contributed by polysaccharides, thereby resulting in a stable, dry, brittle product which is traded as a commodity worldwide (Heath 1981). At the processing facilities the cocoa beans are heated to produce the roasted aroma characteristic of cocoa powders and chocolate that is attributed to the aldehyde products from oxidative deamination of amino acids and from the formation of pyrazines, which are the primary Maillard reaction products (Heath 1981; Rasmussen and Bach 1996; Maga 1982, 1992). The roasting step is followed by dehulling and removing of the cocoa bean shell (Heath 1981; Rasmussen and Bach 1996). The nibs recovered are ground and subjected to heating in alkali to form a rich liquor high in cocoa butter. In the following step, the cocoa butter is removed by high-pressure presses and the pressed cakes are recovered, and ground to form cocoa powders that are dried and blended to customer-desired colour and taste specifications.

Ingredient cost consideration and limited availability of cocoa beans has generated interest in cocoa powder extenders and replacers. Blends of spray-dried malted barley and roasted barley or other roasted grains are used in some cases at a rate of 35–50% as a replacement for cocoa powders (Heath 1981). In other cases, the stillage and yeast recovered from malt spirits is recovered following distillation of ethanol and other fusel oils and concentrated by evaporation followed by spray-drying to produce cocoa powder replacements. Cocoa-substitute compounds can also be produced from a number of yeast species such as *S. cerevisiae*, *S. carlsbergensis*, *C. utilis*, *C. tropicalis*, or *Brettanomyces* genera that are preferably propagated on hop-containing or non-hop-containing media and combined with a reducing sugar in an aqueous slurry and heated to high temperature under pressure. Alternative processes that use low-moisture yeast cell preparations that are obtained following spray-drying are heated to elevated temperatures with dry heat to over 250°C in an oven to produce a desired product with a roasted flavour (Liggett 1978). More recently, genetic engineering approaches have been used to clone and produce cocoa-flavour precursor peptides in yeasts or bacteria that when mixed with amino acids and saccharides in the fermentation production medium and heated from 100 to 200 ‘C for 1–60 min can result in the production of cocoa flavours that can be used in food, pharmaceutical, or cosmetic products (Rasmussen and Bach 1996). The lipid extracts from oleaginous yeast have been analysed and evaluated for use as cocoa butter equivalent. The commercial use of oleaginous yeast to produce cocoa butter equivalent while attractive remains untapped (Ratledge 1997).

10.2.4.3.5 Fermented Edible Meats and Edible Meat Flavours

The production of meat flavours from products prepared from yeast extracts has been covered extensively (Van Pottelsberghe de la Potterie 1972) in patents and in a

number of publications (Halasz and Lasztity 1991; Huynh-Ba et al. 2003; Nagodawithana 1992; Peppler 1979; Reed 1981). The use of yeasts in the starter cultures to prepare fermented yeast products, yeast extracts for their flavours, or to support growth of microbial flora used to ferment meats such as sausages or products derived thereof, or other whole-cell or cell wall yeast has been described (Bolumar et al. 2003; Durá et al. 2002; Encinas et al. 2000; Samelis et al. 1994). Meaty flavour or notes can be achieved in non-meat-derived products by processing of aqueous yeast or yeast hydrolysed or non-hydrolysed extracts by treatments that utilize heat in the presence of sugars – monosaccharide or oligosaccharide (preferably xylose or lactose) – with/without other amino acids such as methionine, cysteine, or cystine. The process described produces an improved, durable flavour (Van Pottelsberghe de la Potterie 1972). The sources of carbohydrate can be varied and consist of coffee wastes or other ground plants, oats, rye, or barley. The aqueous medium may also contain a carboxylic acid (lactic, malic, succinic, palmitic, stearic, oleic, or their mixture). After the reaction the medium is evaporated to a paste or converted into a powder. The final formulation consists of hydrolysed plant protein free of cysteine, sodium guanylate, malic acid (or a mixture of lactic, palmitic, and succinic acids), xylose, and water with pH set at 2.5–3.0. This mixture is refluxed for approximately 100 h and concentrated to a paste or to a powder under vacuum. The products possess excellent roast beef aroma, and are suitable for use as food additives.

A number of yeasts contribute significantly to the flavour of fermented meat products and meat-flavoured products and these frequently consist of strains of *D. hansenii* (teleomorph of *C. famata*), *D. kloeckeri*, *Y. lipolytica* (perfect form of *C. lipolytica*), *Citeromyces matritensis* (teleomorph of *C. globosa*), *Trichosporon ovoides* (formerly *T. beigelii*) and several other species of *Candida* (*C. intermedia* or *C. curvata*, *C. parapilosis*, *C. zeylanoides*), *Pichia*, *Cryptococcus*, and *Rhodotorula* (Bolumar et al. 2003; Encinas et al. 2000; Ingram and Simonsen 1980). These yeasts are known to secrete lipases and/or proteases which contribute to flavour by offsetting and modifying the acidic pH produced by mixed bacterial starter culture activities through the degradation of lipids to produce free fatty acids and glyerol and the breakdown of nitrogenous compounds to amino acids with release of ammonia. Several recently published reports describe the isolation and characterization of several such enzymes from *D. hansenii* (Bolumar et al. 2003; Durá et al. 2002). In one of these reports, Bolumar et al. (2003) described the first isolation and characterization of a yeast prolyl and of an arginyl aminopeptidase from *D. hansenii*. In a second report, Durá et al. (2002) described the production of a glutaminase by the same yeast. This yeast is typically dominant in meat fermented products at all stages of sausage manufacturing and is known for its high salt tolerance and its production in addition to proteases, lipases and peroxidase activity. The isolation, characterization, and overproduction of the previously mentoned two groups of enzymes have also been reported for *Y. lipolytica* (Nicaud et al. 2002). Both of these yeasts also contribute to significant flavour development of fermented dairy products such as cheeses.

10.2.4.3.6 Soy-Derived Flavour Products

Soybean products are characterized by a mealy and fatty flavour that is bland (Heath 1981; Kinsella and Damodaran 1980). By comparison yeast-fermented soy

products such as miso prepared from soybean paste or other fermented miso products from rice or barley, soy sauce (shoyu), and other fermented soybean protein hydrolysates have complex flavour and aroma profiles (Hamada et al. 1991; Komai et al. 1987; Kumari and Singh 1990; Sarkar et al. 1994; Sasaki 1996). These flavour and aroma profiles vary with the methods used to prepare the raw materials and other ingredients included, microbial flora used, and the ripening or aging process employed. In the production of soy sauce the high concentration of sodium chloride (more than 12% w/v) used favours the growth of salt-tolerant yeast, such as *Z. rouxii*, *Torulopsis etchellsii*, and *C. versatilis* or *Torulopsis versatilis* (Chien 1974; Dahlen et al. 2001; Halasz and Lasztity 1991; Oro 2001; Sugawara 2001). Other less tolerant yeast genera can be used provided that the initial NaCl level is reduced (below 5% w/v) and these include *S. cerevisiae*, *Hansenula* sp., and *Pichia* sp. (Kayahara et al. 1980; Suezawa et al. 2003; Taiyoji and Watanabe 2002). Industrial yeast strains with improved soy sauce aroma and flavour have been developed and used commercially (Kusumegi et al. 1992; Lee and Kim 1993). The salt-tolerant yeasts used in soy sauce production elaborate a number of flavour compounds which includ: 4-hydroxy-2 (or 5)-ethyl-5 (or 2)-methyl-3 (2*H*)-furanone (HEMF), 2,5-dimethyl-4-hydroxy-3(2*H*)-furanone (DMHF), 3-methyl-1-butanol, and volatile alkyl phenolics such as 2-phenylethanol and 4-ethyl guaiacol (4-EG). HEMF has a strong sweet cake-like aroma with a threshold value of less than 0.04 ppb (Sugawara 2001). HEMF is a strong antioxidant that has been shown to exert an anticarcinogenic effect on benzo[α]pyrene-induced mouse forestomach neoplasia (Sugawara 2001). It is also effective in preventing radiation hazards and has an important physiological function as well as being an aroma component (Sugawara 2001). HEMF was formed during the cultivation of yeast by using a precursor of HEMF which may have been produced by the amino-carbonyl reaction of pentose with amino acids during heating (Sugawara 2001). DMHF is found in many fruits such as strawberries and pineapple (Dahlen et al. 2001). While dilute solutions of DMHF exhibit a strawberry or pineapple-like flavour, in the concentrated form DMHF solutions have a caramel-like aroma (Dahlen et al. 2001). DMHF is a secondary metabolite that is produced by the soy-sauce-fermenting yeast *Z. rouxii* after the addition of D-fructose-1, 6-diphosphate to YPD nutrient media (Dahlen et al. 2001). DMHF concentrations in the range 5–10 g/L have been shown to partially and completely inhibit the growth of *Z. rouxii* cells (Dahlen et al. 2001). While 4-EG is an important aroma in soy sauce, when present in excess over several milligrams per litre it gives an off-odour (Oro 2001). A process for continuous production of 4-EG by the salt-tolerant *C. versatilis* in an airlift reactor has been reported (Hamada et al. 1990, 1991). In this process, large amounts of 4-EG(more than 20 ppm) were produced by immobilized yeast cells for up to 40 days with 1–3 ppm of 4-EG, which is the optimal level in conventional soy sauce, produced within 0.5 h (Hamada et al. 1990). Good soy sauce flavour profiles were reported recently with the salt-tolerant yeasts *C. versatilis* and *Z. rouxii* immobilized on a poly(ethylene oxide) resin in a continuous stirred reactor (Van der Sluis et al. 2001). The production of a soy sauce with quality comparable to that produced by the conventional method was demonstrated in a controlled fermentation process using a genetically modified strain of the red yeast *C. versatilis* in which an inducible ferulic acid

decarboxylase was deleted and replaced with a gene coding for a constitutive enzyme (Suezawa et al. 2003).

The selection and use of improved strains of *Z. rouxii* for production of miso from barley, soybean, wheat, or non-salted rice has been reported (Kasumegi et al.1997, 2001; Kayahara et al. 1980; Matsuda and Yamamoto 1999; Taiyoji and Watanabe 2002; Yoshikawa et al. 1990). Mutants of *Z. rouxii* produced more than twofold aromas of higher alcohols such as isoamyl alcohol, propyl alcohol, isobutyl alcohol, butyl alcohol, and/or β-phenethyl alcohol (Kasumegi et al. 1997, 2001, Kayahara et al. 1980; Matsuda and Yamamoto 1999). The use of wine yeast strains that produce ethanol in the presence of high levels of sodium chloride, yielded miso flavour profiles that are characteristic of the wine yeast used (Kawamura and Kawano 1999). The effect of ethanol addition on the formation of fatty acid esters from the degradation of glycerides present in raw materials that are usually formed during miso fermentation was investigated (Ohnishi 1983). It was found that the ratio of ethyl fatty acid esters formed was determined by the level of ethanol present. This suggests that the production of ethanol and other higher alcohols by yeasts is not only responsible for the production of aroma substances but also for the rate at which lipids are metabolized and for flavour development during miso fermentation (Ohnishi 1983). At reduced salt concentration during the production of non-salted rice miso by *Z. rouxii*, ethanol addition (4%) at 45°C leads to increased sugar and protein metabolism, resulting in bitter flavour and light colour development (Taiyoji and Watanabe 2002).

10.2.4.3.7 Other Fermented Foods and Beverages

Yeasts are also responsible for the complex flavours of many other raw materials, foods, and beverages which include a great number of distilled spirits, other fermented fruit and cereal drinks such as wines, ciders, and sake (Fukuda et al. 1998a, b; Furukawa et al. 2003a, b; Lambrechts and Pretorius 2000; Nykanen 1986; Romano et al. 1999), fermented syrups, and caramel or almond flavours such as salicyl flavour aldehyde produced by the methylotrophic yeast *C. methanolovescens* (Van den Bremt et al. 2000, 2001), fermented dairy products such as kefir and yogurt, and other foods characterized by roasted and fragrant flavours which include tea, vanilla beans, and coffee. Some of the yeasts used in some of these applications are listed with the food type in Table 10.4.

10.2.5 Vitamins

Owing to their ability to incorporate ingredients present in fermentation media, yeast cells are an important source of proteins, vitamins, and minerals (Halasz and Lasztity 1991; Mosiashvili et al. 1971). Among the vitamins and other enzyme cofactors that are accumulated and/or synthesized by yeast are thiamine (vitamin B_1), nicotinic acid containing enzyme cofactors NAD and NADP and their reduced forms, pyridoxine (vitamin B_6), pantothenic acid, or CoA precursor (Peppler 1967), cyanocobalamin or vitamin B_{12}, biotin, folic acid (pteroylglutamic acid) or folacin, and riboflavin or vitamin B_2 (Drewek and Czarnocka-Roczniakowa 1986; Halasz and Lasztity 1991; Oura and Suimalainen 1982). Examples of these are the yeast *Kloeckera apiculatas*, which

Table 10.4 Yeast used in main fermented foods

Fermented food	Yeast employed in process
Alcoholic beverages	*Saccharomyces cerevisiae* and other species; *Schizosaccharomyces* (rum)
Breads and cakes	*S. cerevisiae* and *S. exigus*; *Candida krusei* and *C. tropicalis*; *Pichia* and *Hansenula anomala*
Beers	*S. cerevisiae* and *S. uvarum* (ex *S. carlsbergensis*)
Ciders	*S. cerevisiae* and *S. uvarum*; *Hansenisapora valbyensis*; *Metschnikowia pulcherrima*
Coffee	*S. cerevisiae*
Cocoa	*C. krusei*, *C. famata* and *C. holmii*;
Fermented food	Yeast employed in process *P. membranaefaciens*, *S. chevalieri*
Fermented milks	*S. cerevisiae* and *C. kefir* (for kefir)
Fresh, semihard, pressed, or mould cheeses	*Kluyveromyces* sp., *Debaryomyces hansenii*, *S. unisporus*, *Candida* sp., *Pichia* sp., *Yarrowia lipolytica*, *Clavispora lusitaniae*, *Trichosporoninkin*, *Torulospora delbrueckii*
Fermented meat products	*D. hansenii*, *Candida* sp., *Rhodotorula rubra*
Wines	*S. cerevisiae*

Modified from Cristiani and Monnet (2001)

is known to accumulate thiamine up to a tenth of its dry weight, whereas some strains of the yeasts *S. cerevisiae* and *S. uvarum* (*carlsbergensis*) during ethanol production can release thiamine following treatment with UV light (Halasz and Lasztity 1991). *S. cerevisiae* is also the principal source of CoA and the first important commercial preparations of this coenzyme were made from this yeast (Peppler 1967). Some strains of *S. carlsbergenesis* have also been shown to contain vitamin D_2 and vitamin D_3 as well as 25 hydroxy forms of these two vitamins (Halasz and Lasztity 1991). Ergosterol, a precursor to the aforementioned forms of vitamin D, is an important constituent of yeast cell membrane lipids (Fazekas and Sebok 1959; Tanaka et al. 1971; Xue et al. 2002). Increasing yield of ergosterol in yeast cultures was shown to be influenced by the addition of vitamin B_1 and thioglycolic acid to enhance cell mass in aerated cultures (Fazekas and Sebok 1959). It was concluded that the combined addition of vitamin B_1 and thioglycolic acid enhances sterol production in the cells, most likely by the conversion of pyruvic acid to sterols (Fazekas and Sebok 1959). The cultural conditions for ergosterol production by yeasts were also investigated by using an *n*-alkane mixture as the sole carbon and energy source (Tanaka et al. 1971). Among the yeasts tested, several strains of *C. tropicalis* were shown to produce a relatively large amount of ergosterol (Tanaka et al. 1971). *n*-Alkanes with carbon numbers that range from C10 to C14 and from C17 to C18 were good substrates for ergosterol production by this yeast. The addition of a nonionic detergent (0.02%) and a natural nutrient (0.1%) was also effective. Aeration and the pH of the medium also affected the production of ergosterol. Under the optimal conditions employed, a strain of *C. tropicalis* designated as pK 233 produced ergosterol in a yield of approximately 71 mg/L of broth or

5.8 mg/g of dry cells after 5-day cultivation in a medium containing a mixture of *n*-decane to *n*-tridecane as the sole carbon sources. More recently, a method for transforming ergosterol into vitamin D_2 in yeast was described by Xue et al. (2002). In this method UV irradiation of ergosterol containing yeast grown under optimal conditions with molasses as the carbon source resulted in cells that contain 15,000 IU/g (Xue et al. 2002). Clinical observations showed that the yeast cells generated have good preventive and curative effects for rickets (Xue et al. 2002). Other forms of lipid-soluble vitamins and their precursors that are present in yeast are the vitamin E tocopherols, and vitamin A and its precursor, β-carotene.

In addition to the B vitamins consisting of B_1, B_6, and B_{12}, yeasts are also important sources of flavins derived from riboflavin or vitamin B_2, the water-soluble vitamin C or ascorbic acid (Hancock et al. 2001), and multiple forms of coenzyme Q. The production of yeast cell mass with a predetermined level of vitamin B_1 potency was demonstrated by the incorporation and assimilation of vitamin B_1 from a water-clear vegetable extract with a sugar source provided as the carbon source and used as a growth medium (Gorcia and Levine 1942). Improvement of the biological synthesis of the vitamin B_1 in yeast was shown through the incorporation of vitamin B_1 precursors into the fermentation medium (Harrison 1942). The addition of a suitable thiazole derivative, such as 4-methyl-5-β-hydroxyethylthiazole, and a pyrimidine derivative containing a cyano radical, such as 2-methyl-5-cyano-6-aminopyrimidine, in equimolar quantities to the fermenting mash, preferably toward the end of the process under vigorous aeration at 28–30°C at a pH of 5.5–6.0, resulted in the formation of a methylene linkage between the pyrimidine ring and the nitrogen of the thiazole. Other ethylthiazole derivatives can be used in the aforementioned reaction that yielded vitamin B_1 as a product (Harrison 1942). The production of vitamin B_1 for use in beverages and in pharmaceutical applications was described by Silhankova (1978). In this patent, a vitamin B_1 producing strain of *S. cerevisiae* designated DBM 159 was shown to produce normal levels of ethanol while producing an elevated level of vitamin B_1 (approximately 10 mg/L) in a molasses-containing medium (Silhankova 1978). The formation of vitamin B_6 by several genera of yeasts was examined in hydrocarbon-containing media with vigorous aeration by Tanaka and Fukui (1967). Among the genera studied of *Candida*, *Rhodotorula*, and *Hansenula*, a strain of *C. albicans* exhibited the excellent vitamin B_6 producing ability (300–400 μg/L) in a synthetic medium containing hexadecane as the sole carbon source. The addition of corn steep liquor and an appropriate nonionic detergent, such as Tween 85 or Span 60, stimulated yeast growth and vitamin B_6 production (Tanaka and Fukui 1967). The cell and vitamin yields increased with the rate of aeration. In a medium containing glucose as the carbon source, the vitamin B_6 formed was secreted into the fermentation broth prior to achieving maximum cell density. In cells grown with hydrocarbons as the sole source of carbon, vitamin B_6, mainly in the form of pyridoxal phosphate, accumulated inside the cells (Tanaka and Fukui 1967).

The formation of coenzyme Q, cytochrome c, and flavins by yeasts grown on mixed hydrocarbons was studied using several strains of *Candida* and *Pichia* by Teranishi et al. (1971). These strains included a strain of *C tropicalis* designated as pK 233. The time-course changes in the coenzyme Q, cytochrome c, and flavine contents in the yeast cells were studied during growth on hydrocarbons. The effects of medium

constituents, additional purines, and detergents on flavine production and the type of flavines produced were also investigated. *C. tropicalis* pK 233 was shown to grow well on C9–C14 alkanes under the conditions employed. Undecane was most suitable for cell growth and flavine production. A hydrocarbon mixture of four alkanes which was enriched in *n*-undecane was also suitable as the sole carbon source. Several nitrogen sources, namely NH_4NO_3, $(NH_4)_2SO_4$, and $NH_4H_2PO_4$ were all shown to be effective for flavine production by this yeast. The amount of flavines produced by *C. tropicalis* pK 233 was about 5 mg/L of broth under the conditions employed. The time-course study suggested an interesting correlation between the intracellular contents of coenzyme Q, cytochrome c, and flavines during growth (Teranishi et al. 1971).

The production of protein–vitamin concentrates with yeasts in media containing lipids as the carbon source was described by Szechenyi et al. (1973). Media containing lipids such as grease, lard, rancid and waste fats, or tallow, that are emulsifiable at 30–40°C, were fermented with yeasts (*C. guilliermondii*, *C. utilis*, *S. fragilis*, etc.) in the presence of NH_4^+ salts and (or) urea at pH 4.5–5.0. The cell mass recovered from the fermentation broth had high protein (more than 50%) and vitamin content, which included vitamins B_2, B_6, and B_{12}, nicotinic acid, and pantothenic acid. The cell mass was useful as a food or feed additive without considerable purification. Thus, a medium containing animal-derived lipids or their by-products was shown to yield a 75% dry yeast (based on the lipid consumed) that contained greater than 50% protein, 7% lipids, and 6% ash, as well as vitamin B_1 greater than or equal to 3 mg/100 g, vitamin B_2 greater than or equal to 5 mg/100 g, pantothenic acid greater than or equal to 20 mg/100 g, vitamin B_6 greater than or equal to 1.5 mg/100 g, and ergosterol at a concentration greater than or equal to 3 mg/100 g.

The increased demand for vitamins and enzyme cofactors as food additives and supplements has stimulated research to increase the content of these important components in yeasts intracellularly and in some cases as secreted extracellular products. The development of yeast strains with increased vitamin and enzyme cofactors using classical strain selection and/or genetic engineering has resulted in the development of commercial processes for the production of vitamin B_2, riboflavin (Dmytruk et al. 2004; Lim et al. 2001; Protchenki et al 2000; Stahmann et al. 2000; Voronovsky et al. 2002, 2004). The *C. famata* (*D. hansenii*) strains described are among the most flavinogenic microorganisms developed so far and unlike strains of *Pichia* (*Candida*) *guilliermondii* which concentrate vitamin B_2 intracellularly secrete riboflavin into the growth medium.

The genetic engineering of strains of *S. cerevisiae* that can synthesize the fat-soluble vitamins E and A and its precursor, β-carotene, has been described in great detail in a recent patent application (Millis et al. 2000). The cited patent describes the production of the previously mentioned vitamins by fermentative biosynthesis of intermediates using genetically engineered microorganisms followed by chemical synthesis (Millis et al. 2000). The invention provides methods of producing vitamin E (α-TOH and γ-tocopheryl esters), vitamin A (retinol), or β-carotene. The methods comprise using a biological system with enhanced synthesis of farnesol or geranylgeraniol intermediates to shift microbial metabolism away from sterol biosynthesis via genetic inactivation of the squalene synthase ERG9 gene or by inactivation of squalene synthase by zaragozic acid in a strain with a functional ERG9 gene.

Geranylgeraniol biosynthesis is further enhanced in strains overexpressing any of four different cloned geranylgeranyl pyrophosphate synthase genes from several microbial and plant sources, and these include (1) BTS1 gene from *S. cerevisiae*, (2) crtE gene from *Erwinia uredovora*, (3) a1-3 gene from *Neurospora crassa*, and (4) ggs gene from *Gibberella fujikuroi*. The overexpression of the hydroxymethyl-CoA reductase and/or the ERG20 gene which encodes farnesyl pyrophosphate synthase in *S. cerevisiae* also leads to enhanced biosynthesis of fermentative intermediates. The overexpression of multiple isoprenoid pathway genes or an alternative pathway (Rohmer pathway) was investigated in strains that have an erg9 mutation and elevated levels of hydroxymethylglutaryl-CoA reductase. Further chemical conversion of the fermentation products, farnesol or geranylgeraniol, into α-TOH, γ-tocopheryl ester, vitamin A, or β-carotene was described in some detail (Millis et al. 2000).

The genetic engineering of yeast strains that can synthesize the water-soluble vitamin C or ascorbic acid is described in another recent patent application (Berry et al. 1999). The synthesis of vitamins D_2 and D_3 from ergosterol by *S. cerevisiae* and *C. tropicalis* has long been described in some detail in a number of publications (Fazekas and Sebok 1959; Rao and Raghuntha 1942; Subbotin et al. 1974; Tanaka et al. 1971; Xue et al. 2002). The accumulation and secretion of thiamine or vitamin B_1 in beer wort following treatment of *S. cerevisiae* and *S. carlsbergenesis* has been confirmed at the laboratory and pilot-plant scale and the production of thiamine from its precursors by these two yeasts has been recognized for some time (Bakhchevanska et al. 1984; Popova et al. 1982; Silhankova 1978, 1985). The potential use of several yeasts for production of the vitamin B_{12} has been assessed and *Z. bailii* and fodder yeast were recognized as promising yeasts for its production on industrial media and agricultural residues (Bykhovskii et al. 1972; Mosiashvilli et al. 1971; Popova et al. 1971; Trofimenko and Cheban 1970). The production of vitamin B_6 or pyridoxine has been demonstrated on hydrocarbon-containing media with several yeasts, which include several *Candida*, *Rhodotorula*, and *Hansensula* (Tanaka and Fukui 1967).

10.3 Concluding Remarks and Future Outlook

The economic importance of yeasts to the food and beverage industries continues to outweigh all other commercial uses of yeasts. The revived interest in naturally derived products and the increased concerns about products from animal sources has generated renewed interest in food and nutraceutical uses of yeasts. Among the many advantages of yeasts are their ease of production and for several yeast genera the favourable regulatory environment. New strains of yeasts enhanced through genetic breeding to improve production of food ingredients and for use as supplements provide added promise to biotechnologists and consumers alike (Maraz 2002). It is without doubt that the full potential of the commercial value of yeasts has not been fully realized. With new nutraceutical applications under development the 2–4 % projected annual growth in sales for all yeast products will result in markets that exceed US $20 billion by the end of this decade. The continued expansion in the use of *Saccharomyces* and other genera of so-called nonconventional yeasts represents the rise of yeast biorefineries that aim to capture the full potential of these important biocatalysts.

References

Abbas CA (2001) Industrial applications of nonconventional yeast. Plenary presentation abstract, ISSY 2001, Lviv, Ukraine

Abbas CA (2003) Emerging biorefineries and biotechnological applications of nonconventional yeast: now and in the future. In: Jacques KA, Lyons TP, Kelsall DR (eds) The alcohol textbook, 4th edn. Nottingham University Press, Nottingham, UK, pp 171–191

Abbas CA (2004) Harnessing the metabolic repertoire of nonconventional yeast for biotechnological applications. Session presentation abstract, ICY 2004, Rio de Janerio, Brazil

Afanas'ev I, Ostrachovich E, Deeva I, Korkina L (2000) Protective effects of antioxidants on iron-induced free radical-mediated damaging processes in humans and animals. In: Roussel A, Anderson RA Favier AE (eds) Trace elements in man and animal, vol 10. Plenum, New York, USA, pp 115–118

Akita O, Obata T (1991) Breeding of sake yeast producing a large quantity of aroma and brewing fragrant sake. Kagaku To Seibutsu 29(9):586–592

An GH (1996) Characterization of yellow mutants isolated from the red yeast *Phaffia rhodozyma* (Xanthophyllomyces dendrorhous). J Microbiol Biotechnol 6(2):110–115

An GH, Jang BG, Suh OS, Kim CJ, Song KB (2001) Iron(III) decreases astaxanthin production in *Phaffia rhodoxyma* (*Xanthophyllomyces dendrorhous*). Food Sci Biotechnol 10(2):204–207

Anderson DF, Day EA (1966) Quantitation, evaluation, and effect of certain microorganisms on flavor components of blue cheese. J Agric Food Chem 14(3):241–245

Annan NT, Poll L, Sefa-Dedeh S, Plahar W A; Jakobsen M (2003) Volatile compounds produced by *Lactobacillus fermentum*, *Saccharomyces cerevisiae* and *Candida krusei* in single starter culture fermentations of Ghanaian maize dough. J Appl Microbiol 94(3):462–474

Arai S (1980) The bitter flavour due to peptides or protein hydrolysates and its control by bitterness-masking with acidic oligopeptides. In: Charalambous G (ed) The analysis and control of less desirable flavors in foods and beverages. Academic, New York, USA, pp 133–147

Archibald F (2003) Oxygen toxicity and the health and survival of eukaryote cells: a new piece is added to the puzzle. Proc Natl Acad Sci USA 100(18):10141–10143

Arikawa Y, Yamada M, Shimosaka M, Okazaki M, Fukuzawa M (2000) Isolation of sake yeast mutants producing a high level of ethyl caproate and/or isoamyl acetate. J Biosci Bioeng 90(6):675–677

Arfi K, Tache R, Spinnler H E, Bonnarme P (2003) Dual influence of the carbon source and L-methionine on the synthesis of sulphur compounds in the cheese-ripening yeast *Geotrichum candidum*. Appl Microbiol Biotechnol 61(4):359–365

Armstrong DW (1986) Selective production of ethyl acetate by *Candida utilis*. ACS Symp Ser 317:254–265

Asano T, Kawadu M, Kurose N, Tarumi S, Kawakita S (2000) Effect of the FAA1 gene disruption of sake yeast on the accumulation of ethyl caproate in sake mash. J Biosci Bioeng 89(6):609–611

Ashida S, Ichikawa E, Suginami K, Imayasu S (1987) Isolation and application of mutants producing sufficient isoamyl acetate, a sake flavor component. Agric Biol Chem 51(8):2061–2066

Bakhchevanska S, Popova Z, Popova Y (1984) Fermentation of beer wort by vitamin B1 fortified yeast. Plovdiv 31(1):223–231

Bardi L, Cocito C, Marzona M (1999) *Saccharomyces cerevisiae* cell fatty acid composition and release during fermentation without aeration and in absence of exogenous lipids. Int J Food Microbiol 47(1/2):133–140

Bastin P, Dillemans M, Van Nedervelde L, Boivin P, Debourg A (2002) Evaluation of the potent antioxidant effect of *Saccharomyces cerevisiae* extracts during beer production. Cerevisia 27(1):27–32

Becker JVW, Armstrong GO, Van der Merwe MJ, Lambrechts MG, Vivier MA, Pretorius IS (2003) Metabolic engineering of *Saccharomyces cervisiae* for the synthesis of the wine-related antioxidant resveratrol. FEMS Yeast Res 4(1):79–85

Bednarski W, Adamczak M, Tomasik J, Plaszcyk M (2004) Application of oil refinery waste in the biosynthesis of glycolipids by yeast. Bioresour Technol 95:15–18

Bekers M, Krauze I, Zeile M, Viesturs U, Chatskii PA, Kluna G (1972) Carotenoids containing concentrates. Ger Patent Appl DE 70-2059387 19701202

Bel Rhlid R, Blank I, Steen CG (1999) Process for the preparation of flavouring compositions and use of these compositions in bakery products. US Patent 6,432,459

Benítez T, Gasent-Ramírez JM, Castrejón, Codón AC (1996) Development of new strains for the food industry. Biotechnol Prog 12:149–163

Berry A, Running JA, Severson DK, Burlingame RP (1999) Vitamin C (L-ascorbic acid) production in microorganisms and plants genetically engineered for increased sugar epimerase activity. PCT Int Appl WO 9964618 A1 19991216

Berry DR (1989) Manipulation of flavor production by yeast: physiological and genetic approaches. In: Piggott JR, Paterson A (eds) Distilled beverage flavour. Harwood, Chichester, UK, pp 299–307

Berry DR (1995) Alcoholic beverage fermentations. In: Lea AGH, Piggott JR (eds) Fermented beverage production. Blackie, Glasgow, UK, pp 32–44

Bhosale P, Gadre RV (2001a) β-Carotene production in sugarcane molasses by a *Rhodotorula glutinis* mutant. J Ind Microbiol Biotechnol 26(6):327–332

Bhosale P, Gadre RV (2001b) Optimization of carotenoid production from hyper-producing *Rhodtorula glutinis* mutant 32 by a factorial approach. Lett Appl Microbiol 33(1):12–16

Bhosale PB, Gadre RV (2001c) Production of β-carotene by a mutant of *Rhodotorula glutinis*. Appl Microbiol Biotechnol 55(4):423–427

Bogdanovskaya ZN (1973) Effect of several factors of the medium on the growth of feed of yeasts and the synthesis of carotenoids. In: Samtsevich SA (ed) Mikroorganizmy-Produtsenty Biol. Aktiv Veshchestv Nauka I Tekhnika, Minsk, USSR, pp 72–77

Bogdanovskaya ZN, Zalashko MV (1982) Protein–vitamin preparations from peat hydrolysates. In: Lishtvan II (ed) Nov Protessy Prod Pererab. Nauka I Tekhnika., Minsk, USSR, pp 174–179

Bolumar T, Sanz Y, Aristoy MC, ToldráF (2003) Purification and properties of an arginyl aminopeptidase from *Debaryomyces hansenii*. Int J Food Microbiol 86:141–151

Bon JA, Leathers TD, Jayaswal RK (1997) Isolation of astaxanthin-overproducing mutants of *Phaffia rhodozyma*. Biotechnol Lett 19(2):109–112

Bykhovskii VY, Pantskhava ES, Zairseva NI, Pchelkina VV, Fedorov SA, Golubovskii SA, Yarovenko (1972) Vitamin B12 production. USSR Patent 359264 19721203

Buzzini P (2000) An optimization study of carotenoid production by *Rhodotorula glutinis* DBVPG 3853 from substrates containing concentrated rectified grape must as the sole carbohydrate source. J Ind Microbiol Biotechnol 24(1):41–45

Buzzini P, Martini A, Cappelli F, Pagnoni UM, Davoli P (2003) A study on volatile organic compounds (VOCs) produced by tropical ascomycetous yeasts. Antonie van Leeuwenhoek 84(4):301–311

Calo P, de Miguel T, Velázquez JB, Villa TG (1995) Mevalonic acid increases trans-astaxanthin and carotenoid biosynthesis in *Phaffia rhodozyma*. Biotechnol Lett 17(6):575–578

Cang Y, Lu L, Wang G (2002) Antioxidant role of carotenoids in *Rhodotorula sp*. and screening of carotenoid hyperproducing yeasts. Junwu Xitong 21(2):84–91

Cannizzaro C, Rhiel M, Marison I, von Stockar U (2003) On-line monitoring of *Phaffia rhodozyma* fed-batch process with in situ dispersive Raman spectroscopy. Biotechnol Bioeng 83(6):668–680

Chae HJ, Joo H, In MJ (2001) Utilization of brewer's yeast cells for the production of food-grade yeast extract. Part 1: effects of different enzymatic treatments on solid and protein recovery and flavor characteristics. Bioresour Technol 76(3):253–258

Chang JE, Yoshino U, Tsugo T (1972) Cheese ripened mainly with yeast. V. Changes of flavour components in cheese during ripening. Nippon Chikusan Gakkaiho 43(10):561–566

Chao KC, Ridgway JA (1979) Modifying the flavor of yeast. US Patent 4,166,135

Chen B, Liu Z (1999) Study of biosynthesis of carotneoids using *Rhodotorula* sp. 8. Qinghua Daxue Xuebao Ziran Kexueban 39(10):101–103

Cheng Q, Rouviere PE, Tao L (2004) Mutant carotenoid-producing microorganisms and their use in carotenoid fermentation. PCT Int Appl WO 2004056974

Chien HC (1974) Treatment of soybeans with yeast. US Patent 3,810,997

Coghe S, Benoot K, Delvaux F, Vanderhaegen B, Delvaux FR (2004) Ferulic acid release and 4-vinylguaiacol formation during brewing and fermentation: Indications for feruloyl esterase activity in *Saccharomyces cerevisiae*. J Agric Food Chem 52(3):602–608

Combs GF Jr, Clark LC, Turnbull BW (2002) Cancer prevention by selenium. Met Ions Biol Med 7:600–603

Cooper EJ, Peppler HJ (1959) Yeast extraction. US Patent 2,904,439

Cremer J, Vatou V, Braveny I (1999) 2,4-(Hydroxyphenyl)-ethanol, an antioxidative agent produced by *Candida* spp., impairs neutrophilic yeast killing in vitro. FEMS Microbiol Lett 170(2):319–325

Cristiani G, Monnet V (2001) Food micro-organisms and aromatic ester synthesis. Sci Aliments 21(3):211–230

Cruz JM, Dominguez JM, Dominguez H, Parajo JC (1999) Solvent extraction of hemicellulosic wood hydrolysates: A procedure useful for obtaining both detoxified fermentation media and polyphenols with antioxidant activity. Food Chem 67(2):147–153

Dahlen T, Hauck T, Wein M, Schwab W (2001) 2,5-Dimethyl-4-hydroxy-3(2H)-furanone as a secondary metabolite from D-fructose-1,6-diphosphate metabolism by *Zygosaccharomyces rouxii*. J Biosci Bioeng 91(4):352–358

De Baets S, Vandamme EJ (1999) Yeasts as producers of polysaccharides with novel application potential. SIM News 49(6)321–328

del Rio L, Corpas J, Sandalio L, Palma J, Barroso J (2003) Plant peroxisomes, reactive oxygen metabolism and nitric oxide. IUBMB Life 55(2):71–81

Dias L, Pereira-da-Silva S, Tavares M, Malfeito-Ferreira M, Loureiro V (2003) Factors affecting the production of 4-ethylphenol by the yeast *Dekkera bruxellensis* in enological conditions. Food Microbiol 20:377–384

Dickinson JR (2003) The formation of higher alcohols. In: Smart KA (ed) Brewing yeast fermentation performance, 2nd edn. Blackwell, Oxford, UK, pp 196–205

Dmytruk KV, Abbas CA, Voronovsky AY, Kshanovska BV, Sybirna KA, Sybirny AA (2004) Cloning of structural genes involved in riboflavin synthesis of the yeast *Candida famata*. Ukr Biochem J 76:78–87

Do TQ, Schultz JR, Clarke CF (1996) Enhanced sensitivity of ubiquinone-deficient mutants of *Saccharomyces cerevisiae* to products of autoxidized polyunsaturated fatty acids. Proc Natl Acad Sci USA 93(15):7534–7539

Doll M (2002) Yeast-renaissance of an old natural remedy. Erfahrungsheilkunde 51(5):354–357

Drewek Z, Czarnocka-Roczniakowa B (1986) Microbiological processes in folacin synthesis in kefir. Acta Aliment Pol 12(1):39–45

Dufosse L, Feron G, Perrin C, Djian A, Spinnler HE (2000) Bioconversion of ricinoleic acid into γ-decalactone: Optimisation of the production using the yeasts *Sporidiobolus salmonicolor* and *Sporidiobolus ruinenii*. In: Schieberle P, Engel KH (eds) Frontiers of flavour science. Deutsche Forschungsanstalt für Lebensmittelchemie, Garching, Germany pp 389–393

Dufosse L, Blin-Perrin C, Souchon I, Feron G (2002) Microbial production of flavors for the food industry. A case study on the production of γ-decalactone, the key compound of peach flavor, by the yeasts *Sporidiobolus sp*. Food Sci Biotechnol 11(2):192–202

Durá MA, Flores M, Toldrá F (2002) Purification and characterization of a glutaminase from *Debaryomyces spp*. Int J Food Microbiol 76:117–126

Eden A, Van Nedervelde L, Drukker M, Benvenisty N, Debourg A (2001) Involvement of branched-chain amino acid aminotransferases in the production of fusel alcohols during fermentation in yeast. Appl Microbiol Biotechnol 55(3):296–300

Edlin DAN, Narbad A, Dickinson JR, Lloyd D (1995) The biotransformation of simple phenolic compounds by *Brettanomyces anomalus*. FEMS Microbiol Lett 125(2/3):311–315

Edwards CG, Beelman RB, Bartley CE, McConnell AL (1990) Production of decanoic acid and other volatile compounds and the growth of yeast and malolactic bacteria during vinification. Am J Enol Vitic 41(1):48–56

El-Dash AAM (1969) The role of free amino acids and primary amino groups in production of bread aroma stimuli. Diss Abstr Int B 30(6):2752

Encinas JP, López-Díaz TM, García-López ML, Otero A, Moreno B (2000) Yeast populations on Spanish sausages. Meat Sci 54:203–208

Endrizzi A, Pagot Y, LeClainche A, Nicaud JM, Belin JM (1996) Production of lactones and peroxisomal β-oxidation in yeasts. Crit Rev Biotechnol 16(4):301–329

Etschmann MMW, Sell D, Schrader J (2003) Screening of yeasts for the production of the aroma compound 2-phenylethanol in a molasses-based medium. Biotechnol Lett 25(7):531–536

Ettayebi K, Errachidi F, Jamai L, Tahri-Jouti MA, Sendide K, Ettayebi M (2003) Biodegradation of polyphenols with immobilized *Candida tropicalis* under metabolic induction. FEMS Microbiol Lett 223(2):215–219

Fabre CE, Blanc PJ, Goma G (1997) Screening of yeasts producing 2-phenylethyl alcohol. Biotechnol Tech 11(7):523–525

Fabre CE, Blanc PJ, Goma G (1998) Production of 2-phenylethyl alcohol by *Kluyveromyces marxianus*. Biotechnol Prog 14(2):270–274

Fadda ME, Mossa V, Pisano MB, Deplano M, Cosentino S (2004) Occurrence and characterization of yeasts isolated from artisanal Fiore Sardo cheese. Int J Food Microbiol 95(1):51–59

Fang TJ, Chiou TY (1996) Batch cultivation and astaxanthin production by a mutant of the red yeast, *Phaffia rhodzyma*. J Ind Microbiol 16(3):175–181

Farbood MI, Willis BJ (1985) Production of γ-decalactone. US Patent 4,560,656

Farid MAR, Azar M (2001) β-Carotene production from *Rhodotorula*. Amirkabir 12(46):228–236

Fazekas AG, Sebok J (1959) Effect of vitamin B1 and vitamin B1 plus thioglycolic acid on the production of ergosterol and the fermentative processes in yeast cultures. Acta Biol Acad Sci Hung 10:141–50

Feron G, Dufosse L, Mauvais G, Bonnarme P, Spinnler HE (1997) Fatty acid accumulation in the yeast *Sporidiobolus salmonicolor* during batch production of γ-decalactone. FEMS Microbiol Lett 149(1):17–24

Fontana JD, Guimaras MF, Martins NT, Fontana CA, Baron M (1996a) Culture of the astaxanthinogenic yeast *Phaffia rhodozyma* in low-cost media. Appl Biochem Biotechnol 57/58:413–422

Fontana JD, Czeczuga B, Bonfim TMB, Chociai MB, Oliveira, Guimaraes MF, Baron M (1996b) Bioproduction of carotenoids: the comparative use of raw sugarcance juice and depolymerized bagasse by *Phaffia rhodozyma*. Bioresour Technol 58:121–125

Fontana JD, Chocial MB, Baron M, Guimaraes M F; Maraschin M, Ulhoa C, Florencio JA, Bonfim Tania MB (1997) Astaxanthinogenesis in the yeast *Phaffia rhodozyma*: optimization of low-cost culture media and yeast cell-wall lysis. Appl Biochem Biotechnol 63/65:305–314

Forbes M, Gyoergy P, Zilliken F (1958) Antioxidant for food products and its preparation from yeast. US Patent 2,865,809

Forman HJ, Rotman EI, Fisher AB (1983) Roles of selenium and sulfur-containing amino acids in protection against oxygen toxicity. Lab Invest 49(2):148–53

Frengova G, Simova E, Beshkova D (2003) Carotenoid production by lactoso-negative yeasts co-cultivated with lactic acid bacteria in whey ultrafiltrate. J Biosci 58(7/8):562–567

Frengova G, Simova E, Beshkova D (2004) Use of whey ultrafiltrate as a substrate for production of carotenoids by the yeast *Rhodotorula rubra*. Appl Biochem Biotechnol 112(3):133–141

Fujii T, Yoshimoto H, Tamai Y (1996) Acetate ester production by *Saccharomyces cerevisiae* lacking the ATF1 gene encoding the alcohol acetyltransferase. J Ferment Bioeng 81(6):538–542

Fukuda K, Watanabe M, Asano K, Ueda K, Ohta S (1990a) Breeding of brewing yeast producing a large amount of β-phenylethyl alcohol and β-phenylethyl acetate. Agric Biol Chem 54(1):269–272

Fukuda K, Watanabe M, Asano K, Ohta S (1990b) Mutants of yeast *Saccharomyces cerevisiae* producing large amounts of the flavor components isobutyl alcohol and isoamyl alcohol. Agric Biol Chem 54(9):2445–2446

Fukuda K, Inoue Y, Wakai Y, Kimura A (1998a) Production of isoamyl acetate by yeasts *Hansenula mrakii* and *Saccharomyces cerevisiae*: role of esterase. Recent Res Dev Ferment Bioeng 1:47–53

Fukuda K, Yamamoto N, Kiyokawa Y, Yanagiuchi T, Wakai Y, Kitamoto K, Inoue Y, Kimura A (1998b) Balance of activities of alcohol acetyltransferase and esterase in *Saccharomyces cerevisiae* is important for production of isoamyl acetate. Appl Environm Microbiol 64(10):4076–4078

Fung DY, Lin CC, Gailani MB (1985) Effect of phenolic antioxidants on microbial growth. Crit Rev Microbiol 12(2):153–83

Furukawa K, Yamada T, Mizoguchi H, Hara S (2003a) Increased ethyl caproate production by inositol limitation in *Saccharomyces cerevisiae*. J Biosci Bioeng 95(5):448–454

Furukawa K, Yamada T, Mizoguchi H, Hara S (2003b) Increased alcohol acetyltransferase activity by inositol limitation in *Saccharomyces cerevisiae* in sake mash. J Biosci Bioeng 96(4):380–386

Garafolo A (1992) The ester synthesizing activity of yeast under different nutrient conditions. Riv Vitic Enol 45(3):41–58

Gatfield IL, Guentert M, Sommer H, Werkhoff P (1993) Some aspects of the microbiological production of flavour-active lactones with particular reference to γ-decalactone. Chem Mikrobiol Technol Lebensm 15(5/6):165–170

Gazi MR, Hoshikuma A, Kanda K, Murata A, Kato F (2001) Detection of free radical scavenging activity in yeast culture. Saga Daigaku Nogakubu Iho 86:67–74

Ghazal NS, Azzazy MA (1996) Assessment of the suitability of some yeast strains for production of microbial lipids. Egypt J Microbiol 31(1):61–70

Girard P, Falconnier B, Bricout J, Vladescu B (1994) β-Carotene producing mutants of *Phaffia rhodozyma*. Appl Microbiol Biotechnol 41(2):183–191

Goss KA, Alharethi R, Laposata M (1999) Fatty acid ethyl ester synthesis in the preparation of scotch whiskey. Alcohol 17(3):241–245

Grbin PR, Henschke PA (2000) Mousy off-flavour production in grape juice and wine by *Dekkera* and *Brettanomyces* yeasts. Aust J Grape Wine Res 6(3):255–262

Groguenin A, Wache Y, Garcia EE, Aguedo M, Husson F, LeDall MT, Nicaud JM, Belin JM (2004) Genetic engineering of the β-oxidation pathway in the yeast *Yarrowia lipolytica* to increase the production of aroma compounds. J Mol Catal B 28(2/3):75–79

Guirnovich ES, Koroleva IF (1971) Synthesis of carotenoids by *Rhodotorula* and *Sporobolomyces* yeasts. Fiziol Biokhim Mikroorg 37–42

Guirnovich ES, Lukashik AN, Koroleva IF (1966) Effect of interchanging darkeness and light on accumulation of carotenoids by *Rhodotorula gracilis*. Issled Fiziol Biokhim Rast 165–170
Halasz A, Lasztity R (1991) Use of yeast biomass in food production. CRC, Boca Raton, FL, USA
Hamada T, Sugishita M, Motai H (1990) Continuous production of 4-ethylguaiacol by immobilized cells of salt-tolerant *Candida versatilis* in an airlift reactor. J Ferment Bioeng 69(3):166–169
Hamada T, Sugishita M, Fukushima Y, Fukase T, Motai H (1991) Continuous production of soy sauce by a bioreactor system. Process Biochem 26(1):39–45
Hancock RD, Viola R (2001) The use of micro-organisms for L-ascorbic acid production: current status and future perspectives. Appl Microbiol Biotechnol 56(5/6):567–576
Hansen J, Kielland-Brandt MC (1996) Inactivation of MET2 in brewers' yeast increases the level of sulfite in beer. J Biotechnol 50(1):75–87
Haramaki N, Marocci L, D'Anna R, Yan LJ, Kobuchi H, Packer L (1995) Bio-catalyzer no. 11 (Bio-Normalizer) supplementation: effect on oxidative stress to isolated rat hearts. Biochem Mol Biol Int 36(6):1263–1268
Harrison HE (1942) Biological synthesis of vitamin B1.US Patent 2,359,521
Harrison JS (1970) Miscellaneous products from yeast. In: Rose AH, Harrison JS (eds) The yeasts, vol III. Academic, London, UK pp 529–545
Heath HB (1981) Source book of flavors. AVI sourcebook and handbook series. AVI, Westport, CT, USA
Hecquet L, Sancelme M, Bolte J, Demuynck C (1996) Biosynthesis of 4-hydroxy-2,5-dimethyl-3(2H)-furanone by *Zygosaccharomyces rouxii*. J Agric Food Chem 44(5):1357–1360
Hosono A, Tokita F (1970) Effects of *Brevibacterium linens* and yeasts on the development of volatile flavor substances in Limburger cheese. I. General properties of yeasts isolated from Limburger cheese and the production of volatile flavor substances by these yeasts. Nippon Chikusan Gakkaiho 41(3):131–137
Hurrell RF (1982) Maillard reaction in flavour. In: Morton ID, Macleod AJ (eds) Food flavours part A. Introduction. Developments in food science 3A. Elsevier, Amsterdam, The Netherlands, pp 399–437
Huynh-Ba T, Matthey-Doret W, Fay LB, Rhlid RB (2003) Generation of thiols by biotransformation of cysteine-aldehyde conjugates with baker's yeast. J Agric Food Chem 51(12):3629–3635
Imai Y, Yazawa S, Suzuki K, Yamaguchi Y, Shibazaki M, Saito T (1989) Active oxygen-removing Q-1047R and its fermentative and synthetic manufacture. Jpn Kokai Tokkyo Koho 12
Imura T, Onaka T, Muromachi H, Kawasaki H (2003) Process for producing bread. US Patent 6,649,198 B2
Ingram M, Simonsen B (1980) Meats and meat products. In: Silliker JH et al (eds) Microbial ecology of food, vol II. Academic, New York, USA, pp 333–409
Jacob Z (1991) Enrichment of wheat bran by *Rhodotorula gracilis* through solid-state fermentation. Folia Microbiol 36(1):86–91
Jacobson KJ, Jolly SO, Sedmak JJ, Skatrud TJ, Wasileski JM (2000) Astaxanthin over-producing strains of *Phaffia rhodozyma*: method for their cultivation and their use in animal feed. US Patent 6,015,684
Janssens L, De Pooter HL, Vandamme EJ, Schamp NM (1987) Production of flavors by the yeast *Hansenula mrakii*. Meded Fac Landbouwwet Univ Gent 52(4B):1907–1913
Johnson EA (2003) A *Phaffia rhodozyma*: colorful odyssey. Int Microbiol 6(3):169–174
Johnson EA, Schroeder W (1995) Astaxanthin from the yeast *Phaffia rhodozyma*. Stud Mycol (38):81–90
Kakizono T, Kakimoto A, Nakajimata Y, Nishio N (2003) Method for screening anti-oxidant high production cells. Jpn Patent JP 2003189893 A2 20030708

Kalle GP, Deshpande SY (1977) Production of processed cheese using a flavor concentrate prepared by cream fermentation. J Food Sci Technol 14(5):207–212

Kasumegi K, Baba M, Yoshida H, Miyagi M (1997) Breeding of *Zygosaccharomyces rouxii* producing enhanced aroma components and its application to barley miso fermentation – note. Seibutsu Kogaku Kaishi 75(5):343–346

Kasumegi K, Baba M, Miyagi M (2001) Breeding of *Zygosaccharomyces rouxii* producing enhanced aroma components and its application to barley miso making. Miso No Kagaku To Gijutsu 49(4):118–122

Kawamukai M (2002) Biosynthesis, bioproduction and novel roles of ubiquinone. J Biosci Bioeng 94(6):511–517

Kawamura D, Kawano K (1999) Miso brewing using alternative yeast species. Nippon Jozo Kyokaishi 94(1):72–76

Kayahara H, Yasuhira H, Mochizuki T (1980) Utilization of yeast in miso maturation. Part 15. Manufacturing of low salt miso by addition of various yeast. Shinshu Miso Kenkyusho Kenkyu Hokoku 21:28–33

Kilian SG, Sutherland FCW, Meyer PS, du Preez JC (1996) Transport-limited sucrose utilization and neokestose production by *Phaffia rhodozyma*. Biotechnol Lett 18(8):975–980

Kim SJ, Kim GJ, Park DH, Ryu YW (2003) High-level production of astaxanthin by fed-batch culture of mutant strain *Phaffia rhodozyma* AJ-6-1. J Microbiol Biotechnol 13(2):175–181

King A, Dickinson JR (2000) Biotransformation of monoterpene alcohols by *Saccharomyces cerevisiae*, *Torulaspora delbrueckii* and *Kluyveromyces lactis*. Yeast 16(6):499–506

Kinsella JE, Damodaran S (1980) Flavour problems in soy proteins; origin, nature, control and binding phenomena. In: Charalambous G (ed) The analysis and control of less desirable flavors in foods and beverages. Academic, NewYork, USA, pp 95–131

Kiss M, Stefanovitsne BE, Venyige T (1998) Production of ethyl oleate aroma ester by using *Saccharomyces bayanus* yeast cell and lipase. Kozmetika 47(6):271–274

Kodama Y, Omura F, Miyajima K, Ashikari T (2001) Control of higher alcohol production by manipulation of the BAP2 gene in brewing yeast. J Am Soc Brew Chem 59(4):157–162

Komai T, Gocho S, Hatae S (1987) Manufacture of miso or soy sauce flavor. Jpn Patent JP 61293367

Kometani T, Morita Y, Kiyama Y, Yoshii H, Matsuno R (1995) Relationship between ethanol consumption rate and prochiral ketone reduction rate in baker's yeast. J Ferment Bioeng 80(2):208–210

Kondo M, Nishimura S, Tanaka N, Flood M (2001) Safety evaluation of phosphodiesterase produced from *Penicillium citrinum*: summary of toxicological data. Regulatory toxicology and pharmacology. Regul Toxicol Pharmacol 33(1):2–11

Koroleva IF, Demina SG, Kosonogova LV, Evdokimova GA (1982) Use of peat oxidates for cultivation of yeasts. In: Lishtvan II (ed) Nov Protessy Prod. Pererab Torfa Conference Proceedings. Nauka I Tekhnika, Minsk, USSR, pp 171–174

Kumari P, Singh S (1990) Microbial profile of soy slurries during ripening. J Food Sci Technol India 27(5):253–255

Kunkee RE, Snow SR, Rous C (1983) Method for reducing fusel oil in alcoholic beverages and yeast strain useful in that method. US Patent 4,374,859

Kurniadi T, Rhlid RB, Fay LB, Juillerat MA, Berger RG (2003) Chemoenzymatic synthesis of aroma active 5,6-dihydro- and tetrahydropyrazines from aliphatic acyloins produced by baker's yeast. J Agric Food Chem 51(10):3103–3107

Kusdiyantini E, Gaudin P, Goma G, Blanc PJ (1998) Growth kinetics and astaxanthin production of *Phaffia rhodozyma* on glycerol as a carbon source during batch fermentation. Biotechnol Lett 20(10):929–934

Kusumegi K, Baba M, Nakahara T, Ogawa K (1992) Breeding of soy sauce yeast by protoplast fusion technique. 1.Construction of protoplast fusants between soy sauce yeast and sake or wine yeast. Nippon Jozo Kyokaishi 87(9):645–650

Lambrechts MG, Pretorius IS (2000) Yeast and its importance to wine aroma: A review. S Afr J Enol Vitic 21 97–129

Larsson S, Quintana-Sainz A, Reimann A, Nilvebrant NO, Jonsson LJ (2000) Influence of lignocellulose-derived aromatic compounds on oxygen-limited growth and ethanolic fermentation by *Saccharomyces cerevisiae*. Appl Biochem Biotechnol 84/86:617–632

Larsson S, Cassland P, Jonsson LJ (2001) Development of a *Saccharomyces cerevisiae* strain with enhanced resistance to phenolic fermentation inhibitors in lignocellulose hydrolysates by heterologous expression of laccase. Appl Environ Microbiol 67(3):1163–1170

Lee EJ, Kim JK (1993) Development of a novel yeast strain which ferments soy sauce by protoplast fusion. J Microbiol Biotechnol 3(1):24–30

Lee SM, Park JW (1998a) Thermosensitive phenotype of yeast mutant lacking thioredoxin peroxidase. Arch Biochem Biophys 359(1):99–106

Lee SM, Park JW (1998b) A yeast mutant lacking thiol-dependent protector protein is hypersensitive to menadione. Biochim Biophys Acta 1382(1):167–175

Lewis MJ, Ragot N, Berlant MC, Miranda M (1990) Selection of astaxanthin-overproducing mutants of *Phaffia-Rhodozyma* with β-ionone. Appl Environ Microbiol 56(9):2944–2945

Liang X, Zhang H (2003) Utilizing maize cobs as fermentation substrate to produce feed yeast rice in astaxanthin by *Phaffia rhodozyma*. J Chin Cereals Oils Assoc 18(5):85–88

Lieske B, Konrad G (1994) Protein hydrolysis: the key to meat flavoring systems. Food Rev Int 10(3):287–312

Liggett JL (1978) Cocoa substitute from yeast. UK Patent Appl GB 2010657 A

Lim SH, Choi JS, Park EY (2001) Microbial production of riboflavin using riboflavin overproducers, *Ashbya gossypii*, *Bacillus subitlis*, and *Candida famata*: an overview. Biotechnol Bioprocess Eng 6:75–88

Liu J, He X, Wang Y,Liu C, Kong Y, Zhang B (2003) The breeding of high glutathione-producing strain and optimization of culture condition Weishengwu Xuebao 43(1):99–103

Machida K, Tanaka T, Taniguchi M (1999) Depletion of glutathione as a cause of the promotive effects of polygodial, a sesquiterpene, on the production of reactive oxygen species in *Saccharomyces cerevisiae*. J Biosci Bioeng 88(5):526–530

Maga JA (1982) Pyrazines in flavour. In: Morton ID, Macleod AJ (eds) Food flavors part A. Introduction. Developments in food science 3A. Elsevier, Amsterdam, The Netherlands, pp 283–323

Maga JA (1992) Pyrazine update. Food Rev Int 8(4):479–558

Maraz A (2002) From yeast genetics to biotechnology. Acta Microbiol Immunol Hung 49(4):483–491

Marova I, Breierova E, Koci R, Friedl Z, Slovak B, Pokorna J (2004) Influence of exogenous stress factors on production of carotenoids by some strains of carotenogenic yeasts. Ann Microbiol 54(1):73–85

Martin AM, Acheampong E, Patel TR (1993) Production of astaxanthin by *Phaffia rhodozyma* using peat hydrolysates as substrate. J Chem Technol Biotechnol 58(3):223–230

Martin N, Berger C, Le Du C, Spinnler HE (2001) Aroma compound production in cheese curd by co-culturing with selected yeast and bacteria. J Dairy Sci 84(10):2125–2135

Mast-Gerlach E, Stahl U (1997) SOD antioxidant in yeast. Hung Szeszipar 45(2):36–39

Matelli HL, Silva IMD, Souza NO; Pomeroy D (1990) Production of β-carotene by a *Rhodotorula* strain grown on sugar cane juice. Biotechnol Lett 12(3):207–208

Matsuda A, Yamamoto S (1999) Development of miso yeasts with high flavor productivity. Ishikawa-ken Kogyo Shikenjo Kenkyu Hokoku (vol date 1998) 48:55–62

Meaden P (1998) Brewers' yeast. International Dairy Federation special issue 9801. Yeasts in the dairy industry: positive and negative aspects. International Dairy Federation, Brussels, Belgium, pp 162–172

Millati R, Niklasson C, Taherzadeh MJ (2002) Effect of pH, time and temperature of overliming on detoxification of dilute-acid hydrolyzates for fermentation by *Saccharomyces cerevisiae*. Process Biochem 38(4):515–522

Millis JR, Saucy GG, Maurina-Brunker J, McMullin TW (2000) Vitamin production by fermentative biosynthesis of intermediates using genetically engineered microorganisms followed by chemical synthesis. PCT Int Appl WO 99-US15264 19990706

Mirsky N, Schachter A, Sussan S (2001) Natural extracted and synthetic antioxidant compositions. PCT Int Appl WO 2001045701 A1

Misawa N (1997) Production of carotenoid by *Escherichia coli* and yeast. Kagaku To Seibutsu 35(1):60–68

Misawa N (1999) Metabolic engineering of *Candida utilis* in carotenoid production. Kemikaru Enjiniyaringu 44(6):447–452

Misawa N, Shimada H (1998) Metabolic engineering for the production of carotenoids in non-carotenogenic bacteria and yeasts. J Biotechnol 59(3):169–181

Misawa N, Shimata H, Miura H, Kondo E (1998) Recombinant production of carotenoids with yeasts expressing HMG-CoA reductase. Jpn Patent 10248575

Miura Y; Kondo K; Shimada H; Saito T; Nakamura K; Misawa N (1998) Production of lycopene by the food yeast, *Candida utilis* that does not naturally synthesize carotenoid. Biotechnol Bioeng 58(2/3):306–308

Mo EK, Kang HJ, Lee CT, Xu BJ, Kim JH, Wang QJ, Kim JC, Sung CK (2003) Identification of phenylethyl alcohol and other volatile flavor compounds from yeasts, *Pichia farinosa* SKM-1, *Pichia anomala* SKM-T, and *Galactomyces geotrichum* SJM-59. J Microbiol Biotechnol 13(5):800–808

Moskowitz GJ (1980) Flavour development in cheese. In: Charalambous G (ed) The analysis and control of less desirable flavors in foods and beverages. Academic, New York, USA, pp 53–70

Mosiashvili G, Marianashvili E, Osipova S, Pataraya M, Sulkhanishivili N (1971) Yeasts as producers of vitamins and enzymes. Vinograd Vinodel (Tbilisi) 19/20:523–553

Nagodawithana T (1992) Yeast-derived flavors and flavor enhancers and their probable mode of action. Food Technol 46(11):138–144

Nicaud JM, Madzak C, van den Broek P, Gysler C, Duboc P, Niederberger P, Gaillardin C (2002) Protein expression and secretion in the yeast *Yarrowia lipolytica*. FEMS Yeast Res 2(3):371–379

Nikolaev PI, Palceva MA, Samokhvalov GI, Vakulova LA, Lopatik MD (1966) Production of carotenoids. USSR Patent 178449 19660122

Nishino T, Ishikawa F (1998) Pharmaceutical, cosmetic, and food antioxidants containing yeasts and a method for evaluation of antioxidant activity of microorganisms. Jpn Patent 10287872 A2 19881027

Noble AC (1978) Aroma. In: Peterson MS, Johnson AH (eds) Encyclopedia of food. AVI, Westport, CT, USA, pp 50–51

Noordam B, Kortes JG (2004) Production of 5′-ribonucleotides by separation of RNA from yeast extracts and conversion of separated RNA and use in food or feed. PCT Int Appl WO 2004067758 A2 20040812

Nykanen L (1986) Formation and occurrence of flavour compounds in wine and distilled alcoholic beverages. Am J Enol Vitic 37(1):84–96

Ohnishi K (1983) Studies on lipids of miso during aging. II. Effect of ethanol on changes of lipids during miso fermentation. Nippon Shokuhin Kogyo Gakkaishi 30(2):88–93

Ok T, Lin NN, Zaima T, Nakajima H (2003) Para-aminobenzaldehyde produced by yeast *Zygosaccharomyces rouxii* and its antioxidant activity. Abstr Gen Meet Am Soc Microbiol 103:0–009

Omori T, Takashita H, Omori N, Shimoda M (1995) High glycerol producing amino acid analogue-resistant *Saccharomyces cerevisiae* mutant. J Ferment Bioeng 80(3):218–222

Omura F, Shibano Y, Fukui N, Nakatani K (1995) Reduction of hydrogen sulfide production in brewing yeast by constitutive expression of MET25 gene. J Am Soc Brew Chem 53(2):58–62

Oro K (2001) Test brewing of soy sauce using after ripening yeast. Kenkyu Hokoku Kagawaken Sangyo Gijutsu Senta (vol date 2000) 1:138–141

Oura E, Suomalainen H (1982) Biotin-active compounds, their existence in nature and the biotin requirements of yeasts. J Inst Brew 88(5):299–308

Padilla S, Jonassen T, Jimenez-Hidalgo MA, Fernandez-Ayala DJM, Lopez-Lluch G, Marbois B, Navas P, Clarke CF, Santos-Ocana C (2004) Demethoxy-Q, an intermediate of coenzyme Q biosynthesis, fails to support respiration in *Saccharomyces cerevisiae* and lacks antioxidant activity. J Biol Chem 279(25):25995–26004

Palagyi Z, Ferenczy L, Vagvolgyi C (2001) Carbon-source assimilation pattern of the astaxanthin-producing yeast *Phaffia rhodozyma*. World J Microbiol Biotechnol 17(1):95–97

Pan TM, Kuo CC (1993) Production of flavor by a mutant of yeast. J Food Process Preserv 17(3):213–225

Papanikolaou S, Chevalot I, Komaitis M, Marc I (2002) Single cell oil production by *Yarrowia lipolytica* growing on an industrial derivative of animal fat in batch cultures. Appl Microbiol Biotechnol 58:308–312

Parajó JC, Santos V, Vázquez M, Cruz JM (1997) Production of carotenoids by *Xanthophyllomyces dendrorhous* growing on enzymatic hydrolysates of prehydrolysed wood. Food Chem 60(3):347–355

Parajó JC, Santos V, Vázquez M (1998a) Production of carotenoids by *Phaffia rhodozyma* growing on media made from hemicellulosic hydrolysates of eucalyptus globulus wood. Biotechnol Bioeng 59(4):501–506

Parajó JC, Santos V, Vázquez M (1998b) Optimization of carotenoid production by *Phaffia rhodozyma* cells grown on xylose. Process Biochem 33(2):181–187

Park C (2003) Fermented health beverage containing natural antioxidants, octacosanol and β-glucan. US Patent Appl II 2003 0039723 A1

Patane M (2004) Methods for producing flavoring agents in *Saccharomyces cerevisiae* using liposomes containing 5-phosphodiesterase. PCT Int Appl WO 2004053118 A1 20040624

Penninckx MJ (2002) An overview on glutathione in *Saccharomyces* versus non-conventional yeasts. FEMS Yeast Rese 2(3):295–305

Peppler HJ (1967) Yeast technology. In: Peppler HJ (ed) Microbial technology. Van Nostrand-Reinhold, Princeton, NJ, USA, pp 145–171

Peppler HJ (1970) Food yeasts. In: Rose AH, Harrison JS (eds) The yeasts, vol III. Academic, London, UK, pp 421–462

Peppler HJ (1979) Production of yeasts and yeast products.In: Peppler HJ, Perlman D (eds) Microbial technology, vol I, 2nd edn. Academic, New York, USA, pp 157–185

Petersen KM, Westall S, Jespersen L (2002) Microbial succession of *Debaryomyces hansenii* strains during the production of Danish surfaced-ripened cheeses. J Dairy Sci 85(3):478–486

Pobedimskii DG, Vinarov AY, Reshetnik OA, Mingaleeva ZS, Gutman AS, Shishkina LN (1988) Influence of antioxidants on the growth of microorganisms.Acta Biotechnol 8(1):55–69

Popova Y, Bakhchevanska S, Popova Z (1971) Effect of some factors upon the biosynthesis of vitamin B_{12} by natural fermentation of waste waters from hydrolytic-yeast production. Plovdiv 18(3):363–70

Popova Y, Illieva M, Bakhchevanska S (1982) Formation of vitamin B1 in brewery yeast in the presence of its precursors. Plovdiv 29(1):89–97

Protchenki OV, Boretsky YR, Romanyuk TM, Fedorovych DV (2000) Oversynthesis of riboflavin by yeast *Pichia guilliermondii* in response to oxidative stress. Ukr Biokhim Zh 72(2):19–23

Pshevorskaya VY, Zabrodskii AG (1972) Effect of several factors on carotene formation by *Rhodotorula* yeasts.Vodoch Prom (14):179–183

Quilter MG, Hurley JC, Lynch FJ, Murphy MG (2003) The production of isoamyl acetate from amyl alcohol by *Saccharomyces cerevisiae*. J Inst Brew 109(1):34–40

Ramirez J, Nunez M L, Valdivia R (2000) Increased astaxanthin production by a *Phaffia rhodozyma* mutant grown on date juice from *Yucca fillifera*. J Ind Microbiol Biotechnol 24(3):187–190

Randez-Gil F, Sanz P, Prieto JA (1999) Engineering baker's yeast: room for improvement. Trends Biotechnol 17(6):237–244

Rao Y, Raghunatha K (1942) Vitamin D and other products of metabolism from yeast. Curr Sci 11:189

Rasmussen S, Bach M (1996) Cocoa flavor precursor peptides, DNA encoding them, processes for producing the peptides, and their use for generating cocoa flavor. PCT Int Appl WO 9638472 A1 1996120

Ratledge C (1997) Microbial lipids. In: Kleinkauf H, Von Döhren (eds) Biotechnology, vol 7, 2nd edn. VCH, Weinheim, Germany, pp 133–197

Ravaglia S, Delfini C (1993) Production of medium chain fatty acids and their ethyl esters by yeast strains isolated from musts and wines. Ital J Food Sci 5(1):21–36

Reed G (1981) Use of microbial cultures: yeast products. Food Technol 35(1):89–94

Reynders MB, Rawlings DE, Harrison STL (1996) Studies on the growth, modeling and pigment production by the yeast *Phaffia rhodozyma* during fed-batch cultivation. Biotechnol Lett 18(6):649–654

Romano P, Marchese R, Laurita C, Saleano G, Turbanti L (1999) Biotechnological suitability of *Saccharomycodes ludwigii* for fermented beverages. World J Microbiol Biotechnol 15(4):451–454

Russell I, Hancock IF, Stewart GG (1983) Construction of dextrin fermentative yeast strains that do not produce phenolic off-flavors in beer. J Am Soc Brew Chem 41(2):45–51

Sakaki H, Nochide H, Nakanishi T, Miki W, Fujita T, Komemushi S (1999) Effect of culture condition in the biosynthesis of carotenoids in *Rhodotorula glutinis* no. 21. Seibutsu Kogaku Kaishi 77(2):55–59

Sakaki H, Nakanishi T, Komemushi S, Namikawa K, Miki W (2001) Torularhodin as a potent scavenger against peroxyl radicals isolated from a soil yeast, *Rhodotorula glutinis*. J Clin Biochem Nutr 30:1–10

Sakaki H, Nochide H, Komemushi S, Miki W (2002) Effect of active oxygen species on the productivity of torularhodin by *Rhodotorula glutinis* no. 21. J Biosci Bioeng 93(3):338–340

Sakato K, Tanaka H (1992) Advanced control of glutathionie fermentation process. Biotechnol Bioeng 40(8):904–912

Samelis J, Stavropoulos S, Kakouri A, Metaxopoulos (1994) Quantification and characterization of microbial populations associated with naturally fermented Greek dry salami. Food Microbiol 11:447–460

Sanchez B, Reverol L, Galindo-Castro I, Bravo A, Rangel-Aldao R, Ramirez JL (2003) Brewer's yeast oxidoreductase with activity on Maillard reaction intermediates of beer. Tech Q Master Brew Assoc Am Commun 40(3):204–212

Sanderson GW (1978) Biogeneis of flavor. In: Peterson MS, Johnson AH (eds) Encyclopedia of food science. AVI, Westport, CT, USA, pp 71–74

Sarkar PJ, Tamang JP, Cook PE, Owens JD (1994) Kinema-a traditional soybean fermented food: proximate composition and microflora. Food Microbiol 11:47–55

Sasaki M (1996) Influence of sodium chloride on the levels of flavor compounds produced by shoyu yeast. J Agric Food Chem 44(10):3273–3275

Schieberle P (1990) Studies on bakers' yeast as source of Maillard-type bread flavor compounds. In: Andre P (ed) Proceedings of the international symposium on Maillard reactions., 4th meeting 1989, Deutsche Forschungsanstalt, Garching, Germany. Birkhaeuser, Basel, Switzerland, pp 187–196

Schwan RF, Wheals AE (2004) The microbiology of cocoa fermentation and its role in chocolate quality. Crit Rev Food Sci Nutr 44(4):205–221

Shih CT, Hang YD (1996) Production of carotenoid by *Rhodotorula rubra* from sauerkraut brine. Food Sci Technol 29(5/6):570–572

Shimada H, Kondo K, Fraser PD, Miura Y, Saito T, Misawa N (1998) Increased carotenoid production by the food yeast *Candida utilis* through metabolic engineering of the isoprenoid pathway. Appl Environ Microbiol 64(7):2676–2680

Shimizu H, Araki K, Shioya S, Suga KI (1991) Optimal production of glutathione by controlling the specific growth rate of yeast in fed-batch culture. Biotechnol Bioeng 38(2):196–205

Shinohara T, Kubodera S, Yanagida F (2000) Distribution of phenolic yeasts and production of phenolic off-flavors in wine fermentation. J Biosci Bioeng 90(1):90–97

Shitazawa A, Sakurai, K, Ogine T, Minetoma T (2002) Astaxanthin-containing yeast or yeast products for use as immunostimulant. Jpn Patent JP 2002080351 A2 20020319

Silhankova L (1978) Vitamin B1 for the beverage and pharmaceutical industries.Ger Patent DE 2702700 19780727

Silhankova L (1985) Yeast mutants excreting vitamin B1 and their use in the production of thiamin-rich beers. J Inst Brew 91(2):78–81

Simova ED, Frengova G, Beshkova DM (2003) Effect of aeration on the production of carotenoid pigments by *Rhodotorula rubra-Lactobacillus casei* subsp. *Casei* co-cultures in whey ultrafiltrate. J Biosci 58(3/4):225–229

Slyusarenko TP, Stabnikova EV, Mironets LI, Storozhuk AA (1976) Stimulation of growth and biosynthesis of carotenoids in *Rhodotorula glutinis* M-214 yeasts. Nauchno-Tekh Sb 22:33–36

Squina FM, Yamashita F, Pereira JL, Mercadante AZ (2002) Production of carotenoids by *Rhodotorula rubra* and *R. glutinis* in culture medium supplemented with sugar cane juice. Food Biotechnol 16(3):227–235

Stahmann KP, Revuelta JL, Seulberger H (2000) Three biotechnical processes using *Ashbya gossypii*, *Candida famata*, or *Bacillus subtilis* compete with chemical riboflavin production. Appl Microbiol Biotechnol 53:509–516

Stam H, Verbiest A, Wesdorp J, Batenburg M, Mooren A (2000) Genetic engineering as a tool to influence the sensory characteristics of yeast extracts: yeast enriched in cysteine. In: Proceedings of the 9th Weurman flavour research symposium. Frontiers of flavour science, Freising, Germany, 22–25 June 1999. Deutsche Forschungsanstalt für Lebensmittelchemie, Garching, Germany, pp 357–360

Steensma A, van Dijck PWM, Hempenius R (2004) A Safety evaluation of phosphodiesterase derived from *Leptographium procerum*. Food Chem Toxicol 42(6):935–944

Subbotin TG, Zotagina SA, Mosyagina NM, Ovchinnikova NP (1974) Enrichment of feed yeasts with vitamin D2. Gidroliz Lesokhim Prom 6:2022

Sucher RW, Sidoti DR, Seeley RD, Robbins EA, Schuldt EH Jr, Newell JA (1974) Yeast protein isolate, yeast glucan, and yeast extract. Ger Patent DE 2359900 19740606

Suezawa Y, Kimura I, Inoue M, Goda N, Kimura F, Sakakibara M, Tanaka M, Inoue K (2003) Test brewing of soy sauce using yeast producing red pigment. Hokoku Kagawa-ken Sangyo Gijutsu Senta (vol date 2002) 3:59–64

Sugawara E (2001) Formation by yeast of the aroma component specific to miso and soy sauce. Foods Food Ingred J Jpn 193:57–65

Suomalainen H, Lehtonen M (1978) Yeast as a producer of aroma compounds. DECHEMA Monogr 82(1693/1703):207–220

Suomalainen H, Lehtonen M (1979) The production of aroma compounds by yeast. J Inst Brew 85(3):149–156

Suzuki T, Sugawara E, Kobayashi H (2003) Hydroxy ethyl or methyl furanone, a flavor component of soy sauce is mutagen or antimutagen? Dual inhibitory and promotive effect and its mechanism in mutation tests. Aroma Res 4(3):280–284

Szechenyi L, Karpati G, Simek F, Vas K (1973) Protein-vitamin concentrates by fermentation of lipids with yeasts. Hung Patent HU 7189 19731027

Taiyoji M, Watanabe S (2002) Brewing tests of non-salted rice miso. Kenkyu Hokoku Niigataken Nogyo Sogo Kenkyusho Shokuhin Kenkyu Senta 36:1–4

Tanaka A, Fukui S (1967) Formation of vitamins and their functions in hydrocarbon fermentation. III. Production of vitamin B6 by several kinds of yeast in hydrocarbon media. Hakko Kogaku Zasshi 45(7):611–616

Tanaka A, Yamada R, Shimizu S, Fukui S (1971) Formation of vitamins and their functions in hydrocarbon fermentation. X. Production of ergosterol by yeasts from hydrocarbons. Hakko Kogaku Zasshi 49(9):792–802

Teranishi Y, Shimizu S, Tanaka A, Fukui S (1971) Formation of vitamins and their functions in hydrocarbon fermentation. VIII. Vitamin B productivity of yeasts grown on hydrocarbons. Hakko Kogaku Zasshi 49(3):213–221

Ter Schure EG, Flikweert MT, Van Dijken JP, Pronk JT, Verrips CT (1998) Pyruvate decarboxylase catalyzes decarboxylation of branched-chain 2-oxo acids but is not essential for fusel alcohol production by *Saccharomyces cerevisiae*. Appl Environ Microbiol 64(4):1303–1307

Tressl R, Bahri D, Kossa M (1980) In: Charalambous G (ed) The analysis and control of less desirable flavors in foods and beverages. Academic, NewYork, USA, pp 293–318

Trofimenko NM, Cheban IA (1970) Production of yeast with a high level of vitamin B12 at the Belets sugar-alcohol plant. Konserv Ovoshchesus Prom 8:15

Uchida N, Suzuki K, Saiki R, Kainou T, Tanaka K, Matsuda H, Kawamukai M (2000) Phenotypes of fission yeast defective in ubiquinone production due to disruption of the gene for p-hydroxybenzoate polyprenyl diphosphate transferase. J Bacteriol 182(24):6933–6939

Udeh KO, Archremowicz B (1997) High-glutathione containing yeast *Saccharomyces cerevisiae*: optimization of production. Acta Microbiol Pol 46(1):105–114

USA Food and Drug Administration (1973) Food additives. Yeast-malt sprout extract. Food Fed Reg 38(91):12397–12398

Van Dam HW (1986) The biotechnology of bakers' yeast: old or new business? In: Blanshard JMV, Frazier PJ, Galliard T (eds) Chemisty and physics of baking. Royal Society of Chemistry, London, pp 117–131

Vandamme EJ (2003) Bioflavours and fragrances via fungi and their enzymes. Fungal Diversity 13:153–166

Van den Bremt K, Van der Plancken I, Delvaux FR, Verachtert H, Derdelinckx G (2000) Production of almond flavoured salicyl aldehyde in batch cultures of *Candida methanolovescens* strains. Meded Fac Landbouwkund Toegepaste Biol Wet Univ Gent 65(3a):287–290

Van den Bremt K, Delvaux FR, Verachtert H, Derdelinckx G (2001) Biogeneration of flavors: performance of *Candida methanolovescens* strains in nonalcoholic beer. J Am Soc Brew Chem 59(2):80–83

Vanderhaegen B, Neven H, Coghe S, Verstrepen KJ, Derdelinckx G, Verachtert H (2003) Bioflavoring and beer refermentation. Appl Microbiol Biotechnol 62(2/3):140–150

Van Der Schaft PH, Ter Burg N, Van den Bosch S, Cohen AM (1992) Microbial production on natural delta decalactone and delta dodecalactone from the corresponding alpha beta-unsaturated lactones in massoi bark oil. Appl Microbiol Biotechnol 36(6):712–716

Van der Sluis C, Stoffelen CP, Castelein SJ, Engbers GHM, ter Schure EG, Tramper J, Wijffels RH (2001) Immobilized salt-tolerant yeasts: application of a new polyethylene-oxide support in a continuous stirred-tank reactor for flavour production. J Biotechnol 88(2):129–139

Van Haecht JL, Dufour JP (1995) The production of sulfur compounds by brewing yeast: a review. Cerevisia 20(1):51–64

Van Pottelsberghe de la Potterie PJ (1972) Flavoring substances with a roast beef aroma. Ger Patent DE 2149682 19720413

Vazquez M, Martin AM (1998) Mathematical model for *Phaffia rhodozyma* growth using peat hydrolysates as substrate. J Sci Food Agric 76(4):481–487

Vazquez M, Santos V, Parajo JC (1998) Fed-batch cultures of *Phaffia rhodozyma* in xylose-containing media made from wood hydrolysates. Food Biotechnol 12(1/2):43–55

Verstrepen KJ, Van Laere SDM, Vanderhaegen BMP, Derdelinckx G, Dufour JP, Pretorius IS, Winderickx J, Thevelein JM, Delvaux FR (2003) Expression levels of the yeast alcohol acetyltransferase genes ATF1, Lg-ATF1, and ATF2 control the formation of a broad range of volatile esters. Appl Environ Microbiol 69(9):5228–5237

Verstrepen KJ, Derdelinckx G, Delvaux FR (2004) Esters in beer – part 3: Why do yeast cells produce fruity flavours: the physiological role of acetate ester synthesis. Cerevisia 29(1):19–29

Visser H, van Ooyen AJJ, Verdoes JC (2003) Metabolic engineering of the astaxanthin-biosynthetic pathway of *Xanthophyllomyces dendrorhous*. FEMS Yeast Res 4(3):221–231

Voronovsky AY, Abbas CA, Fayura LR, Kshanovska BV, Dmytruk KV, Sybirna KA, Sibirny AA (2002) Development of a transformation system for the flavinogenic yeast *Candida famata*. FEMS Yeast Res 2:381–388

Voronovsky AY, Abbas CA, Dmytruk KV, Ishchuk OP, Kshanovska BV, Sybirna KA, Gaillardin C, Sibirny AA (2004) *Candida famata* (*Debaryomyces hansenii*) DNA sequences containing genes involved in riboflavin synthesis. Yeast 21:1307–1316

Wache Y, Aguedo M, Nicaud JM, Belin JM (2003) Catabolism of hydroxyacids and biotechnological production of lactones by *Yarrowia lipolytica*. Appl Microbiol Biotechnol 61(5/6):393–404

Wang SL, Zhang X, Zhang H, Lin K (2001) Effects of some additives on the growth and carotenoids content of *Rhodotorula*. Food Sci Technol (2):20–21

Watanabe M, Fukuda K, Asano K, Ohta S (1990) Mutants of bakers' yeasts producing a large amount of isobutyl alcohol or isoamyl alcohol, flavor components of bread. Appl Microbiol Biotechnol 34(2):154–159

Wei G, Li Y, Du G, Chen J (2003) Application of a two-stage temperature control strategy for enhanced glutathione production in the batch fermentation by *Candida utilis*. Biotechnol Lett 25(11):887–890

Word KM, Pflugfelder RL, Neu JA, Head GP, Patino H (1994) Colorless flavored malt beverage. US Patent 5,294,450

Xue DH, Li XL, Lu XT, Zhang HZ (2002) Study on the vitamin D2 yeast. Jilin Gongxueyuan Xuebao Ziran Kexueban 23(2):7–9

Yang W, Liu J, Wu C, Sun X (1993) Isolation of carotene from red yeast. Shipin Yu Fajiao Gongye (4):24–28

Yoshikawa S, Kondo K, Kuwabara H, Arikawa Y, Kobayashi I, Okazuki M (1990) Breeding of salt-tolerant yeasts by mutation. 1. Isolation of mutants producing sufficient isoamyl alcohol and butyl alcohol, miso flavor components. Nagano-ken Shokuhin Kogyo Shikenjo Kenkyu Hokoku 18: 71–76

Yoshimoto H, Fukushige T, Yonezawa T, Sone H (2002) Genetic and physiological analysis of branched-chain alcohols and isoamyl acetate production in *Saccharomyces cerevisiae*. Appl Microbiol Biotechnol 59(4/5):501–508

Zalaskho LS, Chaika LL, Chizhova ZP (1973) Conditions for the growth and carotenoid formation of the yeasts *Rhodotorula lactis* on whey. In: Samtsevich SA (ed) Mikroorganizmy-Produtsenty Biol Aktiv Veshchestv. Nauka I Tekhnika, Minsk, USSR, pp 51–57

Zeile M (1972) Effect of trace metal complexes on carotenogenesis and the formation of biomass by *Rhodotorula gracilis* yeasts. Mikrobn Biomassy Metab 53–57

Zhuge J, Fang HY, Wang ZX, Chen DZ, Jin HR, Gu HL (2001) Glycerol production by a novel osmotolerant yeast *Candida glycerinogenes*. Appl Microbiol Biotechnol 55(6):686–692

Ziegler E (1972) Beer flavorings for use in foods and beverages. Br Patent GB 1266309 19720308