

Chapter 19

Production and Evaluation of Vinegar Using Nabag as a Raw Material



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19.1 Introduction

Zizyphus spina-christi (L.) is a shrub or evergreen tree called Christ's thorn (Al-Wakeel 2008; Anthony and Dweck 2005) with edible sweet fruits. The plant is recognized to be very heat resistant in addition to its drought tolerance (Paroda and Mal 1989). It is a most significant grown local tree species of Arabia with historical, medicinal and religious benefits (Sameera and Mandakini 2015; Youssef et al. 2011; Adzu et al. 2002).

Nabag is a desert fruit, wild in its origin, and it can be grown with ease. It does not need abundant irrigation due to its desert nature, it lives on the banks of rivers and mountainous areas and spreads widely in the Mediterranean basin and the original home of the buckthorn is the regions of southern Europe, the Himalayas, northern China, North Africa, Sudan, Egypt, Iraq, the Emirates and South America (<https://suna-sd.net/ar/single?id=434642>). Its homeland is the Arabian Peninsula and its fruit is called (Nabag).

The fruits of nabag are sweet in taste and fragrance. They are grown in the winter and collected in the summer. They are planted in fields and gardens, and their fruits are eaten. In addition, its fruits, flowers and leaves are often used to prepare medicinal materials for ailments and diseases, and all these reasons have contributed to surrounding the Sidra tree with an aura of love throughout history.

Nabag fruit has a great position for the inhabitants of the Arabian Peninsula because it is rich in foodstuffs that are linked to stories and narratives, and there are those who believe that those who cut down the Sidr tree preach themselves death because they believe that Sidr is the abode of Paradise.

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Fig. 19.1 *Zizyphus spina-christi*.jpg

The chemical composition (%) of Nabag various parts as investigated by many researchers indicated that the contents of moisture in fruits, seeds, and leaves were (5.4–16.8%), (4–14.89%), and (7.72%), respectively. The main components include polyphenols, carbohydrates, minerals, oil, fatty acid and vitamins. The big number of these constituents were found in the fruits, followed by seed, stem and finally kernel oil (Ahamed 2016; Duke 1985; Adekunle and Adenike 2012; Ibrahim et al. 2015; Ahmed et al. 2015) (Fig. 19.1).

Nabag fruit taste like a combination of dates and apples and are profoundly valued by the Bedouins and were found to have a high energy value. The fruit can be eaten crude or dried for sometime in the future and has a lovely sub corrosive taste, fairly looking like dried apples (Facciola 1990). The seeds were wealthy in protein and the leaves in calcium, iron and magnesium. The food from this plant is a significant wellspring of energy, protein and minerals.

Nabag fruits can be eaten as a food because of their many health benefits, and they can also be used in preparing desserts, pies and drinks. Nabag, or the oil extracted from it, is also used in the cosmetic industry. The followings are the most important benefits of nabag:

1. It contains many nutrients: The nabag oil extract contains several vitamins, minerals, and antioxidants, which help prevent signs of aging and also contribute to the prevention of heart disease and cancer. The seeds and leaves also contain substances that help reduce blood pressure and protect against cardiovascular disease. Nabag fruits, which are used as a kind of food, contain potassium, calcium, magnesium, iron, phosphorous and many vitamins (B1, B2, B6, C, E) that are beneficial to human health.
2. Promote heart health: Nabag fruits oil helps reduce the risk factors associated with heart disease, it helps to reduce blood clotting, reduce blood pressure and cholesterol.
3. Prevention of diabetes: Nabag fruits oil may help prevent diabetes.

4. Skin care: Nabag fruits oil helps the skin to treat wounds, infections, and sunburn. It is also used in many cosmetic products to moisturize the skin and protect it from drying out.
5. Boost immunity: Nabag oil helps protect the human body from various diseases and microbes, and this benefit is attributed to the presence of antioxidants and other substances known as flavonoids.
6. Prevention of cancer diseases: Flavonoids and antioxidants may contribute to the prevention of various cancers, while their efficacy is not comparable to proven and established cancer treatments.
7. Liver health: Some studies have shown that buckthorn may help reduce the level of enzymes associated with liver inflammation.
8. Aids with digestion: Nabag fruit oil may help prevent and treat stomach ulcers.

This study aimed to elaborate on nabag fruit cultivated in Sudan and the manufacture of vinegar product from its edible part, as well as assessing its quality characteristics.

19.2 Vinegar Industry

The vinegar industry is very old, more than 500 years BC, and it was discovered by coincidence when it was observed that the formation of a sour taste and a pungent odor was observed when wine or beer was exposed to the air, resulting in beer and wine being turned into acetic acid by the acetic acid bacteria. Accordingly, vinegar can be defined as the substance resulting from the double fermentation process of suitable raw materials containing starch or sugar, or both, where in the first stage of alcoholic fermentation is produced by yeast, which often follows genus *Saccharomyces*. This yeast works to convert sugars into alcohol, while in the second stage of fermentation, the alcohol is oxidized by the vinegar acid bacteria, under aerobic conditions, to acetic acid, which is usually between 5–8% in vinegar.

Vinegar is obtained from apples, sugar cane, rice, barley, coconut, palm, dates, raisins, honey, and kiwi. The vinegar should be clear, transparent, free from solid sediments or suspended or floated membranes or those containing acetic acid bacteria. Vinegar should be old not freshly prepared so that it has the characteristic flavor of good vinegar and that the acid concentration is not less than 6.0%.

Vinegar is widely used in the food industry to give some products a special, desirable taste. It can also be used in the food manufacturing process as one of the components of the ketchup industry, in addition to its uses for many medicinal purposes. It is considered one of the important chemical reagents, and it is an industrial material used in the manufacture of polyethylene terephthalate (polyester), which is used in the manufacture of soft drinks, photographic films, wood glue, fabrics and fibers.

When making it, it is preferable to use utensils that are not subject to rust or corrosion, in order not reacts with salt or acid solutions formed during fermentation

processes. The most suitable utensils used for pickling operations are wooden containers “barrels” which are preferred to be those manufactured from oak or Sidr wood, and it must be cleaned before use and removed any smells in it. These barrels are washed with a caustic soda solution (1/2%), then with soap and water and washed properly with water before use to remove traces of alkali and when mixed with small amounts of vegetables.

There are many types of vinegar, depending on the raw material used in the manufacture:

1. Fruit vinegar: It is the vinegar resulting from the use of fruit juices such as grape juice, orange, strawberry, etc.
2. Vinegar resulting from the use of starchy materials: such as potatoes or starchy vegetables in general, and in this case the starches must be hydrated into fermentable sugars.
3. Malt vinegar: It is the vinegar obtained from the use of a drenched barley, wheat, corn, or others.
4. Vinegar resulting from sugary solutions: such as molasses and honey.
5. Distilled vinegar: It is vinegar resulting from the use of alcohol obtained from alcoholic residues (beer industry) or from yeast manufacture.

Vinegar is corrosive, and its vapor causes eye irritation, dryness and burning nose, sore throat and lungs. It is a weak acid because it is in standard conditions of temperature and pressure, the dissociating acid is in equilibrium with the non-shaped dissolved in the form of aqueous solutions, in contrast to strong acidity, which dissociate completely (FDA 2007).

In general, vinegar can be produced from any material that contains sufficient quantities of fermentable sugars or alcohol, provided that there is nothing to prevent it from being used in food.

19.3 Fruit Vinegar

Fermented juices from many types of fruits can be utilized to produce vinegar. It is technically doable to produce them from second quality fruit and even waste fruit (Monspart-Sényi 2006). Nonetheless, the fundamental explanation that fruits are not usually used to produce fruit vinegar is their low sugar content. In spite of the likenesses between the processes and the long tradition and knowledge accessible with respect to the elaboration of wine vinegars, this process isn't completely comparable to the production of fruit vinegars. Aside from the differences in sugar concentration between fruits, there are other factors to be considered as well. These factors incorporate the difficult extraction needed to get the juice of certain fruits, which prompts the utilization of commercial pectinolytic enzymes, and the high convergence of organic acids in some fruits, which can obstruct the development of certain microorganisms. It is imperative to take note of that numerous fruit vinegars are made by refining of an alcoholic solution, and the further addition of fruit juice

or fruit puree is accommodated their aromatization. These sorts of “non-natural” fruit vinegar are normally accessible in some Asian nations, for example, China, where the market has no particular guidelines for this kind of item (Chang et al. 2005). Even in Europe, clear regulation of these products doesn't exist.

As of late, various investigations have been led on these products that mainly concentrated on their sensory attributes and their quality parameters, which has been examined by chemical and sensory methods. Some examples incorporate the studies carried out with rabbiteye blueberry (Min-Sheng and Po-Jung 2010), apple (Liu et al. 2008; Sakanaka and Ishihara 2008), lemon, peach (Liu et al. 2008), persimmon (Sakanaka and Ishihara 2008; Ubeda et al. 2011), plum, and strawberry (Ubeda et al. 2011, 2012) vinegars.

Vinegar has many medicinal uses including:

1. It is either considered a generic or an antibiotic, which was known to be used in treatment scabies, strains, chronic ejaculation, scabies, wound treatment, and some types of poisoning, burning and varicose veins.
2. Used to inhibit the growth of cancer cells and solve some success in treatment of cancer.
3. It helps in treating swelling.
4. Helps to reduce haemorrhage and cosmetics.
5. It is involved in the manufacture of airplanes and stoves

19.4 Vinegar Composition and Specification

The components of vinegar generally depend on the nature of the raw material manufactured from it. Specification for natural vinegar:

1. To have a special flavor with the type of raw material from which it is produced
2. That the acid content of vinegar should not be more than 4% (weight / volume).
3. The ash content should not exceed 0.5% (weight / volume).
4. That the percentage of solid materials does not exceed 2%(weight / volume).
5. That the percentage of alcohol does not exceed 0.5% (weight / volume).
6. That the percentage of phosphoric acid does not exceed 0.5% (weight / volume).

19.5 Microorganisms Associated with the Vinegar Production

The microorganisms associated with the elaboration of vinegars are mostly yeasts and Acetic acid Bacteria (AAB). The former are the responsible for the alcoholic fermentation (AF), and the latter are required for the acetification.

19.5.1 *The Yeasts*

The yeasts are the most essential microorganisms during AF because they affect fermentation speed, wine flavor and other wine qualities (Pretorius 2000; Fleet 2003; Loureiro and Malfeito-Ferreira 2003; Jolly et al. 2006). The *Saccharomyces* genus is the most normally utilized genus in beverage industry. The *Saccharomyces* genus has a few novel attributes that are not found in different genera, for example, their higher ability to ferment sugar. This capacity permits them to colonize sugar-rich media and prevail over different yeasts, which are not as tolerant to alcohol. However, most of the non-*Saccharomyces* wine-related species have low fermentation activity (Ciani et al. 2010).

19.5.2 *Acetic Acid Bacteria (AAB)*

AAB are Gram-negative bacillus that is often found in single cell or in pairs. It is compulsively aerobic and thus forms membranes on the surfaces of fermentation tanks. They are not able to form spores, which enables us to eliminate them by pasteurization, and these bacteria are characterized by their tolerance of high acidity, but with a lower percentage of lactic acid bacteria (lactic acid). The optimum temperature for their growth and the production of a good amount of acid ranges between 26–31 °C (Thompson et al. 2001; Nielsen et al. 2007; Yamada and Yukphan 2008).

There are several subdivisions of acetic acid bacteria and one of these is a division Frateur who divided the acetic acid bacteria into four main groups:

1. Oxidans: *Ac. Melanogenum*
2. Suboxydans: *Gluconobacter oxydans*
3. Mesooxydans: *Ac. aceti*, sub sp. *xylinum*
4. Peroxydans.

The most important characteristics that must be met by vinegar bacteria:

1. To be able to produce vinegar acid in the appropriate quantity and speed without oxidizing the resulting acid.
2. To withstand relatively high concentrations of alcohol.
3. That the material is not sticky to prevent clogging of the openings of the fermentation apparatus.

It is worth mentioning here that some strains of vinegar acid bacteria are distinguished by their ability to oxidize the resulting acetic acid to carbon dioxide and water, that is, they are able to cause complete oxidation.

Among the most important of these types with the ability to cause full oxidation:

A. aceti, *A. xylinum*, *A. lancens*. Consequently, these aforementioned types are not desirable in the manufacture of vinegar, as the increase in their number in vinegar

in relation to other types leads to oxidation of the resulting vinegar and the formation of pure cellulose films, which appear as fleshy deposits or membranes.

19.6 Materials and Methods

19.6.1 Preparation of Samples

Nabag (*Zizyphus spina-Christi*) fruits were collected from Wad Medani market. To the Department of Food Engineering and Technology, College of Engineering and Technology University of Gezira. The fruits washed and soaked in water for 24 h, then seeds were removed and the pulp was filtered for vinegar production. The pulp was removed from the other fruit samples from the seeds manually, and then the pulp was crushed to powder and kept for further analyses. The pulp had an average sugar content of 9.4 degree Brix ($^{\circ}\text{Bx}$) and $\text{pH} = 3.9$. The soluble solids were adjusted to 18 $^{\circ}\text{Bx}$ using a sucrose solution. To enhance the sedimentation of the nonfermentable solids, 1 g/L of bentonite was added to the nabag pulp blend (Fig. 19.2).

The following chemicals were utilized in the vinegar production and analyses:

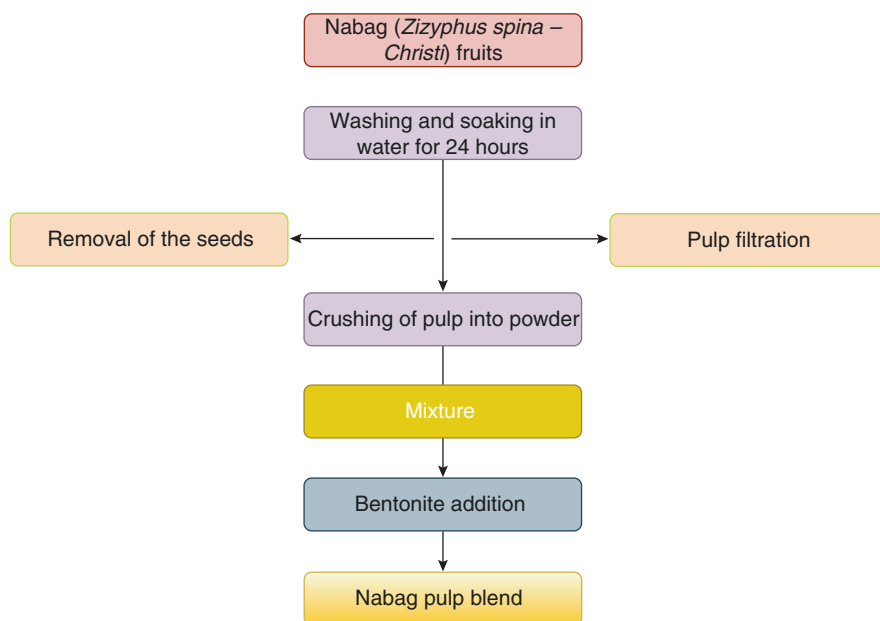


Fig. 19.2 Preparation of Nabag fruit pulp for vinegar production

- Sodium benzoate $C_7H_5O_2Na$ 1.25
- Pheno nphthaline $C_{20}H_{16}O_4$
- Absolute ethanol C_2H_5OH
- Hydrochloric acid HCl 0.02 N
- Boric acid CH_3BO_3
- Ammonium sulphate $(NH_4)_2SO_4$
- Sodium chloride $NaCl$
- Starch
- Acitic acid CH_3COOH
- Copper sulphate
- Calcium sulphate $CaSO_4$
- Sodium hydroxide $NaOH$
- Sulphuric acid H_2SO_4 conc.
- Alfa naphthol $C_{10}H_8O$
- Butanol C_3H_7OH
- Hexane C_6H_{14}
- Potassium chloride KCl
- Calcium chloride $CaCl_2$
- Silica gel
- Orthophosphoric acid
- Potassium sulphate

The yeast, (*Saccharomyces cerevisiae*) at an initial count of 10^7 cells/mL were utilized for the production of ethanol. The yeast was obtained from the local market, manufactured by Turkish Company, and was utilized according to the manufacturer's instructions. The yeast cells were rehydrated in sterile water at $38^\circ C$ for 30 min. and then inoculated into the nabag blend for alcoholic fermentation.

Acetic acid bacteria

19.6.2 Chemical Composition of Nabag Pulp

Nabag fruits were analyzed chemically to determine the contents of moisture, protein and fat according to AOAC (2000) methods. The concentration of free amino nitrogen (FAN) using the formol index method as described by Aerny (1996).

The sugar concentrations (glucose, fructose and sucrose) were measured with enzymatic kits (Boehringer Mannheim, Mannheim, Germany). Titratable acidity was estimated titration with 0.1 N NaOH and phenolphthalein as the indicator.

19.6.3 Fermentation and Vinegar Production

The method described by Paturau (1982) was utilized for the fermentation process and production of vinegar (flow chart 1).

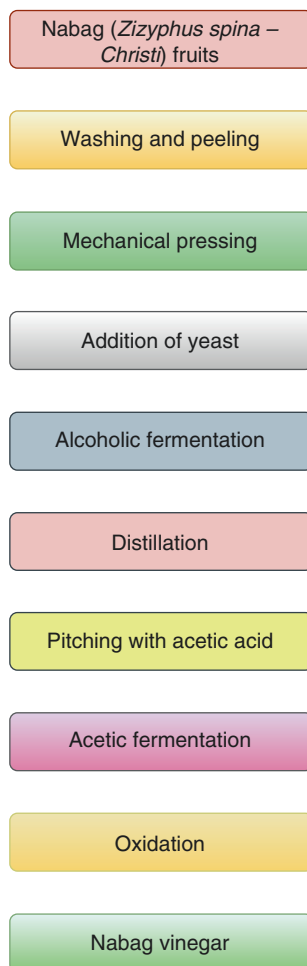
Five kg of nabag fruits were cleaned from extraneous matter, washed and soaked in 10 liter of distilled water for 24 h in stirrer tank, and then seeds were removed from the pulp and filtered. The brix and pH were measured by the refractometer and pH meter, respectively.

Half ml of orthophosphoric acid were added. Then 50 g of yeast were added and well mixed. Then batch fermentation was carried out at room temperature for 72 h in a closed in steel tank. Fermented slurry was kept in the refrigerator at $10-20^\circ C$.

The fermented slurry sample was distilled using a distillation unit, at 78–80 °C. Then produced ethanol was collected weighted and analyzed. After, this, 10 ml of acetic acid was added to the 100 ml of ethanol.

For vinegar production, 10 ml of acetic acid were blended with 100 ml ethanol in a conical flask, and then the flask was closed with a foil paper containing several pores in order to allow oxygen to enter for 72 h (the oxidation process) at a temperature of 37 °C. The vinegar harvest was then weighted and analyzed (Fig. 19.3).

Fig. 19.3 Flow diagram of nabag-vinegar production



19.6.4 Physicochemical Analyses of Ethanol

The pH values of the ethanol was measured using a pH meter (PHS-3C Digital) at ambient temperature according to ICUMSA (1994). The refractive index was estimated using an Abbe bench refractometer (ICUMSA 1998). The ethanol density was measured according to Scann (1971) and was calculated as follows:

$$\text{Density} = \text{weight} / \text{volume}$$

19.6.5 Determination of Ethanol Concentration

Abbe refractometer was used for the determination of the concentration of ethanol. A series of dilutions for absolute alcohol were prepared (10%, 20%, 40%, 60%, 80% and 100%), and the refractive index of the dilutions was recorded, then a curve was plotted. The concentration of the ethanol was determined from the curve (Fig. 19.4):

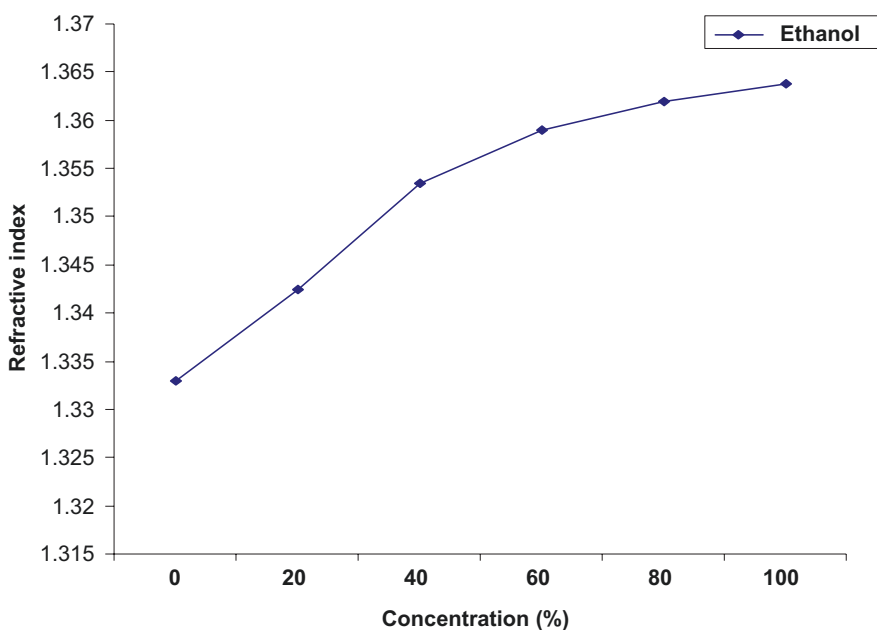


Fig. 19.4 Concentration of ethanol

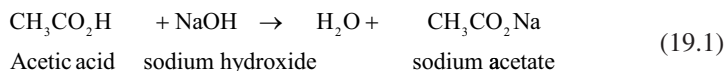
19.6.6 Physicochemical Analyses of Vinegar

The pH of the vinegar was measured using pH- meter (PHS-3C Digital) at ambient temperature (ICUMSA 1994).

The density of vinegar was measured as described by Scann (1971). The flacon (100 ml) was weighted then filled by ethanol, and weighed again, then the density of ethanol was calculated. The weight per volume of flacon.

$$\text{Density} = \text{weight} / \text{volume}$$

The concentration of acetic acid in vinegar to contrast it with the least required concentration of 4 g acetic acid per 100 ml of vinegar. The analytical technique followed utilizes the neutralization reaction between acetic acid and sodium hydroxide, in this strategy: sodium hydroxide solution of 1.0 molarity was contained in a burette, and the acetic acid solution was contained in an Erlenmeyer flask and phenolphthalein was added. Acetic acid reacts with sodium hydroxide, a base, according to the reaction:



This is an example of an acid-base neutralization reaction in which an acid and a base- react to produce water plus a salt.

In the titration strategy, NaOH was added to the acetic acid solution until complete reaction with all of the acid. The point where just enough base has been added to neutralize the acid was called the equivalence point. According to reaction (19.1), one mole of base reacted with one mole of acid. Therefore, at the equivalence point we have the relation.

Moles of base added = moles of acid initially present

Moles of base added = molarity of base x volume of base added and, therefore:

Moles of acid initially present = molarity of base x volume of base added

<http://wwwchem.csustan.edu/consumer/vinegar/analysis.htm> (1999).

19.7 Results

19.7.1 Chemical Composition of Nabag Pulp

Some of the chemical components of the nabag pulp are indicted in Fig. 19.5. The contents of moisture, protein, total sugars, fructose, sucrose, FAN, Titratable acidity and ascorbic acid were found to be $6.8 \pm 0.09\%$, 7.6%, 7.2%, 112, 50.2, 60, 118 (mg/l), 0.76% and 35.56 (mg/100 g), respectively.

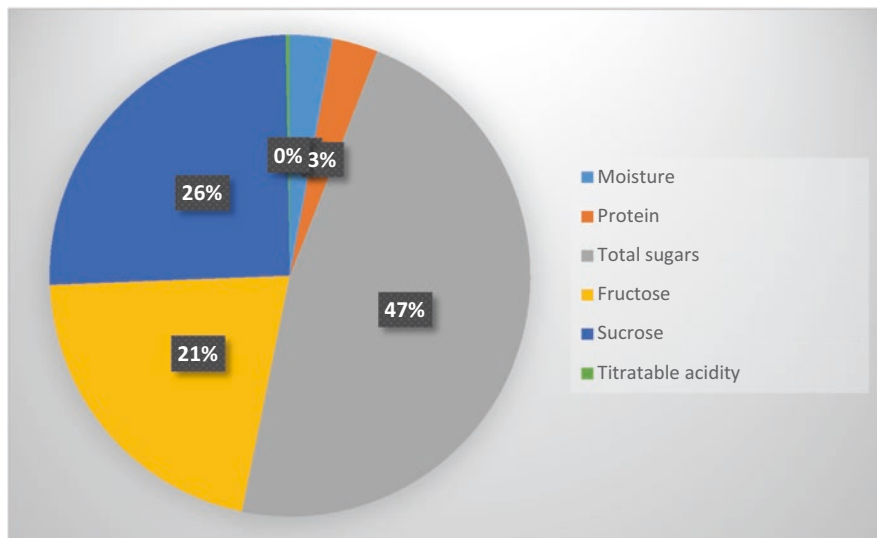


Fig. 19.5 Chemical composition of nabag pulp (per 100 g fruit)

Table 19.1 Physicochemical characteristics of ethanol resulting from nabag fermentation

Character	Value
Concentration	91%
Density	0.83799 at 33 °C g/ml
Refractive index	1.3720
pH	3.7

19.7.2 Physicochemical Properties of Ethanol and Vinegar

Table 19.1 presents some of the physicochemical properties of ethanol produced as nabag pulp fermentation with *Saccharomyces cerevisiae*. The concentration, density and Refractive index of ethanol was 91%, 0.83799 at 33 °C g/ml and 1.3720, respectively.

Table 19.2 shows some of physicochemical characteristics of the vinegar after production. The volume of the produced vinegar was 833 ml/kg of nabag. The vinegar had a density value of, The concentration of acetic acid, pH value and density value of the vinegar was 6.24 g/100 ml, of 2.92 and 0.93344 g/ml at 33 °C, respectively.

Table 19.2 Physicochemical characteristics of vinegar prepared from nabag fruit

Character	Value
Concentration of acetic acid	6.24 g/100 ml
pH	2.92
Density	0.933448 g/ml at 33 °C

19.8 Discussion

In the present study, vinegar was prepared from nabag fruit. All the processes were carried out with fruit pulp. In order to analyse the vinegar process and to prevent side effects caused by wood, we used glass containers glass containers, which were cleaned with boiling water.

Alcoholic fermentation of fruits is affected by the lack of balance between fermentable sugar and accessible nitrogen and the accessibility of various vitamins or minerals (Ribéreau-Gayon et al. 2006). Moreover, the utilization of selected yeast strains is a requirement and makes a critical commitment to the attributes of the final product in beer (Degre 1993). For the alcoholic fermentation., the greater part of the yeasts accessible for starting cultures have been chosen from brewing in light of the fact that they are acceptable performers, have low nutritional requirements, start fermentations rapidly, give great good fermentation rates, and produce secondary metabolites that are auxiliary metabolites that are valued consumers (Degre 1993). In the present study, the fruit utilized (nabag) has a large amount of available nitrogen, considering the fermentable sugar and comparing with that present in numerous fruits used to prepare vinegar. Other nutrients and vitamins are also available in nabag fruits (Hussein 2019).

The concentration of ethanol, density and Refractive index of ethanol was 91%, 0.83799 at 33 °C g/ml and 1.3720, respectively. The initial pH was 5.6 and decreased sharply to 3.7 after 24 h in the alcoholic fermentation. This value stayed steady all through the alcoholic fermentation.

The concentration of acetic acid in vinegar determined in the present study was within the scope of the standard value which was 4–8% (<http://en.wikipedia.org/Acetic acid>, 2011), and lower than the value detailed by the US Food and Drug Administration, Code of Federal Regulations (<http://www.Fda.gov/org/compliance-ref/cpg fod/cpg525-825-2006>) which expressed that vinegar product should contain a minimum of 4% acidity. Typical white distilled vinegar is at least 4% acidity and not more than 7%. Cider and wine vinegars are are normally somewhat more acidic with approximately 5–6% acidity (<http://www.versatile vinegar.org/ faqs>. Html, 2007).

The density of vinegar was lower than both the standard value (0.96 g/ml) and the house hold vinegar utilized for cooking (1.05 g/ml) (<http://en.wikipedia.org/wiki/vinegar>, 2009). It was expressed that density, or mass per unit volume for a typical commercial vinegar with 5% acetic acid content, is about 1.01 g/ml (<http://>

www.apple-cider-vinegar-benefits.com/apple-cider-vinegar-health-benefits.html, 2009).

During vinegar production, the acetification process is still just mostly comprehended. The vast majority of the vinegar is created from alcohol and a blend of supplements in industrial processes in which the seed culture is submerged in an exceptionally circulated air through tank and kept up constantly all through a batch process, with a daily refilling system. Nonetheless, the high quality vinegars are delivered with the conventional surface culture technique. In this strategy the acetic acid bacteria lie on the fluid air surface and produce a biofilm that utilizes oxygen straightforwardly from the air or from the restricted measures of air that go through the wood pores. Thus, most starter cultures in both cases have restricted accessibility and are inadequately characterized (Mas et al. 2007).

The yield of vinegar is satisfactory as it was in every case well over 60% (Bokhari 2013). The entire cycle was performed at the lab level, with such restricting elements as the strength of the press and the recuperation of fruit mash on a small scale. The final product got in this investigation demonstrated great colour and good sensory characteristics, with compensated pungent smell of the unpredictable acidity.

19.9 Conclusion

Since nabag is utilized as conventional food in the Sudan, however nowadays Sudanese individuals are not keen on nabag fruit such a huge amount with exemption for certain individuals who appreciate eating nabag tissue as nibble food particularly the youngsters. Next to this nabag cost is extremely low in the Sudanese market. Every one of these variables roused the specialist to utilize nabag underway of a significant item, for example, vinegar and consequently improve its economical value. The high nutritional and therapeutic values, beside high sugar content favoured its use in the production of vinegar. The volume of vinegar prepared from nabag fruit pulp was equivalent to 828 ml per kg of nabag. It is highly recommended to process vinegar under controlled conditions and increase the production of vinegar at a large – scale level. Vinegar should not be packed in plastic containers for its acidic properties which may lead to serious problems to the consumers.

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