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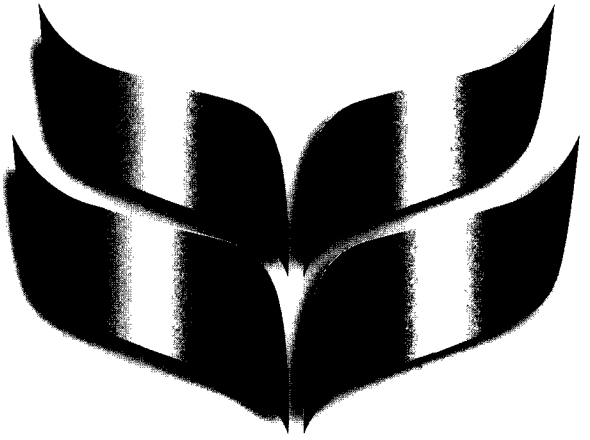

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Production and quality assessment of vinegar prepared from nabag (*Zizyphus spina christi*)

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Abstract

The objective of the study was to produce vinegar from nabag fruit and to evaluate its quality. The average chemical components of nabag fruit were; 6.6 ± 0.09% moisture, 7.0 ± 0.1% protein, 0.6 ± 0.1% oil, 3.0 ± 0.4% ash, 4.0 ± 0.7% fiber and 78.8% carbohydrate. The minerals contents (g/100g) were; 0.012 sodium, 0.36 potassium and 0.25 calcium. The ascorbic acid was found to be 36.28 (mg/100g). Nabag fruit also contained sucrose, glucose and fructose. The nutritional composition of nabag fruit pulp suggests that it can be used in food formulation as industrial raw materials. The alcoholic fermentation experiment was conducted to produce ethanol which was then oxidized to acetic acid depending on nabag sugar concentration. The concentration of the distilled ethanol after fermentation was found to be 89%. After oxidation process, 100 ml vinegar was obtained. The study indicated that the volume of vinegar production was 828 ml per kg of nabag and the concentration of vinegar was equivalent to 6.12% and had a pH value of (2.8).

Introduction

Zizyphus spina-christi is a shrub, sometimes a tall tree. The genus *Zizyphus* belongs to the family (Rhamnaceae) and with about 100 species of deciduous or green trees and shrubs distributed in the tropical and subtropical regions of the world. The Arabs call it nabak or sidr and in English it is called the christ's thorn (Lawton, 1989).

This species is found over the whole Sahelian area from Senegal to Sudan. It commonly grows by seasonal water courses and near water depressions.

All parts of the plant are used by the local people to help maintaining a healthy life style (Adzu *et al.* 2001 and 2002). The ripe fruits are edible and found in large quantities in local market. The seeds inside the fruits are roasted to be eaten. The fleshy part can be dried and pulverized to be baked under the heat of the sun. In Saudi Arabia, it is used for the treatment of ulcers, wounds, eye diseases and bronchitis. The Bedouin use it for the treatment of wounds, skin diseases and as an anti- inflammatory. They also use it as a febrifuge and diuretic. Plant leaves in south Iran are used in Iranian folk medicine as an

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antiseptic, antifungal, anti–inflammatory agent and for healing skin diseases (Amin, 1991; Nafisy, 1989). The fruit taste like a mixture of dates and apples and is highly prized by the Bedouins, have a very high energy value. Fruit can be eaten raw or dried for later use and has a pleasant sub acid taste, somewhat resembling dried apples (Facciola, 1990).

In Sudan, the plant is widely found in the western and central parts (Salih, 1991; Ismail, 1998) and fruits are sold in local markets. The tree is often planted near settlements to provide food, cash and shade (Vogt, 1995).

Vinegar has been known and appreciated as an important food adjunct (condiment and preservative) for a long time as man has been able to practice the arts of brewing and wine making. It may be defined as the product resulting from acidification of alcoholic solutions derived from sugary or starchy raw materials. It could be produced from a wide variety of raw materials, the main requirements being a satisfactory, economic source of alcohol and accessory flavoring constituents (Kofler and Hickey, 1954). Vinegar is made by two distinct biological processes, both the result of the action of harmless microorganisms yeast and *Acetobacter* that turn sugars (carbohydrates) into acetic acid. It contains many vitamins and other compounds not found in acetic acid such as riboflavin, vitamin B_1 and minerals from the starting material that impart vinegar with its distinct flavor. The objectives of the present study were to determine the physical and chemical composition of nabag fruits, to use nabag pulp for the production of vinegar and to assess the quality of the produced vinegar.

Materials and Methods

Materials

Nabag *Zizyphus spina -christi* fruit samples were obtained from Wad Medani local market. All samples were collected in plastic sacks and transported to the Department of Food Science and Technology, University of Gezria. Part of the nabag fruit samples were cleaned from foreign matter, washed and soaked in water for 24 hours in stirrer tank and the pulp was used to remove the seeds and filtered for vinegar production. The other part was obtained by removing the pulp from the seed manually and the pulp was crushed to powder by machine and kept for chemical analyses. All experiments were carried out in triplicates.

Methods of analysis

Physical analysis of nabag

The measurements of nabag fruit physical characteristics were done using Vernier Caliper. The characteristics included, volume and radius, while the weight of a single fruit was determined using a digital balance.

Proximate analysis of nabag

The proximate composition of nabag fruit powder was carried to determine crude protein, crude fat, crude fiber, moisture content, crude oil and ash according to AOAC (1998) methods. Total carbohydrate content was determined by difference (100-% moisture + crude protein + crude fiber + crude fat +ash).

Determination of ascorbic acid

The ascorbic acid (vitamin C) content was found according to the AOAC (2000) procedure using the following titration method. 30 g of pulp sample were blended with reasonable amount of 0.4% oxalic acid, filtered by Whatman No. 1 filter paper. The volume of the filtrate was completed to 250 ml with 0.4% oxalic acid. 20 ml of the filtrate were pipetted into a beaker, titrated with dye solution (0.2g of 2,6-dicholorophenol-indophenol dissolved in 500ml distilled water) till color of the solution changed to faint pink. The ascorbic acid content was calculated by the formula:

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{titer (ml) x dye strength x 100}}{\text{Factor A}}$$

Where:

$$\text{Factor A} = \frac{\text{sample wt. (g) x sample volume for titration (ml)}}{\text{Total volume of sample (ml)}}$$

The dye strength was determined by taking 5ml of standard ascorbic acid (0.05g ascorbic acid/ 250ml 10% oxalic acid solution) in a beaker and titrated with the dye solution to faint pink color.

Determination of minerals

Potassium (K), sodium (Na) and calcium (Ca) determination were carried using flame photometer (model Corning 400) according to AOAC (1970) in which different concentrations (5, 10, 15, 20, 25 ppm) were prepared from stock solutions of Ca, Na and K using the flame photometer, readings were taken and a graph was made. The sample was prepared by weighing 5g sample into a clean pre- dried and weighed porcelain dish. The dish containing the sample was placed in a muffle furnace at 550 °C and left to burn at this temperature for 5 hours. The dish with its content was weighed again after cooling in a desiccator to room temperature and ash content was determined. Ash was then dissolved in distilled water and 10 ml of HCl were added to make 100 ml. The absorption of the sample was measured and the concentration was determined from the calibration curve.

Qualitative analysis of sugar by Thin layer Chromatography (TLC)

Sugars in nabag powder extract were found by TLC as described by AOAC (1970).

Preparation of plates

Thin layer plates of thickness 0.25 and 0.50 mm were prepared using equipment from Shandon Scientific Instrument Ltd. Thirty grams of silica gel (G AND 60 GF 254, MERCK KGaA, Germany) containing 13% $CaSO_4$ were vigorously shaken for about one minute with a volume of distilled water equivalent to twice the weight of the gel and applied to 20×20 cm glass-plates set at the required thickness. The plates were heated in an oven for ½ hr at 110 ^{0}C before cooling in dessicator. The samples were applied to the plates and equilibrated against the developing solvent before chromatographic development.

Preparation of extract

The extract was prepared by taking 10 g of nabag powder in 20 ml of distilled water and allowed to stand for 4 hrs, filtered and a few micro liters were taken from the extract. The standard was prepared by weighing one g of the standard sugar, sucrose, glucose, fructose and maltose in 10 ml of distilled water. A few micro liters were taken and spotted on the plate. The plate was developed in a tank containing the solvent mixture butanol, acetic acid and water. The separation was completed when the solvent front reached 2/3 or 15 cm length. Then, the plate was taken out from the tank and dried in the oven. A coloring agent was sprayed after the drying of the plate and put again in the oven at 100 °C for 3 – 5 min. The spots which appeared were recognized by comparison with the standard sugars.

Fermentation and vinegar production

The methods of fermentation and vinegar production were carried out according to Paturau (1982).

Fermentation

Nabag fruit samples (5.60 kg) were cleaned from foreign matter; washed and soaked in 11 liter of water for 24 hours in stirrer tank. The seeds were removed from the pulp and filtered. The brix and pH were read using the refractometer and pH meter, respectively. The pH was adjusted to 4.8 using sodium hydroxide (1N). One gram of ammonium sulphate, 0.5 ml of orthophosophoric acid were added; then 50g of commercial yeast (from local market) were added, well shaken and the steel tank was closed for fermentation at room temperature for 72 hours. Fermented slurry was kept in the refrigerator at 10-20 °C.

Distillation

Using the distillation unit, the fermented slurry sample was distilled at 78-80 °C. The ethanol distillates was collected, weighed and analyzed.

Vinegar production

Ten ml of acetic acid were added to 100 ethanol in the flask. The flask was closed with a foil paper containing several pores to allow oxygen entry for 72 hours (the oxidation process) at a temperature of 37 °C. The produced vinegar was weighed and analyzed.

Ethanol analysis

Refractive index

The refractive index was determined using an Abbe bench refractometer according to ICUMSA (1998). As the measurement was affected by temperature changes, the sample of ethanol was cooled to correct temperature (20 °C). The refractometer was adjusted to zero with distilled water. A drop of the sample of juice was placed on the refractometer and reading of the refractive index was taken.

The Density

The density (The weight per volume) of ethanol was measured according to Scann (1971) as follows:

The flacon (100 ml) was weighed, filled with ethanol and weighed again and the density of ethanol was calculated.

Density = Weight / volume

Concentration

To determination the concentration of the processed alcohol refractometrically, series of dilutions for absolute alcohol were prepared in the order of 10%, 20%, 40%, 60%, 80% and 100%. Using the Abbe refractometer, the refractive index of the series dilutions was recorded respectively, and the curve was plotted. The refractive index of the sample was found using the same instrument and the concentration of the processed alcohol was found from the curve.

Vinegar analysis

pH measurement

The pH of the vinegar was measured using pH meter (PHS-3C Digital) at ambient temperature (ICUMSA, 1994). The electrode was rinsed and receptacle with portion of vinegar sample. A beaker was filled with the vinegar sample to a depth covering the bulb of glass electrode. Then the system was allowed to equilibrium and pH was read.

Concentration of acetic acid

The purpose of this experiment was to determine the concentration of acetic acid in samples of vinegar and to compare it with the required minimum concentration of 4g acetic acid per 100 ml of vinegar. The analytical method followed used the neutralization reaction between acetic acid and sodium hydroxide. In this method, sodium hydroxide solution of 1.0 molarity was contained in a buret, and the acetic acid solution was contained in an erlenmeyer flask and the phenonapthaline was added. Acetic acid reacts with sodium hydroxide (a base) according to the reaction:

$$\underset{\text{Acetic acid}}{CH_3CO_2H} + \underset{\text{Sodium hydroxide}}{NaOH} \rightarrow H_2O + \underset{\text{Sodium acetate}}{CH_3CO_2Na} \qquad (1)$$

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 method, NaOH was added to the acetic acid solution until just enough base was added to completely react with all of the acid. The point where just enough base was added to neutralize the acid was called the equivalence point. According to reaction (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).

The density of vinegar

The density of vinegar was measured as described by Scann (1971). The flacon (100 ml) was weighted, filled by ethanol and reweighed and the density of ethanol was calculated.

Density = Weight / volume

Results and Discussion

Physical analysis of nabag

The data presented in Table (1) showed some of the physical characteristics of nabag fruit. The fruit was red brown in color and had a globose shape. The fruit net weight, volume and diameter were 0.7108 ± 0.03 g, 1.38 ± 0.09 cm^3 and 1.29 ± 0.06 cm, respectively.

The diameter of nabag fruit (1.29 cm) was relatively lower than the values reported by both Vogt (1995) and Amanuel (1994) who reported values of

2 cm and 1.5cm, respectively.

Table 1. Physical properties of Nabag.

Parameters	Value (means ± SE)
Fruit volume (cm^3)	1.38 ± 0.09
Fruit radius (cm)	1.29 ± 0.06
Weight of single fruit (g)	0.7108 ± 0.03

Chemical composition of nabag pulp

As shown in Table (2), the pulp of nabag contained high amounts of carbohydrates, protein, fiber, ash, moisture and lower values of oil. The moisture content of nabag was 6.6 ± 0.09%; this value was slightly lower than that reported by Salih (1991) who found about 7.6% moisture content. The ash content (3.0 ± 0.4%) was lower than that reported by Salih (1991) of 5.2%. The protein content of nabag was 7.0 ± 0.1% which was greater than the value found by Salih (1991) of 5.6%. The fat content of nabag fruit was (0.6 ± 0.1%); this value was in close agreement to that reported by Salih (1991). The fiber content (4.0 ± 0.7%) was similar to that reported by Salih (1991) who found 4.1% fiber content for nabag. The carbohydrates content of nabag fruit was high (78.8%) and are useful in vinegar production. It needs to be converted by enzymes or acid hydrolysis to obtain a readily fermentable source of hexose sugar. The variation in chemical and physical properties of nabag depends on locality and other environmental conditions. The mineral content of nabag fruit was also presented in Table (2). The sodium content was 0.01g /100g which was similar to the value (0.01g /100 g) obtained by Younes *et al.* (1996) and Mahran *et al.* (1996). The potassium content was 0.36g /100g and this value was lower than the value (1.91 g /100 g) reported by Younes *et al.*(1996) and Mahran *et al.* (1996). As for calcium content, it was found to be 0.25 g/100g which was slightly lower than the value (0.61 g /100 g) reported by Younes *et al.* (1996) and Mahran *et al.* (1996).

A mineral found in vinegar is a vital catalyst in enzyme activity and is involved in the production of energy food. It assists in the uptake of calcium and potassium and required for the formation of healthy bones and teeth (http://www.apple-cider-vinegar-benefitis.com/apple-cider-vinegar-health-benfits.html, 2009). Nabag fruit also has appreciable amounts of vitamin A and C than apples (Anon, 1986). Some vitamins, amino acids and bioflavonoids found in nabag help to prevent cancer and protect the body against damage caused by exposure to chemical toxins (http://www.apple-cider-vinegar-benefitis.com/apple-cider-vinegar-health-benfits.html, 2009).

Ascorbic acid

The ascorbic acid in nabag fruit was about 36.28 mg/100g (Table 2). This value was higher than the that reported by Mahran *et al.* (1996) which was 30 mg/100 g. The Ascorbic acid (vitamin C) contributes to the nutritional value of fruits juices and is essential water-soluble vitamin. It also aids in the formation of liver bile which helps to detoxify alcohol and other substances. It was reported that, ascorbic acid reduces the activity of the enzyme aldose reductase which helps to protect people from diabetes. It may also protect the body against accumulation or retention of the toxic mineral lead. Ascorbic acid in nabag fruits is in amounts four times higher than in citrus fruits.

Table 2. Chemical composition of Nabag pulp (per 100 g fruit).

Parameters		Value
Moisture	(%)	6.6 ± 0.09
Protein	(%)	7.0 ± 0.1
Fat	(%)	0.6 ± 0.1
Ash	(%)	3.0 ± 0.4
Fiber	(%)	4.0 ± 0.7
Carbohydrates	(%)	78.8
Sodium	(g/100g)	0.012
Potassium	(g/100g)	0.36
Calcium	(g/100g)	0.25
Ascorbic acid	(mg/100g)	36.28

Separation of sugars by thin layer chromatography (TLC)

The nabag fruit contains about 4 different sugar compounds including sucrose, fructose, glucose and other unknown sugars. It is known that, the natural sugars converted to alcohol in the first stages of vinegar production is called alcoholic fermentation. Whatever the raw material, it must contain at least 8% of sugar and preferably more, for alcoholic fermentation to occur. After alcoholic fermentation, the regulation indicated that, vinegar must contain at least 4g acetic acid per ml (Kofler and Hickey, 1954). Salih, (1991) found that nabag fruit contained 21.8% starch, 17.4% glucose, 16% fructose, 9.6% sucrose and other sugars.

Ethanol production

Ethanol was produced as the first step of vinegar production using nabag fruit as a raw material. Table (3) shows the different characteristics of the produced ethanol. The concentration, density and refractive index of ethanol were 89%, 0.83799 g/ml at (32 °C) and 1.3630, respectively. The density value was lower than the value 0.94908 g/ml at 30°C reported by Eltayeb,

(2002) and greater than the value 0.807 g/ml reported by Gasm Alla, (2008). It was also greater than the standard density value which was 0.789 g/ml at 15 °C (San, 2000). The refractive index value 1.3630 was lower than the value 1.3638 of the absolute ethanol.

Table 3. Physicochemical characteristics of ethanol resulting from Nabag fermentation.

Character	Value
Concentration	89%
Density	0.83799 at 32 °C g/ml
Refractive index	1.3630

Physical and chemical characteristics of vinegar

The volume of the produced vinegar was 833 ml/kg of nabag. Table (4) shows some of the physicochemical characteristics of the vinegar after production. The vinegar had a density value of 0.93344 g/ml at 32 °C; concentration of acetic acid was 6.12 g/100 ml and a pH value of 2.80. The concentration of acetic acid in vinegar determined in the present study was within the range of the standard value which was 4-8% (http:en.wikipedia. org/Acetic acid, 2011) and lower than the value reported by the US Food and Drug Administration, Code of Federal Regulations (http:// www. Fda.gov/org/ compliance- ref/ cpg fod/cpg525-825.html. Accessed, March, 9, 2006) which stated that, vinegar product must 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 typically slightly more acidic with approximately 5-6% acidity (http://www.versatile vinegar.org/ faqs. html, 2007). Acetic acid in vinegar imparts the sour taste; it also possesses cleaning and antiseptic or germ killing properties. The pH of vinegar depends on the concentration of acetic acid. Most commercial distilled white vinegars contain 5% acetic acid and have a pH of 2.4. The pH value 2.8 was greater than the pH value of trade vinegar which was 2.42 (http://www.apple-cider-vinegar-benefitis. com/ apple-cider-vinegar-health-benfits.html, 2009).

The density of vinegar was lower than both the standard value (0.96 g/ml) and the house hold vinegar used for cooking (1.05 g/ml) (http://en,wikipedia. org/wiki/vinegar, 2009). It was stated that density, or mass per unit volume for a typical commercial vinegar with 5% acetic acid content, is about1.01g/ml (http://www.apple-cider-vinegar-benefitis.com/apple-cider-vinegar-health-benfits.html, 2009).

Table 4. Physicochemical characteristics of vinegar prepared from Nabag.

Character	Value
Concentration of acetic acid	6.12 g/100 ml
pH	2.80
Density	0.933448 g/ml at 32°C

Conclusions

- The study explored other avenues of using nabag in the production of vinegar to improve nabag economical value.
- The high sugar concentration in nabag favours 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.

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