

Determination of Some Quality and Safety Parameters for Black Raisin Juice

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Abstract

The aim of the present study was to determine the safety and the quality parameters of raisin juice, such as total soluble solids (TSS), pH, titratable acidity (TTA), kinds and quantity of sugars, phenolic acid compounds, polyphenoloxidase (PPO) enzyme activity, water activity and total microbial counts. The total soluble solids and pH values were significantly decreased after two weeks of storage, while the titratable acidity in most samples were slightly changed. Glucose, fructose and sucrose as well as three kinds of phenolic acids such as gallic, chlorogenic and vanilic acids were determined in all samples. Total microbial contents of the freshly prepared raisin juice and of the raisin juice stored for one and two weeks did not significantly changed. The water activity was slightly decreased, while the polyphenoloxidase activity was significantly decreased, after storage for two weeks. Consequently, it can be said that the freshly prepared raisin juice and the raisin juice stored for one week have superior sensorial properties than the raisin juice stored for two weeks.

Keywords: Quality and safety, black raisin juice, phenolic acid compounds, polyphenoloxidase

1. Introduction

Grape is a non-climacteric fruiting that grows on the perennial and deciduous woody vines of the genus *Vitis* that are mainly found in the moderate zones of the northern hemisphere and plurality divided between America and Asia (Mullins et al., 1992).

Drying is one of the oldest methods used to preserve fruits by reducing moisture contents and reaction rate to increase the shelf life of products. The moisture content is changed with drying period time of samples in open sun drying. Initial moisture content in grape varied between 78% and 80% (w/w), was reduced to the final water content of 22% (w/w). Three methods are used for dehydration of grapes; the first one is a considered, sun or natural dryers, which it is low-cost process also the quality of natural dried fruits is affected, and there is a little likeness to the fresh fruit. The second one is direct solar dryers. In this dryer, the material to be dried is placed in poly net-shad-with or without transparent covers or side panels. The third one is indirect solar dryer, which is the new and more effective technique of product

drying. The drinking of fruit juices could have both positive and negative impact on the health of consumers. Consumer demands safety and good processed foods with higher-quality properties have encouraged better food production (Riahi and Ramaswamy, 2004). Freshly expressed grape juice consists of 70 to 80% moisture and numerous dissolved solids. These soluble solids include many organic and inorganic compounds.

Natural juice is the non-fermented or fermented product obtained from fruit through the physical methods to maintain the basic structure and the quality. The juice can evoke the use of carbon dioxide (Iraqi standard, 1988). Shi et al. (2003) were investigated universal phenolic acids in grapes contain benzoic acids (galic acid, vanillic acid, protocatechuic acid, and p-hydroxybenzoic acids) and cinnamic acids (chlorogenic acid, caffeic acid coumaric acid, ferulic acid, and neochlorogenic acid). Phytochemicals such as phenolic compounds, carotenoids, flavonoids, and vitamins in fruit juices play an important role in disease protection (Gardner et al., 2000). The consumption of polyphenol-load juice improves antioxidant status. (Bub et al., 2003).

Raisin juice consumed fresh fruits and especially is valuable for its tangy taste. It has a low shelf-life during seven days storage in refrigerator; this is because of (1) natural contamination of raisin juice by bacteria and molds, (2) enzymatic activities (Polyphenol oxidase and peroxidase) and these lead to changes in taste and flavor during storage. Storage of raisin juices at refrigerator is not always best method for the prevention of desirable quality of some fruits. Water used for juice preparation can be the main source of microbial contamination. The polyphenol oxidase (PPO) enzyme is highly heat sensitive and its activity reduced during the dehydration process. The residues of this enzyme interfere with the discoloration of the raisins during storage. However, juice properties such as appearance, taste, and odor are made undesirably changed via effects of enzymes such as PPO enzyme, peroxidase, and pectin methylesterase (Awuah, 2007). For these case, there are big losses in their market value demand and product quality (Gauillard and Richard-Forget 1997).

No such research has been carried out in this field, related to Arbil and Sulaymaniyah grapes, which are most widely used in Iraq. In order to develop awareness people about raisin juices, this study efforts to measure phenolic compounds, ascorbic acid, activity of polyphenol oxidase enzyme and total microbial counts as the main parameters of quality and safety of raisin juice which is mechanically extracted.

The objectives of this study were: Characterization of local black raisin (*Vitis vinifera*) juice samples as quality and safety parameters: (1) determination of some physical and chemical properties including pH, brix values, and titratable acidity, (2) quantitative determination of phenolic acids using high-performance liquid chromatography (HPLC) in flesh, seed and juice of local raisins. (3) quantitative determination the activity of PPO in raisin and raisin juices, (4) microbial analysis during normal storage of samples and (5) determination of sensory quality and the acceptability of raisin juice drinks during storage.

2. Experimentals

2.1. Materials

Hand-held Refractometer (RHB-50ATC/0-50% Brix) was purchased from Grandidex company (China). Benchtop pH Meter BP3001 was purchased from Trans Instruments (S) Pte Ltd. (Jalan Kilang Barat, Singapur). UltiMate 3000 HPLC systems and UV-Visible Evolution 200 were purchased from Thermo Fisher Scientific (Waltham, MA USA). 4-Hydroxybenzoic acid, vanillic acids, and galic acids, D - (+) glucose, acetoneitrile, methanol, phosphoric acid, formic acid, potassium phosphate monobasic, caffeic acid, chlorogenic acids, sucrose, maltose monohydrate, D-fructose, ascorbic acid solution, polyphenol oxidase enzyme solution, ethylenediaminetetraacetic acid (EDTA) solution, and potassium dihydrogen phosphate were purchased from Sigma-Aldrich (Darmstadt, Germany). Phenolphthalein and sodium hydroxide solution were purchased from Merck (Darmstadt, Germany). CHROMAFIL XTRA PVDF-20/25 filter was purchased from Macherey-Nagel GmbH & Co. KG (Duren, Germany)

2.2. Methods

2.2.1. Collecting the black raisin samples

Locally black raisin juice made from seed *Vitis vinifera* grapes, four samples of black raisins (black dried grapes) (Tre-rash varieties sample A, B) from Sulaimaniya and (Rashmiree samples C, D) from Arbil were collected from different local market. Two natural black raisin juice was extracted (A and C)

and another two samples of raisin juice (B and D) were purchased from local market and then all samples were used for experiments in order to investigate the quality and safety of locally raisin juices in four bottles were stored at +4°C for two weeks.

2.2.2. Preparation of freshly squeezed raisin juice samples for analysis

The samples were prepared for analysis according to Abdullah's method (2012). 1 kg of raisins was washed in tap water, and soaked in water for some hour, and mixed with 2.5 L of water and were grounded before being squeezed with the cheese-cloth. The prepared raisin juice samples A and C were stored at +4°C in the refrigerator for two weeks until used for analysis..

2.2.3. Determination of moisture content

5 g raisins from each sample (A to D) were dried in an oven at 70 °C for six hours, and then after cooling in a desiccator, the samples were weighed to a constant value. Three replicates of weighing was conducted for each sample. The Equation 1 was used to calculate the moisture content of samples (Anonym, 1992).

$$M_n = \left[(W_w - W_d) / W_w \right] \times 100 \quad (1)$$

in where:

M_n = moisture content (%) of material

W_w = wet weight of the sample, and

W_d = weight of the sample after drying.

2.2.4. Determination of pH values of sample

The pH values of raisin juices were determined using pH meter (Benchtop pH Meter BP3001), at room temperature. Before measurements, pH meter was calibrated using calibration buffers (pH 4, 7 and 10). Raisin juices were tested three times (0, 7 and 14 days), in triplicate during storage in a refrigerator at +4 °C.

2.2.5. Determination of total soluble solids (Brix value)

Total soluble solid (TSS) contents of raisin juice samples were determined as Brix value using a refractometer. To determine the Brix levels of the raisin juice samples, a few drops of the samples were placed on the prism and closed before reading the refractometer scale, then the values at 20 °C were recorded three times for each sample. TSS values of raisin juices were determined three times (0, 7 and 14 days) during storage in a refrigerator at +4 °C.

2.2.6. Determination of total titratable acidity

5 mL of raisin juices were taken for each sample and distilled water was added up to 100 ml in a 250 mL conical flask, then titrated against 0.067 N sodium hydroxide solution using phenolphthalein as indicator. Three replicates for each sample were conducted. The total titratable acidity (TTA) was calculated as g/L tartaric acid according to Equation 2 (Xu et al., 2012) Raisin juices was tested three times (0, 7 and 14 days) during storage in a refrigerator at +4 °C.

$$TTA \text{ (g/L) Tartaric Acid} = \frac{NaOH \text{ (mL)} \times 0.1N \text{ NaOH} \times 0.075 * \times 1000}{\text{Sample volume (mL)}} \quad (2)$$

*= equivalent weight of tartaric acid

2.2.7. Determinations of kinds and quantity of sugar contents

Fructose, glucose, sucrose and maltose contents were determined using HPLC according to Turkish Standard Method (TS, 2008). 7.5 ml of raisin juice and 2.5 ml of methanol were mixed in a flask and shaken. After the mixture was filtered into HPLC vials using filters (pore size 0.25 µm) before HPLC analysis. All samples were analyzed three times (0, 7 and 14 days) during storage at +4 oC.

2.2.8. Determination of phenolic compounds by using HPLC

Determination of quality and quantity of phenolic acids in seeds and flesh of raisins individually as well as in fresh raisin juice, after one week and two weeks from a squeezed raisin juice and stored at +4 °C in the refrigerator until analysis have been done by using HPLC.

2.2.8.1. Sample preparation

A- Seed and flesh. 2 g grounded seed and flesh were taken into the volumetric flasks. 10 mL of distilled water: methanol mixtures (95:5) were added and then were shaken in water bath at 200 rpm and 50 °C for one hour, later were centrifuged at 5000 rpm for 5 min. The extracts were filtered into vials using filters (pore sizes were 0.22 µm) and used for HPLC analysis.

B- Raisin juices. 0.5 mL methanol samples were added into volumetric flasks containing 9.5 ml juices and after then this mixtures were filtered using vials with 0.22 µm filter. The filtrates were used for the analysis with HPLC

Analytical column: Thermo 250mm x 4,6 mm x 5 µm ID, hypersil gold

Flow rate: 0.75 mL / min

Solvent A: Water: Formic Acid (98: 2) **Solvent B:** water: Acetonitrile: Formic Acid (78:20:2)

Column temperature: 28 °C ± 1 °C

Injection volume: 20 µL

Standard stocks:

Gallic acid: 30 ppm (280 nm)

Colorogenic acid: 60 ppm (280 nm)

Caffeic acid: 20 ppm (320 nm)

4-Hydroxy aenzoic Acid: 40 ppm (280 nm)

Vanilic acid: 20 ppm (280 nm)

1 min 0.75 mL/min A %75 B %25.

5 min 0.75 mL/min A %50 B %50

10 min 0.75 mL/min A %25 B %75

12 min 0.75 mL/min A %25 B %75

15 min 0.75 mL/min A %0 B %100 (Slightly modified)

The system components were SPD-M20A diode array detector, SIL-20A HT autosampler, CTO-20A column oven, and DGU-20A5 degas units. Chromatography was performed at wavelengths of 280 nm and 320 nm (Table 1) and a C18 column with 250×4.6×5 mm dimensions was used. Mobile phase A; water/formic acid (98:2), mobile phase B water/acetonitrile/formic acid (78:20:2) and the mobile phase C was regulated as 100% methanol. Injection volume 20 µL, the flow rate was 0.75 mL/min and the temperature was set at 28 °C. As the flow gradients of mobile phases were given above. As a flow program External standards were used in order to the identification of the different phenolic compounds. For example, phenolic acids standards were used at least with 5 different concentrations. All calibration coefficients (R^2) of standard plots were greater than 0.990.

Table 1. Analysis parameters of phenolic acids

Sample Name	UV (nm)	RT (min)	100%	80%	40%	20%	10%
Gallic acid	280	4.187	30 ppm	24 ppm	12 ppm	6 ppm	3 ppm
Cholorogenic acid	280	4.877	60 ppm	48 ppm	24 ppm	12 ppm	6 ppm
Caffeic acid	320	6.797	20 ppm	16 ppm	8 ppm	4 ppm	2 ppm
4-hydroxy benzoic acid	280	6.763	40 ppm	32 ppm	16 ppm	8 ppm	4 ppm
Vanilic Acid	280	9.793	20 ppm	16 ppm	8 ppm	4 ppm	2 ppm

2.2.9. Enzymatic assay of Poly Phenol Oxidase

Method: Continuous Spectrophotometric activity determination.

Reagents:

1. 50 mM potassium phosphate buffer, pH 6.5
2. 5 mM L-3,4-Dihydroxyphenylalanine solution.
3. 2.1 mM L-ascorbic acid solution.
4. 0.065 mM Ethylenediaminetetraacetic acid solution (EDTA).
5. Poly Phenol Oxidase (PPO) enzyme solution (containing 500 - 1000 units/mL of PPO in cold reagent A.)

Procedure: The following reagents were pipetted (in milliliters) into suitable quartz cuvettes (Table 2). They were mixed by inversion and equilibrate to 25°C. Monitor the A_{265 nm} until constant, using a suitable thermostatted spectrophotometer. Then added reagent E. They immediately mixed by inversion and record the decrease in A_{265 nm} for approximately 5 minutes.

The A_{265 nm}/minute is obtained using the maximum linear rate for both the test and blank.

Calculation: Enzyme unit was calculated using Equation 3.

$$\text{Units / mg enzyme} = \frac{\Delta A_{265 \text{ nm}} / \text{min Test} - \Delta A_{265 \text{ nm}} / \text{min Blank}}{(0.001) (\text{mg Enzyme} / \text{RM})} \quad (3)$$

0.001 = the change in A_{265 nm}/minute per unit PPO at pH 6.5 at 25 °C in a 3 mL reaction mixture.

RM = Reaction Mixture (3 mL)

Unit definition: One unit was enzyme amount that caused the change in A_{265 nm} of 0.001 per minute in a 3 mL reaction mix containing L-3,4-dihydroxyphenylalanine and L-ascorbic acid at pH 6.5 at 25°C.

Final assay concentrations: In 3 mL reaction mixture, the final concentrations: potassium phosphate buffer was 50 mM, L-ascorbic acid was 0.17 mM and PPO was 50-100 units (Dawson et al., 1955; Marumo et al., 1986).

2.2.10. Water activity

Measurement of water activity (a_w) was carried out using a water activity meter at 25°C. The raisin samples were first sliced into thin pieces and placed in a small chamber and a few drops of the raisin juices were added into the reading cell. All samples were tested 3 times (fresh, after one week and two weeks) during storage in a refrigerator at +4 °C. The water in the chamber air was measured after reaching equilibrium conditions and the result at 25°C were recorded for each samples (steady state) (Aktas and Polat, 2007).

2.2.11. Total bacterial counts

Total bacterial counts were determined on 0, 7 and 14. days of storage in a refrigerator at +4°C. 10 mL of the samples were diluted with 90 mL of sterile distilled water and mixed well to obtain 10⁻¹ dilution. Serial dilutions 8-10 were prepared and spread plate technique was used on appropriate selective

Table 2. The amounts of reagents and blank

Reagent	Test	Blank
Reagent A (Buffer)	2.60	2.80
Reagent B (L-DOPA)	0.10	0.10
Reagent C (Ascorbic Acid)	0.10	0.00
Reagent D (EDTA)	0.10	0.10
Reagent E (Enzyme solution)	0.10	0.00

media. Duplicate nutrient agar plates were used for each sample and incubated at $37\pm 1^{\circ}\text{C}$ for 48 hours. Plates containing 30-300 CFU/mL were selected for counting 3 times during storage in a refrigerator at $+4^{\circ}\text{C}$ (Braide et al., 2012).

Number of colonies on plate \times Reciprocal of dilution of samples = Number of bacteria/mL

2.2.11. Sensorial evaluation

Sensorial evaluation of raisin juice is very important and gives a reliable opinion to appear the acceptability of new products. Samples were presented to at least ten panelists inside a plastic bottle. Appearance, aroma, taste, sweetness, and texture/mouth-feel were evaluated by the panelists on 0, 7, and 14 days of storage in terms of consumer acceptability. Panelists scored the samples using a five-point hedonic scale where a score of 1 dislike a lot and a score of 5 like a lot (Watts, 1989).

2.2.12. Statistical analysis

The PROC GLM (General Linear Model) procedure (SAS, 1999) was used to analyze the data in this study. Moreover, the Duncan multiple range tests used to a comparison between means for all traits under (≥ 0.05) level.

3. Results and Discussions

3.1. Determination of moisture content in raisins

Determining the moisture content of raisin plays an important role in estimating the shelf life consumables of grape berries. The grape properties directly affect raisin quality. Raisin is not considered as the agricultural perishable products which can be hardly deteriorated by microorganism because of its low content of moisture and water (Christensen, 1995). The obtained data showed that the highest moisture content (16.5%) determined in sample B whereas sample C showed the lowest moisture content (14.9%) (Fig. 1). Moreover, no significant differences observed between moisture content in all samples, except sample C which was significantly different from other samples. This is compatible with who reported the range of moisture content from 11-18% (Christensen, 1995).

3.2. Determination of pH values

One of the crucial quality properties that illuminate the stability of bioactive compounds in fruit juice is pH. Compounds such as organic acids are responsible for providing low pH in fruit juice. The pH can either rise or fall. (Tasnim et al. 2010). Fig. 2 showed that, fresh raisin juice sample A had the highest value of pH (4.23) and sample D had lowest value pH (2.96) in raisin juice sample refrigerated at the end of period (14 days), as well as the maximum differences between the fresh sample and last day of refrigerated sample was (-1.10) in sample D. At the same time, the influence of storage time had dramatic effects on the pH value in all samples; there was a significant decrease in pH value after 1 and 2 weeks. Boulton (1980) reported that the precipitation of potassium bitartrate either during fermentation or stabilization will cause fall of pH. This result agrees with the results obtained in study of Mehmood et al (2008) related to the study of the effect of pasteurization and chemical preservatives on the quality and shelf stability of apple juice stored at ambient temperature for three months. However, similar results were found by Wisal et al (2013). The reason of decrease in the pH value during storage, it is possible to have biochemical reaction taking place during storage periods together with microbial action in the juices (Ibrahim, 2016). Otherwise, these results are a reverse portion of the results obtained by Mgaya-Kilima et al (2014), and Islam et al (2015).

3.3. Determination of TSS

TSS values of raisin juice stored under different storage period were given in Fig. 3. The total soluble solid values were ranged from 18.06% to 20.33% in fresh raisin juice A and B, respectively. However, the maximum value reduction of TSS was reached, while the lowest reduction was reached in samples after two weeks of storage. Moreover, in refrigeration condition, the TSS in all samples was decreased significantly after 2 weeks, but there was no significant difference between most of the samples after 1 week. This finding is consistent with the study of Wisal, et al (2013). They reported that approximately 0.5 Brix of TSS decreased in strawberry juice after being refrigerated for 20 days. Arin and Akdemir (2004) found the same results. TSS decrease at the end of the storage period (2 months). Bull et al. (2004) also investigated that the TSS by Brix of thermally processed Valencia and Navel orange juice.

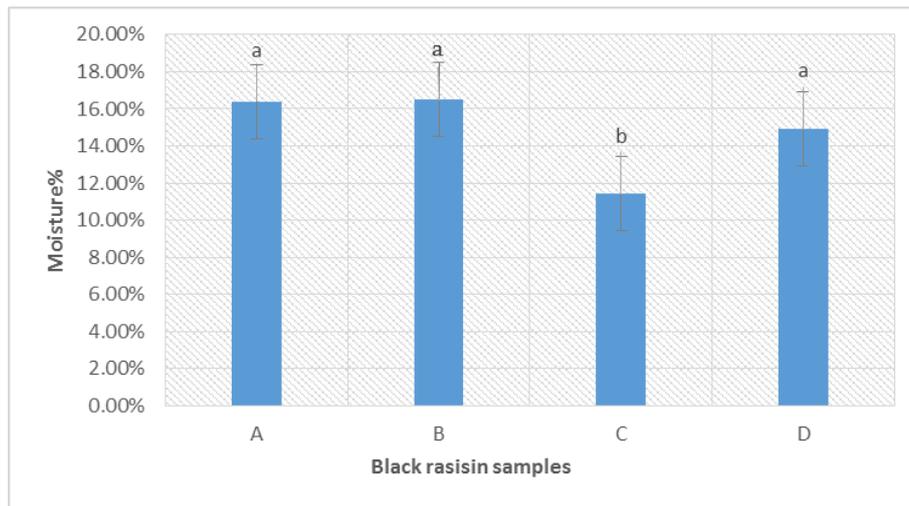


Fig 1. Moisture contents of raisin samples (A-D)

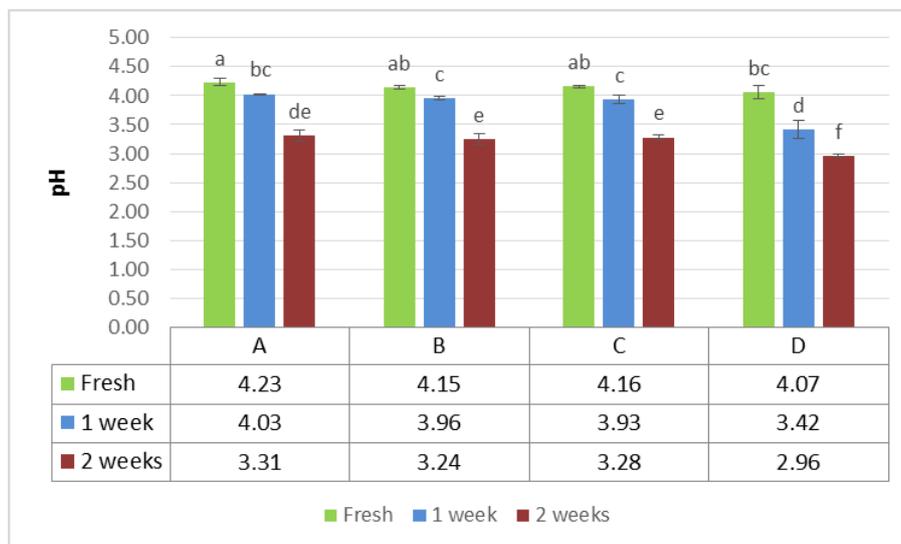


Fig 2. pH values of raisin juices (Samples A-D)



Fig 3. Total Soluble Solids (°Brix) of raisin juices

TSS was not significantly changed during storage time in a refrigerator at +4 °C. On the other hand, this is a contrast with the observation by Mgaya-Kilima (2014) and Bhardwaj and Pandey (2011). Retention or a slight increase in the TSS of the roselle-fruit blends observed during 6 months at both storage temperatures +4 °C and 28 °C. Increase in total soluble solids may be due to break down of polysaccharides into monosaccharide and oligosaccharides that decreased possibly due to fermentation of these sugars into ethyl alcohol, carbon dioxide, and water.

3.4. Determination of TTA

Tartaric acid is most important acidic component in grape or raisin juice, and important standard for grape juice (Kanellis and Roubelakis, 1993). The main organic acid extracts in grapes are tartaric, malic, also little quantity of citric and succinic acid (Kliwer, 1966). The other acids in raisins obtained from different grape varieties return to tartaric, malic, citric, succinic acids during storage. Statistical analysis showed that the storage does not influence the titratable acidity in most samples. The formation of lactic acid, either during the alcoholic fermentation or the malo-lactic fermentation leads to lowering of both the total acidity and the titratable acidity (Boulton 1980). The same author reported that the precipitation of potassium bitartrate either during fermentation or stabilization will reduce both the total acidity and titratable acidity. As shown in Fig. 4, there are a significant difference between most of the fresh samples. The TTA in fresh samples B and D, were determined to be 3.01 g/L and 8.14 g/L, respectively. The difference in TTA between the grape cultivars was expected, this can be due to some factors such as climate, variety, genetic and cultural practice (Dharmadhikari, 2010). High TTA can be an indication of the presence of tartaric acid (Kodur, 2011). As seen in the figure, D sample stored for two weeks showed the most TTA value (9.57 g/L) while fresh B sample had showed the lowest TTA value (3.01 g/L). According to Fig. 5, the TTA values of all samples had increased linearly with decreasing pH values.

3.5. Qualitative and quantitative determinations of sugar contents

One of the most crucial components of fruit products, essential for and also act as a natural food for prolonging of shelf life are sugars (Bhardwaj and Pandey 2011). The sugar content in grape is essential to produce raisins or table grape with an acceptable quality. It has been stated that the initial sugar content before drying should be more than 22° Brix (Amerine, 1981). The raisin juice has a sweet taste; consequently, the major parameters for evaluating the quality of raisin juice are the concentration of sugars and pH acidity. Basically, with trace content of sucrose, glucose, and fructose which are present in grape or raisin juice, as well as there were considered the most soluble in water because there are belonged to reducing sugar as a monosaccharide. More significantly the hexose sugars in raisin juice are converted into alcohol through anaerobic fermentation conducted by yeasts.

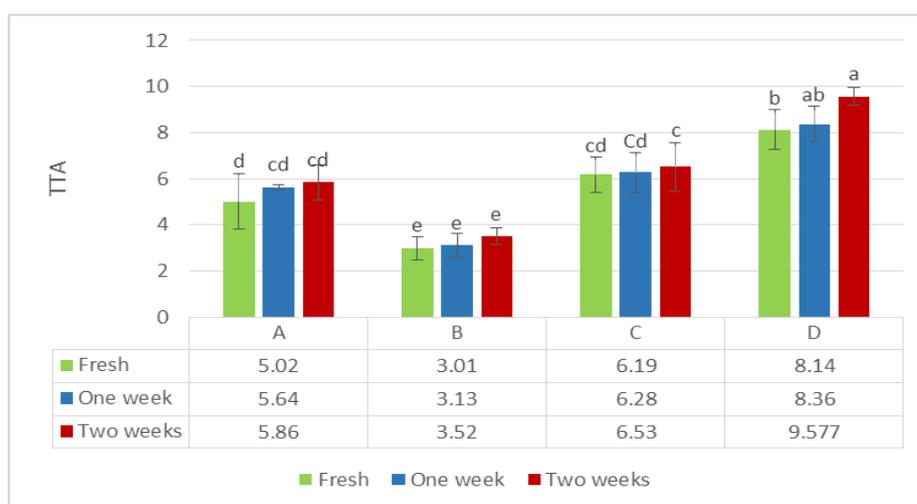


Fig 4. TTA of raisin juices

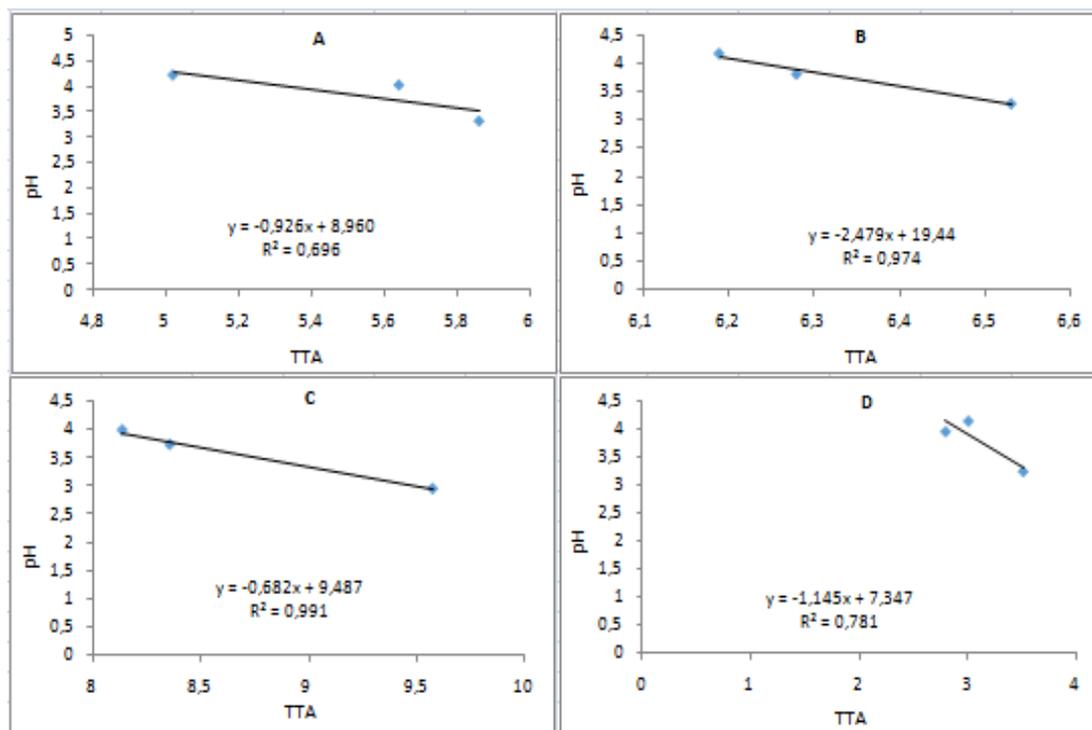


Fig 5. Relationship between pH and TTA.

In the present study, HPLC method was used to determine the quality and quantity profiles and concentrations of sugars in samples are shown in Table 3. Identified three types of sugars in raisin juice which are included glucose (53.52 - 99.127 g/L) and fructose (45.04 -104.80 g/L) as well as the trace amount of sucrose (0-16.67 g/L). These results were consistent with the results of Duran (2014) who observed that glucose and fructose concentration of 8 different grape cultivars were the predominant sugars in grape berries ranging from 89.4 to 144.24 g/L and from 92.1 to 139.4 g/L, respectively. According to the table, glucose/fructose ratio was between 0.97-1.07. Furthermore, the ratio of glucose/(fructose+ sucrose) was between 0.95-1.06. These are similar to results reported by several researchers. Shiraiishi et al.(2010) reported that the ratio of glucose/(fructose + sucrose) is higher than 0.8 depend on types of grapes based on sugar composition. Dai et al (2011) reported that the ratio of glucose to fructose ranged between 0.47-1.12, depend on the maturity of grapes. On the other hand, Elsheikh, et al (2014), illustrated that, the total sugars of Abu Samaka concentrate stored in the refrigerator decreased after 2 months, these illustrate consistent with the obtained results in the present study.

3.6. Determination of phenolic compounds by using HPLC

Refrigeration slows down the chemical and biological processes in foods, and the accompanying deterioration and loss of quality of nutrients. The occurrence of sufficient amount of phytochemical such as carotenoids, flavonoids, and bioactivity of phenolic acid compounds in grape or raisin juice can be standard the quality and high nutrition value. Kahkonen et al., (2012) described that, the grape juice is a major source of bioactive compounds such as phenolic compounds and the consumption of this product is related to several health benefits to consumers. Table 4 summarizes all identification and quantification of phenolic compounds determined in studied raisins (seed and flesh), fresh raisin juice samples and raisin juice refrigerated for 14 days.

In this study, the result showed that chlorogenic acid, gallic, and vanilic acid were detected as a three types of phenolic bioactive compounds in both seeds and flesh of raisins individually. Furthermore, the sum of total phenolic acids quantified in seeds and flesh of raisins varied from 933.57 to 1434.13 mg/ kg and 1060.85 to 2645.59 mg/kg for samples B, D, A and C, respectively.

Table 3. Sugar content of black raisin juices

Samples	Times	Sugar Concentrations (g/L)			Significant Ratios (%)	
		Glucose	Fructose	Sucrose	Glu/Fru	Glu/(Fru+ Suc)
A	Fresh	79.30	83.43	0	0.95	0.95
A	One week	77.69	82.85	0.26	0.93	0.93
A	Two weeks	53.52	45.04	0.89	1.18	1.16
B	Fresh	99.12	104.80	6.52	0.94	0.89
B	One week	94.39	100.51	0.01	0.93	0.93
B	Two weeks	99.10	98.71	0.46	1.00	0.99
C	Fresh	63.15	57.90	0	1.09	1.09
C	One week	62.86	60.05	0.36	1.04	1.04
C	Two weeks	60.05	53.26	0.04	1.12	1.12
D	Fresh	67.37	68.85	16.67	0.97	0.78
D	One week	66.60	70.64	0.16	0.94	0.94
D	Two weeks	56.93	53.15	0.15	1.07	1.06

Table 4. Phenolic acids in seed and flesh of raisin

Phenolic acids	Phenolic Acid Concentrations (mg/kg)							
	A		B		C		D	
	Seed	Flesh	Seed	Flesh	Seed	Flesh	Seed	Flesh
Gallic acid	404.96	532.69	607.78	650.46	699.31	704.01	818.76	745.68
Chlorogenic acid	604.86	428.26	278.09	843.84	592.5	1855.8	599.7	1137.34
Vanilic acid	27.19	99.90	47.70	108.55	15.75	85.78	15.67	77.22
Total	1037.01	1060.85	933.57	1602.85	1307.56	2645.59	1434.13	1960.24

The variation of phenolic concentration in seeds and flesh of raisin is caused from the variety of grapes, drying process to produce raisins and storage period, as described by Hassan et al (2015). The results we obtained in this study are in good agreement with the results obtained by Shi et al (2003) and by Eydurán et al (2015). On the other hand, the results given in Table 5 shows that the same phenolic acid compounds identified with a different concentration in fresh raisin juice and in refrigerated raisin juices for one and two weeks. In terms of gallic, chlorogenic and vanilic acids the highest contents were proved with fresh raisin juice samples D, C and A respectively, which were significantly different from the others. This result is indicated the high nutrient value regarding of bioavailability of phenolic compounds. The increase and reduce levels of this compounds as well as create and degrade the organoleptic qualities of raisin juice for each term of phenolic compounds individually, after one and two weeks of storage were observed. The gallic acid compound was significantly increased in sample A after one week and at the end of storage period of raisin juice, while slightly increased in sample C after both storages as well as sample D was slightly increased after one week and significantly increased after two weeks of storage for the same term of phenolic acids compound (Fig. 6). Hence, the reduce of gallic acid in fresh raisin juice sample B was no significantly difference between one and two weeks of storage. However, the highest increase in the level (17.23) of gallic acid was recorded in sample A after two weeks. In the present study, chlorogenic acid was significantly increased in sample A and B after one week storage,

while the vanilic acid compound was significantly changed in samples A and did not significantly changed in sample B after one and two weeks. At the end of refrigerated

Table 5. Galic, chlorogenic and vanilic acids in raisin juices

Phenolic acids	Phenolic Acid Concentrations (mg/kg)				Storage
	A	B	C	D	
Galic acids	67.99 ± 0.53	52.09 ± 0.10	104.37 ± 0.15	115.63 ± 0.56	Fresh
Chlorogenic acid	67.96 ± 0.67	29.48 ± 0.44	242.87 ± 0.85	132.66 ± 0.20	
Vanilic acid	17.67 ± 0.42	6.61 ± 0.01	13.68 ± 0.65	8.43 ± 0.02	
Total	153.62	88.18	360.91	256.71	
Galic acids	81.38 ± 0.09	50.86 ± 0.74	106.07 ± 0.37	118.28 ± 0.47	One week
Chlorogenic acid	84.28 ± 0.23	33.66 ± 0.29	232.98 ± 0.05	70.37 ± 1.65	
Vanilic acid	12.72 ± 0.02	6.68 ± 0.10	12.75 ± 0.15	7.95 ± 0.17	
Total	178.38	91.19	351.80	196.60	
Galic acids	85.22 ± 0.28	47.48 ± 0.81	110.03 ± 0.20	126.03 ± 0.09	Two weeks
Chlorogenic acid	79.30 ± 0.37	26.99 ± 0.28	232.49 ± 0.01	42.62 ± 0.18	
Vanilic acid	12.99 ± 0.05	6.32 ± 0.02	13.34 ± 0.35	8.31 ± 0.27	
Total	177.51	80.79	355.86	176.96	

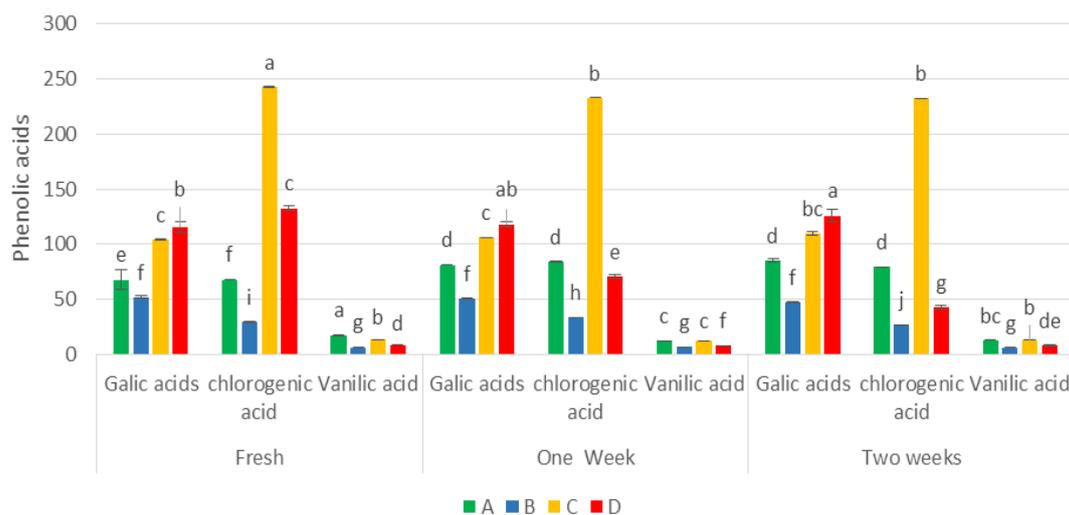


Fig 6. Phenolic acids in raisin juice.

storage period, the variations in the availability of phenolic compounds might be referring to the fermentation and some chemical reaction and enzyme activity as well as the type of grapes used for production of raisins and juices may greatly influence its phenolic composition (Eyduan, et al 2015).

3.7. Assay of PPO activity

One of the main obstacles of undesired of quality of fruit juice results from enzymatic browning, which is caused by the act of polyphenol oxidase enzyme. The freshness of fruit juices can be prolonged for a few days by storing in a refrigerator. Thus, the microbial contamination that reduce the quality, distorting the sensorial evaluation and causes turbidity, browning and sedimenting during storage can be prevented (Lea, 1994). Yemenicioğlu and Cemeroglu (1997) had illustrated that the oxidative enzymes such as polyphenol oxidase and peroxidase are the main factor in reducing the nutritional and sensorial values as well as color and flavor of fresh fruit juice. As shown in Fig. 7, the highest value of polyphenoloxidase enzyme was observed in raw raisin sample D, and it was superior to other samples. The lowest level of PPO was found in sample A. Furthermore, under the current experimental conditions, among fresh raisin juice samples, the maximum value of PPO was found in fresh raisin juice sample A and the minimum value was observed in sample D (Fig. 8). Perhaps the variation in the results is due to the species of the grapes, the acidity, the storage time, the type of drying and juice treatment process. These results are consistent with results obtained in previous studies (Wesche-Ebeling and Montgomery, 1990; Zemel et al., 1990).

3.8. Water activity

Water activity (a_w) indicates how tightly water is bound, structurally or chemically, in food products. he predicts food safety and stability with respect to microbial growth, chemical/biochemical reaction rates, and physical properties are combined the concept of a_w which has been an essential role for food safety. To attempt to preserve the quality and safety or the chemical stability (Non- enzymatic browning reaction) of foods and activation of microorganisms, it's possible by controlling the water activity content in food products. Therefore, the non-enzymatic browning reaction increases with increasing a_w as well as this reaction will reach to the maximum reaction when the range of reaching 0.6 to 0.7. The lowest a_w at which the vast majority of food spoilage bacteria will grow is about 0.90. The a_w of molds

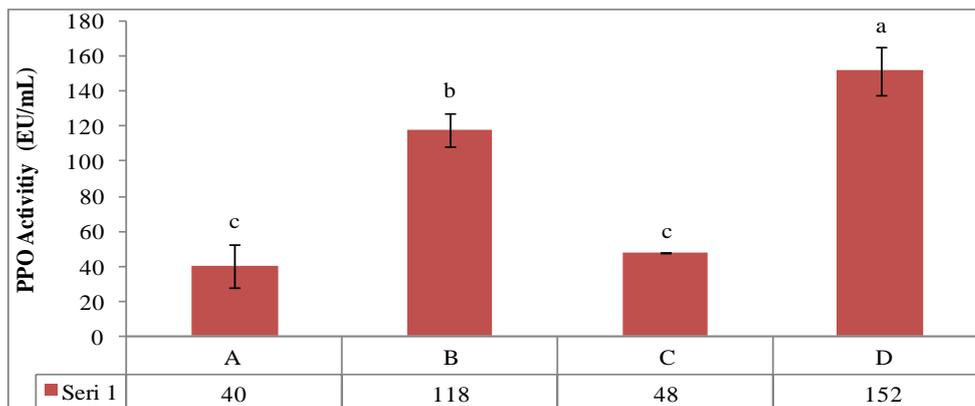


Fig 7. PPO activities in raisins

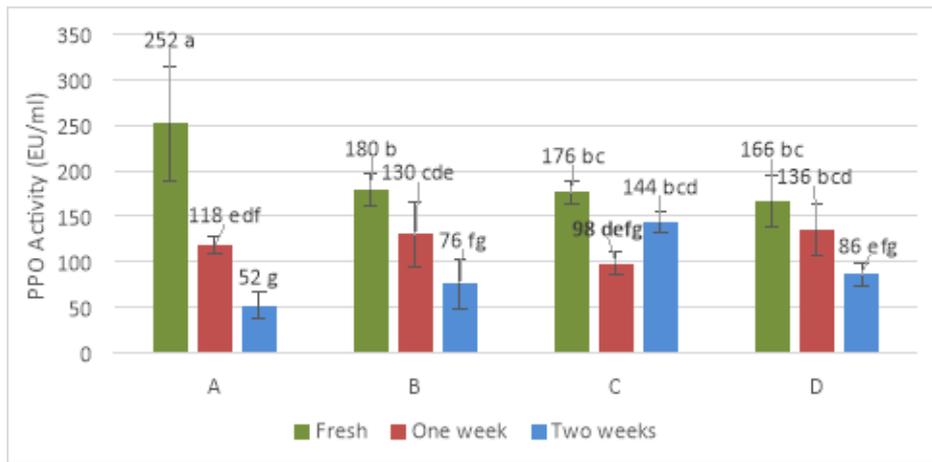


Fig 8. PPO activities in raisin juices

and yeasts growth are about 0.61 with the lower limit for growth of mycotoxigenic molds at 0.78 a_w (Beuchat 1981). The a_w of pure water is 1.00 and the a_w of a completely dehydrated food is 0.00. The a_w of a food on this scale from 0.00 - 1.00 is related to the equilibrium relative humidity of the food on a scale of 0-100%. Thus, % Equilibrium Relative Humidity (ERH) = $a_w \times 100$. The a_w of a food describes the degree to which water is "bound" in the food, its availability to participate in chemical/biochemical reactions, and its availability to facilitate the growth of microorganisms (Mossel and others 1995). The results for the characterization of the raisins and fresh raisin juices as well as raisin juices refrigerated for two weeks are represented in Fig. 9 and Fig. 10, respectively. The contribution to the of raisins and fresh raisins ranged from 0.484-0.519 and 0.949-0.955, respectively in 25°C. The results of a_w in raisins were lower than those found by Mossel et al. (1996), the approximate a_w levels in dried fruits between 0.55-0.80. The difference might be due to the drying process, and juice processing. Furthermore, the a_w of the most of raisin juice products slightly increases with increasing the shelf life of product for one week and at the end of refrigerated storage period. This phenomenon is due to decrease of total soluble solid.

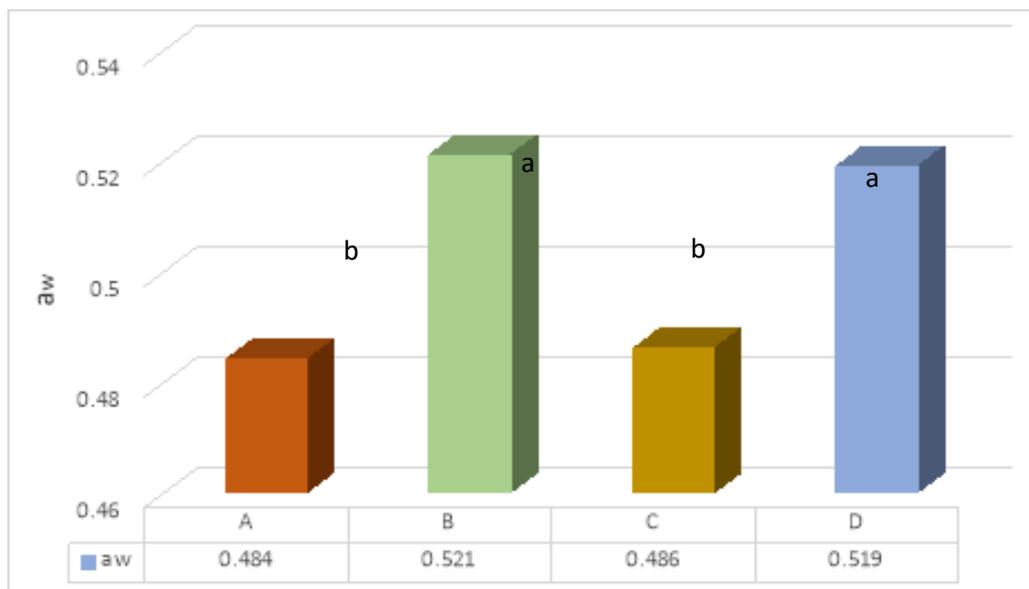


Fig 9. Water activities in raisins

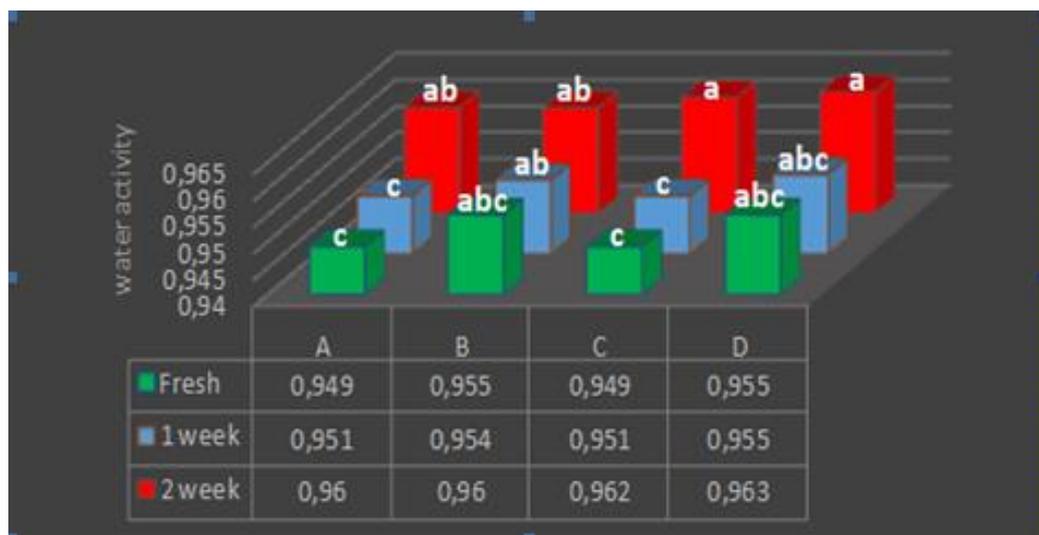


Fig 10. Water activities in raisin juice.

3.9. Total bacterial counts

Ensuring food safety, adequate nutrition content and bioavailability free from preservatives and additives are the curiosities of public health and consumer protection, which have been processed in a fashion to minimize the influence on the original food products (Reed and Grivetti, 2000). Enhancing consumer's health due to inhibition of diseases, it is important the performance of hygienic condition during raisin juice production. Good manufacturing practice (GMP) has influenced the quality and safety of raisin juice. Raisin juice will be contaminated at any points from raw material to end of the production and could be the source of infectious pathogens till to reach the consumers. The microbial population depends on several factors such as pH, a_w true hygienic condition, treatments, and storage condition. The refrigerator is not always the best way to extend the shelf life with retention of the nutrient value of fruit juice.

It can be seen in the histogram that the increase in the number of micro-organisms was during the first week of refrigeration in most samples, then it accelerated at the end of storage period, as well as the results showed the maximum and minimum initial of total count microorganisms in fresh raisin juice were 8.48 and 7.09 CFU/ml, in samples D and B, respectively (Fig. 11). The reason for this result might be due to the degree of achieving hygienic condition during raisin juice processing. Furthermore, there were no significant difference in the population of microorganisms in all fresh samples compares the samples at the middle and the end of storage period in refrigerator or the survival of microorganisms in fresh raisin juice was slightly increased after one and two weeks of storage in refrigerator and these results are in accordance with a previous study done by Elsheikh et al (2014) that investigated the total viable count was nil at zero time of storage and started to increase after 2 months. On the other hand, this results agrees with results obtained by Kaddumukasa (2017) in the microbiological analyses of passion fruit, pineapple, and mango juices in dark and light bottles at 24°C and 4°C were conducted in Kampala, Uganda for 12 days for juices.

3.10. Sensorial evaluation

In this study, sensorial evaluation is used as an essential part of quality control as well as quality assurance of fresh raisin juice and samples refrigerated for two weeks. Sensory analysis was performed with 10 panelists (5 females and 5 males and between 18 and 40 years of age), on first week, second week and third week of storage in terms of consumer acceptability. The evaluated attributes were the intensity of sensory properties was included (appearance, aroma, taste, sweetness and texture mouth feel). Panelists scored the samples using a five-point hedonic scale where a score of 1 extremely dislike and a score of 5 extremely like.

Table 6 shows that the fresh raisin juice samples showed the highest acceptability in all the sensory attributes. This may be related to the freshness of the juice (Akusu, et al., 2016). Further, there was significantly decrease in all sensory characteristics of fresh raisin juice compared to other samples at the

end of storage period under the refrigerated condition, as illustrated in Table 6. This result opposite the work of Adebayo Tayo and Akpeji (2016), who reported that there was no significant difference in taste, aroma, color, and appearance of the probioticated juice samples during 4 weeks storage.

Otherwise, the appearance evaluation of the fresh raisin juice for all samples compared with the samples stored for 1 week exhibited similar trends as well as significantly decrease of flavor, taste and sweetness characteristics in all fresh raisin juice samples compared their counterparts stored for 1 week, except samples B and A. This result agree with study of Akusu et al (2016) who found that sensory evaluation result showed a significant difference in the attributes of colour, flavour, taste and overall acceptability of the orange/pineapple juice blends compared to the reference sample.

Although, the results showed that the texture mouth feel in samples A and C was changed significantly after 1 week, in contrast, a comparison results of the texture mouthfeel of fresh raisin juice in samples B and D to the first storage study were no significant. Furthermore, these results showed significant differences between all fresh raisin juice samples and refrigerated for 14 days, except sample A. Similar results reported by Bhardwaj and Nandal (2014). Their results indicated that flavour, colour and bitterness score of juice blends, decreased with advancement of storage period.

The reasons for the changed the sensory evaluation, might be referring to some chemical, microbial, and enzymatic reactions as described by same authers and the juice blend can be kept conveniently for three weeks without preservatives, in contrast to the results reported by Islam et al (2015).

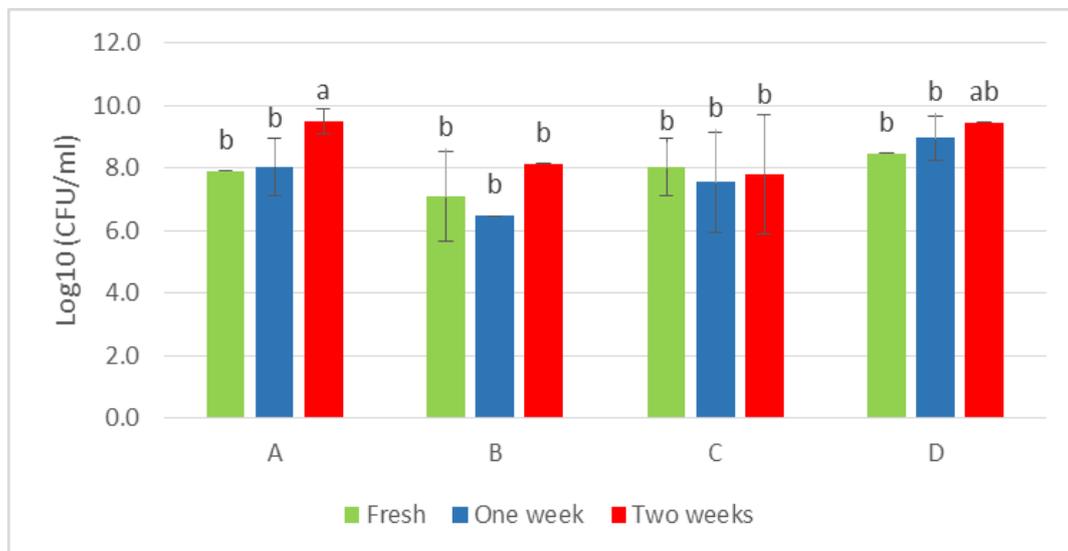


Fig 11. Total bacterial counts

Table 6. Sensorial evaluation of raisin juices

Test Time	Color	Flavor	Taste	Sweetness	Texture / Mouthfeel
A Fresh	4 ± 0.62ab	4.35± 0.52a	3.75± 0.67a	3.40± 0.80 a	3.75± 1.06 a
B Fresh	3.60 ± 0.73abc	3.75 ±0.67abc	3.40± 3.93a	3.05 ± 1.25a	3.30± 0.67ab
C Fresh	3.80 ± 0.88ab	3.80± 1.22ab	3.75± 0.35a	3.35 ± 0.81a	3.80 ± 0.97a
D Fresh	4.25 ± 0.75a	3.85± 1.10ab	3.65± 0.78a	3.50 ± 0.91a	3.20± 1.25ab
A One week	3.35± 0.78bcd	2.60± 0.73de	2.35± 0.85b	1.95 ± 0.76cd	2.5± 1.08b
B One week	3.85± 0.62ab	3.10± 1.24bcd	3.25± 0.97a	2.90± 0.80ab	2.75± 1.07b
C Oneweek	3.55± 0.59abc	2.35± 0.57def	2 ± 0.33bc	2 ± 0.62cd	2.50± 0.81b
D One week	4.05 ± 0.49ab	3± 0.52cd	2.20± 0.71b	2.30± 0.91bc	2.50± 0.57b
A Two weeks	3.1 ± 0.61cd	1.5± 0.52gh	1.30± 0.34ed	1.35 ± 0.52de	1.25± 0.58c
B Two weeks	2.80± 0.53d	1.80± 0.67fgh	1.25± 0.63ed	1.15± 0.78 e	1.55± 0.55 c
C Two weeks	2.70± .58 d	1.25± 0.58 h	0.70± 0.53 e	1.10± 0.51 e	1.35± 0.57 c
D Two weeks	3.90± 0.80 ab	2.20± 0.75efg	1.50 ± 0.47 cd	1.40± 0.56 ed	1.50± 0.57 c

4. Conclusions

In this study, the differences in terms of both quality and safety of the samples were given. As a result it can be said that the fresh raisin juice samples were superior than the stored samples in refrigerator during 1-2 weeks based on quality parameters such as total soluble solids, pH, the quantity of phenolic acids, sensory evaluation, appearance, sweetness and aroma.

Furthermore, in this research three types of sugars which include glucose, fructose, and sucrose as well as three types of essential bioactive phenolic acid compounds which involves gallic, chlorogenic and vanilic acids with different concentrations have been identified.

Consequently, it can be said the variation of phenolic concentration in seeds and flesh of raisin is caused from the variety of grapes, drying process to produce raisins and storage period. Furthermore, the evaluation of bacterial count and water activity showed that the safety of untreated raisin juice samples depends on the good hygienic practices (GMP) and storage condition until to reach the consumers.

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