

**REPUBLIC OF TURKEY
SİİRT UNIVERSITY
INSTITUTE OF SCIENCE**

MASTER'S THESIS

**DETERMINATION OF SOME QUALITY AND SAFETY PARAMETERS OF
BLACK RAISIN JUICE**

**Hawsar Syamand HUSSEIN
(163108002)**

Department of Food Engineering

Thesis Supervisor: Asst. Prof. Dr. Yakup ASLAN

Thesis Co-Supervisor: Prof. Dr. İsa CAVIDOĞLU

Thesis Second Co-Supervisor: Dr. Seerwan Ahmed ABDULLAH

SİİRT - 2018

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THESIS ACCEPTANCE AND APPROVAL

The thesis study titled "Determination of some quality and safety parameters of black raisin juice" prepared by Hawsar Syamand HUSSEIN has been accepted unanimously/by majority by the following jury as a MASTER'S/DOCTORAL THESIS at Siirt University Institute of Science and Technology Department of Food Engineering on 02/02/2018.

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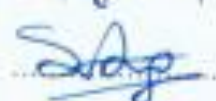
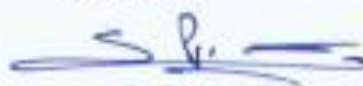
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Hawsar syamand HUSSEIN

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PREFACE

In this thesis, Determination quality and safety of raisin juices First, I would like to express my deepest gratitude to my supervisor Asst. Prof. Dr. Yakup ASLAN for his guidance, patience, understands and excellent support throughout this study program. He showed me different ways to approach a research problem and taught to be persistent to accomplish any goal.

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SIIRT-2018

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ABBREVIATIONS AND SYMBOLS LIST

Abbreviation Description

a_w	: Water activity
C°	: Celsius
CADs	: Coronary artery diseases
CFU	: Colony forming unit
DF	: Dietary fiber
DNA	: Deoxyribonucleic acid
EDTA	: Ethylene diamine tetra acetic acid
ERH	: Equilibrium relative humidity
g	: Gram
GMP	: Good hygienic practices
GMP	: Good manufacturing practice
HPLC	: High performance liquid chromatography
IU	: The international unit of enzyme
kcal	: Kilocalorie
kg	: Kilogram
L	: Liter
LDL	: Low density lipoprotein
LOX	: Lipoxygenase
mg	: Milligram
mL	: Milliliters
MRS	: De Man, Rogosa and Sharpe
N	: Normality
PDA	: Potato dextrose agar
PG	: Polygalacturonase
pH	: -log [H ⁺]
PhC	: Phenolic compounds
PME	: Pectin methylesterase
POD	: Peroxidase
PPO	: Polyphenol oxidase
PPT	: Polyphenol, and tannin
TPC	: Total phenolic compounds
TS	: Turkish Standard
TSA	: Tryptic soy agar
TSS	: Total soluble solid
TTA	: Total titrable acidity
UV	: Ultraviolet
W	: Weight
w.b	: Wet basis
µg	: Microgram
µL	: Microliter



ÖZET

YÜKSEK LİSANS TEZİ

SİYAH KURU ÜZÜM SUYUNUN BAZI KALİTE VE GÜVENLİK PARAMETRELERİNİN BELİRLENMESİ

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Bu tez çalışmasının amacı, toplam çözünen katılar, pH, titrasyon asitliği, şeker çeşitleri ve miktarı, fenolik asit bileşikleri, polifenol oksidaz aktivitesi, su aktivitesi toplam bakteri sayısı ve duyu analizi gibi kuru üzüm suyunun bazı kalite ve güvenlik parametrelerini belirlemektir. Çoğu numunedeki titrasyon asitliği hafifçe değiştirilirken, toplam çözünen katılar ve pH değerleri iki haftalık depolamadan sonra önemli ölçüde azaldı. Bütün örneklerde glukoz, fruktoz ve sükrözün yanı sıra gallik, klorojenik ve vanilik asit gibi üç çeşit fenolik asit tespit edilmiştir. Yeni hazırlanmış kuru üzüm suyunun ve bir ile iki hafta boyunca depolanan üzüm suyunun toplam mikrobik içeriği önemli ölçüde değişmedi. Polifenol oksidaz aktivitesi iki haftalık depolamadan sonra önemli ölçüde azalırken, su aktivitesi biraz azalmıştır. Sonuç olarak, yeni hazırlanmış kuru üzüm suyu ve bir hafta boyunca depolanan kuru üzüm suyunun, iki hafta boyunca depolanan kuru üzüm suyundan daha yüksek kalite ve güvenlik değerlerine sahip olduğu söylenebilir.

Anahtar Kelimeler: Duyusal değerlendirme, fenolik asitler, güvenlik, kalite, polifenol oksidaz, siyah kuru üzüm suyu, su aktivitesi, şeker analizi, toplam bakteri sayısı



ABSTRACT

MASTER'S THESIS

DETERMINATION OF SOME QUALITY AND SAFETY PARAMETERS OF BLACK RAISIN JUICE

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The Degree of Master of Science In Food Engineering

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The aim of the present thesis study was to determine some quality and safety parameters of raisin juice, such as total soluble solids, pH, titratable acidity, kinds and quantity of sugars, phenolic acid compounds, polyphenol oxidase activity, water activity, total microbial counts and sensory evaluation. 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 bacterial count of the freshly prepared raisin juice and of the raisin juice stored for one and two weeks not significantly changed. The water activity was slightly decreased, while the polyphenol oxidase 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 higher quality and safety values than the raisin juice stored for two weeks.

Keywords: Black raisin juices, microbial count, phenolic acids, polyphenol oxidase, quality, safety, sensory evaluation, sugar analysis, water activity



1. INTRODUCTION

Grape is one of the world's biggest fruit crops, showed that grape production all over the world was about 67,221 million tones for 2007 (FAO, 2008). The grape tree is widely planted in north Iraq, where it grows well due to this suitable weather condition and soils. Different varieties belonging to the genus *Vitus vinifera*, known as the European grape or the grape of the very old world, are the most widely followed grape in Iraq. Examples include Rashmiree, Rashmiree Wazha, Bae-dank-seedless, Thomson-seedless, Tre-Rash, Rash-Miow, RashMiow (Elongated), Taefee, Kamali, Soor Ssinaee, Kazhaw, Sarquola, Zarek, Soraw, Halwani, Des-AlAnnz, Awilka, Hejazi-Spee (Anonym, 2005a).

A grape is a non-climacteric fruiting berry that grows on the perennial and deciduous woody vines of the genus *Vitis*. Which 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 slows down 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 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 raisins. 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 dryer. 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 (Mary and Michael, 2003).

The drinking of fruit juices could have both positive and negative impact on the part of body health consumers. Consumer demands safe 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 grape juice through the physical way or from the dilution of concentrated natural juice with water in the appropriate quantity to maintain the basic structure and the limited qualities of 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 (gallic 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).

Besides consumed as fresh fruit, grape could be used to produce several end products such as grape juice, wine, raisin, as well as current. In addition, it is used to produce raisin juice which is one of the common products varieties of sweet grapes are usually processed in raisin industry. The sweet taste of raisin juice is more favorites by the consumers, making it more marketable than grape juice. To enhancing consumers' health, the hygienic condition of grape or raisin juice extracted is extremely important. However, raisin juice contains adequate nutrients that could support microbial growth. Raisin juice contaminated at any point from filed until reach to the consumers. It could be the source of infectious pathogens (Riahi and Ramaswamy, 2004).

Raisin is consumed as fresh fruits and especially is value for its tangy taste. It has a low shelf life of seven days in refrigerator; this is because of (1) natural contamination of raisin juice by bacteria and molds, (2) enzymatic activities (polyphenol oxidase and peroxidase) and to change the taste and flavor during the storage times (Quarmby and Ratkowsky, 1988). Storage of raisin juices at refrigerator is not always best for the prevention of desirable quality of some fruits. Water used for Raisin juice preparation can be the main source of microbial contaminants.

The polyphenol oxidase (PPO) enzyme is highly heated 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 undesirable 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, Erbil and Sulaymaniyah Raisins, which is most widely in Iraq. In demand to raise awareness people about raisin juices, this study effort to measure of phenolic compounds, ascorbic acid, activity polyphenol oxidase enzyme and total microbial as the main parameters of quality and safety of raisin juice which is mechanically extracted and it is consumed.

The objectives of this study were to:

- Characterization of local black raisin (*Vitis vinifera*) juice samples determination of some physical and chemical properties including such as pH, brix values, titratable acidity and Sugar analysis.
- Determination and separation of phenolic acids in local raisins (seeds and flesh), and raisin juices and HPLC Mass for identification quantitative analysis of these components.
- Determination activity of PPO in raisin and raisin juices during normal storage of samples.
- Microbial analysis as a safety parameter for this product.
- Effect on sensory quality and the acceptability of raisin juice during normal storage.

2. LITERATURE REVIEW

2.1. Grape

Grape (*Vitis vinifera* L.) belongs to *Vitaceae* family. It favors a climate which is moderate-warm. It is distributed between 20-40° and 20-50° South and North latitude respectively. The production quantity varies worldwide, in Italy 9,256,814 Million Tone/year, France 6,787,000 Million Tone/year USA 6,414,610 Million Tone/year Iran 2,800,000 Million Tone/year. It is believed that the 700 species and 14000 cultivated varieties spread worldwide (Alleweldt et al., 1991).

The number of the cultivars in Iraq and mostly in the region of northern Iraq is estimated to be around 70 among 100 varieties widespread (Abdul-Qader, 2006). The occupied area of the grape in Iraq is 48000 hectares with the yield 184000 tons (FAO, 2008). The grape is mostly grown in Duhok, Erbil and Sulaymaniyah governorates in Iraq where it grows well in order to suitable climatic state and soils. Grape production provides work and income for hundreds of thousands of families in Erbil, Duhok, and Sulaymaniyah (Anonym, 2005b).

Economically, grape production is important in Duhok, Erbil, and Sulaymaniyah, grapes, are used in a wide range of formats, such as raisins and people prefer to consume their juice naturally. There are several parameters can influence the quality of juice such as level of maturity, grape cultivar and some environmental factors such as climate region, soil quality and vineyard management and harvesting method alongside with the processing technique could influence the quality of grape juice to a large extent (Morris, 1989).

Due to natural and artificial contamination, the shelf life of fresh fruit juice may be reduced to a few days during storage in the refrigerator. Consumers choose food to be natural, safe, and also to provide a range of interesting flavors, colors, textures and aromas in food and to maintain the nutritional quality (Soliva-Fortuny and Martin-Belloso, 2003). The important parts of the grape berry include the flesh, pulp, and seeds. The flesh (skin and pulp) contain an outer layer cover around the grape berry. The major components of the skin are coloring substances (pigments), aromatic, tannins matter, and minerals (Dharmadhikari, 1994).

The commercial food industry can be summarized as a logical basis of steps to produce an acceptable of quality and safety food product from raw materials which allows them to be understood this food, developing products and methods that can help the different steps of production (Mitić et al., 2010).

2.2. Raisin Production in Turkey

In 2017/18 expectation is 295,000 tons, down 5% from May 2016/17 because of climate conditions. Raisin exports for MY2016/17 are expected to be 260,000 tons. For MY 2017/18, raisin exports are expected also be approximately 240,000 MT. The main export market of the well-known Sultana raisins produced in Turkey in Europe. Thirty percent of total grape production is being offered for direct consuming as freshly, thirty-five percent as dried, twenty-twenty five percent for traditional products such as dried fruit pulp, boiled grape juice, and five-ten percent is for alcoholic beverage production. Production of seedless grape is about 1.5 million MT. Twenty percent of seedless grape is being offered for consuming as fresh and the rest as dried. Approximately 1 kg of raisins can be obtained from 4 kg of grapes by drying process (Karabina, 2016).

2.3. Chemical Composition of Raisin Juice

Grape or raisin juices contain a low ash and crude fiber as well as are sources of sugar, vitamins, low protein, and lipid. The composition of black grape juice is similar to other grapes except for crude fiber and oils, which are the main component of the seed, carbohydrates, acids, methyl anthranilate (in *Vitis labruscana*), volatile esters, alcohols, and aldehydes are major flavor components. Glucose and fructose are the main carbohydrates available in grape juice. Additionally, the quality of grape juice depends on carbohydrate, acid content and flavor constituents such as methyl anthranilate and other volatile components, tannins and color compounds. The quality of the juice is determined by the changes that occurred in grapes while growth and maturation (Bates et al., 2001). The primary acids present in the grape juice are tartaric, malic and citric, but other acids can be present as minor components. However, flavor and aroma develop while maturation. On the other hand, anthocyanin pigments located in and near the skin are largely responsible for the color of grape juice. The types and quantity of anthocyanin pigments vary between grape species. It is understandable that some grapes have better color stability due to differences in the type of anthocyanin. There no specific composition for any kind of grape, because of it varies in every year and during

maturation (De Golier, 1978). Generally, carbohydrate content and color density increase while the pH and titratable acidity decrease (Bates et al., 2001).

Basically, the chemical properties of grape juice are changed depending on a variety of grapes, and weather condition as well as processing of juice. Generally, the phytochemical composition of grape juice was investigated as follows: Totals soluble solids 28.85%, protein 0.69%, fiber 1.10%, and fat 0.82%. (Abdrabba and Hussein, 2015). The average pH and brix value are 4.25 and 65.58, respectively. The concentrations of Ca, Na, P, Mg, and Fe are 817, 0.9, 492, 1,704, 60 ppm, respectively (Sani, 2013). Proline that is main amino acid in grape juice, varies between 180 to 320 mg/L (12.5° Brix juice), depending on grape type. These values agree with those determined by Huang and Ough (1989). The other individual amino acids assayed to be below 10%. Moreover, Maillard reaction velocity is also related to the specific amino acid available in juices as well as the type of reducing sugar, and acidity.

Nowadays, different grape-derived products are being offered to the market such as juices, wines, jam, jelly, raisins, syrup, and alcoholic beverages. Furthermore, even byproducts of grape processing (pomace, seeds, skins, seed oil) possess high nutraceutical values and were being offered to the market in different forms such as powders, granulates, concentrated or dried extracts (Barona et al., 2012).

Grape composition varies by kind of dehydration method, especially carbohydrate concentration increases (Franco et al., 2004). Furthermore, total calorie per gram, the density of other components fibers and antioxidant activity significantly increase in compared to fresh fruit as a consequence of drying process. The increased antioxidant activity and browning index are perhaps related to the elevated polyphenol and products of Maillard Reactions such as hydroxyl methyl furfural (Sanz et al., 2001). On the other hand, the increased sugar concentration and heat as results of drying process can also increase the antioxidant activity (Yilmaz and Toledo, 2005; Moreno et al., 2007).

Raisins prepared from dried grapes that have the type (*Vitisvini feral*) manufactured or treated properly to make it fit for raisin or sultanas consumption, exclude dried fruit from dried berries The product has two varieties:

- (a) Seedless - prepared from grape types that are naturally seedless or almost seedless.

(b) Seed-bearing - prepared from the grape that own seed, which may or may not is removed in processing (Iraqi standard, 1991).

Raisins are generally consumed in large quantities in countries and these fruits are consumed a lot often particularly, in Mediterranean countries. Raisins are produced by dehydrating grapes by natural air drying use the heat of the sun or a mechanical act of oven drying (Mary and Michael., 2003). Raisin product in the industry such as bakery, bread, cereals, condiments, confectionery, dairy and snacks (Karabina, 2016). The United States and Turkey are the major producers and exporters of raisins. The moisture content of the raisins after being dried and washed has more than 18%, it will be contaminated with molds, and if the moisture content is less than 11%, it will have an undesirable flavor and have hard mouth fell. The most appropriate moisture content for raisin should be between 14% to 16 % (Karimi, 2015).

Both raisins and grapes provide similar amounts of sugar (19.6 g and 21.4 g, respectively), energy (97.5 kcal in raisins and 93 kcal in grape) also the amount of carbohydrate 25.7 g in raisins 24.6 g in grapes divided almost equally between fructose and glucose due to mature fruits and constantly due to immature fruits with minimal amounts of sucrose. Raisins are high components of potassium and low in sodium, compared to other fruits, they are high in magnesium and iron (Carughi, 2008). The total dietary fiber content of raisins is 3.7 g/100 g (Anonym, 2005a).

Raisin juice contains approximately 70% w/w invert sugar and about 2% w/w proteins. It is also rich in trace elements particularly Ca, Mg, P, Na, and K as well as vitamins such as A, B3, and C (Papadakis. 2006). Phenolic acids, flavanols, and stilbenes are other compounds isolated from whole grapes, juice, or pomace by using enzymatic hydrolysis. Nine phenolic compounds were also temporarily identified and quantified in the whole grape samples. Twelve phenolic compounds had been identified and quantified in grape juice. Their individual concentrations vary between 3 mg/kg to 875 mg/kg of dry weight. The content varied from 0.07 mg/kg to 910 mg/kg dry weight. The concentrations of fifteen phenolic compounds were identified quantified in pomace varies between 2 mg/kg to 198 mg/kg dry matter. According to HPLC analysis of the samples, the main phenolic compounds are gallic acid and (+)-catechin hydrate in grapes and pomace, while cyanidin and petunidin 3-O-glucoside are the primarily anthocyanin glucosides in the juice (Ramirez-Lopez, et al ., 2014).

Maia et al. (2007), investigated that one of the most significant vitamins in grape or raisin juice is considered ascorbic acid (Vit-C), which is interested in the performance of vital organs in the human health body. Furthermore, Vitamin C can be considered an important parameter for the quality of food since its existence might ensure that other components are preserved as Vit-C is sensitive to heat (Sousa, 2014). There are several parameters can influence the quality of juice such as level of maturity, grape cultivar, and environmental factors as climate region, soil quality and vineyard management and harvesting method alongside with the processing technique could impact the quality of grape juice to a large extent (Morris, 1989).

Two models are used for extraction of juice from raisins, hot and cold extraction; both methods have several advantages and disadvantages. Hot break process is commonly used in red fruits such as grapes, cherries, and berries to maximize juice yield and color-flavor extraction. Crushed fruit or mash is heated to 40 °C to 60 °C using a tubular heat exchanger. This stage is called hot break process and is aimed to extract a large amount of color from the skin into juices. The heating also improves the extraction of both phenols and anthocyanins (McLellan and Acree, 1993). The type of enzyme used is critical since some enzymes may have some side effects that could destroy the juice color (Helbig, 2001). In cold extraction process, raisins are washed with distilled water, soaked in pure water for 15 minutes, mixed with some of pure water and ground before being squeezing with the cheese-cloth. approximately 2-3 L of raisin juice can be produced from one kilogram of raisins. The total solid mass of the concentrated raisin juice is approximately 18%. Finally, the prepared juice is then transferred to a clean container and stored at +4 °C (Abdullah, 2012).

During the early age, juice extraction is done manually by the hand squeezing. The storage of juices does not last a long time due to lack of preventive measures; hence fruits were only available for consumption during harvest season. With this position, there were no extraction and prevention of grape juice for future uses (Ikechukwu and Okonkwo, 2014). Extraction is a technique to move a target compound from one phase to other. Extraction of juice by producing the high product juice and protecting the nutritive values are the crucial expectation in the beverage industry. The eventual aim is to extract a lot of product as it can in order to achieve a higher profit. It is even better if the pressure can be exerted till lowest moisture content, which can lower the disposal

costs as well (Yang et al., 2011). The advantages of using an industry or machine, for extraction are time-saving, improvement of capacity, an increase of efficiency and decrease of spoilage and waste (Abulude et al., 2007).

In addition, advantages of minimally processed juice to the consumer is healthy for human body, fresh sensory attraction (flavor, color) and near to self-preparation, but convenient natural form also to manufacturer easy process, adds nutrient value to juice, all juices pass through this step, increase seasonal rotation but disadvantages of minimally processed juice is more cost, shorter shelf life, quality requirement proper storage, higher quality fruit demand and high safety responsibility (Bates, et al. 2001).

2.4. Shelf Life of Raisin Juice

Untreated juice and was stored at refrigerator temperature (+4 °C) and counted on TSA (Tryptic soy agar) for total plate count, PDA (Potato dextrose agar) acidified to pH 3.5 with 10% tartaric acid for yeasts and MRS (De Man, Rogosa and Sharpe) agar for lactic acid bacteria every 2-3 days during 13 days. In this study, the reference for microbiological spoilage was 5000 CFU/mL that was reported by Tran and Farid (2004). Microbiological shelf life results during 13 days for untreated and irradiated (Kaya,2011)

Fresh squeezed white grape juice was spoiled within 10 days at refrigerator because the juice reached to approximately 5000 CFU/ml (3.7 logs CFU/mL) (Tran and Farid, 2004). Same researchers found that, juice treated with 126 mg/cm² UV dose did not spoiled and contained less microbial load than 5000 CFU/mL reported by) as 1.53 log CFU/mL (33.88 CFU/mL) total count, 0.79 log CFU/mL yeasts (6.17 CFU/mL) and 1.06 log CFU/mL (11.48 CFU/mL) lactic acid bacteria after 13 days.

Some factors effects of safety and quality of juice during storage due to raw material, processing, treatment of the product, as well as time and temperature of storage. The initial microbial load of orange, tomato, and carrot juices was 3.5 CFU/mL, 4.5 logs CFU/mL and 5.5 logs 10 CFU/mL respectively (Dede, 2005).

2.4.1. The effects of microorganisms on fruit juice

Most fruit juices suitable nutrients that could support microbial grow. A lot of causes support, prevent or limit the growth of microorganisms in juices, the most important is water activity(a_w), acidity, hygienic conditions and storage temperature and

concentration of preservative (Basar and Rahman, 2007). Fresh fruit juices which have always been beholding as healthy foods may not always be safe due to a load of microorganism. Contamination of juices with microbial pathogenic such as *Escherichia coli* O157: H7 and *Salmonella* were pretended to cause many sickness and fatalities (Bates et al. 2001). A lot of microorganisms, especially acid-tolerant bacteria, and fungi yeasts and molds can be the reason contamination in the fresh juice using fruit as a substrate. Discoloration, off-flavors, off-attar may develop in the produce. The most fruit juices spoilers are yeasts. Low pH of the juices protect the endurance of most types of bacteria and provide a suitable condition for fungi (Tournas et al. 2006). Preservation of fruit juice by refrigeration, pasteurization, and sterilization are common methods used to check microbiological stability by destroying pathogenic microorganisms and to maintain the sensory evaluation such as color, flavor, and taste of juices (Rahman et al., 2011).

There are three kinds of spoilage: a material may go moldy, or it may be fermented with a production of carbon dioxide and alcohol, or it may be acetified as in the production of vinegar. These changes are usually due to three different kinds of microorganisms; mold fungi, yeasts, and bacteria. To produce a juice with no danger of spoilage a process of 5.5 logs microbial reduction is needed (Cemeroglu, 2004).

Gluconobacter is a popular spoilage agent of fruit juices; it is a strict anaerobe, requires free oxygen (Battey and Schaffner, 2001). *Alicyclobacillus* spoilage is becoming an important the case in heat-treated fruit juices. The growth of the microorganism is connected with the product of antiseptic and smoky taints within juice (Jensen and Whitfield, 2003). This microbial species can also grow at a pH range between 2.5 and 6.0. The total aerobic plate count is helpful for signaling the gross microbiological quality of a product and thus is useful for pointing potential spoilage in perishable products. The aerobic plate count is also beneficial for indicating the healthy situation under which the food produced and/or processed (Andrews, 1997).

It is well known that the storage temperature is important it can slow down the rate of Maillard reaction and the growth of spoilage microorganism in the apple juice concentrate and this is important to food quality and safety.

2.4.2. Water activity (a_w)

This is the most important parameter of water in the expression of food safety. a_w is the partial vapor pressure of water in materials classified by the standard state partial vapor pressure of water. Effects of Reduced water activity on Food Safety the average of ensuring chemical and biochemical processes are impacted by the amount of available water.

One of the impacts of reducing the water activity in a food product is to decrease the rate of these reactions (Lytton and McAlliste, 2014). The exception is the occur oxidation of fats where the rate increases to 0.5 and then decreases down to 0.4. Another chemical reaction effect is Maillard Browning which is the maximum range at 0.6-0.7. Most of the enzymes are deactivated at <0.85 . At less than 0.75 bacterial growth is inhibited but some fungi (yeast and molds) may grow, at less than 0.6 all growth is inhibited. Losses of vitamins C, E, B1 when reduced water activity. Generally, bacteria must higher values of a_w for growth than fungi, with gram-negative bacteria having higher require than gram-positive. Most spoilage bacteria 0.90, most spoilage yeasts 0.88, most spoilage molds 0.80, halophilic bacteria 0.75 xerophilic molds 0.61, and osmophilic yeasts 0.61. A full factorial design was followed by the factors considered. The water activity is changed depending on the temperature during their measurements. Tassou (2009), showed that, the water activity levels analyzed were 0.850, 0.880, 0.900, 0.920, 0.940, 0.960, 0.980 and the incubation period at temperatures were 10 °C , 15 °C, 20 °C, 25 °C, 30 °C, 35 °C and 40 °C.

2.5. Quality of Raisin Juice

Fresh fruit juice is a product that tends to contamination more than whole fruits because Its ingredient are more in contact with air and more reachable for microorganisms. Thus, it can be spoiled and degenerated by microorganisms (Bates, et al. 2001). Other quality losses are dissolved oxygen, metal cautions and other components that change taste, aroma, and color in the fruit juice. Nonenzymatic Maillard reaction between reducing sugars and amines in the fruit juice produces brown pigments and leads to undesirable color in the juice. Also, preventing juice from pesticide remains filtration and unable dissolved particles and avoiding metals during juice processing are major rules in order to produce qualified juice (Bates, et al. 2001).

Raisin quality is judged in terms of factors related to appearance, texture, aroma, food value, and cleanliness. properties such as seedlessness, size, and distinctive flavor can be varied dependent, notably in Zante curran (Black corinth) and *Vitis vinifera* (Muscat of Alexandria Characteristics that are for the most part influenced during harvest are insect and mold damage, mechanical damage stickiness (juicing or breakage), embedded sand, caramelization, and water content (Christensen, 1995)

Grapes acidity and pH have a significant impact on wine production. pH plays a main role in winemaking, affecting the following (Zoecklein et al., 1991): color oxidation rate, ability to clarify, biological protein stability and tartrate stability as well as sensory attributes.

Titrateable acidity (TA) the acid concentration of fruit and resultant wine is important to textural/structural balance. TA in grapes normally ranges between 5.0 g/L - 16.0 g/L as tartaric acid; these values are affected by variety, cultural practices, climatic conditions, and maturity. The organic acid concentration of a wine is traceable to four sources. The grape includes tartaric, malic and, to a much lesser extent, citric acid. By comparison to tartaric and malic acids, which are present at ranging from 2.0 g/L to 10 g/L and 1.0 g/L to 8.0 g/L, respectively, citric acid is found in unfermented grapes at 0.2 g/L to 3.0 g/L (Amerine and Ough, 1980).

2.5.1. Nutrition value of raisin juice

The composition of grape juice is water, carbohydrate, protein, fat, ash, calcium, phosphorus, iron, sodium, potassium, thiamine, riboflavin, niacin, and ascorbic acid. (Kaya, 2011). .It is not very different from the grapes, except the difference is that lies in the oil and crude fiber content presented in the seed that is removed from grapes during processing (Somogyi et al., 1996; Bates et al., 2001).

Generally, several factors influence on the quality of fruit juice within grape or raisin juice such as environmental condition on the grape production, variety of grapes, as well as the drying modes to make a raisin, and the methods of extraction and processing. The quality of grape juice is based on acidity, the amount of sugar and flavor substances such as methyl anthranilate, tannins, and color pigments (Bates et al., 2001). It is also reported that aromatic compounds including acids, alcohols, aldehydes, ketones, and esters in the grapes are also affecting the quality of grape juice. Methyl

anthranilate is an aromatic volatile compound responsible for the odor of certain grape species (Somogyi et al., 1996).

Proteins are part of the nutrient content of grape juice and wines. Protein stabilization is provided by adding bentonite and removed during processing after precipitation (Zoecklein, 1991). Fruit juice has fat or protein in the small amount, so these are not attended as a juice ingredient. Although amino acids are present in a little quantity in the fruit juices, they are essential because of their reactions with reducing sugar in the nonenzymatic browning (Maillard Reaction) of the juice (Ting and Deszyck, 1960). Although products of this reaction provide the antioxidant and antimicrobial effect, browning causes the loss of quality of the fruit juices (Burdurlu and Karadeniz, 2003).

Quality of fruit juices is highly affected by the organic acid profile. Major organic acids which compose the 90% or more of the total acidity in grapes are tartaric and malic acids (Soyer et al., 2003). Also, citric and malic acids were reported to be the major acids in citrus fruits (Karadeniz, 2000). It was reported that acetic acid, lactic, citric and malic acids have antimicrobial effects on *E. coli* O157:H7. Additionally, organic acids are also added to fruit juices as acidifiers to prevent it from spoilage of the juice (Soyer et al., 2003).

Fruit juices are known as considerable sources of vitamin C. Ascorbic acid acts as an antioxidant (Dani et al., 2007) Especially citrus fruits and juices are good sources of ascorbic acid, folic acid, vitamin B1, thiamine, and potassium. In addition to these components, potassium, magnesium, calcium, sodium, zinc, copper and iron are the main minerals in fruit juice (Winiarska-Mieczan and Nowak, 2008). Ascorbic acid is one of the most significant vitamins for the performance of vital organs in the human body (Maia et al., 2007), also it cannot be produced in vivo, so it is necessary to intake via diets in particular fruits which are the main sources (Chitarra, 2005). Furthermore, Vitamin C can be considered an important parameter for the quality of food since its existence might ensure that other components are preserved as it-c is sensitive to heat (Sousa, 2014).

Fiber is as dietary and edible parts of plants or analogous carbohydrates that are resistant to digestion and absorption in the human small intestine with complete or partial fermentation in the large intestine. Dietary fiber (DF) refer to a non-starch

polysaccharide, resistance starch, lignin, analogous carbohydrate, and related plant substance classified according to solubility in water after enzymatic treatment (Anonym, 2001). DF in most grape in seed, skins, and pulp after manufacturing of grape juice remains as pomace. After production, this processed raw material in dumps, being a great waste of health-promoting compounds, so it is essential to intake as a dietary (Forte et al., 2008). Widely studying in recent decades were carried out on health benefits of DF and indicate that it possesses very important physiological benefits such as reducing the risks of cardiovascular diseases, hypertension, hyperglycemia, hypercholesterolemia and colorectal problems (Galali, 2014).

2.5.2. Phenolic compounds

The phenol, polyphenol, and tannin (PPT) content of foods, beverages, and plants can be determined by some methods, each with their own advantages and disadvantages. Some easy-to-use and cheaper methods have been developed to measure the total PPT, using reagents such as Folin-Denis. Based on this assay, total phenolic in raisins content ranging from about 9 mg to 12 mg of Gallic acid equivalents per gram of fruit (Xu et al., 2010). Values are higher for dried fruit than the congruent values for fresh fruit because the PPT are concentrated but partially modified during the drying process.

Shi et al. (2003), investigated that, the universal phenolic acids in grapes consist of cinnamic acids (coumaric acid, caffeic acid, ferulic acid, chlorogenic acid, and neochlorogenic acid) and benzoic acids (p-hydroxybenzoic acids, protocatechuic acid, vanillic acid, and gallic acid). Then the raisins which are made from grapes may result in the loss of total phenolic and antioxidants (Brekša et al., 2010). Raisins are derived from grapes and contain some PPT that was originally present in the grape together with some other products formed during the processing. Some PPT such as the procyanidins, appear to be lost during processing, although this may simply be the result of limitations in the extraction and analytical methods used so far. On a wet weight basis, some compounds are present at a higher level in raisins compared to grapes, including caffeoyl tartaric acid, and some quercetin and kaempferol derivatives (Karadeniz et al., 2000). The Folin–Ciocalteu assay is the most widely used procedure for quantification of total phenolics in plant materials. Reduction of phosphomolybdic–phospho–tungstic

acid (Folin–Ciocalteu reagent) to a blue-colored complex in an alkaline solution occurs in the presence of phenolic compounds.

Most of the grape phenolic antioxidants are distributed in grape skins or seeds (Careri et al., 2003). For instance, anthocyanins, resveratrol, and catechins are concentrated in the skin part, while procyanidins are concentrated in grape seeds (Kammerer et al., 2004), 60% of Phenolic compounds are present in the grape seeds; and 40% in the skin. The seeds are rich in monomeric compounds, such as catechins, epicatechin, and epicatechin-3-Ogallate, dimeric, trimeric also tetrameric procyanidins. Whereas the flavonols are the most abundant phenolic compounds in grape skins (Singleton and Esau, 1969). PhC is chemical compounds of raisins that are involved in sensorial properties such as color, flavor, astringency, and bitterness (Fischer and Noble, 1994). These compounds play an effective role as anti cancerogenic and antiviral agents (Kammerer et al., 2004), and inhibit oxidation of human low-density lipoprotein (LDL) in vitro (Teissedre et al., 2011). Flavan-3-ols(monomeric catechins and proanthocyanidins) are another large family of phenolic compounds that is initially in charge of astringency and bitterness, also responsible for the browning reaction in grapes and undergo various chemical reactions with anthocyanins that cause stabilization of color in red wines (Macheix et al., 1991). The consumption of polyphenol-rich juice improves antioxidant status, decreases oxidation of DNA (Deoxyribonucleic acid), and stimulates immune cell functions (Bub et al., 2003).

Grape juices contain vitamin, mineral, phenols, resveratrol content and other compounds grape juices are healthy juices, The daily consumption of grapes give suitable energy for the body with other fruits. Red grape juice has a slightly a lot of advantages because, during maceration processing, the heating is applied providing passage of antioxidant phenols in the skin and seeds to the juice, Consumption of flavonoid-rich grape products may have a significant beneficial effect on brain function and central nervous system (Tasnim et al., 2010).

Phenolic substances have an effect on the color, flavor, and aroma of foods (Anlı, 2006). Flavonoid content of fruit juices, especially grape juice products, reduces the risk of coronary artery diseases (CADs) by inhibiting platelet aggregation (Stein et al. 1999). Nicklas and Kleinman (2008) indicated that consumption of 100% fruit juice helps better nutrient intake. It was also viewed that it does not reason being overweight

in children between the ages of 2 to 11. Grape juice in the diet may have neurocognitive advantages in older adults with early memory decline (Hogan et al., 2010). Polyphenol-rich grape seed extract has a significant capability that is a key neuropathological feature in Alzheimer disease (Ksiezak-Reding et al., 2012). The authors showed that resveratrol was ineffective in this process but rather catechin and epicatechin were involved. Grape polyphenols have the ability to prevent liver because of their anti-inflammatory and antioxidant properties (Nassiri-Asl et al., 2009). Polyphenol-rich grape skin extract has been found to improve liver steatosis and to protect against diet-induced obesity and hepatic steatosis (Park, 2003). It has also been demonstrated that consumption of flavonoid-rich purple grape juice may attenuate cardiovascular diseases and inhibit thrombosis (Albers, 2004).

Flavonoids are the main group of active anticancer constituents in grape products and are concentrated mainly in grape skins and seeds (Hogan et al., 2010). Researchers have shown that grape skin extract possesses chemotherapeutic efficacy against breast cancer with metastases in the model system (Sun, 2011).

White grapes have beneficial effects on women who are susceptible to iron deficiencies. Also, grapes help kidneys in working properly by decreasing the acid in our urine (Aloe, 2008).

Resveratrol (3,5,4 -trihydroxy-stilbene) that occurs as a result of the injury of the fruits is a phenolic compound. Resveratrol which has antioxidant, anti-carcinogen, anti-inflammatory characteristics and prevents cardiovascular diseases. In addition, resveratrol inhibits atherosclerosis by preventing the low-density lipoprotein oxidation (Fremont, 2000).

Grapes include many organic acids such as tartaric, malic and citric acid and they act a medical role in the human health. Minerals in the grape juice such as potassium, calcium, sodium, phosphorus help purification of the livers while, dark grape juice has iron blocking chemicals (Anonym, 2005b). Addition to these benefits, skin protection is provided by grape juice consumption due to high vitamin C content.

Antioxidants are agents which scavenge the free radicals and prevent or reduced greatly the damage caused by them via neutralizing the free radicals before they can attack the cells, also prevent damage of lipids, proteins, enzymes, carbohydrates and DNA (Fang et al., 2002). Food antioxidant is generally applied to those compounds that

interrupt the free radical chain reactions involved in lipid oxidation (Kochhar and Rossel, 1990). There is an increased evidence for the participation of free radicals in the etiology of various diseases like cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases, aging, etc. (Bandyopadhyay et al., 1999).

A number of studies suggest that the high consumption of grape components could be associated with the reduced risk of certain cancers such as breast and colon cancers (Falcao et al., 1994). Proposed mechanisms of potential anticancer effects of grape antioxidants include antioxidant, anti-inflammatory associated with their chemopreventive effects, and antiproliferative activities (Seeram et al., 2005). Grape antioxidants could act as chelating agents help to reduce physiological reactive oxygen species (Sun et al., 2011).

In addition, several types of research have demonstrated the inhibitory effects of whole grape extracts, individual grape antioxidants, or their mixture on cyclooxygenase activity and gene expression (Waffo et al., 2001; Malik et al., 2003). Hence, a study found that grape antioxidants exert an antitumor activity partially related to their immunopotentiating activities through the enhancements of lymphocyte proliferation, and cell cytotoxicity (Seeram et al., 2005).

2.6. Enzymatic Activities in Fruit Juices

Degeneration of fruit and vegetable produces is highly associated with the enzyme activity. The most important enzymes in an evaluation of the quality of fruits and vegetables are polyphenol oxidase (PPO) and lipoxygenase (LOX) are the enzymes that are responsible for the oxidation of the juice. The existence of enzymes sometimes provides a desirable effect in the juice processing, but they are usually essential problems that eventuate the quality loss in the juice (Falguera et al., 2011; Cemeroglu, 2004).

Also, pectinesterase referred to as pectin methylesterase. It was reported that pectinesterase participates the growth or lack of textural characteristics (Vora et al., 1999). Pectin methylesterase (PME) is a de-esterification enzyme and it is the most critical one for the citrus fruit (Ludikhuyze et al., 2003). They found that at 70 °C PME enzyme was totally deactivated in 10 minutes. The inactivation of pectin methylesterase enzyme and its isoenzymes found in orange juice was reported to be provided by juice processing at 90 °C for 1 minute (Nienaber and Shellhammer, 2001).

Some enzymes in vegetable and fruit processing include:

- Polyphenol oxidase (PPO) which is responsible for enzymatic browning
- Lipxygenase (LOX) which induces changes in color, flavor, and nutritional value
- Pectin methyl esterase (PME) which is responsible for cloud destabilization and consistency changes
- Peroxidase (POD) which gives rise to unfavorable flavors.

Enzymes occasionally give coveted effect in the juice processing, but they are usually main troubles that lead to the quality loss in the juice (Falguera, et al. 2011; Cemeroğlu, 2004). Aside from, pectin methylesterase (PME) and polygalacturonase (PG) from pectinases occur in cloudiness and effect on viscosity of the juice (Schilling, et al. 2008; Timmermans et al. 2011). PPO utilize phenolic compounds in the juice and turn into the o-quinones. After a polymerization stage, brown pigments called melanins occur in the juice. These reactions that are known as “enzymatic browning reactions” in the food technology have an adverse impact on the quality of the juice and should be inhibited during processing (Falguera et al. 2011; Cemeroğlu, 2004).

Amylases may also existent in the juice (Falguera et al. 2011). PPO activity profile of various morphological parts of grape was considered as an index to specify its suitability to process in the form of raisins. In the present investigation the presence of residual PPO in different morphological parts of grapes and the effects of pH, temperature and storage conditions on the PPO activity were studied (Ranveer, 2010; Dandwate, 1996).

Residual PPO activity during processing and storage was found to be an important factor affecting the changes in color and external appearance of raisins. The PPO activity of the skin (108) was found more than the flesh (55) which indicates that the PPO is located in the skin than flesh. Also, the PPO activity was found maximum at pH 5.4 and at 25 °C. Raisin sample prepared in the laboratory by Australian cold dip method and stored at low temperature was best with respect to color (Ranveer, 2010).

2.7. Sensorial Evaluation of Raisin Juice

Sensory evaluation plays significant roles in evaluating properties and acceptability of foods and drink products. The sensory evaluation also has widely spread to become a dynamic force in the food business (Chambers IV, 1990). It is known that in fruit juices, for example, the sensorial properties of the final product are the most important quality parameter.

The quality of grapes, as well as wine quality, flavor, stability, and sensorial characteristics depends on the content of compounds from grapes. One of this compounds sugars such as fructose and glucose (Jordão et al, 2015). Sensory evaluation is the major technique used in product development and quality control to measure the flavor of food products and to verify the most suitable sweetener to alternative sucrose in the product of interest (Porto Cardoso and Andre Bolini, 2008). Descriptive analysis can be applied in the areas of new product development, quality control, storage stability and correlation of sensory with physical and chemical tests (Meilgaard et al., 1988). The major disadvantages of this test are the difficulty of getting all panelists to agree to use a given descriptor and the fact that it is time-consuming (Quarmby and Ratkowsky, 1988).

The aroma changes in the juice samples might be related to enzymatic effect (maceration enzyme and endogenous enzymes). Because of the cooking effects which occur during conventional concentration, pulpy cantaloupe juice samples subjected to this method were significantly higher ($p < 0.05$) in heat-induced aroma characters (e.g. raw squash, taro syrup, and cooked squash) than the juice samples subjected to osmotic concentration.

The flavor change in the first two characters was related to the enzyme treatment which may have lead to the formation of low molecular weight compounds. Sucrose inversion occurred in all samples during processing. Complete degradation of ascorbic acid occurred in all samples during processing. The sensory characteristics (aroma and flavor) of pulpy cantaloupe juice concentrate were highly affected by the concentration method (Galeb, 1993).

3. MATERIALS AND METHODS

3.1. Materials

Locally black raisin and raisin juice made from seed *Vitus vinifera* grapes, four samples of black raisins (Tre-rash varieties sample A, B) from Sulaymaniyah and (Rashmiree samples C, D) from Erbil were collected from the different local market. Hand-held Refractometer (RHB-50 ATC/0-50% Brix) was purchased Grandidex company (China). Benchtop pH Meter BP3001 was purchased from Trans Instruments (S) Pte Ltd. (Jalan Kilang Barat, Singapur). UltiMate 3000 HPLC systems with refractive index Detector and Evolution 201 UV-Visible Spectrophotometer were purchased from Thermo Fisher Scientific (Waltham, MA USA). LabTouch Water activity meter was purchased from Novisina (Lachem, Switzerland). 4-Hydroxybenzoic acid, vanillic acids, and gallic acids, D-(+) glucose, acetonitrile, methanol, phosphoric acid, formic acid, potassium phosphate monobasic, caffeic acid, chlorogenic acids, sucrose, maltose monohydrate, D-(-) fructose, ascorbic acid solution, polyphenol oxidase enzyme solution, ethylene diamine tetra acetic 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).

3.2. Methods

3.2.1. Collecting the black raisin and raisin juice samples

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 utilized for experimental in order to investigate the quality and safety of locally raisin juices in the size four bottles were stored at +4 °C for two weeks.

3.2.2. Preparation of freshly squeezed raisin juice samples for analysis

The samples prepared for analysis according to Abdullah's method (2012). 1 kg of raisins was washed in tap water, soaked in water for 15 minutes, mixed with 2.5 L of water and were ground 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.

3.2.3. Measurement of physical and chemical properties

3.2.3.1. Determination of moisture content

5 g of raisins of 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 for each sample was conducted. The Equation 1 is used to calculate the moisture content samples (Anonym, 1992).

$$M_n = ((W_w - W_d) / W_w) \times 100 \quad (1)$$

in where:

M_n = moisture content (%) of mater

W_w = wet weight of the sample, and

W_d = weight of the sample after drying.

3.2.3.2. Determination of pH values of samples

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

3.2.3.3. Determination of total soluble solids (Brix value)

Total soluble solids (TSS) contents of juices were determined as Brix value by 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 then closed as well as read the refractometer scale, the result at (20 °C) was recorded three replicates for each sample. TSS was recorded raisin juices were tested three times (0., 7. and 14. days) during store 4°C.

3.2.3.4. Determination of total titratable acidity (TTA)

5 mL of raisin juices was taken for each sample and added to 100 mL of distilled water 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. 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 store +4°C.

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

*= milliequivalent weight of tartaric acid

3.2.3.5. Determinations of kinds and quantity of sugar contents

Fructose, glucose, sucrose, maltose contents were determined by using high-performance liquid chromatography (HPLC) according to Turkish Standard Method (TS 13359, 2008).

7.5 mL of raisin juices and 2.5 mL of methanol were mixed in a flask and shook. After that was filtered into HPLC vials by using 0.25 μm filters before HPLC analysis. All samples were analyzed three times (0., 7. and 14. days) during storage at +4°C.

3.2.4. 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 by using HPLC.

3.2.4.1. Sample preparation

A- Seed and flesh:

2 g granulated seed and flesh take the volumetric flask. Add to 10 mL 95:5 (distilled water: methanol) after that take the shaking water bath 200 rpm and 50 °C an hour after centrifuged 5 min 5000 rpm. Extract add to the vial with 0.22 μm filter and take the HPLC for analyses.

B- Raisin juices:

9.5 mL juices and 0.5 mL methanol add to volumetric flask add to vial after that add to the vial with 0.22 μm filter. And take the HPLC For analysis.

Analytical column: Thermo 250 mm x 4.6 mm x 5 μm ID, hypersil gold

Flow rate: 0.75 mL /min

Solvent A: 98: 2 (water: formic acid) Solvent B: 78: 20: 2 (water: Acetonitrile: Formic Acid)

Column temperature: 28 °C ± 1°C

Injection volume: 20 µL

Standard stock

Gallic acid: 30 ppm (280 nm)

Chlorogenic acid: 60 ppm (280 nm)

Caffeic acid: 20 ppm (320 nm)

4-Hydroxybenzoic Acid: 40 ppm (280 nm)

Vanillic acid: 20 ppm (280 nm)

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

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

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

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

15 min 0.75 mL/min solvent A %0 solvent 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 and 320 (Table 3.1) and a C18 column with 250 × 4.6×5 µm separation. 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. It is given as the flow gradient.

External standard method In order to the identification of the different phenolic compounds. For example, phenolic concentrations, phenolic substance standards at least 5 different concentrations were found in HPLC system in the same flow program. All standards calibration coefficients obtained in calibration (R^2) were greater than 0.990.

Table 3.1. Analysis conditions 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
Vanillic Acid	280	9,793	20 ppm	16 ppm	8 ppm	4 ppm	2 ppm

3.2.5. Determination of Polyphenol Oxidase (PPO) Enzyme Activity

Method: Continuous Spectrophotometric Rate determination

Reagents:

- A. 50 mM potassium phosphate buffer, pH 6.5 at 25 °C (prepare 100 mL in deionized water using potassium phosphate monobasic. Adjust to pH 6.5 at 25 °C with 1 M NaOH.)
- B. 5 mM L-3,4-dihydroxy phenyl alanine solution (L-DOPA) (Prepare 10 ml of Reagent A using L-3,4-dihydroxy phenyl alanine.)
- C. 2.1 mM L-ascorbic acid solution (Prepare 10 mL of reagent A using L-ascorbic acid and sodium salt).
- D. 0.065 mM EDTA solution (prepare 10 ml of reagent A using EDTA, and disodium salt).
- E. PPO enzyme solution (a solution prepared using raisin or raisin juice containing 500 - 1000 units/mL of PPO in cold reagent A is prepared, immediately before use)

Procedure:

The following reagents were pipetted (in milliliters) into suitable quartz cuvettes (Table 3.2).

They were mixed by inversion and equilibrate to 25°C. Monitor the $A_{265\text{ nm}}$ until constant, using a suitably thermostatted spectrophotometer. Then added reagent E. They immediately mixed by inversion and record the decrease in $A_{265\text{ nm}}$ for approximately 5 minutes.

Table 3.2. The amounts of reagents and blank

	Test	Blank
Reagent A (Buffer)	2.60	2.80
Reagent B (L-DOPA)	0.10	0.10
Reagent C (AscorbicAcid)	0.10	-----
Reagent D (EDTA)	0.10	0.10
Reagent E (samples containing PPO)	unknown	-----

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

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

0.001 = the change in $A_{265 \text{ nm}}$ /minute per unit PPO at pH 6.5 at 25 °C in in a 3 ml reaction mix

RM = Reaction Mix (final volume = 3 ml)

Unit definition:

One unit will cause the change in $A_{265 \text{ nm}}$ of 0.001 per minute at pH 6.5 at 25°C in a 3 ml reaction mix containing L-3,4-dihydroxyphenylalanine and L-ascorbic acid.

Final assay concentrations:

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

3.2.6. Determination of water activity (a_w)

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

3.2.7. Determination of total mesophilic bacterial counts

Total mesophilic bacterial counts were determined on 0., 7. and 14. days of storage at +4°C. Ten milliliters (10 ml) of the samples were diluted with 90 ml of sterile distilled water and mixed well to obtain 10⁻¹ dilution. Serial dilutions 10⁻⁸ were prepared and spread plate technique was used on appropriate selective media. Duplicate nutrient agar plates were used for each sample and incubated at 37±1°C for 48 hours. Plates containing 30-300 CFU/mL were selected for counting 3 times during store 4°C (Braide et al., 2012).

Number of colonies on plate x reciprocal of dilution of samples = Number of bacteria/mL (3)

3.2.8. Sensory evaluation

Sensory analysis of raisin juice is very important and gives a reliable opinion to appear the acceptability of new yields, the sensory evaluation is the major technique used in product development and quality control to measure the food products. Samples were presented to at least ten panelists inside a plastic bottle, appearance, aroma, taste, sweetness, and texture/mouth-feel were evaluated by ten panelists on the first week, second week, and third weeks 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) and (Singh-Ackbarali and Maharaj, 2014).

Dislike = 1, Dislike alittle = 2, Neither like nor dislike = 3, Like a litle = 4, Like alot = 5

3.2.9. Statistical analysis

The PROC GLM (General Linear Model) procedure (SAS, 2002) was used to analyze the data was fitted in the following model:

$$Y_{ijkl} = \mu + A_i + B_j + AB_k + \varepsilon_{ijkl}$$

in where:

Y_{ijkl} = Traits.

A_i , = Sample location effect.

B_i = Type of fruit.

AB = Interaction between A & B .

μ = Population mean.

ϵ_{ijkl} = standard error. It was assumed to be normally and independently distributed with mean zero and variance $\delta^2 e$.

The Duncan multiple at level 0.05% and it used to a comparison between means and \pm standard deviation for all traits under study.





4. RESULTS AND DISCUSSION

4.1. Moisture Content in Raisins

Fresh and refrigerated raisin juices for two weeks were assessed for physiochemical, sensory evaluation and microbial parameters without any treatment in order to investigate the quality and safety of raisin juice for consumption. 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%, in sample B whereas sample C showed the lowest moisture content 14.9% (Figure 4.1). Moreover, no significant differences between moisture content in all samples, except sample C, which it was a significant difference with other samples. This is compatible with who reported the range of moisture content from 11-18% (Christensen, 1995). The Raisins shall have a water content of not less than 13% and not more than 31% for Malaga Muscatel type, 23% for seed-bearing varieties and 18 % for seedless varieties and currants (Anonym,1992).

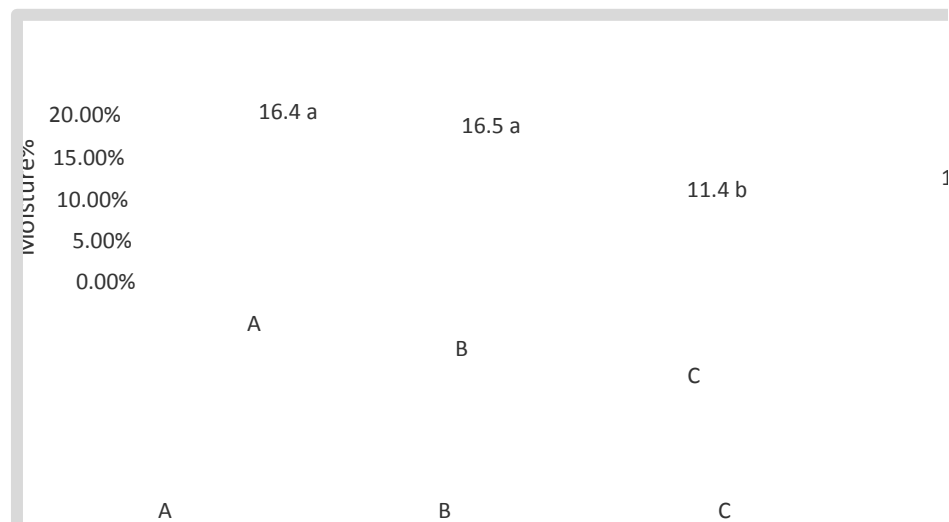


Figure 4.1: Moisture contents of raisin samples.

4.2. pH Value of Raisin Juices

One of the crucial quality properties that illuminate the stability of bioactive compounds in fruit juice is a 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). The obtained data in Figure 4.2 showed that, fresh raisin juice sample A had the highest value of pH (4.23) and lowest value pH (2.96) in raisin juice sample refrigerated at the end of storage period, 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 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 the pH to fall. This agrees with results obtained (Mehmood et al (2008) during the study of the effect of chemical preservatives and pasteurization on the shelf stability and quality of apple juice stored at ambient temperature for three months. However, similar results were found by Wisal et al. (2013), the reason of decrease pH value during storage, it is potential to have biochemical reaction taking place during storage times together with the microbial act in the juices (Ibrahim, 2016). Otherwise, these results are a reverse portion of the results obtained by Mgya-Kilima et al. (2014), and Islam et al. (2015).

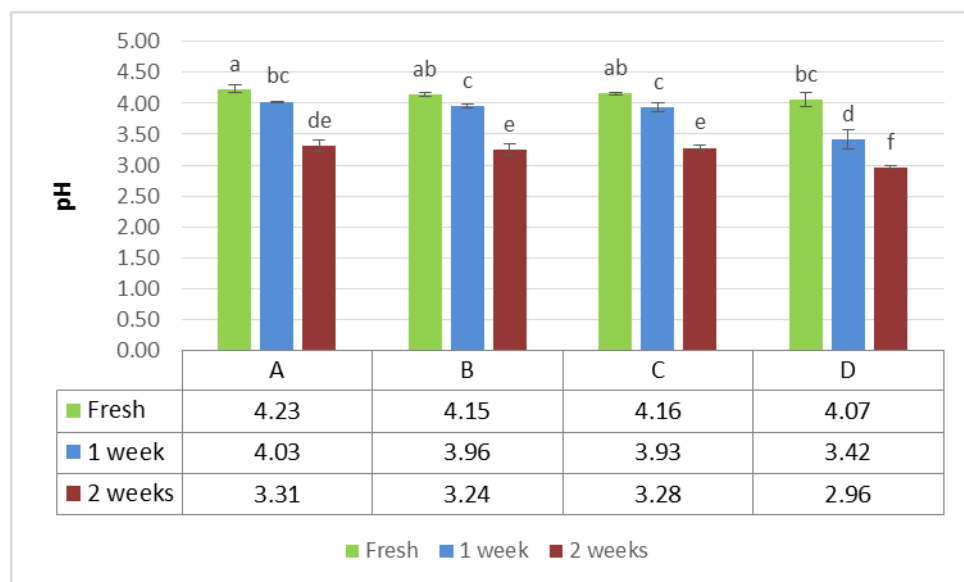


Figure 4.2: pH values of raisin juices (samples: A-D)

4.3. Total Soluble Solids (TSS) of Raisin Juices

TSS of raisin juice stored for the storage period was given in Figure 4.3. The total soluble solid levels ranged from 17.06 to 20.33 in fresh raisin juice C and B, respectively. The obtained results are similar to those reported by Swanepoel et al., (2016) and Islam et al. (2015), for 653 grape juice samples, TSS values were found within the range between 6-25.60 Brix values. However, the results were evaluated; the maximum value reduction of total soluble solid was reached, while the lowest reduction was reached in samples after two weeks of refrigerated. 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) reported that approximately 0.5 Brix of TSS decreased in strawberry juice after being refrigerated for 20 days.

Arin and Akdemir (2004) found that the same results, decrease of TSS 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 did not change significantly during storage time 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 during 6 months at both storage temperatures +4°C and +28°C. Increase in TSS may be due to break down of polysaccharides into oligosaccharides and monosaccharide, while a decrease may be due to fermentation of sugars into carbon dioxide, ethyl alcohol, and water.

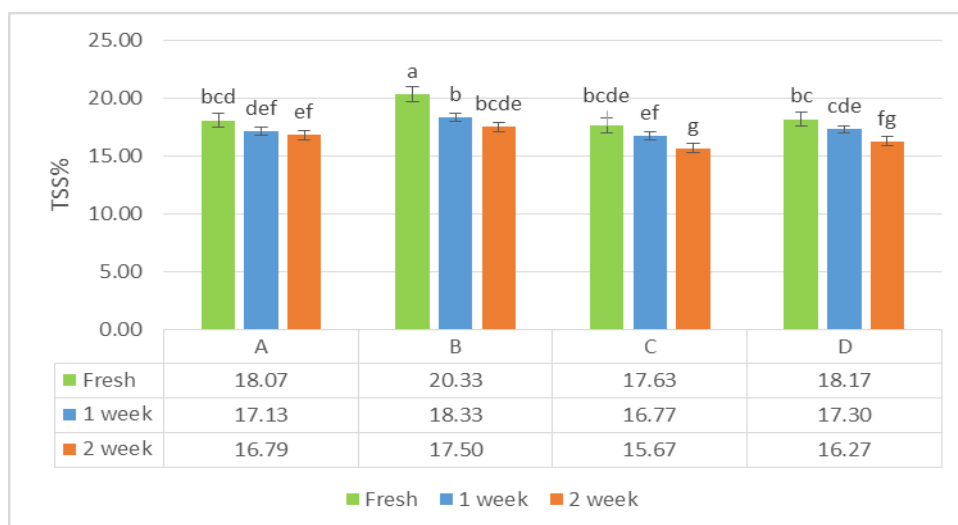


Figure 4.3. Total soluble solids content (Brix value) of raisin juices

4.4. Total Titratable Acidity (TTA) of Raisin Juices

In grape or raisin juice, tartaric acid was most acid in regard to tartaric acid concentration, an important standard for grape juice (Kanellis and Roubelakis, 1993). The main organic acids extract in grapes are tartaric, malic, also little quantity of citric and succinic acid (Kliwer, 1966). The total acidity in raisins from various grape varieties return to the presence of tartaric, malic, citric, succinic and others. Statistical analysis showed that the storage did not influence the titratable acidity to change it in most samples. The formation of lactic, either during the alcoholic fermentation or the malo-lactic fermentation leads to a lowering of both the total acidity and the titratable acidity (Boulton 1980), the same author explained that the precipitation of potassium bitartrate either during fermentation or stabilization will reduce both the total acidity and titratable acidity. Otherwise, it had a significant difference between most of the fresh samples, the amount of TTA between 3.01 g/L to 8.14 g/L in fresh samples B and D respectively, as shown in Figure 4.4. 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 total acidity can be an indication of the presence of tartaric acid (Kodur, 2011). As seen the most acidic in raisin juice sample D with TTA 9.57 g/L after two weeks then sample B with lower TTA value 3.52 g/L in sample B after two weeks. The TTA value of the samples increased linearly with decreasing pH value by experimental methods, which had a strong R^2 as seen in Figure 4.5.

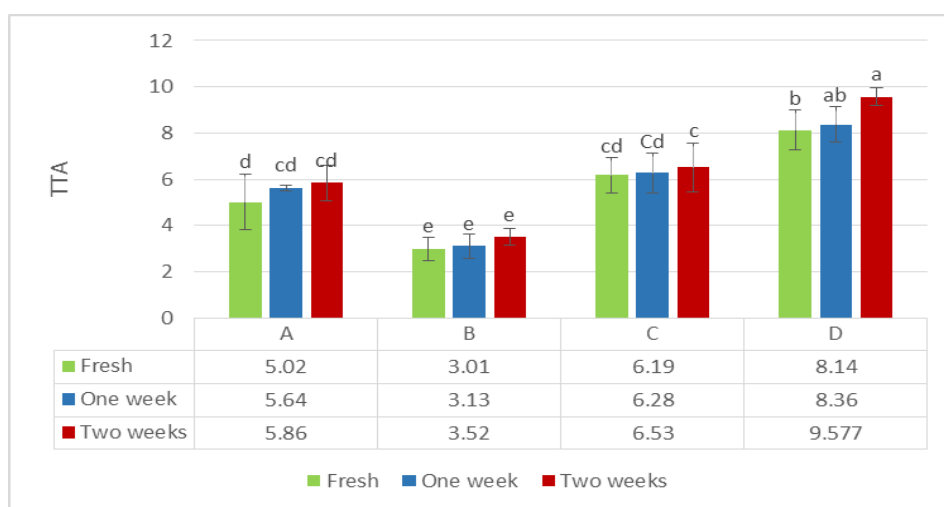


Figure 4.4. Total titratable acidity (TTA) of raisin juices

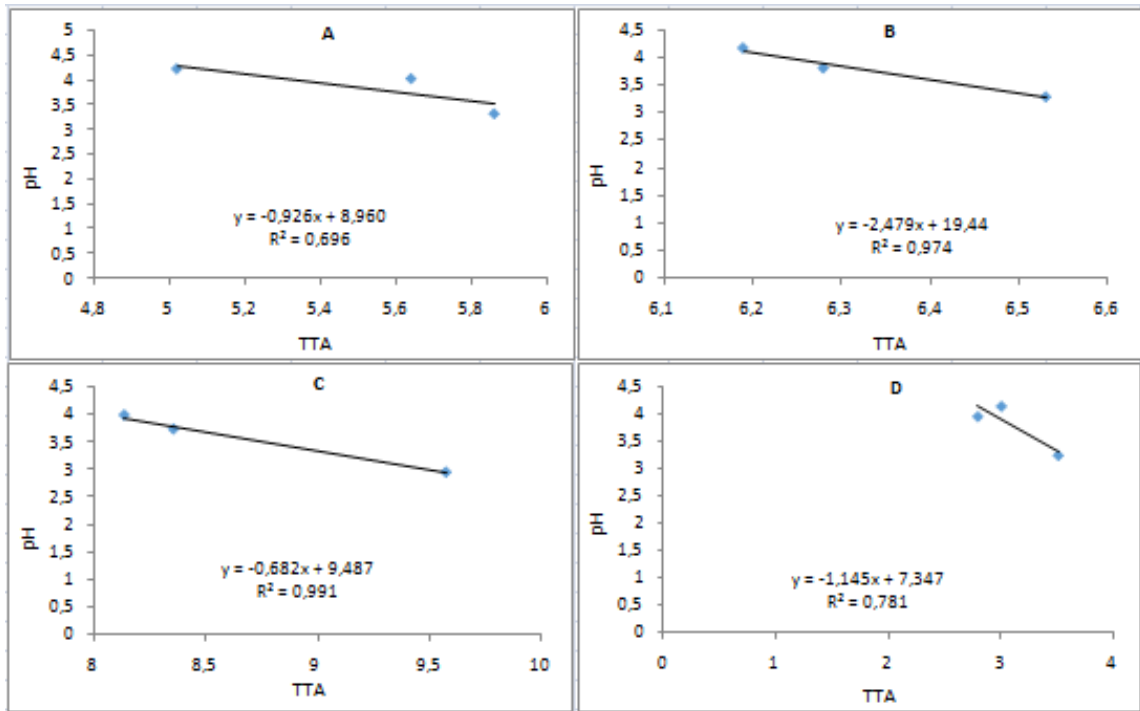


Figure 4.5. The relationship between pH and TTA of raisin juices

4.5. Sugar Contents of Raisin Juices

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 white for table grape above a certain minimum (Amerine, 1980).

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. In the present study, HPLC method was utilized to the determination of quantity and quality of sugars in fresh raisin juice samples and refrigerated condition for two weeks. The profiles and concentrations of sugars current in samples are shown in Table 4.1. Three types of sugars were identified in raisin juice which include glucose (53.523- 99.127 g/L) and fructose (45.046-104.803 g/L) as well

Table 4.1. Sugar content of raisin juices

Samples	Times	Glucose	Fructos	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

as the trace amount of sucrose (0-16.67 g/L). These results were consistent with the results of Duran (2014) who observed glucose and fructose concentration of 8 different grape cultivars were the predominant sugars in grape berries ranging from 89.4 g/L to 144.24 g/L and from 92.1 g/L to 139.44.04 g/L respectively, also glucose/fructose between 0.97-1.08. On the other hand, the results explained, the ratio of glucose/ (fructose + sucrose) between 0.78-1.16, and the ratio of glu/fru between 0.93-1.18. These results are similar to results of several researchers. The ratio of glucose/(fructose+sucrose) between < 0.8 > and depend on types of grapes based on sugar composition Shiraishi et al. (2010). Dai et al. (2011) reported that, the ratio of glucose to fructose between 0.47-1.12, and depends on the maturity of grapes. Although, the authors Elsheikh et al (2014), illustrated that the total sugars of Abu Samaka concentrate stored in the refrigerator decreased after 2 months.

4.6. Some Phenolic Compounds of Raisin Juices

Refrigeration slows down the biological and chemical processes in foods and the accompanying retrogradation and loss of quality and nutrients. The occurrence of sufficient amount of phytochemicals such as carotenoids, flavonoids, and bioactivity of phenolic acid compounds in grape or raisin juice can be standard the quality and high nutrition value. Kähkönen et al., (2012) described that, the grape juice is a main source of bioactive compounds such as phenolic compounds and the consumption of this product is related to several health benefits to consumers. Table 4.2 summarizes all quantification and identification 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 shown that, cinammic acids (chlorogenic acid), and hydroxy benzoic acids (gallic, and vanillic acids) 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 observed which had varied from 933.57 mg/kg to 1434.13 mg/kg and 1060.85 mg/kg to 2645.59 mg/kg for samples B, D, A and C, respectively. The variation of phenolic concentration in seeds and flesh of raisin are belonged to the variety of grapes, drying process to produce raisins and storage period, as described multiple study by (Hassan et al 2012; Giovanell et al., 2014) as well as the current results are in line with those of (Shi et al., 2003; Eyduran et al. (2015) who they were identified same terms of phenolic compounds with extra several terms of bioactive phenolic compounds.

On the other hand, the results in Table 4.3 shows that the same phenolic acid compounds identified with a different concentration in fresh raisin juice and they're refrigerated for one and two weeks. In terms of gallic, chlorogenic and vanillic acids the highest contents were proved with fresh raisin juice samples C, D and A respectively, which were significantly different from the others. The result was indicated the high value of nutrient value regarding of bioavailability of phenolic compounds. Forever, 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

Table 4.2. Phenolic acids in seed and flesh of raisin

	A		B		C		D	
Phenolic acids	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
Vanillic acid	27.19	99.9	47.7	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

Table 4.3. Gallic, chlorogenic and vanillic acids in raisin juices

Phenolic acids	A	B	C	D	
Gallic acids	67.99 ± 8.53	52.09 ± 1.10	104.37 ± 0.15	115.63 ± 4.56	Fresh
Chlorogenic acid	67.96 ± 0.67	29.48 ± 0.44	242.87 ± 0.85	132.66 ± 2	
Vanillic acid	17.67 ± 0.42	6.61 ± 0.01	13.68 ± 0.01	8.43 ± 0.02	
Total	153.62	88.18	360.91	256.71	
Gallic acids	81.38 ± 0.09	50.86 ± 0.074	106.07 ± 0.37	118.28 ± 2.47	One week
Chlorogenic acid	84.28 ± 0.23	33.66 ± 0.29	232.98 ± 0.05	70.37 ± 1.65	
Vanillic acid	12.72 ± 0.02	6.68 ± 0.10	12.75 ± 0.1	7.95 ± 0.17	
Total	178.38	91.19	351.80	196.60	
Gallic acids	85.22 ± 2.28	47.48 ± 0.81	110.03 ± 2.20	126.03 ± 7.09	Two weeks
Chlorogenic acid	79.30 ± 0.37	26.99 ± 0.28	232.49 ± 0.01	42.62 ± 2.18	
Vanillic acid	12.99 ± 0.05	6.32 ± 0.02	13.34 ± 0.02	8.31 ± 0.27	
Total	177.51	80.79	355.86	176.96	

storage for the same term of phenolic acids compound (Figure 4.6). Hence, the reduce of gallic acid in fresh raisin juice sample B was no significant difference between one and two weeks of storage. However, the highest increase level (17.23) of gallic acid was recorded in sample A after two weeks.

In the present study, chlorogenic acids were significantly increased in samples A and B after one week of storage, while the vanillic acid compound was significantly changed in samples A of storage, and no significantly changed in sample B after one and two weeks. at the end of refrigerated storage period, the variation of increase and decrease the availability of phenolic compounds it might be referring to the fermentation and some chemical reaction and enzyme activity as well as the type of grapes used to produce the raisins and juices may greatly influence its phenolic content and composition (Eyduran et al., 2015).

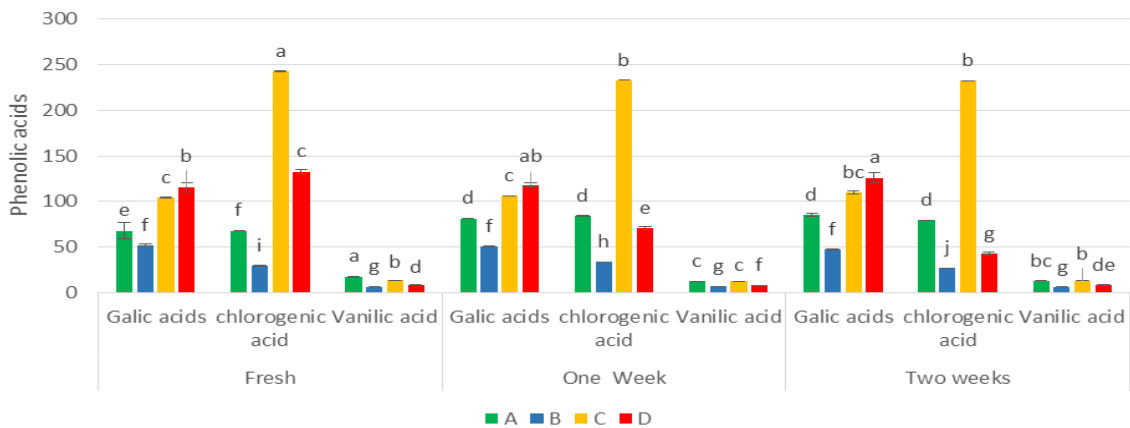


Figure 4.6. Phenolic acids determined in raisin juice.

4.7. PPO Activities in Raisins and Raisin juices

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. Lea (1994) reported that the fresh juice can be prolonged for a few days by storage in a refrigerator from microbial contamination such of juice is reduce the quality such as sensory evaluation which is always turbid, brown in color and tends to sediment on storage. Previously, several authors have been illustrated the oxidative enzymes such as polyphenol oxidase and peroxidase are the main factor in reducing the nutrition value and sensorial value of fresh fruit juice such as color and flavor (Yemenicioğlu and Cemeroglu, 1997).

As seen shown in Figure 4.7, the highest value of polyphenoloxidase enzyme was observed in raw raisin sample D, and it was superior to other samples, therefore the lowest level of PPO was found in sample A. Therefore, under the current experimental conditions, among fresh raisin juice samples, there was a maximum value of PPO investigated in fresh raisin juice sample A. and minimum value was observed in sample D. Perhaps, this variation of results refers to types of grapes, acidity, drying process to raisins, storage period and juice processing. between fresh sample C and refrigerated for the above day's storage under the same condition. These results agree with those formerly (Wesche-Ebeling and Montgomery, 1990). Zemel et al. (1990) reported that PPO activity in apples was gradually inactivated at lower pH values. This may be explained by a low enzyme activity at the pH value of

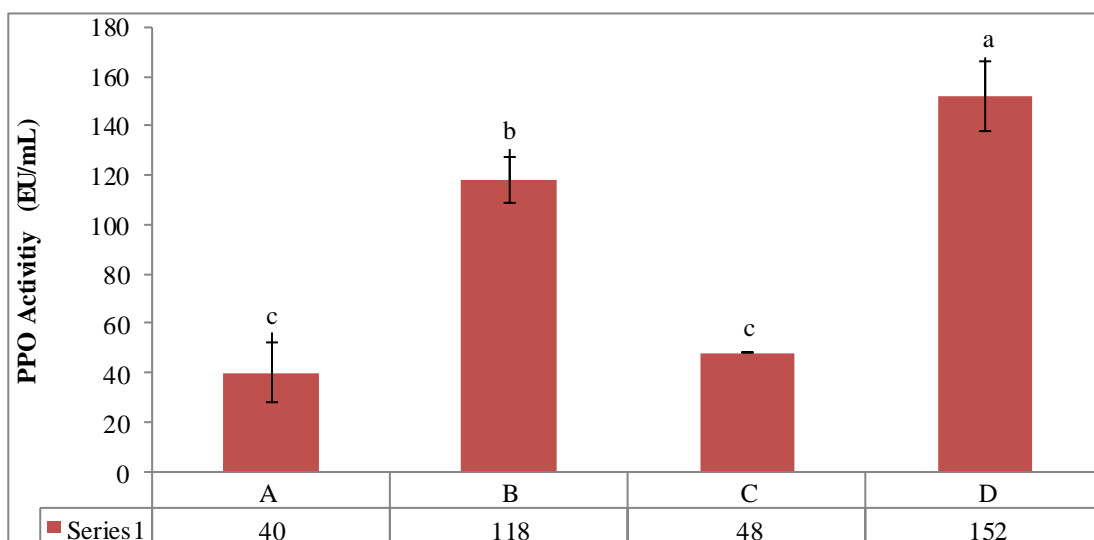


Figure 4.7. PPO activities in raisins

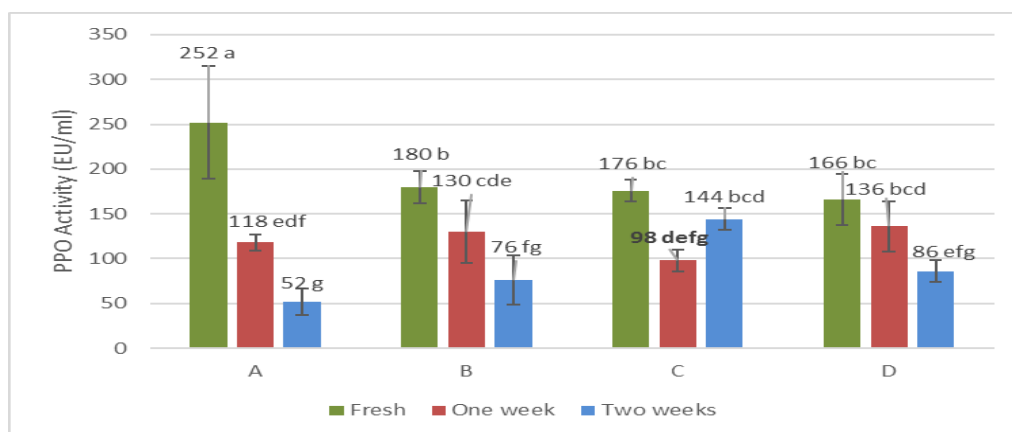


Figure 4.8. PPO activities in raisin juices

the cut orange under study (pH 3.9). The optimum pH for PPO activity is higher; around pH 4.5 similar results have been reported for maturing orange juice (Nagy, 1980) and with storage time (Fellers, 1988).

4.8. Water Activity (a_w) of Raisin Juices

Water activity, (a_w) indicates how tightly water is bound, structurally or chemically, in food products. The predicts food safety and stability with respect to microbial growth, chemical/biochemical reaction rates, and physical properties are combined the concept of water activity (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 controlling the water activity content in food products. Therefore, the non-enzymatic browning reaction increases with increasing water activity as well as this reaction will are reached to the maximum reaction when the range of water activity reaching 0.6 to 0.7.

The lowest a_w at which the vast plurality of food spoilage bacteria will grow is about 0.90. The water activities of yeasts and molds 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 wholly 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 the food on a scale of 0 - 100%. Thus, % Equilibrium Relative Humidity (ERH) = $a_w \times 100$. The a_w of a food defines the degree to which water is "bound" in the food, its

availability to involve in biochemical/chemical reactions, and its availability to ease the growth of microorganisms (Mossel and others 1995).

The results for the water activity characterization of the raisins and fresh raisin juices as well as raisin juices refrigerated for two weeks are represented in Figures 4.9 and Figure 4.10, respectively. The contribution to the water activity of raisins and fresh raisins ranged from 0.484-0.519 and 0.949-0.955, respectively in 25 °C. The results of water activity in raisins were lower than who found it by Mossel et al (1995), the approximate aw levels in dried fruits between (0.55-0.80). The difference might be due to the drying process, and juice processing. Furthermore, the water activity 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 decreased of total soluble solid.

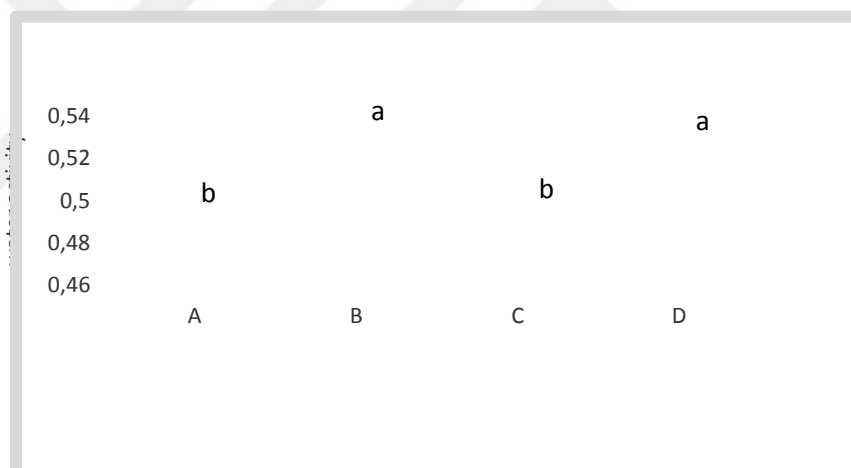


Figure 4.9. Water activities in raisins

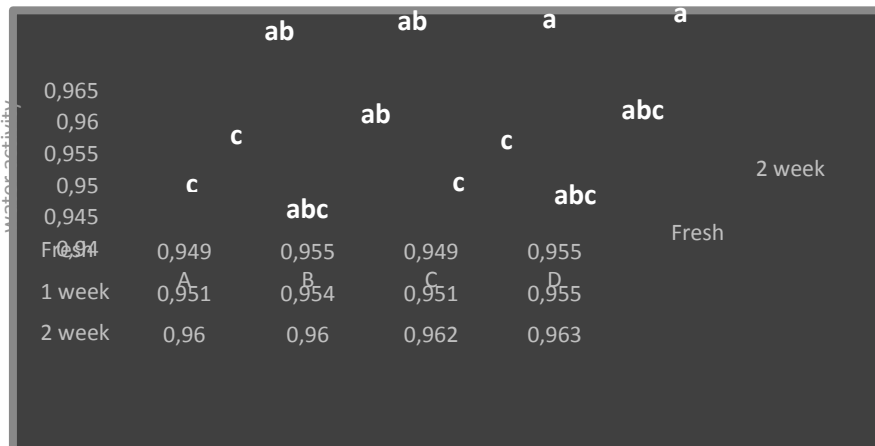


Figure 4.10. Water activities in raisin juice.

4.9. Total Mesophilic Bacterial Counts of Raisin Juices

Ensuring food safety, sufficient nutrition content and bioavailability free from additives and preservatives are the curiosities of public health and consumer protection, which have been processed in a fashion to minimize the impact on the original food products (Reed and Grivetti, 2000). Enhancing consumers' 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, water activity, 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 microorganisms 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 8.5 CFU/mL and 7.1 CFU/mL, in samples D and B, respectively (Figure 4.11). The reason for this result might be due to the degree of achieving hygienic condition during raisin juice processing. Otherwise, there were no significant difference the population of microorganisms in all fresh

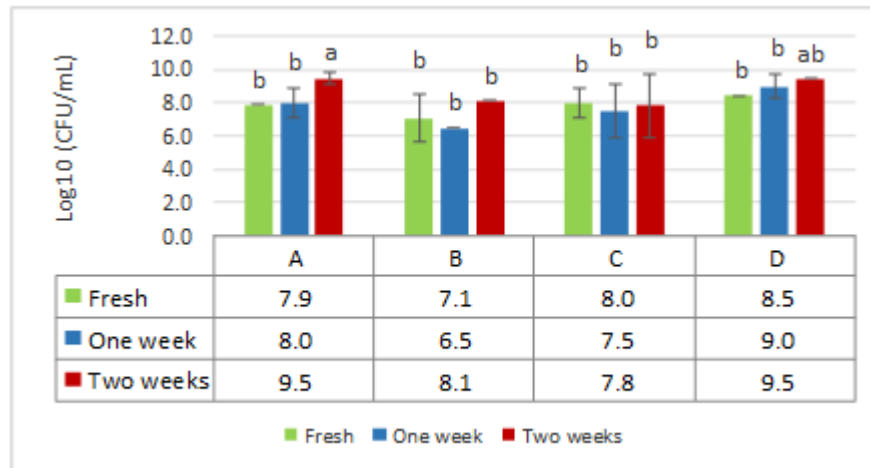


Figure 4.11 Total bacterial counts of raisin juices

samples compares the samples at the middle and the end of storage period under refrigerator storage condition or the survival of microorganisms in fresh raisin juice was slightly increased after one and two weeks of storage under refrigerator storage in most of the samples, and these results are in accordance with a previous study 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. Also, this agrees with results obtained (Kaddumukasa, 2017) 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.

4.9. Sensory Evaluation

In this study, sensory evaluation is used as an essential part of quality control as well as quality assurance of fresh raisin juice and 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 the first week, second week and third weeks 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 4.6 shows that the fresh raisin juice samples showed the high acceptability in all the sensory attributes. This may be related to the freshness of the juice (Akusu, et al., 2016). Hence, he obtained results indicate that there was decreased significantly in

all sensory characteristics of fresh raisin juice compared to study their store at the end of storage period under the refrigerated condition, as illustrated in Table 4.6. 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 Probiotic Pineapple juice samples during 4 weeks storage. Otherwise, the appearance evaluation of the fresh raisin juice for all samples compared the samples stored for 1 week exhibited similar trends as well as decreased significantly of flavor, taste and sweetness characteristics in all fresh raisin juice samples compared theirs stored for 1 week, except samples B A. This result agree with study of Akusu, et al. (2016) who found that sensory analysis result showed a significant difference in the attributes of flavour, colour, taste and overall acceptability of the orange/pineapple juice blends compared to the reference sample. Although, the results observed the texture mouth feel in samples A and C was changed significantly after 2 weeks, 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, the results showed significant differences between all fresh raisin juice samples and refrigerated for 14 days, except sample A. Similar result observed of Bhardwaj and Nandal (2014) results indicated that colour, flavour and bitterness score of juice blends, decreased with advancement of storage period, the reasons for the changed the sensory evaluation, it might be referring to some chemical reaction, microorganisms, and enzyme activities as mentioned same authers and in contrast (Islam et al (2015) described, the juice blend can be kept conveniently for three weeks without preservatives.

Table 4.4. Sensory evaluation of raisin juices

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

5. CONCLUSION AND RECOMMENDATIONS

5.1. Conclusions

The overall aim of this research was to detection of fresh raisin juice and their refrigerated for two weeks. Based on the results of this study, it is concluded that the most important differences among the samples studied were given in terms of the relative of both parameters quality and safety of this product. The fresh raisin juices samples were superior of quality parameters such as total soluble solids, pH, the quantity of phenolic acids as well as sensory evaluation for taste, appearances, sweetness, and aroma on their stored samples under refrigerated for one and two weeks. Moisture and water activity in raisins is very safety standard, however, in this research identified three types of sugars which include glucose, fructose and sucrose, sucrose in fresh raisin juices sample A , C are zero as well as three types of essential bioactive phenolic acid compounds which involves gallic, chlorogenic and vanillic acids with different concentrations, were investigated. Although, regarding safety parameters which include total mesophilic bacteria count, and water activity for untreated raisin juice samples in this study depends on the good manufacturing practices (GMP) and storage condition until to reach the consumers.

5.2. Recommendations

- It is essential good manufacturing practices (GMP) to control or avoid microbial contamination and to be safe the raisin juice industry.
- Monitoring of raw materials, processing and packaging environments which are a simple scientific microbiological control.
- Appropriate training for a producer of raisin juice.
- To improve the raisin juice product, it will be better conversion small-scale production to the large-scale product and chief all requirements and condition of safety and quality of this product.
- It is important to avoid of fraud of raisin juice product by using artificial color and sugars.
- Recommendation for consumers to void of buy or drink of raisin juice when you know the juice refrigerated for more than one week because the quality of this product will be reduced after more than 1 week.
- Raisin juice concentrates are produced by evaporation of juices and their microbiology is greatly influenced by their reduced water activity.



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