



## **Isolation and diagnosis of beta-carotene from carrot juice residues and its use to prolong the shelf life of yogurt**

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### **Abstract:**

Carrots are one of the most consumed vegetables around the world and the production of juice leads to large amounts of carrot juice residues, which is one of the main by-products, which are rich in fiber and high amounts of carotenoids and phenolic compounds that can contribute to improving the quality of food products, in this study yogurt was manufactured using 0, 2.5 and 5 g of carrot juice residues and added to yogurt, The results obtained showed that the estimate of the chemical composition of carrot juice residues for moisture, ash, fat, protein and fiber where the ratios were 67.84, 4.25, 3.42, 2.83, 12.7% respectively and beta-carotene was diagnosed for carrot juice residues by HPLC device, and the total dissolved solids were 18, 25 and 45% respectively, and protein 7.48, 7.77 and 7.90% respectively. Fats of 19.58, 19.77 and 19.84%, respectively, and ash 8.15, 8.33 and 8.48%, respectively, were higher in carrot-residual yogurt samples than in the control sample. As well as the gradual decrease in total humidity 7.11, 7.08 and 7.00% respectively, and pH 4.63, 4.58 and 4.51% during the progress of the storage period accompanied by a rise in pH 0.60, 0.64 and 0.67% higher than the percentages in the comparison sample. The density increased by 0.2, 0.4 and 0.7%, respectively, and the viscosity of 99.87, 105.7 and 113.35%, respectively, in the yogurt samples significantly increased by increasing the concentration of carrot juice residues. As the storage period progressed, the scores for the sensory characteristics of the samples decreased, while the samples with a concentration of 2.5 g obtained the highest scores for the sensory characteristics. These results will be useful for those interested in the food industry in integrating carrots and offal with yogurt to improve the quality and value of food products.

**Keywords:** carrot juice waste, yogurt, beta-carotene

## **Introduction:**

Carotenoids are natural dyes with widespread and have different and important functions, as well as they give many colors, including orange, red, yellow and violet to plants and microorganisms [2,1]. Global trends in functional food production have focused on the development of nutrient-rich products that contain good amounts of bioactive compounds [3], as the expansion of the food industry leads to the use of large quantities of waste or by-products that are subsidized and converted into economically viable products. Waste from the processing of fruits and vegetables contains antioxidants, proteins, dietary fiber, flavor compounds, natural dyes and others, which are extracted, purified, dried, concentrated and reused as functional components in food products, healthcare, pharmaceutical products and cosmetics [5,4]. Natural carotenoids have more interest than artificial colors due to consumer concerns about the harm of chemicals. Carotenoids are a natural source of provitamin A compounds and antioxidants, are anti-inflammatory and enhance the immune response [6]. Carotenoids (hydrocarbon carotenoids) are classified according to their structure into xanthophyll (oxycarotenoids), lutein, cryptoxanthin and zeaxanthin. The types of carotenoids are alpha-carotene, beta-carotene and lycopene [8,7]. Carotenoids are prevalent in different forms in nature, especially in carrots, and carrot juice residues are of high biological value and are extracted from carrot juice [9]. Carrot juice residues are flavoring and antioxidants and give colors to

the foods added to them, as well as the use of artificial colors that give processed foods and enhance the aesthetics of food when the permanent consumption of these industrial dyes leads to health problems such as allergies in the body, neurotoxicity and cytotoxicity, and therefore natural dyes that have been extracted are used safely [10]. By extracting carotenoids from carrot biological residues, preparing carrot waste powder, drying it in a drying oven and applying it to food products such as yogurt [12,11]. Beta-carotene is a fat-soluble plant pigment that turns into vitamin A when the body needs it, and beta-carotene is an inhibitor of active oxygen molecules that cause damage to cells and protects beta-carotene from cancerous diseases and cardiovascular disease [13]. Humidity is important for food manufacturers, as it is one of the factors that show the quality of food, its preservation and resistance to spoilage. It is also important for calculating other food components according to the law of humidity, which expresses the dry weight of a food [14]. Ash is the remaining inorganic residue from the burning of food and this process is called incineration, and there are two types of incineration, dry incineration and wet incineration [15].

Fats play a key role in regulating metabolic function and in the balance of nutrients and calories, adipose tissue is the complex organ leading to a change in organ functions and body physiology [16]. Protein analysis is important in foods based on the unique properties of amino acids and one of the most important methods of measuring protein is the Kjeldahl method [17]. Fibers pay wide attention to their easily formable, abundant

and renewing their natural properties [18]. Yoghurt is part of the diet and is called by several names around the world and it is believed that the word yoghurt originated from the Turkish word *yoğurt*, which means coagulation or condensation, either the health-promoting properties of yoghurt date back to BC. The benefits of lactic acid bacteria have been discovered as yoghurt is fermented milk that is acidified with *bulgaricus Lactobacillus* and *Streptococcus thermophiles*, [19]. Yoghurt is an opportunity to provide an

excellent source of protein and an excellent source of calcium that provides multiple health benefits. More than 80% of yoghurt is introduced in the functional food markets in the production of yoghurt and fortified yoghurt, making the beneficial properties of yoghurt popular worldwide. Fermented dairy products play many roles in human health because they provide large amounts of natural nutritional ingredients and improve Lactic Acid Bacteria with probiotic strains and other lactic acid bacteria. Eating yoghurt leads to non-exposure to food pathogens and improves the immune system and the absorption of lactose and essential minerals. He described the addition of vital ingredients to yoghurt to produce products fortified with several extracts, including apple waste extract [20], hibiscus extract [21], grape seed extract [22], mushroom extract [23] and carrot juice residues extract [24]. The aim of the study is to know the chemical estimation of carrot juice residues, isolate and diagnose beta-carotene and apply it in the yoghurt food

product to prolong the preservation period and improve its properties.

## Materials and methods:

### Sample preparation:

- **Carrot waste:** The imported carrots were obtained from the local markets of Baghdad - Iraq, and the juice was extracted from the carrots by washing the carrots, cutting it and placing it in the pressing machine of the Japanese origin MARSHAL type to extract the juice and separate the carrot pomace from it. And put in a special oven for drying at a temperature of 40 - 50 degrees Celsius until the weight of the dried sample is stable, the dried carrot juice residues were ground and placed in a dark and completely closed box and placed in Refrigerator until use, [25] The extraction rate was 67.84 g of 100 g of carrot juice residues applying the following equation:

$$\text{Percentage of extraction} = \frac{\text{model weight}}{\text{total weight}} \times 100$$

1. Chemical estimation of carrot juice residues:

**Humidity:** The method mentioned in [26] was followed where the samples were dried using a controlled weight of the model up to (5 g) and placed in a known weight in advance and diluted in an electric oven at a temperature of 105 ° C until the weight is stable [27]. Humidity was estimated according to the following equation

$$\% \text{ Moisture} = \frac{\text{Sample weight before drying} - \text{Sample weight after drying}}{\text{Sample weight before drying}} \times 100$$

**Ash:** The method mentioned in [26] was followed, where the percentage of ash was

estimated by burning the carrot sample after placing it in a ceramic lid known in advance weight in the incineration furnace at a temperature of 550 degrees Celsius for a period of 6 hours until the humidity percentage was calculated [28].

Based on the following equation

$\% \text{ Ash} = \frac{\text{Eyelid weight with sample after burning} - \text{empty eyelid weight}}{\text{Sample weight}} \times 100$

**Fat:** The method mentioned in [26] was followed as the percentage of fat in carrots was estimated by taking 1 gram of ground dried carrots and placed in filter paper and wrapped and then placed

in the thimble of the fat extraction device (Soxhlet), the weight of the decanter of the device using a sensitive balance and then add it to 150 ml of solvent (hexane) and the extraction process continued for 16 hours, then collect the solvent from the device and take out the flask and put in an electric oven for half an hour and at a temperature of 80 degrees Celsius to ensure Evaporation of solvent residues from the flask and the survival of fatty substances, then taken out of the oven and left to cool and then weighed using the same balance [29], and the percentage of fat was extracted based on the following equation

$\% \text{ for fat} = \frac{\text{weight of the flask before extraction} - \text{weight of the decanter after extraction}}{\text{weight of the sample}} \times 100$

**Protein:** The method mentioned in [26] was followed where the percentage of protein in carrots was estimated using Kjeldahl device after placing 0.2 grams of carrots in special

digestion tubes of Kildahl device and adding to each test tube 5 ml of concentrated sulfuric acid 95% with two drops of HClO<sub>4</sub> with continued digestion until the solution became clear, after that the digested samples were distilled after adding 10 ml sodium hydroxide 10 carats and collected the liberated ammonia in Vlasque capacity 50 ml contains 25 ml of boric acid 2% with two drops of Bromocresol Green guide Methyl red and then powdered of hydrochloric acid HCL at a concentration of 0.05 standard, and calculated the amount of acid sufficient to change the color of the index from green to red [27], then calculated the percentage of crude protein according to the following equation

$\text{Protein} = \frac{\text{HCL volume consumed} \times \text{standard (0.05)} \times 0.014 \times 6.25}{\text{Sample weight}} \times 100$

**Fiber:** I followed the method mentioned in [26] where a sample was taken weighing 1 gram and put it in Baker to estimate the fiber and clean the acid solution 200 ml that was prepared in certain concentrations, and then put it in the fiber estimator at a high temperature to boil and then it is converted to LO, we wait for half an hour after which it is lifted from the device and filtered with a fidgeting cloth and is deposited and washed by adding 200 ml of the base solution that was prepared in certain concentrations Then it is retrieved on the fiber estimator on the heat source until it boils and then on LO, we calculate half an hour and then filter it with the lid of the fiber estimation and put it in Oven for a whole night, cool the sample and take its weight after drying and then put it in a burner for 6 hours and cool and take its weight after burning [29], and the fibers are

calculated according to the following equation

$$\text{Fiber} = \frac{\text{sample after drying} - \text{sample after burning}}{\text{sample weight}} \times 100$$

### Diagnosis of the active compound **B-Carotene**:

The sample was prepared according to the traditional method except for the saponification procedure. (2.5 g) samples were taken in a vial of (50 ml) and then (25 mm) of anhydrous ethanol KOH with 1N caliber and (0.02 g) of butyl hydroxytoluene BHT were added. The vial was placed in a heating block at 100 ° C for 30 minutes for saponification after saponification, the vial is placed in a dark place and left for 30 minutes at room temperature until cool. The top liquid was transferred from the vial to the 250ml separation funnel through whatman filter paper. 20 ml of saturated sodium chloride solution and 40 ml of petroleum containing 0.22 mmol of BHT were added to the separation funnel. The separation funnel was vigorously shaken for 5 minutes. The top liquid was then collected in a 250 ml round-bottomed vial. The above procedure was repeated again, and the collection of extracts was combined. After alcohol removal Petroleum below 40°C Using a rotary evaporator, the concentration was reconstituted up to 200 ml with a moving phase in a volumetric vial. Finally the sample solution was filtered through a 0.2 µm syringe type filter in a small glass vial for HPLC analysis.

**Beta-carotene analysis** / The beta-carotene concentration was determined using HPLC, a system (SYKAM-German). The C18-ODS column (25 cm 4.6 mm) was used as a

separation column. The moving phase was ethyl acetate: acetonitrile: acetic acid (2:68:30) (volume / volume / volume) with 0.22 mmol BHT and the flow rate was 0.7 ml/min. The detector was an ultraviolet detector set at a wavelength of 450 nm [30].

### • **Food Application:**

**Manufacturing yogurt:** The method mentioned in [31] was followed, where milk was boiled at a temperature of 80 degrees Celsius and left until reaching 37-39 degrees Celsius and distributed in glass cans with a size of 100 ml and add the initiator (curd) on the cans by 5-8 grams, then take three concentrations of dried and ground carrot juice residues at a concentration of (0% - 2.5% - 5%) and add them to the milk cans allocated to them and mix them with milk and mark the cans with the concentration of each can and put the cans in A warm place until coagulation [32].

**Chemical tests:** Moisture, ash, protein and fat were estimated as mentioned earlier.

**pH :** Milk samples were placed in cups weighing 3-5 g of each sample and diluted with distilled water and measured by a PH meter [33].

**Acidity:** Milk samples were placed in glass flasks and red phenolphthalein reagent was placed with them, sodium hydroxide was placed in the buret, and the substance was downloaded from the buret on each flask until the red color disappeared and the blue color appeared (until the electrical neutralization point was reached) and the acidity law was applied to know the acidity of each sample as mentioned by [34].

$$\text{Acidity} = \frac{\text{ml NaOH} \times \text{Equivalent weight of sodium hydroxide}}{\text{Sample weight}}$$

× 100

### Physicochemical Tests :

**Viscosity:** Placing water in the viscometer after the liquid exit hole has been closed and when opened, the time of descent of the liquid is calculated (the water viscosity measurement is calculated first to be compared with the rest of the models to be measured viscosity), [35] After that, the viscosity is measured with the rest of the samples in the same way above and the viscosity law is applied as follows :

$$\text{Viscosity} = \text{water viscosity} / \text{liquid viscosity} \\ = \text{water passage time} \times \text{density} / \text{liquid passage time} \times \text{density}$$

**Density:** Density was calculated by the following law

$$\text{Density} = \text{sample weight (mass)} / \text{volume} [36].$$

### Determination of total dissolved solids :

Total solids are estimated by the Hand refractometer, by placing a drop of the model on the glass prism and observing the percentage of total solids through a small hole located on the other side of the prism, [36].

**Microbiology tests:** microbiology was counted by standard plat count method, sterilization of laboratory instruments is performed, decimal (7 tashif) was prepared to withdraw 1 ml of milk and 9 ml of distilled water and prepare the agricultural medium

Nutrient Agar, petridish dishes were prepared and poured out 1 ml of the seventh dilution where it is withdrawn by Micropipette , and pour the culture medium on it and stir clockwise and counterclockwise in order to make sure that the sample is mixed with the culture medium, we leave it to harden and then enter the dishes into the incubator at a temperature of 35-38 ° C for a whole night, the bacterial colonies that appeared on the dishes are counted [36].

**Sensory evaluation:** The sensory evaluation of yogurt was conducted by a group of professors and experts in the College of Agricultural Engineering Sciences / University of Baghdad and adopted sensory evaluation on the basis of a set of characteristics including (storage age, taste and flavor, texture, acidity, appearance, packaging) [37].

### Results and discussion:

**Chemical tests of carrot juice waste:** Table (1) shows the results of the chemical composition of carrot juice residues and the percentage of moisture was 4.92%, ash 4.25%, fat 3.42%, protein 2.84% and fiber 12.7%, and the results were similar to what was stated by [38], where the humidity was recorded 7.60%, ash 6.38%, fat 3.48%, protein 6.12%, fiber 17.94%, and the chemical composition of carrots varies according to the variety and type.

**Table 1. Chemical properties of carrot juice residues.**

<b>Fiber</b>	<b>Protein</b>	<b>Fat</b>	<b>Ash</b>	<b>Humidity</b>
<b>%</b>	<b>%</b>	<b>%</b>	<b>%</b>	<b>%</b>
12.7	2.84	3.42	4.25	4.92

Figure (1) shows the beta-carotene ratio (12.328  $\mu\text{g}/100\text{ g}$ ), which is consistent with [39] where the beta-carotene ratio was recorded at 11,210  $\mu\text{g}/100\text{ grams}$ , which is also consistent with [40], recording 11,620  $\mu\text{g}/100\text{ grams}$ .

Table (2) shows differences between the moisture, ash, protein, pH and acidity ratios of the yogurt, where the moisture percentage in the comparison treatment A was higher than the rest of the coefficients where it gave 7.11% and treatment B (2.5% carrot juice residues) gave 7.08%, and the lowest percentage was in treatment C (5% carrot juice residues) where it gave 7.00%. The highest percentage of ash and protein was in treatment C (5% carrot juice residues) which gave 8.48 and 7.90% respectively. Compared to comparison A coefficient, which gave the lowest ash and protein ratios, it was 8.15 and 7.48%, respectively. It was found that the highest percentage of pH was in the comparison treatment A which amounted to 4.63%, while the lowest percentage was in the treatment C (5% carrot juice residues) which amounted to 4.51%,

while the acidity was higher in the C treatment (5% carrot juice waste) which amounted to 0.67% and the lowest acidity in the comparison treatment A was 0.60%.

Table (3) shows that the high percentage of viscosity, density and total dissolved solids of yogurt was in the C treatment (5% carrot juice residues) which gave 113.35, 0.7 and 45% respectively, compared to the comparison coefficient A which gave the lowest percentages of 99.87, 0.2 and 18% respectively.

Table 4 shows that the highest number of yogurt colonies in treatment B (2.5% carrot juice residues) was  $107 \times 34$ , compared to treatment C (5% carrot juice residues) which gave the lowest number of colonies at  $107 \times 20$ .

Table (5) showed that treatment B (2.5% carrot juice residues) outperformed the rest of the coefficients for sensory characteristics (taste and flavor - texture - acidity - appearance - packaging) as it gave the highest total of 86.75%, while treatment C (5% carrot juice residues) gave the lowest total of 74%.

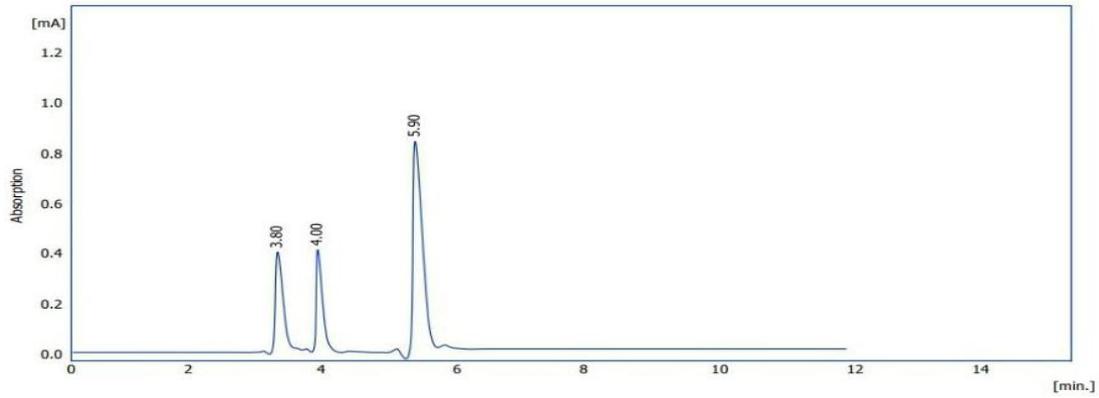


Figure 1 Percentage of beta-carotene in carrot juice residues.

Table 2: Chemical composition of yogurt fortified with carrot juice residues.

idity	Aci	H	p	tein	Pro	ders	cin	idity	Hum	ction	Transa
0	0.6	.63	4	8	7.4	5	8.1		7.11		A
4	0.6	.58	4	7	7.7	3	8.3		7.08		B
7	0.6	51	4.	0	7.9	8	8.4		7.00		C
A Comparison Treatment, B Treatment 2.5 g, C Treatment 5 g											

Table 3: Physical structure of yogurt fortified with carrot juice residues.

Total TDS	Density	Viscosity	Transaction
18	0.2	99.87	A
25	0.4	105.7	B
45	0.7	113.35	C
A Comparison Treatment, B Treatment 2.5 g, C Treatment 5 g			

**Table 4: Microbiology structure of yogurt fortified with carrot juice residues**

Colony-forming unit ((CFU	Variables
$27 \times 10^7$	A
$34 \times 10^7$	B
$20 \times 10^7$	C
A Comparison Treatment, B Treatment 2.5 g, C Treatment 5 g	

**Table (5): Sensory evaluation of yogurt fortified with carrot juice residues.**

Total 00	ackaging	Appearanc e 1 0	Acidity 0	Tissue 3 0	Taste and flavor 4 5	Stored age	Variable
8			.75	2.5	5.75		
6.75				5.75	8		
4			.25	4.25	1.5		
A Comparison Treatment, B Treatment 2.5 g, C Treatment 5 g							

From the results of the above tables, it was found that the increase in the percentage of carrot juice residues powder led to a decrease in moisture and an increase in the percentage of ash, protein, fat, fiber and total dissolved solids.

Differences in moisture for yogurt containing carrot juice residues can be attributed to the properties of total dissolved solids and interactions between water and proteins are essential for the diet due to their effect on the texture and flavor of food [41,42,43], The moisture content is related to the ability of proteins to retain water in the structure of yogurt as well as milk fat molecules have an important role in water retention [44]. to the increase in the percentage of fat is due to the

increase in the proportion of total soluble solids in milk, which leads to an increase in the viscosity of yogurt and thus significantly affects the stability of the tissue of yogurt, and with the increase of powder residues of carrot juice increases the proportion of total soluble solids because the islands contain carbohydrates, which include reducing sugars such as fructose and glucose, where total dissolved solids contribute to the decrease in pH value and increase acidity and only [31], yogurt texture improves when the total dissolved solids content of milk increases. It was found that the addition of carrot juice residues at a concentration of 2.5 g to yogurt led to an increase in the duration of the survival of biobacteria compared to

regular yogurt, and the reason for this is due to the presence of phenolic compounds in carrot juice residues, which have a role in stimulating and improving the growth of primitive bacteria, biobacteria and antioxidant properties [45,46]. [47] reported similar results in his study on the effect of plant extracts to improve the survival of bacterial species in yogurt, where he observed an increase in bacterial populations in yogurt containing plant extracts compared to normal yogurt.

It was also noted at the end of the storage period that the number of biobacteria decreased significantly in the treatments with a concentration of 5 g of carrot juice residues compared to the transactions with a concentration of 2.5 g of carrot juice residues and the measurement treatment and that the acidity increases during the storage period, and this is consistent with what was stated by [34]. when using carrot waste in the manufacture of yogurt. The decrease in the number of biobacteria during the storage period is likely due to increased acidity and the production of hydrogen peroxide by the initiator, resulting in lower consumer acceptance [48].

It was also found that the acidity of yogurt samples containing carrot juice residues during the storage period and a gradual decrease in the pH value were due to the presence of organic and amino acids and phenolic compounds in carrot juice residues [49].

#### **Recommendations:**

1. Expanding the use of carrot juice waste in the food industry, due to its richness

in fiber, carotenoids and phenolic compounds that enhance nutritional value.

- The adoption of 2.5 g of carrot juice residues as an ideal concentration to add to yogurt, as this percentage achieved the highest assessment of sensory qualities while improving nutritional quality.

- Conduct additional studies on the effect of carrot juice residues on other dairy products such as cheese or fermented beverages, to measure the extent to which they improve nutritional value and organoleptic properties.

- Evaluate the shelf life of yogurt fortified with carrot residues, by studying the effect of longer storage on chemical, physical and microbial quality.

- Improve carrot juice residue incorporation techniques to ensure the homogeneity of the final product and prevent any unwanted changes in texture or flavor during storage.

- Encouraging food industries to exploit agricultural residues such as carrot juice as a natural source of nutrients, which contributes to reducing food losses and enhancing environmental sustainability.

- Study consumer acceptance of this type of product and determine the possibility of marketing it widely based on consumer preferences and changes in flavor and texture.

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### عزل وتشخيص البيتا كاروتين من مخلفات عصير الجزر

#### وإستخدامه في إطالة حفظ اليوغرت

عذراء غزوان هاشم شكر

أ.د. إشراق منير محمد

#### الخلاصة :

يعد الجزر من أكثر الخضروات استهلاكاً في جميع أنحاء العالم ويؤدي إنتاج العصير كميات كبيرة من مخلفات عصير الجزر الذي يعتبر أحد المنتجات الثانوية الرئيسية والتي تكون غنية بالألياف وكميات عالية من الكاروتينات والمركبات الفينولية التي يمكن أن تساهم في تحسين جودة المنتجات الغذائية، في هذه الدراسة تم تصنيع اليوغرت بإستخدام 0 و 2.5 و 5 غم من مخلفات عصير الجزر وإضافتها لليوغرت، وأظهرت النتائج التي تم الحصول عليها إن التقدير للتركيب الكيميائي لمخلفات عصير الجزر لكل من الرطوبة والرماد والدهن والبروتين والألياف حيث بلغت النسب 67.84, 4.25, 3.42, 2.83, 12.7% على التوالي وتم , وكان إجمالي المواد الصلبة الذائبة الكلية 18 و 25 و HPLC تشخيص البيتا كاروتين لمخلفات عصير الجزر بواسطة جهاز 45% على التوالي، والبروتين 7.48 و 7.77 و 7.90% على التوالي، والدهن 19.58 و 19.77 و 19.84% على التوالي، والرماد 8.15 و 8.33 و 8.48% على التوالي، كانت أعلى في عينات اليوغرت المدعم بمخلفات الجزر قياساً في عينة المقارنة. فضلاً عن الإنخفاض التدريجي في إجمالي الرطوبة 7.11 و 7.08 و 7.00% على التوالي، والأس الهيدروجيني 4.63 و 4.58 و 4.51% اثناء تقدم فترة التخزين ورافقها ارتفاع في نسبة الحموضة 0.60 و 0.64 و 0.67% أعلى من النسب الموجودة في عينة المقارنة. زادت الكثافة 0.2 و 0.4 و 0.7% على التوالي، واللزوجة 99.87 و 105.7 و 113.35% على التوالي في عينات اليوغرت بشكل ملحوظ بزيادة تركيز مخلفات عصير الجزر. مع تقدم فترة التخزين إنخفضت الدرجات للصفات الحسية للعينات، في حين حصلت العينة ذو تركيز 2.5 غم على أعلى الدرجات للصفات الحسية. ستكون هذه النتائج مفيدة للمهتمين بصناعة الاغذية في دمج الجزر ومخلفاته مع اليوغرت لتحسين جودة وقيمة المنتجات الغذائية.