

## The Effect of Enrichment with *Sargassumillicifolium*, *Ulvalactuca* and *Gracilariacortica* Algae on Physicochemical and Microbial Properties of Processed Fruits (Sour Date Purees and Apple Paste)

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**ABSTRACT:** Currently, processed fruits are consumed as one of the most popular goodies in the country and the position of this product in the food basket of Iranian families is gradually becoming important. The aim of this study was to investigate the effect of *Sargassumillicifolium*, *Ulvalactuca*, and *Gracilariacortica* algae at different concentrations (1.5 and 3%) on the physicochemical (moisture, ash, pH, acidity, SO<sub>2</sub>, amino acid composition, and texture), microbial (total count, coliforms, *Escherichia coli*, *Staphylococcus aureus*, acid resistant bacteria, mold and yeast) of processed fruit. The results showed that the moisture content, ash, insoluble ash, pH, acidity, salt, and sulfur anhydride of different samples were in the range of the national standard of Iran. The moisture content decreased as pH increased during the storage period. Seventeen amino acids including essential and non-essential amino acids such as aspartic acid, glutamic acid, serine, histidine, glycine, threonine, arginine, alanine, proline, tyrosine, valine, methionine, cystine, iso-leucine, leucine, and phenylalanine were identified. The microbial contamination of samples decreased during storage. *Escherichia coli* and *Staphylococcus aureus* counts were not reported in any of the samples. The coliforms, acid-resistant bacteria, mold, and yeast populations of different samples didn't show significant differences ( $p > 0.05$ ). Since the addition of algae did not adversely affect the sensory properties of the processed fruits, they can be used in the formulation of functional products.

**Keywords:** Algae, *Gracilariacortica*, Processed Fruit, *Sargassum Illicifolium*, *Ulvalactuca*.

### Introduction

In many countries, the demand for functional foods is rapidly growing. Functional foods have beneficial physiological effects and reduce the risk of chronic diseases. Functional foods often include probiotics, prebiotics,

polyunsaturated fatty acids, omega-3s, antioxidants, vitamins, minerals, proteins, peptides, and amino acids, in addition to their original nutritional value (Sangwan *et al.*, 2011).

Algae is a rich source of many essential bioactive substances, including antioxidants, minerals, essential amino acids, and fatty acids. In addition, higher

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levels of soluble dietary fiber make it a potential source for *functional* foods and medicinal uses. Therefore, determining the sustainable and wise value of marine resources represents a very attractive platform for the development of new biomaterials that are cost-effective and nature-friendly (Yu-Qing *et al.*, 2016).

Recently new algae including micro and macroalgae have been used as the main source of functional ingredients. Around the world, 8 million tons of seaweed are collected annually, most of which are dedicated to algae that are dumped on the beach (McHugh., 2003). On the southern coast of Iran, algae dumped on the shores of the Oman Sea coast, mainly of the genus *Sargassumillicifolium*, *Ulvalactuca* and *Gracilariacortica*, reach about 2000 tons per year. (Hafeziah *et al.*, 2017; Shaghuli *et al.*, 2017), which can be used in the food industry, medicine, and cosmetics in Iran (Shaghuli *et al.*, 2017).

*Sargassumillicifolium* belongs to the brown algae, which, despite numerous studies, is one of the most systematically complex species. With its wide geographical distribution in tropical regions, sargassum has the most complex classification and is the richest species among brown algae. It is a good source of carbohydrates, vitamins, beta-carotene and some essential amino acids such as arginine, tryptophan and phenylalanine (Guiry, 2009).

*Ulvalactuca* algae are often green in color due to their chlorophyll content. It is known as sea lettuce because of its resemblance to lettuce leaves. The presence of many vitamins in this algae can prevent damage caused by free radicals in the body (Kim *et al.*, 2011). Sea lettuce has long been used as a rich source of nutrients in different applications such as animal feed and medical, cosmetic or agricultural fertilizers (Muralidhar *et al.*,

2010). In Asia, they are used in many soups or salads because of their nutritional value. They are rich in nutrients such as proteins, fats, minerals, and vitamins. High levels of omega-3 and omega-6 fatty acids in sea lettuce increase their nutritional importance (Shaghuli *et al.*, 2017).

*Gracilariacortica* algae is dark red to brown in color and has an elastic texture. *Grassila* contains significant amounts of essential amino acids, fatty acids, minerals, and all vitamins needed for human and animal consumption (Hafeziah *et al.*, 2017). Medically, the presence of antiviral and antitumor compounds has been reported for this genus. This species is widely distributed in the seas of warm regions of the world, including the southern coast of Iran. In the Persian Gulf and the Sea of Oman in southern Iran, 13 species of *Gracylaria* have been identified so far, most of which are distributed on tidal shores. *Gracilariacortica* is one of the most important sources of agar production in the world (McHugh, 2003).

Since these three algae are sources of nutritional components, can be used in the food industry and the production of new functional foods and processed fruits (Rupérez & Saura-calixto, 2001; Ranjbar-Shamsi *et al.*, 2021). Processed fruits are products prepared from the processing of various dried or fresh fruits. In this processing, the fruits are packaged and presented after separation, washing, and adding ingredients including salt, sugar, and citric acid and with or without cooking process according to good manufacturing and production conditions (ISIRI, 2018-2019). Identifying chemical composition and nutrient compounds such as amino acids in processed fruits can help assess the potential of different species for future use in the food industry.

The aim of the present study was to determine the physicochemical and

microbial properties of functional processed fruits enriched with *Sargassumillicifolium*, *Ulvalactuca*, and *Gracilariacortica* algae.

## Materials and Methods

### - Materials

The algae powder of *S.illicifolium*, *U. lactuca* and *G. corticawas* purchased from Algae Development Company (Fars, Iran). A sampling of the algae was performed from March to April 2019, where the algae grow in the rocky areas. The algae powders contained 12-16% moisture, 9-37% protein, 0.3-2.18% fat, and 33-61.5% carbohydrate.

### - Preparation of processed fruits

The formulation of processed fruits is shown in (Table 1). *S.illicifolium*, *U.lactuca*, and *G. corticaalgae* (1.5 and 3% w/w) were added to functional processed fruit formulations (Table 2). First, the fruits were washed by water pressure in the canal. After sorting, the fruits were crushed and then pre-cooked. There were special filters that were responsible for removing the skin of the fruit. This device separated the pure fruit from the skin, seeds and tail. The fruit puree was pumped through steel pipes to large tanks for cooking, where the final cooking takes place. Inorder to maintain the proper quality, flavor and color of the

product, the thermal processing applied in a vacuum at a relatively low temperature of 60 °C and a pressure of 26 inch Hg. Then salt, citric acid and permitted additives (including algae powder) were added to create a good taste of the final product in addition to durability. The process of processed fruit was similar to apple paste. Only in the case of processed fruits, there was no step of crushing and smoothing, and after the concentration operation, the fruit filling operation was performed. Also, the produced processed fruit did not have concentrating steps and was directly imported from the cooking pot to the filling machine by steel pipes and packed. All samples were packed in polypropylene containers and stored at room temperature for 180 days.

### - Chemical properties

The moisture content, pH, ash, insoluble ash, acidity and sulfur dioxide were measured according to AACC, 2000.

### - Amino acid profile

The high performance liquid chromatography (HP Hewlett Packard 1046A model) equipped with a fluorescence detector with a wavelength of 254 nm with a C18 column (25 cm × 4.6 mm) was used to measure free amino acids. The mobile phase consisted of acetate and acetonitrile (Liu *et al.*, 1995).

**Table 1.** Ingredients used in the main formulation of processed fruits

Product	Ingredients	% (w/w)
Sour date purees	Date	71
	Refined edible salt	3
	Citric acid	1
	Water	25.7
Apple paste	Apple	81
	Refined edible salt	1.8
	Citric acid	1
	Sugar	1
	Water	15.2

**Table 2.** The studied treatments

Treatments	<i>S. Illicifolium</i> (% w/w)	<i>U. lactuca</i> (% w/w)	<i>G. cortica</i> (% w/w)
FOControl (sour date purees)	0	0	0
FS <sub>1</sub>	1.5	-	-
FG <sub>1</sub>	-	1.5	-
FU <sub>1</sub>	-	-	1.5
FS <sub>2</sub>	3	-	-
FG <sub>2</sub>	-	3	-
FU <sub>2</sub>	-	-	3
L0 Control (apple paste)	0	0	0
LS <sub>3</sub>	1.5	-	-
LG <sub>3</sub>	-	1.5	-
LU <sub>3</sub>	-	-	1.5
LS <sub>4</sub>	3	-	-
LG <sub>4</sub>	-	3	-
LU <sub>4</sub>	-	-	3

**- Microbial properties**

Microbiological counts were determined by placing a 10 g sample in 90 ml of 0.1% peptone water, followed by homogenization with stomacher. From this dilution, other decimal dilutions were prepared and plated in the appropriate media. Total bacterial counts were determined by the pour plate method, using plate count agar (PCA, Merck, Germany). The plates were incubated at 30 °C for 24-48 h. *Escherichia coli* population was confirmed according to the presence or absence of gas and turbidity of LSB and EC broth (Merck, Germany) and incubated at 37 °C for 24-48 h. *Staphylococcus aureus* population was counted in baird parker agar (Merck, Germany) at 37 °C for 24 h. To count Enterobacteriaceae, VRBG agar (Merck, Germany) and incubation at 35 °C for 48 h were used. For counting of mold and yeast, YGC agar (Merck, Germany) and incubation at 25 °C for 5 days were used. Acid resistant bacterial counts were determined by the pour plate method,

using orange serum agar (OSA, Merck, Germany). The plates were incubated at 30 °C for 5 days. All microorganism counts were reported as log 10 cfu/g (ISIRI, 2018-2019).

**- Statistical analysis**

The experimental results were statistically analyzed using the SPSS statistical package (version 20, SPSS Inc., Woking, Surrey, UK). The statistical significance of samples was performed using analysis of variance (ANOVA) at  $p < 0.05$ . The results were expressed as mean value  $\pm$  standard deviation of three replications.

**Results and Discussion**

**- Chemical properties**

The chemical properties of processed fruit samples containing 1.5 and 3% algae have been shown in (Tables 3 and 4). As can be seen, there was no significant difference between ash, insoluble ash, pH, and acidity in different samples ( $p > 0.05$ ). There is a statistical difference in

moisture, salt, and SO<sub>2</sub> values according to (Tables 3 and 4) (p< 0.05). Changes in the moisture content, pH, and acidity of the processed fruits containing algae during 180 days are shown in (Figure 1, 2 and 3). There was a significant difference between the moisture content of different samples during 180 days (p< 0.05). The moisture content decreased over time. The highest moisture content was observed in the control sample on the first day. The pH value increased during storage. The acidity of all samples except the sample containing *Ulvalactuca* showed an increase and decrease for 90 and 180 days, respectively.

According to the Iranian national standard (ISIRI, No. 3308), the acceptable limit for moisture content of processed fruit is 40% (sour date puree) and 22% (apple paste), which is consistent with the results of our study. Golmakani *et al* stated that the hydrocolloid compounds in the microalgae *Spirulina platensis* maintain moisture in Yazdi cake (christwardana *et al.*, 2013 ,Golmakani *et al.*, 2015).

The processed fruit samples have an acidity within the range of 6%. According to the national standard of Iran, the acidity of apple paste should not be more than 8% by weight of citric acid. These results confirmed that the addition of three algae *Sargassumillicifolium*, *Ulvalactuca* and *Gracilariacortica* had no effect on the

citric acid content of the samples. The pH values confirmed the results obtained from the acidity and complied with the national standard of Iran (2.5-4.5 for apple paste and 3-4 for sour date puree). The trend of pH and acidity changes was very slow due to the buffering capacity of these algae and the presence of proteins, peptides, and amino acids.

According to the national standard of Iran, the salt content of sour date puree and apple paste is 2.9% and 1.8%, respectively which are in agreement with our results. Also, the ash content must be 6% and 5% for sour date puree and apple paste, respectively. The ash content of the samples containing algae showed a slight increase compared to the control sample. This is due to mineral content of these algae, including iron. Studies by Sanchez-Machado *et al.* (Sanchez-Machado *et al.*, 2004) showed that the ash content of two species of *S. illicifolium* and *G. cortica*, is in the range of 23-29%. According to national standard of Iran, the residual SO<sub>2</sub> for apple paste and sour date puree is 200 mg/kg (ISIRI., 2018), all of which are in accordance with the results of this study. Rupérez showed that brown and red algae contained ash and sulfate (Ruprrez, 2002). These results were in agreement with other researchers regarding enriched yogurt with *spirulina* algae (Agustini *et al.*, 2015).

**Table 3.** Chemical properties of processed fruit containing 1.5 % algae

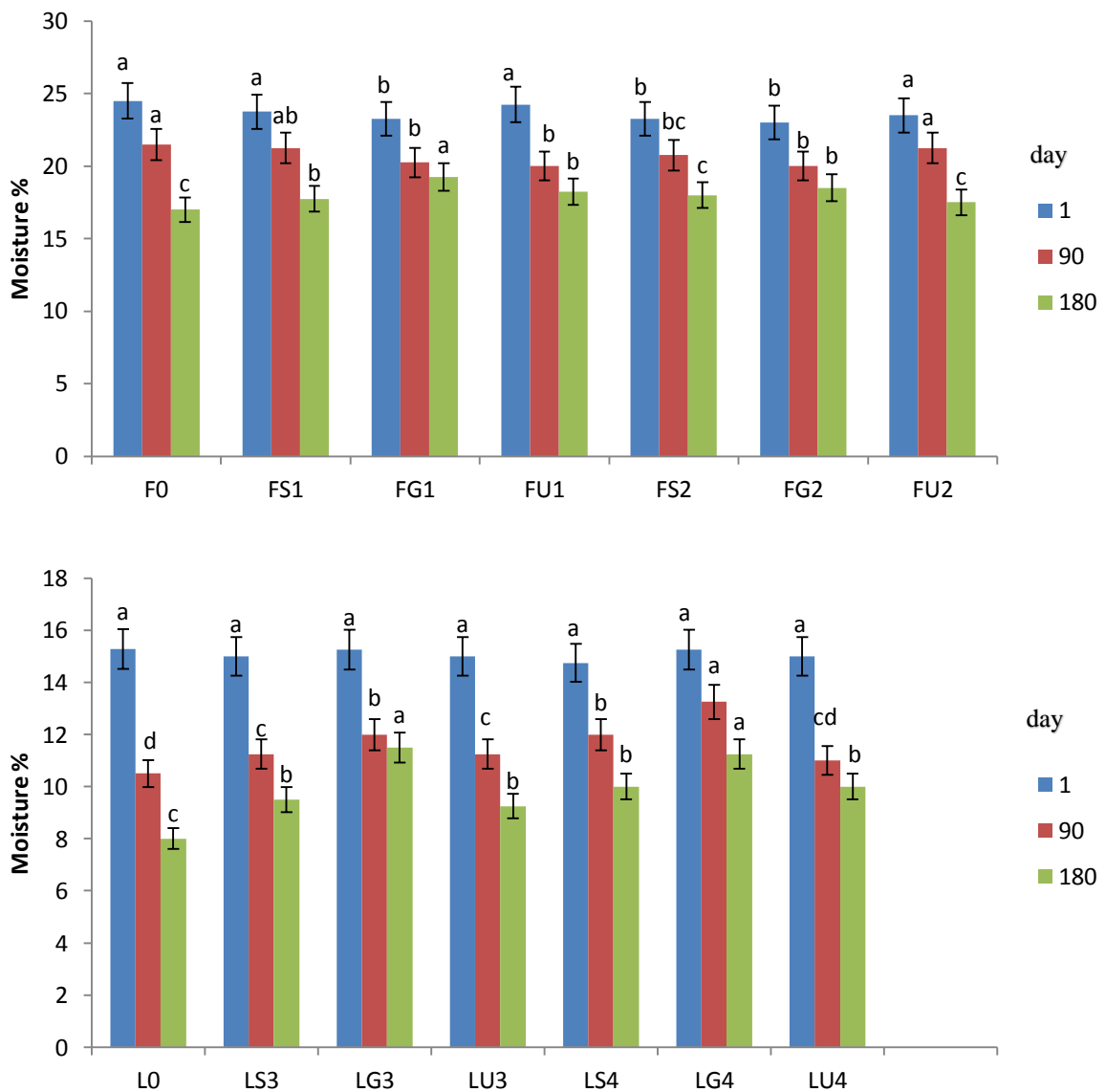
CHEMICAL PROPERTIES	FS <sub>2</sub>	FG <sub>2</sub>	FU <sub>2</sub>	F <sub>0</sub>	LS <sub>4</sub>	LG <sub>4</sub>	LU <sub>4</sub>	L <sub>0</sub>
MOISTURE(%)	25.25±0.35a	24.00±0.70ab	23.5±0.70b	24.5±0.70a	12.75±0.61a	13.75±0.35a	12.50±0.70a	13.5±0.70a
ASH(%)	3.14±0.05b	3.03±0.03b	3.56±0.08a	3.10±0.14a	4.25±0.35a	4.07±0.10a	4.27±0.32a	4.04±0.56a
INSOLUBLE ASH(%)	0.002±0.00a	0.001±0.00a	0.002±0.00a	0.002±0.00a	0.002±0.00a	0.005±0.0a	0.001±0.00a	0.002±0.00a
pH	3.02±0.01a	3.01±0.01a	3.01±0.02a	3.06±0.06a	4.08±0.04a	4.02±0.07a	4.15±0.21a	4.03±0.02a
ACIDITY(%)	6.25±0.35a	6.10±0.44a	6.10±0.14a	6.04±0.56a	6.3±0.42a	6.20±0.28a	5.95±0.07a	6.04±0.12a
SALT(%)	1.44±0.01a	1.36±0.19a	1.11±0.00a	0.75±0.07a	1.20±0.28ab	1.6±0.13a	1.25±0.35ab	0.80±0.00b
SO <sub>2</sub> (PPM)	0.16±0.00a	0.39±0.45a	0.29±0.05a	0.24±0.01a	0.41±0.02a	0.41±0.01a	0.33±0.02b	0.31±0.00b

The different letters in a row are significantly different (p <0.05).

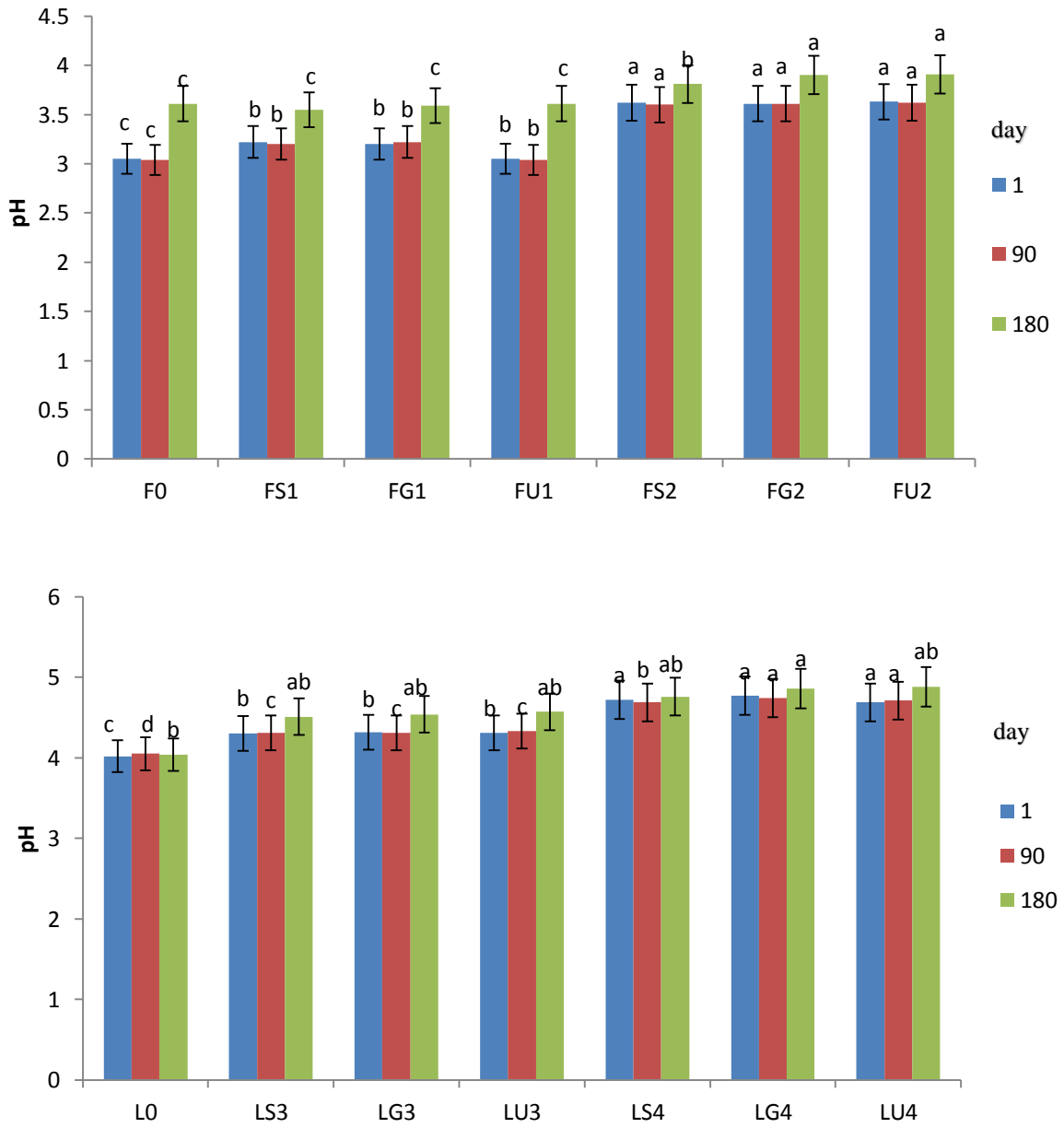
**Table 4.** Chemical properties of processed fruit containing 3% algae

CHEMICAL PROPERTIES	FS <sub>2</sub>	FG <sub>2</sub>	FU <sub>2</sub>	F <sub>0</sub>	LS <sub>4</sub>	LG <sub>4</sub>	LU <sub>4</sub>	L <sub>0</sub>
MOISTURE(%)	25.50±0.07a	24.50±0.70ab	25.5±0.70a	24.5±0.70a	13.50±0.70a	13.50±0.70a	14.50±0.70a	13.50±0.70a
ASH(%)	3.07±0.10a	3.35±0.21a	3.20±0.28a	3.10±0.14a	4.25±0.35a	4.5±0.42a	4.15±0.07a	4.04±0.56a
INSOLUBLE ASH(%)	0.002±0.00a	0.002±0.00a	0.003±0.00a	0.002±0.00a	0.001±0.00a	0.002±0.0a	0.002±0.00a	0.002±0.00a
pH	3.07±0.06a	3.13±0.01a	3.01±0.02a	3.06±0.06a	4.06±0.08a	4.11±0.14a	4.02±0.01a	4.03±0.02a
ACIDITY(%)	6.20±0.28a	6.26±0.36a	6.45±0.07a	6.04±0.56a	6.20±0.28a	6.60±0.28a	6.40±0.56a	6.04±0.12a
SALT(%)	1.43±0.65a	1.22±0.07a	1.11±0.08a	0.75±0.07a	1.75±0.07a	1.40±0.14b	1.70±0.00a	0.80±0.00a
SO <sub>2</sub> (PPM)	0.16±0.07b	0.71±0.11a	0.33±0.01b	0.002±0.00a	0.001±0.00a	0.001±0.00a	0.002±0.00a	0.31±0.00b

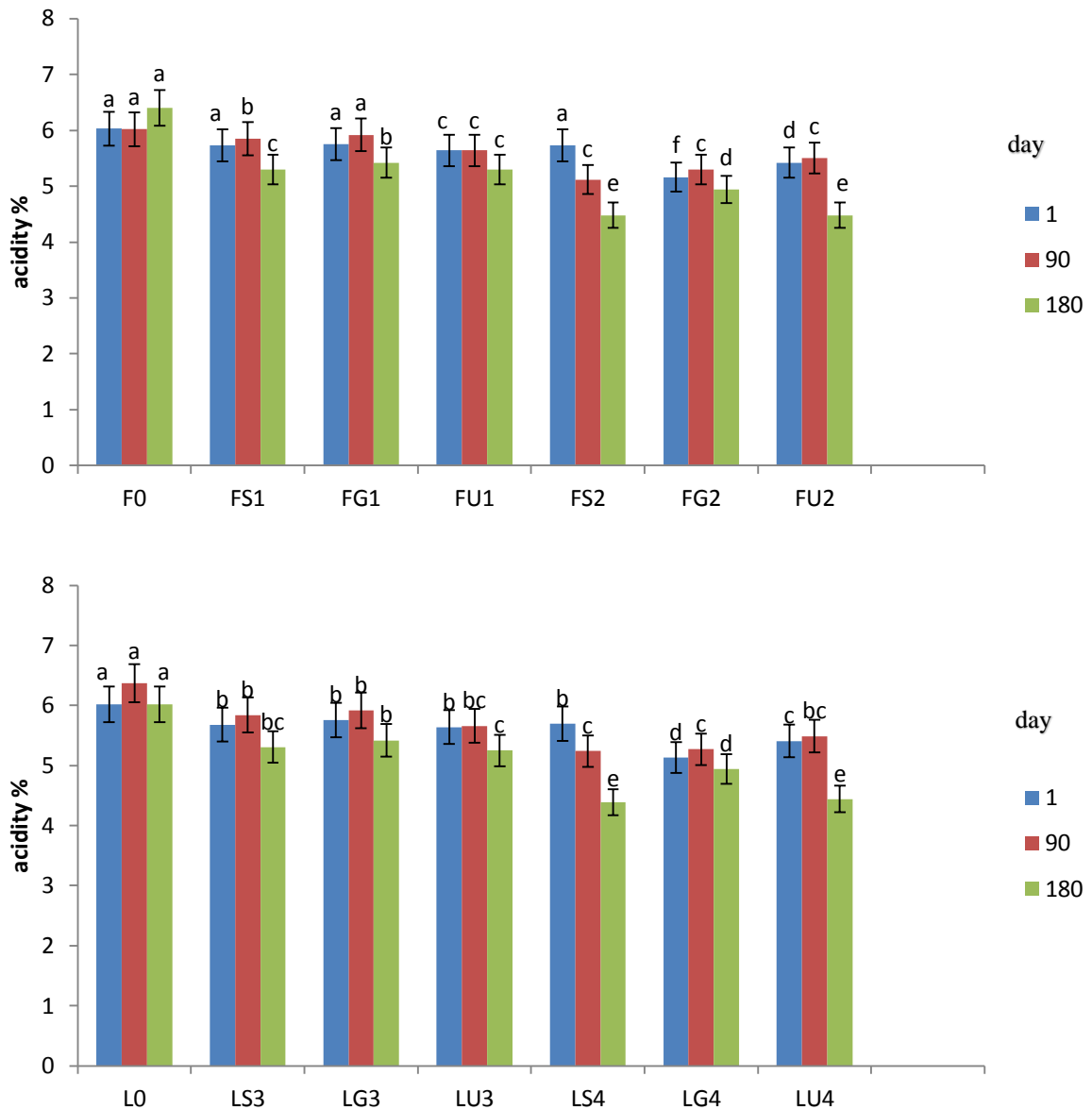
The different letters in a row are significantly different (p <0.05).



**Fig. 1.** Changes in the moisture content of the processed fruits containing algae during 180 days. The different letters are significantly different (p <0.05).



**Fig. 2.** Changes in pH value of the processed fruits containing algae during 180 days. The different letters are significantly different ( $p < 0.05$ ).



**Fig. 3.** Changes in the acidity of the processed fruits containing algae during 180 days. The different letters are significantly different ( $p < 0.05$ ).

**- Amino acid profile**

The amino acid compositions of processed fruits containing 1.5% and 3% of the algae are shown in (Figure. 4). In this study, 17 amino acids including essential and non-essential amino acids such as aspartic acid, glutamic acid, serine, histidine, glycine, threonine, arginine, alanine, proline, tyrosine, valine, methionine, cysteine, isoleucine, leucine,

and phenylalanine were identified. The most amino acids in sour date puree are alanine, proline, iso-leucine, leucine and proline, and in the apple paste are glutamic acid, threonine, alanine, valine, leucine, and lysine. The highest amount of amino acid is related to isoleucine in sour date puree enriched with *S. illicifolium* (1900 mg/100g). Histidine was not observed in the control apple paste and enriched apple



paste by *Ulvalactuca*. Also, threonine and valine were not observed in the control sour date puree.

The previous results showed that these algae have high amount of amino acids, therefore 40% of essential amino acids are

very close to soy protein and egg yolk (Hafezieh *et al.*, 2017 , Valerie *et al.*, 1999). According to previous studies, fifteen amino acids were identified in *S.illicifolium* and *G.cortica* (Pattama and Anong, 2006).

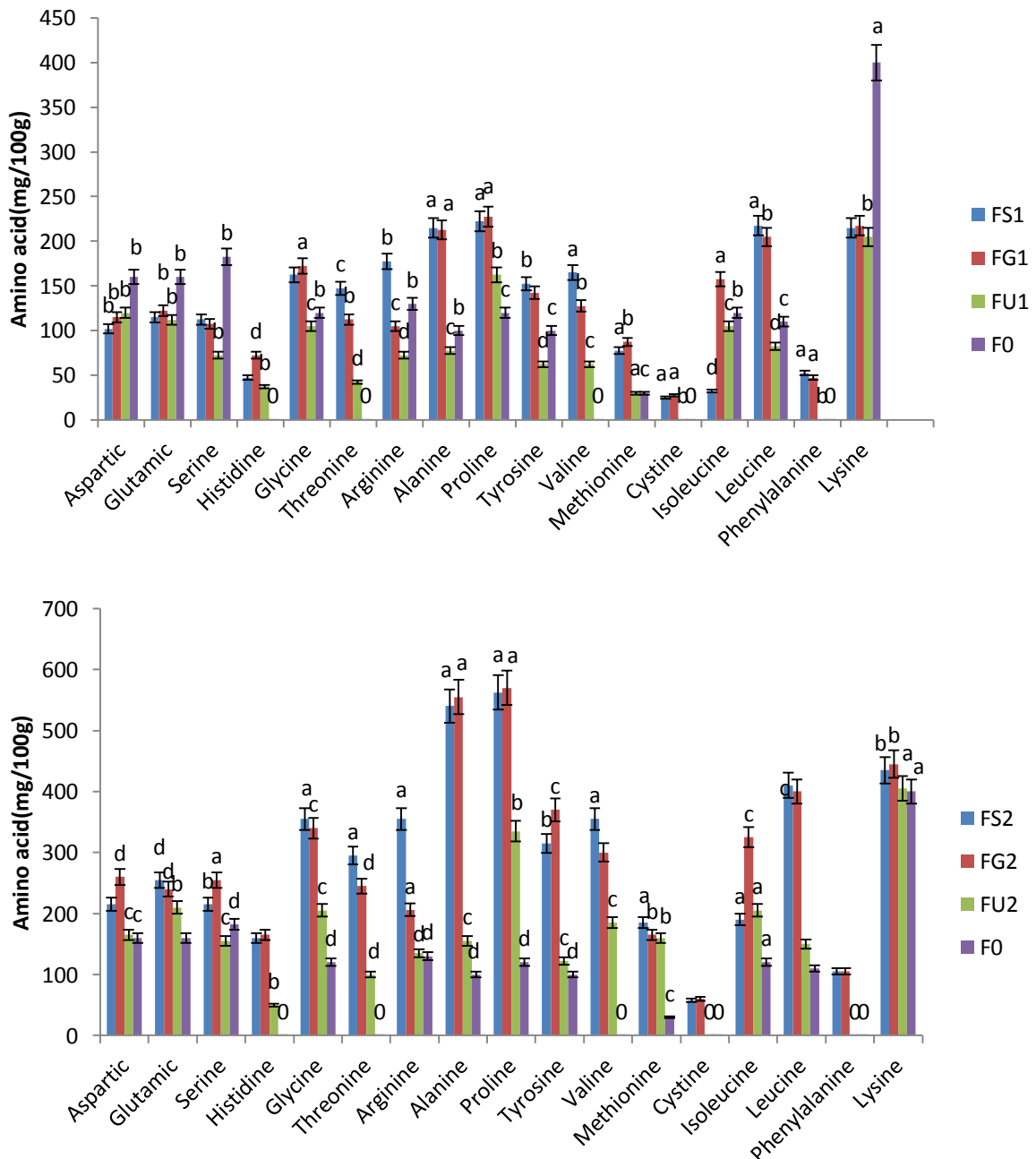
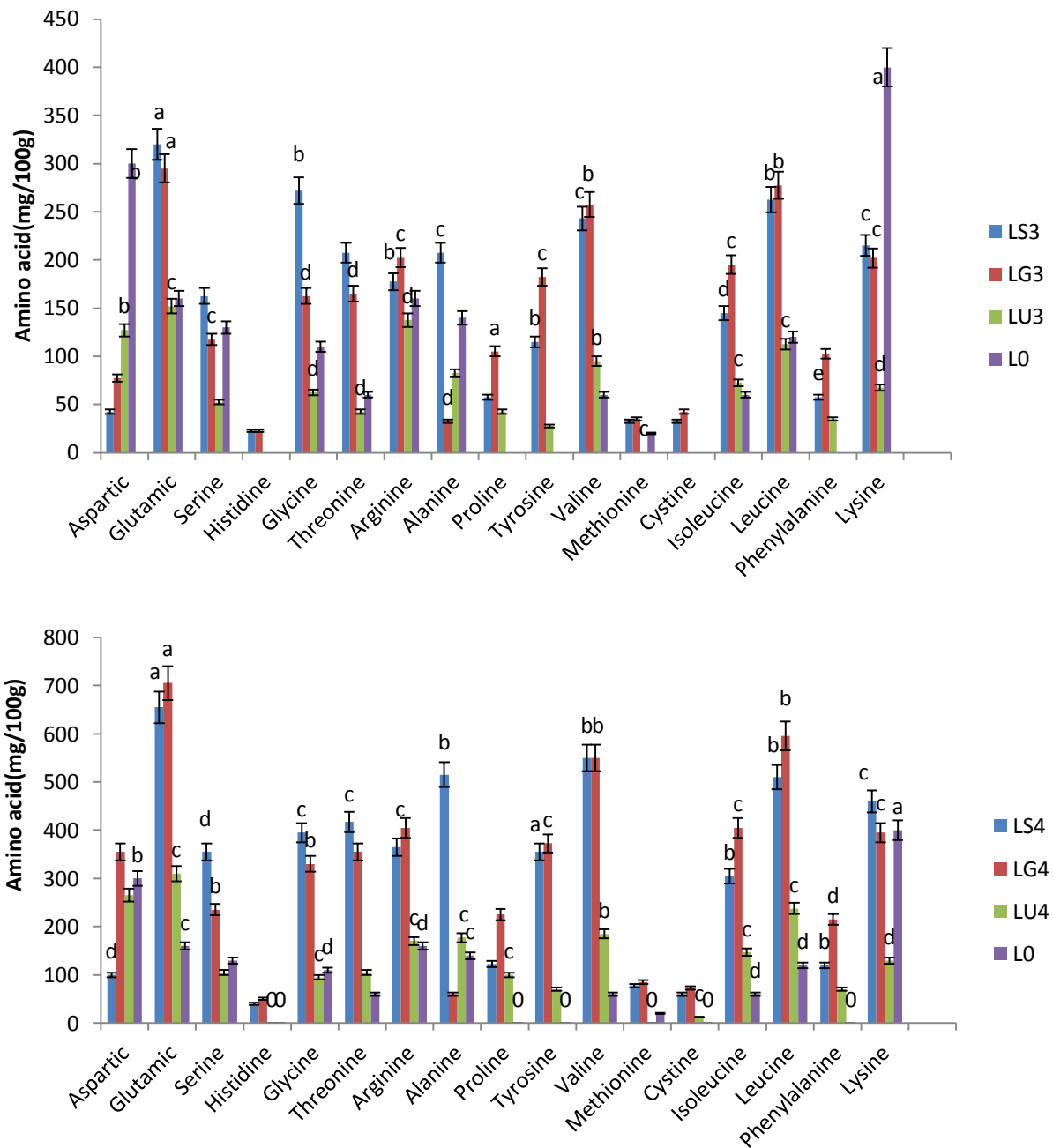


Fig. 4. Amino acid profile of processed fruit. The different letters are significantly different (p < 0.05).



**Fig. 4 (Continted).** Amino acid profile of processed fruit. The different letters are significantly different ( $p < 0.05$ ).

**- Microbial properties**

Microbial evaluation of processed fruits containing 1.5% and 3% algae is listed in (Tables 5 and 6). There was no significant difference between different samples regarding coliforms, acid-resistant bacteria, mold, and yeast ( $p > 0.05$ ). The

growth of *Escherichia coli* and *Staphylococcus aureus* was not reported in all samples. The highest total count was observed in the control processed fruit (113 cfu/g). The total count of samples decreased during 180 days (54-113 cfu/g). The population of coliforms, acid-resistant

bacteria, mold and yeast also showed a slight decrease over time ( $p > 0.05$ ). The contamination of samples was very low and was according to the national standard of Iran (ISIRI, No. 3308). The microbial content of these products is controlled by citric acid.

Various studies confirmed the antifungal, antibacterial, and antiviral properties of algae (AbaEl *et al.*, 2009, Wong, 2001). The compounds that have antimicrobial activity in algae are known as unsaturated fatty acids and steroids (Awad, 2000; Ohta *et al.*, 1995).

**Table 5.** Microbial properties of processed fruit during storage

Storage time	MO	FU <sub>2</sub>	FG <sub>2</sub>	FS <sub>2</sub>	FU <sub>1</sub>	FG <sub>1</sub>	FS <sub>1</sub>	F <sub>0</sub>
Day 1 (cfu/g)	<i>Escherichia coli</i>	-	-	-	-	-	-	-
	mold and yeast	67.00±2.82 <sup>a</sup>	66.50±0.36 <sup>a</sup>	65.50±3.53 <sup>a</sup>	66.00±1.41 <sup>a</sup>	66.25±0.35 <sup>a</sup>	95.25±0.35 <sup>a</sup>	62.00±1.41 <sup>a</sup>
	Total count	64.00±1.41 <sup>c</sup>	55.00±1.41 <sup>b</sup>	97.00±1.41 <sup>a</sup>	66.00±2.82 <sup>b</sup>	57.50±3.53 <sup>bc</sup>	93.00±5.65 <sup>a</sup>	54.00±2.82 <sup>b</sup>
	<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-
	coliform	6.00±0.00 <sup>a</sup>	7.50±0.70 <sup>a</sup>	5.07±0.70 <sup>a</sup>	6.55±1.06 <sup>a</sup>	7.75±0.35 <sup>a</sup>	6.50±0.70 <sup>a</sup>	6.50±0.00 <sup>a</sup>
Day90 (cfu/g)	Acid resistant bacteria	4.00±1.41 <sup>a</sup>	5.00±1.41 <sup>a</sup>	5.00±0.00 <sup>a</sup>	4.50±0.70 <sup>a</sup>	5.50±0.07 <sup>a</sup>	6.50±0.70 <sup>a</sup>	4.00±0.00 <sup>a</sup>
	<i>Escherichia coli</i>	-	-	-	-	-	-	-
	mold and yeast	39.00±1.41 <sup>a</sup>	26.00±1.41 <sup>d</sup>	36.00±1.41 <sup>b</sup>	24.50±0.70 <sup>d</sup>	20.00±0.00 <sup>f</sup>	31.00±1.41 <sup>c</sup>	36.00±0.07 <sup>a</sup>
	Total count	46.50±2.12 <sup>b</sup>	52.00±2.82 <sup>b</sup>	54.50±2.12 <sup>b</sup>	32.50±3.53 <sup>c</sup>	83.50±7.77 <sup>a</sup>	57.50±2.12 <sup>b</sup>	5.00±1.41 <sup>a</sup>
	<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-
Day180 (cfu/g)	coliform	5.50±0.70 <sup>a</sup>	6.00±1.41 <sup>a</sup>	6.50±0.70 <sup>a</sup>	6.00±0.70 <sup>a</sup>	6.00±0.00 <sup>a</sup>	6.50±0.70 <sup>a</sup>	4.50±1.41 <sup>a</sup>
	Acid resistant bacteria	4.50±0.70 <sup>a</sup>	5.50±0.70 <sup>a</sup>	5.00±0.00 <sup>a</sup>	4.50±0.70 <sup>a</sup>	4.50±0.07 <sup>a</sup>	5.00±0.00 <sup>a</sup>	5.00±1.41 <sup>a</sup>
	<i>Escherichia coli</i>	-	-	-	-	-	-	-
	mold and yeast	39.00±1.41 <sup>a</sup>	29.50±1.70 <sup>c</sup>	37.00±1.41 <sup>ab</sup>	41.00±1.41 <sup>a</sup>	34.00±2.82 <sup>b</sup>	39.00±1.41 <sup>a</sup>	52.50±2.21
	Total count	46.00±1.41 <sup>b</sup>	51.00±1.41 <sup>a</sup>	49.00±1.41 <sup>ab</sup>	37.00±1.41 <sup>f</sup>	41.00±1.41 <sup>c</sup>	46.50±2.12 <sup>b</sup>	40.50±0.07 <sup>a</sup>
Day180 (cfu/g)	<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-
	coliform	4.50±0.70 <sup>a</sup>	5.50±0.70 <sup>a</sup>	4.00±1.41 <sup>a</sup>	4.50±0.70 <sup>a</sup>	5.50±0.70 <sup>a</sup>	5.00±1.41 <sup>a</sup>	5.50±1.41 <sup>a</sup>
	Acid resistant bacteria	6.50±0.70 <sup>a</sup>	5.50±0.07 <sup>b</sup>	5.50±0.70 <sup>ab</sup>	7.00±0.00 <sup>a</sup>	5.50±0.70 <sup>ab</sup>	4.50±0.70 <sup>b</sup>	4.50±1.41 <sup>b</sup>

The different letters in a row are significantly different ( $p < 0.05$ ).

**Table 6.** Microbial properties of processed fruit during storage

Storage time	MO	LU <sub>4</sub>	LG <sub>4</sub>	LS <sub>4</sub>	LU <sub>3</sub>	LG <sub>3</sub>	LS <sub>3</sub>	L <sub>0</sub>
Day 1 (cfu/g)	<i>Escherichia coli</i>	-	-	-	-	-	-	-
	mold and yeast	63.00±1.41 <sup>a</sup>	52.00±1.01 <sup>a</sup>	61.00±0.41 <sup>a</sup>	42.50±0.12 <sup>a</sup>	50.00±1.41 <sup>a</sup>	63.00±1.41 <sup>a</sup>	45.50±1.00 <sup>a</sup>
	Total count	112.00±3.53 <sup>a</sup>	111.00±1.41 <sup>a</sup>	112.50±3.53 <sup>a</sup>	60.50±7.07 <sup>a</sup>	105.50±7.07 <sup>a</sup>	111.00±1.41 <sup>a</sup>	113.00±0.70 <sup>a</sup>
	<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-
	coliform	4.50±0.70 <sup>a</sup>	5.00±1.41 <sup>a</sup>	5.50±0.70 <sup>a</sup>	4.50±0.70 <sup>a</sup>	4.50±0.70 <sup>a</sup>	4.00±1.41 <sup>a</sup>	5.00±1.41 <sup>a</sup>
Day 90 (cfu/g)	Acid resistant bacteria	5.00±1.41 <sup>a</sup>	4.50±0.70 <sup>a</sup>	5.50±0.70 <sup>a</sup>	4.75±0.35 <sup>a</sup>	4.50±0.70 <sup>a</sup>	3.50±0.07 <sup>a</sup>	4.50±0.07 <sup>a</sup>
	<i>Escherichia coli</i>	-	-	-	-	-	-	-
	mold and yeast	60.50±3.53 <sup>a</sup>	47.00±3.53 <sup>b</sup>	48.00±2.82 <sup>b</sup>	61.50±4.94 <sup>a</sup>	41.50±2.12 <sup>b</sup>	47.00±1.41 <sup>b</sup>	58.00±3.41 <sup>a</sup>
	Total count	38.00±2.82 <sup>c</sup>	59.00±2.82 <sup>a</sup>	60.00±2.82 <sup>a</sup>	46.50±4.94 <sup>b</sup>	56.50±0.70 <sup>a</sup>	59.00±1.41 <sup>a</sup>	60.00±1.41 <sup>a</sup>
	<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-
Day 180 (cfu/g)	coliform	5.50±2.12 <sup>a</sup>	5.50±0.70 <sup>a</sup>	6.41±1.41 <sup>a</sup>	5.50±2.12 <sup>a</sup>	4.00±1.41 <sup>a</sup>	6.00±1.41 <sup>a</sup>	5.00±1.41 <sup>a</sup>
	Acid resistant bacteria	4.50±2.12 <sup>a</sup>	4.50±0.70 <sup>a</sup>	5.00±1.41 <sup>a</sup>	4.05±2.12 <sup>a</sup>	4.50±2.12 <sup>a</sup>	3.50±0.07 <sup>a</sup>	4.50±2.12 <sup>a</sup>
	<i>Escherichia coli</i>	-	-	-	-	-	-	-
	mold and yeast	38.00±2.82 <sup>a</sup>	37.00±1.41 <sup>a</sup>	41.00±1.41 <sup>a</sup>	37.00±4.42 <sup>a</sup>	38.00±2.82 <sup>a</sup>	39.00±1.41 <sup>a</sup>	40.50±1.41 <sup>a</sup>
	Total count	41.00±1.41 <sup>b</sup>	57.00±3.53 <sup>a</sup>	62.50±3.53 <sup>a</sup>	41.00±1.41 <sup>b</sup>	55.00±4.24 <sup>a</sup>	53.00±9.89 <sup>ab</sup>	45.00±0.07 <sup>a</sup>
Day 180 (cfu/g)	<i>Staphylococcus aureus</i>	-	-	-	-	-	-	-
	coliform	5.00±1.41 <sup>a</sup>	7.00±0.00 <sup>a</sup>	6.50±1.41 <sup>a</sup>	5.50±0.70 <sup>a</sup>	5.50±0.70 <sup>a</sup>	5.50±0.70 <sup>a</sup>	4.50±0.00 <sup>a</sup>
	Acid resistant bacteria	4.50±0.70 <sup>a</sup>	4.50±0.70 <sup>a</sup>	4.50±0.70 <sup>a</sup>	5.00±1.41 <sup>a</sup>	5.00±1.41 <sup>a</sup>	3.50±0.70 <sup>a</sup>	5.00±1.41 <sup>a</sup>

The different letters in a row are significantly different ( $p < 0.05$ ).

## Conclusions

Seaweed such as *S.illicifolium*, *U.lactuca*, and *G. corticaalgae* is an excellent potential source of natural compounds that can be used as a functional food. Our results showed that the chemical properties of the final product were in accordance with the national standard of Iran. No microbial growth was observed in the final products. Essential and non-essential amino acids were identified in the final product. Since the addition of *S.illicifolium*, *U.lactuca*, and *G. corticaalgae* did not have an adverse effect on the sensory properties of the final product, they can be used in the production of functional sour date purees and Apple Paste.

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