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Abstract

Fruits and vegetables are considered essential food materials for human nutrition, as they are very rich in minerals, vitamins, fibers, and contain low levels of calories, proteins, and fat. In particular, the genus Citrus contains many secondary metabolites known to correlate positively with reduced risks of chronic diseases such as cardiovascular disease, diabetes and cancers. Freezing storage is considered the best and most convenient processing and preservation method for fruit juices to extend or lengthen their shelf life and preserve their fresh quality. The main goal of this study was to investigate the influence of freezing storage on the quality parameters of lime juice extracted from two lime fruits obtained from two locations in Khartoum City, Sudan. Fresh mature green and full-ripe yellow lime fruits (*Citrus aurantiifolia* L.) cultivars at optimal maturity with uniform size, shape and without visible infections were obtained from Tuti Island and Algaili farms. The juice was extracted using stainless steel hand-held juice squeezer and filtered through a stainless steel sieve. The juice was divided into two parts. The first part was used directly for different investigations, while the second part was stored in a -20 C freezer for 6 months and used for further investigations. The quality parameters such as physicochemical characteristics and sensory evaluation were determined using standard procedures. The freezing storage period was found to have variable effects on lime juice quality. The viscosity, total soluble solids and Vitamin C content remain unchanged, whereas, the pH decreases and the titrable acidity increases after freezing. The brightness and yellowness values increased, while the greenness value decreased. The juice was acceptable and not significantly affected by freezing according to the panelists' perception. The findings of this study recommend using freezing storage for storing lime juice for a long period of time without negative effects on its quality

Keywords: Freezing, storage, Lime juice, Quality parameters, sensory evaluation

Introduction

The Lime fruit (*Citrus aurantiifoliaL.*) is considered as one of the most important fruit worldwide. It is very rich in many vital natural chemical components such as citric acid, ascorbic acid, minerals, flavonoids and essential oils (Shrivastava and Tyagi, 2013; Uçan *et al.*, 2014). Lime fruits not only have delicious flavors but also, very rich in antioxidants with health benefits (Saeid and Ahmed, 2021). Limes are usually produced for fresh consumption and for production of juices, additives and various other processed products. Parameters such as color consistency and purity, physicochemical properties and vitamin C contents are considered among most important characteristics that indicate the quality and acceptance of food product by consumers (Shatta, 2006) The prices of Lime fruits in Sudan fluctuate due to the shortage of supply during off-season. The freezing storage is regarded as the most convenient and easiest processing and preservation method for fruit juices as it extends and lengthens their shelf life. Moreover, it preserves the fresh quality of juice and reduce sit capital costs (Peneau, 2005; Barbosa *et al.*, 2005).According to Shatta (2006), the quality stability during processing, storage and transportation is one of the most important challenges of food producers. The author mentioned that, studies on the effects of frozen storage and concentration method on the chemical and rheological properties of lime juice are limited and incomplete.

On the other hand, substantial amounts of lime fruits are normally lost as post-harvest losses or due to inefficient storage facilities and lack of industrial utilization of lime fruit in Sudan. Therefore, production of frozen lime juice as ready to use product at commercial level with high quality and nutritional value not only will make the product more available throughout the year and cheaper but also, will add economic value to the raw materials and provide job opportunities in the lime fruits production areas. Therefore, the main goal of this study is to investigate the effects of freezing storage on the quality characteristics and acceptability of frozen lime juice.

Materials and Methods

Materials

Fresh mature green and full-ripe yellow lime fruits (*Citrus aurantiifoliaL.*) cultivars at optimal maturity with uniform size and shape and without visible infections were obtained from Tuti Island and Algaili farms. The fruits were thoroughly washed with water and halved with a sharp stainless steel knife. The juice was extracted using stainless steel hand-held juice squeezer and filtered through a stainless steel sieve. The juice was divided into two parts. The first part was used directly for different investigation, while the second part was stored in a -20 C freezer for 6 months and then used for different investigation.

Methods

Physiochemical methods

Viscosity as centipoises, total soluble Solids (TSS %) and hydrogen ions concentration (pH) of lime juice were measured according the official methods of the AOAC (2010). Whereas, the colorof lime Juice was measured by chama meter calibrated with a white plate and light trap supplied by the manufacturer. The color was expressed by using the CIE L, a, and b color system (CIE, 1976). A total of three spectral readings were taken for each sample expressed as Lightness (L*) values (dark to light), the redness (a*) values (reddish to greenish).The yellowness (b*) values (yellowish to bluish).

Chemical methods

Titrable Acidity (TA)

Titrable acidity was determined according to the A.O.A.C, (2010). An amount of 10 ml of well mixed juice, diluted to 250 ml with neutralized or recently boiled water, titrated with 0.01N NaOH using 0.3 ml phenolphthalein for each 10 ml of the solution to pink end point persisting for 30 seconds. Reported acidity as ml 0.01 N NaOH per 10 gm or 10 ml as required.

Ascorbic acid content

Ascorbic acid content was determined according to Ruck (1963) method in the manner described by El-obied (2003). 30g of the sample were blended with about 100ml of 0.4% oxalic acid (4gms /100ml) for 2minutes in a blender. The blended mixture was made up to 500ml in a volumetric flask with 0.4% oxalic acid and filtered. Ascorbic acid in the filtrate was determined by titrating 20ml of the filtrate against 2,6-dichlorophenol indophenols (0.2g/500ml distilled water) of known strength, ascorbic acid, expressed in mg/ 100g dry matter, was calculated as follows:

 $Ascorbic \ Acid \ (mg/100g) = \frac{\text{Titer} \ (ml) \times \text{Dye strength} \times 100}{\text{Titer}}$

Where the factor = Weight × Sample volume taken for titration Total volume of the sample

The dye was standardized as follows:

50mg of standard ascorbic acid were weighed and made up to volume by 10% oxalic acid in a 250 volumetric flask and 5ml a liquid was diluted with 5ml 10% oxalic acid (50 grams oxalic acid/500m distilled water) and titrated with the dye solution to a pink end point.

Sensory evaluation method

The sensory evaluation was carried out in the fresh and frozen lime juices following the Hedonic scale method which consists of 10 points. The panelists were asked to evaluate the two lime juices with respect to their color, flavor, taste, and purity.

Results and Discussions

Physiochemical Characteristics

Lime juice viscosity (Cp)

Juice viscosity is an important property for all fruits and greatly affects the consumer's acceptability. However, the 6 month freezing storage period didn't change the viscosity significantly in all lime juice samples used in this study (Fig. 1). The results obtained in this study contradict the findings of Jittanit *et al.*, (2013), who found the viscosity values decreasing along the longer storage period under four storage conditions: pasteurization at 85 °C for 30 seconds, or 95°C for 30 seconds, sterilization at 136°C for 4 seconds and vacuum evaporation at temperature range of 68-72°C and vacuum pressure of 97325.35 Pa before pasteurization at 72°C for 15 seconds. The possible reason for this contradiction is that the freezing temperature may have different influence on the viscosity over long storage period.



Fig. 1: Effect of freezing storage on the viscosity of Tuti and Algaili lime juices.

Total soluble solids (T.S.S %)

The total soluble solid content as per-cent in lime juice is mainly organic acids and marginally sugars (Manjunatha *et al.*, 2014). The 6 month of storage period at freezing temperature (-20 °C)didn't have a significant influence in all types of lime juice when compared to each other's or to their fresh lime juices (Fig. 2). This result agrees with Shatta (2006) who reported that

the freezing storage of lime juice after 12 months of storage didn't affect the total soluble solids significantly. This result also agrees with El-Ashwah *et al.*, (1974) who reported than the soluble solid contents remained unchanged in unpasteurized frozen storage lime juice.





Hydrogen ions concentration (pH)

Effects of freezing storage on lime juice hydrogen ions concentration (pH) are presented n Fig. 3. In general, the freezing storage period for lime juices for 6 months was found to have significant decreases in the pH values of lime juice of both green and yellow Tuti and Algaili lime fruits. This finding agrees well with Samad et al., (2019) who found that the storage duration affect the pH value of fruit juice significantly. Also, Giuffre at al. (2017) found a slight decrease in the pH value of blood orange juice during freezing storage. Oppositely, these results disagree with Shatta (2006) who reported that the freezing storage of lime juice after 12 months of storage didn't affect the pH significantly. Also, Gundogdu and Kirziglu (1995) and Molinari and Silva (1998) found no significant differences in lime juice pH after freezing storage. In general, low juice pH indicates high acidity which is one of the major qualitative attributes of the lime juice (Samad et al., 2019). However, the differences in the results obtained in this study and other studies



may be due to the variations in lime fruits varieties and maturity levels.

Fig. 3:Effects of freezing storage on hydrogen ions concentration (pH)of Tuti and Algaili lime juices

Total acidity (g/100g)

The effect of freezing storage on total acidity of lime juices are presented in Fig. (4).the results indicate a slight increase in the total acidity of both lime juices after 6 months of freezing storage. Significant differences were found in total acidity of both green Tuti and yellow Algaili limes. This results greatly agree with Giuffre at al. (2017), who found only 3.94 % increase in total acidity of blood orange juice after 5 month freezing storage. In contrast, Shatta, (2006) reported that the freezing storage of lime juice after 12 months of storage had a significant effect on juice acidity. While, El-Ashwah et al., (1974) reported that, the acidity contents remained unchanged in unpasteurized frozen storage lime juice. Moreover, Samad et al., (2019) reported that the acidity level decline during ripening because the metabolism of citric acid occur which is the main organic acid in citrus. Therefore, the total acidity level in ripen fruits are usually lower than the acids in half ripen and mature green fruits during storage. Furthermore, the decline in acidity during storage with increase in maturity could be due to enhancement of sugar substance in fruits which suppose to boost respiratory action during storage in citrus fruits. This decrease in titratable acidity during storage makes fruits lesser acidic thus it can be more suitable for consumption.



Fig. 4: Effect of freezing storage on total acidity of Tuti and Algaili lime juices

Ascorbic acid content (mg/100ml)

Ascorbic acid content is the most important quality characteristic of fruit juice because it reflects the nutritional value and technological characteristics of the juice. Fig.5 shows the effects of freezing storage on vitamin C content in Tuti and Algaili lime juices. In general, after 6 months of freezing storage, the ascorbic acid content was found to be higher in green lime than yellow lime (p < 0.05) and was higher in Tuti lime than in Algaili lime as indicated in Fig. 5. In fact, the ascorbic acid content after 6 months of freezing storage did not change significantly in all types of lime juices when compared to fresh lime juices (Fig. 5). These results are inconsistent with those of El-Ashwah (1974) who found a significant decrease in ascorbic acid content after 12 months of freezing storage of both pasteurized and unpasteurized lime juices. Also, these results disagree with those obtained by Abd-El-Baki (1980) and Askar (1981). The two authors reported a pronounced degradation in ascorbic acid content during the freezing process and concentration of lime juice.

The decline in ascorbic acid during storage of citrus fruit may be due to the oxidation process or o the pH increment. However, after 40 days storage, the maximum ascorbic acid content was (16.86 mg 100 ml-1) recorded in fruits treated with garlic extracts, followed by ginger extract (15.86 mg 100 ml⁻¹). Whereas, the minimum ascorbic acid content (12.32 mg 100ml⁻¹) Whereas, the minimum ascorbic acid content (12.32 mg 100ml⁻¹).



Fig. 5.Effect of freezing storage on vitamin-C content of Tuti and Algaili lime juices

Color value of lime juice

Lightness value (L*)

Fig. 6 shows the effects of freezing storage on color Lightness value (L*) of lime juice. As a fact, the color characteristic is considered as one of the major parameters that affect the quality characteristics of fruits final products. From the results obtained in this study, higher (L*) color value was observed in lime juice after 6 months freezing storage than in the fresh juice (Fig. 6). This result is disagree with Shatta (2006) who reported that the lightness (L*) values of lime juice gradually decreased during storage time from 55.2 at zero time to 47.7 at the end of storage time. Furthermore, the concentration of fruit juice resulted in lower values of lightness in comparison to that of the control. This may be attributed to the direct effect of heat treatment on sugars. Also, Ziena, (2000) found that, the browning index colors of lime juice gradually increased by cold storage. The changes were higher for refrigerated samples more than frozen ones.



Fig. 6: Effect of freezing storage on Lightness value (L*) of Tuti and Algaili lime juices

Greenness and redness value (a*)

Effects of freezing storage on greenness and redness value (a*) of Tuti and Algaili lime juices are presented in Fig.7. The results showed the color (a*) values of both fresh and frozen juices were negative with a green color and significantly affected during the frozen storage. However, the greenness colour intensity decreased significantly with increasing storage period (Fig. 7). This results agree well with Shatta *et al.*, (2006) who reported decreasing values of (a*) value from - 3.0 to - 1.4.Also, Jittanit *et al.* (2013) reported a decline greenness values during freezing storage oh lime juice. The author attributed the drop to the chlorophyll degradation due to the remaining enzymes and light exposure during storage.



Fig. 7:Effects of freezing storage on greenness and redness color values (a *).

Yellowness to blueness color values (b*)

Fig. 8indicates the effects of freezing storage on the color (b*) values Tuti and Algaili lime juice. The results obtained in this study showed the color (b*) values were significantly affected in both fresh and frozen storage juice (Fig. 8). The yellowness colour intensity increased significantly with increasing storage period. This results are well agree with Jittanit *et al.* (2013) who found an increment in b* values after 7 weeks frozen storage for juice treated with three different pasteurization methods, in contrast, this results was found to be disagree with Shatta, (2006) who reported a decreasing value of redness from 13.8 to 10.8 after 12 months of storage at -20 C.





Table 1: Sensor	v evaluation 1	results of	fresh and	frozen lime	iuices of	Tuti and	Algaili fruits

Sensory evaluation of fresh and frozen lime juices

Sensory evaluation test was conducted in fresh and frozen Tuti and Algaili lime juices. Twenty (20) Panelists from the Dept. of Food Technology, Faculty of Agricultural Technology and Fish Sciences at Al Neelain University(Khartoum, Sudan) were asked to evaluate the different samples of lime juices with respect to their color, flavor, taste; purity and overall quality, following the 10 points hedonic scale as described by (Pimentel *et al.*, 2016).

Table 1: indicates the results of the sensory evaluation test that conducted on both fresh and frozen lime juices of Tuti and Algaili fruits. According to the results obtained in this study, the color, flavors, taste and purity characteristics of both lime juices were not significantly affected during the six months freezing storage period (Table 1). The maximum color, flavor, taste and purity scores ranged from 7.6 to 8.0, 5.5 to 6.4, 6.3 to 7.0 and 7.5 o 7.8, respectively. These results indicate the good stability of lime juice of Tuti and Algaili fruits during frozen storage.

•		Tuti lime		
Green	Yellow	Green	Yellow	
8.00 ±0.33ª	7.60 ± 0.43 ^a	8.30 ± 0.42^{a}	7.80 ± 0.33 °	
6.40 ± 0.34 ^a	6.40 ± 0.31 ^a	5.80 ± 0.33 ^a	5.50 ± 0.52 ^a	
$6.30\pm0.45^{\rm a}$	6.40 ± 0.27 ^a	6.60 ± 0.34 ^a	7.00 ± 0.40 ^a	
7.60 ± 0.37 ^a	$7.50\pm0.86^{\ a}$	7.80 ± 0.37 ^a	7.60 ± 0.45 ^a	
	Green 8.00 ± 0.33^{a} 6.40 ± 0.34^{a} 6.30 ± 0.45^{a} 7.60 ± 0.37^{a}	GreenYellow 8.00 ± 0.33^{a} 7.60 ± 0.43^{a} 6.40 ± 0.34^{a} 6.40 ± 0.31^{a} 6.30 ± 0.45^{a} 6.40 ± 0.27^{a} 7.60 ± 0.37^{a} 7.50 ± 0.86^{a}	GreenYellowGreen 8.00 ± 0.33^{a} 7.60 ± 0.43^{a} 8.30 ± 0.42^{a} 6.40 ± 0.34^{a} 6.40 ± 0.31^{a} 5.80 ± 0.33^{a} 6.30 ± 0.45^{a} 6.40 ± 0.27^{a} 6.60 ± 0.34^{a} 7.60 ± 0.37^{a} 7.50 ± 0.86^{a} 7.80 ± 0.37^{a}	

Values are presented as mean \pm SE (n = 3).

Values with same letters in same row indicate no significant difference at *p*-value 0.05.

Conclusion

According to the results obtained in this study it can be concluded that the freezing storage as a preservation method had variable effects on both Tuti and Algaili lime juices. The viscosity, total soluble solids and vitamin C content remain unchanged, whereas, the pH decreased and the titrable acidity increased after six (6) months freezing storage period. In contrast, the color, flavors, taste and purity characteristics of both lime juices were not significantly affected during the same storage period. On the other hand, the lightness (L*) and yellowness (b*) color values of both lime juices increased significantly with increasing storage period. While, the greenness color (a *) intensity decreased significantly with increasing storage period in comparison with the fresh juice. Finally, the freezing storage of lime juices is a suitable and acceptable preservation method for lime juices.

Recommendations

Further studies are definitely needed to cover the following areas:

- National survey should be conducted to study the different quality characterizes of lime fruits cultivars in Sudan
- Industrial utilization of enzymes in lime juice purifications and processing.
- Commercial and Industrial utilization of freezing process in production of frozen limejuices as ready to use products.
- 4. Industrial production of innovative products from limejuices should be encouraged.

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