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Improving Characteristics of Frozen Yogurt Enriched with Loquat Leaves Extract

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Abstract

Frozen yogurt is a fermented frozen dairy dessert that combines the physical qualities of ice cream with the sensory and nutritional benefits of fermented milk. Frozen yogurt is considered a healthier alternative to ice cream because of its low-fat content. In this study, the frozen yogurt was incorporated with 1% loquat leaf extracts obtained using 30, 70, and 90% ethanol. The final product was evaluated for physical characteristics (pH, total acidity, melting rate and, color), total phenolic content, microbiological analysis, and sensory evaluation. The results obtained illustrated that leaves extract obtained using 90% ethanol has the highest content of flavonoids (1.023 mg QE/g). Pyrogallol was found to be the main phenolic compound in the 70% ethanol extract. All supplemented frozen yogurt samples were effective against all tested microbial strains with 1% loquat extracts. The lowest MIC (minimum inhibitory concentration) values observed with the samples contained 1% of 30% ethanolic loquat leaf extract at concentration of 5.0 µg/µl for Staphylococcus aureus and Escherichia coli. Data also indicated that the control sample recorded the highest pH value (4.67) and was the fastest in melting rate. Furthermore, the a* values of frozen yogurt with 90% ethanol extract were lower, while the b * values were higher. The same samples had a significantly higher amount of total phenolic content by 3.04 times compared to the control sample. The control frozen yogurt registered the highest value of the total plate count (30x102cfu/g).

Keywords: Loquat Leaves, frozen yogurt, melting rate, microbiological analysis.

Introduction

Plants are a great source of natural antioxidants as found in previous research. Plants contain phenolic compounds, flavonoids, tannins, and lignin as well as vitamin A and E and other substances with antioxidant characteristics (Razali et al., 2012). Loquat (*Eriobotrya japonica*) is an Asian fruit belonging to the Rosaceae family. The plant is indigenous to southeast China, where it thrives primarily in subtropical and mild-temperate climates (Cha et al., 2011). It is currently grown in South Africa, South America, Australia, and California, among other places. The evergreen loquat tree has short branches and blooms in the late fall or early winter (Hong et al., 2008).

Additionally, loquat leaves are a traditional Chinese herb with phlegm-transforming and cough-suppressing properties (Lin, 2007& Lin and Tang, 2008).Loquat leaves are frequently used to make loquat tea in Japan (Tan et al., 2014).Triterpenoids and flavonoids, which are derived from leaves, flowers, and fruits, have hypoglycemic, anti-inflammatory, antioxidant, and anticancer properties (Dufour et al., 2007; Ju et al., 2003). The median lethal dose of LLE (leaf aqueous extract) was higher than 5000 mg/kg BW (Khouyaa et al., 2022).

Traditional extraction techniques like solvent extraction are more frequently utilized to isolate bioactive chemicals. The circumstances of the extraction and the solvent polarity determine the extraction yield of bioactive chemicals in this approach. The solvent extraction method is widely utilized for extraction because there have been no studies showing any negative effects on plant bioactive components; however, because of the lengthy extraction procedure, scientists have been looking for alternatives (Dufour et al., 2007& Ju et al., 2003).

Demand for functional and health-promoting foods has increased recently as a result of customers' interest in the nutritional properties of foods, and the food industry has concentrated on revamping traditional meals to maximize nutritional value while retaining or improving the product's flavor (Rodrıguez-Rojo et al., 2012).

A dessert called frozen yoghurt combines the texture of ice cream with the nutritive and healthy properties of yoghurt (Daraei et al., 2021). Its process consists in mixing all ingredients to make natural stirred yoghurt with stabilizers/emulsifiers and sugar, then freezing the mix in a conventional ice cream freezer (Rezaei et al., 2011).

One of the most popular frozen sweets consumed worldwide is frozen yoghurt, yogurt products are increasingly popular, and producers are constantly looking for value-added ingredients to appeal to consumers who are health conscious (Tamime & Robinson, 2007).

Although loquat (*Eriobotrya japonica*) grows well in Egypt, it is not widely known. In 2007, the total area of loquat was about 122.08 ha with production of 1273 tonnes. Cultivars grown include 'El Sukary', 'Advance', 'Premiere', and 'Late Victoria') Elsabagh, 2011).

Hence the present study was carried out to investigate the potential application of loquat leaves extracts as antibacterial activity against some selected spoiling and pathogenic microorganisms and its effect on sensory and physical properties of the produced frozen yoghurt. Moreover, prolonging the shelf-life through inhibiting the spoilage organisms and producing more healthier popular dairy product. The frozen yogurt was formulated with loquat leaves extract based on the acceptable optimal levels and was evaluated according to Physical characteristics, (pH, total acidity, melting rate, color, total phenolics), microbiological analysis, and sensory evaluation as response variables. The benefit of this research is to provide information to the public about frozen yogurt enriched with loquat leaves extract as food products of high nutritional and beneficial health values.

Materials and Methods

Materials

Loquat leaves were collected in May 2020 from a garden in Baheej Village, Burj Al Arab Center - Alexandria. Fresh whole raw buffalo's milk and cow milk were obtained from Dairy department, Faculty of Agriculture, Kafrelsheikh University, Starter culture used in frozen yoghurt was obtained from Faculty of Agriculture, Ain Shams University. Sucrose, vanilla, gelatin, skim milk powder and emulsifier were obtained from local market, at Tanta City, El-Gharbia Governorate, Egypt.

Methods:

Preparation of loquat leaves extracts:

Loquat leaves were dried in the electric oven air at the temperature of 50°C, the loquat leaves were ground and screened through a mesh size of 60. The powder was then weighed, dipped into a mixture of 30, 70, and 90% ethanol (1:20 w/v), and refluxed 3.5 hrs. at 80°C. Ethanol can be recovered through reduced pressure distillation, and then ethanol extract of loquat leaves was dried by rotary evaporator. The extraction was then transferred to a 10 mL volumetric flask and stored at 4 °C in the refrigerator as directed by (Zhang et al., 2018).

Preparation of frozen yogurt

Preparation of plain yoghurt:

Fresh cow milk was heated to 60 °C and homogenized for 15 min. This mixture was then cooled to 45 °C and 0.03% starting culture was added. Poured into plastic cups (100 mL), the inoculated yoghurt mixture was incubated at 45 °C for over 6 hours until coagulation, at which point it was quickly chilled to 4 °C.

Preparation of ice cream mixture:

Fresh cow milk was heated to 60°C and homogenized for 15 min. Following the addition of sugar, butter, skim milk powder, and an emulsifier/stabilizer, the mixture was pasteurized at 85 °C for 5 min. Thereafter mixture was cooled to 10°C and aged for about 18 hr. at 4°C.

Preparation of frozen yoghurt incorporated with loquat leave extracts:

According to Dissanayaka et al., (2019) prepared plain yoghurt mixture and ice cream mixture were thoroughly blended (75% yoghurt and 25% ice cream (w/w)). Extracts of loquat leaves were then added separately at a concentration of 1% (w/w). The resulted mixture was continuously stirred for 5 min to homogenize it. Following that, it was frozen and aerated in an ice cream maker for roughly 15 minutes. The mixture was then transferred to 100 mL plastic cups, hardened at -18 °C, and placed in a deep freezer at -8 °C until further examination. Frozen yoghurt made without extracts of loquat leaves served as the control.

Phytochemical analysis

Total Flavonoids Content (TFC) Assay

Total flavonoids content (TFC) was quantified according to Chang et al., (2002) method as follows:

0.1 mL extract was mixed with 3.90 mL distilled water and 0.3mL sodium nitrite (5%) solution, allowed to react for 5 min. After that, 0.3 mL of 10% aluminum chloride solutions were added. The mixture was given six minutes to continue reacting. After that, 2 mL of 1 mM-1 sodium hydroxide was added to the mixed solution. Finally, 2.4 mL distilled water was added to all samples. The absorbance was red at 510 nm against a sample blank without reaction using Milton Roy (Spectronic 1201) spectrophotometer. The TFC of the extracts are expressed as mg quercetin equivalents (QE) /g extract.

Estimation of total cardiac glycosides content

Cardiac glycosides contents were quantitatively determined according to Solich et al., (1992). For determination of cardiac glycosides, 10 mL extract was combined with 10 mL newly made Baljet's reagent (95 mL of 1% picric acid + 5 mL of 10% NaOH). The mixture was diluted with 20 mL of distilled water after an hour, and the absorbance was calculated at 495 nm.

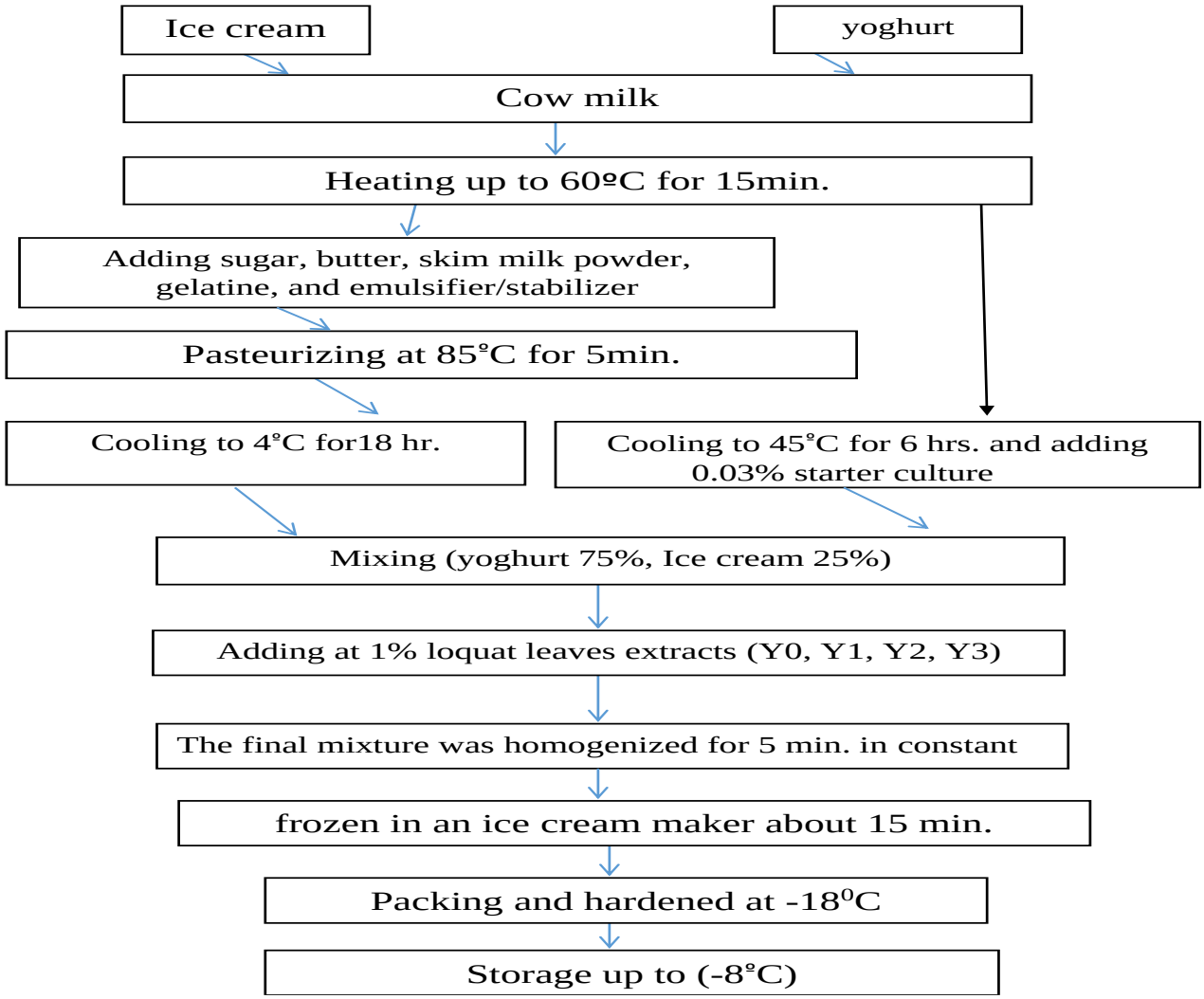

Fig (1) Preparation of frozen yogurt

Total Steroid Content Determination

50 mg extract dissolved in 5 ml ethanol then filtered and 1 mL of extract solution was transferred into 10 ml volumetric flask, Sulphuric acid (4N, 2 mL) and iron (III) chloride (0.5% w/v, 2 mL) were added followed by potassium hexacyanoferrate (III) solution (0.5% w/v, 0.5 mL). The mixture was heated for 30 minutes in a water bath maintained at 70.2 °C with intermittent shaking before being diluted with distilled water to the proper concentration. At 780 nm, the absorbance was measured by Somasundaram et al., (2010).

Estimation of total tannins content:

According to Broadhurst and Jones (1978) technique, the total tannin concentration was assessed in triplicate. As a reference material, tannic acid (SRL-92101, India) was employed. At 500 nm, the absorbance was measured. The amount of total tannins was calculated as mg of tannic acid equivalent/g of dry weight.

Estimation of total saponin content

Total saponin content was determined through spectrophotometry Briefly, 0.5 mL of extract of each sample solution was added to 1 mL of reagent mixture (glacial acetic acid/sulfuric acid 1:1 v/v). The mixture was vortexed, heated to 60 °C for 30 minutes in a water bath, and then allowed to cool. At a

wavelength of 527 nm, the sample's absorbance was determined. Oleanolic acid was used as a standard Lorke, (1983).

Determination of chlorophyll

20 mg of sample was mixed with 25 mL of 80% acetone. The sample was then put into a refrigerator at 4 °C for 4 hours. The material was then centrifuged for 5 minutes at 500 rpm. The supernatant was transferred to 100 mL volumetric flak. The final volume was made up to 50 ml with addition of 80% acetone. The color absorbance of the solution was estimated by a spectrophotometer using 645 and 663 nm wavelength against the solvent. A blank was created using acetone (80%) Hereher et al., (2010).

Equation:

Chl a = 11.75×A663 — 2.35×A645

Chl b =18.61×A645 — 3.96× A663

Where Chl a: Chlorophyll a; Chl b: Chlorophyll b; A663 is the solution absorbance at 663 nm and A645 is the absorption at 645.

The total carotenoid content:

Using a spectrophotometer, the absorbance measured at 450 nm was used to calculate the total carotenoid (TC) content. The samples extracted with ACN: MeOH: THF (50:45:5, V/V/V) and total carotenoid content was calculated based on the calibration curve and expressed as mg β-carotene equivalent (BE) Craft & Soares (1992).

Estimation of total alkaloids content.

The amount of total alkaloids was calculated using the method described in Fazel et al., (2008). At 470 nm, the absorbance was measured. As a benchmark, caffeine (SRL, India) was employed. The amount of total alkaloids was calculated as mg of caffeine equivalent/g of dry weight.

Chromatographic conditions for Quantitative separation of Phenolic

HPLC System

Analysis was performed by HPLC-(Agilent 1100) is composed of a two LC- pumps pump, a UV/Vis detector. C18 column (125 mm × 4.60 mm, 5 µm particle size). Chromatograms were obtained and analyzed using the Agilent Chem Station Mizzi et al., (2020).

Phenolic compounds content

Phenolic compounds content was separated by employing a mobile phase of two solvents 0.1% methanol: phosphoric acid (50: 50 v/v, isocratic mode). The flow rate was adjusted to 1.0 mL/min; the detector was set at 280 nm.

Total Phenolic Content (TPC) Assay

Total phenolic content (TPC) was determined using the Folin-Ciocalteu colorimetric method (Singleton and Rossi, 1965& Singleton et al., 1999). TPC was assayed by using the Folin-Ciocalteau method, with a volume of 3 mL of Folin-Ciocalteau (10%) mixed with five µL (0.05 mL) plant extract and 0.8 mL sodium bicarbonate (7.5%). The reaction mixture was incubated for 30 minutes at room temperature. The absorbance of the mixture was measured at 765nm by means of Milton Roy (Spectronic, 1201) spectrophotometer. Gallic acid equivalents (GAE) were used to express the TPC as mg/g of extract.

Color

Crust and crumb color of various cake samples were evaluated in triplicate using a Chroma meter CR-410 (Konica Minolta, Japan) according to Ayadi et al., (2009), where color was expressed in terms of L*, a* and b*, which indicate lightness, redness/greenness, and yellowness/blueness, respectively.

Physicochemical characteristics of frozen yogurt

A. Determination of pH value

The pH value was determined by blending 10g of frozen yoghurt samples with 100 ml of distilled water for 30 seconds. The pH meter was calibrated with buffer solutions of 4 and 7. At room temperature, the pH measurements were done. A pH meter was used to measure the pH (Model Consort P107) according to Defreitas et al., (1997).

B. Determination of titratable acidity

Titratable acidity of the frozen yoghurt samples was determined according to the

method of (A.O.A.C, 2010). A beaker was filled with approximately 9ml of milk sample or 9g of frozen yoghurt (or frozen yoghurt that had been diluted with 9 mL of water) and 3 to 5 drops of 1% phenolphthalein indicator was added to it. The milk sample was then titrated with NaOH (0.1N) solution until a slight pink hue persisted. The titratable acidity, expressed as % lactic acid, was finally calculated using the following formula:

$$Titratable\ acidity\ \% = \frac{N/10\ NaOH(ml) \times 0.009}{\text{wt of milk sample}} \times 100$$

C. Melting Rate:

The melting rate test was performed following the method of Muse and Hartel (2004), with small modifications. At a constant temperature of 24°C, 60 grams of frozen yoghurt samples were accommodated on a wire mesh screen (2 mm) on a beaker, and the drained volume recording persisted for more than 60 minutes. Results were expressed as time (min) against drained volume (%).

Antimicrobial activity Assay:

The antimicrobial activity was investigated on the tested samples to increase the selectivity of these derivatives towards test microorganisms. All microbial strains were provided from culture collection of the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Cairo, Egypt.

The antimicrobial profile was tested against Gram-positive bacterial species (*Staphylococcus aureus* and *Bacillus cereus*), as well as against Gram negative bacterial species (*Escherichia coli*, and *Salmonella typhimurium*) using a modified well diffusion method as follows.

100 µl of the tested bacteria were grown in 10 mL of their selective fresh media until they reached a count of approximately 108 cells/ml. 100 µl of microbial suspension was spread onto agar plates corresponding to the broth in which they were maintained and tested for susceptibility by well diffusion method. 100 µL of each sample (at 20 mg/mL) was added to each well (6 mm diameter holes cut in the agar gel). The plates were incubated for 24-48 hr. at 37°C. After incubation, the microorganism's growth was observed.

The resulting inhibition zone diameters were measured in millimeters and used as criterion for the antimicrobial activity. If an organism is placed on the agar, it will not grow in the area around the well if it is susceptible to the chemical. This area of no growth around the disc is known as a "Zone of inhibition" or "Clear zone". The size of the clear zone is proportional to the inhibitory action of the compound under investigation. Solvent controls (DMSO) were included in every experiment as negative controls. DMSO was used for dissolving the tested compounds and showed no inhibition zones, confirming that it has no influence on growth of the tested microorganisms. Positive controls were also performed using gentamycin as standard antibacterial drug.

All the biologically active samples were subjected to determinate the MIC (Minimum Inhibitory concentration) by the broth microdeletion method. After incubation, the lowest concentration showing complete inhibition of growth was recorded as the MIC of the respective sample.

Determination of total bacterial, yeasts & molds count

Total bacterial count was determined using nutrient agar medium. Yeasts & mold counts were determined using potatoes dextrose agar medium (Difco-Manual, 1984). The media were autoclaved at 121 °C for 15 min. Ten grams of sample were mixed with 90 mL of sterile water in a blender, under sterile conditions, to give 1/10 dilution. Serial dilutions were prepared using distilled water. One mL of the dilution was transferred to a sterile, Petri-dish. Approximately 15 mL of agar medium was poured into the petri-dish which containing the sample.

For yeast & mold medium, 1ml of sterile 10% tartaric acid solution was used in each plate to give the medium a final pH 3.5. The sample and agar medium were mixed thoroughly by rotating the plate several times, and then counterclockwise. When the media solidified, the plates of bacteria medium were inverted and incubated at 37 °C for 48 hr. while the plates of yeast & mold medium were incubated at 25 °C for 5 days. Following the appropriate length of incubation, the colonies were countered by using 3 replicates. The data was expressed as colony forming unit cfu/ gm.

Microbiological analysis lactic acid bacteria count

Lactic acid bacteria were counted by pour plating in MRS agar. After 48 hours of anaerobic incubation at 36 °C, count each colony on the plate in accordance with Devriese et al., (1992).

Psychrophilic bacteria

Using nutrient agar media, psychophilic bacteria were found. The plates were incubated for five days at 7 °C (Difco, 1984).

Sensory evaluation of frozen yoghurt

According to An-cheol and Young-Ho (2010),10 members of the dairy science department at the faculty of agriculture at Kafrelsheikh University evaluated the sensory qualities of frozen yoghurt samples. For the categories of colour, flavour, texture, sweetness, sourness, and overall acceptability, each panelist was asked to provide a score between 0 and 10.

Statistical analysis

The statistical analysis was carried out using SPSS. Statistical software (version 11.0 SPSS inc., Chicago, USA), the results were expressed as mean. Data were subjected to analysis of variance (ANOVA). The differences between means were tested for significance using Duncan's test at ($p \leq 0.05$) according to Armitage and Berry (1987).

Results and Discussion

Phytochemical content in loquat leaves

Phytochemical constituents possess various protective and therapeutic effects (Mir et al., 2013). The flavonoid from loquat leaves not only have antioxidant activity, but also have anti-inflammatory activity (Maher et al., 2015).

The results of phytochemical constituents of loquat leaves extracted by different concentrations of ethanol are presented in **Table (1)**. Results of flavonoids content indicated that leaves extracted with 90% ethanol has the highest flavonoids content (1.023 mg QE/g extract), followed by leaves extracted with 70% ethanol (0.863 mg QE/g extract) and the lowest was for the leaves extracted with 30% ethanol (0.714 mg QE/g extract).

In this study, the extract from loquat leaves has the highest enrichment of flavonoids as ethanol extraction increased. Similar results were reported by Huang et al., (2017) who remarked that, the preparation of crude extract from loquat leaves have high contents of total terpenes and flavonoids the yields of flavonoids and total terpenes in precipitate are 1.22% and 3.57%, respectively.

These results showed that, Chlorophyll-a was found to be 0.67, 1.04 and 1.33 for loquat leaves extracted with 30, 70 and 90% ethanol, respectively. Meanwhile, chlorophyll-b was to be 0.563, 0.882 and 0.951 mg/g. The content of chlorophyll-a was dominated over chlorophyll-b being by 1.40 times higher. chlorophylls a and b content of loquat leaves extracted with 90% ethanol showed the highest content compared with loquat leaves extracted with 30 and 70% ethanol.

Carotenoids found in loquat are thought to contribute to the fruit's colour and health-promoting qualities (Azqueta & Collins, 2012; Zhou et al., 2011). In the current study, leaves extracted with 90% ethanol demonstrated higher carotenoid content than that of all samples. The change tendency of carotenoids content in loquat leaves extracted by different concentration from ethanol were the same to flavonoids as mentioned earlier. The

carotenoids content decreased with leaves extracted by 30% ethanol showing the lower content. The total saponin contents of loquat leaves were ranging between 0.587 and 0.318 mg/g extract across the various solvent extracts studied.

Data in the same table showed that the content of total alkaloids recorded 0.825 mg/g for leaves extracted with 90% ethanol while, it recorded 0.608 and 0.643 mg/g for the leave´s extracts with 30 and 70% ethanol, respectively.

Table (1) also showed high content of terpenoids in loquat leaves and their values were 2.14, 2.82 and 3.09 % for 30,70 and 90% ethanol, respectively. The steroids content was 0.543 mg/g in leaves extracted with 90% ethanol followed by leaves extracted with 70% and 30% ethanol (0.517 and 0.302 mg/g) respectively.

Table (1). Phytochemicals content (mg/g) in loquat leaves extracted by different concentrations of ethanol.

Compounds	30% ethanol	70% ethanol	90% ethanol
Total Flavonoids	0.714±0.038	0.863±0.069	1.023±0.097
Chlorophyll-a	0.670±0.21	1.040±0.18	1.330±0.25
Chlorophyll-b	0.563±0.129	0.882±0.244	0.951±0.135
Carotenoids	0.247±0.021	0.425±0.039	0.821±0.073
Glycosides	0.155±0.019	0.184±0.032	0.218±0.024
Saponin	0.318±0.026	0.456±0.038	0.587±0.061
Alkaloids	0.608±0.036	0.643±0.059	0.825±0.061
Terpenoids	2.140±0.52	2.820±0.46	3.090±0.57
Steroids	0.302±0.046	0.517±0.095	0.543±0.091

Concentration of phenolic acids in loquat leaves:

The antioxidant activity and possible health benefits of phenolic acids account for the steadily rising interest in them (Qiu et al., 2010). Different phenolic compounds were found in leaves extracts when analyzed by HPLC. Hydroxycinnamic acid derivatives such as caffeic were detected in all loquat leaves extracts. Phenolic acids consist of two subgroups, i.e., the hydroxybenzoic and hydroxycinnamic acids. Hydroxybenzoic acids include gallic, Salicylic and syringic acids, which in common have the C6-C1 structure. Hydroxycinnamic acids, on the other hand, are aromatic compounds with a three-carbon side chain (C6-C3), with caffeic, ferulic and sinapic acids being the most common (Bravo, 1998).

The contents of phenolic acids in loquat leaves extracted with different concentration from ethanol are shown in **Table (2) & fig. (2)**. Major phenolic compound in leaves extracted with 70% ethanol was the pyrogallol.

Leaves extracted with 70% ethanol had higher pyrogallol (17.53 µg /mL) and syringic contents (8.23 µg/mL) than leaves extracted with 90 and 30% ethanol. However, leaves extracted with 90% ethanol had higher amounts of caffeic (7.16 µg/mL) than leaves extracted with 70 and 30% ethanol (2.55 and 2.46 µg/mL). There are several hydroxycinnamic acid derivatives, primarily esters of trans cinnamic acids (caffeic, p-coumaric, and ferulic acids), quinic acid, and flavonoid glycosides like quercetin and kaempferol glycosides. were reported by Ferreres et al., (2009) in leaves from 6 loquat cultivars grown in Brazil.

The results in **Table 2** illustrated that salicylic, p-coumaric and ferulic were not detected in the leaves extracted with 30% ethanol, but benzoic, catechol, syringic, pyrogallol, Gallic, ellagic, cinnamic and caffeic were found at the levels of (0.97, 3.22, 1.14, 0.69, 2.14,10.95, 8.36, 2.46 µg/mL) respectively and ellagic reveled the highest value (10.95 µg/mL). While, both catechol and ellagic were not detected and Pyrogallol registered the highest number in the leaves extracted with 70% ethanol. Also, ellagic, p-

coumaric and ferulic were not found in the leaves extracted with 90% ethanol and Gallic had the highest value (10.87 µg/mL).

Table (2). Concentration of phenolic acids (µg/ml) in loquat leaves extracted with different concentration of ethanol:

Compound	loquat Leaves Extract					
	30% ethanol		70% ethanol		90% ethanol	
	RT	Concentration µg/ml	RT	Concentration µg/ml	RT	Concentration µg/ml
Benzoic	15.0	0.97	15.0	2.55	15.1	8.22
Catechol	4.2	3.22	ND	ND	3.7	4.05
Syringic	5.1	1.14	5.0	8.23	5.0	6.11
Pyrogallol	9.0	0.69	9.2	13.37	9.0	2.66
Gallic	9.8	2.14	10.0	1.22	10.0	10.87
Ellagic	12.8	10.95	ND	ND	ND	ND
Salicylic	ND	ND	12.0	5.16	12.2	9.65
Cinnamic	7.0	8.36	7.0	3.06	7.0	2.75
***p*-coumaric**	ND	ND	6.0	2.14	ND	ND
Ferulic	ND	ND	11.2	4.87	ND	ND
Caffeic	8.0	2.46	8.2	2.55	8.0	7.16

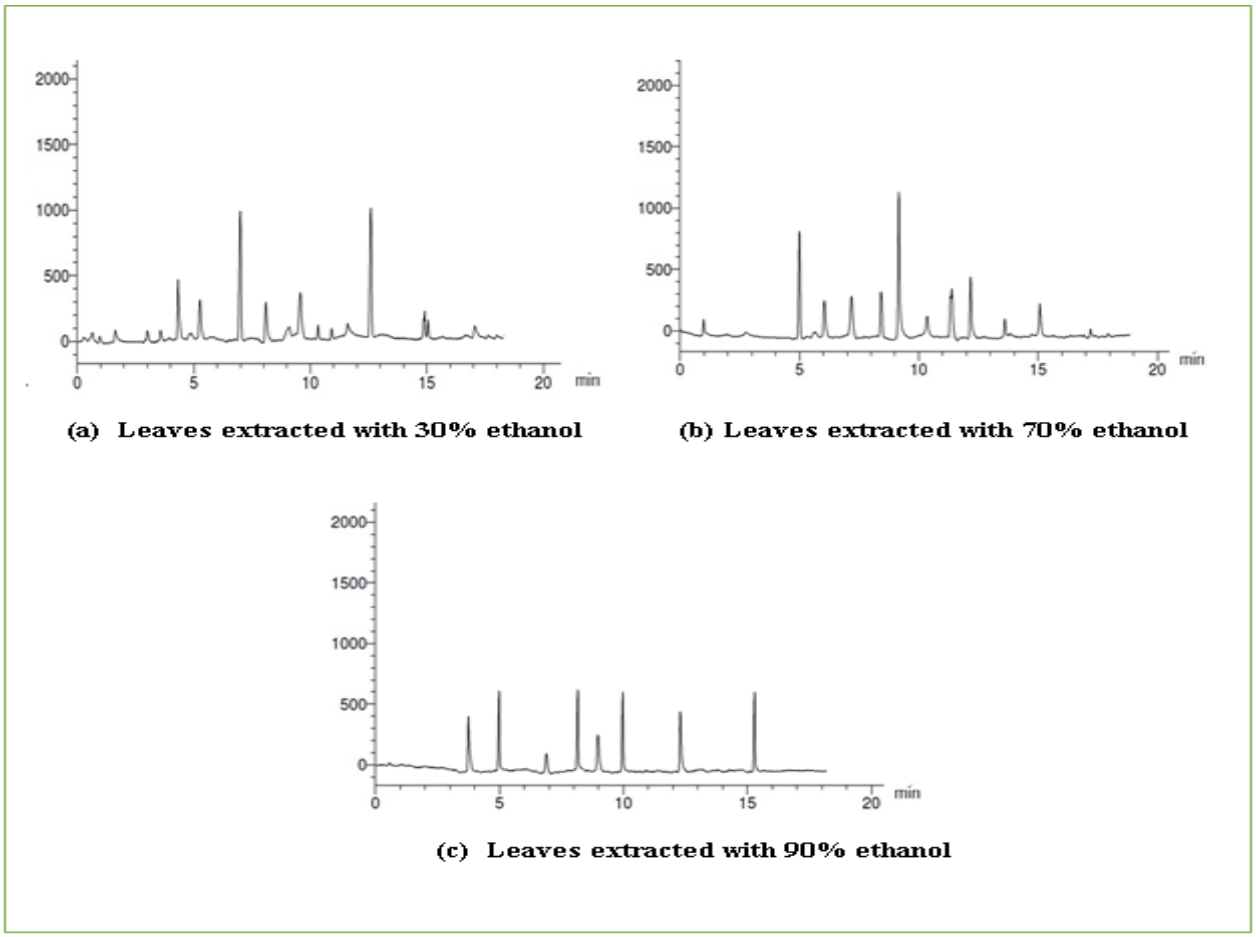

Fig (2) Concentration of phenolic acids (µg/ml) in loquat leaves extracted with different concentration of ethano

Inhibition effect of loquat leaves extracted with different concentrations of ethanol against tested microorganisms.

The loquat leaves extracted using 70% and 90% ethanol showed comparable antibacterial activity with *Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli* **(Table 3)**. Moreover, the controls showed activity against tested microorganisms at 1.95 µg/µl gentamycin. This result agrees with Abdou et al. (2011) who found Eriobotrya japonica leaf and branch extracts exhibit antibacterial activity against ESBL-producing *Escherichia coli* and *Klebsiella pneumonia*. They also discovered that the individual MIC ranged between 2.5 and 80 µg/µl.

Table (3). Inhibition effect of loquat leaves extracted with different concentrations of ethanol against tested microorganisms:

Sample code	loquat leave extracts			Control
Tested microorganisms	30 % Ethanol	70 % Ethanol	90 % Ethanol	
Gram Positive Bacteria:				*Gentamycine*
***Staphylococcus aureus* ATCC 25923**	5.0	10.0	10.0	1.95
***Bacillus cereus* RCMB 027 (1)**	10.0	10.0	10.0	1.95
Gram Negatvie Bacteria:				*Gentamycine*
***Escherichia coli* ATCC 25922**	5.0	10.0	10.0	1.95

Physical properties of frozen yogurt:

a. pH and total acidity of frozen yoghurt

The pH of the frozen yoghurt during storage at -8 °C is presented in **Table 4**. The initial pH at zero time was 4.51, 4.56 and 4.45 for frozen yoghurt enriched with 1% loquat leaves extract using 30, 70 and 90% ethanol respectively. The highest and lowest pH was observed in control sample (4.67) and frozen yoghurt enriched with 1% loquat leaves extract using 90% ethanol (4.45), respectively. Chamber et al., (1979) reported that, Yogurt's ideal pH lies between 3.27 and 4.53. Increased storage duration caused a significant decrease in pH treatments.

After 21 days of storage, pH values recorded 4.20, 4.33, and 4.16 for frozen yoghurt supplemented with 1% loquat leaves extract using 30, 70 and 90% ethanol respectively, while the control sample was 4.45. significant decrease was observed among treated samples and the control. The pH values of frozen dairy products were also often found to decrease with increasing content of probiotics or with storage in other studies. Ozdemir et al., (2005) reported that, pH values decreased from 4.62 at 1 day of storage to 4.06 at 30 days of storage for their plain yoghurt ice cream. However, changes in the pH of ice cream type of frozen yoghurt during 6 months of storage were small in the study Inoue et al. (1998).

Titratable acidity is also presented in Table (4). Recorded values at zero time of frozen yoghurt enriched with 1% loquat leaves extract using 30% ethanol were observed to be 0.80 %. According to ISI (1974), Lactic acid acidity should not exceed 0.8 percent. After 21 days of cold storage, registered data illustrated nonsignificant increase in titratable acidity by increasing cold storage up to the end storage period since these values ranged from 0.65 to 0.80%, from 0.80 to 0.95%, from 0.70 to 0.85% and from 0.90 to 1.5% in the formulas Y0, Y1, Y2 and Y3, respectively. Titratable acidity found to be increased meanwhile, pH decreased with the time after 3 weeks mainly due to sugar fermentation and conversion of lactose to lactic acid. Significant increase in titratable acidity was also seen by increasing the used solvent at the level of all storage periods for the treatments compared to the control. Similar findings were reported by (Tamime and Robinson, 1999; Shin et al. 1991; Salwa et al., 2001) for yoghurt. On the other hand, Significant with steadily increase was shown by increasing the used solvent (ethanol) in comparing with the control sample. This increase ranged from 0.65 up to 0.90% respectively.

Table (4). pH and total acidity of frozen yoghurt enriched with loquat leaves extracts during storage at (-8 °C)

Treatment	Storage period, days			
	0	7	14	21
		pH		
Y0	4.45[a]	4.50[a]	4.60[a]	4.67[a]
Y1	4.20[c]	4.37[b]	4.40[b]	4.51[c]
Y2	4.33[b]	4.38[b]	4.51[c]	4.56[b]
Y3	4.16[d]	4.29[c]	4.34[d]	4.45[d]
		Total acidity		
Y0	0.80[d]	0.75[d]	0.70[d]	0.65[d]
Y1	0.95[b]	0.90[b]	0.85[b]	0.80[b]
Y2	0.85[c]	0.80[c]	0.75[c]	0.70[c]
Y3	1.50[a]	1.00[a]	0.95[a]	0.90[a]

Where: Y0 control frozen yogurt, Y1 frozen yogurt enriched with 1% leaves extract using 30% ethanol, Y2 frozen yogurt enriched with 1% leaves extract using 70 ethanol, Y3 frozen yogurt enriched with 1% leaves extract using 90% ethanol.

b. Melting rate of frozen yoghurt

Melting rate is the time it takes for an ice cream to completely melt at room temperature is referred to as melting time. Melting time which was calculated at room temperature. The melting percent of the frozen yogurts is presented in **Table (5)**. Obtained data indicated that the control sample recorded the fastest melting rate in comparing with frozen yoghurt enriched with loquat leaves extract which showed higher decrease that was observed with frozen yogurt enriched with 1% loquat leaves extract using 30% ethanol which had the highest melting resistance at the beginning and end of the melting time. However, control sample had the lowest melting resistance at the level of 10 up to 60 min.

The melting rate (10%-40%) is considered satisfactory, when they are let to block melting through two hours at 25˚C (Cheng et al., 2015).

c. Color parameters of frozen yogurt

Color is one of the most important sensory attributes as it helps us to reject or accept food. Table (6) shows the index values of different color parameters, L*, a*, and b* (L*, were measured. The parameter a* takes positive values for reddish colors and negative values for the greenish ones, whereas b* takes positive values for yellowish colors and negative values for the bluish ones. L* is an approximate measurement of luminosity.

Table (5). Melting rate of frozen yogurt enriched with loquat leaves extract.

Treatment	Melting rate (min.)					
	10	20	30	40	50	60
Y0	15.0[a]	31.0[a]	50.0[a]	68.0[a]	80.0[a]	90.0[a]
Y1	05.0[c]	11.0[c]	25.0[d]	42.0[c]	54.0[d]	65.0[d]
Y2	06.0[c]	20.0[b]	42.0[c]	61.0[ab]	70.0[b]	85.0[b]
Y3	08.0[b]	31.0[a]	46.0[b]	59.0[b]	63.0[c]	76.0[c]

Where: Y0 control frozen yogurt, Y1 frozen yogurt enriched with 1% leaves extract using 30% ethanol, Y2 frozen yogurt enriched with 1% leaves extract using 70 ethanol, Y3 frozen yogurt enriched with 1% leaves extract using 90% ethanol

Table (6). Colour parameters of frozen yoghurt enriched with loquat leaves extracts.

Sample	L*	a*	b*
Y0	43.19	4.11	6.75
Y1	37.95	3.65	6.71
Y2	33.65	3.85	9.86
Y3	30.74	1.68	9.86

Where: Y0 control frozen yogurt, Y1 frozen yogurt enriched with 1% leaves extract using 30% ethanol, Y2 frozen yogurt enriched with 1% leaves extract using 70 ethanol, Y3 frozen yogurt enriched with 1% leaves extract using 90% ethanol

The L* values decreased depended on the concentration of ethanol. Additionally, the a* values of frozen yogurt enriched with 1% loquat leaves extract using 90% ethanol yoghurts were lower, and b* values were higher than those in the control frozen yoghurt. The chlorophyll and flavonoid pigments in loquat leaves turn the yoghurt a dark green and yellow color (Choe et al., 2010).

Table (6) showed that lightness L* and a* were higher in control frozen yoghurt and decreased in frozen yoghurt samples up to the treatment with 90% ethanol concentration. On the contrary, b* value was increasing from 6.75 for control frozen yoghurt to 9.86 in frozen yoghurt samples fortified with 1% loquat leaves extract using 90% ethanol. Wallace and Giusti (2008) reported that, Fruits and vegetable extracts and powders could be a functional component in food. In our research, adding loquat leaf extract to yoghurt resulted in a product with an appealing, stable colour and steady quality, obviating the requirement for industrial colourant extraction.

Total phenolic contents of frozen yoghurt:

Regardless that dairy products especially (yoghurt and yoghurt products) have a high nutritive value, they are poor sources for phenolic compounds. The majority of phenolic chemicals in the human diet come from plants (O'connell and Fox, 2001). Therefore, the combination of plant raw materials with various yoghurt products could be nutritionally beneficial**. Table (7)** shows that frozen yoghurt enriched with 1% loquat leaves extract using 90% ethanol had a significantly higher amount of total phenolic content by 3.04 times compared with the control sample) without additives (. The highest total phenolic amount was established for frozen yoghurt enriched with 1% loquat leaves extract using 90% ethanol (33.29 mg/g), followed by the samples with 1% loquat leaves extract using 70 and 30% ethanol amounts of this element (24.12 and 14.72 mg /g, respectively) at zero time. As our study has shown, these plant raw materials are excellent sources of phenolic compounds for enrichment of frozen yogurt. Loquat leaves are known as rich in phenolic compounds, the total phenolics and total flavonoids in the leaves of the diploid loquat cultivars as well as in the flower buds were identical. For instance, the leaves of Xiangzhong had the greatest total phenolic content, at 77.61 mg GAE/g DW (Liu et al., 2019).

The TPC of yoghurt samples ranged from 9.21 to 28.85 mg/g after 7 days of storage. Particularly, the frozen yoghurt enriched with 1% loquat leaves extract using 70% ethanol showed 2.4-fold higher in TPC values than that in the control frozen yoghurt. Furthermore, the control frozen yoghurt and frozen yoghurt treatments showed considerable decrease in TPC during storage ($p \leq 0.05$). The highest content of TPC was 11.17, 16.49 and 17.58 mg/g in frozen yoghurt enriched with 1% loquat leaves extract using 30, 70 and 90% ethanol after 21 days of storage.

Table (7). Total phenolic contents (mg/g) of frozen yogurt enriched with loquat leaves extract during storage at -8 °C

Sample Code	Storage period (days)			
	0	7	14	21
Y0	10.93±0.59	9.21±0.37	6.70±0.24	4.82±0.36
Y1	14.72±0.64	13.96±0.72	12.03±0.29	11.17±0.31
Y2	24.12±1.46	22.73±0.99	18.55±0.91	16.49±0.17
Y3	33.29±1.97	28.85±1.23	20.37±0.15	17.58±0.72

Where: Y0 control frozen yogurt, Y1 frozen yogurt enriched with 1% leaves extract using 30% ethanol, Y2 frozen yogurt enriched with 1% leaves extract using 70 ethanol, Y3 frozen yogurt enriched with 1% leaves extract using 90% ethanol

Microbiological analysis of frozen yoghurt

Microbiological profile (total plate count, moulds & yeasts, Lactic acid bacteria and

Psychrophilic bacteria) of the frozen yoghurt and frozen yoghurt enriched with loquat leaves extract at different concentration of ethanol solvent during storage periods at -8 0C are illustrated in **Table (8)**. The highest value of total plate count was obtained for the control (30x102cfu/g) while, the lowest value was obtained with the treated samples at the level extraction using 90% ethanol (3x102cfu/g) followed by frozen yoghurt sample using 70% ethanol (25x102cfu/g) and with 30% ethanol respectively at zero time. The loquat leaves extract led to decrease the total plate count of frozen yoghurt.

Table (8). Changes in microbiological profile of frozen yoghurt enriched with loquat leaves extract at different solvent concentration during storage period at -8 °C

Treatment	Storage period (days)			
	Zero	7	14	21
		Total plate count		
Y0	30 $x10^2$	22 $x10^2$	18×10^2	15×10^2
Y1	27 $x10^2$	25×10^2	$20x10^2$	$20x10^2$
Y2	25 $x10^2$	19 $x10^2$	$18x10^2$	18 $x10^2$
Y3	$3x10^2$	2 $x10^2$	ND	ND
		Moulds & Yeasts		
Y0	$14x10^2$	$12x10^2$	11 $x10^2$	$10x10^2$
Y1	$12x10^2$	$11x10^2$	$9x10^2$	$9x10^2$
Y2	$12x10^2$	$8x10^2$	$8x10^2$	$7x10^2$
Y3	$4x10^2$	$3x10^2$	$1x10^2$	$1x10^2$
		Lactic acid bacteria		
Y0	1.9×10^7	1.6×10^7	1.3×10^7	2.7×10^6
Y1	2.5×10^7	2.4×10^7	2.0×10^7	3.8×10^6
Y2	2.8×10^7	2.7×10^7	2.4×10^7	4.1×10^6
Y3	3.1×10^7	3.1×10^7	2.9×10^7	4.5×10^6
		Psychrophilic bacteria		
Y0	ND	ND	ND	ND
Y1	ND	ND	ND	ND
Y2	ND	ND	ND	ND
Y3	ND	ND	ND	ND

Where: Y0 control frozen yogurt, Y1 frozen yogurt enriched with 1% leaves extract using 30% ethanol, Y2 frozen yogurt enriched with 1% leaves extract using 70 ethanol, Y3 frozen yogurt enriched with 1% leaves extract using 90% ethanol

The total plate count was lower in frozen yoghurt enriched with loquat leaves extract. Total plate count of all the samples decreased during storage period up to 21 days at -8^{o}C. The gradual decline in the total plate count of all samples may be due to destruction of microorganisms at low temperature storage. The production of ice crystals during storage may have damaged the cell wall of microorganisms leading to lysis of cell by feedback exhibition (Davidson et al., 2000). The other reason might be the accumulation of toxic metabolites which could further enhance cell lysis by feedback exhibition. The outcomes fell within the permitted bounds of the ISI specification, which calls for a maximum of 250 log10 cfu/mL (Sukumar, 1997).

This reduction in the total count also related to higher phenolic compounds and the presence of phytochemicals (tannin, alkaloids, Glycosides, Saponin, Terpenoids, Steroids), contained in loquat leaves extract that act as antimicrobial agent. as pointed in the study performed by (Aiyegoro and Okoh, 2009). They reported that the phytochemical screening of the plant extracts revealed the presence of

high concentrations of bioactive compounds including tannin, terpenoids, alkaloids, etc., these phytochemicals have been proved to possess biocidal and inhibitory activities against a wide range of microorganisms. This reduction in also may explain as a result of mechanical freezing effect on the microbial cell walls resulting irruption of the cells microorganisms.

Lactic acid bacterial count: The dairy products, particularly frozen yoghurt is a good vehicle to transfer probiotics to the human intestinal tract. Consuming probiotic bacteria through dairy food products is the best strategy to restore the balance of the intestinal microflora. The preservation of cultures in frozen yoghurt is crucial to maintaining the product's beneficial health characteristics (Tamine and Robinson, 1999). **Table (8)** showed lactic acid bacteria counts in the four trials of the frozen yoghurt. The initial count of probiotic in frozen yoghurt sample enriched with 1% loquat leaves extract using 70% ethanol was 2.8×107 cfu/g meanwhile that of sample enriched with 1% loquat leaves extract using 90% ethanol was 3.1×107 cfu/g. The counts of probiotic in the two formulae decreased gradually during storage. After three weeks, the counts of probiotic in frozen yogurt enriched with 1% loquat leaves extract using 70 and 90% ethanol were 4.1 and 4.5 x106 cfu/g, respectively. However, the bacterial contents of the all sample were still higher than 1.0x106 cfu/ml, the number regulated by FDA (2008) for the probiotic products. Lopez et al. (1998) observed only a slight decline in lactic acid bacteria in three batches (pH = 4.32, 5.09, and 5.53) of commercial frozen yogurt held at -23°C for a year.

Lopez et al. (1998) studied the persistence of LAB (ST and LB) in commercial frozen yoghurt kept at -23 °C for 60 weeks. Initial counts for ST and LB were 7.57–7.58 log CFU/g and 4.29–6.79 log CFU/g, respectively. They discovered that LAB remained stable throughout storage and came to the conclusion that the shelf-life time might be extended past 60 or 67 weeks.

On the other hand, psychrophilic bacteria were non detected in all frozen yogurt samples whether fresh or throughout the storage period (21 days).

Sensory evaluation of frozen yogurt

Sensory scores for the color, flavor, texture, sweet taste, sour taste and over all acceptability of the samples are shown in **Table (9)**. The incorporation of loquat leaves extracts with frozen yoghurt significantly influenced the color functions of frozen yogurt. The color of treated samples was darkened by loquat leaves extract at the level of 90% ethanol and color scores were lowered from (9.30 and 9.0) in frozen yogurt enriched with 1% loquat leaves extract using 30% ethanol and control frozen yogurt, respectively to 8.40 frozen yogurt enriched with 1% loquat leaves extract using 90% ethanol. From the same table, it was observed that both texture and sour test improved and scored the highest score compared to the other parameters.

The flavor of all four mixes was acceptable. However, the flavor of control sample and frozen yogurt enriched with 1% loquat leaves extract using 30, 70 and 90% ethanol was rated 9.95, 9.60, 9.35 and 9.05, respectively showing significant decrease by increasing the used solvent.

Texture scores of the control and the treated samples are also shows by Table (9), the samples with 90 and 70% ethanol extract recorded the highest scores (9.0 and 8.95), with not significant in differences between them, while the lowest score was for control sample (8.05). Joung et al. (2016) studied the organoleptic qualities of yogurt containing Korean traditional plant extracts (Nelumbo nucifera leaf (NN) and DK) Diospyros kaki Thumb.). They discovered that herbal yoghurt has superior viscosity and textural qualities over plain yoghurt in terms of its ability to hold onto water, giving it a higher grade.

Control frozen yoghurt was the sweetest product with the score of 8.10. followed by frozen yogurt sample with extract using 30% ethanol (8.0). Meanwhile, the frozen yogurt sample using extract 70% ethanol had a higher score (7.90) of sweetness than that with 1% loquat leaves extract using 90% ethanol (7.85). Possibly the higher acid content of loquat leaves extract masked the perception of sweetness.

The Sour test for frozen yogurt sample with 1% loquat leaves extract using 90% ethanol scored the highest score compared to the control and other samples (7.50). Meanwhile, the extracted samples using 70 % and 30% ethanol were statistically similar and were higher than that of control frozen yoghurt.

It was also observed that the highest overall acceptance score was for the control frozen yoghurt, which was significant ($p \leq 0.05$), and the lowest was for frozen yoghurt with the extract using 90% ethanol. The results indicated that adding loquat leaves extract to frozen yogurt was significantly decreasing the overall acceptance in comparison to the control sample ($p \leq 0.05$). This might be due to the reduction in the acceptability of sweet taste and increased sour taste of frozen yogurts, which led to a reduction in overall acceptance.

Conclusions

In conclusion, Loquat leaves extract obtained using 90% ethanol has showed the highest flavonoids content (1.023 mg QE/g). Pyrogallol was found to be the major phenolic compound in the 70% ethanol extract. The loquat leaves extracted using 70% ethanol showed comparable antibacterial activity with Staphylococcus aureus, Bacillus cereus and Escherichia coli. The addition of the loquat leaves extract to frozen yogurt increased the Total phenolic contents from10.93 to 33.29mg/g total acid ranged from 0.65 to 0.90% and lowered the pH value 4.67 - 4.45 and improved quality characteristics of frozen yogurt.

Table (9). Sensory evaluation of yogurt enriched with loquat leaves extract.

Treatment	Color	Flavor	Texture	Sweet test	Sour test	Overall acceptability
Y0	9.0b	9.95a	8.05c	8.10a	6.60c	8.90a
Y1	9.30a	9.60b	8.80b	8.0ab	6.85b	8.40b
Y2	8.70c	9.35c	8.95a	7.90b	7.0b	8.30c
Y3	8.40d	9.05d	9.0a	7.85b	7.50a	8.05d

Where: Y0 control frozen yogurt, Y1 frozen yogurt enriched with 1% leaves extract using 30% ethanol, Y2 frozen yogurt enriched with 1% leaves extract using 70 ethanol, Y3 frozen yogurt enriched with 1% leaves extract using 90% ethanol

Figure 3. Color parameters of frozen yoghurt enriched with loquat leaves extracts

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