

## Production of Functional Beverages from Whey and Permeate Containing Kumquat Fruit

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Received: 5 March, 2017

Revised: 4 April, 2017

Accepted: 19 April, 2017

### ABSTRACT

The objective of the present study was to utilize whey and permeate as the by-products of the cheese industry after mixing with kumquat purée and/or paste for producing functional beverages. Physical, chemical, physicochemical, microbiological and sensory properties of kumquat fruit and its purée and paste were studied. The results indicated that kumquat purée and paste are good sources of  $\beta$ -carotene, vitamin C, minerals such as calcium, potassium and magnesium, total phenolic acids and flavonoids. In addition, they have high antioxidant capacity. Physicochemical, microbiological and sensory properties of the prepared functional beverages during cold storage periods were determined. The obtained results indicated that ascorbic acid and  $\beta$ -carotene of all beverages were higher than those of whey and permeate only, with a high level of total phenols, flavonoid content and antioxidant activity. Generally, sensory evaluation of the prepared beverages showed that the addition of purée and paste of kumquat fruit to whey and permeate increased the overall acceptability of these beverages. Total count was in permissible limit while the coliform and moulds & yeasts counts were not detected in all beverages during storage at  $4\pm 1^\circ\text{C}$  for 28 days. Beverages containing purée and paste of kumquat fruit could be recommended as new acceptable functional products.

**Key words:** *Whey, permeate, kumquat, purée, paste and functional beverages*

### INTRODUCTION

Whey is a by-product obtained from cheese manufacture. Depending on the type of casein coagulation, whey can be sweet or acid. Composition and properties of whey depend on the technology of cheese manufacture and the quality of milk used for cheese production (Tratnik, 1998). According to its average composition, whey is approximately 93% water and contains about 50% of total solids present in the milk of which lactose is the main constituent (Beucler *et al.*, 2005). Minerals and milk fat are also present but in less amounts. However, whey composition is very variable and significantly depends on the technology of whey production.

Permeate is an important by-product of the treatment of milk by using ultra filtration process in cheese industry. It contains lactose as the major constituent in addition to water soluble vitamins and salts of milk (Menrad *et al.*, 2000). Therefore, permeate can be considered as a solution of nutritious significance. Many efforts have been made for the development of beverages with addition of fruit concentrates in order to produce a drink with

acceptable sensory properties especially flavour (Koffi *et al.*, 2005).

Citrus fruits have been considered a valuable part of a healthy and nutritious diet and it is well established that some of the nutrients in citrus prompted health and provide protection against chronic disease. Citrus fruits generally have the highest antioxidant activity among all fruit classes and may protect against cancer and heart disease (Adibelli *et al.*, 2009).

Kumquats (*Fortunella margarita*) are fruits belonging to the *Citrus* genus. It means 'gold orange' in China (Morton, 1987). The deep-orange fruits are small ovals, with two to five seeds or sometimes seedless, not very juicy, ranging from acid to sub acid, and are pleasantly flavoured (Hsueh, 2001). Fresh kumquats taste just like other citrus fruits; they can be eaten completely, including their skin. Despite this, there have only been few studies on kumquat, in contrast to the other major citrus fruits such as lemon and orange (Sadek *et al.*, 2009, Parashar *et al.*, 2014). Kumquats are also an excellent source of natural antioxidants such as ascorbic acid, carotenoids, flavonoids and

essential oils, which eliminate the harmful effects of free radicals (Güney *et al.*, 2015).

The aim of the present study was to determine the physical, physicochemical, and microbiological properties of kumquat fruits. The possibilities of mixing kumquat purée and paste with whey and /or permeate to produce functional beverages were studied. The second target of the present work is to study the physicochemical, microbiological, and sensory properties of the prepared functional beverages during cold storage periods at  $4\pm 1^\circ\text{C}$ .

## MATERIALS AND METHODS

### Materials

Fresh whey (93.35 % moisture, 0.78 % protein, 0.30% fat, 4.66% total sugars and 0.86% ash) and permeate (93.90 % moisture, 0.02 % protein, 0.22% fat, 4.99% total sugars and 0.83% ash) were obtained from Animal Production Research Institute, Agriculture Research Center, Dokki, Giza. Kumquat fruits (5 kg) were obtained from the local market, Alexandria. All chemicals and reagents used in the present study were of analytical grade and purchased from El-Gomhouria Co., while chemicals used in HPLC methods were of HPLC grade. Freeze dried DVS (direct to vat set) yoghurt cultures containing *S. salivarius* subsp. *thermophilus* and *L. delbrueckii* subsp. *bulgaricus* strains were obtained from Chr. Hansen Inc. Laboratories, Denmark, by Misr Food Additives (MIFAD).

## Methods

### Physical methods

Shape, skin colour, pulp colour, peel, pulp and juicy center of kumquat fruits were visually described. Number of fruits/ kg, average fruit weight (g/fruit), average fruit volume ( $\text{cm}^3$ ) and number of seeds / one fruit were determined as mentioned by Kramer & Twigg (1970). Measurements of the three major perpendicular dimensions of the fruit, namely length, width and thickness were carried out with vernier calipers (Kanon Instruments, Japan) reading to 0.01 mm. In addition, pulp, peel and the seeds of kumquat fruits were weighed by a top loading balance (model: D0001-HR120, AQD company, Limited EC).

### Technological methods

#### Preparation of kumquat purée and paste

Kumquat fruits (*Fortunella margarita*) were washed using tap water then drained. Fruits were cut into two halves by stainless steel knife and their seeds were removed manually.

Kumquat fruit purée was prepared by blending the halves in a blender (Kenwood major titanium, Japan) and filled into glass jars, then heated at  $90^\circ\text{C}$  for 10 min in a water bath and stored at ambient temperature (Fig. 1).

Kumquat fruit paste was prepared by boiling purée with constant stirring to prevent burning, and mixed with sugar until the concentration

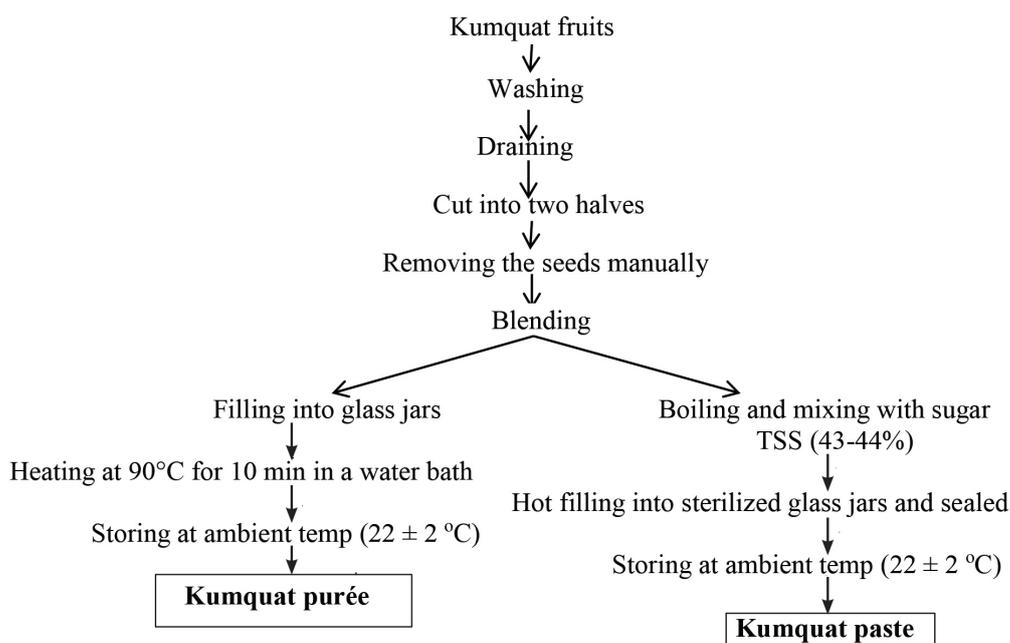


Fig. 1: Flow sheet for preparing kumquat purée and paste

of total soluble solids in the final product reached around (43-44%) according to the Egyptian Standard Specification for preserved fruit products (Codex Stan 129-2005), then hot filled into sterilized glass jars and sealed. Finally the paste was stored at ambient temperature (Fig. 1).

#### Preparation of functional beverages

Cooled whey and permeate were poured into 6 sterilized glass bottles, then heat treated at 85°C for 15 min. Then, the bottles were cooled to fermentation temperature (42°C) and immediately inoculated by adding 2% (v/v) of yoghurt cultures (*S. thermophilus* and *L. bulgaricus*), in each glass bottles. About 25% (w/v) of kumquat purée or paste were added to each mix to produce the following six samples: C<sub>1</sub>: 100% whey (control 1), T<sub>1</sub>: 75% whey+25% kumquat purée; T<sub>2</sub>: 75% whey+25% kumquat paste; C<sub>2</sub>: 100% permeate (control 2), T<sub>3</sub>: 75% permeate+25% kumquat purée and T<sub>4</sub>: 75% permeate+25% kumquat paste. The six samples were incubated at 42°C /4-5 hr. The final mixtures were poured into sterilized glass jars and stored at 4°C±1 for 28 days. This experiment was carried out in triplicate.

#### Chemical methods

##### Extraction and identification of essential oils from the fresh kumquat peels

Distillation using a Clevenger-type apparatus was used for essential oils extraction. The individual kumquat fruits were washed with tap water, then separated into peels and flesh manually. The fresh peels (100g) were homogenized for 2 min with distilled water and placed into a round-bottom flask. The process of distillation took 3 hr according to Board, (2003). The obtained essential oils were stored in a dark glass bottle at -20°C till GC/MS analysis.

Gas Chromatography-Mass Spectroscopy (GC/MS) analysis was performed separately with a Hewlett Packard model 5890. Gas chromatograph equipped with 5 series Mass selective detector 8644 (HP) with flame ionization detector (FID) on a fused silica 132 capillary column DB-5 (25 m in length, 0.32 mm i.d., and 0.5 mm film thickness) was used. The oven temperature was maintained at 60°C for 2 min after injection, and then programmed at 4°C/min to 270°C. The split injector temperature was 270°C and MS conditions were kept at 280°C and 42eV. The percentage of major

constituents of essential oil was estimated by measuring the peak area of the different compounds of the chromatogram according to Gunther & Joseph (1978).

##### Proximate chemical composition

Moisture, crude protein, crude ether extract, lactose, crude fiber, ash and total reducing and non-reducing sugars were determined according to the AOAC (2007) unless otherwise stated. Nitrogen free extract was calculated by difference. Titratable acidity as % citric acid for kumquat purée and paste and as % lactic acid for whey, permeate and functional beverages was determined by titration with 0.1N NaOH solution according to the method given in the AOAC (2007). Energy value was calculated using the universally acceptable conversion factors by multiplying protein and carbohydrates by 4.00 and fat by 9.00 Kcal/g.

##### Mineral composition

Minerals including K, Ca, Mg, Na, Fe and Mn were determined according to the AOAC (2007) method.

##### Bioactive compounds

##### Determination of total phenols, flavonoid content and antioxidant activity

One g sample was mixed with 10 ml of 80 % methanol and stirred at room temperature for 24 hr and filtered. Total phenols, flavonoid content and antioxidant activity were determined in the methanolic extract.

The total phenolic contents as % gallic acid of the purée and paste of kumquat fruits and beverage extracts were determined by Folin-Ciocalteu reagent after extracting with 80% methanol according to the method of Lim *et al.* (2006). Total flavonoid content as % quercetin of purée and paste of kumquat fruit extracts was determined according to the method of Ordon *et al.* (2006).

Antioxidant activity of the samples after extracting with 80% methanol was determined by scavenging the radicals with 2,2-diphenyl-1-picrylhydrazyl-hydrate (DPPH) as described by Brand-Williams *et al.* (1995) and expressed as percentage inhibition of the DPPH radical.

##### Identification of vitamin C by HPLC

Vitamin C content was analyzed using HPLC as described by Romeu-nadal *et al.* (2006). Ascorbic acid was identified by comparing the retention

time of the sample peak with that of the ascorbic acid standard at 254 nm. Quantification was carried out using external standardization.

#### Identification of $\beta$ -carotene by HPLC

$\beta$ -carotene was determined in kumquat purée and paste using HPLC according to the method of Chinosa *et al.* (2005). Two different detectors, one UV detection (785 UV/VIS detector) and one using fluorescence detection (Perkin-Elmer Luminescence spectrometer LC 300) were used in the HPLC analysis. The data were collected and integrated with a Gynko Soft Chromatography DS Version 5.30. Detection wavelength was set at 450 nm with flow rate of 1.5 ml/min.

#### Physicochemical analysis

Colour of kumquat fruit purée, paste and functional beverage samples was observed visually and measured with a Hunter Lab colorimeter (Ultra scan vis, USA) as outlined by Piggott, (1984). pH value was determined using glass electrode pH meter (Persica model pH 900, Switzerland) as described in the AOAC (2007). The content of total soluble solids (TSS) at 20°C expressed as °Brix was determined using a digital refractometer (Hanna, HI 96811, Germany) as described in the AOAC (2007).

#### Microbiological analysis

The resultant kumquat purée, paste and functional beverages were microbiologically examined for their total bacterial count, mould & yeasts and coliform according to the American Public Health Association (A.P. H. A., 1992).

#### Sensory properties:

The sensory properties of fresh and stored functional beverages from different treatments with the control sample were assessed by 10 persons of the staff members of Dairy Research Department and Food Technology Research Institute, Egypt. The beverage samples were scored for colour, taste, flavour, and overall acceptability. Scores

were based on a hedonic scale of 1 to 9 where: 1 = extremely dislike (very bad) and 9 = extremely like (excellent) (Walts *et al.*, 1989).

#### Statistical analysis

All data were expressed as mean values  $\pm$  SD. Statistical analysis system (SAS) software program (SAS Institute 2004) was performed using two-way analysis of variance (ANOVA) followed by using t Tests (LSD) at  $P \leq 0.05$  being considered statistically significant difference.

## RESULTS AND DISCUSSION

#### Fruit evaluation

##### Physical properties

As shown in Fig. (2) and Table (1), the shape of the fruits was round or oval. Skin colour was deep orange while the pulp colour was bright orange-yellow. Peel taste was sweet and edible, while the pulp was juicy and sweet. Juicy center was slightly acidic. Koyasako & Bernhard (1983) mentioned that the fruits are usually eaten raw as a whole fruit together with the peel, excluding the seeds. The peel is sweet and edible with a typical aroma due to the presence of flavonoids and terpenoids. This description agreed well with that found by Hsueh (2001). The round-oval fruit is orange-yellow and the flesh is sour, and the fruit is eaten together with the peel.

The data in Table (1) revealed that the number of fruits, average fruit weight and volume were 106.27 fruits/kg, 9.41 g/fruit and 5.66 cm<sup>3</sup>/fruit, respectively. Number of seeds were 3.20/one fruit. With regard to the fruit dimensions, the data declared that fruit length (3.09 cm) was longer than its width (1.97 cm), while width and thickness had almost equal values tending to have an oblate shape. These results are not agree with those of Jalilantabar *et al.* (2013) who found that the average value of length, width and thickness for the kumquat fruits were 3.95, 2.57 and 2.51 cm, respective-



Fig. 2: General appearance of whole fruits, seeds, purée and paste of kumquat

ly. Güney *et al.* (2015) mentioned that the fruit is much smaller and ovular, being approximately the size and shape of an olive. The results in the same Table showed that the peel had the highest percentage (60.37%) followed by the pulp (37.67%) and the seeds (1.96%).

**Table 1: General properties of kumquat fruits**

Parameter	Description
<b>Appearance properties</b>	
Shape	Round or oval
Skin colour	Deep orange
pulp colour	Bright orange-yellow
Peel	Sweet and edible
pulp	Juicy and sweet
Juicy center	Slightly acidic
General properties	Value *
Number of fruits/ kg	106.27 ±1.67
Average fruit weight (g/fruit)	9.41±4.14
Average fruit volume (cm <sup>3</sup> /fruit )	5.66 ±1.80
Number of seeds/one fruit	3.20±0.92
<b>Fruit dimensions (cm)</b>	
Length	3.09±0.42
Width	1.97±0.26
Thickness	1.96±0.27
<b>Weight composition (%)</b>	
pulp	37.67±4.19
Peel	60.37±6.33
Seeds	1.96±2.15

### Chemical composition of purée and paste of kumquat fruits

#### Proximate chemical composition

The proximate chemical composition of kumquat purée and paste on fresh and dry weight basis are shown in Table (2). It could be noted that the moisture content in kumquat purée (83.21%) was higher than that found in kumquat paste (51.97%), while the total solids in purée (16.79%) was lower than that of kumquat paste (48.03%). The kumquat purée contained 9.11% crude protein which higher than that found in kumquat paste which recorded 2.48% on dry weight basis. The crude ether extract content of kumquat purée and paste were found to be very low, being 1.01 and 0.13%, respectively. Crude fiber in kumquat purée was 7.33% which is higher than that of kumquat paste and recorded

1.87%. The result also showed that kumquat purée and paste contained 3.22 and 0.85% ash on dry weight basis, respectively. Mousa (1998) found that the kumquat fruits contain 83.73% moisture, 0.41% crude ether extract, 1.86% protein, 0.82% ash and 2.75% crude fiber. From the same Table, it could be noted that nitrogen free extract in kumquat purée and paste were 79.33 and 94.67%, respectively. The energy value in kumquat paste was 389.77 kcal/100g which is higher than that of kumquat purée being 362.85 kcal/100g. These results are mainly due to the addition of sugar during the preparation of this paste. The results in Table (2) also showed that the total and non-reducing sugars in the kumquat paste were 86.07 and 45.10%, respectively which were higher than that found in kumquat purée being 71.47 and 13.22%, respectively, while the reducing sugars in the kumquat purée were 58.25% which was higher than that found in kumquat paste being 40.97% on dry weight basis. The titratable acidity as % citric acid of kumquat purée and paste were 1.89 and 1.12%, respectively.

#### Mineral composition

Table (2) also showed that K, Na, Ca, Mg, Fe and Zn content in kumquat purée were 907.92, 717.87, 270.46, 1.43, 5.00 and 1.13 mg/100g on dry weight basis, respectively. Lower values were recorded for the paste. These findings are in agreement with those reported by Mousa (1998). In general, these results are in accordance with those reported by the USDA National Nutrient Database (2014), in which 100 g of raw kumquat fruit contains 71 kcal of energy, 1.88 g protein, 0.86 g fat, 15.9 g carbohydrate, 0.52g ash, 9.36g total sugars, 6.50g dietary fiber, 62 mg Ca, 19 mg P, 0.86 mg Fe, 186 mg K, 20 mg of Mg, 0.17 mg Zn and 43.90 mg ascorbic acid.

#### Bioactive compounds of kumquat purée and paste

##### Total phenolic, flavonoid content and antioxidant activity

The data in Table (3) showed that kumquat purée and paste had high amount of phenolic content being 393.89 and 309.00 mg/100g, respectively. These values are higher than those reported by Ramful *et al.* (2011) who mentioned that the total phenolic content in kumquat fruits was 169.4 mg/100g fresh weight.

Also, it could be noted that kumquat purée and paste had considerable amount of flavonoid content being 17.58 and 14.23 mg/100g, respectively. How-

**Table 2: Chemical composition and mineral content of kumquat purée and paste (fresh and dry weight basis)**

Component	Kumquat purée		Kumquat paste	
	(fresh weight basis)	(dry weight basis)	(fresh weight basis)	(dry weight basis)
Moisture (%)	83.21±0.49	-	51.97±0.85	-
Total solids (%)	16.79±0.49	100	48.03±0.85	100
Crude protein (%)	1.53±0.35	9.11±2.11	1.19±0.15	2.48±0.33
Crude ether extract (%)	0.17±0.06	1.01±0.42	0.06±0.03	0.13±0.06
Crude fiber (%)	1.23±0.14	7.33±0.85	0.90±0.32	1.87±0.67
Ash (%)	0.54±0.24	3.22±0.17	0.41±0.06	0.85±0.13
N-free extract (NFE) * *(%)	13.32±0.21	79.33±1.26	45.47±0.85	94.67±0.88
Energy value (Kcal /100g)	60.93±2.88	362.85±15.86	187.18±2.55	389.77±4.59
Total sugars (%)	12.00±0.35	71.47±2.08	41.34±1.27	86.07±2.64
Reducing sugars (%)	9.78±0.20	58.25±0.84	19.68±0.79	40.97±1.64
Non-reducing sugars (%)	2.22±0.49	13.22±2.91	21.66±0.53	45.10±1.11
Titrateable acidity as % citric acid	1.12±0.01	-	1.89±0.03	-
Minerals (mg/100g)				
K	152.44	907.92	140.27	292.05
Na	120.53	717.87	112.67	234.58
Ca	45.41	270.46	43.7	90.98
Mg	0.24	1.43	0.23	0.48
Fe	0.84	5	0.82	1.71
Zn	0.19	1.13	0.16	0.33

\* Mean of three replicates ± SD except β-carotene and Ascorbic acid.

\*\* Calculated by difference      ND: Not Detected

**Table 3: Bioactive compounds of kumquat purée and paste (fresh and dry weight basis).**

Component	Kumquat purée *		Kumquat paste*	
	(fresh weight basis)	(dry weight basis)	(fresh weight basis)	(dry weight basis)
Total phenolic content (mg/100g)**	393.89±0.80	2345.99±4.79	309.00±0.30	645.35±3.96
Total flavonoids (mg/100g) ***	17.58±0.80	104.74±4.33	14.23±1.19	29.64±2.48
Antioxidant activity (%)	56.66±0.06	-	42.74±0.43	-
Ascorbic acid (mg/100g)	65.97	392.91	33.52	69.79
β-carotene (mg /100 g)	205	1220.96	152.07	316.61

\* Mean of three replicates ± SD

\*\* Gallic acid equivalent

\*\*\* *Quercetin equivalent*

ever, the total flavonoids are found to be higher than those reported by Wang *et al.* (2007). The data in Table (3) also showed that kumquat purée and paste had relatively high percentage of antioxidant activity being 56.66 and 42.74%, respectively. These results confirmed the possibility of using kumquat purée and paste as a natural antioxidant source. Jayaprakasha *et al.* (2012) found that kumquat fruits (*Fortunella margarita*) have high radical scavenging capacities

and strong antioxidant activity, and consumption of these fruits may be of health-promotion.

#### Vitamin C and β-carotene

The data in Table (3) showed that ascorbic acid content was 65.97 mg/100g in kumquat purée while it was 33.52 mg/100 g in the paste. Concerning β-carotene, it could be noted that the kumquat purée had 205 mg/100g, while the kumquat paste had 152.07 mg/100g.

### Chemical compounds of the essential oils in the fresh kumquat peel

Twenty-five components in the essential oils were identified by GC-MS and shown in Table (4). The major compounds were limonene (68.82%), myrcene (10.91%), *p*-Mentha-1, 5-dien-8-ol (5.46%), Carvone (3.34%), camphene (2.31%), *p*-Mentha-2, 8-dien-1-ol (1.19%),  $\alpha$ -selinene (0.97%) and  $\alpha$ -pinene (0.63%). Interestingly, the given results are consistent with those of Choi (2005), Wang *et al.* (2012) and Peng *et al.* (2013) who identified similar components in the essential oils of kumquat. The limonene content of *Fortunella margarita* peel oil was comparatively less than that reported for kumquat (*F. japonica*) peel oil (Choi, 2005) and kumquat (*F. crassifolia*) peel oil (Wang *et al.*, 2012).

**Table 4: Main components of the essential oil of kumquat fresh peels**

No.	Components	Area (%)
1	Limonene	68.82
2	Myrcene	10.91
3	<i>p</i> -Mentha-1,5-dien-8-ol	5.46
4	Carvone	3.34
5	Camphene	2.31
6	<i>p</i> -Mentha-2,8-dien-1-ol	1.19
7	$\alpha$ -Selinene	0.97
8	$\alpha$ -Pinene	0.63
9	trans-Myrtenyl acetate	0.59
10	3,4-Dimethyl styrene	0.58
11	Linalool	0.54
12	$\beta$ -Elemene	0.48
13	$\alpha$ -Copaene	0.48
14	Spathulenol	0.48
15	Piperitenone	0.48
16	Perillyl acetate	0.36
17	Perillaldehyde	0.35
18	Carveol	0.33
19	Isopropyl cinnamate	0.28
20	$\gamma$ -Terpinene	0.26
21	Bicyclogermacrene	0.26
22	2,7-Dimethyl-1,6-octadione	0.25
23	cis-Myrtenyl acetate	0.24
24	<i>p</i> -Cymene	0.21
25	Octyl acetate	0.2

\* Means of three determinations  $\pm$ S.D

Previous studies showed that d-limonene inhibits lipid peroxidation and prevents free radical-induced damage (Devi *et al.*, 2004). The  $\alpha$ -myrcene content of the essential oil was higher than that reported for kumquat (*F. japonica*) peel oil, oval kumquat and *F. japonica* peel oil (Choi, 2005, Wang *et al.*, 2012, Peng *et al.*, 2013). The  $\alpha$ -pinene content of the essential oil was similar to that reported for *F. japonica* peel oil (Choi, 2005). However, the contents of myrcene, camphene and  $\alpha$ -selinene were found to be higher than those reported for *F. japonica* peel oil (Choi, 2005, Schirra *et al.*, 2008).

### Physicochemical properties of kumquat purée and paste

Physicochemical properties of kumquat purée and paste were shown in Table (5). Total soluble solids and pH values in kumquat Purée and paste were 15%, 2.85 and 43%, 2.60, respectively. The high percentage of TSS in kumquat paste is mainly due to the addition of sugar during the production of the paste as mentioned previously.

**Table 5: Physicochemical properties of kumquat purée and paste (fresh weight basis)**

Physicochemical properties	Kumquat purée	Kumquat paste
Total soluble solids *( Brixo)	15 $\pm$ 0.03	43 $\pm$ 0.08
pH value*	2.85 $\pm$ 0.05	2.60 $\pm$ 0.13
Hunter Lab measurements		
<i>L</i>	58.35	44.53
<i>a</i>	17.99	15.3
<i>b</i>	51.96	32.53
<i>C</i>	54.98	35.94
<i>H</i> <sup>o</sup>	70.9	64.82

\* Mean of three replicates  $\pm$  SD (on fresh weight basis)

(L): lightness, (+a): redness, (+b): yellowness, (*H*<sup>o</sup>): Hue angle and (C): saturation

All the hunter lab measurements including L (lightness), a (redness), b (yellowness), C (saturation) and *H*<sup>o</sup> (Hue angle) were higher in kumquat purée than that found in kumquat paste

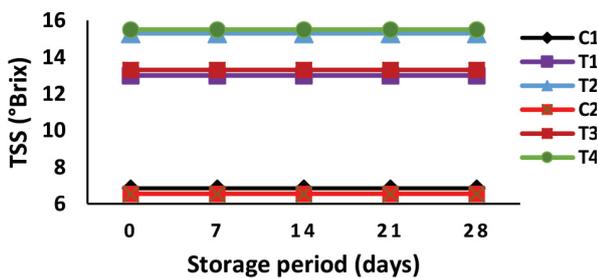
### Microbiological analysis

The total bacterial count was 110 and 100 cfu/g in kumquat purée and paste, respectively while coliform and moulds & yeasts were not detected.

**Effect of storage period on physicochemical properties of functional beverages**

**TSS values**

The effect of adding kumquat purée and paste on TSS values of different functional beverages during storage periods at 4±1°C are presented in Fig. (3). The TSS of the beverages increased with the addition of kumquat purée and paste. The TSS content of freshly prepared beverage samples C<sub>1</sub>, T<sub>1</sub>, T<sub>2</sub>, C<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> were 6.85, 13.0, 15.30, 6.55, 13.30 and 15.50 °Brix, respectively. The values of TSS for different beverages were stable during storage periods.



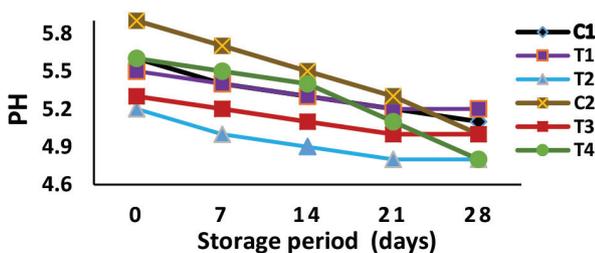
**Fig. 3: Changes of TSS in different functional beverages during storage period**

**pH values**

Addition of kumquat purée and paste affected the pH values of the prepared functional beverages (Fig. 4). It can be noted that with increasing the storage period, the pH values of the prepared functional beverages significantly decreased. This may be due to the increment of acidity as well as the formation of lactic acid and acidic amino acids during the storage period. Similar results have also been reported by Kalra *et al.* (1991) and Sikder *et al.* (2001).

**Colour**

The effect of adding kumquat purée and paste to whey and permeate beverages on colour values (*L*, *+a*, *+b*, *H<sup>o</sup>* and *C*) are showed in Table (6). It can be observed that both *L* (lightness) and *+b* (yellowness) increased with addition of kumquat purée and



**Fig. 4: Changes of pH in different functional beverages during storage period**

**Table 6: Colour of functional beverages**

Sample	Colour value	<i>L</i>	<i>+a</i>	<i>+b</i>	<i>C</i>	<i>H<sup>o</sup></i>
<b>Whey</b>						
C <sub>1</sub>		27.41	-0.34	4.61	4.62	94.28
T <sub>1</sub>		40.60	3.47	21.71	21.99	80.92
T <sub>2</sub>		35.80	4.47	15.64	16.26	74.04
<b>Permeate</b>						
C <sub>2</sub>		28.69	-1.81	2.97	3.48	121.27
T <sub>3</sub>		40.15	3.33	22.32	22.57	81.52
T <sub>4</sub>		33.72	4.37	11.11	11.13	93.76

(*L*): lightness, (*+a*): redness, (*+b*): yellowness, (*H<sup>o</sup>*): Hue angle and (*C*): saturation.

C<sub>1</sub>: 100% whey (control 1); T<sub>1</sub>: 75% whey+25% kumquat purée ; T<sub>2</sub>: 75% whey+25% kumquat paste; C<sub>2</sub>: 100% permeate; T<sub>3</sub>: 75% permeate+25% kumquat purée and T<sub>4</sub>: 75% permeate+25% kumquat paste.

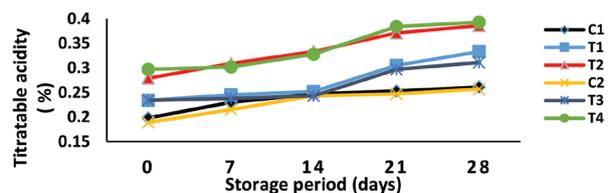
paste in whey and permeate. On the other hand, *+a* (redness) and *C* (saturation) increased in comparison with the control samples. Further, *H<sup>o</sup>* (Hue angle) decreased comparing with the control samples.

**Titrateable acidity**

The effect of storage period on titrateable acidity of functional beverages was evaluated and the results are presented in Fig. (5) It can be noted that there was a gradual increment in titrateable acidity with increasing the storage period up to 28 days. This increment is mainly due to conversion of lactose to lactic acid. The results are in agreement with those of Soliman *et al.* (1995), Sikder *et al.* (2001).

**Effect of storage period on chemical composition of functional beverages**

The effect of adding kumquat purée and paste during storage period on the chemical composition of functional beverages was assessed and the results are presented in Table (7). The results revealed that the addition of kumquat purée and



**Fig. 5: Changes of titrateable acidity in different functional beverages during storage periods**

paste to whey and permeate to produce functional beverages led to a reduction of moisture content. The highest values were observed in fresh (zero time) C<sub>1</sub> and C<sub>2</sub> (93.35 and 93.90%), respectively, while the lowest values were recorded in T<sub>2</sub> and T<sub>4</sub> at zero time (83.37 and 83.75%), respectively. A

significant difference at ( $P \leq 0.05$ ) was observed in moisture content between C<sub>1</sub>, T<sub>1</sub> and T<sub>2</sub> also for C<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>. These results also showed no significant difference at ( $P \geq 0.05$ ) in moisture content for all beverages during storage periods.

**Table 7: Chemical composition of functional beverages during storage periods**

Components	Storage period (days)	Whey			Mean	Permeate			Mean
		C1	T1	T2		C2	T3	T4	
Moisture content (%)	0	93.35±0.06	90.28±0.18	83.37±0.13	89.00a	93.90±0.53	90.12±0.10	83.75±0.22	89.26A
	14	93.33±0.08	90.24±0.08	83.33±0.06	88.96a	93.83±0.03	90.10±0.03	83.72±0.31	89.22A
	28	93.28±0.26	90.19±0.11	83.31±0.26	88.92a	93.80±0.03	90.06±0.05	83.68±0.09	89.18A
Mean		93.32a	90.24b	83.34c	LSD=0.15	93.84A	90.09B	83.71C	LSD=0.22
Crude protein (%)	0	0.78±0.01	0.44±0.02	0.33±0.01	0.52a	0.02±0.01	0.39±0.09	0.30±0.01	0.24A
	14	0.79±0.03	0.45±0.01	0.34±0.01	0.53a	0.03±0.01	0.40±0.07	0.31±0.01	0.25A
	28	0.80±0.02	0.46±0.01	0.35±0.01	0.53a	0.03±0.01	0.42±0.05	0.32±0.02	0.26A
Mean		0.79a	0.45b	0.34c	LSD=0.01	0.03C	0.40A	0.31B	LSD=0.04
Fat *(%)	0	0.30±0.02	0.21±0.05	0.19±0.04	0.24a	0.22±0.3	0.21±0.02	0.19±0.04	0.21A
	14	0.31±0.04	0.22±0.04	0.19±0.01	0.24a	0.22±0.09	0.22±0.05	0.19±0.02	0.21A
	28	0.31±0.02	0.22±0.04	0.20±0.05	0.23a	0.23±0.04	0.22±0.03	0.20±0.05	0.22A
Mean		0.32a	0.22b	0.19b	LSD=0.03	0.22A	0.21A	0.20A	LSD=0.04
Ash %	0	0.86±0.01	0.78±0.01	0.75±0.01	0.80a	0.83±0.04	0.77±0.01	0.73±0.01	0.78A
	14	0.86±0.02	0.78±0.01	0.75±0.01	0.80a	0.83±0.05	0.76±0.02	0.73±0.01	0.77A
	28	0.85±0.01	0.77±0.01	0.74±0.01	0.79a	0.82±0.04	0.76±0.01	0.72±0.02	0.77A
Mean		0.86a	0.78b	0.75c	LSD=0.01	0.83A	0.77B	0.73C	LSD=0.03
Total sugars (%)	0	4.66±0.06	8.01±0.31	14.38±0.19	9.02a	4.99±0.03	8.41±0.12	14.55±0.47	9.31A
	14	4.64±0.06	7.94±0.25	14.24±0.45	8.94a	4.93±0.07	8.37±0.19	14.48±0.29	9.26A
	28	4.62±0.08	7.84±0.28	14.20±0.18	8.89a	4.90±0.10	8.31±0.11	14.42±0.72	9.21A
Mean		4.64c	7.93b	14.27a	LSD=0.24	4.94C	8.36B	14.48A	LSD=0.22
Reducing sugars (%)	0	4.66±0.06	2.21±0.15	3.88±0.07	3.58a	4.99±0.03	2.43±0.11	3.93±0.36	3.78A
	14	4.64±0.06	2.27±0.15	3.93±0.8	3.61a	4.93±0.07	2.44±0.13	3.94±0.24	3.78A
	28	4.62±0.08	2.32±0.15	3.95±0.03	3.63a	4.90±0.10	2.48±0.13	3.97±0.40	3.77A
Mean		4.64a	2.26c	3.92b	LSD=0.10	4.94A	2.45C	3.94B	LSD=0.16
Non reducing sugar (%)	0	0	5.80±0.18	10.50±0.18	5.43a	0	5.97±0.01	10.62±0.48	5.53A
	14	0	5.68±0.36	10.31±0.49	5.33a	0	5.93±0.21	10.54±0.09	5.49A
	28	0	5.52±0.38	10.25±0.16	5.25a	0	5.83±0.23	10.46±0.28	5.43A
Mean		0c	5.66b	9.35a	LSD=0.26	0C	5.91B	10.54A	LSD=0.21
Energy values (K.cal)	0	24.47±0.36	35.73±1.40	60.56±0.46	40.26a	22.89±0.23	37.09±0.47	61.14±1.51	40.09A
	14	24.54±0.47	35.55±1.29	60.04±1.67	40.04a	21.83±0.49	37.04±0.73	60.90±1.17	39.92A
	28	24.48±0.06	35.17±1.35	60.00±1.13	39.88a	21.80±0.29	36.84±0.66	60.78±1.27	39.81A
Mean		24.50c	35.48b	60.20a	LSD=1.05	21.89C	36.99B	60.95A	LSD=0.85

C1: 100% whey (control 1); T1: 75% whey+25% kumquat purée ; T2: 75% whey+25% kumquat paste; C2: 100% permeate; T3: 75% permeate+25% kumquat purée and T4: 75% permeate+25% kumquat paste.\*fat content was determined by Gerber centrifuge method (BIS, 1977)

The highest value of crude protein content at zero time was observed in C<sub>1</sub> (0.78%). This value decreased to 0.44 and 0.33% in T<sub>2</sub> and T<sub>3</sub>, respectively, while the lowest value of crude protein content was recorded in C<sub>2</sub> (0.02). This value increased to 0.30 and 0.39% in T<sub>4</sub> and T<sub>3</sub>, respectively. No significant difference was observed ( $P \geq 0.05$ ) in crude protein content for all beverages during storage periods. These results are in agreement with those reported by Mona *et al.* (2005).

The results in Table (7) also showed that the treatment (T<sub>1</sub> and T<sub>3</sub>), (T<sub>2</sub> and T<sub>4</sub>) contained the same value of fat content (0.21 and 0.19%), respectively. No significant difference at ( $P \geq 0.05$ ) was noted between T<sub>1</sub> and T<sub>2</sub> also, T<sub>3</sub> and T<sub>4</sub> in their fat content during storage periods. The fat content was almost more or less the same.

The highest content of ash was observed in C<sub>1</sub> and C<sub>2</sub> (0.86 and 0.83%) while the lowest content was noted in T<sub>2</sub> and T<sub>4</sub> (0.75 and 0.73%), respectively. A significant difference at ( $P \leq 0.05$ ) was observed between C<sub>1</sub>, T<sub>1</sub> and T<sub>2</sub> also C<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub> while no significant difference at ( $P \geq 0.05$ ) was observed on ash content during storage periods.

The results in Table (7) also revealed that the addition of kumquat purée and paste to whey and permeate beverages led to an increment of total and non-reducing sugars and decrement of reducing sugars. The highest value of total and non-reducing sugars was recorded in T<sub>2</sub> and T<sub>4</sub> (14.38, 10.50 % and 14.55, 10.62%), respectively, while the lowest values of total sugars were observed in C<sub>1</sub> and C<sub>2</sub> (4.66 and 4.99%), respectively. Also, it was observed that the treatment C<sub>1</sub> and C<sub>2</sub> did not contain any reducing sugars. Total sugar content was slightly decreased and no significant difference at ( $P \geq 0.05$ ) was observed during storage periods in all beverages. This is in conformity with Sirohi *et al.* (2005) who found that total sugar content in whey-based mango herbal beverage did not show any significant variation with storage. However, the results of the present study are in a contradiction with those of Kumar & Manimegalai (2005) who observed a decrease in the total sugar content during storage of ready to serve (RTS) from bitter gourd and whey-based papaya beverage. However, non-reducing sugars were decreased during the storage period probably due to low hydrolysis of sucrose as shown by concomitant reduction in total sugars. During storage, inversion of sucrose occurs with a corresponding increase in the contents of the

reducing sugars, glucose and fructose (Kornvalai *et al.*, 2008). Similar results have been reported by Krishnaveni *et al.* (2001) for jack fruit beverages.

The data in the same Table indicated that the high value of energy in fresh beverages was recorded in T<sub>4</sub> and T<sub>2</sub> (61.14 and 60.56 Kcal/100g), respectively followed by T<sub>3</sub> and T<sub>1</sub> (37.09 and 35.73 Kcal/100g), respectively, while the lowest values of energy were recorded in C<sub>1</sub> and C<sub>2</sub> (24.47 and 22.89 kcal/100g), respectively. These results may be due to the addition of kumquat paste and purée to these beverages. A significant difference at ( $P \leq 0.05$ ) was observed between the control and the treated samples, while no significant difference at ( $P \geq 0.05$ ) was noted in all treated samples during storage periods.

### Effect of storage period on ascorbic acid and $\beta$ -carotene contents of functional beverages

Fig. (6) shows the values of ascorbic acid content of functional whey and permeate beverage samples. The results indicated that ascorbic acid content increased in the functional whey and permeate beverage as a result of adding kumquat paste and purée as compared to the control samples. The results revealed that ascorbic acid decreased in all samples during storage periods. These results are in accordance with those reported by Hernandez-mendoza *et al.* (2007). The obtained values of ascorbic acid content in T<sub>1</sub> and T<sub>3</sub> were 15.41 and 15.38 mg/100g, in functional whey and permeate beverage samples, respectively. These values were higher than those of T<sub>2</sub> and T<sub>4</sub> being 8.48 and 8.29 mg/100g respectively. Ascorbic acid of beverage samples were found to be 15.11, 8.18, 15.05 and 8.01 mg/100g for T<sub>1</sub>, T<sub>2</sub>, T<sub>3</sub> and T<sub>4</sub>, respectively throughout 14 days of storage period, then their values decreased. In the present study, ascorbic acid content was not detected in all the control samples. According to these results, consumption of

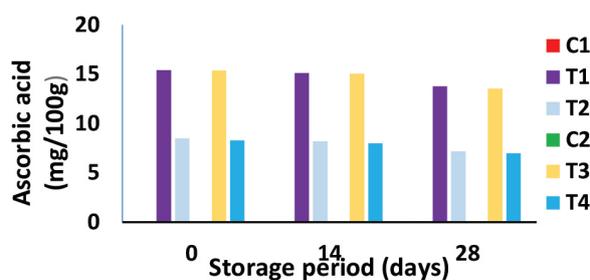


Fig. 6: Ascorbic acid (mg/100g) of different functional beverages during the storage period

wey and permeate with kumquat paste and purée can contribute in covering the daily requirement of vitamin C.

On the other hand,  $\beta$ -carotene increased in all prepared beverages as compared with the control samples (Fig. 7). The prepared samples T<sub>1</sub> and T<sub>3</sub> had the highest increment (50.12 and 50.22 mg/100gm) followed by T<sub>2</sub> and T<sub>4</sub> (35.57 and 35.65 mg/100g) in fresh treated samples at zero time.  $\beta$ -carotene was almost stable in all beverages during storage period. In the present study,  $\beta$ -carotene was not detected in all control samples during storage period.

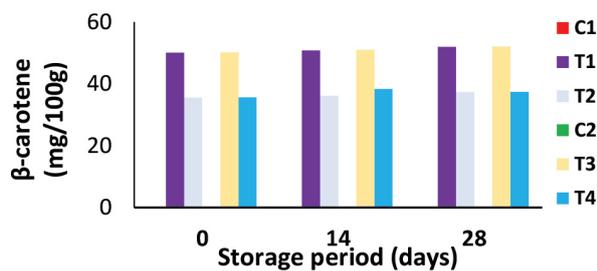


Fig. 7:  $\beta$ -carotene (mg/100g) of different functional beverages during the storage period

**Total polyphenols and antioxidant activity**

The data in Figs. (8 and 9) indicated that samples containing 25% kumquat purée and paste (T<sub>1</sub>, T<sub>3</sub>, T<sub>2</sub> and T<sub>4</sub>) had higher total polyphenols and antioxidant activity than the control samples C<sub>1</sub> and C<sub>2</sub>. The data also, indicated that a gradual decrement in total polyphenols and antioxidant activity was noted after 14 and 28 days of storage. These results are in agreement with those reported by Sreerupa *et al.* (2014) who mentioned that fruit juices contained less amount of antioxidant after 28 days of storage than that found in the fresh ones.

From the previous results, it can be concluded that the addition of kumquat purée and paste to whey and permeate can improve its content of

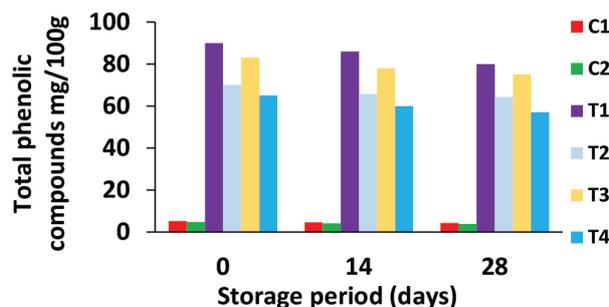


Fig. 8: Total phenolic compounds of different functional beverages during the storage period

phenolic compounds which play an important role as an antioxidant besides its essential oil (Table 2) that had high antioxidant activity

**Mineral content**

The data presented in Table (8) illustrated the mineral content of the functional beverages during storage for 28 days. The obtained data showed that all the studied minerals increased with the addition of kumquat purée and paste. In general, adding kumquat purée and paste are accompanied by high levels of Na, Ca, Zn, Mg, and Fe of the prepared functional beverages which is mainly due to the high content of these elements in the raw materials used.

**Microbiological properties of functional beverages**

Total bacterial count (log cfu/ml) at zero time and during storage of functional beverages containing kumquat purée and paste are presented in Fig. (10). The obtained results indicated that the log of total bacterial count decreased in all beverage samples compared to the control samples (C<sub>1</sub> or C<sub>2</sub>). This may be due to the antibacterial effect of essential oils, phenolic and flavonoid compounds present in kumquat (Table 2). During storage, the count gradually increased until the end of the storage period. This might be due to that these functional beverages contain lactose, minerals and proteins which may lead to enhance the growth of bacteria. Similar results are recorded by Wang *et al.* (2012) who reported that kumquat possessed the essential oils which can be considered as an effective capacity to control the total count of viable bacteria.

The coliform and moulds & yeasts counts were not detected in all the beverages either at zero time or during storage period at 41 $\pm$ C for 28 days. This is due to good hygienic conditions during manufacture and storage. Mona *et al.* (2005)

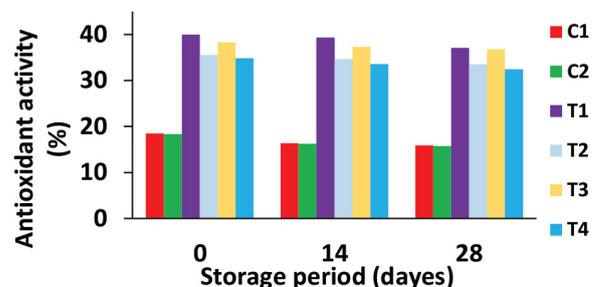
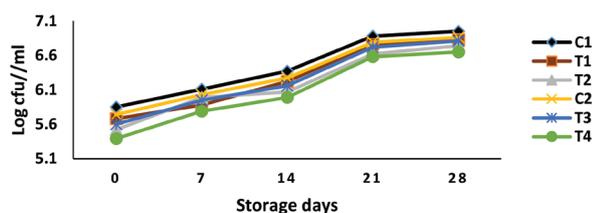


Fig. 9: Antioxidant activity (%) of different functional beverages during the storage period

**Table 8: Mineral content (mg/100g) of fresh and stored functional beverages**

Element	Storage period (days)	Whey			Permeate			
		C1	T1	T2	C2	T3	T4	
K	0	115.6	120.91	118.37	107.32	114.35	112.84	
	28	114.18	118.22	118.29	107.05	113.5	110.28	
Na	0	48.44	57.68	52.37	49.69	57.31	56.34	
	28	47.55	56.55	51.61	49.34	56.96	55.67	
Ca	0	42.18	43.25	42.45	45.41	46.62	44.62	
	28	42.04	42.24	42.04	45.25	43.21	43.18	
Mg	0	0.27	0.27	0.27	0.25	0.26	0.24	
	28	0.26	0.25	0.26	0.24	0.26	0.23	
Fe	0	0.07	0.47	0.46	0.09	0.45	0.44	
	28	0.07	0.46	0.45	0.08	0.44	0.43	
Zn	0	0.04	0.13	0.12	0.05	0.11	0.1	
	28	0.04	0.12	0.11	0.05	0.1	0.09	

C1: 100% whey (control 1); T1: 75% whey+25% kumquat purée ; T2: 75% whey+25% kumquat paste; C2: 100% permeate; T3: 75% permeate+25% kumquat purée and T4: 75% permeate+25% kumquat paste



**Fig. 10: Total bacterial count (log cfu/ml) of functional beverages**

obtained similar results for ice cream and stirred yoghurt with mango and strawberry. Thus, it is evident that the pasteurization followed by storage at low temperature would be sufficient to keep the samples for 28 days.

### Sensory properties of functional beverages

Sensory attributes including colour, taste, odour and overall acceptability of the studied functional beverages during storage are presented in Table (9). It could be observed that all the studied attributes were accepted by the panelists even during storage periods except for the odour score. These results agreed well with those reported by Castro *et al.* (2013). The decrement in the odour score during storage could be possibly due to some losses of volatile aromatic substances as shown by Thakur & Barwal (1998). From the aforementioned data, it can be concluded that the addition of fresh kumquat purée and paste to whey and permeate increased the overall acceptability of the prepared functional beverages.

### CONCLUSION

The addition of kumquat purée and paste had positive effect on the physical, chemical, sensory and antioxidant characteristics of whey and permeate beverage products. Kumquat purée and paste can be used as an easily accessible source of natural antioxidants and as a potential food supplement. Moreover, kumquat purée and paste could be used in whey and permeate beverage products as a source of pleasant flavour. The highest overall acceptability score was obtained for sample T<sub>2</sub> and T<sub>4</sub>. Therefore kumquat may be used as a suitable source of natural additives in whey and permeate functional beverages to enhance flavour, nutritional value and natural colour.

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**Table (9): Sensory properties of functional beverages during storage periods**

Sample	Storage period (days)	Colour	Taste	Odour	Overall acceptability
C1	0	7.70±0.48	7.60±0.52	7.30±0.48	7.40±0.97
	14	7.40±1.07	7.50±0.53	7.50±0.53	7.10±0.97
	28	7.40±1.07	7.30±0.48	7.20±0.63	6.90±0.57
T1	0	8.40±0.69	7.70±0.48	8.60±0.52	8.20±1.03
	14	8.25±0.89	7.40±0.52	8.00±0.66	7.85±0.62
	28	8.10±0.56	7.60±0.52	7.80±0.79	7.60±0.52
T2	0	8.60±0.51	8.40±0.52	8.40±0.70	8.50±0.52
	14	8.40±0.70	8.20±0.79	7.90±1.10	8.2±0.78
	28	8.20±0.79	7.90±0.56	7.70±0.48	8.0±0.47
Significance of treatment effect					
Treatment (A)		*	*	*	*
Storage (B)		NS	NS	NS	NS
A x B		NS	NS	NS	NS
C2	0	7.7±1.25	7.6±1.07	7.7±0.82	7.5±1.08
	14	7.2±0.42	7.4±0.42	7.5±0.53	7.1±0.56
	28	7.3±0.48	7.5±7.50	7.7±0.67	6.8±0.63
T3	0	8.3±0.95	7.9±0.87	8.40±0.52	8.3±0.95
	14	8.3±0.48	7.6±0.84	8.1±1.10	8.00±0.94
	28	8.1±0.88	7.4±0.52	7.7±0.67	7.70±0.48
T4	0	8.4±0.51	8.7±0.48	8.3±0.95	8.7±0.48
	14	8.3±1.06	8.4±1.07	8.00±1.05	8.6±0.46
	28	8.1±0.73	8.00±0.47	7.30±0.48	8.0±0.47
Significance of treatment effect					
Treatment (A)		*	*	NS	*
Storage (B)		NS	NS	NS	*
A x B		NS	NS	NS	NS

NS, not significant; \*: Significant difference at  $P \leq 0.05$

C1: 100% whey (control 1); T1: 75% whey+25% kumquat purée ; T2: 75% whey+25% kumquat paste; C2: 100% permeate; T3: 75% permeate+25% kumquat purée and T4: 75% permeate+25% kumquat paste.

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## إنتاج مشروبات وظيفية من شرش اللبن Permeate المحتوية على فاكهة الكمكوات

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الهدف من هذه الدراسة هو الاستفادة من مخلفات صناعة اللبن المثلثة في شرش اللبن وال permeate في إنتاج مشروبات وظيفية بعد خلطها ببيوريه وعجينة فاكهة الكمكوات، تم تقييم فاكهة الكمكوات من النواحي الفيزيائية والكيميائية والفيزيوكيميائية والميكروبيولوجية وأظهرت النتائج أن فاكهة الكمكوات تعتبر مصدراً جيداً للمركبات الفينولية و الفلافونويدات و مضادات الاكسدة و البيتا كاروتين و فيتامين ج وبعض المعادن مثل الكالسيوم والبوتاسيوم والماغنسيوم. كذلك تم دراسة الخواص الفيزيائية والكيميائية والميكروبيولوجية والخواص الحسية للمشروبات الوظيفية خلال فترات التخزين على درجة حرارة التبريد حيث أظهرت النتائج أن محتواها عال من فيتامين ج والبيتا كاروتين كان مرتفعاً وذلك بالمقارنة بمشروب شرش اللبن وال permeate الخالي من فاكهة الكمكوات وكذلك محتواها من المركبات الفينولية والفلافونويدات ومضادات الأكسدة أعلى مقارنة بالكونترول. كما أظهرت نتائج التقييم الحسى أن إضافة بيوريه وعجينة فاكهة الكمكوات إلى شرش اللبن وال permeate تزيد من درجة تقبل هذه المشروبات. وكان العد الكلي في الحدود المسموح بها في حين أظهر الكشف عدم وجود الكوليفورم والفطريات والخمائر في جميع المشروبات أثناء التخزين على  $4 \pm 1$  درجة مئوية لمدة ٢٨ يوماً. ولذلك يوصى باستخدام فاكهة الكمكوات في إنتاج مشروبات وظيفية أكثر تقبلاً.