Physicochemical and Microbiological Properties of Papaya Functional Beverages Based on Sweet Cheese Whey

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Abstract: An experiment was conducted to produce and evaluate Papaya functional beverages based on sweet cheese whey during refrigerated storage for 30 days. Papaya pulp and sweet whey were mixed in proportion of 0:100, 10:90, 20:80, 30:70, 40:60 and 50:50 (w/w) to create 6 blends, respectively. Assessment of their chemical constituents, physical properties, microbiological examination as well as organoleptic evaluation was carried out directly after preparing and during storage for one month at refrigerator temperature (5°C ± 2°C). The results revealed that the acidity of beverages samples were gradually increased during storage periods; meanwhile, the loss percentage of ascorbic acid contents was increased by increasing the storage time. Lactose and calcium contents of the beverages samples were slightly decreased. Bacterial counts and yeast & molds counts were also gradually decreased through storage period. On the other hand, the prepared functional papaya-whey beverages could be successfully storage at the refrigerator temperature for the period of one month without significant changes in their sensory attributes. Concerning sensory properties, the blend 5 gained the highest organoleptic scores followed by blend 6; and no flavor or color defects were observed in any of all blends during the storage period however the total acceptability were between varied.

[Mohamed, A.G.; Hayam M. Abbas and Abeer F. Zayan. **Physicochemical and Microbiological Properties of Papaya Functional Beverages Based on Sweet Cheese Whey.** *Life Sci J* 2014;11(3s):257-263]. (ISSN:1097-8135). http://www.lifesciencesite.com. 39

Keywords: Papaya pulp, Sweet cheese whey, Papaya- whey blends, Functional beverages.

1. Introduction

Present dietary scenario necessitates exploring the possibility of incorporating novel ingredients in commonly consumed foods rather than developing new food product**, (Aleem *et al*., 2012).** Food or beverage which has physiological benefits beyond basic nutrition is being preferred now a day to reduction risk of chronic diseases **(Boghani *et al.*, 2012).** Fruits are excellent sources of phytochemicals which are essential for human health and relished by consumers in all seasons and they are rich sources of nutritional and bioactive compounds which confer immense health benefits (**Balaswamy *et al.*, 2013**). Of the wide variety of tropical fruits, papaya occupies a primary position owing to its wide availability and nutritional value. Each and every part of papaya plant from root to shoot is used for medicament purposes. Ripe papaya fruit is used in jam, jelly, marmalade, puree, wine, nectar, juice, frozen slices, etc.

Papaya (***Carica papaya L.***) fruit belongs to species in genus *Carica* of the plant family *Caricaceae*, widely distributed throughout the tropics **(Boghani *et al.*, 2012).** Papaya is a good source of vitamins, particularly vitamin A & C, dietary fiber and minerals (calcium and iron) as well as provides flavor, aroma and texture to the pleasure of eating **(Nakasone & Paull, 1998**). Fully ripened papaya fruits are usually eaten fresh as the enzymes in the fruit produce calm, smoothing feelings in the stomach. Papaya is known for its fine and natural laxative virtue which aids digestion. Furthermore, Papaya fruits are rich in enzymes called papain and chymopapain that break down the proteins from the food_ a person eats_ into amino acids and therefore help digestion. The anti-inflammatory properties and high antioxidant content of papaya is known to prevent cholesterol oxidation and can be used in preventive treatments against strokes, heart attacks, diabetes, heart disease and blood pressure **(Eno *et al.*., 2000**). **Zaman *et al.* (2006)** reported Papaya is as an excellent source of ascorbic acid and a good source of carotene & riboflavin. The major carotenoid is cryptoxanthin. Carotenoids are responsible for the flesh color of papaya fruit mesocarp. Red-fleshed papaya fruits contain five carotenoids, *viz.* beta-carotene, beta-cryptoxanthin, beta-carotene-5-6-epoxide, lycopene and zeta-carotene. Yellow-fleshed papaya contains only three carotenoids, *viz*. beta-carotene, beta-cryptoxanthin and zeta-carotene. On the other side, Papaya contains a low level of fatty acids. Palmitic acid and linolenic acid are the two major fatty acids in papaya. Fatty acid composition was changed during fruit ripening and no significant difference is observed in lipid composition with maturity of papaya fruits (Nakasone and Paull (1998). The edible portion of fruit contains macro-minerals include sodium, potassium, calcium, magnesium and phosphorus. The micro-minerals include iron, copper, zinc, manganese and selenium (**Zaman *et al.*, 2006).** Regarding to the anti-nutrients constituents; peel and pulp of ripe papaya fruits contain low amounts of anti-nutrients like tannin (10.16 mg/100 g of dry matter), phytate (3.29 mg/100 g of dry matter) and oxalate (1.89 mg/100 g of dry matter) creating incompatibility problems as reported by **Srividya and Ramachandran (2012**).

Whey is the aqueous fraction produced in large amounts as a sub-product of the cheese-making process which categorized as sweet whey, being produced at the rate of large amounts from cheese producing (**Adam *et al.,* 2004 and Mallik and Kulkarni, 2010**). In **1998,** The world production of whey cheese is estimated as 118 million tons. This amount is equivalent to approximately 7 million tons of solids, of which 10% are proteins; **Wit, 1998).** The environmental question and the various health benefits of whey cheese proteins for humans (immunomodulation, antimicrobial, antiviral, anti-carcinogenic, and antiulcer activities as well as protection of the cardiovascular system) and the presence of essential sulfur amino acids that had both nutritive value and antioxidant activity, make the use of this compound as an ingredient in food products a question of priority, and thus it is being more used by the food industry **(Sienkiewicz and Riedel 1999; and Sgarbjieri, 2004).)**. Whey and its biological components have proven its effects in treatment of cervical chronic diseases like cancer, cardiovascular, HIV etc. As it is nutritionally too rich it can also be used in beverages of infant; geriatric and athletic foods **(Devera, 2005).**

Over the years, various approaches have been taken in an effort to transform a large volume of cheese whey into products suitable for use as food (**Smithers *et al.,* 1996; and Mirjana *et al.,* 2004).** Numerous procedures have been developed for improving its characteristics aiming to enable its direct utilization by human as (concentrates, juices, syrups pulps and nectars). It recognized as a genuine thirst quencher, light refreshing healthful and nutritious (**Prendergast, 1985**).

Whey based fruits beverages are more suitable for health as compared to other drinks (**Sarvana Kumar, 2005; and Yaday *et al.,* 2010)** Fruit beverages are well enjoyed by all age groups of the society (**Balaswamy *et al.,* 2013).** Blended drinks are good alternative for development of new products to provide benefit of taste, nutrition as well as medicinal properties **(Srividya & Ramachandran, 2012)**. Therefore**,** the aim of this study was to produce functional papaya-whey beverages by blending sweet cheese whey (as a by- product) and papaya pulp (as a good source of fiber, ascorbic acid and calcium) and evaluate the physicochemical, microbiological and organoleptic quality of the resultant products during refrigerated storage (5 ±2°C) for 30 days.

2. Materials and Methods

Main Materials

Papaya fruits (***Carica papaya L.)*** were bought from Horticultural Research Institute Agriculture Research Center, Giza, Egypt. Fruits were picked at ripening period in September 2012. Fresh sweet Ras cheese whey was obtained from the Agricultural Secondary School, Shibin El-Kom, Minoufia, Egypt.

Preparation of papaya pulp

Papaya fruits were washed, hand peeled, cut into pieces then mixed in a blender and filtered through a cheese cloth. The mixed pulp was heated in a stainless steel container at 90°C for 3 min to inactivate pectin enzymes, cooled rapidly to 20°C then filled into polyethylene bags **(Boghani *et al.,* 2012**). The fruit pulp were stored at -18°C until used.

Preparation of papaya-whey beverage

Homogenized Papaya pulp and sweet whey were mixed in proportion of 0:100, 10:90, 20:80, 30:70, 40:60 and 50:50 (w/w) to create 6 blends as showed in Table 1. Carboxyl methyl cellulose (CMC), citric acid and 0.1% potassium sorbets were added at rate of 0.2, 0.2 and 0.1%, respectively. The total soluble solids (TSS) content was adjusted to 18.0% in all blends using sucrose. All blends were filled in white glass bottles, tightly closed, pasteurized at 65°C for 30 min, then filled at the same temperature, cooled and stored at refrigerator temperature (5°C ± 2°C) for 30 days.

Table 1: The blends of papaya pulp and sweet whey mix.

Materials (%)	Blend 1	Blend 2	Blend 3	Blend 4	Blend 5	Blend 6
Papaya pulp	0	10	20	30	40	50
Sweet cheese whey	100	90	80	70	60	50

Methods

Chemical Analysis

Total solids (TS), fat, protein, ash, calcium, and total soluble solids (TSS) and titratable acidity contents as well as ascorbic acid were determined according to **AOAC (2000).** Lactose content was determined according to **Nickerson *et al.* (1979).** The pH value was measured using digital pH meter (HANNA, Instrument, Portugal) with glass electrode.

Microbiological examination

The total bacterial counts were estimated using the appropriate media as recommended by the **APHA (1978).** Total yeast and molds counts were enumerated by surface plating on malt extract agar (Oxoid) with 0.01% chloramphenicol as bacterial inhibitor and incubated aerobically at 25ºC for 2-3 days (**Harrigan & Mac-Cance, 1976).**

Color parameters

Hunter L, a and b parameters of Papaya-whey beverage blends were measured using a spectro-colorimeter (Tristimulus Color Machine) with the CIE lab color scale (Hunter, Lab Scan XE - Reston VA, USA) in the reflection mode.

Sensory Evaluation

Twenty expert judges were selected from staff member of Dairy Science Dept., National Research Center, Egypt, to evaluate flavor (60 points), appearance & body (30 points) and color (10 points) of the beverages samples according to **(Deka *et al.,* 1984).**

3. Results and Discussion

1. Chemical composition of both papaya pulp and sweet cheese whey

Chemical composition of both papaya pulp and sweet cheese whey are presented in Table (2). It could be noticed that fresh papaya pulp contains 87.5% moisture, 0.42% ash, 0.14% fat and 0.61% protein; while sweet cheese whey contained 93.7% moisture, 0.65% ash, 0.05 % fat and 0.80% protein. TSS and titratable acidity were 10.5 % and 0.31% in papaya pulp and 5.70% and 0.32% in sweet cheese whey, respectively. The ascorbic acid and calcium contents in papaya pulp were 65.6 mg/100 g and 21 mg/100g respectively. However, the corresponding values in sweet cheese whey were 18.0 mg/100 g and 58 mg/ 100 g. It could be noticed that papaya pulp contained the higher content of TSS, fat and ascorbic acid while the sweet cheese whey contained the higher content of protein, fat and calcium. The main constituent of *Carica papaya* fruit is water like other fruits and the major components of papaya dry matter are carbohydrates (**USDA, 2009).** A similar observation was found by **Zaman *et al* (2006).**

Table (2): Chemical composition of both papaya pulp and sweet cheese whey

Constituents	Papaya pulp	Sweet whey
Moisture (%)	87.50	93.70
Total solids (%)	12.50	6.30
Total soluble solids; TSS; (%)	10.50	5.70
Protein (%)	0.61	0.80
Fat (%)	0.14	0.050
Ash (%)	0.42	0.65
Lactose (%)	-	4.69
Titratable acidity (%)	0.31	0.32
Ascorbic acid (mg/100 g)	65.6	18.0
pH	4.22	5.60
Calcium (mg/100 g)	21.0	58.0

Changes in chemical composition of functional Papaya-whey beverage during storage

Changes in total solids

Changes in chemical constituents of papaya - whey beverage samples stored at refrigerator temperature (5°C ± 2°C) up to one month are presented in Table (3). Results show that TS were 18.0% in all papaya - whey beverage samples at zero time and then slightly noticeable changes were observed during storage being 17.60 % after 1 month of storage. **Jakhar *et al.* (2012)** reported results pertaining to chemical changes during storage which revealed that total soluble solids increased gradually during storage and these increases might be attributed in conversion of polysaccharides and other constituents of juice into sugar.

Changes in pH and acidity

It could be noticed that, freshly, the acidity value was slightly decreased by increasing of papaya pulp ratio till the third blend, then fixed and began to increased in blend 5 & 6. (Table 3).The acidity content of functional papaya-whey beverage blends was increased gradually during the storage periods and the incremental percentages of acidity varied between 16.67 and 28.12%. On the other hand, the pH values of functional papaya-whey beverage samples were decreased gradually during the storage periods. This noticeable decrease of pH values and the increases in acidity percent could be attributed to the acid production during storage due to the conversion of lactose to lactic acid and formation of organic acids. Similar increase in acidity of beverages during storage had been reported by **Pawar *et al.* (2004).** The pH of beverage could be correlated inversely with the acidity of product and found to decrease with increase in storage. These results are also in agreement with data reported by **Sikder *et al.* (2001) and Ritika *et al.* (2010).**

Changes in ascorbic acid content

In fresh samples, it could be observed a noticeable increase in ascorbic acid content as papaya pulp ratio increased in the blends. Their values were 18.0, 20.0, 26.0, 29.5, 30.30 and 34.30 mg / 100 g respectively (Table 3). Data indicated that the loss of ascorbic acid content was increased with increasing the storage period for 30 days. This loss could be due to the oxidation process during storage. Similar results were reported by **EL-Gharably *et al.* (2004); Andriano *et al.* (2009); Mallik & Kulkarni (2010) and Boghani *et al.* (2012).**

Changes in lactose content

Logically, lactose contents were decreased in the blends which had high portions of papaya pulp and low ratio of whey as whey is the main source of lactose (Table 3). Their values were 4.69,4.22,3.75,3.28,2.81 and 2.45 respectively for the 6 blends.

Lactose content of functional Papaya-whey beverages blends during refrigerated storage for 30 days was presented also in Table 3. It could be noticed a slight reduction in lactose contents was occurred among all tested samples, since lactose levels of the papaya-whey beverage samples stored to 30 days were lower than those at the beginning of storage.

Changes in calcium content

The calcium content of functional Papaya-whey beverage samples during refrigerated storage for 30 days showed a slight reduction in calcium content among all tested samples (Table 3). It could be noticed that calcium contents were decreased in the fresh blends as whey ratio decreased Their values were 58.0, 54.3, 50.6,46.9,43.2 and 39.5 mg / 100 g for blends 1,2,3,4,5 and 6 respectively.The calcium content of the stored beverages was lower than those at zero time. These results are in agreement with those reported by **Rizk *et al.* (2012).** The reduction level in calcium content at the end of storage period could be arranged in increasing order as follow blend 3 had the lowest calcium content (0.32%) followed by blend 1 (0.51%), blend 2 (0.55%), blend 5 (0.78%), blend 6 (0.90%) and blend 4 (1.03%), respectively. These results are also in agreement with **Abeid *et al.* (2001) and** Rizk ***et al.* (2012).**

Table (3): Changes in chemical constituents of functional Papaya-whey beverage blends during refrigerated storage for 30 days (5 ± 2ºC).

Constituents	Storage Period (days)				Storage Period (days)				Storage Period (days)			
	0	7	15	30	0	7	15	30	0	7	15	30
	Blend1				Blend2				Blend3			
Total solids%	18.00	17.90	17.80	17.70	18.0	18.0	17.90	17.80	18.0	17.90	17.80	17.75
Titratable acidity%	0.32	0.36	0.38	0.41	0.31	0.33	0.36	0.38	0.29	0.32	0.34	0.36
Ascorbic acid mg/100g.	18.0	15.20	13.20	10.40	20.0	17.0	15.0	11.50	26.0	23.50	19.0	14.50
pH value	5.30	5.27	5.11	4.90	5.20	5.0	4.75	4.50	5.00	4.76	4.40	4.10
Calcium mg/100g	58.0	58.0	57.9	57.8	54.3	54.3	54.2	54.1	50.6	50.6	50.4	50.4
Lactose mg/100g	4.69	4.49	4.44	4.35	4.22	4.12	4.13	4.10	3.75	3.65	3.55	3.45
Ash%	0.65	0.65	0.65	0.64	0.64	0.64	0.63	.063	0.62	0.62	0.61	0.61

Blend 1(0% papaya + 100% sweet whey); Blend 2(10%papaya + 90% sweet whey); Blend 3(20% papaya + 80% sweet whey). All analyses were carried out in triplicates.

Table (3): Continued

Constituents	Storage Period (days)				Storage Period (days)				Storage Period (days)			
	0	7	15	30	0	7	15	30	0	7	15	30
	Blend4				Blend5				Blend6			
Total solids %	18.0	17.95	17.90	17.80	18.00	17.90	17.80	17.70	18.00	17.80	17.70	17.60
Titratable acidity%	0.29	0.31	0.33	0.34	0.30	0.32	0.34	0.36	0.30	0.32	0.33	0.35
Ascorbic acid mg/100g	29.50	25.60	22.30	16.20	30.30	25.29	20.24	18.40	34.30	30.50	26.40	20.0
pH value	4.90	4.60	4.24	4.00	4.80	4.50	4.20	3.90	4.75	4.32	4.10	3.65
Calcium mg/100g	46.9	46.9	46.8	46.7	43.2	43.2	43.2	43.0	39.5	39.5	39.4	39.4
Lactose mg/100g	3.28	3.27	3.26	3.25	2.81	2.71	2.61	2.51	2.35	2.25	2.15	2.05
Ash%	0.61	0.61	0.60	0.59	0.60	0.60	0.59	0.58	0.58	0.58	0.57	0.57

Blend 4(30% papaya + 70% sweet whey); Blend 5(40% papaya + 60% sweet whey); Blend 6 (50% papaya + 50% sweet whey). All analyses were carried out in triplicates.

Microbiological examination

Total bacterial counts and yeast & mold counts of functional Papaya-whey beverage blends were freshly estimated and during one month of storage at refrigerator temperature (5°C ± 2°C). As shown in Table 4, both bacterial as well as yeast & mold counts were decreased with increasing the storage period since bacterial counts were 6.5, 6.10, 5.32, 5.10, 4.27 and 4.21 x 10^4 at zero time, while after one month of storage they became 0.97, 0.84, 0.60, 0.55, 0.42 and 0.32x 10^4 for blend 1, 2, 3, 4, 5 and 6, respectively. Papaya pulp contains anti microbial agent which decreased the microbial counts (**Boghani *et al.* (2012)**

Regarding for yeast & mold counts; the initial counts before storage were 6.23, 6.75, 6.90, 7.11, 7.47 and 7.58 x 10^2 whereas after one month of storage they were 0.50, 0.63, 0.72, 0.82, 0.89 and 0.95 x 0^2 for blend 1, 2, 3, 4, 5 and 6, respectively.

The change in bacterial counts were : -85.10, -86.23, - 88.72, -89.22, -90.16 and -92.23% whereas the corresponding decreasing values for yeast and molds counts were -92.10, -90.67, -89.57, -88.47, -88.49 and -87.46%. It could be included that both bacterial and yeast & mold counts were gradually decreased during storage as the effect of potassium sorbets which added and the antimicrobial agent of papaya fruit. These results were similar to the data reported by **Sikder *et al.* (2001) and Ritka *et al.* (2010).**

Table(4): Changes in total bacterial counts and yeast & molds counts of functional Papaya-whey beverages samples during refrigerated storage for 30 days (5 ± 2ºC).

functional Papaya-whey beverage	Total bacterial counts ($X 10^4/g$)				Change (%)	Yeast and molds counts ($X 10^2 / g$)				Change (%)
	Storage period (days)					Storage period (days)				
	0	7	15	30		0	7	15	30	
Blend 1	6.50	4.62	2.45	0.97	- 85.10	6.33	4.93	1.90	0.50	- 92.10
Blend 2	6.10	3.24	1.95	0.84	- 86.23	6.75	4.97	1.97	0.63	- 90.67
Blend 3	5.32	2.86	1.56	0.60	- 88.72	6.90	5.11	2.33	0.72	- 89.57
Blend 4	5.10	2.32	1.30	0.55	- 89.22	7.11	5.32	2.72	0.82	- 88.47
Blend 5	4.27	2.23	1.26	0.42	- 90.16	7.47	5.49	2.85	0.89	- 88.49
Blend 6	4.12	2.20	1.15	0.32	- 92.23	7.58	5,66	2.94	0.95	- 87.46

Blend 1(0% papaya + 100% sweet whey); Blend 2(10%papaya + 90% sweet whey); Blend 3(20% papaya + 80% sweet whey); Blend 4 (30% papaya + 70% sweet whey); Blend 5(40% papaya + 60% sweet whey); Blend 6(50% papaya + 50% sweet whey).

Changes in color parameters

Color parameters of functional Papaya-whey beverage samples during refrigerated storage for 30 days are presented Table (5). The whiteness and yellowish degree of all Papaya-whey beverage blends was higher than that of control. Whiteness degree of Papaya-whey beverage blends slightly increased with increasing the percentage of Papaya pulp and with storage time increased. A similar, but more marked a gradual increase in yellowish degree of Papaya-whey beverage with increasing the amount of Papaya pulp. A similar observation was found by **Andrian *et al.*, 2009** and **Boghani *et al.* (2012)**

Increase in yellowish degree of Papaya-whey beverage blends might be due to the natural yellow color of papaya fruit. Inversely, by increasing the proportion of Papaya pulp in functional Papaya-whey beverage blends, the degree of greenish decreased. As storage periods increased; whiteness and yellowish degree of Papaya-whey beverage blends slightly increased, while greenish slightly decreased at the same rate within blends. Such an effect has been reported by **(Sikder *et al.*, 2001).**

Table(5): Color parameters of functional Papaya-whey beverage samples during refrigerated storage for 30 days (5 ± 2ºC)

functional Papaya-whey beverage	L		a		b	
	Fresh	1 month	Fresh	1 month	Fresh	1 month
Blend 1	30.88	31.15	- 4.49	- 4.30	7.03	7.56
Blend 2	36.19	36.54	- 4.38	- 4.22	11.99	12.18
Blend 3	36.86	37.01	- 3.91	- 3.25	15.10	15.66
Blend 4	37.13	37.80	- 3.07	- 2.96	18.33	18.96
Blend 5	37.81	37.99	- 2.76	- 2.96	19.57	20.11
Blend 6	38.69	39.10	- 2.96	- 2.20	21.42	21.77

L, darkness from black (0) to white (100); a, color red (+) to green (-); b, color yellow (+) to blue

Sensory evaluation

The sensory quality profile of beverages is a prime factor to consider the marketability of the product. Sensory evaluation of beverages prepared with different blends of papaya pulp and sweet whey at zero time and after one month of storage at 5°C ± 2°C were illustrated in Table (6). Functional Papaya - whey beverages prepared with 40% papaya pulp and 60% sweet whey (blend 5) gained the highest scores for color, appearance and flavor at zero time and after one month of storage at 5°C ±2°C. This treatment was the most acceptable blend followed by blend 6; while other blends gained the lowest scores.Similar data dealt with development and storage of papaya beverages and their quality evaluation were reported by **Boghani *et al.* (2012), Srividya & Ramachandran, (2012) and Balaswamy *et al.* (2013).**

Table (6): Sensory evaluation of functional Papaya-whey beverages samples during refrigerated storage for 30 days (5 ± 2ºC)

Functional Papaya-whey beverages	Sensory Evaluation at zero time of storage			Total scores (100)	Sensory Evaluation after one month of storage (5±2ºC)	
	Color (10)	Appearance (30)	Flavor (60)		Color (10)	Appearance (30)
Blend1	4.90	11.80	22.00	38.70	4.40	10.00
Blend2	6.40	13.00	28.00	47.40	6.00	11.00
Blend3	7.60	19.00	38.00	64.60	7.00	16.00
Blend4	8.60	27.00	54.00	89.60	8.00	23.00
Blend5	9.70	29.50	59. 50	98.70	9.50	27.50
Blend6	9.60	29.00	58.00	96.60	9.20	26.00

Blend 1(0% papaya + 100% sweet whey); Blend 2(10%papaya + 90% sweet whey); Blend 3(20% papaya + 80% sweet whey); Blend 4(30% papaya + 70% sweet whey); Blend 5(40% papaya + 60% sweet whey); Blend 6 (50% papaya + 50% sweet whey).

Conclusion

From the previous results, it could be concluded that functional Papaya-whey beverages can be successfully prepared specially at the higher level of adding Papaya pulp with optimum sensory characteristics. Beverages can be stored at refrigeration temperature up to 30 days without any defects in their sensory properties. All acceptability was improved by storage. The product can prove a nutritionally with agreeable flavor, energy providing due to whey proteins and papaya pulp; in addition to its delicious test and attractive yellow color.

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3/22/2014