

Preparation of New Carbonated Beverages Based on Hydrolyzed Whey by Fruit and Some Herbs

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Abstract In an attempt to develop new carbonated beverages, whey was hydrolyzed by lactase enzyme at pH 6.6. Whey beverages were prepared by blending sugar (10%), fruit juice or/and herbs extract to hydrolyzed whey. The hydrolyzed whey beverages were carbonated by injecting CO₂ at a pressure of 15 psi/15sec. The carbonated whey beverages were analyzed for chemical, physical, microbiological and evaluated for its sensory properties. The TS, ash, total carbohydrates, and pH values had recorded significant ($P < 0.05$) between all treatments in carbonated beverages. But the protein and fat contents showed no significant between all treatments. From the results, it is clear that T8 treatment recorded the significantly high value of viscosity compared to T3 treatment which had the lowest values. Energy value of carbonated whey beverages ranged from 56.85 to 61.21 kcal 100g⁻¹ sample. The total bacterial counts were ranged from 0.95 to 1.04 log₁₀ CFU mL⁻¹ in all treatments. The acceptability value in carbonated whey beverages had an increasing trend with a treatment containing green tea, lemon and/or peppermint. Higher acceptability values were obtained for the T8 sample and minimum acceptability scores were attained for the T1 sample of carbonated whey beverages.

Keywords: hydrolyzed whey, carbonated whey beverages, chemical analysis, Energy value, sensory properties, physical analysis

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1. Introduction

Whey is one of the major by-products produced from the cheese and casein industry. Properties and composition of whey types depending on the production methodology and vary of end product. It contains about 50% of the milk solids, containing lactose, whey proteins, salts, a low level of lipid and most of the water-soluble vitamins [1]. The milk sugar is about 75% of whey total solids [2].

Most plain, carbonated, alcoholic and non-alcoholic whey beverages have been successfully developed and marketed all over the world. There is various whey beverages already have achieved success in India, Europe, Switzerland, Poland, Netherland and Japan market. Recently whey and partially hydrolyzed lactose syrup-based and whey and herbal extract containing non-fermented nutritious soft drinks have been developed [3,4].

Several studies investigated the variance in antioxidative and anti-mutagenic activity between tea with or without the addition of milk. Serafini et al. [5] recorded that total antioxidant activity did not decrease due to the addition of tea to milk, but the polyphenols were rather unsuitable for absorption as the polyphenol-protein complexes were resistant to gastric hydrolysis. Other studies did not find a

variance in the antioxidant activity before or after the addition of tea to milk [6].

Citrus fruits have contained high contents of antioxidant activity, which may be protected against cataracts, heart diseases, cancer, degeneration of the macular area of eyes and infection. Lemon juice has the highest flavonoids (800-1500 mg L⁻¹), especially flavoneglycosides and flavanone [7]. Peppermint (*Mentha piperita*) is one of the most widely consumed single ingredient herbal teas. The phenolic compounds of the leaves contain several flavonoids. Peppermint leaves obtain a considerable amount of pre-sources of vitamin A [8]. Therefore, this work was carried out to produce carbonated whey beverages from carbonated whey fortified with citrus fruit juice (lemon juice) and herbs (peppermint and green tea) and study their quality properties *i.e.* chemical, physical, microbiology, and sensory properties.

2. Materials and Methods

2.1. Materials

Ras cheese whey was provided by the Dairy Science Department, Faculty of Agriculture, Benha Univ., Egypt. The main composition of whey was 6.62% total solids, 0.53% ash, 0.25% fat, 1.37% protein, 0.21% acidity and

4.34% total carbohydrate. Lactase enzyme (Maxilact[®] L X 5000) was purchased from DSM Food Specialties BV, Netherlands, production site: DSM Food Specialties, USA, INC. Fine sugar cane was obtained from the local market. Lemon fruit and peppermint were purchased from the local market, Qaluibia, Egypt. Green tea was purchased from ISIS company for food processing, El-Horry, Heliopolis, Cairo, Egypt. Carbonated water (CO₂) was purchased from the Ice soda (Isoda), Egypt.

2.2. Methods

2.2.1. Preparation of fruit juice and herb extracts

Lemon fruit was washed and cut into "two pieces" and then squeezed to produce lemon juice. The lemon juice was filtered to separate the seeds and other fine materials through a muslin cloth. The clear lemon juice was ready to use. Meanwhile, peppermint extract was prepared from fresh mint leaves as follows: the leaves were mixed in a blender grinder (1500 rpm) and filtrated through a muslin cloth. On the other hand, the green tea extract was prepared by mixing tea leaves at a rate of 200g/l; it was heated at 90°C for 10 min then filtrated, cooled and kept at 5°C until use.

2.2.2. Preparation of whey beverages

Whey was filtrated through cheesecloth and pH was adjusted to 6.6 by KOH (1N) and then pasteurized at 72°C/15sec and cooled at 37°C for lactose hydrolysis. An enzyme of lactase was added at the ratio of 0.4 ml L⁻¹ and incubated at 37°C for 5 h with intermediate interval stirring each 30 min. After incubation, enzyme inactivation was done by heating at 80°C/5 min and cooled at 37°C. The sugar was added to hydrolyzed whey at a rate of 10%, lemon juice, and green tea extract were added at a rate of 5% and peppermint was added at a rate of 6% with stirring. The beverages were packaged in glass bottles (1000 ml). The beverages were pasteurized at 72°C/15sec; cooled at room temperature and stored at 5°C in the refrigerator. The carbonation of whey beverages was carried out by injection of CO₂ at a pressure of 15 psi/15sec in bottles and rapidly cooled to 5°C. The carbonated whey beverages were analyzed for physicochemical, microbiological and sensory properties.

2.2.3. Chemical analysis

Total solids, fat, protein, ash and titratable acidity contents of the produced carbonated whey beverages were determined according to the methods described by [9]. The pH values were determined according to [10]. Vitamin A content was determined according to procedure of [11]. The total carbohydrates (CHO) were calculated by difference as follows:

$$\text{CHO \%} = \text{Total solids \%} - (\text{Fat} + \text{Protein} + \text{Ash}) \%$$

2.2.4. Physical analysis

The viscosity was measured using a Brookfield Engineering lab DV-III ultra rheometer in the carbonated whey beverages according to [12]. A colorimeter (Konica Minolta, model CR400) was used for assessment of color parameters of carbonated whey beverages, which directly provide the parameters L* (measures the lightness,

ranging from 0 (black) to 100 (white), a* value ranges from -100 (greenness) to +100 (redness) and b* value ranges from -100 (blueness) to +100 (yellowness) according to [13]. Total soluble solids were examined according to [14] by using Abee-refractometer.

2.2.5. Energy value (EV)

Total calories (kcal) in the carbonated whey beverages were determined according to a 100 g sample using Atwater values for fat (9 kcal g⁻¹), protein (4.02 kcal g⁻¹) and carbohydrate (3.87 kcal g⁻¹) according to [15].

$$\text{EV} = [(\text{fat contents} \times 9) + (\text{protein} \times 4.02) + (\text{Carbohydrate} \times 3.87)]$$

2.2.6. Microbiological examinations

Total viable bacteria, psychrophilic bacteria, aerobic spore-forming bacteria, coliform bacteria, and yeasts & molds counts were enumerated according to [16,17,18,19] and [20], respectively.

2.2.7. Sensory evaluation

Sensory evaluation of carbonated whey beverages was applied by 10 experienced panelists from the staff-members of Dairy Sci. Department, Fac. of Agric., Benha Univ., Egypt. The treatments of carbonated whey beverage were evaluated for taste (10 points), odor (10 points), consistency (10 points), appearance (10 points), color (10 points), sweetness (10 points), acidity (10 points) and overall acceptability (10 points).

2.2.8. Statistical analysis

All statistical analysis for the obtained results was carried out according to [21].

3. Results and Discussion

3.1. Chemical composition of carbonated whey beverages

Table 1 shows the chemical composition of carbonated whey beverages with fruit juice, peppermint or/and green tea extracts. The total solids content in carbonated whey beverages ranged from 15.00±0.01 to 15.80±0.03%. Ash contents varied from 0.45±0.02 to 0.52±0.01%. Fat content in carbonated whey beverages varied from 0.25±0.01 to 0.30±0.01%. This may be due to the very low content of fat in the used whey as a base of carbonated whey beverages. The protein content ranged from 1.26±0.01 to 1.30±0.02%. In carbonated whey beverages, the acidity ranged from 0.13±0.00 to 0.32±0.01% as lactic acid. Total carbohydrates levels in carbonated whey beverages varied from 12.79±0.04 to 13.80±0.09%. The TS, ash and total carbohydrates levels had shown significant (P < 0.05) between all treatments, while protein and fat contents had no significant differences between all treatments. Generally, the addition of fruit juice, peppermint or/and green tea extracts in carbonated whey beverages had a significant model term for TS, ash and total carbohydrates. Similar chemical composition levels of the whey beverage in terms of TS and acidity were recorded by [22] in the preparation of

lactose hydrolysis whey-based beverage. Also, these results agree with those obtained by [23] and [24] shown that total solids content ranged from 10.2 to 17.2% and acidity varied from 0.31 to 0.60% in hydrolyzed whey beverages.

3.2. Vitamin A content of carbonated whey beverages

The vitamin A content of the different carbonated whey beverages is shown in Figure 1. The obtained data cleared that vitamin A of all treatments ranged from 10.89 ± 0.05 to 231.23 ± 0.32 IU $100g^{-1}$, respectively. Also, it can be seen from the results that T5 recorded the highest values ($P < 0.05$) of vitamin A followed by T7 and T3 compared to the other treatments. Rizk [23] shown that vitamin A content ranged from 15 to 4248 IU $100g^{-1}$ of raw materials used in beverages. Farag et al. [8] reported that

peppermint and green tea are an important source of vitamin A, and it improves immune system.

3.3. Physical properties of prepared carbonated whey beverages

3.3.1. pH and total soluble solids (TSS) values

The changes in pH and TSS values in carbonated whey beverages are shown in Figure 2a and b. The pH values ranged from 3.84 ± 0.01 to 6.24 ± 0.01 in all samples (Figure 2a). Minimum pH values were obtained in treatment T4, T6, and T8 which had contained lemon juices. Thus lemon juice contributes significantly to a decrease in pH values of the treatments containing it. On the other hand, TSS values varied from 18.27 ± 0.03 to 18.60 ± 0.04 in carbonated whey beverages (Figure 2b). These variations may be due to added lemon juice which differs in their properties.

Table 1. Gross chemical composition (%) of carbonated whey beverages supplemented by fruit juice and herbs extract

Treatments	TS	Ash	Fat	Protein	Acidity	CHO*
T1	15.71 ± 0.02^a	0.52 ± 0.01^a	0.30 ± 0.01^a	1.30 ± 0.02^a	0.14 ± 0.00^c	13.45 ± 0.05^b
T2	15.80 ± 0.03^a	0.50 ± 0.01^a	0.30 ± 0.01^a	1.27 ± 0.01^a	0.13 ± 0.00^c	13.80 ± 0.09^a
T3	15.36 ± 0.01^b	0.47 ± 0.02^b	0.30 ± 0.02^a	1.26 ± 0.01^a	0.14 ± 0.00^c	13.00 ± 0.06^c
T4	15.31 ± 0.03^b	0.48 ± 0.01^b	0.25 ± 0.01^a	1.27 ± 0.02^a	0.32 ± 0.01^a	12.79 ± 0.04^f
T5	15.00 ± 0.01^b	0.50 ± 0.01^a	0.30 ± 0.02^a	1.29 ± 0.02^a	0.14 ± 0.00^c	12.86 ± 0.05^e
T6	15.11 ± 0.02^b	0.45 ± 0.02^b	0.25 ± 0.01^a	1.26 ± 0.02^a	0.27 ± 0.01^a	13.15 ± 0.04^d
T7	15.09 ± 0.02^b	0.48 ± 0.01^b	0.25 ± 0.02^a	1.26 ± 0.01^a	0.20 ± 0.00^b	13.07 ± 0.04^c
T8	15.22 ± 0.02^b	0.50 ± 0.01^a	0.30 ± 0.01^a	1.28 ± 0.02^a	0.29 ± 0.00^a	13.14 ± 0.04^d
LSD	0.272	0.043	0.102	0.121	0.043	0.044

CHO*= Total carbohydrates %

T1= Hydrolyzed whey as control

T2= Carbonated hydrolyzed whey

T3= Carbonated hydrolyze whey with green tea extract (5%)

T4= Carbonated hydrolyzed whey with lemon juice (5%)

T5= Carbonated hydrolyzed whey with mint extract (6%)

T6= Carbonated hydrolyzed whey with green tea extract (2.5%) and lemon juice (2.5%)

T7= Carbonated hydrolyzed whey with green tea extract (2.5%) and mint extract (3%)

T8= Carbonated hydrolyzed whey with lemon juice (2.5%) and mint extract (3%)

a-f: There is no significant difference ($p > 0.05$) between any two means, within the same column have the same superscript letter.

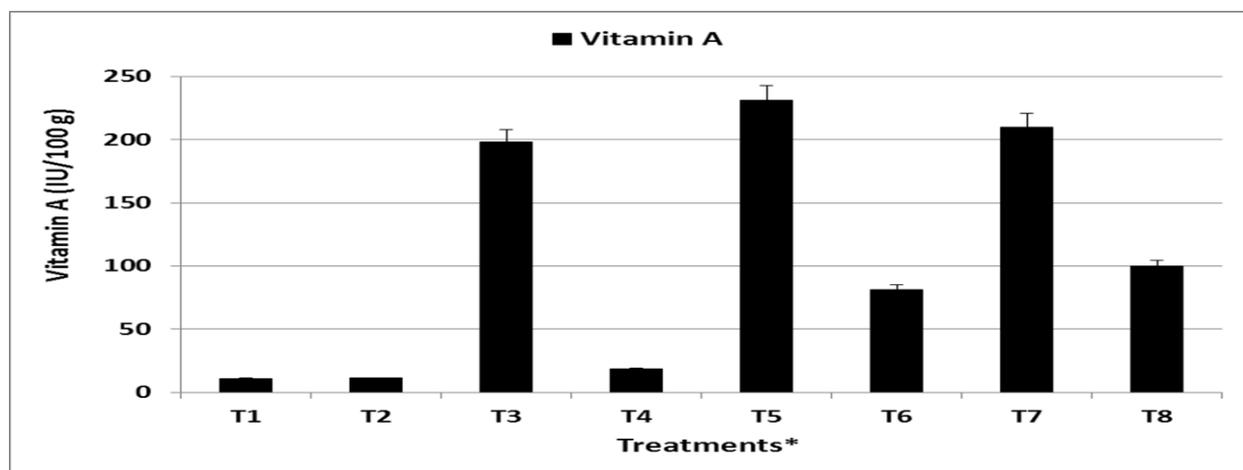


Figure 1. Vitamin A content of carbonated whey beverage samples supplemented by fruit juice and herbs extract (*See footnote Table 1.)

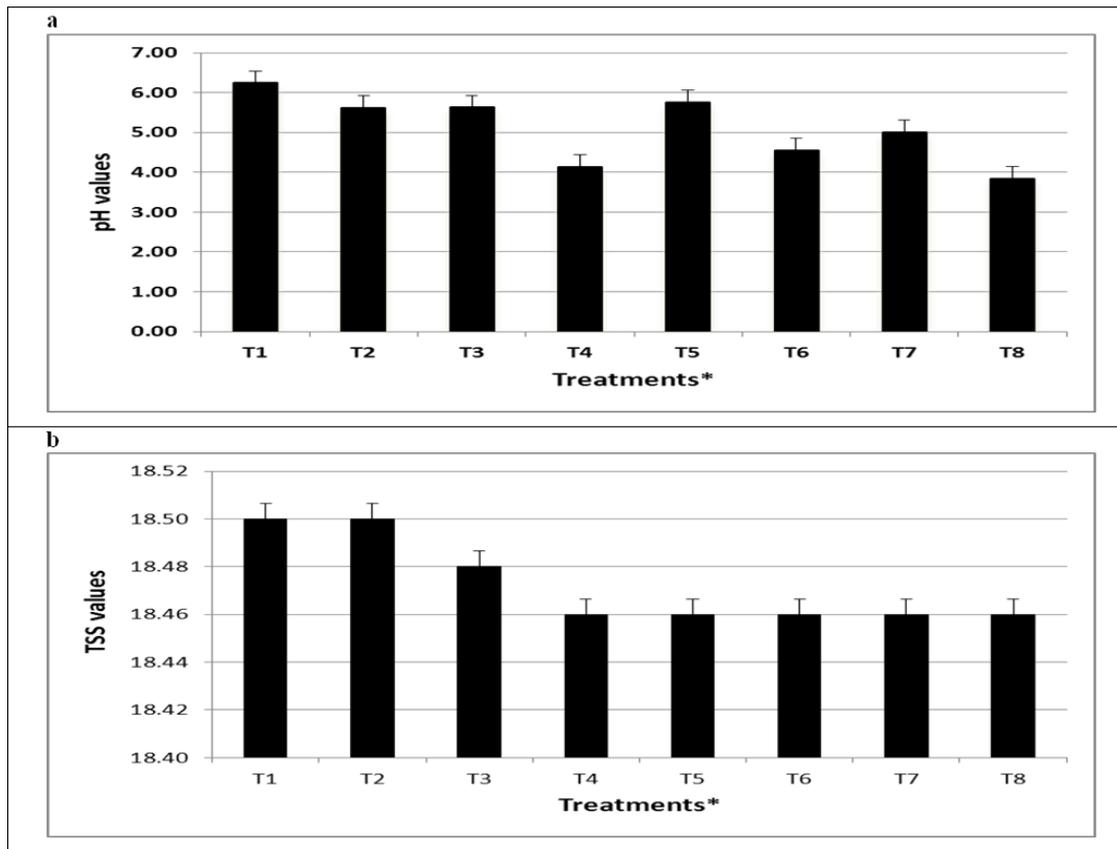


Figure 2. The pH and TSS values of carbonated whey beverage samples supplemented by fruit juice and herbs extract (*See footnote Table 1.)

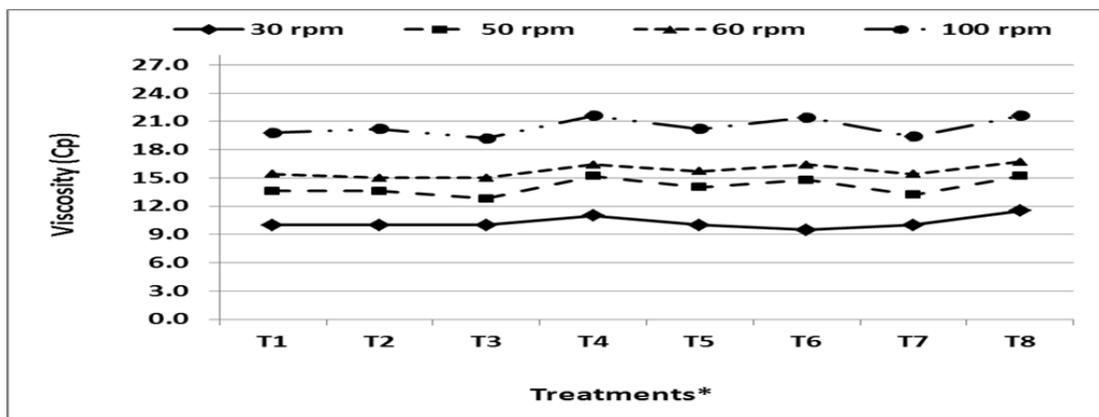


Figure 3. Viscosity values in carbonated whey beverage treatments supplemented by fruit juice and herbs extract (*See footnote Table 1.)

Statistically, carbonated whey beverages containing lemon juice showed lower values of pH compared to other treatments, while TSS contents have no significant differences ($P > 0.05$) between all treatments. A similar trend of composition properties in the beverage of TSS and pH values was reported by [22].

The levels on the regression coefficient in carbonated whey beverages for physicochemical characteristics reflected that fruit juice, peppermint or/and green tea extracts had a negative effect on TS and acidity content in all treatments. TSS values did not show correlations with fruit juice, peppermint or/and green tea extracts in all treatments.

3.3.2. Viscosity of carbonated whey beverages

The changes in viscosity values in carbonated whey beverages under different speeds of viscometer in this work are presented in Figure 3. The viscosity values

ranged from 10.0 ± 0.00 to 11.5 ± 0.01 cP at 30 rpm at 5°C in carbonated whey beverage treatments. Also, it was recorded 19.2 ± 0.01 to 21.6 ± 0.02 cP at 100 rpm at 5°C in all samples. The values of viscosity significantly increased with increasing of the viscometer speed at 5°C . From the previous results, it is clear that sample T8 recorded the significantly high value of viscosity compared to other treatments. But, sample T3 had the lowest values of viscosity in carbonated whey beverages.

The obtained results are in the same line with those reported by [22] of viscosity value in the preparation of lactose hydrolyzed whey beverages. These results agree with those recorded by [24] that showed the effect of sugar, lemon juice, lemon flavor and CMC levels on the viscosity values of hydrolyzed whey beverages. The similar observations had been found out in whey beverages containing lemon, guava, and mint [23].

3.3.3. Colour index of carbonated whey beverages

The effect of carbonated whey beverages incorporation with green tea, lemon and/or peppermint on the color index is shown in Table 2. Results revealed that values of L* (Lightness), a* (Redness) and b* (Yellowness) ranged from 35.07±0.07 to 44.83±0.08; 1.22±0.00 to 12.29±0.01 and 5.51±0.01 to 20.97±0.08, consequently for all treatments. It can be noted that adding green tea, lemon and/or peppermint significantly decreased L* values, while significantly increased b* values compared to control sample (T1). So that, sample T2 significantly recorded the highest b* values followed by sample T3, while sample T1 was the lowest treatment. Thus, sample T5 followed by sample T6 had significantly the lowest L* values while control samples (T2 and T1) significantly gained the highest L* values. On the other hand, b* values significantly increased with the addition of green tea, lemon and/or mint. This may be explained that green tea, lemon and mint extracts are rich in pigments (β -carotene and chlorophyll). Similar trends were reported by [13].

Table 2. Colour index in carbonated whey beverage treatments supplemented by fruit juice and herbs extract

Treatments*	Colour index		
	L	a	b
T1	40.91±0.12 ^d	4.77±0.02 ^c	5.51±0.01 ^e
T2	44.83±0.08 ^a	12.29±0.01 ^a	20.97±0.08 ^a
T3	41.33±0.11 ^d	8.54±0.02 ^b	14.11±0.03 ^c
T4	42.86±0.14 ^b	5.50±0.01 ^c	6.40±0.03 ^f
T5	35.07±0.09 ^e	2.97±0.01 ^d	12.20±0.04 ^d
T6	39.09±0.12 ^f	5.08±0.02 ^c	12.34±0.03 ^d
T7	40.02±0.16 ^c	1.22±0.00 ^e	9.41±0.01 ^e
T8	42.04±0.17 ^c	8.37±0.02 ^b	14.59±0.02 ^b
LSD	0.326	0.211	0.181

*See footnote Table 1.

a-g: Values with the same superscript letter are not a significant difference ($P > 0.05$) in the same column.

Table 3. Energy values of carbonated whey beverages supplemented by fruit juice and herbs extract

Treatments*	Protein (g)	Fat (g)	CHO** (g)	Energy value (Kcal)
T1	1.30	0.30	13.45	59.98
T2	1.27	0.30	13.80	61.21
T3	1.26	0.30	13.00	58.08
T4	1.27	0.25	12.79	56.85
T5	1.29	0.30	12.86	57.65
T6	1.26	0.25	13.15	58.21
T7	1.26	0.25	13.07	57.90
T8	1.28	0.30	13.14	58.70

*See footnote Table 1. ** Total Carbohydrates

3.4. Energy value (EV) of carbonated whey beverages

Energy value of carbonated whey beverages has presented in Table 3. It is excellent that both treatments have very high values of EV (biological value) with high sensory properties. To develop the technology of such dairy beverages, it is important to understand some technological factors that affect the liquid of basic methodological processes. EVs of carbonated whey beverages were calculated from the percentage of fat,

protein and total carbohydrate contents. Results revealed that EVs of carbonated whey beverages ranged from 56.85 to 61.21 kcal 100g⁻¹ sample. Also, it can be seen from the results that T2 recorded the highest values of EV followed by T1 and T8 compared to the other treatments.

3.5. Microbiological characteristics of carbonated whey beverages

Table 4 shows the variations of total viable bacteria (TV), psychrophilic bacteria (PS), aerobic spore-forming bacteria (ASF), yeasts & moulds (YM) and coliform bacteria (CF) counts in carbonated whey beverages. The results indicated that the TV counts ranged from 0.95 to 1.04 log₁₀ CFU ml⁻¹ in all treatments; and PS counts varied from 0.10 to 0.48 log₁₀ CFU ml⁻¹. The ASF counts were ranged from 0.10 to 0.15 log₁₀ CFU ml⁻¹ in all samples. On the other hand, the obtained data cleared that yeasts & moulds and coliform bacteria counts were not detected in all carbonated whey beverage treatments. This might be attributed to high hygienic practices through manufacture, besides the perfect heat treatment of whey. Also, may be due to the role of added CO₂. Rizk [23] reported that the total viable counts ranged from 1.38 to 1.55 log₁₀ CFU ml⁻¹ in beverages, while yeasts and molds number were not detected in all treatments. Silva e Alves et al. [25] found that the total aerobic mesophilic bacteria and total aerobic psychrotrophic bacterial counts were <1 log₁₀ CFU ml⁻¹ in probiotic carbonated whey beverages. These results were in a harmony with [18] and [26].

Table 4. Microbiological quality of carbonated whey beverage treatments supplemented by fruit juice and herbs extract

Treatments*	Log ₁₀ CFU ml ⁻¹				
	TV	PS	ASF	YM	CF
T1	1.00	0.18	0.10	ND	ND
T2	0.95	0.30	0.12	ND	ND
T3	0.93	0.48	0.13	ND	ND
T4	0.95	0.10	0.15	ND	ND
T5	1.00	0.30	0.10	ND	ND
T6	1.04	0.11	0.12	ND	ND
T7	0.98	0.48	0.13	ND	ND
T8	0.95	0.40	0.12	ND	ND

TV= Total viable bacteria PS= Psychrophilic bacteria
ASF= Aerobic spore-forming bacteria YM= Yeasts & molds
CF= Coliform bacteria ND= Not detected

*See footnote Table 1.

3.6. Sensory evaluation of carbonated whey beverages

The sensory properties of carbonated whey beverages with green tea, lemon and/or peppermint are shown in Figure 4. The results showed that the odor values varied from 9.00±0.02 to 9.55±0.03 of all carbonated whey beverages. Maximum ($P < 0.05$) odor value (9.55±0.03) was recorded for sample T6 and minimum ($P < 0.05$) odor value (9.00±0.02) was found for sample T1 and T2. Maximum ($P < 0.05$) taste score (9.60±0.02) was attained for sample T6 treatment and the minimum taste score (9.00±0.03) was reflected in sample T1 and T2. Judges had minimum ($P < 0.05$) preference of appearance value (9.00±0.02) in sample T1, T2, T3, T4, and T5 while

maximum ($P < 0.05$) value was obtained for sample T6. Mouthfeel scores ranged from 9.00 ± 0.03 to 9.50 ± 0.04 of all carbonated whey beverages. The sweetness values of carbonated whey beverages varied from 8.40 ± 0.03 to 9.45 ± 0.04 . It can be observed that all samples significantly enhanced sourness values. The acceptability value in carbonated whey beverages had an increasing trend with a treatment containing green tea, lemon and/or peppermint. Higher acceptability values were obtained for sample T8 and minimum acceptability scores were attained for sample T1 of carbonated whey beverages. Also, the percentage of lemon and sugar in carbonated whey beverages increased the odor, taste, sourness and sweetness scores. From the results, it could be observed that the addition of green tea, lemon and/or mint to whey increased the overall acceptability of beverage. Similar sensory scores of the whey beverage in terms of flavor, consistency, color and appearance and sweetness were noticed by [24] in the preparation of lactose hydrolyzed whey beverages. Rizk [23] found the beverage with lemon in all treatments shown significantly high values for all

sensory evaluated (color, taste, flavor, and acceptability) compared with mint. These results were in harmony with [27].

4. Conclusions

From this study, it could be concluded that carbonated whey beverages supplemented with lemon as well as mint could be recommended as new functional carbonated whey beverages. The total bacterial counts ranged from 0.95 to 1.04 \log_{10} CFU ml⁻¹ in all treatments and there is no detection for either coliform bacteria or yeast & molds. The acceptability value in carbonated whey beverages had an increasing trend with treatments containing green tea, lemon and/or peppermint. Higher acceptability values were obtained for treatment T8 and minimum acceptability score was obtained for treatment T1. It was concluded that it could be successfully incorporated in fruit juice and some herbs extract with carbonation in carbonated whey beverages production.

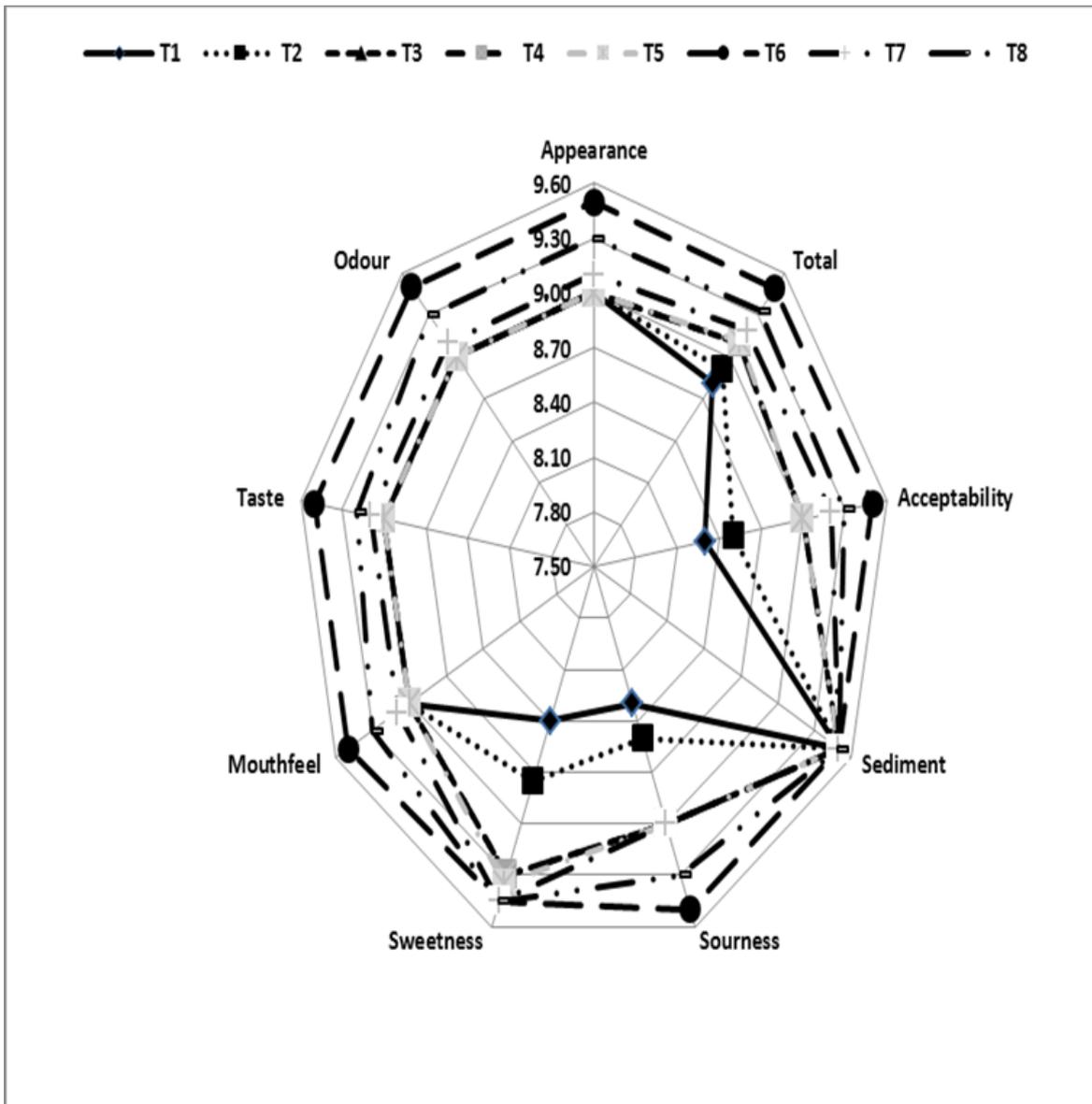


Figure 4. Sensory evaluation of carbonated whey beverage treatments supplemented by fruit juice and herbs extract (*See footnote Table 1.)

Disclosure Statement

The authors declare that there is no conflict of interest.

Compliance with Ethical Requirements

This original research does not contain any article including human members or animals performed by any of the authors.

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