



## Chemical Characterizations of Carbonated Whey Beverages Fortified with Fruit Juice and Some Herbs Extract



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**I**N THIS STUDY, sweet whey was obtained from manufacturing Ras cheese. Lactose was hydrolyzed by lactase ( $\beta$ -D-galactosidase) at pH 6.6. Hydrolyzed whey beverages were produced by mixing sugar (10%), fruit juice, or/and herbs extract to hydrolyzed whey. The whey beverages were carbonated by injecting CO<sub>2</sub>. The carbonated hydrolyzed whey beverages were evaluated for their amino acids, vitamin C, total antioxidant, total phenol compounds, and minerals. Higher hydrolysis in whey sugar was obtained at pH 6.6 with a  $\beta$ -D-galactosidase level of 0.4% after incubation for 300 min at 37°C (P<0.05). The content of leucine was the highest followed by lysine and threonine in all beverage treatments (P<0.05). Results indicated that carbonated whey beverages containing lemon (S4) had the highest ascorbic acid content, total antioxidant activity, and total phenolic compounds content. The level of calcium was the biggest (P<0.05) in carbonated whey beverages. Generally, carbonated whey fortified with lemon juice, green tea extract, and/or peppermint extract had high contents of major bioactive compounds compared with control samples (S1 and S2).

**Keywords:** Lemon juice, Herbs extract, Carbonated whey beverages, Amino acids, Ascorbic acid, Antioxidant activity, Total phenolic, Minerals.

### Introduction

Whey is the liquid that remains after milk has coagulated. Whey is a by-product of cheese or casein manufacturing. It is a greenish-yellow color or typically in blue shade relying upon the quality and kind of milk used (Grba et al., 2002). Whey contains about 50% of the milk solids together with 100% of the milk sugar and 20% of the protein. The milk sugar makes up concerning 75% of the total whey solids (Siso, 1996). Also, lactose intolerance is a clinical syndrome that exhibits characteristic signs and symptoms when eating foods containing lactose (a disaccharide). Usually, after consuming lactose, lactase will be found in the brush margin of the small intestine to hydrolyze it into glucose and galactose. Lactase deficiency caused by primary or secondary cause's clinical symptoms. The severity of the disease varies from person to person. Lactose is found in dairy products, milk products and mammalian milk (Mattar et al., 2012). It is propertied as industrial

effluent, with a maximum biochemical oxygen demand (BOD) due to its high percents of organic substances, making it the foremost polluting by-product of the dairy industry (Banaszewska et al., 2014). However, despite the attainable polluting effect, whey may also have great applicability as an ingredient within the dairy industry due to its nutritional profile.

There are many types of whey-based beverages containing plain, carbonated, and fruit-flavored that have been successfully developed and marketed everywhere. The benefits of the whey proteins will simply be increased by the beverages producers into various products, *i.e.* the pH value ranged from 2 to 10 (Gottschalk, 2005). Today dairy beverages have become one of the widely spread carriers that can deliver bioactive nutrients and phytonutrients, which are beneficial to health. Adding bioactive nutrients and vitamins to dairy and food stuffs are supplied in many countries' to combat deficiency diseases (Wrick, 2003).

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The liquid of green tea is that it's rich in phenols, catechin, especially epigallocatechin gallate (EGCG), which may be a powerful antioxidant; in addition to inhibiting the growth of cancer cells, it can also kill cancer cells. It is conjointly been effective in lowering LDL cholesterol levels, inhibiting the abnormal formation of blood clots, lowering platelet aggregation, lipid regulation, and inhibition of proliferation and migration of smooth muscle tissue. Inhibiting the abnormal formation of blood clots takes on another importance when you consider that thrombosis is that the leading reason behind heart attacks and stroke. Any of these factors may be promising in reducing cardiovascular diseases. The major and most chemo-preventive compound in green tea chargeable for these biochemical or pharmacological effects is (-)-epigallocatechin-3-gallate (Katiyar and Elmets, 2001).

For along time, citrus fruits (lemon or orange) have been regarded as an important part of a healthy and nutritious diet, and it is well known that certain nutrients in citrus can promote health and provide protection for chronic diseases (Adibelli et al., 2009). Citrus fruits usually have the highest antioxidant activity of all fruit types that could protect against cancer, heart diseases, cataracts, degeneration of the region of eyes, and infection. Citrus juices, notably lemon juice, contain maximum contents of flavonoids (800-1500 mg l<sup>-1</sup>), particularly flavone and flavone glycosides (González et al., 2008). Among the flavones, hesperidin, and eriocitrin (flavones) (such as 90% in lemon), (flavonoids) are the most present components. Moreover, further minor flavonoids identified within lemon juice are iso/limocitrol 3- $\beta$ -glucoside and limocitrin 3- $\beta$ -glucoside (Chornomaz et al., 2013).

Peppermint (*Mentha piperita*) is a celebrated aromatic and medicinal herbs that are utilized within traditional and people medicines within the world for the antimicrobial and antioxidant characteristics. Peppermint is one among the foremost wide consumed single material herbal tea; the polyphenolic components of the peppermint leaves contain many flavones. Peppermint leaves show a considerable level of pre-sources of vitamin A. Vitamin A promotes supports the immune system and healthy skin (Frag et al., 2003).

Food produced from plants (fruits and vegetables) abounds with natural biologically

active components, such as phenols, vitamin C or carotenoids, anthocyanins, flavonoids, have their antioxidant activities, are of high value to human health. It is thought that an adequate amount of antioxidant activities supplied with one's diet induces immunological processes and increases the defensive abilities of cells in a proper way (Kalt, 2005).

The biochemical compounds such as anthocyanins, carotenoids, flavones, polyphenols, and vitamin C... etc., have potential health roles in the decreasing cardiovascular, platelet aggregation, blood pressure of disease, and a role in modulation of cholesterol synthesis and absorption (Li, 2008). Moreover, fruits and vegetables contain protein-bound polysaccharides which may increase the levels of serum insulin, lower the blood glucose concentrations and improve tolerance of glucose and hence could be developed as new anti-diabetic agents (Li et al., 2005). This work aims to study the physicochemical composition of carbonated whey beverages fortified with fruit and some herbs and their nutritional value by determining amino acid profiles, minerals, vitamin C, total antioxidant, and total phenolic compounds.

## **Materials and Methods**

### *Materials*

Whey (Ras cheese) was obtained by the Faculty of Agriculture, Benha Univ., Egypt. The enzyme  $\beta$ -D-galactosidase (Maxilact® LX 5000) was obtained from DSM Food Specialties BV, Netherlands, and production site: DSM Food Specialties, USA, INC. Fine sugar was obtained from the local market, Cairo, Egypt. Lemons and peppermint (*Mentha piperita*) were obtained from the local market, Cairo, Egypt. Green tea was obtained from the ISIS Company for Food Processing, El-Horrey, Heliopolis, Cairo, Egypt. Carbonated water was purchased from the Ice soda (Isoda), Egypt (Facebook/Isoda.Egypt, T: 01001355244). Potassium hydroxide (KOH) as food grade was obtained from MIFAD Company in Badr Industrial City, Cairo, Egypt.

### *Methods*

#### *Preparation of fruit juice and herbal extracts*

Lemon and peppermint were washed thoroughly, lemon was cut into "two pieces" and then lemon was squeezed and filtered to separate the seeds and sediment. Mint was prepared from fresh leaves. The leaves were blended in a mixer grinder and filtrated by muslin cloth. Green tea (15 g) was added into a beaker containing 40 ml heated water (90°C) and covered by the aluminum foil for 10 min. The green tea water extract was filtered then cooled and stored in a refrigerator at 5°C (Atallah and Gemiel, 2020).

### Preparation of whey beverages

Whey beverages were prepared as illustrated in Fig 1. The carbonated beverages were analyzed for physico-chemically and their nutritional value by determining amino acid profiles, minerals, vitamin C, total antioxidant, and total phenolic compounds.

### Physico-chemical analysis

Total solids, fat, ash, and total nitrogen levels were measured according to the methods illustrated by AOAC (2012). The pH and titratable acidity were measured according to the methods described by BSI (2010). The calculation method of total carbohydrates (CHO) is as follows:

$$\text{Carbohydrates\%} = \text{Total solids\%} - (\text{fat} + \text{protein} + \text{ash}) \%$$

Total soluble solids (TSS) were determined by hand Abee-Refractometer and the values were expressed as °Brix at 25°C.

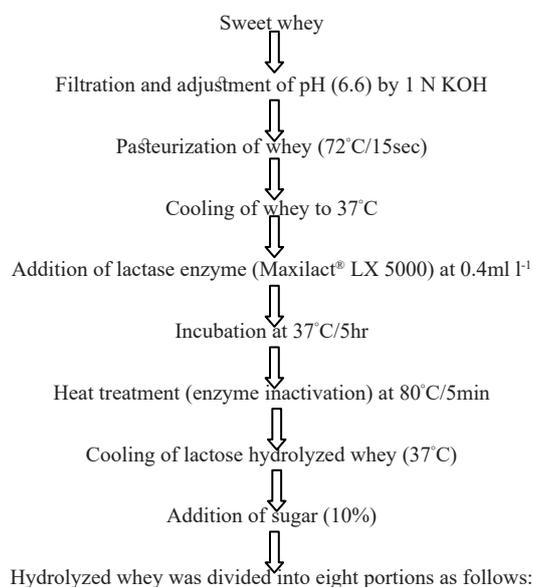
### Sugars analysis by HPLC of whey hydrolysis

Samples were prepared according to the methods illustrated by Karkacier et al. (2003). 20 µl of each prepared sample was injected into the HPLC (Shimadzu Class-VPV 5.03 (Kyoto, Japan) equipped with a refractive index RID-10A Shimadzu detector, LC-16ADVP binary pump and DCou-14A, Guard column Sc-LcShodex, and heater set at 80°C). Prepare stock standard solutions for lactose, glucose, and galactose. Each sugar solution was prepared to be 2 mg ml<sup>-1</sup> with deionized water. Each solution was stoppered and sonicated until completely dissolved. These solutions were stored in glass vials in the refrigerator (Ahmed et al., 2017). The percentage of lactose hydrolysis is calculated as follows:

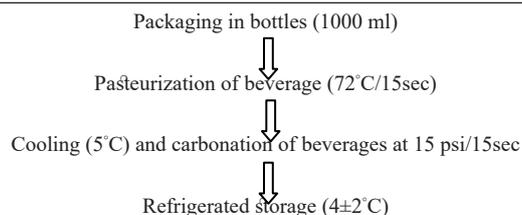
$$\text{The percent lactose hydrolysis} = \frac{(X - Y)}{X} * 100$$

X= lactose content in whey before hydrolysis

Y=lactose content in the whey after hydrolysis



- 1- Hydrolyzed whey as control (S1)
- 2- Carbonated hydrolyzed whey (S2)
- 3- Carbonated hydrolyzed whey with green tea extracts (5%) (S3)
- 4- Carbonated hydrolyzed whey with lemon juice (5%) (S4)
- 5- Carbonated hydrolyzed whey with mint extracts (6%) (S5)
- 6- Carbonated hydrolyzed whey with green tea extracts (2.5%) and lemon juice (2.5%) (S6)
- 7- Carbonated hydrolyzed whey with green tea extracts (2.5%) and mint extracts (3%) (S7)
- 8- Carbonated hydrolyzed whey with lemon juice (2.5%) and mint extracts (3%) (S8)



**Fig.1. Preparation of functional carbonated whey beverages.**

#### *Determination of amino acid profile*

Amino acid profile of carbonated beverages was performed following the protocol of Walsh and Brown (2000).

#### *Determination of total phenolic contents and antioxidant activity*

##### *Preparation of extracts*

Fifteen grams of each sample was mixed with 30 ml methanol: water (60:40 v/v) and set at 4°C overnight. The solution was then passed through Whatman No.1 to collect the filtrate and concentrated using a rotary evaporator at 40°C. The solution was centrifuged and the supernatant was adjusted to 25ml. An aliquot of its extracts was used to quantify total phenolic and antioxidant activity (DPPH radical).

##### *Total phenolic content*

Total phenolic compounds were determined by the Folin–Ciocalteu as described by Shiriet al. (2011). Gallic acid is expressed as a standard and the results are applied as mg gallic acid equivalent (GAE 100 g<sup>-1</sup>).

##### *Total antioxidant activity*

The ability of the extract to scavenge DPPH free radicals was measured by the method described by Prieto et al. (1999). The free radical scavenging activity of each extract is applied as percent DPPH radical scavenging effect using the following equation:

$$\% \text{ Antioxidant activity} = \frac{[(\text{absorbance control}) - (\text{absorbance sample})]}{(\text{absorbance control})} \times 100.$$

##### *Ascorbic acid determination*

The ascorbic acid level was measured by visual titration, using 2, 6-dichlorophenol method, and applied as mg 100 ml<sup>-1</sup> carbonated beverage according to the method described in AOAC (2000).

##### *Mineral contents*

The mineral level was measured by the method described by AOAC (2000). 5 g of the obtained ash of sample was dissolved in 5 ml concentrated HCl (36%), and the volume was completed to 50 ml by deionized water. The dilutions were injected into the atomic absorption spectrophotometer (PERKIN ELMER 3300) to estimate the levels of Ca, Mg, Na, P, Fe, and Zn.

##### *Statistical analysis*

The significant differences between the results were measured by analysis of variance (ANOVA). One-way analysis of variance was calculated on three triplicate parameters. The analysis of variances was calculated at P<0.05.

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Where there was significant, a low significant difference (LSD) test is determined to record the treatments between which there were variances. All statistical analysis for the obtained results was carried out using MSTATC software, version 1.42. The values of different measurements were described as the means with LSD.

## **Results and Discussions**

### *Whey composition*

Results of chemical analysis of whey used in the preparation of carbonated whey beverages showed that total solids, ash, fat, protein, acidity, and total carbohydrate contents were 6.66, 0.59, 0.30, 1.37, 0.24, and 4.16 g 100g<sup>-1</sup>, respectively. These data are very consistent with the findings of Singh et al. (2014). Likewise, Sakhaleet al. (2012) presented the chemical properties of liquid whey were 0.19, 0.45, 5.73, and 6.12% for fat, protein, solids not fat (SNF) and total solid contents, consequently.

### *Sugar contents and lactose hydrolysis*

Sugar contents were determined during incubation times at 37°C and pH 6.6 using 0.4% lactase enzyme (Table 1). Lactose content in whey ranged from 0.83 to 1.89 mg ml<sup>-1</sup> during incubation times. Glucose contents varied from 0.37 to 1.25mg ml<sup>-1</sup> during incubation times in whey. Galactose contents ranged from 1.48 to 0.60 mg ml<sup>-1</sup> in whey during incubation times. The contents of lactose, glucose, and galactose found in the whey analyzed varied widely during incubation times. Higher content (P<0.05) of lactose was obtained during incubation time at zero min, while lower content was found during incubation time at 300 min. Greater contents (P<0.05) of glucose and galactose were found in whey during incubation time at 300 min. Lactose contents decreased in whey with an increase in the incubation times, while glucose and galactose contents increased in whey with an increase in the incubation time. This may be due to lactose hydrolyzing by lactase after incubation. Singh et al. (2014) have recorded similar trends.

Lactose hydrolysis in whey using a 0.4% lactase enzyme was determined during incubation times at 37°C and pH 6.6. Lactose hydrolysis in whey was increased by increasing the incubation time from 60 min to 300 min (Table 1). The percent of hydrolysis of whey varied from 28.57 to 95.24 % after 60 min and 300 min of incubation, respectively. Suresh and Jayaprakasha (2004) found that the highest hydrolysis of lactose in whey was at a temperature of 37°C.

Also, Suresh and Jayaprakasha (2004) observed that the maximum hydrolysis of lactose in whey was at pH 6.5. The optimization of pH was very important in obtaining lactose hydrolysis of whey (Jelen, 1993). Singh et al. (2014) obtained maximum hydrolysis (88.2-89.2%) of lactose with Maxilact L-2000 enzyme concentration of 0.4% while, minimum hydrolysis (59.2-63%) was observed at the enzyme concentration of 0.1% in cheese whey.

#### *Physico-chemical composition*

The effect of the addition of green tea, lemon, and/or peppermint on physicochemical properties in carbonated whey beverages is shown in Table 2. The data show that total solids of carbonated beverages were varied from 15.04 to 15.94%. The ash levels in carbonated whey beverages ranged from 0.42 to 0.54%. In carbonated whey beverages, the acidity ranged from 0.11 to 0.35% as lactic acid. The pH levels of all samples varied from 3.45 to 6.37. Adding lemon caused a decrease in the pH of the treatments containing it, a respective that helps to stabilize the ascorbic acid, as the data revealed. The total carbohydrates contents in carbonated whey beverages varied from 12.71 to 13.64%. Total soluble solids in carbonated whey beverages varied from 19.10 to 19.47. The protein content was ranged from 1.31 to 1.34% in carbonated whey beverages. Fat content in carbonated whey beverages was varied from 0.20 to 0.30%. ANOVA studies of TS, ash, total carbohydrates, and pH values in carbonated hydrolysis whey beverages had shown significances ( $P < 0.05$ ) between all treatments, while TSS value showed no significant

differences between all treatments. These results are in the same line as those found by Rizk (2016). A similar trend of composition properties in the beverage of total solids, total soluble solids, and acidity was reported by Suresh and Jayaprakasha (2004) in the preparation of lactose hydrolysis whey-based beverage. Singh et al. (2014) found that the total solids content in hydrolyzed whey beverages ranged from 10.2 to 17.2%. The acidity levels ranged from 0.31 to 0.60% as citric acid in lactose hydrolyzed whey.

#### *Amino acid profiles of beverages*

The content of amino acid profiles in the analyzed samples varied widely between carbonated whey beverages treatments (Table 3). The data show that all samples contained eight essential amino acids; it's contained nine non-essential amino acids. The data in Table 3 showed that leucine is the major essential amino acid followed by lysine and threonine in all samples. Maximum leucine content (96.82 mg 100 ml<sup>-1</sup>) was obtained for treatment S5 and minimum leucine content (55.81 mg 100ml<sup>-1</sup>) was attained for treatment S1. On the other hand, non-essential amino acids contents show that glutamic acid was the highest followed by aspartic acid. Maximum glutamic acid content (163.94 mg 100ml<sup>-1</sup>) was attained for treatment S5 and minimum glutamic acid content (106.06 mg 100ml<sup>-1</sup>) was obtained for treatment S1. From the statistical analysis, it is cleared that there were significant differences between treatments of whey beverages for essential and non-essential amino acids ( $P < 0.05$ ). The glutamic acid content is showed higher ( $P < 0.05$ ) values followed by leucine and lysine contents in whey beverages.

**TABLE 1. Effect of incubation times on lactose, glucose, and galactose contents in whey.**

Incubation times (min)	mg ml <sup>-1</sup>			%
	Lactose	Glucose	Galactose	Lactose hydrolysis
zero	1.89 <sup>a</sup>	0.37 <sup>f</sup>	0.60 <sup>f</sup>	---
60	1.65 <sup>b</sup>	0.52 <sup>e</sup>	0.75 <sup>e</sup>	28.57 <sup>f</sup>
120	1.59 <sup>c</sup>	0.65 <sup>d</sup>	0.88 <sup>d</sup>	46.03 <sup>e</sup>
180	1.49 <sup>d</sup>	0.77 <sup>c</sup>	1.00 <sup>c</sup>	52.91 <sup>d</sup>
240	1.24 <sup>f</sup>	1.01 <sup>b</sup>	1.24 <sup>b</sup>	78.84 <sup>c</sup>
300	0.83 <sup>g</sup>	1.25 <sup>a</sup>	1.48 <sup>a</sup>	95.24 <sup>a</sup>
360	1.31 <sup>e</sup>	1.05 <sup>b</sup>	1.25 <sup>b</sup>	89.42 <sup>b</sup>
Mean	1.43	0.80	1.03	65.17

<sup>a-f</sup> There is a significant difference between any two means, within the same column have the same superscript letter (Duncan's test  $P < 0.05$ )

**TABLE 2. Physico-chemical composition of carbonated whey beverages.**

Samples	TS *	Ash %	Acidity %	CHO**	Protein %	Fat %	pH value	TSS***
S1	15.80 <sup>ab</sup>	0.54 <sup>a</sup>	0.11 <sup>c</sup>	13.48 <sup>b</sup>	1.33 <sup>a</sup>	0.20 <sup>a</sup>	6.37 <sup>a</sup>	19.27 <sup>a</sup>
S2	15.94 <sup>a</sup>	0.50 <sup>ab</sup>	0.15 <sup>bc</sup>	13.64 <sup>a</sup>	1.34 <sup>a</sup>	0.20 <sup>a</sup>	5.69 <sup>b</sup>	19.47 <sup>a</sup>
S3	15.45 <sup>bc</sup>	0.48 <sup>b</sup>	0.17 <sup>c</sup>	13.05 <sup>c</sup>	1.32 <sup>a</sup>	0.30 <sup>a</sup>	5.64 <sup>b</sup>	19.37 <sup>a</sup>
S4	15.40 <sup>cd</sup>	0.48 <sup>b</sup>	0.35 <sup>a</sup>	12.71 <sup>f</sup>	1.32 <sup>a</sup>	0.30 <sup>a</sup>	3.45 <sup>d</sup>	19.10 <sup>a</sup>
S5	15.04 <sup>d</sup>	0.49 <sup>ab</sup>	0.15 <sup>bc</sup>	12.79 <sup>e</sup>	1.32 <sup>a</sup>	0.20 <sup>a</sup>	5.87 <sup>b</sup>	19.03 <sup>a</sup>
S6	15.18 <sup>cd</sup>	0.47 <sup>b</sup>	0.31 <sup>a</sup>	12.89 <sup>d</sup>	1.32 <sup>a</sup>	0.20 <sup>a</sup>	4.12 <sup>c</sup>	19.07 <sup>a</sup>
S7	15.16 <sup>cd</sup>	0.48 <sup>b</sup>	0.14 <sup>bc</sup>	13.04 <sup>e</sup>	1.31 <sup>a</sup>	0.20 <sup>a</sup>	5.78 <sup>b</sup>	19.10 <sup>a</sup>
S8	15.30 <sup>cd</sup>	0.48 <sup>b</sup>	0.33 <sup>a</sup>	12.86 <sup>d</sup>	1.32 <sup>a</sup>	0.20 <sup>a</sup>	3.89 <sup>c</sup>	19.10 <sup>a</sup>
Mean	15.41	0.42	12.69	0.21	1.32	0.22	5.10	19.19

TS\* = Total solid % CHO\*\*= Total carbohydrates %

TSS\*\*=Total soluble solids

S1= Hydrolyzed whey as control

S2= Carbonated hydrolyzed whey

S3= Carbonated hydrolyzed whey with green tea extracts (5%)

S4= Carbonated hydrolyzed whey with lemon juice (5%)

S5= Carbonated hydrolyzed whey with mint extracts (6%)

S6= Carbonated hydrolyzed whey with green tea extracts (2.5%) and lemon juice (2.5%)

S7= Carbonated hydrolyzed whey with green tea extracts (2.5%) and mint extracts (3%)

S8= Carbonated hydrolyzed whey with lemon juice (2.5%) and mint extracts (3%)

<sup>a-f</sup> There is a significant difference between any two means, within the same column have the same superscript letter (Duncan's test  $P < 0.05$ )

Hulmi et al. (2010) documented essential amino acids contents in whey protein isolate for isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptophan, and valine as 61, 122, 102, 33, 30, 68, 18 and 59 mg g<sup>-1</sup>, respectively. These results are in the same line as those found by Etzel (2007), who showed the highest content of leucine as 118 mg g<sup>-1</sup> whey protein isolate followed by lysine with a mean content of 95 mg g<sup>-1</sup>. Yasmin et al. (2013) recorded the same trend.

Amongst branched-chain amino acids, leucine plays an important role in dietary protein metabolism. The breakdown of leucine takes place in the liver and skeletal muscles (Layman, 2003). It undergoes transmutation in the muscles by transferring into glutamine or alanine that ultimately converts to glucose in the liver through gluconeogenesis; a unique pathway for the maintenance of blood glucose level (Borsheim et al., 2003). Therefore, dietary proteins rich in essential amino acids (especially leucine) have health benefits, while diets or formulations containing proteins from other sources usually do not have this effect (Wolfe, 2002).

#### Mineral contents

The changes in mineral contents of carbonated

whey beverages are cleared in Table 4. The Ca content was significantly different ( $P < 0.05$ ) between all carbonated whey beverages, as it ranged from 699.65 to 747.24 ppm. The P contents ( $P < 0.05$ ) of all carbonated whey beverages were varied between 511.00 to 564.00 ppm. The trace elements Mg, Fe, and Zn contents differed significantly ( $P < 0.05$ ) in the prepared carbonated whey beverages as they varied from 51.14 to 60.15, 16.96 to 21.34, and 6.23 to 12.18 ppm, respectively. The minerals contents are cleared that there were significant variances ( $P < 0.05$ ) between all treatments of carbonated whey beverages. The calcium element was the highest ( $P < 0.05$ ) followed by phosphorus and sodium elements in carbonated whey beverages. Rizk (2016) showed that negligible changes in Mg, Na, and K for a lemon beverage while Guava beverage showed an increase in Ca content after mixing permeate with Guava fruit. The factors that contribute to higher availability of Ca from milk include lactose, P, and vitamins (El-sayed et al., 2007). The compounds of whey minerals expounded that low sodium to potassium ratio is essential for blood pressure maintenance (Smithers, 2015). Wit (2001) cleared that the level of sodium, potassium, calcium; magnesium, and chloride in whey were 50, 150, 60, 10, and 110 mg 100g<sup>-1</sup>, respectively.

TABLE 3. Amino acid profiles of carbonated whey beverages.

Amino acids	Carbonated hydrolyzed whey beverages*							
	S1	S2	S3	S4	S5	S6	S7	S8
Essential amino acids (mg 100ml <sup>-1</sup> )								
<b>Histidine</b>	10.63 <sup>c</sup>	11.81 <sup>bc</sup>	10.47 <sup>c</sup>	13.84 <sup>ab</sup>	15.63 <sup>a</sup>	11.26 <sup>bc</sup>	13.95 <sup>ab</sup>	14.74 <sup>a</sup>
<b>Isoleucine</b>	37.34 <sup>h</sup>	39.08 <sup>d</sup>	37.41 <sup>g</sup>	38.83 <sup>e</sup>	54.37 <sup>a</sup>	38.12 <sup>f</sup>	45.89 <sup>c</sup>	46.60 <sup>b</sup>
<b>Leucine</b>	55.81 <sup>h</sup>	66.36 <sup>c</sup>	58.76 <sup>g</sup>	67.08 <sup>d</sup>	96.82 <sup>a</sup>	62.92 <sup>f</sup>	77.79 <sup>c</sup>	81.95 <sup>b</sup>
<b>Lysine</b>	53.56 <sup>h</sup>	63.47 <sup>d</sup>	56.99 <sup>g</sup>	62.07 <sup>d</sup>	86.56 <sup>a</sup>	59.53 <sup>f</sup>	71.78 <sup>c</sup>	74.32 <sup>b</sup>
<b>Methionine</b>	11.39 <sup>f</sup>	12.01 <sup>e</sup>	6.51 <sup>g</sup>	32.11 <sup>a</sup>	16.30 <sup>d</sup>	19.31 <sup>c</sup>	11.41 <sup>f</sup>	24.21 <sup>b</sup>
<b>Phenylalanine</b>	16.38 <sup>g</sup>	20.00 <sup>c</sup>	14.29 <sup>h</sup>	21.17 <sup>d</sup>	31.42 <sup>a</sup>	17.73 <sup>f</sup>	22.86 <sup>c</sup>	26.30 <sup>b</sup>
<b>Threonine</b>	39.77 <sup>h</sup>	46.96 <sup>f</sup>	39.91 <sup>g</sup>	58.92 <sup>a</sup>	56.06 <sup>c</sup>	49.42 <sup>d</sup>	47.99 <sup>c</sup>	57.49 <sup>b</sup>
<b>Valine</b>	38.83 <sup>h</sup>	46.32 <sup>d</sup>	40.05 <sup>c</sup>	39.50 <sup>g</sup>	56.45 <sup>a</sup>	39.78 <sup>f</sup>	48.25 <sup>b</sup>	47.98 <sup>c</sup>
Non-essential aminoacids (mg 100ml <sup>-1</sup> )								
<b>Alanine</b>	35.53 <sup>c</sup>	54.39 <sup>a</sup>	34.45 <sup>h</sup>	34.78 <sup>f</sup>	49.88 <sup>b</sup>	34.62 <sup>g</sup>	42.17 <sup>d</sup>	42.33 <sup>c</sup>
<b>Arginine</b>	13.52 <sup>d</sup>	13.13 <sup>e</sup>	12.35 <sup>f</sup>	10.49 <sup>h</sup>	18.75 <sup>a</sup>	11.42 <sup>g</sup>	15.55 <sup>b</sup>	14.62 <sup>c</sup>
<b>Aspartic acid</b>	81.07 <sup>c</sup>	83.85 <sup>d</sup>	71.77 <sup>f</sup>	66.19 <sup>h</sup>	109.53 <sup>a</sup>	68.98 <sup>g</sup>	90.65 <sup>b</sup>	87.86 <sup>c</sup>
<b>Cystein</b>	17.56 <sup>g</sup>	21.34 <sup>b</sup>	18.21 <sup>e</sup>	17.37 <sup>h</sup>	22.48 <sup>a</sup>	17.79 <sup>f</sup>	20.35 <sup>c</sup>	19.93 <sup>d</sup>
<b>Glutamic acid</b>	106.06 <sup>h</sup>	125.08 <sup>c</sup>	106.71 <sup>g</sup>	125.93 <sup>d</sup>	163.94 <sup>a</sup>	116.32 <sup>f</sup>	135.33 <sup>c</sup>	144.94 <sup>d</sup>
<b>Glycine</b>	11.33 <sup>f</sup>	14.89 <sup>b</sup>	10.07 <sup>h</sup>	11.66 <sup>e</sup>	17.43 <sup>a</sup>	10.87 <sup>g</sup>	13.75 <sup>d</sup>	14.55 <sup>c</sup>
<b>Proline</b>	34.33 <sup>g</sup>	38.99 <sup>d</sup>	36.22 <sup>c</sup>	34.10 <sup>h</sup>	50.13 <sup>a</sup>	35.16 <sup>f</sup>	43.18 <sup>b</sup>	42.12 <sup>c</sup>
<b>Serine</b>	22.59 <sup>g</sup>	27.14 <sup>f</sup>	22.50 <sup>h</sup>	39.69 <sup>a</sup>	38.31 <sup>c</sup>	31.10 <sup>d</sup>	30.41 <sup>c</sup>	39.00 <sup>b</sup>
<b>Tyrosine</b>	19.38 <sup>d</sup>	19.04 <sup>c</sup>	5.84 <sup>h</sup>	19.62 <sup>e</sup>	25.13 <sup>a</sup>	12.73 <sup>g</sup>	15.49 <sup>f</sup>	22.38 <sup>b</sup>

\*See footnote Table 2.

<sup>a-h</sup> There is a significant difference between any two means, within the same row have the same superscript letter (Duncan's test  $P < 0.05$ )

From the data of mineral, it could be observed that the produced carbonated whey beverages can be considered a good source for some of these minerals. Micro-elements are fundamental for development and advancement, wound mending, immunity, and other physiological procedures (Miller, 2000). These data are in the same line with those reported by Miller (2000) and Atallah (2015).

*Ascorbic acid, total phenolic (TPC), and total antioxidant activity (TA)*

*Ascorbic acid (Vitamin C)*

Carbonated whey beverages containing fruit and some herbs can be considered a source of ascorbic acid which is an important vitamin in the human diet (Fig. 2). The ascorbic acid values in carbonated whey beverages containing lemon (21.00 mg 100 g<sup>-1</sup>) are higher than that of containing green tea (11.00 mg 100 g<sup>-1</sup>) and mint (10.50 mg 100 g<sup>-1</sup>). Also, vitamin C level

was a significant difference ( $P < 0.05$ ) between all samples. Carbonated whey beverages containing lemon were high in ascorbic acid content. Rizk (2016) found that the ascorbic acid contents were 40, 54, and 33 mg 100 g<sup>-1</sup> respectively, in lemon permeate, guava permeates and peppermint permeates. Zulueta et al. (2007) tested the commercial beverages and found that the ascorbic acid level varied from 9.32 to 53.9 mg 100ml<sup>-1</sup>. Pareek et al. (2014) showed that the nutrient analysis for samples and most acceptable from the fruit-based carbonated whey drink presented a general increase in nutrients. Vitamin C was ranged from 45.0 to 45.1 mg 100g<sup>-1</sup> of fruit-based carbonated whey drinks. These data are in the same line with those reported by Dilipkumar and Yashi (2014). Fruit and vegetable have always been considered an essential part of a healthy diet, concerning its  $\beta$ -carotene and vitamin C (Rimm et al., 1996 and Li, 2008).

**TABLE 4. Mineral contents (ppm) of carbonated whey beverages**

Treatments*	Ca	Na	P	Mg	Fe	Zn
S1	733.06 <sup>b</sup>	510.91 <sup>b</sup>	535.50 <sup>bc</sup>	54.87 <sup>f</sup>	21.34 <sup>a</sup>	12.18 <sup>a</sup>
S2	747.24 <sup>a</sup>	523.48 <sup>a</sup>	522.00 <sup>cd</sup>	51.14 <sup>h</sup>	19.88 <sup>b</sup>	10.80 <sup>b</sup>
S3	734.74 <sup>b</sup>	500.28 <sup>cd</sup>	538.00 <sup>bc</sup>	53.09 <sup>g</sup>	18.26 <sup>c</sup>	7.88 <sup>c</sup>
S4	733.63 <sup>b</sup>	514.55 <sup>b</sup>	564.00 <sup>a</sup>	57.52 <sup>c</sup>	18.92 <sup>c</sup>	6.23 <sup>h</sup>
S5	699.65 <sup>d</sup>	489.66 <sup>c</sup>	511.00 <sup>d</sup>	60.15 <sup>a</sup>	16.96 <sup>b</sup>	6.86 <sup>f</sup>
S6	734.19 <sup>b</sup>	507.42 <sup>bc</sup>	551.00 <sup>ab</sup>	55.30 <sup>c</sup>	18.59 <sup>d</sup>	7.05 <sup>c</sup>
S7	717.20 <sup>c</sup>	494.97 <sup>de</sup>	524.50 <sup>cd</sup>	56.62 <sup>d</sup>	17.61 <sup>g</sup>	7.37 <sup>d</sup>
S8	716.64 <sup>c</sup>	502.11 <sup>cd</sup>	537.50 <sup>bc</sup>	58.83 <sup>b</sup>	17.94 <sup>f</sup>	6.54 <sup>g</sup>
Means	727.04	505.42	535.44	55.94	18.686	8.11

\*See footnote Table 2.

<sup>a-h</sup> There is a significant difference between any two means, within the same column have the same superscript letter (Duncan's test  $P < 0.05$ )

#### Total phenolic (TP) content

As shown from data (Fig. 3), the TP of carbonated whey beverages were between 12.17 to 17.96 mg GAE100g<sup>-1</sup>. The results demonstrated that S4 had the highest content of TP followed by S8 and S5, as they contain lemon, lemon & mint, and mint which have higher contents of TP. Levels of TP were a significant difference ( $P < 0.05$ ) in different samples. Statistically, carbonated whey beverages with added lemon or lemon & mint or mint had significantly higher concentrations of TP ( $P < 0.05$ ) while, S1, and S2 treatments presented lower concentrations. Zulueta et al. (2007) observed that the level of total phenolic in the treatments varied widely between samples. The total phenolic content varied from 26.5 to 99.8 mg GAE100ml<sup>-1</sup>. Also, beverages contained apple or mango respectively showed lower contents of phenolic compounds (50.3 and 48.8 mg GAE100ml<sup>-1</sup>, respectively), while the beverage contained apple had good contents of phenolic compounds.

#### Total antioxidant activity (TA)

The antioxidant activity of carbonated whey beverages with green tea, lemon, and/or peppermint are described in Fig 4. The TA levels of the different beverage treatments ranged from 41.05 to 52.72%. The highest percentage ( $P < 0.05$ ) was that corresponded to treatment S4, which also had high ascorbic acid and a high TPC content. These results are in accordance with those given by Rizk (2016). Also, they link up with the data observed by Zulueta et al. (2007) who recorded that the antioxidant activity of the beverage samples ranged from 3.60 to 0.61 mmolTrolox/l.

With regard to the compound of the treatments of The TA of carbonated whey beverages are significantly varied ( $P < 0.05$ ). Antioxidant activities were high in the treatments containing lemon followed by those containing peppermint, while the antioxidant activities were low in S1 and S2 treatments. These results are in agreement with those reported by Arnao et al. (1998) when they compared the antioxidant contents of lemon, orange and grapefruit juices, as they recorded that the antioxidant activity of the lemon sample was about 33% bigger than that of orange or grapefruit samples.

#### Conclusion

The results obtained showed that maximum hydrolysis was after 300 min with an enzyme level of 0.4% at pH 6.6 and 37°C. The vitamin C, antioxidant activity, and total phenolic levels were the biggest in carbonated whey beverages containing lemon (S4) followed by treatment S5 containing peppermint. It was concluded that it could be successfully incorporated fruit and herbs with carbonation. Carbonated whey beverages supplemented with lemon juice as well as peppermint extract could be recommended as new functional carbonated whey beverages.

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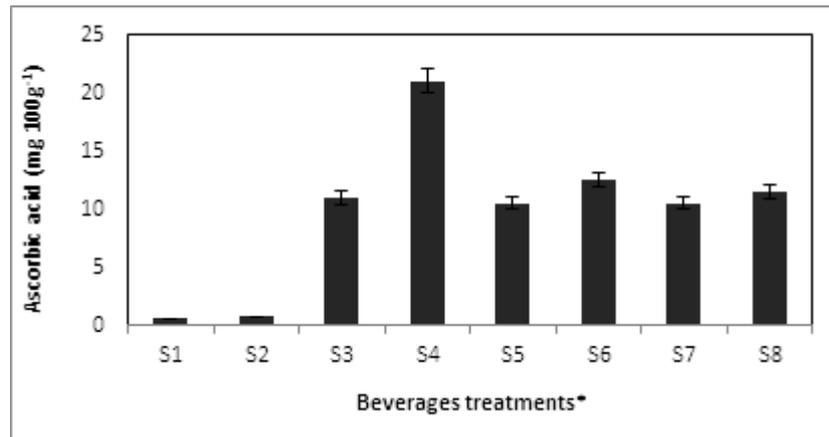


Fig. 2. The ascorbic acid content of carbonated whey beverages.

For treatment identification; See footnote Table 2.

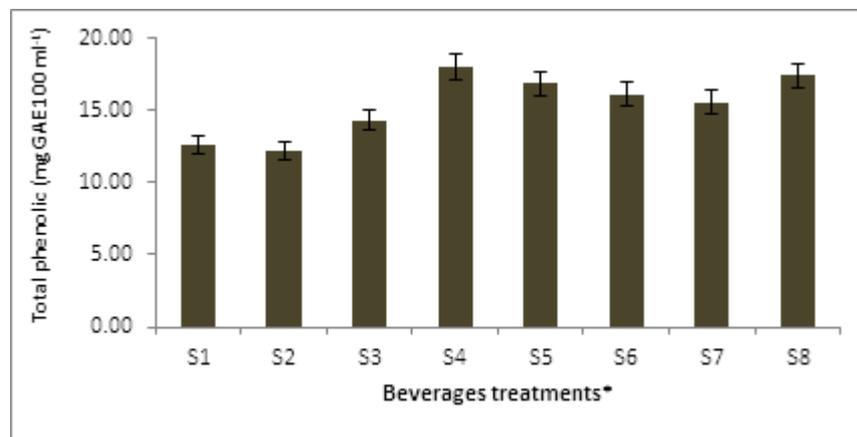


Fig. 3. Total phenolic content of carbonated whey beverages.

For treatment identification; See footnote Table 2.

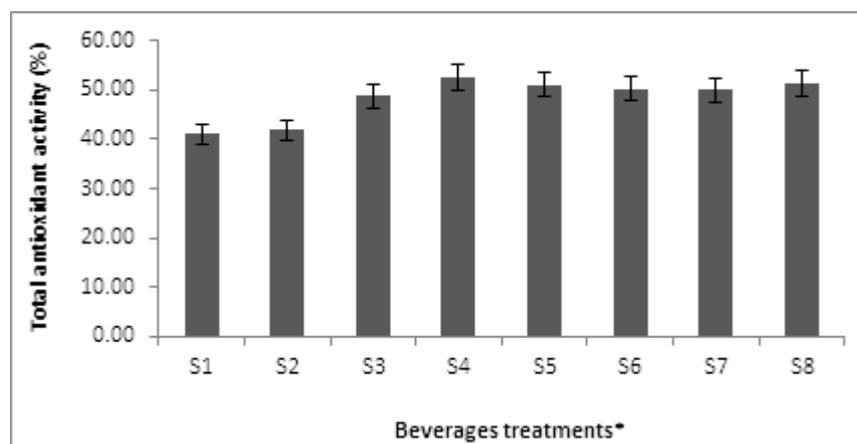


Fig. 4. Total antioxidant activity content of carbonated whey beverages.

For treatment identification; See footnote Table 2.

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## الخصائص الكيميائية لمشروبات الشرش الغازية المدعمة بعصير الفاكهة وبعض مستخلصات الأعشاب

في هذه الدراسة، تم الحصول علي الشرش الحلو من صناعة الجبن الراس. وتم تحليل سكر اللاكتوز الموجود بالشرش باستخدام أنزيم بيتا جالاکتوزيداز ( $\beta$ -D-galactosidase) علي قيمة الـ pH 6.6. وقد تم تحضير مشروبات الشرش المحللة بواسطة خلط الشرش المحل مع السكر (١٠٪) وعصير الفاكهة أو/و مستخلصات الأعشاب. ثم تم تحضير مشروبات الشرش الغازية عن طريق عملية الكربنة بواسطة ضخ وحقن المشروبات بغاز ثاني اكسيد الكربون ( $CO_2$ ). وقد تم تقييم مشروبات الشرش الغازية المنتجة من خلال تقدير الأحماض الأمينية وفيتامين س (vitamin C) ومضادات الأكسدة الكلية والمركبات الفينولية الكلية والمحتوي المعدني. وقد وجد أن أقصى تحلل في سكر اللاكتوز بالشرش كان علي قيمة pH 6.6 ونسبة أنزيم ٤,٠٪ بعد التحضين علي ٣٧م° ولمدة زمنية ٣٠٠ دقيقة. وكان محتوى حمض الليوسين (leucine) هو الحمض الأميني الرئيسي الاساسي متبوع بحمض الليسين (lysine) ثم السريونين (threonine) في كل معاملات مشروبات الشرش الغازية. وقد أوضحت النتائج أن مشروبات الشرش الغازية المحتوية علي عصير الليمون (S4) كانت أعلى محتوى من حمض الاسكوريك و النشاط الكلي لمضادات الأكسدة والمركبات الفينولية الكلية. ووجد أن محتوى الكالسيوم كان الأعلى يليه الفوسفور ثم الصوديوم في مشروبات الشرش الغازية. بصفة عامة وجد أن مشروبات الشرش الغازية المدعمة بعصير الليمون و مستخلص الشاي الأخضر و/ أو مستخلص النعناع كانت أعلى محتوى من المركبات النشطة حيوية الرئيسية مقارنة بعينات الكنترول (S1 and S2).