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Polyphenols, physiochemical and microbiological properties of functional carbonated milk and whey based beverages enriched with mango and *Lactobacillus plantarum*

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Abstract

Probiotic-based beverages with milk or whey-based formulations are referred to functional beverages that contain milk proteins, minerals, vitamins, and probiotics. The present research aimed to produce functional carbonated beverages fortified with mango pulp and probiotics. Investigate the polyphones, chemical, microbiological and sensory attributes during cold storage. For this proposal, six available treatments of milk and whey-based beverages fortified with mango pulp and *Lactobacillus plantarum* NRC AM10 were produced with and without dry ice injection. The results showed that a decrease in pH values was observed in all samples, with or without CO_2 addition. There was no significant difference (p<.0.001) in total solids and fat contents during the storage period. Furthermore, the viscosity of the treated fermented milk was higher than that of the treated fermented whey, either with or without CO2. As fortification with mango pulp, fundamental ratios of phenolic compounds like rutin, ferulic acid, daidzein, querectin, cinnamic acid and kaempferol were found in all treated samples. The viability of *L. plantarum* ranged from 10.25 to 10.62 log CFU/ml and was still above 10^6 log CFU/ml for all beverage samples after 21 days of storage. On the other hand, the counts of psychrotrophic bacteria were significantly lower ($p \leq 0.05$) in CO_2-treated samples compared to samples without CO_2. In addition, coliform bacteria mold and yeasts were not detected because of the good aseptic conditions during manufacture. Finally, the carbonated functional beverages from milk or whey were a good probiotic delivery vehicle for new healthy products.

Keywords: Polyphenols, carbonated beverages, mango pulp, dry ice, phenolic compounds.

1. Introduction

The demand for functional dairy products with health benefits has become the main concern of the factory owners to provide everything new in the different markets [1]. Competition and differences in the production of a novel milk product became the focus of the industry. It is noteworthy to state that dairy products fortified with functional ingredients are important functional products widely consumed due to their numerous health benefits [2]. Whey is the by-product produced during cheese making and contains proteins, lactose, vitamins, and minerals, which cause environmental pollution. Recently, the prominence of the functional components of whey was recognized, which created a wide scope for dealing with it and producing various products. Whey proteins are not coagulated under acidic conditions and are known as "fast proteins" because they reach the intestine quickly after entering the digestive tract. The slower hydrolysis of whey compared to milk casein allows greater

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absorption along the small intestine [3]. Fruits like mango do not contain any allergenic compounds, so they are a good choice for dairy beverages fortified with probiotics. It comprised a lot of nutrients, vitamins, active compounds and dietary fibers that gained anti-cancer, anti-diabetic, anti-hyperlipidemia, and antioxidant characterizations [4]. The increased demand for new dairy products fortified with fruits is the result of increased consumer awareness of the importance of these sources rich in biologically active substances that have an effective role in improving the health of the human body [5]. The functional nutritional drinks division includes different types of energy and sports drinks. These beverages are an excellent source of nutrients and biologically active substances that are palatable, attractive, and profitable. These drinks enhance immunity and have several positive impacts on human health because they contain vitamins, minerals, polyphenols and fiber [6]. The dairy products and beverage markets are witnessing strong competition, with the current traditional products gradually falling. The availability of certain determinants in the marketing product is one of the important factors to be accepted by the consumer, such as sensory quality, an appropriate price, influencing thirst and giving the product a healthy energy character. The use of materials that were previously waste such, as whey, which has effective nutritional properties and a distinctive value, can be included in sports drinks [7]. The supplementation of milk and whey beverages with probiotics is a novel trend to add nutritional value to these drinks and increase the currently available functional dairy beverages in markets worldwide [8]. The Food and Agriculture Organization and the World Health Organization define probiotics as "live microorganisms that, when taken in adequate quantities, confer a health benefit on the consumer." [9, 10]. Innovative functional dairy products with living microorganisms like probiotics have been widely demonstrated in fermented milk products for enhancing organoleptic properties, prolonging shelf life and improving health benefits [11, 12]. Milk drinks containing whey include many health benefits, such as the prevention of cancer and an increase in antioxidant and antimicrobial factors [13].

. One of the most widely used types of probiotics is *L. plantarum,* which produces some antimicrobial substances that have been demonstrated against members of pathogenic bacteria like *Bacillus cereus*, *Escherichia coli*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium, Pseudomonas aeruginosa* and *Shigella flexneri* [10, 14]. In addition, the inhibition activities of *L. plantarum* against most common contaminants, such as molds and yeasts, are also significant [15, 16, 17]. Recently, carbon dioxide (CO_2) has been used as an antimicrobial agent in the beverage industry. There has been an increase in the interest, consumption, and availability of carbonated dairy beverages in recent years. It was found that carbonation leads to the dissolution of carbon dioxide, which also gives the product a shimmering appearance and a refreshing taste. Many explanations have been given for the action of carbon dioxide as an inhibitor of microbial growth, including that the interference of CO_2 in biochemical pathways may lead to a large degree of slowing down the growth of microbes [18]. While the scientist [19] stated that, the displacement of oxygen by carbon dioxide leads to obstruction of the growth of aerobic bacteria in the product. In addition, the presence of carbon dioxide in an aqueous medium leads to the formation of carbonic acid, which liberates H+, which lowers the pH and increases the acidity of drinks, causes stress in the microbial environment and affects the physiological activity of microbes. CO_2 is generally recognized as safe (GRAS) [20], and it can be used to promote the shelf life of dairy products. The use of the carbonation process in milky beverages works to improve thirst quenching and a sense of refreshing quality and causes the opening of new markets for dairy beverages as a healthy alternative to soft drinks to attract a new generation of youth [21]. Carbonated drinkable yogurts may be a good option that would be available on the market for those carbonated beverages [22]. In addition, carbonated whey beverages are products with great consumer acceptability, and whey can be one of the raw materials used in the manufacture of this product. The carbonation process has no negative effects on dairy products [23]. Hence, the purpose of this work was to produce functional carbonated milk and whey-based beverages fortified with mango pulp and *L. plantarum* NRC AM 10. Chemical analysis, apparent viscosity, polyphenol determination, sensory evaluation and microbiological examinations were assessed during the fresh, 7, 15 and 21 days of cold storage.

2. Materials and Methods

2.1. Materials

The dry ice (carbon dioxide) was purchased from DIFFCO$_2$ Company and shipped to the laboratory in aclosed foam box to prevent it from sublimatingduring transport. The microbial media used in this study were all obtained from Oxide and Himedia companies. Fresh mango fruits were purchased from local market, Giza, Egypt. The strain of *L. plantarum* NRC AM 10 was previously isolated, characterized, and identified by [24]. Buffalo milk and sweet whey were obtained from Faculty of Agriculture, Cairo University, Giza, Egypt.

2.2. Methods

2.2.1. Mango juice preparation

Fresh mango fruits were washed with tap water, dried, and peeled with a sharp knife to remove the seeds. The mango pulp was homogenized well with a hand blender, then the mango juice was pasteurized according to the method by [25] cooled down immediately, and stored in a sterile glass beaker at 4º C for preparing milk and whey beverages.

2.2.2. Fermentation of milk and whey

Lactobacillus plantarum NRC AM10 was activated in MRS broth for 48 h at 37º C. A conical flask containing 1.5 liters of reconstituted sterilized milk and another flask containing sterilized sweet whey were inoculated with 2 % of the previous activated culture and incubated at 37^o C for 24 hours. Fermented milk and whey were used in preparing beverages with mango flavor.

2.2.3. Preliminary trail

Three ratios of mango juice were put in fermented beverages to choose the appropriate ratio. The first three treatments used fermented milk as a base fortified with three different ratios of mango juice (10, 20 and 30%) in ascending order. The second group of treatments used fermented whey as a base fortified with the same ratios of mango juice as mentioned before. The third group consisted of fermented milk as a base fortified with three different mango juice ratios (10, 20, and 30%) in ascending order with CO$_2$ gas (1.5%). The fourth group was identical to the third, but milk was replaced with whey. All treatments had stabilizers CMC (0.3%), ascorbic acid (0.3%) and 3% sugar in the manufacturing process. The favorable ratio of mango juice that had been the consensus opinion of the panel experts was 30%. Therefore, this ratio was applied to all treatments in this trial.

2.2.4. Manufacture of functional carbonated milk and whey-based beverages

Six functional beverage treatments were formulated as shown in Table (1) according to [25] with some modifications. Fixed ratios of mango juice (30%), sugar (3%), CMC (0.3%) and ascorbic acid (0.3) were used.

Table 1: Functional milk and whey-based beverage formulations with and without CO$_2$ injection fortified with mango juice and *Lactobacillus plantarum.*

Treatments	Formulations				
T_1	66.4% Fermented milk	30% mango	3% sugar	0.3% CMC	0.3% ascorbic acid
T_2	66.4% Fermented whey	30% mango	3% sugar	0.3% CMC	0.3% ascorbic acid
T_3	33.2% F. milk + 33.2% F. whey	30% mango	3% sugar	0.3% CMC	0.3% ascorbic acid
T_4	66.4% Fermented milk (CO$_2$)	30% mango	3% sugar	0.3% CMC	0.3% ascorbic acid
T_5	66.4% Fermented whey (CO$_2$)	30% mango	3% sugar	0.3% CMC	0.3% ascorbic acid
T_6	33.2% F. milk + 33.2% F. whey (CO$_2$)	30% mango	3% sugar	0.3% CMC	0.3% ascorbic acid

After preparing all beverage treatments under aseptic conditions, the final products were filled into sterilized falcon tubes (50 ml) then stored at 7 °C for 21 days. Beverage samples were taken for analysis when fresh and after 7, 15 and 21 days.

2.3. Chemical analysis

The total solids (TS) and fat contents were measured according to methods described by [26]. The pH value was assessed using a digital pH-meter with combined glass-Calomel electrode (HANNA).

2.3.1. Apparent viscosity

The treated samples were gently stirred 5 times clockwise with a plastic spoon before viscosity measurements. Apparent viscosity was measured at 5±2ºC using a Brookfield digital viscometer (Model DV-II, Canada) fitted with spindle-4. The treated samples were subjected to selected shear rates ranging from 3.0 to 50.0 S1 for the upward curve. Apparent viscosity was expressed as Pascal (Pas).

2.3.2. HPLC analysis for phenolic compounds

Analysis of phenolic compounds were carried out using an Agilent 1260 series. The separation was carried out using an Eclipse C18 column (4.6 mm x 250 mm i.e., 5 μm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rateof 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A); 0–5 min (80% A); 5-8 min (60% A); 8-12 min (60% A); 12-15 min (82% A); 15-16 min (82% A) and 16-20 (82%A). The multi-wavelength detector was monitored at 280 nm. The injection volume was 5 μl for each of the sample solutions. The column temperature was maintained at 40 °C.

2.4. Microbiological analysis

2.4.1. Determination of viable counts of *L. plantarum*

The resultant beverage samples (10 ml) were homogenized in 90 ml of sterile physiological saline (0.85 % NaCl w/v), then the homogenate was serially diluted up to 10^8 according to [28]. One milliliter from each dilution was plated onto duplicate sterile petri dishes; after that, de Mann Rogosa Sharpe (MRS) agar was poured on the plates, which were incubated anaerobically at 37°C for 48 h. The result was expressed as colony forming units per ml (log CFU/ml).

2.4.2. Mould and yeast counts

Mould and yeast count of all samples were determined by using potato dextrose agar (oxiod) according to [29].The plates were incubated at 25 °C for 3 days and the result was expressed as log colony-forming units per ml (log CFU/ml).

2.4.3. Psychrotrophic enumeration

Psychrotrophic bacteria were enumerated according to Standard Methods for the Examination of Dairy Products [30]. The plates were incubated aerobically at 7^o C for 10 days.

2.4.5. Coliforms count

Total coliform counts were enumerated on violet red bile agar medium (VRBA) according to the method described by [31]. The plates were anaerobically incubated at 37^o C for 48 h.

2.5. Sensory evaluation

The organoleptic properties of functional milk and whey-based beverages were assessed by 20 panelists of the experienced staff members of Dairy Department National Research Center. Samples were evaluated periodically by regular-score panelists when fresh and after 7, 15 and 21 days of cold storage for flavor (50 points), body and texture (40 points) and appearance (10 points) as mentioned by [27].

2.6. Statistical analysis

Statistical analysis for obtained data was carried out using analysis of variance (ANOVA) and Duncan tests with the Statistical Analysis System [32]. A probability of P < 0.001 was used to establish the statistical significance.

3. Results and Discussion

3.1. pH Values

Values of pH were estimated in Table 2 for functional fermented beverages during the storage period of 3 weeks. It was observed that as the storage period increased, the pH values decreased for all treated samples. Carbonation did not significantly (p<.0.001) vary the initial pH values compared to treated samples without CO_2. pH values at fresh time were

4.99, 4.79, 5.02, 4.98, 4.65 and 5.0 for T_1, T_2, T_3, T_4, T_5 and T6, respectively. While it was observed that they were 4.51, 4.26, 4.36, 4.57, 4.43 and 4.43 for the same order of treatments at the end of the storage period, the reduction in pH values may be a result of the growth of probiotic bacteria during the trial period. The pH **v**alues were within the range of the fermented milk beverages mentioned by [33, 4]. Whereas [21] revealed that pH values for control and carbonated beverages were 4.51 and 4.47, respectively. After 12 weeks of storage, the pH values reached 3.83 for carbonated fermented drinks. The author declared that the acidification had been controlled by carbon dioxide due to an overlap with internal enzymatic equilibrium. Increasing the solubility of the gas under refrigeration improves this function.

Table 2: pH Values of functional milk and whey-based beverage formulations with and without CO_2 injection fortified with mango juice and *L. plantarum* during storage period.

Treatments	**Storage periods**			
	Fresh	7 days	14 days	21 days
T_1	4.99±0.0088192^{Aa}	4.75±0.0145297^{Cb}	4.52±0.0115470^{Cc}	4.51±0.0088192^{Bc}
T_2	4.79±0.0057735$^{\mathbf{Ba}}$	4.62±0.0145297^{Db}	4.22±0.0115470^{Ec}	4.26±0.0088192^{Ed}
T_3	5.02±0.0057735$^{\mathbf{Aa}}$	4.98±0.0176383^{ABa}	4.42±0.0152753^{Db}	4.36±0.0088192^{Dc}
T_4	4.98±0.0176383^{Ab}	5.02±0.0088192^{Aa}	4.62±0.0115470^{Bc}	4.57±0.0115470^{Ad}
T_5	4.65±0.0145297^{Ca}	4.63±0.0176383$^{\mathbf{Da}}$	4.42±0.0145297$^{\mathbf{Db}}$	4.43±0.0202759$^{\mathbf{Cb}}$
T_6	5.00±0.0145297^{Aa}	4.94±0.0208167$^{\mathbf{Bb}}$	4.72±0.0145297$^{\mathbf{Ac}}$	4.43±0.0145297$^{\mathbf{Cd}}$

The data were expressed as mean of three replicates. Means with the same capital letter between rows (effect of treatments) are not significantly different ($p \leq 0.05$). Means between columns (effect of storage) with the same small letters are not significantly different ($p \leq 0.05$).

3.2. Total solids and Fat contents

The carbonization process did not affect the total solid contents of CO_2 treatments compared to the free ones, as shown in Table 3. The short storage period had no difference in total solid contents. The only significant difference (p<.0.001) observed was between the total solids of fermented milk and the total solids of fermented whey-based beverages. Functionally fermented milk had the highest total solids when fresh and after 21 days of storage. This was expected because the total solids content of milk is higher than the total solids content of whey [34]. It was stated that the total solids of fresh yogurt beverages supplemented with carrot products were 13.41, 15.47 and 15.80%, respectively. The same trend was noticed in the fat contents of all treatments, as mentioned in Table 4. The lowest fat contents were for the fermented whey, followed by the mixture between fermented milk and whey, then the fermented milk, in an ascending order.

Table 3: Total solids contents of functional milk and whey-based beverage formulations with and without CO_2 injection fortified with mango juice and *L. plantarum* during storage period.

Treatments	**Storage periods**			
	Fresh	7 days	14 days	21 days
T_1	17.75±0.0461880^{Aa}	17.74±0.0491031$^{\mathbf{Aa}}$	17.74±0.0230940$^{\mathbf{Aa}}$	17.74±0.0230940$^{\mathbf{Aa}}$
T_2	9.80±0.0519615$^{\mathbf{Ca}}$	9.79±0.0173205^{Ca}	9.82±0.0433333$^{\mathbf{Ca}}$	9.82±0.0433333^{Ca}
T_3	13.59±0.0981495$^{\mathbf{Ba}}$	13.61±0.0288675$^{\mathbf{Ba}}$	13.62±0.0173205$^{\mathbf{Ba}}$	13.61±0.0173205$^{\mathbf{Ba}}$
T_4	17.75±0.0088192^{Aa}	17.78±0.0548736^{Aa}	17.79±0.0692820$^{\mathbf{Aa}}$	17.77±0.0692820^{Aa}
T_5	9.82±0.0519615^{Ca}	9.77±0.0548736^{Ca}	9.81±0.0491031^{Ca}	9.81±0.0491031^{Ca}
T_6	13.62±0.0721880^{Ba}	13.62±0.0202759^{Ba}	13.62±0.0433333^{Ba}	13.62±0.0433333^{Ba}

The data were expressed as mean of three replicates. Means with the same capital letter between rows (effect of treatments) are not significantly different ($p \leq 0.05$). Means between columns (effect of storage) with the same small letters are not significantly different ($p \leq 0.05$).

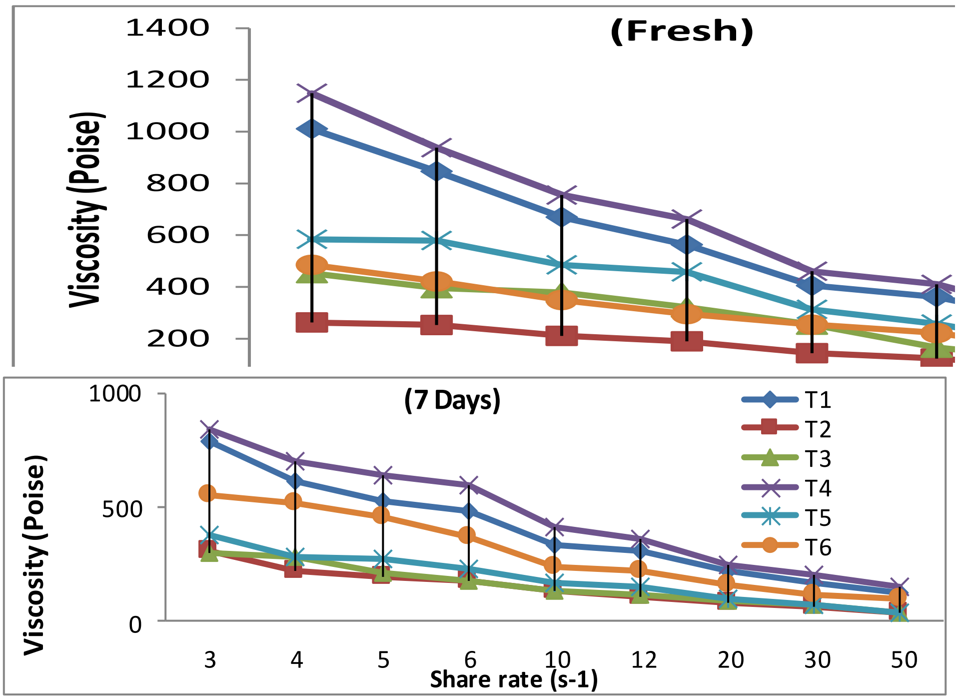
Table 4: Fat contents of functional milk and whey-based beverage formulations with and without CO_2 injection fortified with mango juice and *L. plantarum* during storage period.

Treatments	Storage periods			
	Fresh	7 days	14 days	21 days
T_1	3.65±0.0577350^{Aa}	3.57±0.0288675^{Aa}	3.60±0.0^{Aa}	3.60±0.0288675^{Aa}
T_2	0.10±0.0^{Ca}	0.10±0.0^{Ca}	0.10±0.0^{Ca}	0.10±0.0^{Ca}
T_3	1.97±0.0288675^{Ba}	1.85±0.0288675^{Bb}	1.90±0.0^{Bab}	1.89±.0.0^{Bab}
T_4	3.50±0.0577350^{Aa}	3.56±0.0288675^{Aa}	3.55±0.0288675^{Aa}	3.57±0.0288675^{Aa}
T_5	0.10±0.0^{Ca}	0.10±0.0^{Ca}	0.10±0.0^{Ca}	0.10±0.0^{Ca}
T_6	1.86±0.0288675^{Ba}	1.90±0.0^{Ba}	1.86±0.0288675^{Ba}	1.85±0.0288675^{Ba}

The data were expressed as mean of three replicates. Means with the same capital letter between rows (effect of treatments) are not significantly different ($p \leq 0.05$). Means between columns (effect of storage) with the same small letters are not significantly different ($p \leq 0.05$).

3.3. Apparent viscosity

The viscosity of all treated samples, with or without carbon dioxide gas, is declared in Fig. 1. It was noted that the apparent viscosity had a direct relationship to the total solids of the treated sample. The total solids of fermented milk had higher ratios than the solids of fermented whey. Thus, all whole fermented milk samples or mixes with fermented milk were higher in viscosity than those of the fermented whey-based beverage formulations, either with or without CO_2. As indicated before, pH degrees were not influenced by carbonation injection, so the viscosity did not significantly impact acidity. The viscosity of carbonated-treated samples was higher compared to treated samples free from CO_2 injection. As time passed, the apparent viscosity of all samples was reduced, either with or without CO_2. Anyhow, viscosity is in a straight line with density, and dissolution of CO_2 may cause density variations of 2-3%, as stated by [35]. In addition, [36] demonstrated that the viscosity of carbonated flavoured whey drinks decreased during cold storage for 30 days.



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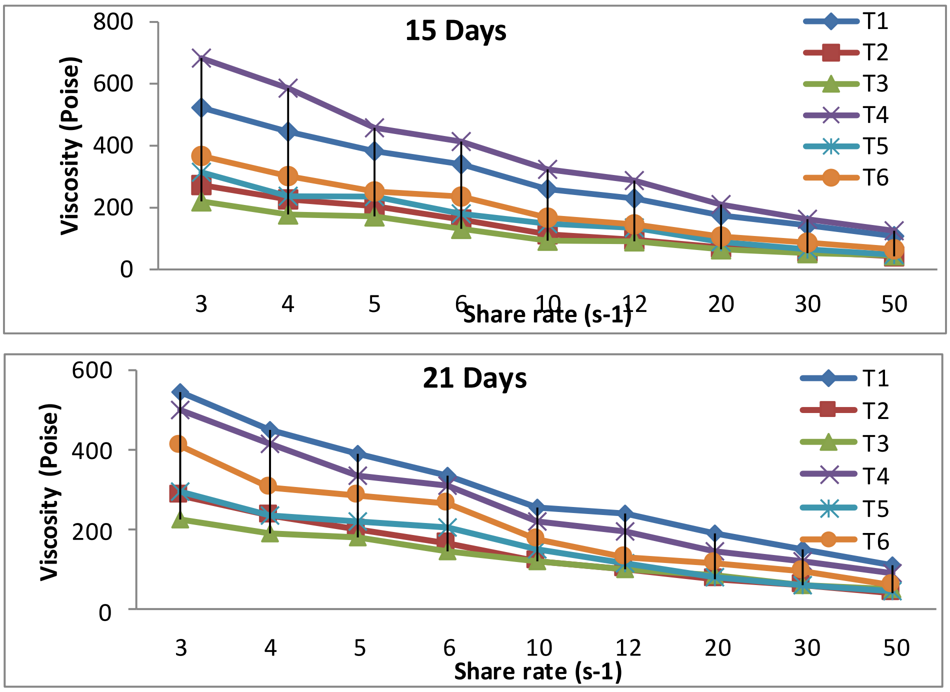

Fig 1: Apparent viscosity of functional milk and whey-based beverage formulations with and without CO2 injection fortified with mango juice and L. plantarum during storage period.

3.4. Polyphenolic compounds

Polyphenol compounds of functional milk and whey-based beverage formulations with and without CO_2 injection fortified with mango juice and *L. plantarum* were analyzed when fresh and at the end of the storage time. The results at fresh have been presented in Figure 2. It was observed that the most abundant phenolic compounds detected in all treatments were gallic, chlorogenic, catechin, daidzein, cinnamic, querectin, apigenin, and kaempferol. Moreover, the ratios of chlorogenic acid were higher in fermented beverages without CO_2 than in carbonated fermented beverages, whether fermented milk or whey. The concentrations were (21.71, 10.54 and 9.10 µg/ml) for T_1, T_2 and T_3, and (13. 20, 3.18 and 6.78 µg/ml) for T_4, T_5 and T_6. Chlorogenic acid is considered a paramount member of the hydroxyl Cinnamic acids and aromatic in nature. It is responsible for the enzymatic browning of fruits like apples and pears; it is formed with a combination of caffeic and quinic acids [37]. Whereas, catechin had high ratios for the first three treatments at fresh time (11.45, 10.06 and 4.98 µg/ml), the last three treatments had lower ratios (5.38, 5.65 and 4.23 µg/ml). Catechin belongs to the tannin group, which is observed in fruits, grains and legumes. In general, there were considerable amounts of phenolic compounds in all fermented beverages, such as rutin, ferulic acid, daidzein, querectin, cinnamic acid and kaempferol. As mentioned in many studies, these phenolic compounds and flavonoids, such as quercetin, which is found mainly in fruits, followed by kaempferol, had a great scavenger activity against ROS (superoxide) and reactive nitrogen species (nitric oxide and peroxynitrite) [38].

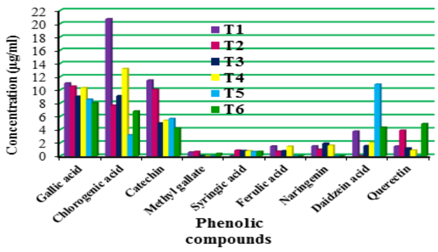

Fig. 2: Phenolic compounds in functional milk and whey-based beverages fortified with mango juice and L. plantarum at fresh time.

After 21 days, the polyphenol compounds were measured and exhibited in Figure 3. The data showed that, some compounds were increased at the end, such as chlorogenic acid, methyl gallate, rutin and querectin. Other phenolic compounds were reduced after storage, like gallic acid, catechin, naringenin, daidzein, syrinigic acid and apigenin. While a few compounds disappeared after 21 days, such as askaempferol, cinnamic acid, coumaric acid, and ferulic acid, it could be because of the storage period, the use of some polyphenols by bacteria, or an increase in the acidity of beverages.

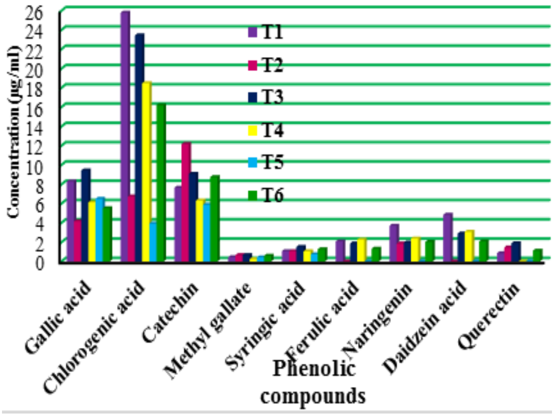

Fig. 3: Phenolic compounds in functional milk and whey-based beverage fortified with mango juice and L. plantarum after 21 days.

3.5. Viability of *L. plantarum* in beverages during storage

Counts (log CFU/ml) of *L. plantarum* in fermented milk and whey beverages are presented in Table 5. The counts of *L. plantarum* were nearly the same in all beverage treatments during storage times. The counts in T_1 and T_2 were significantly ($p \leq 0.05$) increased by one log cycle, whereas they were increased by two log cycles in T_3, T_4, T_5 and T_6. Furthermore, after 21 days of storage, the counts ranged from 10.25 to 10.62 log CFU/ml, so the counts were still above the 10^6 log CFU/ml for all beverage samples that are recommended for the health benefits of probiotics. Therefore, carbonated fermented milk or whey can be developed as novel dairy functional beverages. Our results were in agreement with those reported by [39, 40], who reported that fermented functional dairy food supplemented with probiotics plays an important role in protecting and delivering these strains during intestinal harsh conditions. As reported in a similar study by [12], they concluded that *L. pentosus* B329 and *L. plantarum* 820 still had a high survival rate in sour milk during the storage period.

Table 5: Viability of *L. plantarum* (log CFU/ml) of functional milk and whey-based beverage formulations with and without CO_2 injection fortified with mango juice and *L. plantarum* during storage period.

Treatments	Storage time (days)			
	Fresh	7	15	21
T_1	9.72 ± 0.0057735^{Ad}	10.00 ± 0.0^{Ac}	10.68 ± 0.0173205^{Ba}	10.55 ± 0.0173205^{b}
T_2	9.65 ± 0.0173205^{Bd}	9.85 ± 0.0173205^{Bc}	10.56 ± 0.0260342^{Ca}	10.39 ± 0.0173205^{b}
T_3	8.95 ± 0.0173205^{Cd}	9.88 ± 0.0208167^{Bc}	10.53 ± 0.0173205^{CDa}	10.25 ± 0.0288675^{b}
T_4	8.65 ± 0.0288675^{Dd}	9.75 ± 0.0173205^{Dc}	10.52 ± 0.0115470^{CDa}	10.35 ± 0.0145297^{b}
T_5	8.41 ± 0.0230940^{Ed}	9.79 ± 0.0260342^{DCc}	10.48 ± 0.0115470^{Da}	10.30 ± 0.0230940^{b}
T_6	8.62 ± 0.0115470^{Dd}	9.83 ± 0.0173205^{BCc}	10.75 ± 0.0^{Aa}	10.62 ± 0.0115470^{b}

The data were expressed as mean of three replicates. Means with the same capital letter between rows (effect of treatments) are not significantly different ($p \leq 0.05$). Means between columns (effect of storage) with the same small letters are not significantly different ($p \leq 0.05$).

3.6. Mold and yeast, and psychrotrophic count

Mold and yeasts were not detected in all treatments, whether fresh or during cold storage, because of the high aseptic conditions during the product's manufacture. In addition, [32] mentioned that the addition of CO_2 might be able to inhibit the spoilage of microorganisms in the beverage during storage. Meanwhile, the counts of psychrotrophic bacteria at zero time were recorded at 4, 2.10, 2.61, 2.30, 1.85 and 1.90 log CFU/ml in treatments T_1, T_2, T_3, T_4, T_5 and T_6 respectively Table 6. The counts in all treatments were increased by three log cycles by extending the storage time. It was recognized that the counts of psychrotrophic bacteria were significantly lower ($p \leq 0.05$) in CO_2 treated samples compared to samples without CO_2. These results may be due to the addition of *L. plantarum as a* probiotic biopreservative. Many studies have exhibited the impact of consuming *L. plantarum* containing fermented milk on the functionality of the host.

Table 6: Psychrotrophic counts (log CFU/ml) of functional milk and whey-based beverage formulations with and without CO_2 injection fortified with mango juice and *L. plantarum* during storage period.

Treatments	Storage time (days)			
	Fresh	7	15	21
T_1	4.00±0.1154701^{Ab}	5.10±0.0577350^{Aa}	5.26±0.0230940^{Aa}	5.14±0.0115470^{Aa}
T_2	2.10±0.0666667^{Cd}	4.97±0.0115470^{Ab}	5.11±0.0057735^{Ba}	5.18±0.0115470^{Aa}
T_3	2.61±0.0066667^{Bd}	5.00±0.0^{Ac}	5.17±0.0152753^{ABa}	5.12±0.0115470^{Ab}
T_4	2.30±0.0115470^{Cb}	5.10±0.0^{Aa}	5.20±0.1000000^{ABa}	5.18±0.0173205^{Aa}
T_5	1.85±0.0288675^{Eb}	5.00±0.1154701^{Aa}	5.14±0.0115470$^{B\ a}$	5.10±0.0577350^{Aa}
T_6	1.90±0.0115470^{DEc}	5.03±0.0^{Ab}	5.12±0.0115470^{Ba}	5.11±0.0384419^{Aa}

The data were expressed as mean of three replicates. Means with the same capital letter between rows (effect of treatments) are not significantly different ($p \leq 0.05$). Means between columns (effect of storage) with the same small letters are not significantly different ($p \leq 0.05$).

3.7. Coliform counts

The obtained results confirmed that coliforms were not detected in all fermented beverage samples during the storage period because of high aseptic conditions during the product's manufacture and storage, as found by [41].

3.8. Sensory properties

Sensory evaluations of fermented beverages were displayed in Table 7. It was noticed that samples that contained whey had the highest significant scores in flavor, either with CO_2 or not. Whey gave the beverages a special flavor, and the addition of carbon dioxide gave them a refreshing feeling. In fresh time, the treatments T_2 and T_5 recorded 43 and 47 flavor scores, respectively. The flavor scores significantly ($p \leq 0.05$) decreased after 21 days of storage.

Table 7: Sensory evaluation scores of functional milk and whey-based beverage formulations with and without CO2 injection fortified with mango juice and L. *plantarum*.

Treatments	Body & Texture (40)		Flavor (50)		Appearance (10)	
	Fresh	21 days	Fresh	21 days	Fresh	21 days
T1	38±0.58^{Aa}	36.00±0.58^{Aa}	43.00±0.58^{Ba}	32.00±1.155^{BCc}	9.00±0.0^{Aa}	7.00±0.58^{Ab}
T2	39±0.58^{Aa}	34.00±0.58^{Bc}	47.00±0.58^{Aa}	38.00±1.155^{Ac}	9.00±0.0^{Aa}	7.00±0.0^{Ac}
T3	37±0.58^{Ba}	35.00±0.58^{ABa}	44.33±0.67^{Ba}	35.00±1.155^{ABc}	9.00±0.0^{Aa}	6.00±0.58^{Ac}
T4	37±0.33^{ABa}	30.67±0.33^{Cd}	44.00±0.58^{Ba}	31.00±0.58^{Cd}	9.00±0.0^{Aa}	6.00±0.58^{Ac}
T5	39±0.58^{Aa}	31.00±0.58^{Cd}	47.33±0.33^{Aa}	35.00±1.155^{ABc}	9.00±0.0^{Aa}	6.00±0.58^{Ab}
T6	38±0.58^{ABa}	34.00±0.58^{Bb}	43.66±0.89^{Ba}	33.00±0.58^{BCc}	9.00±0.0^{Aa}	6.00±0.0^{Ab}

The data were expressed as mean of three replicates. Means with the same capital letter between rows (effect of treatments) are not significantly different ($p \leq 0.05$). Means between columns (effect of storage) with the same small letters are not significantly different ($p \leq 0.05$).

The reduction in flavor scores could be due to the increase in acidity, which gives the product its sour taste. Carbonated fermented milk beverages at the end of the trail had the lowest flavor score compared to the rest of the samples. In addition, there were significant differences between all samples in body and texture scores after 21 days of storage; it was observed that fermented milk mixed with whey T_4 gained the lowest body and texture evaluations. Despite the fact that the fermented milk beverage samples gained lower flavor scores, they had high body and texture scores because of the higher total solids compared to fermented whey beverages. On the other hand, there were no significant differences in appearance between all samples when fresh and at the
end of storage.

4. Conclusion

The present study showed that, six treatments of functional milk and whey-based beverages fortified with mango juice and *Lactobacillus plantarum* NRC AM10 were produced successfully with and without CO_2 injection. The acid carbonation process had no significant effect on the chemical composition of all fermented milk and whey-based beverages. Moreover, the differences were significantly noticeable between fermented milk and fermented whey during the storage period. The count of *L. plantarum* in functional carbonated fermented beverages has a high survival rate during the storage period. Coliform, mould and yeast counts were not detected in all treatments. Samples treated with CO_2 had lower counts of psychotropic bacteria compared to samples without CO_2. Finally, it can be concluded that carbonated fermented milk or whey supplemented with *L. plantarum* can be developed as novel functional beverages and possess pronounced healthy alternative and refreshing taste for many people, especially youth.

5. Declaration of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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