Middle East Journal of Agriculture Research
Volume: 11 | Issue: 02| April – June| 2022

EISSN: 2706-7955 ISSN: 2077-4605
DOI: 10.36632/mejar/2022.11.2.36
Journal homepage: www.curresweb.com
Pages: 563-573

Functional Dairy Beverages Production Using Certain Dairy By-Products Enriched With Pumpkin (*Cucurbita Maxima* L.) Pulp

Amal I. El-Dardiry [1], Amro Abdelazez [2,3], Amira S. El-Rhmany [1], Ghada A. Abo ali [1] and Lamiaa A. Kadoum [1]

[1]Department of Dairy Chemistry, Animal Production Research Institute, Agriculture Research Centre, Dokki, Giza 12618, Egypt
[2]Institute of Microbe and Host Health, Faculty of Agriculture and Forestry, Linyi University, Linyi 276005, China
[3]Department of Dairy Microbiology, Animal Production Research Institute, Agriculture Research Centre, Dokki, Giza 12618, Egypt

Received: 20 April 2022 **Accepted:** 05 June 2022 **Published:** 15 June 2022

ABSTRACT
The dairy industry has huge amounts of by-products, and the indiscriminate disposal can lead to significant environmental pollution. However, it contains many valuable nutritional compounds that can be recycled. Pumpkin pulp is a plant with good functional properties due to its high content of antioxidants, vitamins, minerals, and other active ingredients that can boost immunity and enhance human health. The study aims to create new functional beverages prepared from some by-products such as sweet buttermilk, sweet whey, and milk permeate enhanced with pumpkin pulp (*Cucurbita maxima* L.). The natural by-product was prepared by flavoring the different by-products with 50 % pumpkin pulp, 4 % sugar, and 0.2 % carboxymethyl cellulose and stored at 4±1ºC for 30 days. Chemical analysis, sensory evaluation, antioxidant activity, and microbiological assessment were determined. The findings revealed that the by-products supplemented with pumpkin pulp had a significant level of total carbohydrates, fibers, total phenolic components, antioxidant activity, vitamins C, and mineral content. The bacterial total count was reduced. Sensory evaluation indicated that functional beverages containing pumpkin pulp demonstrated superior sensory properties when compared to control treatments. We recommend fortifying the by-products with pumpkin pulp due to its high nutritional value and sensory properties.

Keywords: Functional beverages; Dairy by-products; Pumpkin pulp; Antioxidant activity

1. Introduction
Functional foods have recently gained favor in the health and wellness communities. They are also known as nutraceuticals, which are highly nutritious and linked to a variety of powerful health benefits such as preventing disease, avoiding nutrient deficits, and supporting appropriate growth and development. As a result, functional foods include foods enhanced with vitamins, minerals, probiotics, or fiber. Fruits, vegetables, nuts, seeds, and grains are all examples of nutrient-dense foods (Baker *et al.,* 2022). It can provide organoleptic properties, physicochemical properties, and preservation properties. Fortification of food processing by-products reduces the overall cost of the final product, providing a substantial contribution to the development of functional food (Guldiken *et al.,* 2021).

Dairy by-products are an environmental problem if they are disposed of indiscriminately, as the cheese industry of all kinds represents the most famous industries producing milk by-products (Costa *et al.,* 2021). Sweet whey and acidic whey are two types of whey products that can be obtained. Sweet whey is made from the enzymatic precipitation of casein in the manufacturing of certain cheeses such as cheddar, mozzarella, and Swiss, whereas acidic whey is produced from cottage cheese, cream, and ricotta cheese (Costa *et al.,* 2021).

Corresponding Author: Amal I. El- Dardiry, Department of Dairy Chemistry, Animal Production Research Institute, Agriculture Research Centre, Dokki, Giza 12618, Egypt

Buttermilk is a popular and nutritious drink for people who suffer from lactose intolerant, and it is made from churned unfermented milk cream (Burns *et al.,* 2010). It also contains a lot of proteins, vitamins, milk salts, and other nutrients. Although there is a broad variety of buttermilk on the market, they cannot be termed nutritious beverages owing to the inclusion of sugar, flavors, and preservatives, which may cause more significant health issues. As a result, preparing buttermilk with natural flavor and lactic acid bacteria that add nutrients is highly suggested to decrease the unpleasant flavor and improve customer acceptability (Gebreselassie and Beyene 2016).

Milk permeate is a by-product of the dairy industry with low bacterial contamination. It is obtained during the production of milk protein and contains lactose, minerals, and whey proteins with a pH similar to milk. However, its organoleptic properties are unattractive to customers. Although it cannot be recommended as a stand-alone high-value nutritious supplement. As a result, fermentation with certain lactic acid bacteria strains can improve its properties (Zokaityte *et al.,* 2020).

Pumpkin (*Cucurbita maxima* L.) is a plant simple to grow that is also used as a functional vegetable and has therapeutic and medicinal properties (Dinu *et al.,* 2016). It is rich in carotenoids, a precursor of vitamin A, a powerful antioxidant such as polyphenols and carotenoids, and a natural colorant found mostly in pulp and peels. As well as, other bioactive components such as minerals, and vitamin C are particularly important for human health (Kulczyński and Gramza-Michałowska 2020). Additionally, Pumpkin is consumed fresh as well as in the form of preserves such as soups, smoothies, and juices. Furthermore, Pumpkin flesh is used for bread, cakes, cookies, chocolates, and sweets. Also, Flowers, leaves, fruits, peel, and seeds are all edible components of the plant (Kim *et al.,* 2012). Based on dry weight, pumpkin pulp contains 3 g/100 g protein, 2.3 g/100 g fat, 171.9 mg/g carotenoid, and 66 g/100 g carbohydrate. Moreover, it included 9-10 mg/100 g of vitamin E and 2-10 mg/100 g of vitamin C (Kulczyński and Gramza-Michałowska 2020). The study proposes to investigate various milk by-products combined with pumpkin pulp to produce low-cost functional beverages that improve human health.

2. Material and Methods

2.1. Materials

Sweet whey protein was extracted from mozzarella cheese and milk permeate was previously produced from buffalo milk using the ultrafiltration technique of the Carbo-sep ultrafiltration equipment (SFEC, France), Cairo University, Egypt. Sweet buttermilk was obtained from butter that was produced from buffalo milk provided by the animal production research institute, agriculture research center, Egypt. Sodium carboxymethyl cellulose (CMC) was obtained from Misr Food Additives (MIFAD, Egypt). A commercial-grade of granulated sugar and pumpkin fruit was obtained from the local market. in Egypt.

2.1.1. Preparation of pumpkin pulp

Ripe pumpkins weighing between 2-3 kg were used and composition properties were demonstrated in Table 1. It was washed with tap water, and distilled water then dried. With sharp knives, the pumpkin fruits were peeled, seeds were removed, and the pulp was sliced into 2-3 mm thick pieces. Pumpkin pulp was steam-cooked for 10 min at 80°C (Patel *et al.,* 2020). The cooked pumpkin pulp was blended using a hand blender (Braun, Germany). Following that, the steam-cooked pumpkin pulp was poured into clean sanitized glass bottles 250 ml, then the bottle was heated at 110°C for 25 min, and chilled for the next step of by-product beverage production.

2.1.2. Preparation of the functional beverages

Fresh buffalo buttermilk, sweet whey, and milk permeate were mixed with 4% (v/v) sucrose and 0.2 % carboxymethyl cellulose (CMC) (Sigma-Aldrich, Germany) at 40°C, then 50 % of prepared pumpkin pulp was added to each beverage, then boiled to 85°C in a water bath for 15 min and swiftly cooled to 4±1°C before being stored for 30 days in sanitized glass bottles 250 ml for the end of experiments (Atallah 2015).

2.1.3. Chemical composition of by-products and pumpkin pulp

The chemical composition of each by-product beverage and pumpkin pulp, such as protein, fat, ash, fiber, dry matter, and minerals including (K, Mg, Fe, Cu, P, and Zn) were assessed using the Association of Official Analytical Chemists recommendations (AOAC 2012), and β- Carotene (Barros and Ferreira 2010). Furthermore, pH was determined with a pH meter (Hanna, Italy). The carbohydrate content was calculated as described by the following equation:

$$Carbohydrates\,\% = 100 - (protein\ + \mathrm{fat} + \ \mathrm{fiber}\ + \mathrm{ash}\ + \mathrm{moisture})$$

2.1.4. The viscosity of by-products and pumpkin pulp

The viscosity of by-product beverages was determined using (Brookfield DV- E viscometer) and spindle 4 at rpm 20 in 200 ml of by-product samples. The temperature was maintained at 25ºC, and the viscosity was measured in centipoises (cp).

2.1.5. Flow time. Flow time was measured through a 50-ml pipette (Arbuckle 1986).

Microbiological evaluation

Total bacterial count and molds and yeast count were determined according to standard methods for the examination of dairy products (APHA 2004).

Antioxidant and phenolic content assessment

The antioxidant composition, total phenols, total flavonoids, and Vit. C contents were determined (Lee *et al.,* 2021).

Sensory evaluation

About 12 experts (5 males and 7 females ages ranging from 32 to 60 years) working as researchers and professors at the animal production research institute, Egypt were surveyed as sensory evaluation test participants. In a 26±2°C environment, about 30 mL beverage samples in 60 mL plastic cups were labeled with three numerical numbers and delivered to committee members in random order for comparison between beverage samples, and others as the control without pumpkin pulp addition. All volunteers confirmed that they had no dairy allergies or other health issues. Descriptive analysis was used to explore variations in the sensory characteristics of by-product beverage samples. Additionally, color (10) and appearance (30), flavor (60), and overall acceptability (100) were scored (Patel *et al.,* 2020). There was a three-minute break between samples. To refresh the assessors' palates, water was offered. All beverage samples were kept at 4°C for 30 days, with by-product beverages free of pumpkin serving as the reference standard.

Statistical analysis

The mean ± standard deviation (Mn±SD) was used to express all values. For each test, a minimum of three separate experiments were performed. Two-way analysis of variance was used to evaluate the statistical significance of data comparisons (ANOVA). Statistical significance was defined as $p<0.05$. Statistical analysis was performed using SAS system software (version 9.1, SAS Institute, Cary, NC, USA) to compute F values and compare means using Duncan's multiple range test.

3. Results and Discussion

3.1. Chemical composition of by-products and pumpkin pulp

Table 1. shows the chemical composition of sweet buttermilk (SBM), sweet whey (SW), milk permeate (MP), and pumpkin pulp (PP) used to manufacture functional beverages. The results were not statistically significant at $p<0.05$. PP had the greatest dry matter (DM) of 13.47±0.01 %, ash of 2.04±0.11%, total carbohydrates of 8.70±0.21 %, fiber of 1.88±0.03%, and pH of 7.23±0.21. The lowest amounts of DM and fat were seen in SW (6.28±0.21, 0.21±0.12 %) and MP (6.42±0.42, 0.45±0.11%), respectively. SBM had the greatest protein amount (4.42±0.02 %). SW and PM exhibited a small similarity of 0.68±0.32 and 0.24±0.43 %, respectively. These results agreed El-Dardiry, A. I. (2017); Gab-Allah,R. H. and El-Dardiry, A. I. (2016).

Table 1: Chemical analysis of by-products and pumpkin used in the preparation of functional beverages.

Treatments	DM	Fat	Protein	Ash	Fiber	Total Carbs
SBM	11.16±0.01^{B}	1.16±0.1^{A}	4.42±0.22^{A}	0.75±0.12^{B}	ND	4.83±0.21^{C}
SW	6.28±0.21^{D}	0.21±0.12^{C}	0.68±0.23^{B}	0.54±0.21^{C}	ND	4.85±0.11^{C}
MP	6.42±0.42^{C}	0.45±0.11^{B}	0.24±0.13^{D}	0.38±0.21^{D}	ND	5.35±0.14^{B}
PP	13.47±0.62^{A}	0.28±0.11^{C}	0.57±0.14^{C}	2.04±0.16^{A}	1.88±0.18^{A}	8.7±0.78^{A}

Sweet Buttermilk (SBM); Sweet Whey (SW); Milk Permeate (MP), and Pumpkin pulp (PP). Different lowercase superscript letters show a significant difference ($p< 0.05$). ND: Not detected

3.2. Chemical composition of functional beverages

The chemical composition of functional beverages during the 30 days of storage at 4±1ºC was determined. Table 2. shows the antioxidant components of functional beverages and pumpkin pulp. PP had the greatest quantities of β-carotene, vitamin C, flavonoids, and total phenols (10.67±1.23, 12.11±0.32, 3.14±0.22, and 42.98±0.12 mg/100 g) respectively. Furthermore, the antioxidant activity of PP was determined 81.92±1.23 mg/100 g however this was not investigated in the SBM, SW, or MP assays.

Table 2: The antioxidant composition of by-products and pumpkin pulp used in prepared functional beverages.

Antioxidants composition (mg/100g)	SBM	SW	MP	PP
β-Carotene	ND	ND	ND	10.67±1.23^{A}
Antioxidant activity	18.23±0.21^{B}	7.98±0.32^{D}	11.04±0.34^{C}	81.92±1.23^{A}
Total phenols	ND	ND	ND	42.98±0.12^{B}
Total flavonoids	ND	ND	ND	3.14±0.22^{D}
Vit. C	ND	ND	ND	12.11±0.32^{A}

Sweet Buttermilk (SBM); Sweet Whey (SW); Milk Permeate (MP), and Pumpkin pulp (PP). Different lowercase superscript letters show a significant difference ($p< 0.05$). ND: Not detected

Several studies have looked into the carotenoid content of pumpkin, but little is known about the phenolic content and antioxidant activity of pumpkin. As a result, the findings agreed with those of (Panato and Muller 2022), who stated that fruits and vegetables, such as pumpkin, have a high concentration of carotenoids, vitamins, and dietary fiber and are commonly recognized as an essential component of a healthy diet.

Table 3. showed the chemical composition of functional beverages SBM, SW, and MP as controls for SBM_{C1}, SW_{C2}, and MP_{C3}, and after pumpkin pulp addition as treatments for SBM_{T1}, SW_{T2}, and MP_{T3}. The results obtained were presented both fresh and after 30 days of storage. Fresh $SBM_{C1\ and\ T1}$ was the highest DM values at 14.88±0.05 and 15.99±0.01 % respectively. Whereas $SBM_{C1\ and\ T1}$ after 30 days of storage were 14.98±0.02, and 16.12± 0.04 % respectively. On the other hand, fresh $SW_{C2,\ and\ T2}$ showed the lowest DM content 10.22±0.22, and 13.66±0.2% respectively. While $SW_{C2,\ and\ T2}$ after 30 days of storage were 10.27±0.2, and 13.70±0.2% respectively. Also, $SBM_{C1\ and\ T1}$ showed the highest fat levels in freshly treated at 1.11±0.05, and 0.68±0.2% respectively as well, it was 1.11±0.11, and 0.69±0.21% after 30 days of storage. While $SW_{C2\ and\ T2}$ had high similarity results in both fresh and after 30 days of storage 0.20±0.2, and 0.23±0.2% respectively.

SBM had greater protein and ash levels as compared to the other treatments. In the same comparison, MP receives the lowest score in both controls and after pumpkin addition. While the results for carbohydrates varied, the highest values for $PM_{C3\ and\ T3}$ with fresh carbohydrates were 9.33±0.43 and 10.81±0.2 %, respectively. While it was 9.32±1.12% and 10.76±3.11% after 30 days of storage, respectively. $SBM_{C1\ and\ T1}$ had the lowest carbohydrate levels while fresh at 9.33±0.43% and 30 days of storage had 10.81±0.2%. Furthermore, fresh $SBM_{C1\ and\ T1}$ had the greatest pH values of 6.4±0.05 and 6.82±0.02, and during 30 days of storage, they showed 6.01±0.01 and 6.50±0.14, respectively, whereas $PM_{C3\ and\ T3}$ had the lowest pH values of 5.86±0.21 and 6.48±0.3, and during 30 days of storage, 5.47±0.16 and 6.12±0.31, respectively.

Table 3: Chemical composition of functional beverages fortified with pumpkin pulp (Mean±SD).

Chemical composition %	SBM		SW		MP	
	Fresh					
	C_1	T_1	C_2	T_2	C_3	T_3
Dry matter	14.88±0.05^{Bd}	15.99±0.1^{Ad}	10.22±0.22^{Fd}	13.66±0.2^{Dd}	10.35±0.02^{Ed}	13.72±0.02^{Cd}
Fat	1.11±0.05^{Ad}	0.68±0.2^{Bc}	0.20±0.2^{Fd}	0.23±0.2^{Ec}	0.43±0.2^{Cd}	0.35±0.2^{Dc}
Protein	4.59±0.02^{Ad}	2.39±0.2^{Bd}	0.64±0.3^{Cd}	0.61±0.1^{Dd}	0.22±0.4^{Fd}	0.39±0.5^{Ed}
Ash	0.71±0.01^{Dd}	1.33±0.2^{Ad}	0.51±0.3^{Ed}	1.23±0.34^{Bd}	0.36±0.65^{Fd}	1.15±0.1^{Cd}
Fiber	ND	1.01±0.2^{Ac}	ND	1.01±0.4^{Ad}	ND	1.01±0.2^{Ac}
Total Carbs	8.47±0.05^{Fd}	10.6±0.2^{Bd}	8.86±0.02^{Ed}	10.56±0.2^{Cb}	9.33±0.43^{Dd}	10.81±0.2^{Ad}
pH	6.4±0.05^{Ca}	6.82±0.02^{Aa}	6.13±0.03^{Da}	6.64±0.2^{Ba}	5.86±0.21^{Ea}	6.48±0.3^{Ca}
	10 days					
	C_1	T_1	C_2	T_2	C_3	T_3
Dry matter	14.95±0.02^{Bc}	16.07±0.27^{Ac}	10.24±0.2^{Fc}	10.27±0.2^{Dc}	13.67±0.02^{Ec}	13.73±0.02^{Cc}
Fat	1.11±0.02^{Ac}	0.68±0.01^{Bc}	0.20±0.3^{Fc}	0.23±0.11^{Eb}	0.43±0.05^{Cc}	0.35±0.5^{Dc}
Protein	4.61±0.01^{Ac}	2.40±0.02^{Bc}	0.65±0.1^{Cc}	0.62±0.2^{Dc}	0.23±0.05^{Fc}	0.40±0.2^{Ec}
Ash	0.72±0.05^{Dc}	1.33±0.2^{Ac}	0.52±0.05^{Ec}	1.24±0.03^{Bc}	0.37±0.3^{Fc}	1.16±0.02^{Cc}
Fiber	ND	1.01±0.01^{Ac}	ND	1.01±0.05^{Ac}	ND	1.01±0.1^{Ab}
Total Carbs	8.46±0.05^{Fc}	10.62±0.5^{Cc}	8.86±0.01^{Ec}	10.55±0.2^{Bc}	9.33±0.05^{Dc}	10.81±0.1^{Ac}
pH	6.34±0.5^{Cb}	6.68±0.01^{Ab}	6.02±0.02^{Db}	6.49±0.03^{Db}	5.73±0.01^{Eb}	6.32±0.05^{Cb}
	20 days					
	C_1	T_1	C_2	T_2	C_3	T_3
Dry matter	14.94±0.22^{Bb}	16.09±0.73^{Ab}	10.25±0.24^{Fa}	13.68±0.2^{Db}	10.38± 0.02^{Eb}	13.72±0.02^{Cb}
Fat	1.11±0.05^{Ab}	0.69±0.22^{Bb}	0.2±0.05^{Fb}	0.23±0.1^{Ea}	0.43±0.05^{Cb}	0.35±0.01^{Db}
Protein	4.63±0.5^{Ab}	2.42±0.02^{Bb}	0.66±0.01^{Cb}	0.63±0.15^{Db}	0.25±0.5^{Fb}	0.41±0.1^{Eb}
Ash	0.73±0.05^{Db}	1.34±0.1^{Ab}	0.53±0.12^{Eb}	1.24±0.11^{Bb}	0.37±0.01^{Fb}	1.16±0.13^{Cb}
Fiber	ND	1.01±0.12^{Ab}	ND	1.01±0.04^{Aa}	ND	1.01±0.15^{Ab}
Total Carbs	8.46±0.21^{Eb}	10.62±0.04^{Bb}	8.86±0.1^{Eb}	10.55±0.01^{Cb}	9.32±0.12^{Db}	10.7±0.11^{Ab}
pH	6.2±0.11 ^{Cc}	6.60±0.01 ^{Ac}	5.88±0.51 ^{Dc}	6.36±0.05 ^{Bc}	5.61±0.1^{Ec}	6.22±0.01^{Cc}
	30 days					
	C_1	T_1	C_2	T_2	C_3	T_3
Dry matter	14.98±0.02^{Ba}	16.12± 0.04^{Aa}	10.27±0.2^{Fa}	13.70±0.2^{Da}	10.40±0.01^{Ea}	13.73±0.04^{Ca}
Fat	1.11±0.11^{Aa}	0.69±0.21^{Ba}	0.20±0.12^{Ea}	0.23±0.01^{Da}	0.43±0.15^{Ca}	0.35±0.11^{Ca}
Protein	4.70±0.03^{Aa}	2.44±0.13^{Ba}	0.67±0.11^{Ca}	0.64±0.12^{Da}	0.26±0.11^{Fa}	0.42±0.01^{Ea}
Ash	0.73±0.11^{Da}	1.34±0.01^{Aa}	0.53±0.03^{Ea}	1.24±0.15^{Ba}	0.38±0.14^{Fa}	1.17±0.51^{Ca}
Fiber	ND	1.01±0.13^{Aa}	ND	1.01±0.15^{Aa}	ND	1.01±0.01^{Aa}
Total Carbs	8.43±0.31^{Fa}	10.61±2.1^{Ba}	8.85±0.14^{Ea}	10.55±2.11^{Ca}	9.32±1.12^{Da}	10.76±3.11^{Aa}
pH	6.01±0.01^{Dd}	6.50±0.14^{Ad}	5.65±0.02^{Ed}	6.24±0.11^{Bd}	5.47±0.16^{Fd}	6.12±0.31^{Cd}

Sweet Buttermilk (SBM); Sweet Whey (SW); Milk Permeate (MP), and Pumpkin pulp (PP). $C_{1,2,3}$ treatments are controlled without fortified pumpkin pulp. $T_{1,2,3,}$ treatments with fortified 50 % pumpkin pulp; ND: Not detected; A, B, C, D: Means with the same letter in the same character assessed for each treatment during the storage period are not significantly different ($p< 0.05$). a,b,c,d: Means with the same letter in the same character assessed among treatments in the same storage period are not significantly different ($p<0.05$).

The DM results agreed with (Atallah 2015) who suggested that the slight increase in DM during storage might be due to moisture loss during cold storage. Furthermore, the obtained findings were consistent with those (Varzakas and Verpoort 2016) who investigated the chemical composition of their products enriched with pumpkin pulp, papaya pulp, and apple pulp, resulting in lower fat and protein content than raw milk. Furthermore, the increased total carbohydrate load was due to the higher sugar concentration used in product manufacturing. In addition, the obtained results were consistent with those (Barakat and Hassan 2017) who investigated that the inclusion of pumpkin pulps decreased the pH of pumpkin yogurt significantly ($p<0.05$). Also, it corresponded with those of (Men *et al.,* 2021) who stated that pumpkin is high in dietary fiber, carotene, minerals, and vitamins. In addition, it agreed with (Patel *et al.,* 2020) who reported that pumpkin pulp has a high carotene concentration of 3 mg/100g.

3.3. The vitamin and mineral content analysis

Table 4. showed the vitamin C and mineral content of functional beverages during control treatment and after pumpkin pulp addition. The results revealed no significant difference ($p<0.05$) across treatments. $SBM_{C1 \text{ and } T1}$ had the highest vitamin C concentrations of 3.02±0.01 and 7.71±0.01 mg/100g, respectively. While $MP_{C3 \text{ and } T3}$ had the highest concentration with 2.10±0.01 and 7.25±0.03 mg/100g. Furthermore, the mineral content of the treatments differed considerably ($p<0.05$). SBM also has the greatest amounts of K, Mg, Ca, and Zn. While MP had the greatest amounts of Fe and Cu. Furthermore, SW had the greatest amount of Na in both the control and pumpkin pulp additions.

Minerals and vitamins are necessary for growth, development, wound healing, immunity, and other physiological functions. Furthermore, the results were entirely consistent with (Unal *et al.,* 2022) who demonstrated that pumpkin, like other fruits and vegetables, is high in vitamins and minerals.

Table 4: The vitamin and minerals content mg/100g of functional beverages fortified with pumpkin pulp (Mean±SD).

Component (mg/100g)	Treatments					
	SBM		SW		MP	
	C_1	T_1	C_2	T_2	C_3	T_3
Vit. C	$3.02±0.01^{A}$	$7.71±0.01^{A}$	$2.41±0.11^{A}$	$7.40±0.32^{A}$	$2.10±0.01^{B}$	$7.25±0.03^{A}$
Na	$16.02±0.03^{A}$	$9.21±0.01^{AB}$	$21.46±0.14^{B}$	$11.93±0.02^{A}$	$18.27±0.21^{B}$	$10.33±0.14^{AB}$
K	$101.28±0.11^{B}$	$211.14±0.1^{B}$	$13.78±0.23^{A}$	$167.39±0.32^{AB}$	$12.56±0.11^{A}$	$166.78±0.\ 33^{A}$
Mg	$2.34±0.21^{A}$	$6.89±0.31^{A}$	$2.18±0.33^{A}$	$6.81±0.01^{A}$	$2.07±0.01^{A}$	$6.75±0.01^{A}$
Ca	$92.96±0.31^{A}$	$57.65±0.41^{A}$	$46.63±0.32^{AB}$	$33.48±0.01^{A}$	$57.38±0.23^{A}$	$38.86±0.02^{B}$
Fe	$0.04±0.01^{A}$	$0.39±0.01^{AB}$	$0.26±0.01^{C}$	$0.50±0.31^{A}$	$0.32±0.41^{AB}$	$0.53±0.01^{A}$
Cu	$0.02±0.11^{A}$	$0.08±0.01^{C}$	$0.04±0.11^{C}$	$0.09±0.04^{A}$	$0.05±0.15^{C}$	$0.09±0.05^{A}$
P	$59.47±0.11^{A}$	$45.73±0.41^{A}$	$24.75±0.4^{A}$	$28.38±0.11^{C}$	$27.98±0.03^{A}$	$29.99±0.02^{C}$
Zn	$0.05±0.41^{C}$	$0.18±0.01^{A}$	$0.04±0.12^{A}$	$0.18±0.23^{B}$	$0.03±0.51^{B}$	$0.17±0.01^{A}$

Sweet Buttermilk (SBM); Sweet Whey (SW); Milk Permeate (MP), and Pumpkin pulp (PP). $C_{1,2,3}$ treatments are controlled without fortified pumpkin pulp. $T_{1,2,3,}$ treatments with fortified 50% pumpkin pulp; A, B, C, D: Means with the same letter in the same character assessed for each treatment during the storage period are not significantly different ($p< 0.05$).

3.4. Antioxidant activity of functional beverages

Table 5. showed the antioxidant activity of all functional beverages including pumpkin was higher than in the control samples. However, during storage periods antioxidant activity was significantly different ($p<0.05$) among all treatments.

The total phenol content of functional beverages in a pumpkin was higher than that in control samples ($p<0.05$). Furthermore, the total phenol level in the fresh functional beverages varied from 0.002±0.01 to 22.67±0.11 mg/100g. While stored for up to 30 days increased from 0.002±0.01 to 22.32±0.22 mg/100g.

The findings agreed with those of (Ozer and Ozturk 2022) who indicated that pumpkin is a good source of antioxidants, total phenols, total flavonoids, and vitamin C, all of which are important nutrients in the human diet. They also observed that antioxidant activity levels decreased somewhat during cold storage. It also agreed with (Barakat and Hassan 2017) who found that adding pumpkin pulp to yogurt enhanced the antioxidant content.

Table 5: Total phenolic content (equivalent mg of Gallic acid/100 g) and antioxidant activity (%) of functional beverages supplemented with pumpkin pulp (Mean ± SD).

Storage period/ days	Total phenol (equivalent mg Gallic acid/100gm)					
	SBM		SW		MP	
	C_1	T_1	C_2	T_2	C_3	T_3
0	1.00±0.21 ^{Da}	22.67±0.11 ^{Aa}	0.08±0.23^{Ea}	21.32±0.22^{Ba}	0.02±0.23^{Fa}	20.90±0.44^{Ca}
10	0.82±0.01 ^{Db}	22.48±0.30 ^{Ab}	0.07±0.24^{Eb}	21.15±0.21^{Bb}	0.01±0.21^{Fb}	20.71±0.54^{Cb}
20	0.70±0.07 ^{Dc}	22.37±0.21^{Ac}	0.04±0.31^{Ec}	21.03±0.40^{Bc}	0.005±0.21^{Fc}	20.62±0.21^{Cc}
30	0.52±0.25 ^{Dd}	22.32±0.22^{Ad}	0.02±0.32^{Ed}	20.94±0.24^{Bd}	0.002±0.01^{Fd}	20.51±0.45^{Cd}
	Antioxidant activity (%)					
	SBM		SW		MP	
	C_1	T_1	C_2	T_2	C_3	T_3
0	17.12±0.27^{Da}	46.37±0.29^{Aa}	11.18±1.01^{Ea}	43.89±0.31^{Ba}	11.05±2.21^{Fa}	43.16±3.2^{Ca}
10	17.01±0.21^{Db}	46.31±0.23^{Ab}	11.04±1.24^{Eb}	43.83±2.21^{Bb}	10.92±1.21^{Fb}	43.09±3.01^{Cb}
20	16.95±0.34^{Dc}	46.27±0.25^{Ac}	10.99±0.31^{Ec}	43.79±0.11^{Bc}	10.86±0.21^{Fc}	43.04±2.21^{Cc}
30	16.91±0.22^{Dd}	46.25±0.01^{Ad}	10.94±0.11^{Ed}	43.76±3.31^{Bd}	10.80±0.23^{Fd}	43.01±1.27^{Cd}

Sweet Buttermilk (SBM); Sweet Whey (SW); Milk Permeate (MP), and Pumpkin pulp (PP). $C_{1,2,3}$ treatments are controlled without fortified pumpkin pulp. $T_{1,2,3,}$ treatments with fortified 50% pumpkin pulp; A, B, C, D: Means with the same letter in the same character assessed for each treatment during the storage period are not significantly different ($p< 0.05$). a,b,c,d: Means with the same letter in the same character assessed among treatments in the same storage period are not significantly different ($p<0.05$).

3.5. Microbiological evaluation of functional beverages

Microbiological analysis displayed that there was a significant difference ($p<0.05$) between functional beverage treatments and control samples during cold storage (Table 6). The highest total count of bacteria was MP_{C3} showed 4.75±0.09 log cfu/ml after 30 days of storage while the lowest count was SBM_{T1} at zero time showed 4.09±0.05 log cfu/ml. Notably, there are no fungus or molds were detected in any of the treatments during the storage period.

Table 6: Microbiological evaluation (log cfu/mL) of functional beverages supplemented with pumpkin pulp (Mean±SD).

Storage period/days	SBM		SW		MP	
	Total bacterial count log cfu/mL					
	C_1	T_1	C_2	T_2	C_3	T_3
0	4.14±0.02^{Bd}	4.09 ±0.05^{Fd}	4.13±0.01^{Cd}	4.10 ±0.02^{Ed}	4.18±0.028^{Ad}	4.11±0.21^{Dd}
10	4.19 ±0.03^{Cc}	4.17 ±0.04^{Ec}	4.21 ±0.001^{Bc}	4.16 ±0.23^{Fc}	4.29±0.52^{Ac}	4.18 ±0.41^{Dc}
20	4.34 ±0.21^{Cb}	4.23 ±0.03^{Fb}	4.36 ±0.32^{Bb}	4.26 ±0.06^{Eb}	4.41±0.22^{Ab}	4.30±0.11^{Db}
30	4.53±0.61^{Ca}	4.32 ±0.04^{Fa}	4.56±0.24^{Ba}	4.34 ±0.06^{Ea}	4.75 ±0.09^{Aa}	4.51 ±1.01^{Da}

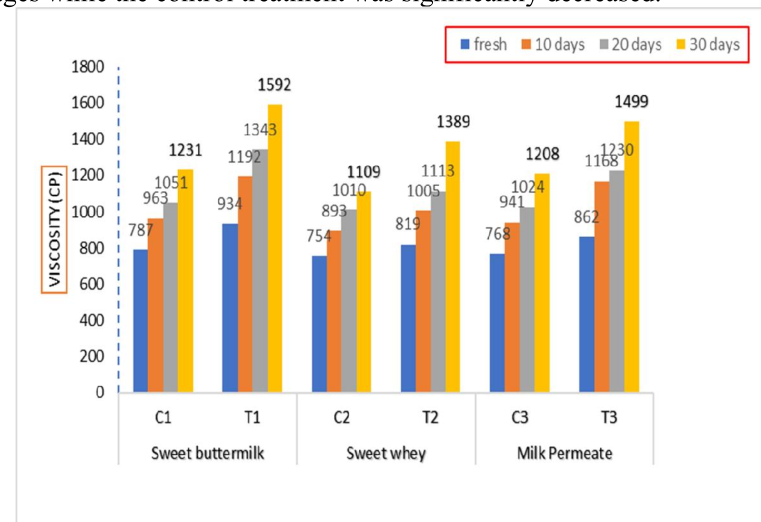
Sweet Buttermilk (SBM); Sweet Whey (SW); Milk Permeate (MP), and Pumpkin pulp (PP). $C_{1,2,3}$ treatments are controlled without fortified pumpkin pulp. $T_{1,2,3,}$ treatments with fortified 50% pumpkin pulp; A, B, C, D: Means with the same letter in the same character assessed for each treatment during the storage period are not significantly different ($p< 0.05$). a,b,c,d: Means with the same letter in the same character assessed among treatments in the same storage period are not significantly different ($p<0.05$).

The total number of bacteria in dairy products plays a key role in the safety improvement and quality enhancement of the final product (De Paula *et al.,* 2021). The findings were consistent with those of (Jantapaso and Mittraparp-arthorn 2022) who indicated that bioactive compounds are based on phenolic compounds (flavonoid, phenols) that had antimicrobial activity on bacterial viability.

Yeasts and molds are indicators of general hygiene during the production process of functional beverages, whether fresh or stored, which may cause health problems in the future. The results obtained are consistent with (Atallah 2015) who demonstrated that yeasts and molds were not detected during treatments, whether fresh or during cold storage, due to good hygienic conditions during the manufacturing and storage process.

3.6. Viscosity and flow time assessment

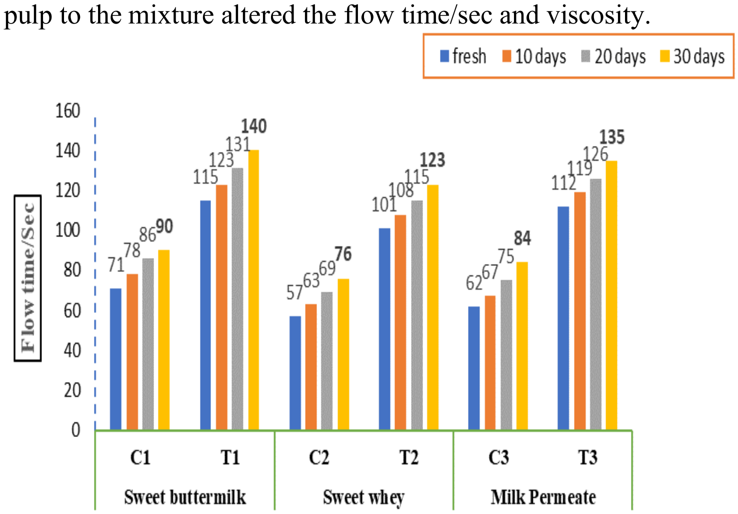
In comparison to the control samples, pumpkin fruits enhanced the viscosity of the produced beverages. Figure 1. demonstrates that functional beverages containing pumpkin pulp had higher total solids levels, which may explain our findings. The findings were strikingly similar to those (Atallah 2015) who discovered that adding fruit pulp such as papaya or guava considerably enhanced the viscosity of beverages while the control treatment was significantly decreased.



Control, $C_{1,2,3}$ and by-products treatments after pumpkin pump addition $T_{1,2,3}$

Fig. 1: Viscosity (cp) of functional beverages fortified with pumpkin pulp

Pumpkin beverages may have the highest viscosity owing to the presence of fiber stabilizers, which have favorable functional properties such as texture, gelling, thickening, emulsification, and stability in dietary fiber-enriched diets (Men *et al.,* 2021). Viscosity is a measurement of a fluid's internal friction; it may also bind water, which might improve product consistency by increasing water-binding capacity (Caili *et al.,* 2007). The results also revealed that the viscosity of the control sample buttermilk was greater than the other control samples such as sweet whey or milk permeate. This might be due to an increase in the number of solids in the buttermilk. Additionally, Figure 2. showed the flow time and it displayed that SBM_{T1} had higher flow time values during fresh or cold storage compared to the other treatments and the control. Moreover, the effect of adding pumpkin and storage time was significant ($p<0.05$). the results are in agreement with (Barakat and Hassan 2017) who reported that adding pumpkin pulp to the mixture altered the flow time/sec and viscosity.



Control, $C_{1,2,3}$ and by-products treatments after pumpkin pump addition $T_{1,2,3}$

Fig. 2: Flow time/Sec of functional beverages fortified with pumpkin pulp

3.7. Sensory evaluation

There were no significant variations in sensory assessment across samples $p<0.05$ (Table 7). A taste panel gave all functional beverages containing pumpkins an extraordinarily high score for acceptability. The organoleptic scores of all treatments improved after 10 days of cold storage. There were no differences in sensory measures across the treatments after 20 days of storage. While, after 30 days of cold storage, a similar trend occurred for all of the tested items, with slight decreases in the obtained score. The beverage samples containing pumpkin pulp achieved the highest overall score ranging from 91±0.08 to 98±0.76 during storage. Given the potential benefits of functional beverages containing pumpkin pulp, they might be utilized to mask any flavor or aroma concerns. The findings were consistent with those (Atallah 2015) who explored whether adding fruit pulp to products improves sensory evaluation.

Table 7: Sensory evolution of functional beverages fortified with pumpkin pulp.

Storage period/days	SBM		SW		MP	
	Color (10)					
	C_1	T_1	C_2	T_2	C_3	T_3
0	$8±0.01^a$	$10±0.21^a$	$8±0.11^a$	$10±0.31^a$	$8±0.03^a$	$10±0.04^a$
10	$8±0.21^b$	$10±0.31^a$	$8±0.01^b$	$10±0.32^a$	$8±0.41^b$	$10±0.21^a$
20	$7±0.04^a$	$10±0.02^a$	$8±0.07^b$	$10±0.21^a$	$8±0.15^a$	$10±0.31^a$
30	$7±0.06^a$	$10±0.04^a$	$7±0.02^a$	$10±0.07^a$	$7±0.11^a$	$10±0.31^a$
	Appearance (30)					
	C_1	T_1	C_2	T_2	C_3	T_3
0	$28±0.03^a$	$30±0.01^a$	$25±0.01^a$	$30±0.21^a$	$28±0.32^{ab}$	$30±0.01^a$
10	$28±0.02^a$	$30±0.21^a$	$25±0.03^b$	$30±0.30^a$	$27±0.01^b$	$30±0.41^a$
20	$25±0.01^a$	$30±0.06^a$	$24±0.21^a$	$28±0.51^a$	$25±0.84^b$	$30±0.06^a$
30	$15±0.01^c$	$28±0.05^a$	$12±0.01^{ab}$	$25±0.03^b$	$15±0.01^a$	$26±0.81^a$
	Flavor (60)					
	C_1	T_1	C_2	T_2	C_3	T_3
0	$45±0.34^a$	$58±0.31^a$	$43±0.54^b$	$57±0.01^a$	$44±0.91^b$	$58±0.05^a$
10	$45±0.06^a$	$58±0.01^a$	$43±0.05^{ab}$	$57±0.41^a$	$42±0.01^c$	$58±0.03^a$
20	$40±0.01^c$	$57±0.51^b$	$42±0.61^a$	$56±0.02^a$	$40±0.07^b$	$57±0.03^c$
30	$40±0.04^a$	$55±0.23^a$	$40±0.01^b$	$54±0.01^c$	$40±0.01^{ab}$	$55±0.01^b$
	Total (100)					
	C_1	T_1	C_2	T_2	C_3	T_3
0	$81±0.23^b$	$98±0.02^a$	$76±0.41^{ab}$	$97±0.91^c$	$80±0.71^a$	$98±0.76^a$
10	$81±0.43^a$	$98±0.91^a$	$76±0.76^b$	$97±0.70^a$	$77±0.01^c$	$98±0.01^a$
20	$72±0.51^{ab}$	$97±0.63^a$	$72±0.23^c$	$93±0.66^a$	$73±0.88^a$	$97±0.31^b$
30	$62±0.01^c$	$93±0.07^a$	$59±0.01^a$	$88±0.31^b$	$62±0.91^a$	$91±0.08^a$

Sweet Buttermilk (SBM); Sweet Whey (SW); Milk Permeate (MP), and Pumpkin pulp (PP). $C_{1,2,3}$ treatments are controlled without fortified pumpkin pulp. $T_{1,2,3,}$ treatments with fortified 50% pumpkin pulp; A, B, C, D: Means with the same letter in the same character assessed for each treatment during the storage period are not significantly different ($p< 0.05$). a,b,c,d: Means with the same letter in the same character assessed among treatments in the same storage period are not significantly different ($p<0.05$).

4. Conclusion

Combining pumpkin pulp with milk by-products such as buttermilk and sweet whey, or milk permeates in a 1:1 ratio with 4 % sugar, can efficiently generate a functional nutritious beverage. Because of its high antioxidant activity, total phenols, total flavonoids, ascorbic acid, and carotenoids, pumpkin pulp improved the flavor and nutritional value of the functional beverage. Furthermore, the sensory assessment of functional beverages was quite acceptable. Generally, pumpkin is highly recommended as a flavor enhancer and public health support ingredient in functional beverage production. Based on the chemical composition, microbiological examination, sensory functions, and antioxidant activity of the final product, recycling of dairy by-products reduces the total cost of functional beverages as well as improves the environmental impact.

References

AOAC., 2012.Official methods of analysis of the association of official analytical chemists (19th ed.)," Washington D.C.USA: AOAC International., 9-13.

APHA., 2004. Standard methods for the examination of dairy products.American Public Health Association (Eds.), 327- 404.Washington, DC

Arbuckle, W.S., 1986.Ice cream, 4th Ed., the AVI publishing Co.Inc.West, Port.Connecticut, USA.

Atallah, A.A., 2015. Development of new functional beverages from milk permeate using some probiotic bacteria and fruits pulp.Egyptian Journal of Dairy Science, 43:25-39.

Baker, M.T., P. Lu, J.A. Parrella, and H.R. Leggette, 2022. Consumer Acceptance toward Functional Foods: A Scoping Review.International Journal of Environmental Research and Public Health, 19:1217.

Barakat, H., and M.F. Hassan, 2017. Chemical, nutritional, rheological, and organoleptical characterizations of stirred pumpkin-yoghurt.Food and Nutrition Sciences, 8:746.

Barros, L., A.M. Carvalho, and I.C. Ferreira, 2010. Leaves, flowers, immature fruits and leafy flowered stems of Malva sylvestris: a comparative study of the nutraceutical potential and composition.Food and Chemical Toxicology, 48:1466 -1472.

Burns, P., F. Molinari, A. Beccaria, R. Paez, C. Meinardi, J. Reinheimer, and G. Vinderola, 2010. Suitability of buttermilk for fermentation with *Lactobacillus helveticus* and production of a functional peptide-enriched powder by spray-drying.Journal of applied microbiology,109:1370 -78.

Caili, F., T. Haijun, C. Tongyi, L. Yi, and L. Quanhong, 2007.Some properties of an acidic protein-bound polysaccharide from the fruit of pumpkin.Food chemistry, 100: 944-947.

Costa, C., N.G. Azoia, L. Coelho, R. Freixo, P. Batista, and M. Pintado, 2021.Proteins derived from the dairy losses and by-products as raw materials for non-food applications.Foods, 10:135.

De Paula, A.C.L., J.D. Medeiros, G.D.R. Fernandes, V.L. da Silva, and C.G. Diniz, 2021. Microbiome of industrialized Minas Frescal Cheese reveals high prevalence of putative bacteria: A concern in the one health context.LWT, 139:110791.

Dinu, M., R. Soare, G. Hoza, and A.D. Becherescu, 2016.Biochemical composition of some local pumpkin population.Agriculture and agricultural science procedia, 10:185-191.

El-Dardiry, A.I., 2017." Effect of using orange on the physico-chemical properties of ricotta cheese." Egypt.J.Agric.Res., 95 (1): 299- 318.

Gab-Allah, R.H., and A.I. El-Dardiry, 2016. Supplementation of Buttermilk with Kaki Fruit (*Diospyros Kaki).*Egyptian J. Dairy Sci., 44 (12):77-88.

Gebreselassie N., F. Abay, and F. Beyene, 2016. Biochemical and molecular identification and characterization of lactic acid bacteria and yeasts isolated from Ethiopian naturally fermented buttermilk.Journal of food science and technology, 53:184-96.

Guldiken, B., Z. Gulsunoglu, S. Bakir, G. Catalkaya, E. Capanoglu, and M. Nickerson, 2021. Innovations in functional foods development.In Food Technology Disruptions.73-130.Academic Press.

Jantapaso, H., and P. Mittraparp-Arthorn, 2022. Phytochemical composition and bioactivities of aqueous extract of Rambutan (*Nephelium lappaceum* L.cv.*Rong Rian*) Peel.Antioxidants, 11:956.

Kim, M.Y., E.J. Kim, Y.N. Kim, C. Choi, and B.H. Lee, 2012. Comparison of the chemical compositions and nutritive values of various pumpkin (Cucurbitaceae) species and parts.Nutrition research and practice, 6: 21-27.

Kulczyński, B., A. Sido, and A. Gramza-Michałowska, 2020. Antioxidant potential of phytochemicals in pumpkin varieties belonging to *Cucurbita moschata* and *Cucurbita pepo* species.CyTA-Journal of Food, 18: 472-84.

Lee, H.S., M.W. Song, K.T. Kim, W.S. Hong, and H.D. Paik, 2021. Antioxidant effect and sensory evaluation of yogurt supplemented with hydroponic ginseng root extract.Foods, 10:639.

Men, X., S.I. Choi, X. Han, H.Y. Kwon, G.W. Jang, Y.E. Choi, M.P. Sung, and O.H. Lee, 2021. Physicochemical, nutritional and functional properties of *Cucurbita moschata*.Food Science and Biotechnology, 30:171-83.

Ozer, H., C. Yılmaz, and B. Ozturk, 2022. The influence of cultivation system and modified atmosphere packaging on quality attributes of tomato fruit during cold storage.Biological Agriculture & Horticulture, 1-13.

Panato, K., and C.M.O. Muller, 2022. Drying kinetics and physicochemical and technological properties of pumpkin purée flour dried by convective and foam-mat drying.Journal of Food Processing and Preservation, 46:e16264.

Patel, A.S., A.R. Bariya, S.N. Ghodasara, J.A. Chavda, and S.S. Patil, 2020.Total carotene content and quality characteristics of pumpkin flavoured buffalo milk.Heliyon, 6:e04509.

Unal, K., A.S. Babaoğlu, N. Erdem, and N.M. Dilek, 2022.The effect of pumpkin powder on the physicochemical, emulsification and textural properties of beef. Journal of Food Processing and Preservation, e16728.

Varzakas, T., G. Zakynthinos, and F. Verpoort, 2016. Plant food residues as a source of nutraceuticals and functional foods. Foods, 5:88.

Zokaityte, E., D. Cernauskas, D. Klupsaite, V. Lele, V. Starkute, P. Zavistanaviciute, and E. Bartkiene, 2020. Bioconversion of milk permeate with selected lactic acid bacteria strains and apple by-products into beverages with antimicrobial properties and enriched with galactooligosaccharides. Microorganisms, 8:1182.