Article

Enrichment of Fermented Milk Drinks with *Mespilus germanica* and *Crataegus azarolus* Fruit Extracts

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Abstract: The aim of the present study was to select the optimal extraction conditions for two underutilized wild fruits of the *Rosaceae* family (*Mespilus germanica* and *Crataegus azarolus*) in order to investigate the possibility of utilizing their potential bioactive properties by developing novel fermented milk drinks enriched with fruit extracts, with functional properties. The total phenolic and flavonoid contents of the extracts, as well as their antioxidant and antidiabetic activities, were evaluated, and based on the results, the optimal extraction conditions were selected. The technological characteristics (i.e., fermentation conditions) and microbiological and bioactive properties of the final products were evaluated over refrigerated storage for 28 days. The findings of the study showed that the incorporation of *Mespilus germanica* or *Crataegus azarolus* extracts had a positive influence on the bioactive properties of the end-products, decreased fermentation times and maintained high viable populations of lactic acid bacteria. Hence, it can be concluded that *Mespilus germanica* or *Crataegus azarolus* extracts can be exploited in the enrichment of an added-value fermented milk drink.

Keywords: *Mespilus germanica*; *Crataegus azarolus*; phenolic compounds; antioxidant activity; antidiabetic; bioactive fermented milk drink

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1. Introduction

Consumers' demand for healthy foods has increased significantly in recent years; hence, there is interest in developing novel foods with distinct health benefits. Currently, a great number of novel functional foods are available, with dairy products representing an important segment of this market.

Fermentation is the oldest and most important method of food processing, which leads to natural preservation. During the fermentation process, complex organic molecules and complex substances are converted into other simpler molecules through the action of various enzymes. These substances are likely to provide the final product with antimicrobial properties due to the production of antimicrobial metabolites, such as bacteriocins, but also desirable organoleptic characteristics due to the properties of specific microorganisms [1]. In fact, some of these microorganisms may also provide benefits for human health, either because of their competition with various pathogenic bacteria or because of their possible probiotic activity.

The effects of fermentation on the technological, organoleptic and nutritional properties of milk drinks are well-established. Fermented milk drinks are not regarded as a rich source of polyphenols and antioxidants; hence, the production of novel enhanced dairy drinks using the extracts of medicinal plants/fruit or even their by-products have recently attracted more attention in order to meet the demands of health-conscious consumers [2,3].

There are few studies reporting on the development of bioactive milk-based drinks enriched with plant extracts, such as strawberry pulp (*Fragaria × ananassa*), thistle (*Silybum marianum* L.), hawthorn (*Crataegus monogyna*), sage (*Salvia officinalis* L.), marjoram (*Origanum vulgare* L.), lemon balm (*Melissa officinalis* L.), mint (*Mentha piperita* L.), lavender

(*Lavandula angustifolia* L.) and rosemary (*Rosmarinus officinalis* L.). In these studies, the addition of plant extracts to milk significantly increased the concentration of bioactive components, with high total phenolic and flavonoid contents and, therefore, high antioxidant activity [4,5]. In addition of the improvement of the physicochemical properties, the rheological properties were also improved in the enriched products compared to the controls. Some of those milk drinks were fermented, and the lactic acid bacteria used for fermentation were viable throughout their shelf life. In general, when a fiber addition was made (e.g., inulin), the lactic acid bacteria improved not only the antioxidant activity but also the antidiabetic activity of the developed fermented milk [4].

The *Rosaceae* family comprises well-known fruits such as apples (*Malus domestica*), peaches (*Prunus persica*), strawberries (*Fragaria* × *ananassa*), etc., as well as some underutilized wild fruits, such as common medlar (*Mespilus germanica*) and Mediterranean hawthorn (*Crataegus azarolus*). Their extracts contain bioactive compounds, such as anthocyanins and flavonoids, and therefore, they may promote human health due to their antioxidant, antimicrobial, anticancer and anti-inflammatory activities. In fact, several of these extracts were used in traditional medicine by people in Southeastern Europe, Turkey and Iran, mainly because of their antidiabetic, diuretic and antidiarrheal actions, as well as the fact that they can remove stones from the kidneys and bladder, thus improving the performance of the liver and kidneys [6–9]. In addition, some species of the *Rosaceae* family are officially referred to as herbal medicines in the pharmacopoeias of many countries like France, China, England and Germany, since they enhance myocardial contraction and conduction, protect against ischemia, have a calming effect by reducing oxidative stress, limit arrhythmia, improve the flow of coronary vessels and are effective for urogenital problems [9,10]. Both plant species, *Crataegus azarolus* and *Mespilus germanica*, are typical climacteric fruits which have gained value for human consumption and commercial importance in recent years, attracting researchers to study their chemical and nutritional compositions. Both fruit plants are native to the Mediterranean countries and their fresh fruits are consumed during the autumn season. Moreover, in Cyprus, they are utilized as in jams and jellies. To the best of our knowledge, no previous studies have focused on the use of these two fruit extracts as ingredients for the enrichment of fermented milk products. The addition of fruit extracts as ingredients in fermented milk products may improve their chemical, nutritional, and health-promoting bioactivities.

Considering the above aspects, the objective of this study was to select the optimal extraction parameters for the utilization of the bioactive properties of two fruits of the *Rosaceae* family, *M. germanica* and *C. azarolus*, and evaluate the effects of the addition of their extracts, at different concentrations in fermented milk drinks, on their microbiological and bioactive properties during storage.

2. Materials and Methods

2.1. Materials

Fresh jersey cow milk with 6.1% fat and 4.8% protein contents was supplied by Pantziaros Farm in Athienou, Larnaca (data by the supplier). Yo-mix 505 LYO 50 DCU yogurt culture (*Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*) was supplied by Danisco (Copenhagen, Denmark). *C. azarolus* fruits and *M. germanica* fruits were purchased from local fruit markets. Chicory root fiber (inulin) was purchased from Cosucra (Pecq, Belgium).

2.2. Preparation of Fruit Extracts

In order to study the properties of *C. azarolus* and *M. germanica*, the fruits were first washed, and their kernels were removed. Then, the fruit flesh was instantly frozen with the use of liquid nitrogen and grinded with a pestle and mortar for 5 min.

The extraction was performed under different conditions (Table 1) in order to investigate the antioxidant and antidiabetic properties of the extracts, and they were then optimized for their total phenolic content (TPC) and antidiabetic properties. During the

extractions, the mixtures were subjected to continuous shaking for 30 min with an orbital shaking incubator at 300 rpm (Lab Companion Si-600) in order to achieve homogeneity. All apparatuses used for the extractions were sterilized before use.

Table 1. Extraction conditions and codes.

Fruit Extract		T, °C	t, min	Fruit/Water Ratio, *w/v*
Crataegus azarolus	M1	60	30	1:10
	M2	60	60	1:10
	M3	60	30	1:20
	M4	60	60	1:20
	M5	80	30	1:10
	M6	80	60	1:10
	M7	80	30	1:20
	M8	80	60	1:20
Mespilus germanica	P1	60	30	1:10
	P2	60	60	1:10
	P3	60	30	1:20
	P4	60	60	1:20
	P5	80	30	1:10
	P6	80	60	1:10
	P7	80	30	1:20
	P8	80	60	1:20

The extracts were filtered and the supernatants were collected prior to their storage at a temperature of −20 °C until further analysis.

2.3. Total Acidity, TA

The TA of the fruit extracts was determined via direct titration using 0.1 M NaOH, using phenolphthalein as an indicator, until the pH reached 8.2. We used the milliliters of NaOH required to calculate TA, which is expressed as grams of malic acid per liter [11].

2.4. Total Soluble Sugars, TSS

To determine the TSS, 50 µL of diluted extract was added to 750 µL of anthrone reagent, followed by incubation at 100 °C for 10 min. Then, 150 µL of the mixture was transferred to a microplate, and the absorption was measured at 625 nm with a Multiskan™ GO Microplate Photometer (Thermo Fisher, Oslo, Norway). For the blank sample, 80% ethanol was used, and a standard curve of d-glucose was prepared. The results are expressed as mg d-glucose per 100 g of dry weight fruit [12].

2.5. Total Phenolic Content, TPC

The TPC was calculated according to the photometric method of Folin–Ciocalteu [13,14]. More specifically, 50 µL of diluted extract was mixed with 50 µL of Folin–Ciocalteu reagent (1:5 v/v) and 100 µL of sodium hydroxide solution (0.35 M) in each well of a microplate. The mixtures were incubated for 3 min, and the phenols were determined using a Multiskan™ GO Microplate Photometer (Thermo Fisher, Oslo, Norway) at 760 nm. A standard curve of gallic acid was prepared, and the results were expressed as mg gallic acid equivalents (GAE) per 100 g of dry weight fruit.

2.6. Total Flavonoid Content, TFC

The TFC was determined by mixing 100 µL of distilled water, 10 µL of $NaNO_2$ (50 g/L) and 25 µL of the diluted extracts. The mixtures were then incubated for 5 min, followed by the addition of 15 µL of $AlCl_3$ (100 g/L). The mixtures were incubated for another 6 min, and then 50 µL of NaOH 1 M and 50 µL of distilled water was added. The mixtures were then shaked for 30 s and the absorption was measured at 510 nm on Multiskan™ GO Microplate Photometer (Thermo Fisher, Oslo, Norway). A standard curve of rutin was

prepared, and the results were expressed as mg rutin equivalents (RE) per 100 g of dry weight fruit [15].

2.7. Antioxidant Properties

2.7.1. Ferric Reducing Antioxidant Power, FRAP

The FRAP measurements were determined by reacting 20 μL of the diluted extracts with 180 μL of FRAP solution (300 mM acetate buffer at pH = 3.6, 10 mM of TPTZ solution and 20 mM of ferric chloride solution in a ratio of 10:1:1, *v*/*v*/*v*) in each well of a microplate. After mixing the samples with the FRAP solution, the mixtures were left for 6 min at 37 °C, and then the absorption was measured at 595 nm with a Multiskan™ GO Microplate Photometer (Thermo Fisher, Oslo, Norway). A standard curve of iron sulfate was prepared, and the results were expressed as mmol $FeSO_4$ per 100 g of dry weight fruit [15].

2.7.2. 1,1-Diphenyl-2-picrylhydrazy (DPPH)

The radical scavenging activity was measured using a microplate DPPH assay [9]. More specifically, 150 μL of diluted extract was mixed with 100 μL of methanol and 100 μL DPPH methanolic solution (0.2 mM). The mixtures were then incubated for 30 min in the dark, and the absorbance was read at 515 nm using a Multiskan™ GO Microplate Photometer. The results were expressed as a percentage of free radical scavenging capacity.

$$\%\ \mathrm{DPPH} = \left(\frac{A_{515\mathrm{nm}}^{\mathrm{blank}} - A_{515\mathrm{nm}}^{\mathrm{sample}}}{\Delta A_{515\mathrm{nm}}^{\mathrm{blank}}} \right) \times 100 \quad (1)$$

2.8. Antidiabetic Properties

2.8.1. Inhibition of α-Glucosidase

For the determination of the inhibition of the enzyme α-glucosidase from the samples, 50 μL of the sample was mixed with 100 μL of α-glucosidase solution (1.0 U/mL) in 0.1 M phosphate buffer (pH = 6.9). Then, the mixture was incubated at 25 °C for 10 min, and 50 μL of 5 mM p-nitrophenyl-α-d-glucopyranoside solution (in 0.1 M phosphate buffer, pH = 6.9) was added. The mixture was then incubated again at 25 °C for 5 min, and then the absorption was measured at 405 nm [16] with a Multiskan™ GO Microplate Photometer (Thermo Fisher, Oslo, Norway). The inhibitory effect of α-glucosidase was expressed as:

$$\%\mathrm{inhibition}_{\alpha-\mathrm{glucosidase}} = \left(\frac{\Delta A_{405\mathrm{nm}}^{\mathrm{control}} - \Delta A_{405\mathrm{nm}}^{\mathrm{sample}}}{\Delta A_{405\mathrm{nm}}^{\mathrm{control}}} \right) \times 100 \quad (2)$$

2.8.2. Inhibition of α-Amylase

To determine the potential of the samples for α-amylase inhibition, the protocol described in [15] was followed, with some modifications. More specifically, 50 μL of the sample was mixed with 50 μL of α-amylase solution (1.0 U/mL in 0.02 M sodium phosphate buffer, pH = 6.9) and 10 μL of sodium phosphate buffer (0.02 M, pH = 6.9). The mixtures were incubated for 10 min at 37 °C. Then, 90 μL of 0.5% *w*/*v* starch solution was added, and the mixtures were incubated for 20 min at 37 °C. The reaction was completed by adding 100 μL of 3,5-dinitrosalicylic acid (DNS) solution of 5 mM to the mixtures and heating them in a water bath for 15 min at 95 °C. The mixtures were then left until they reached an ambient temperature. Then, 30 μL of each sample was transferred to a microplate, followed by dilution with 90 μL of deionized water. The absorption was measured at 540 nm using a Multiskan™ GO Microplate Photometer (Thermo Fisher, Oslo, Norway). The inhibitory effect of α-amylase was expressed as:

$$\%\mathrm{inhibition}_{\alpha-\mathrm{amylase}} = \frac{\left[\left(Ac^{+} - Ac^{-} \right) - \left(As - Ab \right) \right]}{\left(Ac^{+} - Ac^{-} \right)} \times 100 \quad (3)$$

where Ac^+ is the absorbance of 100% enzyme activity (only the solvent with the enzyme), Ac^- is 0% enzyme activity (only the solvent without the enzyme), As is the test sample (with the enzyme), and Ab is the blank (test sample without the enzyme).

2.9. Production of Enriched Fermeneted Milk Drink

2.9.1. Preparation of Freeze-Dried Extracts (FDEs)

The fruit extracts were freeze-dried using a commercial freeze dryer (Zirbus, Bad Grund, Germany) to produce freeze-dried extracts (FDEs). Table 2 shows the operating conditions of the freeze dryer used for the freeze drying of extracts.

Table 2. Freeze dryer operating conditions.

Stage	Pressure, Pa	T, °C	Time, s
D01	25	−65	550
D02	20	−65	600
D03	15	−65	800
D04	15	−67	999

2.9.2. Fermentation Process

Jersey cow milk was pasteurized at 72 °C for 15 s, cooled to 40 °C and then supplemented with inulin (2.0% *w*/*v*) and the freeze-dried extracts in two different concentrations, 1.5% *w*/*v* and 3.0% *w*/*v*, [17,18], for each fruit. The mixture was homogenized using an Ultra Turrax (IKA, Staufen, Germany) for 2 min at 5000 rpm and then for 3 min at 1500 rpm.

Next, the milk was inoculated with a 1.5% reactivated YO-MIX 505 yogurt culture (*Streptococcus salivarius* ssp. *thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*). The inoculated milk samples were kept at 40 °C to reach pH 5.2, and the fermented milk drinks were kept at 4 °C for 28 d.

2.10. Effect of the Storage of the Final Products on Microbial and Bioactive Properties

In order to determine the effect of cold storage on the microbial and bioactive properties of the final products, several parameters were tested on different storage days for each product. On days 1, 7, 14, 21 and 28, the pH and total lactic acid bacteria (MRS Medium (ISO 15214: 1998 [19]) were determined. Additionally, the bioactive properties (i.e., total phenolic and flavonoid contents and antidiabetic capacity) of the products were determined on the 1st, 14th and 28th days of storage.

2.11. Statistical Analysis

All measurements were made in triplicate, and the statistical analysis was carried out using the XLSTAT software 2023 5.1.1410.0 at a significance level at $p < 0.050$. All data were analyzed with one-way ANOVA (Tukey test), and all values were expressed as an average ± SD.

3. Results and Discussion

3.1. Physicochemical Characteristics of Fruit Extracts

The physicochemical characteristics of fruits are important indicators of their quality, i.e., maturation can be influenced by species and harvesting location.

The titratable acidity of the *C. azarolus* fruit extracts (expressed in malic acid g/L) ranged from 0.046 ± 0.005 g/L to 0.106 ± 0.005 g/L. The titratable acidity of the M3 and M7 fruit extracts was the lowest (0.046 ± 0.005 g/L), whereas M5 and M6 fruit extracts showed the highest acidity, with a value of 0.106 ± 0.005 g/L. The titratable acidity of the *M. germanica* fruit extracts was lower compared to the *C. azarolus* fruit extracts. The highest level of titratable acidity was observed in samples P5 and P6, with a value of 0.060 ± 0.009 g/L, and the lowest level (0.029 ± 0.005 g/L) was observed in sample P3.

The results regarding total soluble sugars were expressed as mg d-glucose per 100 g of dry weight fruit. The highest TSS of the fruit extracts was found in the *C. azaro-*

lus M4 and M8 samples, with values of 93.19 ± 0.09 mg d-glucose/100 g of dry fruit and 93.01 ± 0.13 mg d-glucose/100 g dry respectively, and for *M. germanica* P4 sample (55.63 ± 3.02 mg d-glucose/100 g dry). The lowest TSS was observed in sample M2 for *C. azarolus* (17.01 ± 0.30 mg d-glucose/100 g dry) and sample P1 for *M. germanica* (7.92 ± 0.06 mg d-glucose/100 g dry).

3.2. Phenolic Composition of Fruit Extracts

Phenolic compounds are secondary metabolites derived from plant tissues which exhibit a variety of biological effects, including antioxidant and antimicrobial activities due to their redox properties and chemopreventive properties.

In this study, two fruits, *C. azarolus* fruits and *M. germanica*, were extracted under different conditions (see Table 1) in order to determine their phenolic and flavonoid contents, as well as their antioxidant and antidiabetic properties. The results of the TPC and TFC contents of the *C. azarolus* and *M. germanica* fruits are presented in Table 3.

Table 3. TPC, TFC and antioxidant activity of fruit extract samples.

Fruit Samples		TPC, mg_{GAE}/100 g Fruit	TFC, mg_{RE}/100 g Fruit	Antioxidant Activity	
				FRAP, mmol $FeSO_4$/100 g Fruit	DPPH, %
Crataegus azarolus	M1	51.52 ± 4.32 [d]	82.96 ± 6.22 [c]	3998.70 ± 266.39 [e]	86.4 ± 0.0 [cd]
	M2	64.40 ± 14.12 [bcd]	24.33 ± 1.22 [e]	6770.44 ± 879.03 [de]	87.7 ± 1.0 [bc]
	M3	53.15 ± 10.63 [cd]	56.75 ± 4.54 [d]	14744.50 ± 226.27 [a]	92.5 ± 2.0 [ab]
	M4	69.82 ± 2.24 [abcd]	182.10 ± 8.15 [a]	13499.62 ± 1658.20 [ab]	97.4 ± 0.0 [a]
	M5	78.00 ± 0.72 [abc]	176.64 ± 5.72 [a]	5214.36 ± 546.33 [de]	85.0 ± 2.0 [cd]
	M6	94.30 ± 15.55 [a]	140.25 ± 12.19 [b]	5611.56 ± 881.41 [de]	81.0 ± 1.0 [d]
	M7	78.60 ± 5.77 [abc]	209.06 ± 31.47 [a]	10767.44 ± 197.17 [bc]	87.7 ± 4.0 [bc]
	M8	87.58 ± 8.08 [ab]	137.62 ± 13.04 [b]	8389.47 ± 1257.84 [cd]	94.1 ± 1.0 [a]
Mespilus germanica	P1	19.95 ± 4.62 [c]	107.16 ± 14.07 [b]	5151.23 ± 303.71 [b]	91.8 ± 1.0 [bc]
	P2	35.26 ± 8.51 [bc]	130.61 ± 13.55 [b]	6314.64 ± 280.30 [b]	90.9 ± 1.0 [c]
	P3	46.41 ± 9.09 [abc]	167.40 ± 30.00 [b]	4150.35 ± 618.07 [b]	97.3 ± 1.0 [a]
	P4	34.59 ± 6.51 [bc]	91.01 ± 8.27 [b]	12115.88 ± 259.32 [a]	97.8 ±0.0 [a]
	P5	70.01 ± 13.74 [a]	432.18 ± 59.31 [a]	5871.71 ± 1056.16 [b]	93.5 ± 1.0 [b]
	P6	59.10 ± 9.36 [ab]	539.40 ± 67.97 [a]	2167.40 ± 313.59 [b]	92.5 ± 1.0 [bc]
	P7	53.52 ± 5.37 [ab]	156.68 ± 25.22 [b]	12972.12 ± 554.99 [a]	97.9 ± 0.0 [a]
	P8	65.33 ± 7.62 [a]	664.66 ± 113.23 [a]	13415.89 ± 873.80 [a]	97.8 ± 0.0 [a]

Data expressed as the mean ± SD (n = 3). Different superscript letters in the same column indicate significant differences ($p < 0.05$).

The total phenol contents ranged from 51.52 ± 4.32 mgGAE/100 g fruit to 94.30 ± 15.55 mgGAE/100 g fruit for *C. azarolus*, being higher than the total phenol content of *M. germanica*. The highest content of phenolic compounds was observed in samples M6 (to 94.30 ± 15.55 mgGAE/100 g fruit) for *C. azarolus* and P5 (70.01 ± 13.74 mgGAE/100 g fruit) for *M. germanica*.

The highest flavonoid content among the *C. azarolus* extracts was observed in M7, followed by the M4 and M5 extracts. As for the *M. germanica* extracts, the highest value was observed in extract P8, followed by P6 and P5. Also, the *M. germanica* extracts tended to have higher contents of flavonoids in general, compared to the extracts of *C. azarolus*.

A few studies have reported on the total phenolic and flavonoid contents of *C. azarolus* and *M. germanica* extracts from several sources. The total amount of phenolic compounds for *C. azarolus* flower was found to range from 18.88 to 27.59 mg_{GAE}/g, and the total amount of flavonoids was in the range of 4.68 to 8.96 mg/g dry weight [10], while phenolic extracts prepared from the leaf and fruit peel/pulp of yellow and red azarole showed that the yellow azarole was significantly richer in polyphenols than the red fruit species. The highest total phenol content was observed in *C. azarolus* leaves (4006.27 ± 112.17 mg_{GAE}/100 g), followed by the fruit peel (2023.21 ± 47.05 mg_{GAE}/100 g) [20].

In a recent study, it was reported that the TPC and TFC values for *M. germanica* were 16.7 ± 0.3 mg_{GAE}/g and 2.30 ± 0.07 mg_{RUE}/g, respectively [21], while the total phenolic content in the methanolic extract of medlar leaves was 380.58 mg_{GAE}/g of dry extract and the total flavonoid content of the extract was 75.16 mg_{QE}/g of dry extract [22]. In another study, [23], the total phenolic content of fruits of *M. germanica* varied from 164 to 227 mgGAE/100 g. The highest flavonoid content was observed in *M. germanica* fruits collected from the north of Iran (0.90 mg/g dry extract), followed by *M. canescens* leaf (0.53 mg/g dry extract). The reported literature results show great variability, as factors such as fruit species, fruit ripeness stage, extraction conditions and methodologies play an important role.

3.3. Antioxidant Activities of Fruit Extracts

Antioxidant compounds can exert their effects through various mechanisms. The antioxidant activities of *C. azarolus* and *M. germanica* extracts were assessed using the DPPH radical scavenging activity and FRAP (Table 3). The highest antioxidant capacity was observed in M3 extracts (14,744.50 ± 226.27 mmol $FeSO_4$/100 g fruit) for *C. azarolus* extracts, followed by M4 extract (13,499.62 ± 1658.20 mmol $FeSO_4$/100 g fruit), while for *M. germanica*, the highest antioxidant activity was observed for P8 (13,415.89 ± 873.80 mmol $FeSO_4$/100 g fruit), followed by P7 (12,972.12 ± 554.99 mmol $FeSO_4$/100 g fruit) and P4 (12,115.88 ± 259.32 mmol $FeSO_4$/100 g fruit). Free radical scavenging capacity was very high in all the *C. azarolus* extracts, with M4 (97.4% ± 0.0) and M8 (94.1% ± 1.0) extracts having the highest capacity, while in *M. germanica* extracts, the extracts with the highest percentages were P3 (97.3% ± 1.0), P4 (97.8% ± 0.0), P7 (97.9% ± 0.0) and P8 (97.8% ± 0.0).

The antioxidant capacity of *M. germanica* and *C. azarolus* has previously been discussed. *C. azarolus*' antioxidant activity was 0.79 ± 0.10 mmol Fe^{2+}/g of dry extract using the FRAP assay. Both fruit extracts exhibited notable antioxidant activities due to the presence of phenolic compounds [24]. The highest antioxidant activity was observed in the leaves of *C. azarolus*, followed by the peel and pulp extracts, while syrup exhibited the lowest activity [20]. Similarly, the antioxidant activity of the methanolic extract of *M. germanica* leaves using the DPPH assay reported a 69.43% radical inhibition in vitro [22].

The high antioxidant activity of both fruit extracts is associated with the high content of polyphenols and flavonoids. It was also pointed out that this activity may also be influenced by the presence of some other compounds with high antioxidant activity, such as Vitamin C, pigments and tocopherols [25]. The results of this study showed that both fruit extracts can play an important role as a natural antioxidant source and could potentially provide health-promoting effects to consumers.

3.4. Antidiabetic Properties of Fruit Extracts

Globally, diabetes mellitus has emerged as a major metabolic disorder due to hyperglycemia. The main enzymes involved in breaking down carbohydrates in the human body are α-amylase and α-glucosidase; therefore, targets for the development of potential diabetic treatments could include the inhibitors of these enzymes. The highest % inhibition of α-glucosidase was observed in the M2, M5 and M6 extracts, with values of 76.05% ± 3.10, 85.33% ± 2.00 and 87.79% ± 8.00, respectively. All *M. germanica* extracts inhibited α-glucosidase activity at the level of almost 100%. Regarding the inhibition of α-amylase, the highest activity was observed in the M6 and P6 extracts, with values of 16.43% ± 2.34, 37.86% ± 1.47, respectively. Our results are in accordance with other studies demonstrating the high antidiabetic activity of medlar and hawthorn fruit extracts [7,23,26]. The antidiabetic activity of the medlar and hawthorn fruit extracts observed in this work could be attributed to the high contents of total phenolic compounds and flavonoids, as the literature reports that flavonoids such as quercetin, rutin, luteolin, quercetin-3-O-α-l-rhamnopyranoside and epicatechin gallate, as well as phenolic acids (p-hydroxycinnamic acid, protocatechuic acid, caffeic acid, syringic acid, ferulic acid, and ellagic acid), inhibit α-Glc [7].

The selection of the optimal extraction conditions for each fruit was performed based on the results for the TPC and the antidiabetic properties of each extract. More specifically, it seems that the M6 extract has the highest phenolic content and the greatest antidiabetic activity compared to the other seven extracts of the same fruit. Therefore, the selected optimal extraction conditions for *C. azarolus* were those of M6 extract: 80 °C, 60 min and 1:10 *w*/*v* (fruit/water). Correspondingly, P6 extract had the highest antidiabetic activity compared to the other seven extracts of the same fruit, while in terms of the total phenolic content, its value was not the highest, but it was quite high and satisfactory. Therefore, the selected optimal extraction conditions for *M. germanica* were those of the P6 extract: 80 °C, 60 min and 1:10 *w*/*v* (fruit/water).

3.5. Production of Enriched Fermeneted Milk Drink

It is well-known that milk and fermented dairy produces do not contain polyphenols. Therefore, it would be useful to enrich them by utilizing sources of plant origin in order to improve their nutritional and functional values.

Five different fermented milk drinks where prepared: (a) Sample 1 (S1)—no addition of FDE (Control); (b) Sample 2 (S2), containing the FDE of *M. germanica* at 1.5% *w*/*v*; (c) Sample 3 (S3), containing the FDE of *M. germanica* at 3% *w*/*v*; (d) Sample 4 (S4), containing the FDE of *C. azarolus* 1.5% *w*/*v*; and (e) Sample 5 (S5), containing the FDE of *C. azarolus* of 3% *w*/*v*. The fermented milk drinks were incubated at 40 °C until the pH reached 5–5.2.

It took approximately 4 h to achieve the desired pH value in S1 (control, pH 6.25), while for the samples containing the FDE of *M. germanica*, namely, S2, with pH 6.1, and S3, with pH 5.9, times of 3.5 h and 2.5 h were required to achieve pH values of 5.19 ± 0.02 and 5.14 ± 0.05, respectively. The extracts S4, with pH 5.9, and S5, with pH 5.5, required times of 3 h and 1 h to obtain pH values of 5.20 ± 0.01 and 5.12 ± 0.01, respectively. The fermentation time was significantly shorter at all the concentrations of the milk drinks containing FDEs compared to the control. The main reason for this was the lower initial pH when the fruit FDE was added to the milk. Moreover, increasing the concentration of the FDE decreased the fermentation time.

The viability of the lactic-acid-bacteria-population-fermented milk drinks during the 28 days of cold storage is shown in Figure 1. One day after production, the population of lactic acid bacteria varied significantly between samples within a range from 4.9 to 6.4 $\log_{10}$ cfu/mL. During storage, the LAB population increased, which corresponds with other studies which observed that plant substances showed increased microbial populations [27]. On the last day of storage (Day 28), the population ranged between 7.3 and 7.4 $\log_{10}$(cfu/mL) in all the samples, without the values differing significantly. These results are in agreement with the Codex Alimentarius commission [28], which states that LAB counts added as a starter culture must be >10^6 cfu/mL.

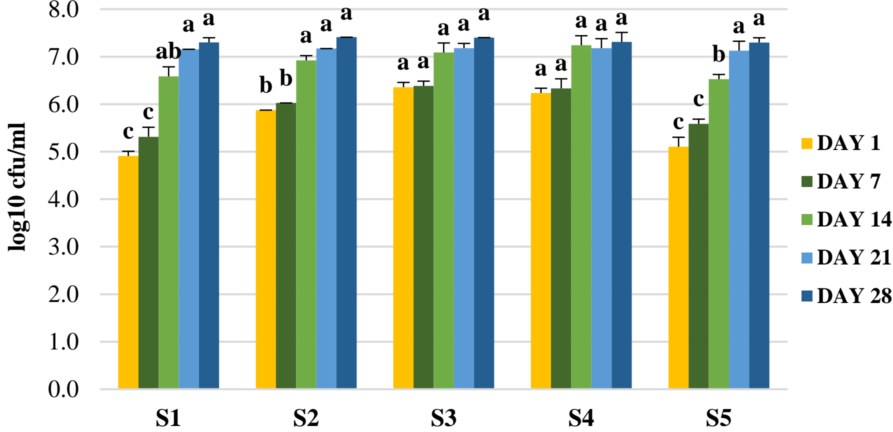

Figure 1. Lactic acid bacteria in enriched fermented milk drinks. Mean values with different letters. (a–c) were statistically different within the same day ($p < 0.05$). S = sample.

As shown in Figure 2, the value of pH gradually decreased significantly during cold storage. The pH of all the samples, on the first day after production, had similar values of 4.91 ± 0.01 to 5.18 ± 0.01. The highest drop in pH was observed on day 14 for all the samples. Moreover, the amount of *M. germanica* and *C. azarolus* FDE extracts had no effect on the changes in pH during cold storage. At the end of cold storage, the pH of the fermented milk drinks ranged from 4.38 ± 0.01 to 4.48 ± 0.01. During the cold storage, no significant differences in pH values were observed between the fermented milk drinks containing FDE extracts and the control. This indicated that the FDE of both fruits had no effect on the post-acidification rate. Similar trends in pH changes during the cold storage of fermented dairy products were observed by other authors, who added plant/fruit extracts and inulin [4].

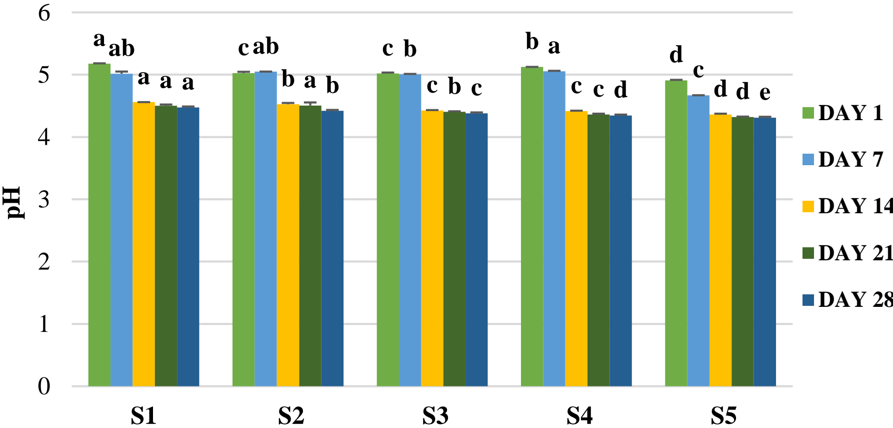

Figure 2. pH of final products during their storage at 6 °C. Mean values with different letters. (a–e) were statistically different within the same day ($p < 0.05$). S = sample.

As for the contents of phenolic components and flavonoids and the antidiabetic capacity of the products, these were determined on the 1st, 14th and 28th days of storage. The incorporation of the FDE of *M. germanica* and *C. azarolus* significantly increased both the TPC and TFC contents compared to the control fermented milk drink during the 28 days of cold storage, as shown in Figures 3 and 4. The increase in the TPC and TFC contents of the samples supplemented with FDE is due to the presence of plant-specific phytochemical compounds such as flavonoids and phenolic compounds. Moreover, the TPC content of the control is associated with the phenolic compounds created during milk protein proteolysis [29].

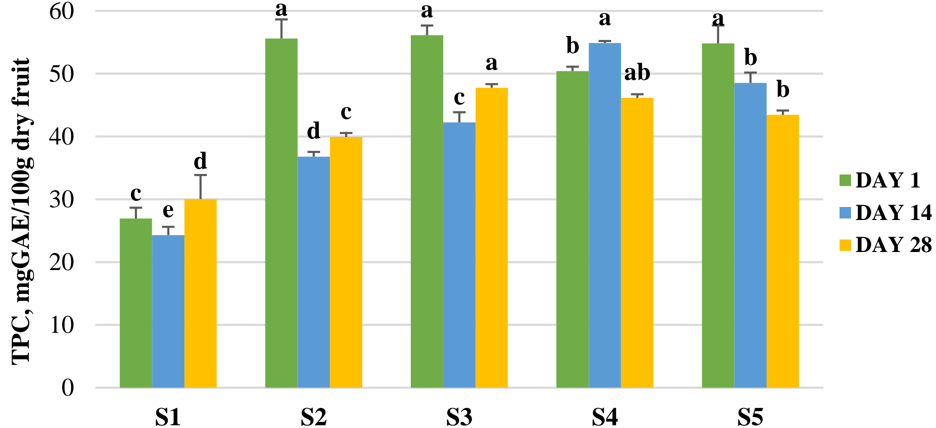

Figure 3. TPC of the final products. Mean values with different letters. (a–e) were statistically different within the same day ($p < 0.05$). S = sample.

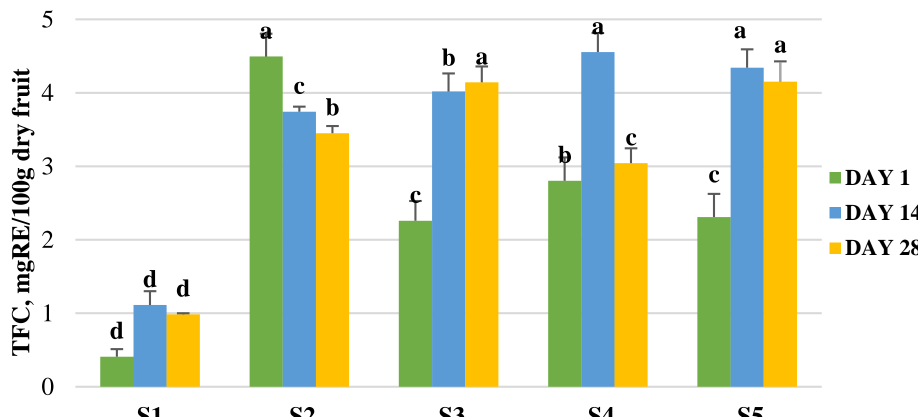

Figure 4. TFC of the final products. Mean values with different letters. (a–d) were statistically different within the same day ($p < 0.05$). S = sample.

The percentage of FDE extracts added into the dairy drinks did not significantly affect the TPC and TFC. The TPC content decreased during cold storage in both the control and fermented milk drinks supplemented with FDE extracts. Such a decrease in TPC could be due to the slow decomposition of phenolic compounds by LAB and the generation of aromatic acids such as phenyl propionic, acetic and benzoic acid during refrigerated storage. On the other hand, the TFC for all the samples, except sample S2, was higher on day 14 and day 28 compared to day 1. Our results indicate that both *C. azarolus* and M. *germanica* could be a good source for the production of novel bioactive fermented milk drinks.

The inhibitions of α-amylase and α-glucosidase by the beverage formulations during cold storage are illustrated in Figures 5 and 6. Both samples containing the FDE extracts of *M. germanica* and *C. azarolus* at two different concentrations showed higher α-amylase inhibition compared to the control during the cold storage period.

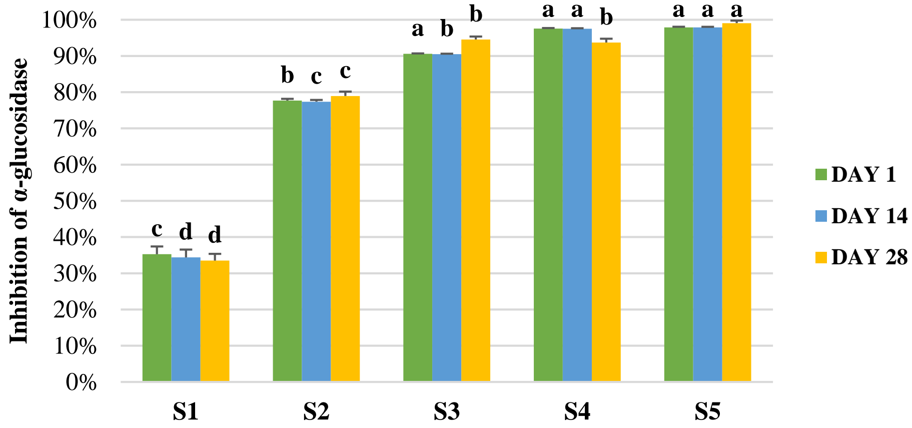

Figure 5. Inhibition of α-glucosidase in the final products. Mean values with different letters. (a–d) were statistically different within the same day ($p < 0.05$). S = sample.

The highest a-amylase inhibition was observed in S3 on day 1, with a % inhibition of 85.60% ± 3.07. There was a slight decrease in α-amylase inhibition after 14 days of cold storage, which remained stable until the end of the storage period (day 28). The breakdown of phenolic compounds and/or the interaction between milk protein and polyphenol might be responsible for the decrease in α-amylase inhibitory activity during the cold storage period [30]. Insoluble molecules can develop as a result of the interaction between phenolic compounds and proteins, which can negatively affect the in vivo bioavailability of both phenolics and proteins [31].

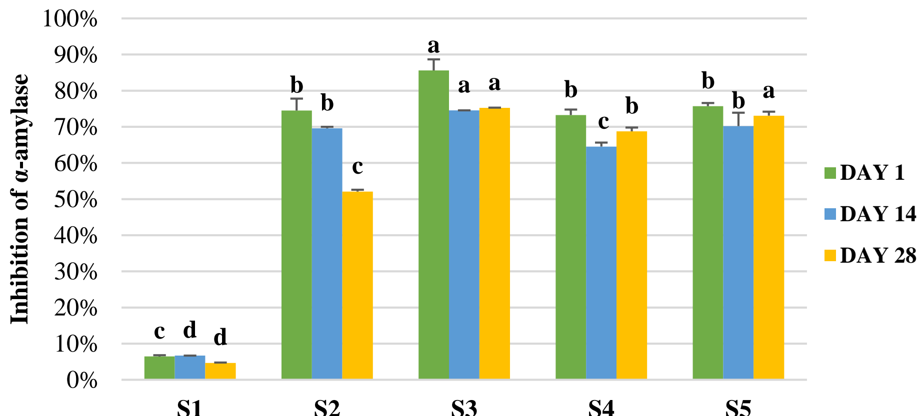

Figure 6. Inhibition of α-amylase in the final products. Mean values with different letters. (a–d) were statistically different within the same day ($p < 0.05$). S = sample.

Regarding the α-glucosidase inhibitory activity, this was also higher in the fermented milk drinks containing FDE extracts of *M. germanica* and *C. azarolus* compared to the control (Figure 5). The inhibition of α-glucosidase for in the samples remained stable during the 28 days of cold storage. The α-glucosidase activity in milk is essential for enhancing glucose absorption. Fermented dairy products have α-glucosidase inhibitory activities, and therefore, their consumption has been linked with a lowered risk of type 2 diabetes and helps to reduce post-prandial hyperglycemia [32]. FDE extracts can be added to milk to boost the inhibition of α-glucosidase and prevent the decline in this enzyme's inhibitory activity during the cold storage period. The present study showed that the addition of FDE extracts of *M. germanica* and *C. azarolus* at different concentrations positively enhances the natural ability of fermented dairy products to inhibit α-amylase and α-glucosidase activities as compared to a control.

4. Conclusions

The results of the present study demonstrated that the addition of freeze-dried *M. germanica* and *C. azarolus* extracts and fiber (inulin) during the fermentation of milk to produce a fermented milk drink significantly increased the total phenolic and flavonoid contents, the antioxidant capacity and the antidiabetic activity of the end-products. These functional properties, as well as the lactic acid bacteria population, remained viable during refrigerated storage for up to 28 days.

The promising results of this study support a potential strategy for developing non-conventional, added-value dairy products by utilizing local plant material. The health-promoting properties (in vivo) as well as the technological and sensory characteristics of the products should be investigated further.

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