Abstract: Nowadays, there is a growing consumer demand for non-dairy functional foods due to several health issues related to milk and dairy consumption and increasing vegetarianism. Following that trend, in the present study emmer-based beverages were developed after flour gelatinization, fortification with fruit juices (blueberry, aronia, and grape) and fermentation with the potential probiotic strain Lactiplantibacillus plantarum 2035. The produced beverages were subjected to a 4-week storage at 4 °C. The addition of juices significantly affected the physicochemical characteristics of the beverages, while resulting in increased red color. Total phenolic content (22.3–31.9 mg gallic acid equivalents 100 g−1) and antioxidant activity (94–136 µmol Trolox equivalents 100 g−1) were significantly higher in the case of aronia juice followed by blueberry and grape juice. All beverages showed high values of apparent viscosity and water-holding capacity. Lactiplantibacillus plantarum 2035 retained high viable counts during storage especially in beverages with fruit juices (>10^8 cells g−1 up to 21st day) revealing a positive effect of the juices. The obtained results show that emmer-based beverages fortified with fruit juices (aronia, blueberry, and grape) have a great potential as carriers of probiotics, prebiotics and other functional compounds and may be served as an ideal alternative to dairy products.

Keywords: total phenolic content; antioxidant activity; viscosity; color; Triticum turgidum ssp. dicoccoides

1. Introduction

The interest of consumers in the development of new functional or/and health-enhancing foods and their incorporation in a healthy diet is increasing and dairy products are leading in the field [1]. Lactose intolerance, milk protein allergies as well as high content of fat and cholesterol are considered health risks of dairy products intake [2,3]. Although lactose intolerance may be easily overcome with the addition of starter cultures like kefir [4], the growing trend of vegetarianism makes the development of non-dairy functional foods a necessity. Non-dairy beverages are useful alternatives [5] and among them, cereal-based beverages have a huge potential since they offer substantial amounts of vitamins, minerals, proteins, carbohydrates, fiber, oligosaccharides, antioxidants and prebiotics [6]. Although there are numerous traditional cereal-based beverages available, especially in Asia and Africa, their industrialization is difficult, and studies are needed to evaluate several technological characteristics [3].

Emmer wheat (Triticum turgidum ssp. dicoccoides) is the tetraploid progenitor of domesticated wheat and nowadays it is cultivated in low quantities, mainly due to low grain yields and poor bread-making properties [7]. However, in recent years this trend has been changing due to numerous health benefits associated with emmer wheat, such as high phytochemical content, and high antioxidant and anti-hyperglycemic properties [8]. Fermentation with lactic acid bacteria (LAB) is an ideal strategy to improve the functionality
and bioactivity of emmer, also revealing its prebiotic potential. Therefore, an increase in studies using emmer wheat is observed in products like Frankfurters [9], pasta [10], beer [11] and beverages [12,13]. However, the use of emmer wheat flour for the development of potential functional/probiotic fermented beverages is limited and, therefore, more studies are needed.

Among LAB, *Lactiplantibacillus plantarum* (former *Lactobacillus plantarum*) is one of the most studied species that has numerous applications in food industry as probiotic and/or starter microorganism. An important feature of this microorganism is its presence in several environments, in contrast to other well-known *Lactobacilli*, such as dairy products, vegetables, cereals, meat, silage, wine, gastrointestinal, vaginal, and urogenital tracts [14]. This characteristic provides the capability to easily adapt in different ecosystems. *L. plantarum* strains have been also used in novel and traditional functional foods and beverages with improved nutritional and technological features [15–17]. Among *L. plantarum* strains, *L. planatrum* 2035 presents excellent technological and functional characteristics and it has been successfully used in yogurt and fermented milk production, also presenting an antagonistic effect against *Listeria monocytogenes* [18,19]. Previous research documented its probiotic properties in vitro including among other things high adhesive capacity and immunostimulatory activity demonstrated by the dorsal mouse air pouch system [20], while it has been successfully used in a rat model and survived transit through the gastrointestinal tract, exhibited transient distinct adhesion to the intestinal mucosa, and modulated the systemic immune response [21].

The aim of the present study was the development of an emmer-based fermented beverage using a potential probiotic *L. plantarum* strain. In order, to further increase the functional properties of the beverage, several juices (grapes, aronia and blueberry) were added in the fermentation mixture, based on the numerous health benefits associated with juices and their characteristics that facilitate LAB/probiotics growth [2,22,23]. Furthermore, it is a usual practice to combine plant-based beverages with fruit products [24]. The novel emmer-based beverages were stored at 4 °C for up to 4 weeks and their physicochemical characteristics, color, viscosity, functional characteristics, and *L. plantarum* 2035 survival, were evaluated.

2. Materials and Methods

2.1. Food Materials

Emmer flour (organic *Triticum dicoccum* whole wheat; BDL organics, Greece) with the following characteristics (per 100 g): protein 12 g, carbohydrates 71.1 g (of which sugars 2.8 g), fat 2.8 g, and dietary fibers 15.7 g, was used. Water used to produce the emmer beverages was natural mineral water (Epirus Kostilatas Springs, Arta, Greece) with pH 7.7, total hardness 96 mg L\(^{-1}\) CaCO\(_3\) and conductivity 168.9 µS cm\(^{-1}\) at 20 °C. Pasteurized aronia juice (Aronia Konstantinidis, Greece; 126 g L\(^{-1}\) sugar) and blueberry juice (Zoi, Greece; 100 g L\(^{-1}\) sugar) were obtained from the market (natural juice without preservatives and added sugars). Furthermore, red grape juice (220 g L\(^{-1}\) sugar) was also used after dilution of concentrated juice with water.

2.2. Microorganism and Culture Media

*Lactiplantibacillus plantarum* 2035 (from the collection of the Laboratory of Food Microbiology and Hygiene, Department of Food Science and Technology, Aristotle University of Thessaloniki, Greece) was used for the production of emmer beverages. Its activation and growth was performed from a glycerol stock culture, which was stored at −80 °C, as described by Coda et al. [13] with some modifications. The strain was activated by two successive subcultures in De Man, Rogosa and Sharpe (MRS) broth, following incubation at 30 °C overnight. Cells were harvested by centrifugation at 6000× g for 5 min and then washed twice with Ringer’s solution and re-suspended in sterile water before added to the beverages.
2.3. Emmer Beverages Production

In order to develop the beverage formulation, preliminary experiments for emmer beverage composition using various emmer flour concentrations (40–300 g kg\(^{-1}\)) and amounts of juice (100–200 g kg\(^{-1}\)) were conducted. The selected composition was chosen based on appearance, texture, and preference for the final product. The produced emmer beverages consisted of (per 100 g): emmer flour (8 g), water (72 g) and juice (20 g). The production procedure of the beverages is described in Figure 1 and was as follows: firstly, emmer flour was mixed with water and left at 95 °C for 10 min for gelatinization. After cooling at 40 °C, the juice was added and homogenized. Inoculation of \(L.\) plantarum 2035 followed, and the mixture was left for 2 h at 30 °C to ferment. Then, cooling took place at 10 °C in an ice/water bath, and finally the produced beverages were stored at 4 °C for 28 days. Four beverages were produced including emmer with aronia juice (AEB), emmer with blueberry juice (BEB), emmer with grape juice (GEB) and control emmer beverage (CEB), with water instead of juice, for comparison reasons. Three batches of the produced beverages were prepared and analyzed.

![Figure 1. Protocol for the manufacture of beverages supplemented with juices from grapes and berries.](image)

2.4. Analyses

2.4.1. Total Acidity and pH

The pH of the beverages was measured using a portable, electronic pH-meter (SensoDirect pH 110, AQUALYTIC, Dortmund, Germany). Titratable acidity was determined by titration with NaOH. Specifically, 10 g of beverage and 90 mL of distilled water were homogenized using a magnetic stirrer. Titration with 0.1 mol L\(^{-1}\) NaOH followed until pH reached the value of 8.3 and the results were expressed as % w/w lactic acid.

2.4.2. Viscosity and Water Holding Capacity

Viscosity of the beverages was determined at 20 °C using a Brookfield viscometer (model DV-II+, Stoughton, MA, USA) equipped with a LV4 spindle. A sample of 150 g beverage was used to immerse the spindle and three readings were recorded after 15 s at different speeds (0.5, 1, 2.5, 4, 5, 10 and 20 rpm) [25,26]. The water holding capacity (WHC) was defined as: \(\text{WHC} (%) = 100 \left(\frac{B - \text{WE}}{B}\right)\), where \(\text{WE}\) = water expelled in g and \(B\) = initial beverage sample in g [12]. Water expelled (WE) quantity was obtained and weighed after centrifugation of 20 g of beverage (B) at 438 × g for 10 min at 20 °C.

2.4.3. Color

Color characteristics \((L^*, a^*, b^*, C^*, \text{and hue})\) were determined by a colorimeter [27].

2.4.4. Preparation of Beverages Extracts

Water/salt-soluble extracts were prepared according to Coda et al. [13] with some modifications. More specifically, 7.5 g of beverage and 30 mL of 50 mmol L\(^{-1}\) Tris-HCl (pH 8.8) were mixed. The mixture was placed at 4 °C for 1 h, while being vortexed every 15 min. Centrifugation at 12,000 × g for 20 min followed, and the supernatant was kept and used for the determination of reducing sugars. Moreover, for the determination of total phenolic content and free radical-scavenging activity, 3 g of beverage and 30 mL of 80% methanol solution were mixed for 30 min using a magnetic stirrer. The mixture was then centrifuged at 6000 rpm for 20 min and the supernatant was retained and used for the analyses.
2.4.5. Free Radical-Scavenging Activity (FRSA) and Total Phenolic Content (TPC)

Free radical-scavenging activity (FRSA) and total phenolic content (TPC) was measured using the methanolic extract of the beverage and the free radical DPPH• (2,2 diphenyl-1-picrylhydrazyl) and the Folin–Ciocalteu method, respectively [27]. TPC expressed as mg gallic acid equivalents (GAE) 100 g\(^{-1}\), using a calibration curve (10–60 \(\mu\)g GAE mL\(^{-1}\); \(R^2 = 0.9971\)), while FRSA as \(\mu\)mol Trolox equivalents (TE) 100 g\(^{-1}\), using a calibration curve (0.01–0.12 \(\mu\)M Trolox; \(R^2 = 0.9995\)).

2.4.6. Reducing Sugars

Reducing sugars (RS) were determined using the DNS (3,5-dinitrosalicylic acid) method, using Tris-HCl beverage extract and D-glucose as a standard [27].

2.4.7. Microbiological Analysis

Ten grams of each beverage sample were mixed with 90 mL of sterile \(\frac{1}{4}\) strength Ringer’s solution using a vortex. Serial decimal dilutions were prepared, and were plated on MRS agar. The counts of \(L.\) plantarum 2035 were enumerated after incubating the plates at 30°C for 72 h. The results were expressed as colony-forming units (CFU) g\(^{-1}\) of sample.

2.5. Statistical Analysis

Statistical analysis was performed using Statistica version 5.0 (StatSoft Inc., Tulsa, OK, USA). Experimental data (triplicate experiments and duplicate or triplicate samples) were evaluated for their significance (\(p < 0.05, 0.01\) and 0.001) with analysis of variance (ANOVA), and Tukey’s honest significant difference (HSD) test.

3. Results

3.1. Acidification Trend

Figure 2 presents the acidification trend in the course of emmer beverage fermentation. The initial pH values of beverages were affected significantly (\(p < 0.001\)) by the incorporation of the added juices. The low pH values of the juices led to initial pH of 3.99 for BEB, 4.69 for AEB and 5.61 for GEB. The maximum rate of pH decline occurred in GEB and then in CEB. In AEB and BEB a slight reduction was observed, which may be attributed to the lower initial pH affecting the starter culture. GEB presented the higher pH reduction mainly due to the high initial pH and the higher content of available sugars.

![Figure 2. Acidification kinetics during beverage fermentation. (CEB: Control Emmer Beverage; GEB: Emmer Beverage with Grape juice; BEB: Emmer Beverage with Blueberry juice; AEB: Emmer Beverage with Aronia juice).](image-url)
3.2. Physicochemical Characteristics and Antioxidant Activity

During storage, a downward trend of pH values was observed as a result of an expected post-acidification effect (Table 1). In all cases, the effect of storage time was significant ($p < 0.001$). A continuous decrease in pH detected in all beverages up to day 21 and then a slight, but not significant ($p > 0.05$) increase was observed. GEB and BEB resulted in similar pH values at the end of storage (28 days), in the range 3.35–3.37, followed by AEB with pH 3.56. CEB presented the highest pH value of 5.44. This is attributed to the initial drastic reduction of pH due to the addition of each juice. These results are in accordance with a previous study that reported values of pH below 4.00 after 28 days of storage in beverages using mixtures of cereals, soy and grape must [12]. Similar observations were reported in the case of acidity. More specifically, significant ($p < 0.001$) differences in acidity were observed between CEB and fruit beverages. Among fruit beverages, BEB resulted in the highest acidity (0.83% $w/w$), while GEB and AEB presented similar values (0.56 and 0.58% $w/w$, respectively).

The ability of *L. plantarum* to undergo continuous fermentation even at 4 °C for up to one month has been also reported in other studies with cereal beverages [28,29]. In the present study the highest decrease of pH compared to the initial value, was observed in the case of GEB and this may be attributed to the higher initial pH compared to other juices and the higher sugar content. In the case of BEB and AEB the reduction was lower probably due to the lower pH of juices that negatively affected the activity of *L. plantarum*. The high pH of CEB is attributed to the short fermentation period of 2 h. Indeed, in several studies with cereal beverages and *L. plantarum*, a fermentation from 2 days at 30 °C [29] up to 6 days at 35 °C [28] is required to reach a pH below 4.0. The pH value (ideally between 3.5 and 4.5) and the buffering capacity of the food play important roles in the stability of probiotics in the gastric tract [30]. Therefore, the pH observed in the cereal beverages of the present study and the reported protective role of cereals on *L. plantarum* strains [30] support the development of a functional probiotic cereal-based beverage fortified with fruit juices.

#### Table 1. Effect of storage on pH values, acidity and reducing sugars of the beverages.

| Analyses                  | Days         | CEB          | GEB          | AEB          | BEB          |
|---------------------------|--------------|--------------|--------------|--------------|--------------|
| **pH**                    | Initial      | 6.64 ± 0.01 $^{c,d}$ | 5.61 ± 0.08 $^{c,c}$ | 4.69 ± 0.06 $^{e,b}$ | 3.99 ± 0.08 $^{d,a}$ |
|                           | After 2 h fermentation | 5.79 ± 0.06 $^{b,d}$ | 4.15 ± 0.07 $^{d,b}$ | 4.42 ± 0.03 $^{d,c}$ | 3.80 ± 0.03 $^{c,d,A}$ |
|                           | 1            | 5.71 ± 0.01 $^{b,c}$ | 3.98 ± 0.08 $^{d,b}$ | 4.09 ± 0.02 $^{c,b}$ | 3.72 ± 0.05 $^{c,a}$ |
|                           | 7            | 5.69 ± 0.06 $^{b,c}$ | 3.66 ± 0.06 $^{c,AB}$ | 3.82 ± 0.02 $^{b,b}$ | 3.52 ± 0.06 $^{b,a}$ |
|                           | 14           | 5.66 ± 0.04 $^{b,c}$ | 3.56 ± 0.01 $^{b,c}$ | 3.57 ± 0.04 $^{a,b}$ | 3.36 ± 0.03 $^{a,b,A}$ |
|                           | 21           | 5.35 ± 0.05 $^{a,c}$ | 3.32 ± 0.02 $^{a,A}$ | 3.51 ± 0.04 $^{a,b}$ | 3.29 ± 0.01 $^{a,A}$ |
|                           | 28           | 5.44 ± 0.06 $^{a,c}$ | 3.37 ± 0.03 $^{a,b,A}$ | 3.56 ± 0.01 $^{a,b}$ | 3.35 ± 0.04 $^{a,b,A}$ |
| **Acidity (% w/w lactic acid)** | Initial      | 0.03 ± 0.00 $^{e,a}$ | 0.08 ± 0.02 $^{a,AB}$ | 0.16 ± 0.01 $^{b,b}$ | 0.30 ± 0.03 $^{c,c}$ |
|                           | 1            | 0.04 ± 0.00 $^{e,a}$ | 0.28 ± 0.00 $^{b,b}$ | 0.32 ± 0.02 $^{b,b}$ | 0.53 ± 0.03 $^{b,c}$ |
|                           | 7            | 0.04 ± 0.00 $^{e,a}$ | 0.39 ± 0.01 $^{b,c}$ | 0.38 ± 0.07 $^{b,c}$ | 0.58 ± 0.08 $^{b,c}$ |
|                           | 14           | 0.04 ± 0.00 $^{e,a}$ | 0.50 ± 0.01 $^{b,d}$ | 0.49 ± 0.01 $^{c,d}$ | 0.74 ± 0.01 $^{c,c}$ |
|                           | 21           | 0.07 ± 0.00 $^{e,a}$ | 0.56 ± 0.02 $^{b,d}$ | 0.54 ± 0.00 $^{d,b}$ | 0.82 ± 0.00 $^{d,c}$ |
|                           | 28           | 0.07 ± 0.00 $^{e,a}$ | 0.56 ± 0.00 $^{b,c}$ | 0.58 ± 0.02 $^{d,b}$ | 0.83 ± 0.04 $^{d,c}$ |
| **Reducing sugars (% w/w glucose)** | Initial      | 0.21 ± 0.04 $^{e,a}$ | 4.62 ± 0.18 $^{c,c}$ | 2.27 ± 0.04 $^{d,b}$ | 2.12 ± 0.16 $^{c,b}$ |
|                           | 1            | 0.09 ± 0.01 $^{e,a}$ | 3.99 ± 0.09 $^{b,c}$ | 1.54 ± 0.08 $^{c,b}$ | 1.53 ± 0.04 $^{b,b}$ |
|                           | 7            | 0.02 ± 0.00 $^{e,a}$ | 3.90 ± 0.07 $^{b,c}$ | 1.38 ± 0.04 $^{b,c}$ | 1.43 ± 0.06 $^{a,b}$ |
|                           | 14           | 0.03 ± 0.01 $^{e,a}$ | 3.84 ± 0.03 $^{b,d}$ | 1.30 ± 0.01 $^{a,b}$ | 1.38 ± 0.01 $^{a,b,c}$ |
|                           | 21           | 0.02 ± 0.00 $^{e,a}$ | 3.58 ± 0.22 $^{b,c}$ | 1.27 ± 0.00 $^{a,b}$ | 1.26 ± 0.06 $^{a,b}$ |
|                           | 28           | 0.02 ± 0.01 $^{e,a}$ | 2.76 ± 0.03 $^{a,c}$ | 1.15 ± 0.07 $^{a,b}$ | 1.21 ± 0.03 $^{a,b}$ |

*Means within a column at the same analysis with different lowercase superscripts differ significantly ($p < 0.05$); Means within a row with different uppercase superscripts differ significantly ($p < 0.05$); Values are mean ± SD of three independent experiments; (CEB: Control Emmer Beverage; GEB: Emmer Beverage with Grape juice; BEB: Emmer Beverage with Blueberry juice; AEB: Emmer Beverage with Aronia juice).*
The residual sugar, as another important parameter of the fermentation process, was also monitored. The initial sugar content of the emmer beverages was significantly affected by the added juices. The variation of the juices’ sugar content (10 °Brix, 12 °Brix, 22 °Brix for blueberry, aronia, grape juice respectively) resulted in the observed differences between the initial sugar content of the beverages. In detail, the sugar content was 2.12% w/w for BEB, 2.27% w/w for AEB and 4.62% w/w for GEB. For CEB, the sugar content was 0.21% w/w, which is similar to other studies with fermented emmer beverages [13]. Storage significantly (p < 0.001) affected the sugar content of all beverages and a continuous decrease was observed in all cases. The increased sugar content of fruit emmer beverages may explain the lower pH and increased acidity of GEB, AEB and BEB, since the *L. plantarum* cells had more sugars available to ferment and produce acids. In CEB the majority of initial reducing sugar content (0.21% w/w) was depleted during fermentation, and after the 1st week of storage it remained stable at low levels (0.02–0.03% w/w). The WHC values of the beverages were in the range 95.50–99.00% and they were similar to those reported for beverages made of a mixture of cereals, soy, and grape must [12]. During storage, the values of WHC remained stable in the case of CEB and GEB, while a slight reduction was observed in the other two beverages (AEB and BEB).

FRSA and TPC of the produced emmer-based beverages fortified with fruit juices are presented in Table 2. The TPC was significantly (p < 0.001) affected by the addition of juices. The highest and the lowest content were observed for AEB and CEB, respectively. The TPC values were similar for AEB and BEB and significantly higher compared to GEB and CEB. The fortification with fruit juices caused a 6-fold (grape juice) to 8-fold (aronia and blueberry juice) increase in TPC. The free radical DPPH• values, in terms of % inhibition and µmol Trolox equivalents 100 g\(^{-1}\), are shown in Table 2 which presents the in vitro antioxidant activity of beverages at the end of storage. Similarly to the TPC, the highest and the lowest antioxidant activities were observed for AEB and CEB, respectively. Previous studies, with yogurt-like beverages made of a mixture of cereals, soy, and grape must, verified the aforementioned observed results [12].

Table 2. Antioxidant activity and total phenolic content of the beverages after 28 days of storage.

| Beverage       | DPPH• % Inhibition | DPPH• µmol TE 100 g\(^{-1}\) | Total Phenolic Content mg GAE 100 g\(^{-1}\) |
|----------------|-------------------|------------------------------|-----------------------------------------------|
| CEB            | 3 ± 2\(^{\text{a}}\) | 12 ± 3\(^{\text{a}}\)       | 3.8 ± 0.3\(^{\text{a}}\)                      |
| GEB            | 52 ± 3\(^{\text{b}}\) | 94 ± 4\(^{\text{b}}\)       | 22.3 ± 2.2\(^{\text{b}}\)                     |
| AEB            | 77 ± 2\(^{\text{d}}\) | 136 ± 4\(^{\text{d}}\)      | 31.9 ± 0.7\(^{\text{c}}\)                     |
| BEB            | 65 ± 4\(^{\text{c}}\) | 116 ± 6\(^{\text{c}}\)      | 30.0 ± 1.7\(^{\text{c}}\)                     |

\(^{\text{a-d}}\) Means within a column at the same analysis with different superscripts differ significantly (p < 0.05); Values are mean ± SD of three independent experiments; (CEB: Control Emmer Beverage; GEB: Emmer Beverage with Grape juice; BEB: Emmer Beverage with Blueberry juice; AEB: Emmer Beverage with Aronia juice; DPPH•: 2,2-diphenyl-1-picrylhydrazyl free radical scavenging method; TE: Trolox equivalents; GAE: Gallic acid equivalents).

### 3.3. Viscosity

Viscosity is a significant parameter that affects a product’s shelf life and consumer acceptance, while it is vital for the optimization of the production process, selection of equipment and the industry operational design [25,31]. Furthermore, viscosity in beverages is related to satiety and postprandial hunger and affects postprandial carbohydrate and lipid metabolism [32,33]. For example, in a cereal beverage the reduction of viscosity led to attenuation of several beneficial effects [33]. Therefore, the viscosity of the produced beverages was recorded during storage. Since viscosity was dependent of rotational speed, the beverages of the present study were non-Newtonian and the so-called apparent viscosity was measured. A non-Newtonian behavior was also verified, in other studies, when the rheological behavior of non-dairy vegetable beverages [34] or cereal fermented beverages [25] was analyzed.
Employing the rotational speed and the apparent viscosity values to a power law model (Ostwald-de Waele), the consistency index (K) and the flow behavior index (n) were obtained (Table 3). While the flow properties of the beverages were characterized by the parameters n and K, the apparent viscosity values at 5 rpm, where used to compare the beverages. Changes in apparent viscosity of the beverages are presented in Figure 3.

**Table 3.** Power law model fitting data of flow behavior index (n), the consistency index (K) and regression coefficient ($R^2$) of beverages during storage at 4 °C.

| Analyses          | Days | CEB            | GEB            | AEB            | BEB            | Significance |
|-------------------|------|----------------|----------------|----------------|----------------|--------------|
| **Consistency index K (Pa s)** |      |                |                |                |                |              |
|                   | 1    | 10.07 ± 1.41   | 12.31 ± 1.41   | 14.86 ± 1.41   | 13.17 ± 1.41   | **           |
|                   | 7    | 7.39 ± 1.24    | 11.93 ± 1.00   | 8.31 ± 1.41    | 6.35 ± 1.41    | *            |
|                   | 14   | 14.32 ± 0.06   | 18.25 ± 2.22   | 8.90 ± 1.41    | 11.06 ± 1.34   | *            |
|                   | 21   | 11.85 ± 1.39   | 7.14 ± 0.88    | 18.80 ± 0.04   | 14.02 ± 0.84   | **           |
|                   | 28   | 15.56 ± 1.41   | 21.10 ± 1.41   | 35.20 ± 2.83   | 32.88 ± 2.83   | **           |
| **Flow behavior index n** |      |                |                |                |                |              |
|                   | 1    | 0.34 ± 0.03    | 0.37 ± 0.03    | 0.26 ± 0.03    | 0.31 ± 0.01    | *            |
|                   | 7    | 0.39 ± 0.01    | 0.31 ± 0.02    | 0.21 ± 0.01    | 0.29 ± 0.03    | *            |
|                   | 14   | 0.37 ± 0.00    | 0.37 ± 0.01    | 0.24 ± 0.01    | 0.32 ± 0.03    | *            |
|                   | 21   | 0.25 ± 0.03    | 0.26 ± 0.05    | 0.32 ± 0.05    | 0.39 ± 0.02    | ns           |
|                   | 28   | 0.36 ± 0.03    | 0.26 ± 0.03    | 0.32 ± 0.13    | 0.29 ± 0.01    | ns           |
| **Correlation coefficient $R^2$** |      |                |                |                |                |              |
|                   | 1    | 0.982 ± 0.002  | 0.996 ± 0.002  | 0.953 ± 0.004  | 0.995 ± 0.001  | ***          |
|                   | 7    | 0.972 ± 0.002  | 0.977 ± 0.002  | 0.995 ± 0.003  | 0.991 ± 0.001  | **           |
|                   | 14   | 0.998 ± 0.001  | 0.991 ± 0.010  | 0.953 ± 0.004  | 0.997 ± 0.001  | **           |
|                   | 21   | 0.993 ± 0.004  | 0.966 ± 0.013  | 0.995 ± 0.003  | 0.997 ± 0.001  | *            |
|                   | 28   | 0.993 ± 0.002  | 0.996 ± 0.001  | 0.996 ± 0.003  | 0.995 ± 0.001  | ns           |

a–c Means within a column at the same analysis with different lowercase superscripts differ significantly ($p < 0.05$); A–C Means within a row with different uppercase superscripts differ significantly ($p < 0.05$); Values are mean ± SD of three independent experiments; (CEB: Control Emmer Beverage; GEB: Emmer Beverage with Grape juice; BEB: Emmer Beverage with Blueberry juice; AEB: Emmer Beverage with Aronia juice); * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; ns: not significant.

**Figure 3.** Apparent viscosity values, at 5 rpm, of beverages during storage (CEB: Control Emmer Beverage; GEB: Emmer Beverage with Grape juice; BEB: Emmer Beverage with Blueberry juice; AEB: Emmer Beverage with Aronia juice).
The K values of all beverages showed the trend of viscosity during storage; with higher K values indicating an increase in viscosity [25,26]. The addition of juice significantly ($p < 0.01$) affected the initial K value of the beverages resulting in higher values compared to the control beverage. The $R^2$ values ranged from 0.953 to 0.998 for all samples. The flow behavior index ($n$) value indicates the difficulty degree of shear thinning, i.e., the pseudoplastic degree [35]. The $n$ values of all samples ranged between 0.21 and 0.39 suggesting that all beverages had a pseudoplastic behavior, also revealing slight differences during storage. This pseudoplastic behavior was also previously observed in the case of a cereal beverage produced with rice and pine nut [26] and a rice flour beverage fermented with LAB [36].

The juice/emmer beverages had the highest viscosity values, compared to the control beverage at the beginning of storage and up to the end (Figure 3). The addition of juices had a significant effect in the viscosity of the produced beverages, especially at the end of storage ($p < 0.001$). In the present study, a reduction of viscosity values was initially recorded, followed by a constant increase, reaching its highest values at the end of storage. A similar tendency in viscosity values was reported by Wang, et al. [37] in their seven-week storage study of an oat-based beverage.

The different viscosity of beverages during storage can be attributed to the rearrangement of proteins [38] or to the interactions of the different structural variations of pectin with the beverage components [35]. For example, the degradation/solubilization of pectin or the interaction between the protein and pectin content of the juices with β-glucan of the emmer flour, may have resulted in the fluctuations of the viscosity values during storage [26,39]. According to Gebruers et al. [40] emmer contains about 3–4 g kg$^{-1}$ of β-glucan (dry weight), which varies according to variety. Therefore, the presence of β-glucans in the added emmer flour may also be associated with the increase of viscosity. This result is supported by other studies including an orange beverage [38] and a high protein beverage [41] supplemented with oat β-glucan.

Moreover, the increase in viscosity may be caused by starch gelatinization [42], or by the addition of probiotic bacteria [43]. Russo et al. [44] also reported that *L. plantarum* Lp90 improved its probiotic potential and affected positively the viscosity of oat beverages.

The apparent viscosities of the samples in this study were affected by the storage temperature; a result also previously reported by Lee and Rhee [26]. An increasing tendency in viscosity, was also reported for rice bran beverages with strawberry and cocoa during a seven-week refrigeration storage [42], and for carrot beverages during a 28-day refrigeration storage [35].

### 3.4. Color Characteristics

Color is a primary characteristic that affects consumers’ preferences [45] and is used as a quality indicator during processing and storage. Anthocyanins is a class of flavonoids that is responsible for the color and different hues in berries, providing, more specifically, the reddish-purple pigment in them [46]. All emmer beverages produced were measured for color characteristics, and the results ($L^*$, $a^*$, $b^*$, $C^*$, and $\text{hue}$) during 4 weeks of storage are presented in Table 4. The $L^*$ coordinate indicates lightness, with the CEB being the lightest compared to the other samples, since the addition of juices caused the produced beverages to darken in color. Storage significantly ($p < 0.001$) affected the lightness of all beverages and an increase was observed after 28 days of storage, compared to day 0. This result was also confirmed by Gonzalez-Molina et al. [47], and it was attributed to the precipitation of various beverage components during storage. Perez-Vicente et al. [48] also reported an increase in color lightness of pomegranate juice after 160 days of storage. The use of different juice affected significantly ($p < 0.001$) the lightness and after 28 days of storage the lowest value was observed in the case of BEB (31.09) followed by GAB (46.13) and AEB (52.91).
An increase of $a^*$ value (redness) of the beverages was observed by the incorporation of juices, with the more intense that of blueberry juice. In detail, the $a^*$ value was increased significantly ($p < 0.001$) from 0.64 in the case of CEB, to 3.74, 5.68, and 10.55 for GEB, AEB, and BEB, respectively, indicating an increase in red color. Alteration in the color coordinate $a^*$ during storage, showed a significant ($p < 0.001$) increase for all beverages with juice, and a decrease ($p < 0.01$) for CEB. Therefore, CEB became less reddish and more greenish, whereas the red color was enhanced in the beverages containing juice during storage. The $a^*$ value remained higher for all beverages compared to CEB, which was expected since the addition of berries provided a reddish color to the beverage. At the end of storage (day 28), similar values were detected for GEB and AEB (9.38 and 10.31, respectively), and significantly higher ($p < 0.001$) in the case of BEB (19.37). An increase in $a^*$ value was also reported in a recent study with the same juices added in yogurts [21].

Simultaneously, the color coordinate $b^*$ was positive for all samples at the end of storage revealing the domination of yellow color. In general, the fortification of beverages with fruit juices caused a decrease of the color coordinate $b^*$ with the blueberry juice addition causing the highest reduction. These results can be supported by another study where, compared to the control, a more dark, red and less yellow yogurt produced by the addition of aronia juice [49]. Barba et al. [50] made similar observations for $a^*$ and $b^*$ color parameters of blueberry juice during refrigeration storage for 56 days. Similar results are also reported by Pereira et al. [51] for a fermented beverage of cashew apple juice, which were attributed to the pH reduction during storage. Furthermore, Sakamoto et al. [52] reported that changes in color might be due to changes in proteins or pectin contained in juices. Generally, color and pigment stability are influenced by many conditions and

### Table 4. Effect of storage on color characteristics of the beverages.

| Analyses | Days | CEB | GEB | AEB | BEB |
|----------|------|-----|-----|-----|-----|
|          |      | $L^*$ |     |     |     |
| 0        | 54.61 ± 0.07 | 39.36 ± 0.08 | 43.20 ± 0.01 | 29.15 ± 0.07 |
| 1        | 65.40 ± 0.23 | 53.91 ± 0.19 | 48.28 ± 0.03 | 31.74 ± 0.04 |
| 7        | 64.64 ± 1.18 | 50.09 ± 0.87 | 49.57 ± 3.69 | 35.28 ± 3.45 |
| 14       | 66.63 ± 1.75 | 54.57 ± 1.25 | 54.27 ± 0.65 | 32.68 ± 1.50 |
| 21       | 64.02 ± 0.98 | 47.50 ± 2.85 | 56.12 ± 0.04 | 34.49 ± 0.04 |
| 28       | 63.61 ± 0.87 | 46.13 ± 0.07 | 52.91 ± 0.07 | 31.09 ± 0.19 |
|          |      | $a^*$ |     |     |     |
| 0        | 0.64 ± 0.01 | 3.74 ± 0.02 | 5.68 ± 0.02 | 10.55 ± 0.09 |
| 1        | 0.51 ± 0.02 | 12.28 ± 0.05 | 6.56 ± 0.01 | 14.70 ± 0.04 |
| 7        | 0.09 ± 0.39 | 9.81 ± 0.24 | 7.71 ± 0.28 | 17.00 ± 2.21 |
| 14       | 0.07 ± 0.23 | 12.01 ± 0.2 | 8.06 ± 0.43 | 23.34 ± 1.52 |
| 21       | 0.13 ± 0.12 | 9.98 ± 0.11 | 9.16 ± 0.54 | 21.45 ± 0.60 |
| 28       | 0.30 ± 0.40 | 10.31 ± 1.02 | 9.38 ± 0.41 | 19.37 ± 0.29 |
|          |      | $b^*$ |     |     |     |
| 0        | 7.40 ± 0.01 | 1.04 ± 0.01 | 5.60 ± 0.02 | 2.11 ± 0.05 |
| 1        | 9.76 ± 0.10 | 6.23 ± 0.05 | 5.84 ± 0.01 | 3.03 ± 0.03 |
| 7        | 7.15 ± 0.35 | 2.81 ± 0.05 | 7.60 ± 1.02 | 3.50 ± 0.40 |
| 14       | 7.94 ± 0.09 | 4.53 ± 0.39 | 7.75 ± 0.59 | 5.75 ± 0.50 |
| 21       | 6.77 ± 0.39 | 2.41 ± 0.14 | 9.13 ± 1.43 | 4.90 ± 0.04 |
| 28       | 9.57 ± 0.03 | 3.87 ± 0.65 | 10.0 ± 0.39 | 4.96 ± 0.06 |
|          |      | $C^*$ |     |     |     |
| 0        | 7.42 ± 0.01 | 3.88 ± 0.02 | 7.98 ± 0.01 | 10.76 ± 0.07 |
| 1        | 9.77 ± 0.10 | 13.77 ± 0.07 | 8.79 ± 0.01 | 15.01 ± 0.03 |
| 7        | 7.16 ± 0.35 | 10.21 ± 0.24 | 10.84 ± 0.91 | 17.36 ± 2.24 |
| 14       | 7.94 ± 0.09 | 12.84 ± 0.26 | 11.18 ± 0.72 | 24.04 ± 1.62 |
| 21       | 6.77 ± 0.39 | 10.27 ± 0.14 | 12.94 ± 1.40 | 22.00 ± 0.58 |
| 28       | 9.38 ± 0.04 | 11.01 ± 1.18 | 13.70 ± 0.57 | 19.99 ± 0.39 |
|          |      | $\text{hue}$ |     |     |     |
| 0        | 85.05 ± 0.07 | 15.49 ± 0.14 | 44.59 ± 0.16 | 11.31 ± 0.34 |
| 1        | 87.03 ± 0.13 | 26.89 ± 0.11 | 41.69 ± 0.05 | 11.66 ± 0.13 |
| 7        | 89.42 ± 3.09 | 15.97 ± 0.19 | 44.40 ± 2.82 | 11.63 ± 0.20 |
| 14       | 89.51 ± 1.64 | 20.65 ± 1.58 | 43.85 ± 0.65 | 13.81 ± 0.49 |
| 21       | 88.96 ± 0.98 | 13.58 ± 0.64 | 44.66 ± 2.82 | 12.88 ± 0.43 |
| 28       | 88.22 ± 2.39 | 20.36 ± 1.42 | 46.84 ± 0.12 | 14.35 ± 0.16 |

a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u,v,w,x,y,z{a,b,c,d,e,f,g,h,i,j,k,l,m,n,o,p,q,r,s,t,u,v,w,x,y,z} Means within a column at the same analysis with different superscripts differ significantly ($p < 0.05$); Values are mean ± SD of three independent experiments; (CEB: Control Emmer Beverage; GEB: Emmer Beverage with Grape juice; AEB: Emmer Beverage with Blueberry juice; BEB: Emmer Beverage with Aronia juice).
parameters such as pH, light, oxidation, time, temperature as well as non-enzymatic and enzymatic browning [46].

The aforementioned results produced an increase in chroma for all beverages at the end of storage, making them significantly ($p < 0.001$) brighter from the production day. Similar results were obtained by other authors, who also provided an explanation through the increased concentration of polymeric pigments that stabilize the color, during large periods of storage, as evidenced in fruit juices and red wines [53,54]. Among beverages, BEB presented the higher values of chroma at the end of storage, followed by AEB, GEB and finally CEB. Therefore, BEB is characterized as the brightest, while CEB as the dullest.

Regarding the hue coordinate, all values are located in the first quadrant ($0^\circ$ to $90^\circ$), therefore between red-orange to yellow-orange [55]. The values of hue angle provide details of different colors, as follows: $0^\circ$ = red-purple, $90^\circ$ = yellow, $180^\circ$ = bluish-green and $270^\circ$ = blue [56]. The hue values were significantly affected ($p < 0.001$) by the addition of fruit juices to beverages. The hue value was significantly ($p < 0.001$) increased after storage in all beverages, with a less intense increase observed for AEB. The hue angle showed a less than $5^\circ$ variation between the 1st and the 28th day of storage for all samples, indicating the maintenance of the characteristic color.

### 3.5. Starter Culture Viability

Ensuring the viability of probiotics at fermentation and during the storage period is a vital concern for the development of probiotic fermented products. Table 5 presents changes in *L. plantarum* 2035 viable cell counts during refrigerated storage of the emmer beverages. The initial counts of *L. plantarum* 2035 were, in all cases, higher than 8 log CFU g$^{-1}$, which are also similar to those reported in other cereal beverages, based on oat, barley and malt flour, inoculated with *L. plantarum* [57]. The acid-resistant nature of *L. plantarum* 2035 can explain the high viable counts, present in all beverages, that were retained after 28 days of storage. The capability of this microorganism to survive in sufficient numbers even at low temperatures has also been previously demonstrated during yogurt and fermented emmer beverage production [13,18].

| Table 5. Viability (log CFU g$^{-1}$) of *L. plantarum* 2035 during storage of the beverages. |
|-----------------------------------------------|
| **Days** | **CEB** | **GEB** | **AEB** | **BEB** | **Significance** |
|---------|--------|--------|-------|------|-----------------|
| Initial | 8.08 ± 0.03 $^{aA}$ | 8.08 ± 0.03 $^{aA}$ | 8.08 ± 0.03 $^{aA}$ | 8.08 ± 0.03 $^{aA}$ | ns |
| 1       | 8.46 ± 0.10 $^{aA}$ | 8.54 ± 0.10 $^{aA}$ | 8.91 ± 0.15 $^{bB}$ | 8.55 ± 0.06 $^{dC}$ | ** |
| 7       | 8.26 ± 0.08 $^{bA}$ | 8.45 ± 0.06 $^{cAB}$ | 8.60 ± 0.10 $^{cB}$ | 8.40 ± 0.08 $^{dAB}$ | ** |
| 14      | 8.06 ± 0.11 $^{bA}$ | 8.25 ± 0.14 $^{bAB}$ | 8.46 ± 0.09 $^{cB}$ | 8.31 ± 0.11 $^{bAB}$ | < |
| 21      | 7.32 ± 0.16 $^{bA}$ | 8.00 ± 0.11 $^{bB}$ | 8.24 ± 0.05 $^{bB}$ | 8.17 ± 0.06 $^{bC}$ | *** |
| 28      | 7.18 ± 0.14 $^{bA}$ | 7.61 ± 0.18 $^{bB}$ | 8.04 ± 0.14 $^{bC}$ | 7.95 ± 0.15 $^{bBC}$ | *** |
| Significance | *** | *** | *** | *** | |

$^{a-d}$ Means within a column at the same analysis with different lowercase superscripts differ significantly ($p < 0.05$); $^{A-C}$ Means within a row with different uppercase superscripts differ significantly ($p < 0.05$); Values are mean ± SD of three independent experiments; (CEB: Control Emmer Beverage; GEB: Emmer Beverage with Grape juice; BEB: Emmer Beverage with Blueberry juice; AEB: Emmer Beverage with Aronia juice); $^* p < 0.05; ^{**} p < 0.01; ^{***} p < 0.001; ns: not significant.

The viability of *L. plantarum* 2035 during storage presented the same trend in all cases (with and without fruit juice addition). More specifically, an increase up to the 1st day of storage, was observed, followed by a continuous slight reduction. More specifically, after the addition of culture the numbers of *L. plantarum* 2035 increased 0.39 to 0.83 log CFU g$^{-1}$ during the 1st day, and then a reduction of 1.3 log CFU g$^{-1}$ in CEB and 0.6–0.9 log CFU g$^{-1}$ in the fruit beverages until the end of the storage period was observed.

After 28 days of storage the numbers of *L. plantarum* 2035 were significantly ($p < 0.001$) higher in the beverages fortified with fruit juices (7.61–8.04 log CFU g$^{-1}$) compared to CEB (7.18 log CFU g$^{-1}$). An explanation could be the increased content of phenolic compounds and reducing sugars that may enhance the viability of *L. plantarum* 2035.

The viability of *L. plantarum* 2035 in beverages during storage is considered very important since this strain has excellent functional characteristics, including high adhesive
capacity as previously documented in vitro [20]. In addition, *L. plantarum* 2035 has been successfully used in a rat model and survived transit through the gastrointestinal track, exhibited transient distinct adhesion to the intestinal mucosa, and modulated the systemic immune response [21].

Nowadays, information about the minimum effective concentrations of probiotics is still insufficient; however, it is generally accepted that probiotic products should have a minimum concentration of $10^6$ CFU mL$^{-1}$ or g$^{-1}$ and that a daily intake of $10^8$–$10^9$ probiotic microorganisms should be achieved, in order to exert a probiotic action in the consumer [58]. Similarly, Health Canada and the Italian Ministry of Health have established, among other requirements for probiotic foods, a minimum number of viable cells ($10^9$ CFU) administered per serving or per day respectively [59]. In the present study, the numbers of *L. plantarum* 2035 in all beverages during production and refrigerated storage (even after 28 days) were above 7 log CFU g$^{-1}$. Of note, the use of juice led to even higher viabilities of *L. plantarum* 2035 cells, up to 7.61, 7.95 and 8.04 log CFU g$^{-1}$ for GEB, BEB and AEB, respectively. Therefore, the requirement for a daily intake of $10^8$–$10^9$ probiotic microorganisms, may be easily achieved with the consumption of 10–100 g of emmer beverages.

4. Conclusions

The possibility of producing a novel non-dairy functional beverage using emmer flour, fruit juices and potential probiotic lactic acid bacteria is clearly demonstrated in the present study. This new beverage combines the benefits of cereals and juices and provides a nutritious alternative to dairy products. The conclusions of the present study are presented below:

- Total phenolic content and antioxidant activity were significantly increased by the addition of juices.
- The probiotic cells maintained a high viability throughout 28-day storage.
- Lower pH values (3.35–3.56 compared to 5.44 in control), higher viscosity and increased red color of the emmer-based beverages fortified with fruit juices were observed.

Novel cereal-based products with enhanced functional characteristics can be designed and developed based on the promising results of the present study. More research is undoubtedly needed in terms of sensory evaluation and consumer acceptability as well as probiotic cells survival during digestion of the produced beverages. Moreover, using in vivo studies could provide valuable information on the potential health benefits of these beverages.

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