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(RESEARCH ARTICLE)

Functional probiotic yoghurt production with black mulberry (*Morus nigra* L.) juice concentrate fortification

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Abstract

In this study, probiotic yoghurts (PY_X and PY_Y) were produced with cow's milk with 13 % dry matter standardized concentration by 3 % skim milk powder addition and Black Mulberry (*Morus nigra* L.) juice concentrate (BMJC) fortification at different ratios (1 % v/v and 2 % v/v) One sample was produced as plain yoghurt (PY). The samples were stored at +4 °C±1 for 14 days. Microbiological, sensory properties as well were analyzed at the 1st, 5th, 10th and the 14th days of the storage. The relation between BMJC fortification and viability and numerical increase of probiotics were significant ($p<0.05$). The increase in BMJC level improved the mentioned parameters. In the study, the relation between BMJC fortifications and the microbiological of yoghurts were significant. Conclusively, 1 % (w/v) and 2 % (w/v) BJC fortification improved the functional properties of yoghurt samples.

Keywords: Black Mulberry; *Morus nigra*; Probiotic yoghurts

1. Introduction

Yogurt, the best carrier of probiotics, traditionally is manufactured using *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp. bulgaricus (L. bulgaricus)* as starter cultures [1]. Probiotics are referred to as 'live microorganisms, which when administered in adequate amounts confer a health benefit on the host [2]. The majority of commercial probiotics are Lactobacillus and bifidobacteria species used in products such as yogurt, milk powder and frozen desserts [3-4]. They produce short-chain fatty acids and improve the intestinal microbial balance, resulting in the inhibition of bacterial pathogens, reduction of colon cancer risk, improving the immune system and lowering serum cholesterol levels [4]. Probiotic dairy product should contain at least 10^6 -10^7 cfu/mL of viable probiotic bacteria at the time of consumption [5]. However, in fermented products various probiotic lactobacilli and bifidobacteria show the decline in their viability during storage [6]. Today there has been increase in trend to fortify the dairy product with fruits and fruit parts to improve their nutritional value and the taste.

Foods based on fruit and vegetables, such as fruit and vegetable juices, represent a new potential carrier and source of probiotic microorganisms [7-8]. Raw and fermented vegetables also represent an excellent vehicle for probiotics due to their natural structure that allows the easy availability of useful nutrients for microbial growth [8-9]. Some operations such as peeling and cutting performed on minimally processed products can favor the availability of nutrients, such as sugars, vitamins, and minerals needed for probiotic growth [9]. Moreover, most fruits and vegetables contain prebiotic ingredients that promote the growth of beneficial microorganisms. But not all probiotic strains added to fruits and vegetables give good results in terms of survival. In fact, numerous factors influence microbial growth, such as salt, acidity, and pH. The viability of probiotic microorganisms in food matrices depends on several factors, such as storage temperature, oxygen levels, pH, and the presence of competitor microorganisms, which must all be carefully evaluated

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before they are added to foods [10]. Also in fruit and vegetable juices, the tolerance to acidity is particularly important. These juices are already naturally acidic; the fermentation process increases the acidity. Nevertheless, results the presence of proteins, minerals, fiber, and other nutrients promotes the survival of microorganisms, introducing a new concept of symbiotic food [11]. Therefore, vegetable and fruit juices (like carrot juice, melon juice) have been tested to assess their suitability to be used as a carrier of probiotics. In the research results carrot and melon juices is rich in sucrose, glucose, and fructose, possible sources of carbon for bifidobacteria. However, in this case, only glucose and sucrose have been used by microorganisms [12-13].

The fermentation of fruit-based probiotic products requires proper selection of microbial strains, the type of fruit juice with appropriate physicochemical properties and cultivation conditions for the optimal growth. The optimum cultivation factors include the water activity, processing and storage temperature, oxygen content and mechanical stress [14]. Results revealed that fruit juices might be suitable to be a medium to cultivate probiotic lactic acid bacteria. This is mainly due to the favorable acidic pH of fruit juices, typically between 2.5 and 3.7 for the growth of probiotics [15]. Possibly, the low pH and high acidity of fruit juices appear to be good for the survival of probiotic strains, even though they are not growing in the juice. The strains are able to resist the acidic condition after transported from stomach to intestine. Their impressive acidic tolerance makes them eligible to be developed into functional supplements in fruit juices [15]. Fruit juices are also good sources of saccharides for the growth of probiotic bacteria, and rich in phenolic compounds for the inhibition of pathogenic microorganisms [3, 16]. Dairy-based probiotic foods and drinks are one of the industrial scaled production of probiotic products in the market. Several researchers explained about the physicochemical composition of milk which is rich in protein and substantial lipid amount could be a protective matrix for probiotics [17].

Mulberry (Morus species) juice obtained from black mulberry (BM, (*Morus nigra* L) contains rich source of bioactive compounds including phenolic substances like flavonoids and anthocyanins with high antioxidant activity [18]. Flavonoids have long been recognized to possess anti-inflamma-tory, antioxidant, antiallergic, hepatoprotective, antithrombotic, antiviral and anticarcinogenic activities. This phytonutrient protects our body from the harmful oxidation of free radicals. Specifically, mulberry contains cyaniding 3-glucoside, which epidemiological studies confirm lowers the risk of many degenerative diseases such as chronic arthritis and antherosclerosis. Cyaniding 3-glucoside protects the body against cardiovascular disease and diabetes. Mulberry along with improving blood circulation can help people who suffer from heart palpitations. Fresh mulberry fruits are rich in protein (2.5 %), total fat (2 %), vitamins (especially vitamin C level 61 %, vitamin K level 6.5 %), carbohydrates (7.8-9 %) and mineral, such as Zn, Mn, Fe, Ca that are indispensable for the human body. In addition, mulberry fruits are also rich in pectin and fibrin. Ascorbic acid content is as high as 20mg/100g in fresh fruit [19]. Mulberry fruits are an excellent source of the antioxidants resveratrol, zeaxanthin, litein and to a lesser extent the alpha and beta carotene. Isolation of lactic acid bacteria (LAB) from fruits and vegetables have frequently been reported [20]. Mulberry (Morus spp.) is an important fruit in Turkey, with a production rate of 74,600 tonnes in 2013. Approximately, 95 % of the mulberry trees grown in Turkey are Morus alba (white mulberry), while the remaining are *Morus rubra* (red mulberry) (3 %) and M. nigra (black mulberry) (2 %) [21]. Black mulberry juice is a potential source of anthocyanins that have high antioxidant activity and thus many health benefits [22,23]. However, black mulberry (BM) phytochemicals, mainly anthocyanins, are labile to heat treatment and storage depending on temperature, light and pH [24]. Black mulberry juice is well-known not only for its nutritional quality and distinctive flavor, but also as a good source of several bioactive phytonutrients [25] In general, the fruits of Mulberry were evaluated as a rich source of carbohydrates and sugars (respectively sucrose, glucose, fructose) [26].

In this study, probiotic yoghurts were produced with cow's milk with 13 % dry matter standardized concentration by 3 % skimmed milk powder addition and black mulberry juice concentrate (BMJC) fortification at different ratios (1 % v/v and 2 % v/v). Yoghurt samples were stored for 14 days at +4 °C±1, and microbiological analysis were conducted on the 1st, 5th, 10th and 14th days of the storage.

2. Material and methods

2.1. Material

Raw cow's milk (CoM) used in the study was obtained from Ege University Department of Animal Science, black mulberry (*Morus nigra L.*) used in the production of black mulberry juice concentrate (BMJC) was obtained from a local producer in, Aydın (Turkey), skim milk powder (SMP) was obtained from Pınar Sut Inc. (Turkey), probiotic yoghurt culture YO-MIX 205 (*Str. thermophilus + Lb. bulgaricus, + Lb.acidophilus*) (Danisco-FRANCE) and freeze-dried yoghurt culture BIFI (*Bifidobacterium ssp.*) was obtained from CSL laboratories (Strade per Merlino, 3- 26839, Italy). Probiotic yoghurt production was conducted in Ege University Dairy Technology Pilot Plants.

2.2. Black mulberry juice concentrate (BMJC) production

In BMJ production, black mulberry was washed with water, separated to their pieces and pressed in press (Bucher-Guyer, Niederweningen, Switzerland) under 0.3-0.5 bar for 5 second and the juice was clarified. In the clarification of BMJ, 0.5 g/L (Sigma-Aldrich) gelatin which was determined as a result of preliminary trials was added to the black mulberry juice and waited for 15 minutes. Then 0.3 g/L bentonite was added (Sigma-Aldrich) and kept at water bath at 50ºC for 30 minutes. Clarification process was applied to the black mulberry juice and cooled to room temperature. Black mulberry juice was filtered through a filtration system consisting of cheesecloth and filter paper and separated from the sediments. Then it was concentrated (BMJC) to 62° Brix value in a laboratory type rotary evaporator (SCILOGEX RE 100-Pro/20 - 280 rpm) at 85±1 °C and stored at 4 °C ± 1. Total dry matter, water soluble dry matter (° Brix), pH and titratable acidity of BMJ were determined on the 0th day of the storage.

2.3. Probiotic yoghurt (PY) production

In this study, probiotic yoghurts (PY_X and PY_Y) were produced with cow's milk with 13% dry matter standardized concentration by 3% skimmed milk powder addition and black mulberry (*Morus nigra L.*) juice concentrate (BMJC) fortification at different ratios (1% v/v and 2 % v/v) and 6% starter culture (*Str. thermophilus + Lb. bulgaricus+ Lb. acidophilus* and *Bifidobacterium ssp.*) mixture (1:1). Cow's milk was divided into 3 batches in yoghurt production. BMJC was added before pasteurization in order to maintain the degradation of anthocyanins [27] and make a lesser effect on the color properties of yoghurt samples. Accordingly, the 1st batch was the plain batch while the 2nd batch was fortified with only 1% (w/v) BMJC (X) and the 3rd batch was fortified with 2% (w/v) BMJC (Y). The batches were then homogenized with Ultra Turrax Blender (at 1200 rpm for 40 seconds) (IKA, Merc, Germany) and pasteurized at 85 °C for 20 minutes. Then the samples were cooled to 42-43 °C and inoculated with 6% (v/v) starter culture. The samples were distributing to plastic cups (200 g) and left to incubation. The incubation was ended at 4.60 pH (4.5 hours) and PY, PY_X and PY_Y probiotic yoghurt samples were obtained. Samples were stored for 14 days at 4 °C±1, and microbiological analysis were conducted on the 1st, 5th, 10th and 14th days of the storage.

2.4. Microbiological analysis

Starter culture counts of the yoghurt samples were performed according to International Dairy Federation standard method [28-29]. *L. acidophilus* and *Bifidobacterium spp.* Counts were determined according to International Dairy Federation standard methods [30-31].

2.5. Statistical analysis

Samples were examined with 3 parallels and 2 repetitions. SPPS version 15 (IBM SPSS Statistics) statistical analysis software was used for analyses. Significance according to analysis of variance (ANOVA) was tested according to the Duncan multiple comparison test at $p < 0.05$ level.

3. Results and discussion

In the study, in CoM, dry matter was determined 11.23%, fat 2.87%, protein 3.01%, lactose 2.85%, ash 1.49%, lactic acid 0.128%, pH 6.50, and viscosity was 2.72 cp (20 °C). Total dry matter content of BMJ at the 0th day was determined as 15.10% while water soluble dry matter was 13.19°Brix, pH was 4.02, titratable acidity was 1.328%.

3.1. Microbiological properties

Changes in *L. bulgaricus, S. thermophilus, L. acidophilus* and *Bifidobacterium spp*. levels in PY samples are given in Figure 1. In PY samples, development and viability of starter cultures improved as the BMJC levels increased. Indeed, probiotic content in PY_Y during storage was higher compared to that in PY_X. The relation between fruit concentrate level and the probiotic levels was significant ($p<0.05$). This also had a positive effect on the development and viability of probiotics. It was reported that serum separation decreased as the fiber ratio in yoghurt production increased [32]. It was also reported that low serum separation helped the preservation of the symbiotic relationship between the starter cultures and the viability [4]. In the study, the samples with the highest probiotic contents were sorted as PY_Y, PY_X and PY. Our study results were compatible with the studies reporting that starter cultures show a better development in the presence of some sugars (such as glucose, maltose) [33], and that the relation between this development and the fruit concentrate levels was significant [34-35]. It was reported that black mulberry juice contains high levels of sukroz and glucose and lower levels of fructose [26]. However, Sanchez et al. [36] reported that the predominant sugar was fructose (~61 %) followed by glucose (~39 %), while sucrose was presented only at trace level in mulberry fruits sampled from Spain and they also found big genotypic differences among clones for different sugars. Ozgen et al. [23] declared that fructose and glucose contents of 14 black and red mulberry genotypes ranged from 4.86 to 6.41 g/100 mL, and 5.50 to

7.12 g/100 mL, respectively. The present genotypes illustrated wide variability for the sugars, which may be ascribed to genetic factors, cultural applications, and ecological conditions (light, temperature, and humidity etc.) as also referred in the previous studies [37].

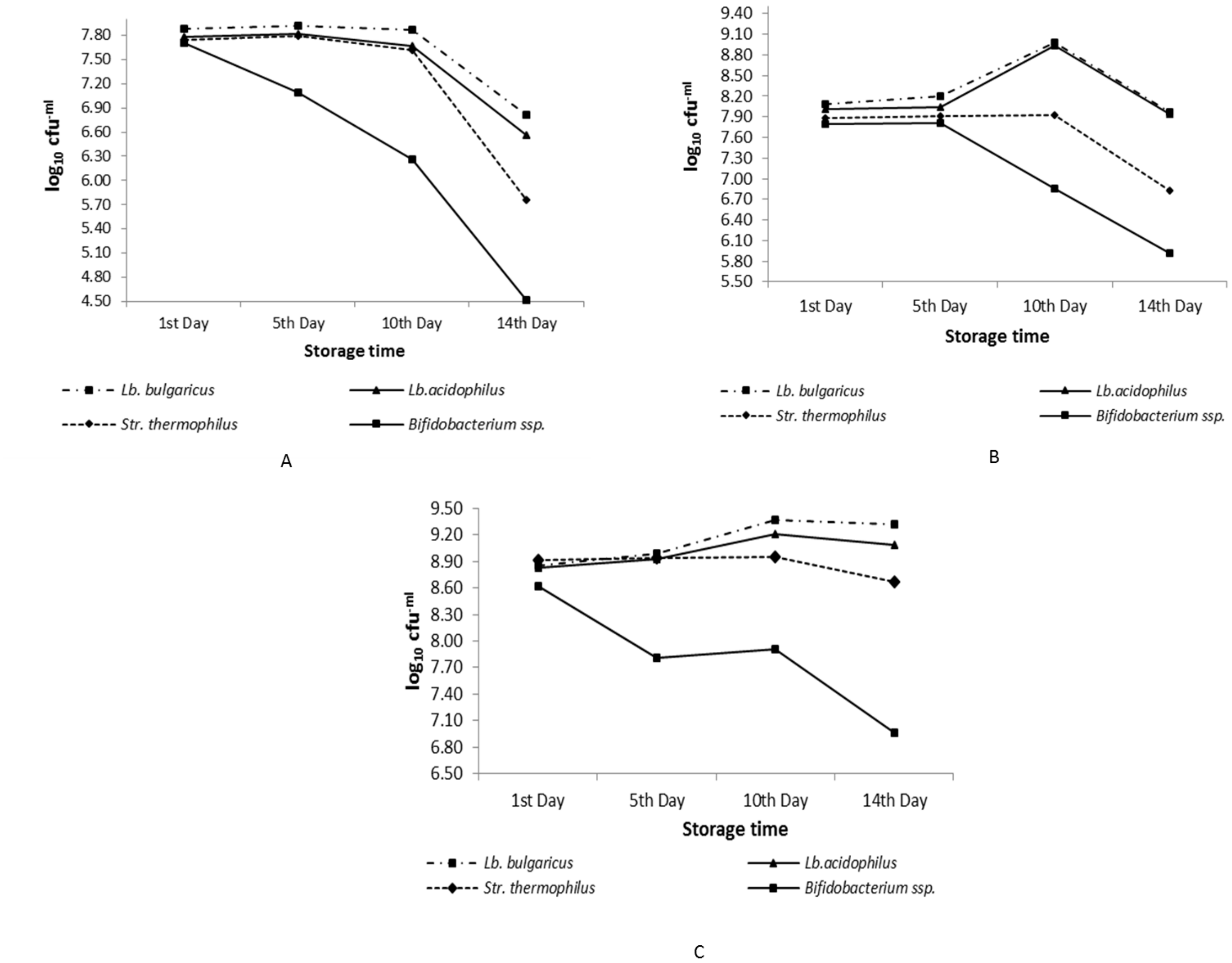

Figure 1 *L. bulgaricus, L. acidophilus, Bifidobacterium spp.* and *S. thermophilus* counts in PY (A); PY_X (B) and PY_Y (C) samples during storage

In general, probiotics in PY, PY_Y and PY_X increased from the 1st day of the storage. The highest increase in PY_Y and PY_X was determined at the 10th day while it was determined at the 5th day in PY. *Bifidobacterium spp*. level in PY decreased to 7.09 Log_{10} cfu/ml at the 5th day. Probiotic levels decreased after the 10th day in PY_Y and PY_X and after the 5th day in PY. The highest decrease was determined in PY, PY_X and PY_Y samples, respectively. The increase in PY_Y was higher than that in PY_X, whereas the decrease was lower. The increase in probiotics levels in PY was lower than those in PY_Y and PY_X, however the decrease was higher. Probiotics with the highest viability were sorted as *L. acidophilus* and *Bifidobacterium spp.* Microorganism level at the 1st day in PY was 7 Log_{10} cfu/ml, while it was 8 Log_{10}cfu/mlin PY_Y. *L. bulgaricus, L. acidophilus* levels in PY_X was 8Log_{10}cfu/ml, while *S. thermophilus* and *Bifidobacterium spp*. level was 7Log_{10}cfu/ml. The level of probiotics in PY_X was higher compared to that of PY. *Bifidobacterum spp.* levels in the final products were low in PY (4.52 Log_{10} cfu/ml) and PY_X (5.26 Log_{10} cfu/ml).

4. Conclusıon

In this study, 1 % (w/v) and 2 % (w/v) BMJC fortification used in the yoghurt production improved the textural, sensory and microbiological properties compared to those of PY. Higher levels of BMJC fortification improved these properties. The viability of probiotics in the final product increased with BMJC fortification. Conclusively, it was determined that probiotic yoghurt production with increased functionality is possible.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors have not declared any conflict of interests.

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