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Production of a potentially synbiotic fermented Cornelian cherry (*Cornus mas* L.) beverage using *Lactobacillus paracasei* K5 immobilized on wheat bran

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ABSTRACT

The aim of this study was the production of a novel, potentially synbiotic beverage by fermentation of Cornelian cherry juice using *Lactobacillus paracasei K5* cells, free or immobilized on wheat bran. Fermentations were carried out for 24 h followed by cold storage of the juices for 4 weeks. The fermentation performance of the free or immobilized *L. paracasei* K5 was monitored and proved by residual sugar and organic acids analysis, while the concentration of ethanol was maintained at low levels (0.2–0.7% v/v). The total phenolics content of the juice fermented with immobilized cells, after fermentation and during the whole cold storage period, was higher (205–287 mg GAE/100 ml) compared to the juice fermented with free cells (188–241 mg GAE/100 ml) and the juice that was used as control. The viability of the immobilized cells was maintained at higher levels compared to free cells (9.74 and 8.58 log cfu/ml, respectively) at the 4th week of storage. The results show that *L. paracasei* K5 cells immobilized on wheat bran can be successfully used to produce novel, synbiotic Cornelian cherry beverages with high nutritional value.

1. Introduction

There is currently an upsurge of industrial and scientific interest in developing functional foods, containing bioactive compounds or probiotic microorganisms, such as lactic acid bacteria. Functional foods are foods that can promote human health besides covering conventional nutritional needs (Neffe-Skocińska et al., 2018). In particular, probiotics are defined as live microorganisms that when administered in adequate amounts can confer various health benefits to the host (George Kerry et al., 2018). Probiotics are mainly delivered through fermented dairy products; however issues such as lactose intolerance, allergy to milk proteins, high fat and high cholesterol content, including the globally expanding vegetarian movement, have driven research to study new vehicles for the delivery of probiotics (Gupta and Bajaj, 2016; Lebaka et al., 2018). Specifically, fruit (Ephrem et al., 2018), vegetables (Martins et al., 2013), cereals (Gupta and Bajaj, 2016), as well as meat (Pasqualin Cavalheiro et al., 2015) have been proposed as successful food matrices candidates for the delivery of probiotics.

Specifically, the extensive interest on the development of fruit juice-based functional beverages with probiotics is mainly due to the (i) higher contents of vitamins, antioxidants and polyphenols, (ii) high amount of sugars that can be metabolized by the probiotic bacteria, and (iii) their appealing character to all age groups because they are generally recognized as healthy and refreshing foods (Danesi and Ferguson, 2017; Ephrem et al., 2018; Tuorila and Cardello, 2002). In addition, fruit juices do not contain cholesterol and allergens, such as lactose and casein, and can protect human health against several chronic and degenerative diseases (Wootton-Beard and Ryan, 2011).

Several fruit juices have been applied as substrates for lactic acid fermentation by probiotics such as pomegranate (Di Cagno et al., 2017), mulberry (Kwaw et al., 2018), elderberry (Ricci et al., 2018), apple (Roberts et al., 2018), orange (Espirito-Santo et al., 2015), various tropical fruit (Maldonado et al., 2017), etc. On the other hand, fruit juices are generally considered as a difficult matrix for the growth and survival of probiotics, e.g. due to their very low pH values. This is very crucial since the minimum concentration of probiotics in food products should be at least 10^6 cfu/g until the end of shelf life, in order for their health benefits to be delivered to the consumer (Neffe-Skocińska et al., 2018). Various solutions to this problem have been proposed such as the application of cell immobilization techniques, which can promote cell viability, activity, and functionality (Ephrem et al., 2018; Terpou et al., 2017b).

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Among fruit, Cornelian cherry (*Cornus mas L.*) juice has been moderately examined (Nematollahi et al., 2016; Nouska et al., 2016), and is currently receiving growing attention. Cornelian cherry contains significant amounts of phenolic compounds and vitamins that exhibit a wide range of biological and pharmacological properties, including antimicrobial activity against various pathogens, as well as anti-inflammatory, antimalarial, antihistamine, antidiabetic and antiatherosclerotic activities (Dinda et al., 2016; Moldovan et al., 2016). Therefore, the Cornelian cherry juice has the potential to be used for the production of functional food beverages (Nematollahi et al., 2016; Nouska et al., 2016).

The aim of this study was to develop a novel beverage by fermentation of Cornelian cherry juice with a potentially probiotic strain (*Lactobacillus paracasei* K5), free or immobilized on wheat bran (as a potential prebiotic carrier). The fermentation efficiency of the biocatalysts and their effect on the product composition were examined by analysis of residual sugars, organic acids, ethanol, total phenolics content, and sensory evaluations. The effect of each process on cell viability during fermentation and cold storage (4 °C for 4 weeks), was also evaluated.

2. Materials and methods

2.1. Microorganism

The novel, potentially probiotic strain *Lactobacillus paracasei* K5, priory isolated from dairy products (Plessas et al., 2017) was used. It was grown under anaerobic conditions at 37 °C for 24–48 h in MRS broth. Wet biomass was harvested by centrifugation (Sigma 3K12, Bioblock Scientific, France) at 5000 rpm for 10 min at 25 °C. All media were autoclaved at 120 °C and at 1–1.5 atm for 15 min prior to use.

2.2. Immobilized biocatalyst preparation

Delignified wheat bran (DWB) was prepared and used for the immobilization of L. *paracasei* K5 cells as described previously by Terpou et al. (2018b). Specifically, for the immobilization, 5 g of DWB were mixed with 1 g of harvested *L. paracasei* K5 cell mass in 500 ml MRS broth and were incubated at 37 °C for 48 h. The biocatalyst (DWB with naturally entrapped *L. paracasei* K5 cells) was washed with sterile 1/4 strength Ringer's solution for removal of free cells. The biocatalyst was frozen to − 44 °C by a cooling rate of 5 °C/min and was freeze-dried for 48 h at 5–15 mbar and at − 45 °C on a FreeZone 4.5 Freeze-Drying System (Labconco, Kansas City, USA).

2.3. Cornelian cherry juice fermentation

Ripe Cornelian cherry fruits were purchased from a local market in Orestiada (N. Greece). They were carefully selected and were blended with the addition of deionized water at a proportion 1:1 (Nouska et al., 2016). The juice was separated by pressing using a cheese cloth and was pasteurized at 80 °C for 5 min. The initial sugar concentration of the juice was 55 g/l and the pH value was adjusted to 3.5 by addition of 0.1 M NaOH sol. Afterwards, 2 g (dry weight) of free *L. paracasei* K5 cells mass (free cells; FC) or 2 g (dry weight) of the immobilized biocatalyst (immobilized cells; IC) were suspended per 100 ml of fermentation substrate. The substrates were allowed to ferment at 30 °C for 1 day and then the flaks were kept at 4 °C for 28 days. The fermentations were carried out in triplicate.

2.4. Ethanol and residual sugar analysis

Ethanol and residual sugar (glucose, fructose and sucrose) were determined by high performance liquid chromatography on a Shimadzu HPLC system (Shimadzu, Kyoto, Japan) consisting of a SCR-101N stainless steel column, a LC-9A pump, a CTO-10A oven set at 60 °C and a RID-6A refractive index detector. Ultra-pure water obtained by a Milli-Q water purifying system (resistivity 18.2 MΩ cm^{-1}) was used as mobile phase with a flow rate of 0.8 ml/min, and 1-butanol (0.1% v/v) was used as internal standard. Samples were filtered through 0.2 µm microfilters, before injection. Ethanol (% v/v) and residual sugar (g/L) concentrations were calculated using standard curves. Samples were collected at various time intervals (at 24 h and weekly until the 4th week of storage). All results are presented as means of at least three repetitions plus standard deviations.

2.5. Organic acid analysis

The analysis of lactic and acetic acid was carried out on an ion-exchange HPLC Shimadzu system consisting of a Shim-pack ICA1 column, an LC-10 CE pump, a CTO-10A oven, and a CDD-6A conductivity detector. A solution of 2.5 mM phthalic acid and 2.4 mM tris (hydroxymethyl) aminomethane (pH 4.0) was used as mobile phase (1.2 ml/min). The column temperature was 40 °C. The sample dilution was 5% v/v, and the injection volume was 60 µL. Determinations were carried out by means of standard curves.

2.6. Total phenolics

Total phenolics content was determined based on a colorimetric reaction using the Folin-Ciocalteu reagent (Singleton and Rossi, 1965). In brief, 1 ml of Folin-Ciocalteu reagent (10% w/v) was added to 0.2 ml of the prepared Cornelian cherry juice, followed by the addition of 1.2 ml of aqueous Na_2CO_3 (7.5% w/v). The mixture was left in the dark for 90 min. The absorbance of the blue colored solution, versus a blank solution, was monitored at 760 nm on a UV–vis spectrophotometer (Shimadzu, Kyoto, Japan). The total phenolics concentration of the samples was determined in triplicate, with the aid of a gallic acid standard curve, and the results were expressed as mg gallic acid equivalents (mg GAE/100 ml juice).

2.7. Microbiological analysis

The viability of *L. paracasei K5* cells was determined on acidified MRS agar (Merck, Germany) after incubation at 37 °C for 48–72 h (Plessas et al., 2017). Yeasts and fungi were enumerated on Potato Dextrose agar (PDA) (Merck, Germany) after incubation at 30 °C for 72 h. Coliforms were enumerated on Violet Red Bile agar (LabM, UK) after incubation at 30 °C for 24 h (Terpou et al., 2018a). Cell counts were expressed as log of colony forming units (CFU) per ml of juice. The results are presented as means of three repetitions plus standard deviations.

2.8. Sensory evaluation

Sensory properties (aroma, taste, and overall quality) of the non-fermented and fermented Cornelian cherry juices with free (FC) and immobilized (IC) L. *paracasei K5,* were evaluated as described previously by Plessas et al. (2008). The assessment was carried out by a panel of 30 non-trained laboratory members at days 1, 7, 14, 21 and 28.

2.9. Statistical analysis

The data obtained from the chemical analysis, the evaluation of cell viability and the sensory analysis of the non-fermented and fermented juices were analysed by Analysis of Variance (ANOVA), followed by Duncan's post hoc multiple range test, to determine any statistically significant differences among the various treatments. The analysis was performed using the IMB SPSS v20 (IBM Corp.) software at the alpha level of 5%.

3. Results and discussion

In this study, a novel, potentially probiotic strain *L. paracasei* K5, recently isolated from dairy products (Plessas et al., 2017), was used to produce a functional beverage by fermentation of Cornelian cherry juice. The stain was used as freely suspended starter culture, as well as immobilised on delignified wheat bran, to evaluate the possibility to produce a symbiotic, nutritious beverage by combination of a probiotic species with a prebiotic immobilization carrier. The fermentation performance of the biocatalysts was evaluated by analysis of the residual sugar, organic acids, and ethanol, as well as the total phenolics content of the fermented juices. The impact of immobilization on the survival of *L. paracasei* K5 in the fermented juice during storage at 4 °C, was also evaluated, as it is a critical parameter to be taken into account in the production of probiotic products. In addition, consumer-oriented sensory evaluations of the juices were carried out after 24 h of fermentation and during the 4 weeks of storage.

3.1. Ethanol, organic acids and residual sugar concentrations

The results of the chemical analysis of fermented Cornelian cherry juices by free (FC) and immobilized (IC) *L. paracasei K5* cells are presented in Table 1. The results showed that both free and immobilized cells showed good lactic acid fermentation ability in the juices, since the sugar concentration was continuously decreased and the organic acids were increased at all the time periods studied (Table 1). Specifically, the sugar concentration was reduced by approximately 56% (24.2 g/L), in the case of FC and by 68% (17.8 g/L) in the case of IC, at the 4th week of storage (statistically significant difference). Therefore, it seems that immobilization enhanced the fermentation activity of the *L. paracasei* K5 cells, as also reported by other studies involving Lactobacilli immobilized on wheat bran (Terpou et al., 2017a, 2018b). In addition, the residual sugar concentrations for FC were significantly higher at all the studied time periods compared to IC.

The concentration of lactic acid was increased (statistically significant) every week of analysis, reaching its maximum value at the 4th week of storage (105.4 mg/100 ml for FC, and 167.8 mg/100 ml for IC). Acetic acid was detected in the juices only after the 24 h of fermentation, with the maximum values achieved at the 4th week (7.7 mg/100 ml for FC and 10.9 mg/100 ml for IC). Is should be underlined that lactic and acetic acid production during the fermentation of Cornelian cherry juice by IC was at statistically higher levels compared to FC.

Ethanol production was observed in all cases, but at very low levels. Specifically, at the 4th week of storage, the ethanol concentration of fermented Cornelian cherry was increased from 0.2% v/v to 0.7% v/v and 0.9% v/v, for FC and IC, respectively, while no statistically significant differences were observed between the two types of biocatalysts.

Table 1
Analysis of sugars, organic acids and ethanol in Cornelian cherry juices fermented by free (FC) and immobilized (IC) *L. paracasei* K5 cells.

Parameter	Time	*L. paracasei* K5 biocatalyst	
		FC	IC
Sugars (g/l)	24 h	48.4 ± 0.2$^{A, a}$	47.2 ± 0.1$^{B, a}$
	Week 1	44.0 ± 0.2$^{A, b}$	39.8 ± 0.2$^{B, b}$
	Week 2	39.8 ± 0.1$^{A, c}$	30.4 ± 0.2$^{B, c}$
	Week 3	29.7 ± 0.1$^{A, d}$	23.7 ± 0.1$^{B, d}$
	Week 4	24.2 ± 0.2$^{A, e}$	17.8 ± 0.5$^{B, e}$
Lactic Acid (mg/100 ml)	24 h	14.7 ± 0.3$^{B, e}$	26.8 ± 0.2$^{A, e}$
	Week 1	45.9 ± 0.3$^{B, d}$	49.8 ± 0.2$^{A, d}$
	Week 2	68.3 ± 0.2$^{B, c}$	79.1 ± 0.2$^{A, c}$
	Week 3	90.6 ± 0.2$^{B, b}$	99.3 ± 0.2$^{A, b}$
	Week 4	105.4 ± 0.3$^{B, a}$	167.8 ± 0.3$^{A, a}$
Acetic Acid (mg/100 ml)	24 h	ND	ND
	Week 1	1.0 ± 0.3$^{B, d}$	2.9 ± 0.2$^{A, d}$
	Week 2	3.6 ± 0.2$^{B, c}$	6.1 ± 0.3$^{A, c}$
	Week 3	5.9 ± 0.2$^{B, b}$	8.0 ± 0.3$^{A, b}$
	Week 4	7.7 ± 0.3$^{B, a}$	10.9 ± 0.2^{A,a}
Ethanol (% v/v)	24 h	0.2 ± 0.1$^{A, b}$	0.2 ± 0.1$^{A, b}$
	Week 1	0.3 ± 0.1$^{A, b}$	0.3 ± 0.1$^{A, b}$
	Week 2	0.3 ± 0.1$^{A, b}$	0.4 ± 0.1$^{A, b}$
	Week 3	0.4 ± 0.1$^{A, b}$	0.4 ± 0.1$^{A, b}$
	Week 4	0.7 ± 0.1$^{A, a}$	0.9 ± 0.1$^{A, a}$

Different superscript letters in the rows (A-B) at the same time of storage, and in the columns (a-d) for each parameter examined, indicate statistically significant differences (Two-Way ANOVA; Duncan's multiple range test; $p < 0.05$).

Table 2
Determination of total phenolics content of non-fermented (NFC) and fermented Cornelian cherry juice with free (FC) and (IC) immobilized L. *paracasei K5* after 24 h of fermentation at 30 °C and during storage at 4 °C for 4 weeks.

Time	Total phenolics content (mg GAE/100 ml)		
	NFC	FC	IC
24 h	169.2 ± 11.5$^{B, a}$	188.1 ± 11.4$^{B, b}$	205.4 ± 10.2$^{A, b}$
Week 1	166.0 ± 10.4$^{C, a}$	201.2 ± 14.1$^{B, b}$	287.1 ± 10.2$^{A, a}$
Week 2	143.1 ± 10.1$^{C, b}$	241.1 ± 10.2$^{B, a}$	274.1 ± 10.2$^{A, a}$
Week 3	141.7 ± 11.1$^{C, b}$	208.2 ± 11.8$^{B, b}$	269.3 ± 23.5$^{A, a}$
Week 4	143.0 ± 10.2$^{C, b}$	200.8 ± 11.3$^{B, b}$	228.5 ± 10.2$^{A, b}$

Different superscript letters in the rows (A-B) at the same time of storage, and in the columns (a-d) for each parameter examined, indicate statistically significant differences (Two-Way ANOVA; Duncan's multiple range test; $p < 0.05$).

3.2. Total phenolics content

The total phenolics content (TPC) of fruits and fruit juices is an important characteristic, since phenolics are considered bioactive substances with positive impact on various physiological processes related to human health (Maus et al., 2016). The results obtained in this study show that the TPC of the Cornelian cherry juice fermented with both FC and IC, was increased (statistically significant) compared to the non-fermented juice (NFC) that was used as control (Table 2). This observation agrees with other studies that reported the enhancement of the TPC of juices by lactic acid fermentation (Mousavi et al., 2013; Sabokbar and Khodaiyan, 2016; Valero-Cases et al., 2017). For example, it was observed that lactic acid fermentation promoted the conversion of the simple phenolic compounds and the depolymerization of phenolics with higher molecular weights by the function of various enzymes such as β-glycosidase (Mousavi et al., 2011)

In this study, statistically significant differences were also observed in the TPC values between the juices fermented by FC and IC. Specifically, the use of IC resulted in higher TPC in the fermented juice, compared to FC (Table 2), reaching its highest value at the 3rd week of storage (269.3–287.1 mg GAE/100 ml). The corresponding TPC values for the juice fermented by FC were lower (200.8–241.1 mg GAE/100 ml). Moreover, immobilization enhanced the survival of the *L. paracasei K5* cells indicating that higher viable cell populations can lead to increased enzymatic functions, and consequently to higher rates of degradation of conjugated forms of phenolics, as also concluded by Mousavi et al. (2011).

3.3. Cell survival

The results for the viability rates of the *L. paracasei* K5 cells and the possible spoilage by yeasts, fungi and coliforms, after fermentation and during the four weeks of storage, are presented in Table 3. In all cases, the viability of *L. paracasei* K5 was maintained over the critical limit of 10^6 cfu/ml required for probiotic products. These results are in agreement with those obtained in a preceding study regarding the fermentation of Cornelian cherry juices with different pH values using non-

Table 3
Viability of free (FC) and immobilized (IC) *L. paracasei K5* cells in the fermented Cornelian cherry juices after fermentation (24 h at 30 °C) and over 4 weeks of storage at 4 °C.

Time	Cell counts (log cfu/ml)			
	FC	IC	Yeasts & fungi	Coliforms
0 h	11.0 ± $0.1^{A, a}$	11.0 ± $0.1^{A, a}$	0	0
24 h	10.6 ± $0.1^{A, b}$	10.7 ± $0.1^{A, a}$	0	0
Week 1	9.9 ± $0.2^{B, c}$	10.9 ± $0.2^{A, a}$	0	0
Week 2	9.2 ± $0.2^{B, d}$	10.3 ± $0.2^{A, b}$	0	0
Week 3	8.6 ± $0.1^{B, e}$	10.3 ± $0.1^{A, b}$	0	0
Week 4	8.6 ± $0.2^{B, e}$	9.7 ± $0.1^{A, c}$	0	0

Different superscript letters in the rows (A-B) at the same time of storage, and in the columns (a-d) for each parameter examined, indicate statistically significant differences (Two-Way ANOVA; Duncan's multiple range test; $p < 0.05$).

Table 4
Preliminary (consumer) sensory evaluation of non-fermented (NFC), and fermented Cornelian cherry juice with free (FC) and immobilized *L. paracasei K5* (IC) after 24 h of fermentation at 30 °C and during storage at 4 °C for 4 weeks.

Storage time	Substrate	Aroma	Taste	Overall quality
24 h	NFC	8.0 ± 0.2^{b}	8.1 ± 0.1^{b}	8.0 ± 0.1^{b}
	FC	8.5 ± 0.2^{a}	8.9 ± 0.2^{a}	8.7 ± 0.2^{a}
	IC	8.6 ± 0.1^{a}	8.8 ± 0.2^{a}	8.6 ± 0.1^{a}
Week 1	NFC	7.1 ± 0.1^{b}	7.1 ± 0.2^{b}	7.0 ± 0.1^{b}
	FC	8.0 ± 0.1^{a}	8.1 ± 0.1^{a}	8.1 ± 0.2^{a}
	IC	8.1 ± 0.1^{a}	8.0 ± 0.1^{a}	8.0 ± 0.2^{a}
Week 2	NFC	6.5 ± 0.2^{b}	6.4 ± 0.2^{b}	6.5 ± 0.1^{b}
	FC	7.5 ± 0.1^{a}	7.5 ± 0.2^{a}	7.5 ± 0.1^{a}
	IC	7.6 ± 0.1^{a}	7.4 ± 0.1^{a}	7.6 ± 0.2^{a}
Week 3	NFC	6.0 ± 0.1^{b}	6.1 ± 0.1^{b}	6.1 ± 0.2^{b}
	FC	7.0 ± 0.2^{a}	7.0 ± 0.1^{a}	7.1 ± 0.1^{a}
	IC	7.1 ± 0.1^{a}	7.2 ± 0.1^{a}	7.3 ± 0.1^{a}
Week 4	NFC	5.8 ± 0.1^{b}	5.9 ± 0.1^{b}	6.0 ± 0.1^{b}
	FC	6.6 ± 0.2^{a}	6.6 ± 0.1^{a}	6.5 ± 0.1^{a}
	IC	6.5 ± 0.1^{a}	6.5 ± 0.1^{a}	6.6 ± 0.3^{a}

Different superscript letters in a column at the same time of storage indicate statistically significant differences (ANOVA, Duncan's multiple range test, $p < 0.05$).

immobilised *L. paracasei K5* and *L. plantarum* ATCC 14917 cells (Nouska et al., 2016). The substrate pH is a critical factor that influences cell viability in fruit juices value (Nematollahi et al., 2016). In this study, the initial pH value of the juice was 2.7–2.9, at which the cell survival was very low (data not shown). Therefore, the pH of the juices was adjusted to 3.5 as also indicated in the work of Nematollahi et al. (2016). In addition to pH adjustment, cell immobilization was employed to enhance the cell viability during fermentation and cold storage.

Regarding cell immobilization techniques, the nature of the immobilization support also plays an important role. Wheat bran can be considered as a potential prebiotic material since it contains about 50% dietary fiber among other components (20% protein, 7% ash, 4% lipids), while it has been successfully used as carrier of lactic acid bacteria for the production of various fermented dairy products (Terpou et al., 2018a, 2018b; 2017a). The protective effect of wheat bran in this study, is shown by the fact that after the 1st week of cold storage the viability of IC was higher (statistically significant) compared to FC, while the viability of IC was decreased by about 12% (9.74 log cfu/ml) only after the 4th week. On the other hand, the viability of FC was decreased by 22% (8.58 log cfu/ml). As a result, wheat bran can be proposed as a suitable immobilization carrier to protect lactic acid bacteria in juices, while providing at the same time a prebiotic character to the product.

It is also noteworthy that no coliforms, yeasts or fungi were detected during fermentation and during the 4 weeks of storage at 4 °C.

3.4. Sensory evaluation

Interesting results were also obtained by the sensory evaluation that was performed by non-trained testers (consumers). The evaluation of the non-fermented and fermented juices, with FC and IC, was based on a preference scale for aroma, taste, and overall quality (Table 4). It can be observed that lactic acid fermentation improved the organoleptic properties of Cornelian cherry juice, based on the consumer preference scores that were higher for both types of biocatalysts. This result is in agreement with the observations of Nematollahi et al. (2016), who reported that there was no off-flavor development in Cornelian cherry juice containing probiotics compared to control samples. However, in the same study it is mentioned that different outcomes may be observed when different probiotic species are used. The determination of specific volatile compounds, related to aromas, produced during the fermentation of Cornelian cherry by *L. paracasei* K5 will be very informative in the future.

4. Conclusions

The obtained results showed that the novel *L. paracasei* K5 strain, free or immobilized on DWB, can be successfully used to produce a functional Cornelian cherry beverage with potential synbiotic properties. Enhanced viability of *L. paracasei K5* during the storage period at 4 °C was observed when the strain was immobilized; therefore, DWB can exert a protective effect on the probiotic species used as well as prebiotic effects for the consumer. The total phenolics content of the fermented juices, after fermentation and during storage, was also increased compared to the control samples. Finally, the fermented juices were readily accepted by the tasters, receiving higher scores compared to the control samples.

References

Danesi, F., Ferguson, L.R., 2017. Could pomegranate juice help in the control of inflammatory diseases? Nutrients 9.
Di Cagno, R., Filannino, P., Gobbetti, M., 2017. Lactic acid fermentation drives the optimal volatile flavor-aroma profile of pomegranate juice. Int. J. Food Microbiol. 248, 56–62.
Dinda, B., Kyriakopoulos, A.M., Dinda, S., Zoumpourlis, V., Thomaidis, N.S., Velegraki, A., Markopoulos, C., Dinda, M., 2016. Cornus mas L. (cornelian cherry), an important European and Asian traditional food and medicine: ethnomedicine, phytochemistry and pharmacology for its commercial utilization in drug industry. J. Ethnopharmacol. 193, 670–690.
Ephrem, E., Najjar, A., Charcosset, C., Greige-Gerges, H., 2018. Encapsulation of natural active compounds, enzymes, and probiotics for fruit juice fortification, preservation, and processing: an overview. J. Funct. Foods 48, 65–84.
Espirito-Santo, A.P., Carlin, F., Renard, C.M.G.C., 2015. Apple, grape or orange juice: which one offers the best substrate for lactobacilli growth? — A screening study on bacteria viability, superoxide dismutase activity, folates production and hedonic characteristics. Food Res. Int. 78, 352–360.
George Kerry, R., Patra, J.K., Gouda, S., Park, Y., Shin, H.-S., Das, G., 2018. Benefaction of probiotics for human health: a review. J. Food Drug Anal. 26, 927–939.
Gupta, M., Bajaj, B.K., 2016. Selection criteria for probiotics and potential of cereal based food products as novel probiotic-carriers. Curr. Nutr. Food Sci. 12, 157–174.
Kwaw, E., Ma, Y., Tchabo, W., Apaliya, M.T., Wu, M., Sackey, A.S., Xiao, L., Tahir, H.E., 2018. Effect of lactobacillus strains on phenolic profile, color attributes and antioxidant activities of lactic-acid-fermented mulberry juice. Food Chem. 250, 148–154.
Lebaka, V.R., Wee, Y.J., Narala, V.R., Joshi, V.K., 2018. Chapter 4 - Development of new probiotic foods—a case study on probiotic juices. In: Grumezescu, A.M., Holban, A.M. (Eds.), Therapeutic, Probiotic, and Unconventional Foods. Academic Press, pp. 55–78 (Therapeutic, Probiotic, and Unconventional Foods).
Maldonado, R.R., Araújo, Ld.C., Dariva, L.Cd.S., Rebac, K.N., Pinto, I.Ad.S., Prado, J.P.R., Saeki, J.K., Silva, T.S., Takematsu, E.K., Tiene, N.V., Aguiar-Oliveira, E., Buosi, R.E., Deziderio, M.A., Kamimura, E.S., 2017. Potential application of four types of tropical fruits in lactic fermentation. LWT 86, 254–260.
Martins, E.M.F., Ramos, A.M., Vanzela, E.S.L., Stringheta, P.C., de Oliveira Pinto, C.L., Martins, J.M., 2013. Products of vegetable origin: a new alternative for the consumption of probiotic bacteria. Food Res. Int. 51, 764–770.
Maus, I., Koeck, D.E., Cibis, K.G., Hahnke, S., Kim, Y.S., Langer, T., Kreubel, J., Erhard, M., Bremges, A., Off, S., Stolze, Y., Jaenicke, S., Goesmann, A., Sczyrba, A., Scherer, P., König, H., Schwarz, W.H., Zverlov, V.V., Liebl, W., Pühler, A., Schlüter, A., Klocke, M., 2016. Unraveling the microbiome of a thermophilic biogas plant by metagenome and metatranscriptome analysis complemented by characterization of bacterial and archaeal isolates. Biotechnol. Biofuels 9, 171.

Moldovan, B., Filip, A., Clichici, S., Suharoschi, R., Bolfa, P., David, L., 2016. Antioxidant activity of Cornelian cherry (Cornus mas L.) fruits extract and the in vivo evaluation of its anti-inflammatory effects. J. Funct. Foods 26, 77–87.
Mousavi, Z.E., Mousavi, S.M., Razavi, S.H., Emam-Djomeh, Z., Kiani, H., 2011. Fermentation of pomegranate juice by probiotic lactic acid bacteria. World J. Microbiol. Biotechnol. 27, 123–128.
Mousavi, Z.E., Mousavi, S.M., Razavi, S.H., Hadinejad, M., Emam-Djomeh, Z., Mirzapour, M., 2013. Effect of fermentation of pomegranate juice by lactobacillus plantarum and lactobacillus acidophilus on the antioxidant activity and metabolism of sugars, organic acids and phenolic compounds. Food Biotechnol. 27, 1–13.
Neffe-Skocińska, K., Rzepkowska, A., Szydłowska, A., Kołożyn-Krajewska, D., 2018. Chapter 3 - Trends and possibilities of the use of probiotics in food production. In: Holban, A.M., Grumezescu, A.M. (Eds.), Alternative and Replacement Foods. Academic Press, pp. 65–94 (Alternative and Replacement Foods).
Nematollahi, A., Sohrabvandi, S., Mortazavian, A.M., Jazaeri, S., 2016. Viability of probiotic bacteria and some chemical and sensory characteristics in cornelian cherry juice during cold storage. Electron. J. Biotechnol. 21, 49–53.
Nouska, C., Kazakos, S., Mantzourani, I., Alexopoulos, A., Bezirtzoglou, E., Plessas, S., 2016. Fermentation of Cornus mas L. Juice for functional low alcoholic beverage production. Curr. Res. Nutr. Food Sci. 4, 119–124.
Pasqualin Cavalheiro, C., Ruiz-Capillas, C., Herrero, A.M., Jiménez-Colmenero, F., Ragagnin de Menezes, C., Martins Fries, L.L.,, 2015. Application of probiotic delivery systems in meat products. Trends Food Sci. Technol. 46, 120–131.
Plessas, S., Bekatorou, A., Gallanagh, J., Nigam, P., Koutinas, A.A., Psarianos, C., 2008. Evolution of aroma volatiles during storage of sourdough breads made by mixed cultures of Kluyveromyces marxianus and Lactobacillus delbrueckii ssp. bulgaricus or Lactobacillus helveticus. Food Chem. 107, 883–889.
Plessas, S., Nouska, C., Karapetsas, A., Kazakos, S., Alexopoulos, A., Mantzourani, I., Chondrou, P., Fournomiti, M., Galanis, A., Bezirtzoglou, E., 2017. Isolation, characterization and evaluation of the probiotic potential of a novel Lactobacillus strain isolated from Feta-type cheese. Food Chem. 226, 102–108.
Ricci, A., Cirlini, M., Levante, A., Dall'Asta, C., Galaverna, G., Lazzi, C., 2018. Volatile profile of elderberry juice: effect of lactic acid fermentation using L. plantarum, L. rhamnosus and L. casei strains. Food Res. Int. 105, 412–422.
Roberts, D., Reyes, V., Bonilla, F., Dzandu, B., Liu, C., Chouljenko, A., Sathivel, S., 2018. Viability of Lactobacillus plantarum NCIMB 8826 in fermented apple juice under simulated gastric and intestinal conditions. LWT 97, 144–150.
Sabokbar, N., Khodaiyan, F., 2016. Total phenolic content and antioxidant activities of pomegranate juice and whey based novel beverage fermented by kefir grains. J. Food Sci. Technol. 53, 739–747.
Singleton, V.L., Rossi, J., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. Am. J. Enol. Vitic. 16, 144–158.
Terpou, A., Bekatorou, A., Bosnea, L., Kanellaki, M., Ganatsios, V., Koutinas, A.A., 2018a. Wheat bran as prebiotic cell immobilisation carrier for industrial functional Feta-type cheese making: chemical, microbial and sensory evaluation. Biocatal. Agric. Biotechnol. 13, 75–83.
Terpou, A., Bosnea, L., Kanellaki, M., Plessas, S., Bekatorou, A., Bezirtzoglou, E., Koutinas, A.A., 2018b. Growth capacity of a novel potential probiotic Lactobacillus paracasei K5 strain incorporated in industrial white brined cheese as an adjunct culture. J. Food Sci. 83, 723–731.
Terpou, A., Bekatorou, A., Kanellaki, M., Koutinas, A.A., Nigam, P., 2017a. Enhanced probiotic viability and aromatic profile of yogurts produced using wheat bran (Triticum aestivum) as cell immobilization carrier. Process Biochem. 55, 1–10.
Terpou, A., Gialleli, A.I., Bosnea, L., Kanellaki, M., Koutinas, A.A., Castro, G.R., 2017b. Novel cheese production by incorporation of sea buckthorn berries (Hippophae rhamnoides L.) supported probiotic cells. LWT - Food Sci. Technol. 79, 616–624.
Tuorila, H., Cardello, A.V., 2002. Consumer responses to an off-flavor in juice in the presence of specific health claims. Food Qual. Prefer. 13, 561–569.
Valero-Cases, E., Nuncio-Jáuregui, N., Frutos, M.J., 2017. Influence of fermentation with different lactic acid bacteria and in vitro digestion on the biotransformation of phenolic compounds in fermented pomegranate juices. J. Agric. Food Chem. 65, 6488–6496.
Wootton-Beard, P.C., Ryan, L., 2011. Improving public health?: the role of antioxidant-rich fruit and vegetable beverages. Food Res. Int. 44, 3135–3148.