Effect of lactic acid fermentation on the phytochemical, volatile profile and sensory attributes of mulberry juice

EMMANUEL KWAW – YONGKUN MA – WILLIAM TCHABO – MAURICE T. APALIYA – LULU XIAO – MENG WU

Summary

This study sought to assess the effect of lactic acid fermentation on the phytochemical, volatile and sensory attributes of mulberry juice. Moreover, the study also categorized and modelled the quality of the lactic acid fermented mulberry juice (LFMJ). The results showed that mulberry juice facilitated the growth of *Lactobacillus plantarum*, *Lb. acidophilus* and *Lb. paracasei* with similar growth capacity in the sample at 37 °C. The fermentation process significantly ($P < 0.05$) improved the phytochemical, sensory and volatile properties of LFMJ. There were significant ($P < 0.05$) differences in the volatile composition of the resultant LFMJ. However, the volatiles, in particular aldehydes, decreased drastically whereas new esters, ketones, phenols and acids were metabolized. Odour-activity values indicated that odourants that had significant impact on LFMJ were esters. Furthermore, the partial least squares regression model was more adequate in predicting the quality of the LFMJ than the principal component regression. It can be concluded that *Lb. plantarum* was the most suitable strain for fermentation of mulberry juice.

Keywords

mulberry; fermentation; lactic acid bacteria; odour activity value; sensory attribute; quality rating

Mulberry belongs to the genus *Morus* from the *Moraceae* family and has been reported to be used in Chinese traditional medicine in the treatment of fever, diabetes, obesity, blood pressure, urinal disorders, protection of liver from damage, atherosclerosis, inflammation, and to strengthen body joints among others [1]. In recent years, mulberry-derived products (jams, jellies, wine, juice and dried fruits) have been developed and commercialized in order to preserve [2] its health benefits, which are associated with its rich phytochemical properties [1].

Lactic acid fermentation could be possible means to process and commercialize mulberry fruit products, since the process is considered cost-effective, energy-efficient and a valuable biotechnology that contributes to the microbial safety, organoleptic and nutraceutical properties of foods [3]. Besides, its application in the fruit industry has been reported as the easiest and utmost approach to help increase consumers' consumption of fruits [4]. Different strains of lactic acid bacteria (LAB) have been isolated and/or used in fruit fermentation [4, 5]. Various LAB have the ability to enhance the polyphenol and volatile profile of foods during fermentation [6–9]. Polyphenols are active ingredients found in many medicinal plants that control wide range of enzymatic activities and cell receptors. Besides, the health benefits of polyphenols depend on the amount consumed and on their bioavailability [10]. Furthermore, polyphenols are known to have an impact on sensory qualities of products [11]. As reported previously, LAB strains can significantly alter the volatile profile of fermented beverages due to their ability to produce diverse enzymes [12]. LAB have been employed to produce various fermented foods such as wine, yoghurt, kefir,

Emmanuel Kwaw, Department of Food Science and Engineering, School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, China; Department of Hotel, Catering and Institutional Management, School of Applied Sciences and Arts, Cape Coast Technical University, 18 Bonkus Road, P. O. Box AD50, Cape Coast, Ghana.
Yongkun Ma, William Tchabo, Maurice T. Apaliya, Lulu Xiao, Meng Wu, Department of Food Science and Engineering, School of Food and Biological Engineering, Jiangsu University, 301 Xuefu Road, Zhenjiang 212013, China.

Correspondence author:
Yongkun Ma, tel.: +86 13913439511; e-mail: mayongkun@ujs.edu.cn

cheese and several others. However, to the best of our knowledge, there is no comprehensive study on lactic acid fermentation of mulberry fruit juice. Hence, understanding the alterations that polyphenolic and volatile compounds undergo during lactic acid fermentation would be very useful in the production and quality assessment of fermented beverages.

In view of this, the study aimed at investigating the effect of lactic acid fermentation on phytochemical, volatile and sensory characteristics of mulberry juice.

MATERIALS AND METHODS

Materials

Tái wān 1 háo (*Morus nigra*) was obtained from a farm in Zhenjiang, Jiangsu Province, China. Pectinex UF was obtained from Novozymes (Bagsvaerd, Denmark), *Lb. plantarum* (ATCC SD5209), *Lb. acidophilus* (ATCC SD5212) and *Lb. paracasei* (ATCC SD5275) were purchased from DuPont China (Shanghai, China). DeMan, Rogosa and Sharpe (MRS) broth and MRS agar were purchased from Sigma-Aldrich (St. Louis, Missouri, USA), pure volatile standards from Sigma-Aldrich. Analytical grade chemicals were purchased from Sinopharm Chemical Reagent (Shanghai, China).

Starter culture preparation

LAB strains were activated by culturing in MRS broth at 37 °C for 18 h and sub-cultured twice in MRS broth for 24 h at 35 °C. Thereafter, the cultures were centrifuged (Anke KA–1000; Beckman Coulter, Brea, California, USA) at 2000 ×*g* for 10 min. The microbial cells were harvested and washed using 0.1% sterile NaCl. A hemocytometer version XB-K-250 (Jianling Medical Device, Danyang, China) was used to estimate the inoculum concentration. The inoculum was adjusted to 10^7 CFU·ml^{-1} using sterile distilled water.

Beverage formulation and fermentation

Frozen mulberry fruits were thawed at 4 °C for 8 h and macerated using Hurom slow juicer (Roland Products, Los Angeles, California, USA). Subsequently, ascorbic acid (1 g·kg^{-1}) was added to minimize oxidation and the must was treated with Pectinex UF enzyme (0.01 l·kg^{-1}, Novozymes) according to the method described by Tchabo et al. [13] with slight modification. Briefly, four samples made up of 350 g must and enzyme were each put into 500 ml Erlenmeyer flask and immersed in ultrasonic bath (20 °C). Samples were sonicated for 15 min at a frequency of 34 kHz, power of 60 W and pulse duration of 10 s on and 5 s off. These conditions, according to Engmann et al. [14], allow for microbial safety. The samples were centrifuged (Avanti J-25, Beckman Coulter) at 24000 ×*g* for 15 min at 4 °C. The individual inoculants (*Lb. plantarum*, *Lb. acidophilus* and *Lb. paracasei*) were inoculated into three separate Erlenmeyer flasks (500 ml) containing 200 ml of the clarified juice. Samples were kept in a rotary incubator (IS-RDD3; Crystal Technology and Industries, Jiangsu, China) at 150 ×*g* and 37 °C for 36 h. The lactic acid fermented juice (LFMJ) samples were sterilized by ultrasonication as described previously. The control was mulberry juice treated under the same conditions but without LAB.

Microbiological analysis

The population dynamics of microorganisms was monitored in the samples at different times (0, 3, 6, 12, 18, 24, 30 and 36 h) during incubation. Specifically, fermented samples (1 ml) were aseptically transferred to 9 ml sterile distilled water. The resulting suspensions were serially diluted in the same diluent and 1 ml of the sample of the appropriate dilution pour-plated on MRS agar. Plates were incubated at 37 °C for 48 h and colony forming units estimated.

Determination of pH, titratable acidity and total soluble solids

Changes in pH and titratable acidity (*TA*) expressed in gram of lactic acid per litre of fermented juice were determined according to Nielsen [15] using PHS-3C Precision pH/mV meter (LIDA Instrument, Shanghai, China). Total soluble solids (*TSS*) were determined using digital refractometer (Beijing Yaxingtai Electrical Equipment, Beijing, China).

Total phenolic concentration

Total phenolic concentration (*TPC*) was determined by Folin-Ciocalteu method as described by Figueroa et al. [16] with some modifications. Briefly, 200 μl of the samples, 2 ml Folin-Ciocalteu reagent (Shanghai Labaide Biotechnology, Shanghai, China) and 2 ml of sodium carbonate (75 g·l^{-1}) were dispensed into a test tube and vortexed for 15 s. The mixture was allowed to stand at 25 °C for 20 min, after which absorbance was measured at 760 nm using UV spectrophotometer (Model UV-1600; Beijing Rayleigh Analytical Instrument, Beijing, China). *TPC* was expressed in terms of milligram of gallic acid equivalent per millilitre of juice.

Total anthocyanin concentration

The pH differential method described by TCHABO et al. [17] was employed in the determination of the total anthocyanin concentration (*TAC*). Briefly, two buffer solutions, KCl (0.25 mol·l^{-1}) at pH 1 and CH_3COONa (0.4 mol·l^{-1}) at pH 4.5 were prepared. A volume of 100 μl of the sample was dispensed into two sets of tubes. One set of the sample was adjusted to 10 ml with KCl buffer while the the other set with CH_3COONa solution. Absorbance was measured at 510 nm and 700 nm using UV spectrophotometer (UV-1600) against a blank (water and reagents). *TAC* was calculated using the following equation and expressed as equivalent of milligram of cyanidin 3-glucoside per millilitre of juice:

$$TAC = [(A_1 - A_2) - (A_3 - A_4)] \times \frac{MW \times DF \times 10^2}{\varepsilon \times L} \quad (1)$$

where A_1 is absorbance at 510 nm at pH 1.0, A_2 is absorbance at 700 nm at pH 1.0, A_3 is absorbance at 510 nm at pH 4.5, A_4 is absorbance at 700 nm at pH 4.5, *MW* is molecular weight of cyanidin-3-glucoside (449.2 g·mol^{-1}); *DF* is dilution factor (100); *L* is path length (1 cm); ε is molar extinction coefficient for cyanidin-3-glucoside (26 900 l·mol^{-1}·cm).

Total flavonoid concentration

The total flavonoid concentration (*TFC*) was determined using aluminium chloride colorimetric assay as described by TCHABO et al. [13] with slight modifications. The juice (1 ml) was diluted with 4 ml of distilled water, after which 0.3 ml $NaNO_2$ (50 g·l^{-1}) was added and vortexed for 1 min. The mixture was allowed to stand for 5 min, after which 1 ml of $AlCl_3$ (100 g·l^{-1}) was added, mixed and allowed to stand for another 5 min. Afterwards, 2 ml NaOH (1 mol·l^{-1}) was added and the final volume was adjusted to 10 ml with 2.4 ml distilled water. The mixture was allowed to stand at 25 °C for 10 min with 2 min periodic shaking. The absorbance of the mixture was measured at 510 nm using UV spectrophotometer (UV-1600). *TFC* was expressed as milligram of rutin equivalents per millilitre juice.

Volatile compounds analysis

The analysis of volatile compounds was performed using the headspace gas chromatography-mass spectrometry method.

Solid phase micro-extraction

Extraction of the volatile compounds was performed using the method described by ZHU et al. [18] with slight modifications. Briefly, 1.5 g of NaCl was added to 5 ml of the sample in a 15 ml glass vial to enhance extraction efficiency. Mixture was spiked with 10 μl 2-octanol (800 μg·l^{-1}) as an internal standard. Vial was sealed with silicone septum and the mixture was equilibrated at 40 °C for 20 min. A 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane fibre (Supelco, Bellefonte, Pennsylvania, USA) was exposed to the headspace for 30 min at 40 °C with continuous stirring at 2.5 Hz. Thereafter, the fibre was removed and desorption was carried out by inserting the fibre into the injection port of the Agilent 6890N-5973B gas chromatograph (Agilent Technologies, Santa Clara, California, USA) coupled with a mass spectrometer detector (MSD) for 5 min at 250 °C.

Gas chromatography analysis

Separation of the volatile compounds was performed with Agilent J&W DB-WAX gas chromatography (GC) column, 60 m × 0.25 mm × 0.25 μm film thickness (Agilent Technologies, Santa Clara, California, United States). The chromatographic conditions were set slightly different from those of BUTKHUP et al. [19]. They were as follows: injection mode was splitless, injection temperature was 250 °C, carrier gas was He at 1 ml·min^{-1}, detectors temperature was 250 °C, temperature program was 50 °C for 10 min, increased to 150 °C at 6 °C·min^{-1}, then raised to 200 °C at 8 °C·min^{-1} and held for 7 min, mass spectrometry (MS) scan range was 33–350 atomic mass units (AMU), energy was 70 eV, source temperature was 230 °C and quadrupole temeprature was 150 °C.

Identification and semi-quantification of volatile compounds

The identification of volatile compounds was performed with MSD and was based on comparison of the GC retention times with those obtained at the same chromatographic conditions and the mass spectra with library databases (Wiley Spectral Library and NIST Library 2005 v 2.0, National Institute of Standards and Technology, Gaithersburg, Maryland, USA). The linear retention indices (*RI*) of the compounds were calculated using a series of *n*-alkanes (C5-C25) run under same conditions using the expression of BIANCHI et al. [20]. Semi-quantification of the volatile compounds was carried out by the internal standard technique using MSD. The concentrations were estimated from the peak area of the total chromatographs as a ratio of the target ion of the individual volatile compound with reference to the spiked internal standard. The odour activity value (*OAV*) for each volatile compound was calculated by dividing the concentration by odour threshold [21].

Sensory analysis

The sensory analysis was conducted using 27 untrained panellists (14 males and 13 females) made-up of students and staff of the School of Food and Biological Engineering (Jiangsu University, Zhenjiang, China). The panellists were presented with coded samples and water (to rinse their mouth after tasting each sample). Each panel evaluated samples (10 ml) for colour, taste, aroma, mouthfeel, flavour and overall acceptability using a 9-point hedonic scale (1 = dislike extremely; 2 = dislike very much; 3 = slightly dislike; 4 = dislike 5 = neither like nor dislike, 6 = like; 7 = slightly like; 8 = like very much; 9 = like extremely) according to the sensory analysis guidelines [17].

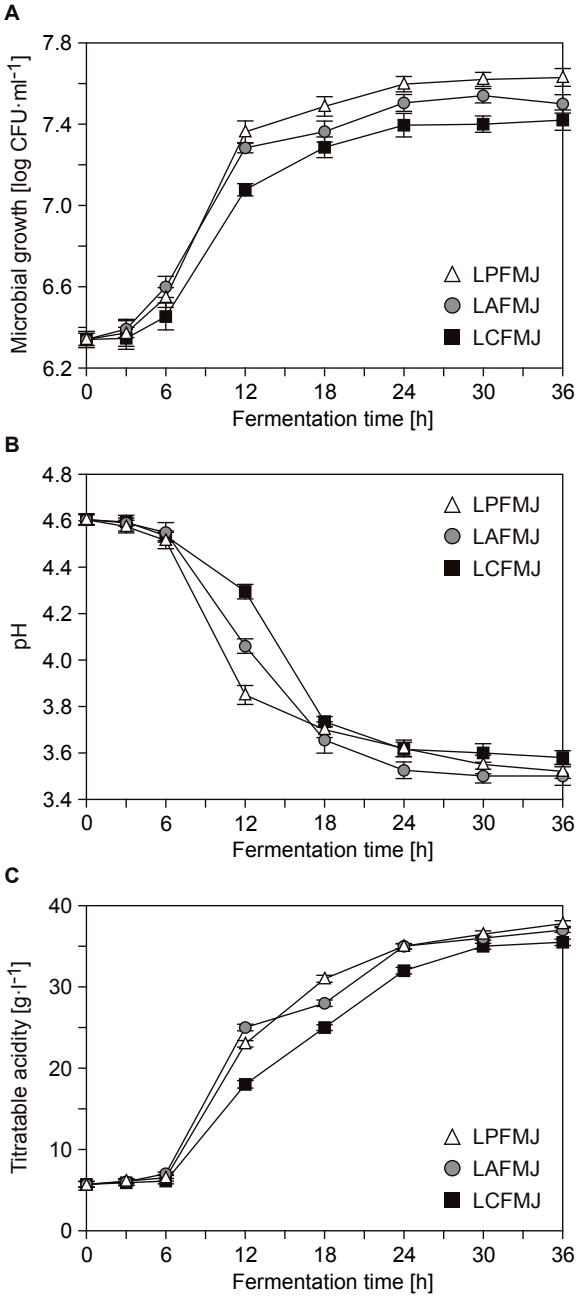

Fig. 1. Microbial growth and physico-chemical changes during mulberry juice fermentation.

A – microbial growth, B – changes in pH , C – changes in titratable acidity.
LPFMJ – mulberry juice fermented using *Lb. plantarum*, LAFMJ – mulberry juice fermented using *Lb. acidophilus*, LCFMJ – mulberry juice fermented using *Lb. paracasei.*

Statistical analysis

All treatments and analyses were carried out in triplicates and results were presented as mean ± standard deviation. The analysis of variance (ANOVA) was performed using OriginPro version 2015 (OriginLab, Northampton, Massachusetts, USA). The means were compared at $P < 0.05$ significance level using Tukey's test. The Radar plot was created using OriginPro version 2015 (OriginLab). Principal components analysis (PCA), partial least squares regression (PLSR) and principal component regression (PCR) were performed using XLSTAT 2016 software (Addinsoft, Paris, France).

RESULTS AND DISCUSSION

Microbial growth and physico-chemical kinetics during fermentations

The microbial growth, pH and *TA* during the fermentation of the mulberry juice are shown in Fig. 1. The results showed that mulberry juice was suitable for the growth of all the LAB (Fig. 1A). The growth curves of the different LAB (*Lb. plantarum, Lb. acidophilus* and *Lb. paracasei*) (Fig. 1A) demonstrated similar growth capacities in the sample at 37 °C with exponential growth occurring between 6 h and 12 h of incubation. It was however observed that *Lb. plantarum* grew better (to 7.63 ± 0.04 log CFU·ml^{-1}) than *Lb. acidophilus* (to 7.50 ± 0.05 log CFU·ml^{-1}) or *Lb. paracasei* (to 7.42 ± 0.05 log CFU·ml^{-1}) after 36 h of incubation.

Acidification of the juice, as a result of acid production during growth, was considerable for all the LAB, as they lowered the pH values to less than 3.6 after 36 h of incubation (Fig. 1B). However, *Lb. acidophilus* showed higher acidification with a pH value of 3.50 ± 0.04 after 36 h of incubation, whereas those of *Lb. plantarum* and *Lb. paracasei* were 3.52 ± 0.04 and 3.58 ± 0.03 respectively. This difference can be ascribed to different nutritional requirements and the raw material utilization rate by LAB [22]. The change in *TA* values (Fig. 1C) had similar trends to those of mi-

crobial growth curve (Fig. 1A). LAB produce lactic, acetic and other organic acids during fermentation, which explains the similar trend of Fig. 1A and Fig. 1C. The production of the acids resulted in the decrease in pH (Fig. 1B). The capability of a microorganism to survive and grow in foods is more dependent on pH than on *TA* [23]. The ability of LAB to grow at such low pH may be due to their heterogeneous characteristics that permit them to survive in various ecological niches. These growth patterns are similar to those reported in literature [24, 25].

Effect of fermentation on phytochemicals

The influence of the LAB fermentation on the phytochemical properties of mulberry juice is presented in Tab. 1. The results show significant difference ($P < 0.05$) in *TPC* among the samples. Comparison of LFMJ and unfermented mulberry juice (CON) shows that LAB enhanced the phytochemical composition of the samples during fermentation [6–9]. The potential mechanisms could be attributed to β-glucosidase produced by the bacteria, which is capable of hydrolysing isoflavone β-glycosides to aglycones [6, 8]. Besides that, pH strongly interferes with extraction of phenolic compounds [26] hence the decrease in pH during fermentation might have influenced extraction of phenolic compounds leading to higher *TPC* in LFMJ than in CON.

Although LAB are known for their esterase activities [27], the higher *TPC* of mulberry juice fermented using *Lb. plantarum* (LPFMJ) than the other fermented samples could be attributed to LAB ability to produce esterase that is more potent at hydrolysing ester bonds of glycosides, to release soluble conjugated or insoluble bounded phenolic compounds from cell walls of the plant [28, 29].

Similar trend was observed in the *TPC* and *TAC* (Tab. 1). However, there was no significant difference ($P < 0.05$) in *TAC* of LPFMJ and mulberry juice fermented using *Lb. acidophilus* (LAFMJ). The high *TAC* of the fermented samples may be due to the ability of the LAB to produce enzymes that can hydrolyse complex polyphenols into simpler anthocyanins [10]. Anthocyanins are also stable at low pH [8], Fig. 1B, also shows similar pH values for *Lb. plantarum* and *Lb. acidophilus*. This could explain the insignificant difference in *TAC* of LPFMJ and LAFMJ (Tab. 1). There were significant differences among *TFC* values (Tab. 1) with the fermented samples having higher values than the control. According to Svensson et al. [30], contents of phenolic acid esters, phenolic acids and flavonoid glucosides increase during lactic fermentation. This could explain the high *TFC* in the fermented samples compared to the control. The variation in *TFC* of the fermented samples is due to differences in the ability of individual LAB strains to metabolize various flavonoid compounds [30, 31].

Tab. 1. Phytochemical properties of fermented mulberry beverage produced using different lactobacilli.

Sample	*TPC* [mg·ml^{-1}]	*TAC* [mg·ml^{-1}]	*TFC* [mg·ml^{-1}]
CON	5.46 ± 0.03^{d}	0.77 ± 0.01^{c}	3.15 ± 0.02^{d}
LPFMJ	8.27 ± 0.13^{a}	1.25 ± 0.01^{a}	4.19 ± 0.01^{a}
LAFMJ	7.15 ± 0.10^{b}	1.24 ± 0.00^{a}	3.96 ± 0.04^{b}
LCFMJ	6.43 ± 0.07^{c}	1.11 ± 0.01^{b}	3.68 ± 0.02^{c}

Data expressed as mean ± standard deviation. Values in the same column with different superscripts are significantly different at $P < 0.05$.
CON – unfermented mulberry juice, LPFMJ – mulberry juice fermented using *Lb. plantarum*, LAFMJ – mulberry juice fermented using *Lb. acidophilus*, LCFMJ – mulberry juice fermented using *Lb. paracasei.*
TPC – total phenolic concentration (expressed as milligram of gallic acid equivalents per millilitre of juice), *TAC* – total anthocyanin concentration (expressed as milligram of cyanidin 3-glucoside equivalent per millilitre of juice), *TFC* – total flavonoid concentration (expressed as milligram of rutin equivalents per millilitre juice).

Volatile profile

The results on the volatile compounds, as extracted by headspace solid-phase microextraction (HS-SPME), are presented in Tab. 2. The results depict significant differences between the fermented samples and control. A total of 42 volatile compounds were identified. They were made up of 18 esters, 10 alcohols, 6 aldehydes, 4 ketones, 2 acids and 2 volatile phenols. Thirty-two (32) of these volatile compounds were identified in LPFMJ, 22 in LAFMJ, 20 in mulberry juice fermented using *Lb. paracasei* (LCFMJ) and 18 in CON, with a concentration of 84.57 ± 1.02 mg·l^{-1}, 85.66 ± 0.83 mg·l^{-1}, 86.79 ± 0.79 mg·l^{-1} and 78.00 ± 1.12 mg·l^{-1}, respectively. The differences may be due to the metabolism and release of active and pleasant flavours by the individual LAB strains during fermentation [32]. Besides, Bleve et al. [33] ascertained a positive correlation between microbial growth of LAB and volatile profile. Hence, the variation in volatile composition may be due to the microbial growth (Fig. 1A). The results also showed an increase in the concentrations and metabolism of more volatile compounds in the fermented samples, whereas 1-pentanol, hexanal, heptanal and *cis*-2-hexenal, which were abundant in CON, were not detected after the

Tab. 2. Concentration of volatile compounds identified in unfermented and fermented mulberry juice.

Code	RI_{exp}	RI_L	Compound	Concentration [mg·l^{-1}]				Odour descriptors
				CON	LPFMJ	LAFMJ	LCFMJ	
Alcohols								
AL1	932	932	Ethyl alcohol	4.84 ± 0.13	4.88 ± 0.15	3.67 ± 0.10	3.21 ± 0.10	Alcohol, floral, ripe apple, sweet
AL2	1103	1103	2-Methyl-1-propanol	0.31 ± 0.01	0.34 ± 0.02	0.36 ± 0.02	0.28 ± 0.01	Apple, bitter, cocoa, plastic, wine
AL3	1215	1217	3-Methyl-1-butanol	3.60 ± 0.10	3.08 ± 0.09	3.98 ± 0.10	2.73 ± 0.12	Burnt, gasoline, bitter, cocoa, malt
AL4	1254	1255	1-Pentanol	0.35 ± 0.01	ND	ND	ND	Balsamic, fruit, green, pungent
AL5	1361	1362	1-Hexanol	1.99 ± 0.06	0.25 ± 0.01	0.36 ±0.02	0.51 ± 0.03	Banana, flower, grass, herb, resin
AL6	1428	1430	1-Heptanol	ND	0.17 ± 0.01	0.17 ± 0.01	ND	Green, wood, chemical, putrid
AL7	1490	1492	2-Ethylhexanol	1.58 ± 0.05	0.46 ± 0.03	0.29 ± 0.01	0.49 ± 0.01	Green, rose
AL8	1549	1547	1-Octanol	ND	0.10 ± 0.01	0.11 ± 0.01	ND	Bitter almond, burnt matches, fat, floral
AL9	1882	1882	Phenylmethanol	ND	0.32 ± 0.02	ND	ND	Boiled cherries, moss, roasted, rose
AL10	1930	1932	2-Phenylethyl alcohol	0.86 ± 0.02	1.09 ± 0.04	ND	0.94 ± 0.04	Fruit, honey, lilac, rose, wine
			Subtotal	13.53 ± 0.38 [a]	10.69 ± 0.38 [b]	8.94 ± 0.27 [c]	8.16 ± 0.31 [d]	
Aldehydes								
AD1	715	716	Ethanal	0.20 ± 0.01	0.08 ± 0.01	0.06 ± 0.00	ND	Green apple, ether, floral, nut
AD2	1060	1061	Hexanal	0.69 ± 0.05	ND	ND	ND	Green, fresh, fat, oil
AD3	1163	1163	Heptanal	1.71 ± 0.08	ND	ND	ND	Citrus, fat, green, nut, rancid
AD4	1189	1189	*cis*-2-Hexenal	0.46 ± 0.01	ND	ND	ND	Fat, rancid
AD5	1366	1367	1-Nonanal	ND	0.04 ± 0.00	ND	ND	Floral, green, lemon, paint, fat
AD6	1556	1556	Benzaldehyde	1.02 ± 0.02	0.27 ± 0.01	0.14 ± 0.01	ND	Bitter almond, burnt sugar, cherry
			Subtotal	4.08 ± 0.17 [a]	0.39 ± 0.02 [b]	0.20 ± 0.01 [c]	0.00 ± 0.00 [d]	
Esters								
ES1	813	813	Methyl acetate	4.41 ± 0.12	0.85 ± 0.02	0.90 ± 0.02	1.52 ± 0.08	Ester, green, sweet
ES2	823	822	Ethyl Acetate	35.89 ± 0.10	8.64 ± 0.11	10.41 ± 0.06	15.85 ± 0.06	Aromatic, brandy, contact glue, grape
ES3	985	985	Isobutyl acetate	ND	0.37 ± 0.01	0.29 ± 0.02	0.42 ± 0.01	Apple, banana, floral, herb, plastic
ES4	1045	1046	Butyl acetate	ND	0.07 ± 0.00	0.12 ± 0.01	0.20 ± 0.01	Apple, banana, glue, pungent, sweet
ES5	1057	1056	Ethyl butyrate	0.40 ± 0.01	ND	ND	ND	Apple, ester, green apple, kiwi, strawberry
ES6	1125	1125	Isopentyl acetate	33.41 ± 0.06	33.97 ± 0.11	33.43 ± 0.15	33.72 ± 0.03	Apple, banana, glue, pear
ES7	1136	1137	Butyl isobutyrate	ND	0.09 ± 0.01	ND	ND	–
ES8	1181	1180	Amyl acetate	ND	ND	0.58 ± 0.02	0.65 ± 0.02	Banana, fruit, sweet
ES9	1234	1236	Ethyl hexanoate	ND	0.06 ± 0.00	ND	ND	Apple peel, brandy, gum, overripe fruit

Tab. 2. *continued*

Code	RI_{exp}	RI_L	Compound	Concentration [mg·l^{-1}]				Odour descriptors
				CON	LPFMJ	LAFMJ	LCFMJ	
ES10	1255	1254	3-Methyl-2-butenyl acetate	ND	0.25 ± 0.02	ND	0.28 ± 0.01	Putty, unpleasant
ES11	1267	1269	Hexyl acetate	ND	2.69 ± 0.08	1.85 ± 0.04	1.81 ± 0.02	Apple, banana, fruit, grass
ES12	1370	1370	Heptanyl acetate	ND	0.11 ± 0.01	0.07 ± 0.01	ND	Floral, fresh
ES13	1387	1387	Ethyl hexyl acetate	ND	0.13 ± 0.01	ND	ND	Burnt plastic, citrus, fat, fruit, tar
ES14	1403	1405	Hexyl butanoate	ND	ND	ND	0.08 ± 0.01	Apple peel, citrus, fresh, toothpaste
ES15	1436	1438	Ethyl octanoate	ND	ND	0.07 ± 0.01	ND	Apricot, brandy, fat, floral, pineapple
ES16	1646	1647	Ethyl benzoate	ND	0.08 ± 0.01	ND	ND	Chamomile, celery, fat, flower, fruit
ES17	1728	1727	Benzyl acetate	ND	0.20 ± 0.02	ND	ND	Boiled vegetable, fruit, honey, jasmine
ES18	1811	1812	Phenethyl acetate	9.85 ± 0.09	24.34 ± 0.12	25.29 ± 0.10	18.44 ± 0.11	Flower, honey, rose, tobacco
			Subtotal	53.96 ± 0.38 ^{c}	71.85 ± 0.53 ^{a}	72.20 ± 0.44 ^{ab}	72.97 ± 0.36 ^{b}	
Acids								
AC1	1451	1452	Acetic acid	2.16 ± 0.08	0.51 ± 0.03	0.57 ± 0.03	0.89 ± 0.02	Acid, fruit, pungent, sour, vinegar
AC2	2060	2060	Octanoic acid	ND	0.49 ± 0.02	ND	ND	Cheese, fat, grass, oil, sweat
			Subtotal	2.16 ± 0.08 ^{a}	1.00 ± 0.05 ^{b}	0.57 ± 0.03 ^{c}	0.89 ± 0.02 ^{d}	
Volatile phenols								
VP1	2086	2088	Methoxy-4-allylphenol	ND	0.19 ± 0.01	ND	ND	Burnt, clove, smoke, spice
VP2	2316	2315	2,4-Di-*tert*-butylphenol	ND	ND	3.75 ± 0.08	3.53 ± 0.05	–
			Subtotal	0.00 ± 0.00 ^{a}	0.19 ± 0.01 ^{b}	3.75 ± 0.08 ^{c}	3.53 ± 0.05 ^{d}	
Ketones								
KE1	982	980	2-Pentanone	ND	ND	ND	1.12 ± 0.04	Ether, sweet
KE2	1165	1167	2-Heptanone	ND	0.07 ± 0.01	ND	0.12 ± 0.01	Blue cheese, fruit, green, nut, spice
KE3	1372	1374	Nonan-2-one	ND	0.07 ± 0.01	ND	ND	Fragrant, fruit, green, hot milk, soap
KE4	1788	1788	Metaxylohydroquinone	ND	0.31 ± 0.01	ND	ND	Boiled apple, floral, fruit, honey, tea
			Subtotal	0.00 ± 0.00 ^{a}	0.45 ± 0.03 ^{b}	0.00 ± 0.00 ^{a}	1.24 ± 0.05 ^{c}	
			Total	78.00 ± 1.12 ^{a}	84.57 ± 1.02 ^{b}	85.66 ± 0.83 ^{c}	86.79 ± 0.79 ^{d}	

Data expressed as mean ± standard deviation
RI_{exp} – experimental retention index, RI_L – retention index from the literature [17, 19, 20]. Odour descriptors were obtained from literature [17, 20, 21].
CON – unfermented mulberry juice, LPFMJ – mulberry juice fermented using *Lb. plantarum*, LAFMJ – mulberry juice fermented using *Lb. acidophilus*, LCFMJ – mulberry juice fermented using *Lb. paracasei.* ND – not detected.

fermentation. In addition, there was a decrease in some volatile compounds in LFMJ compared to CON [22].

Odour activity values and aromatic profile

The odour activity (*OAV*) of the samples is shown in Tab. 3. *OAV* has to do with the concentration of the volatile compounds (Tab. 2) and their corresponding odour threshold values (Tab. 3) that gives the samples their core aromatic notes. Volatile compounds interact with human receptors at equal or greater concentrations than the odour threshold to create a response. Eighteen (43 %) out of the 42 volatile compounds identified contributed significantly to the aroma of the samples. LFMJ, compared to CON, had higher total *OAV* (Tab. 3) making them have distinct aromatic characteristics. This implies that lactic acid fermentation positively impacted on the aroma active compounds. Naturally, odourants with high *OAV* are most likely to be significant, though aroma synergy and suppression exist. According to Eduardo et al. [34] and Añón et al. [35], odourants with $OAV > 1$ are significant and those with $OAV < 1$, but > 0.2 may have synergistic impact on the aroma of the product. The results show that the odourants which had significant impact on the aroma of CON were hexanal, ethyl acetate,

Tab. 3. Odour activity values of potent odourant compounds in lactic acid fermented mulberry juice.

Compound codes	Odour activity values				Odour threshold [$\mu g \cdot l^{-1}$]	Aroma series
	CON	LPFMJ	LAFMJ	LCFMJ		
Alcohols						
AL2	0.01	0.01	0.01	0.01	40 000	Fruity, chemical
AL3	0.12	0.10	0.13	0.09	30 000	Chemical
AL5	0.25	0.03	0.04	0.06	8000	Fruity, floral
AL6	0.00	0.07	0.07	0.00	2500	Vegetative
AL8	0.00	0.11	0.12	0.00	900	Nutty
AL10	0.09	0.11	0.00	0.09	10 000	Fruity, chemical, floral
Subtotal	0.47	0.43	0.37	0.25		
Aldehydes						
AD1	0.40	0.17	0.13	0.00	500	Fruity, floral, microbiological
AD2	1.97	0.00	0.00	0.00	350	Floral, chemical, fruity
AD5	0.00	28.29	0.00	0.00	1.3	Floral, chemical, fruity
Subtotal	2.37	28.46	0.13	0.00		
Esters						
ES2	4.79	1.15	1.39	2.11	7500	Fruity, microbiological
ES3	0.00	0.23	0.18	0.26	1600	Fruity
ES5	0.99	0.00	0.00	0.00	400	Fruity
ES6	21.32	212.30	208.97	210.75	160	Fruity
ES9	0.00	0.80	0.00	0.00	80	Fruity, floral
ES11	0.00	4.01	2.76	2.70	670	Fruity, floral
ES16	0.00	0.17	0.00	0.00	500	Floral, chemical, fruity
ES18	5.47	13.52	14.05	10.24	1800	Floral
Subtotal	32.57	232.18	227.35	226.06		
Acids						
AC2	0.00	0.97	0.00	0.00	500	Microbiological
Subtotal	0.00	0.97	0.00	0.00		
Total	35.41	262.04	227.85	226.31		

Volatile compounds were coded as indicated in Tab. 2. Odour threshold were obtained from literature [34, 38]. Aroma series were obtained from literature [36, 37].
CON – unfermented mulberry juice, LPFMJ – mulberry juice fermented using *Lb. plantarum*, LAFMJ – mulberry juice fermented using *Lb. acidophilus*, LCFMJ – mulberry juice fermented using *Lb. paracasei.*

isopentyl acetate and phenethyl acetate, while those with possible synergy impact were 1-hexanol, acetaldehyde and ethyl butyrate. Those that contributed significantly to the aroma of LPFMJ were ethyl acetate, isopentyl acetate, hexyl acetate, phenethyl acetate and 1-nonanal, whereas octanoic acid, ethyl hexanoate and isobutyl acetate had interactive effect on its aroma.

In LAFMJ, ethyl acetate, isopentyl acetate, hexyl acetate and phenethyl acetate were significant odourants.

The significant odourants in LCFMJ were ethyl acetate, isopentyl acetate, hexyl acetate and phenethyl acetate, with isobutyl acetate having possible synergy impact.

Aromatic series of the lactic acid fermented mulberry juice

The aromatic series permits tentative approximation of olfactive profile of samples based on the generic descriptors obtained from literature [36, 37]. As shown in Fig. 2, the main features of the aroma profile of the fermented samples were microbiological, nutty, vegetative, chemical, floral and fruity. Besides, fermentation reduced the chemical and microbiological aromatic series of the samples (Fig. 2). Fermentation using *Lb. plantarum* and *Lb. acidophilus* generated some nutty and vegetative aroma in LPFMJ and LAFMJ. On the other hand, the fruity and floral aroma increased significantly in all the fermented samples compared to the control.

Sensory assessment of samples

The radar plot of the sensory assessment (Fig. 3) shows that fermentation impacted positively the sensory attributes of the samples compared to the control. This may be due to the organic acids and the volatile compounds produced by LAB during the fermentation [22]. LFMJ samples even though differed in colour, flavour, taste, aroma, mouthfeel and overall acceptability, all samples were slightly liked by the panel with least mean score value being 7.03 (mouthfeel score for CON) and 7.37 (taste score for LCFMJ). The result showed that LPFMJ was scored higher by the panel in terms of aroma, colour, taste and overall acceptability. Its high aroma score was in line with its aromatic profile (Fig. 2) and its colour from its high *TAC* (Tab. 1) compared to the other samples. LAFMJ was scored higher in mouthfeel and flavour.

Principal component analysis

Principal component analysis (PCA) was performed to establish the key features of each sample using phytochemical composition (Tab. 1), sensory attributes (Fig. 3) and volatile attributes (Tab. 2) of the samples. The first two principal components (PC1 and PC2) were able to explain 86.6 % of the total variance. PC1 accounted for 62.5 % of the total variance and clearly differentiated between CON and the fermented samples

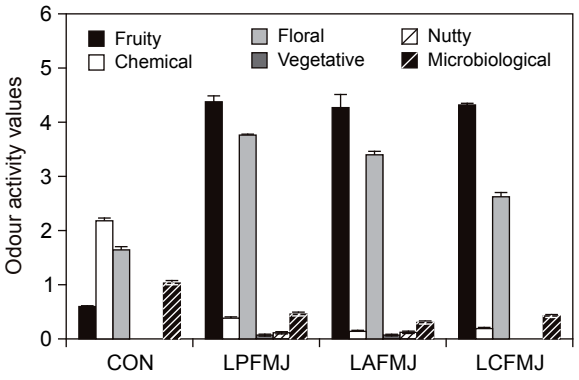

Fig. 2. Aromatic profile of lactic acid fermented mulberry juice.

Values for floral, fruity and microbiological aromatic series are presented as 5th, 50th and 5th part of the real values, respectively.
CON – unfermented mulberry juice, LPFMJ – mulberry juice fermented using *Lb. plantarum*, LAFMJ – mulberry juice fermented using *Lb. acidophilus*, LCFMJ – mulberry juice fermented using *Lb. paracasei.*

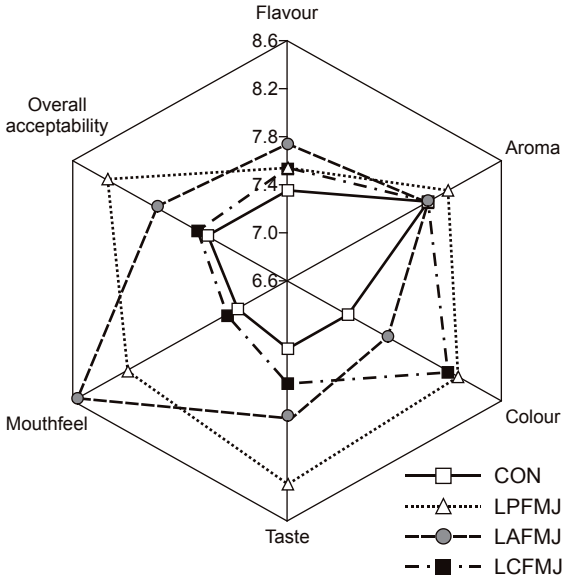

Fig. 3. Sensory assessment of mulberry beverages.

CON – unfermented mulberry juice, LPFMJ – mulberry juice fermented using *Lb. plantarum*, LAFMJ – mulberry juice fermented using *Lb. acidophilus*, LCFMJ – mulberry juice fermented using *Lb. paracasei.*

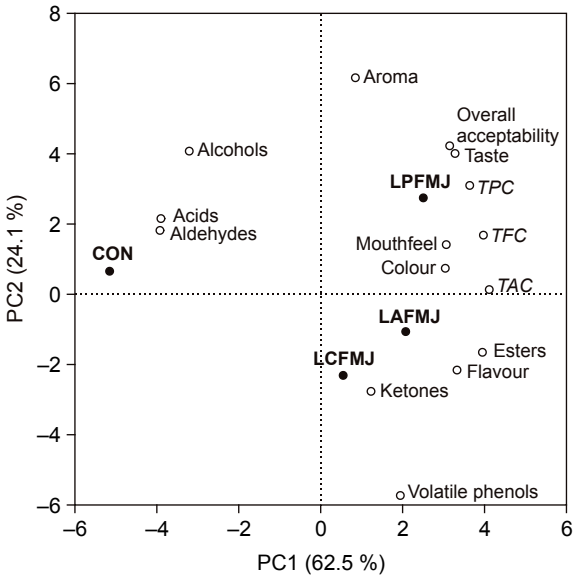

Fig. 4. Principal component analysis of the phytochemical, sensory attributes and volatile compounds of lactic acid fermented mulberry juice.

PC – principal component.
CON – unfermented mulberry juice, LPFMJ – mulberry juice fermented using *Lb. plantarum*, LAFMJ – mulberry juice fermented using *Lb. acidophilus*, LCFMJ – mulberry juice fermented using *Lb. paracasei, TPC* – total phenolic concentration, *TAC* – total anthocyanin concentration, *TFC* – total flavonoid concentration.

Tab. 4. Factor loadings of the first three principal components.

Attributes	PC1	PC2	PC3
TPC	0.886	0.463	0.017
TAC	0.998	0.018	–0.061
TFC	0.967	0.254	–0.028
Flavour	0.810	–0.325	–0.489
Colour	0.737	0.108	0.667
Taste	0.796	0.605	0.018
Mouthfeel	0.747	0.213	–0.629
Aroma	0.210	0.929	0.303
Overall	0.768	0.635	–0.087
Alcohols	–0.778	0.614	–0.135
Aldehydes	–0.950	0.272	–0.153
Esters	0.961	–0.252	0.113
Acids	–0.941	0.322	0.103
Volatile phenols	0.473	–0.865	–0.170
Ketones	0.298	–0.421	0.857

PC – principal component, *TPC* – total phenolic concentration, *TAC* – total anthocyanin concentration, *TFC* – total flavonoid concentration.

(LPFMJ, LAFMJ and LCFMJ) (Fig. 4). PC2, which accounted for 24.1 % of the variance, distinguished CON and LPFMJ from LAFMJ and LCFMJ. With reference to the loading values of each attribute, the samples were split into three groups and their features as illustrated in Fig. 4. The first group, on the positive side of PC1 and negative side of PC2, was made up from LAFMJ and LCFMJ, for which their esters, ketones, flavour and volatile phenols were typical. The second group was situated on the negative side of PC1 and positive side of PC2, and contained CON characterized by its volatile attributes (acids, aldehydes and alcohol). The last group, situated on the positive side of PC1 and PC2, was LPFMJ. This sample was characterized by *TFC*, *TAC*, *TPC*, mouthfeel, colour, aroma and taste. Although LPFMJ was characterized by mouthfeel (Fig. 4), its mouthfeel score (Fig. 3) was slightly lower than that of LAFMJ (Fig. 3). This variation could be explained by the subjective nature of sensory analysis performed.

Modelling analysis

The linear models PCR and PLSR were used to develop a predictive model for the overall quality rating of LFMJ. The coefficient α terms were fitted by PLSR and PCR using the PCA matrix of the factor score for CON (PC1: –5.15; PC2: 0.65; PC3: –0.32), LPFMJ (PC1: 2.51; PC2: 2.74; PC3: 0.71), LAFMJ (PC1: 2.08; PC2: –1.07; PC3: –2.11) and LCFMJ (PC1: 0.56; PC2: –2.32; PC3: 1.72) as the predictor variables and overall acceptability score (Fig. 3) as the predicted variable using the formula:

$$Q = \alpha_0 + \sum_{i=1}^{3} \alpha_i K_i \quad (2)$$

where Q is the predicted response (overall quality rating). α_0 and α_i are the regression coefficients for the intercept and the variables of the model, respectively. K_i is the predictor variable (principal components related to the phytochemicals, sensory attributes and volatile profile of the samples; Tab. 4).

The reliability of the models was evaluated using the root mean square error (*RMSE*) and coefficient of determination (R^2). The results revealed that the PCR model was adequate in the prediction with a high R^2 (0.99) and low *RMSE* (0.07) (Tab. 5). On the other hand, the PLSR model was more adequate in predicting the overall quality of LFMJ, with R^2 of 1 and *RMSE* of 0.00. Furthermore, PC1 was completely related to the phytochemical properties (*TPC, TAC* and *TFC*),

sensory attributes (flavour, colour, taste, mouthfeel and overall acceptability) and the volatile attributes (acids, esters, alcohol and aldehydes; Tab. 4). PC2 was associated with aroma and volatile phenols, whereas PC3 was linked to ketones (Tab. 4). The main contributors to the quality of LFMJ based on standardized coefficients (Tab. 5) were *TAC, TFC*, aldehydes, esters, acids and aroma. However, *TPC*, flavour, volatile phenols and ketones were correlated. Hence, the overall quality rating of LFMJ was predicted as:

$$OQR = 7.71 + 0.12\alpha_1 + 0.16\alpha_2 - 0.03\alpha_3 \qquad (3)$$

where *OQR* is overall quality rating, α_1 is regression coefficient for *TPC*, *TFC*, *TAC*, flavour, colour, taste, acids, esters, mouthfeel, overall acceptability, alcohol and aldehydes, α_2 is standardized coefficient for aroma and volatile profile, α_3 is standardized coefficient for ketones.

Tab. 5. Statistical outcomes of the tested models.

Model	R^2	*RSME*	*SC*	*RC*
PCR	0.99	0.07	$\beta_1 = 0.77$ $\beta_2 = 0.64$	$\alpha_0 = 7.71$ $\alpha_1 = 0.12$ $\alpha_2 = 0.16$
PLSR	1.00	0.00	$\beta_1 = 0.77$ $\beta_2 = 0.64$ $\beta_3 = -0.09$	$\alpha_0 = 7.71$ $\alpha_1 = 0.12$ $\alpha_2 = 0.16$ $\alpha_3 = -0.03$

PCR – principal components regression; PLSR – least squares regression, R^2 – coefficient of determination, *RMSE* – root mean square error; *SC* – standardized coefficient; *RC* – regression coefficient, β_1, β_2, β_3 – standardized coefficients of the models, α_0, α_1, α_2, α_3 – regression coefficients of the models.

CONCLUSION

In the present study, mulberry juice was fermented using three strains of LAB. The study contains also characterization of the volatile compounds produced by LAB during fermentation and analysis of growth kinetics of LAB, pH and *TA* during fermentation. It also assessed the phytochemical composition, sensory attributes, categorized and modelled the quality rating of the samples. The results showed that LAB were able to improve the phytochemical compositions of the mulberry juice. Moreover, the fermentation did improve the volatile profile of the juice and enhanced its sensory acceptability. With reference to the performance, the strain of *Lb. plantarum* was observed to be the most suitable for fermentation of mulberry juice and may have the ability for a possible industrial application in the production of lactic acid fermented mulberry juice. Further work on optimizing the fermentation conditions and in vitro as well as in vivo functionality of the fermented mulberry juice is highly recommended.

REFERENCES

1. Wang, Y. – Xiang, L. – Wang, C. – Tang, C. – He, X.: Antidiabetic and antioxidant effects and phytochemicals of mulberry fruit (*Morus alba* L.) polyphenol enhanced extract. PLOS One, *8*, 2013, Article e71144. DOI: 10.1371/journal.pone.0071144.
2. Duyen, N. T. – Minh, N. P. – Dao, D. T. A.: Application of natural fermentation to ferment mulberry juice into alcoholic beverage. International Journal of Scientific and Technology Research, *2*, 2013, pp. 339–346. ISSN: 2277-8616. <http://www.ijstr.org/final-print/nov2013/Application-Of-Natural-Fermentation-To-Ferment-Mulberry-Juice-Into-Alcoholic-Beverage.pdf>
3. Tripathi, M. – Giri, S.: Probiotic functional foods: Survival of probiotics during processing and storage. Journal of Functional foods, *9*, 2014, pp. 225–241. DOI: 10.1016/j.jff.2014.04.030.
4. Di Cagno, R. – Coda, R. – De Angelis, M. – Gobbetti, M.: Exploitation of vegetables and fruits through lactic acid fermentation. Food Microbiology, *33*, 2013, pp. 1–10. DOI: 10.1016/j.fm.2012.09.003.
5. Garcia, E. F. – Luciano, W. A. – Xavier, D. E. – da Costa, W. C. – de Sousa Oliveira, K. – Franco, O. L. – de Morais Júnior, M. A. – Lucena, B. T. – Picão, R. C. – Magnani, M. – Sarela, M. – de Souza, E. L.: Identification of lactic acid bacteria in fruit pulp processing byproducts and potential probiotic properties of selected *Lactobacillus* strains. Frontiers in Microbiology, *7*, 2016, Article 1371, pp. 1–11. DOI: 10.3389/fmicb.2016.01371.
6. Gan, R.-Y. – Shah, N. P. – Wang, M.-F. – Lui, W.-Y. – Corke, H.: *Lactobacillus plantarum* WCFS1 fermentation differentially affects antioxidant capacity and polyphenol content in mung bean (*Vigna radiata*) and soya bean (*Glycine max*) milks. Journal of Food Processing and Preservation, *14*, 2016, Article e12944. DOI: 10.1111/jfpp.12944.
7. Kachouri, F. – Ksontini, H. – Kraiem, M. – Setti, K. – Mechmeche, M. – Hamdi, M.: Involvement of antioxidant activity of *Lactobacillus plantarum* on functional properties of olive phenolic compounds. Journal of Food Science and Technology, *52*, 2015, pp. 7924–7933. DOI: 10.1007/s13197-015-1912-2.
8. Kaltsa, A. – Papaliaga, D. – Papaioannou, E. – Kotzekidou, P.: Characteristics of oleuropeinolytic strains of *Lactobacillus plantarum* group and influence on phenolic compounds in table olives elaborated under reduced salt conditions. Food Microbiology, *48*, 2015, pp. 58–62. DOI: 10.1016/j.fm.2014.10.016.
9. Zhao, D. – Shah, N. P.: Changes in antioxidant

capacity, isoflavone profile, phenolic and vitamin contents in soymilk during extended fermentation. LWT – Food Science and Technology, *58*, 2014, pp. 454–462. DOI: 10.1016/j.lwt.2014.03.029.
10. Scalbert, A. – Morand, C. – Manach, C. – Rémésy, C.: Absorption and metabolism of polyphenols in the gut and impact on health. Biomedicine & Pharmacotherapy, *56*, 2002, pp. 276–282. DOI: 10.1016/S0753-3322(02)00205-6.
11. Sun-Waterhouse, D. – Wadhwa, S. S.: Industry-relevant approaches for minimising the bitterness of bioactive compounds in functional foods: a review. Food and Bioprocess Technology, *6*, 2013, pp. 607–627. DOI: 10.1007/s11947-012-0829-2.
12. Sun, S. Y. – Gong, H. S. – Liu, W. L. – Jin, C. W.: Application and validation of autochthonous *Lactobacillus plantarum* starter cultures for controlled malolactic fermentation and its influence on the aromatic profile of cherry wines. Food Microbiology, *55*, 2016, pp. 16–24. DOI: 10.1016/j.fm.2015.11.016.
13. Tchabo, W. – Ma, Y. – Engmann, F. N. – Zhang, H.: Ultrasound-assisted enzymatic extraction (UAEE) of phytochemical compounds from mulberry (*Morus nigra*) must and optimization study using response surface methodology. Industrial Crops and Products, *63*, 2015, pp. 214–225. DOI: 10.1016/j.indcrop.2014.09.053.
14. Engmann, F. N. – Ma, Y. – Zhang, H. – Yu, L. – Deng, N.: The application of response surface methodology in studying the effect of heat and high hydrostatic pressure on anthocyanins, polyphenol oxidase, and peroxidase of mulberry (*Morus nigra*) juice. Journal of the Science of Food and Agriculture, *94*, 2014, pp. 2345–2356. DOI: 10.1002/jsfa.6612.
15. Nielsen, S. S. (Ed.): Food Analysis Laboratory Manual. 2nd edition. New York : Springer, 2010. ISBN: 9781441914620. DOI: 10.1007/978-1-4419-1463-7.
16. Figueroa, F. – Marhuenda, J. – Zafrilla, P. – Martínez-Cachá, A. – Mulero, J. – Cerdá, B.: Total phenolics content, bioavailability and antioxidant capacity of 10 different genotypes of walnut (*Juglans regia* L.). Journal of Food and Nutrition Research, *55*, 2016, pp. 229–236. ISSN: 1336-8672. <http://www.vup.sk/en/download.php?bulID=1894>
17. Tchabo, W. – Ma, Y. – Kwaw, E. – Zhang, H. – Li, X.: Influence of fermentation parameters on phytochemical profile and volatile properties of mulberry (*Morus nigra*) wine. Journal of the Institute of Brewing, *123*, 2017, pp. 151–158. DOI: 10.1002/jib.401.
18. Zhu, H. – Zhu, J. – Wang, L. – Li, Z.: Development of a SPME-GC-MS method for the determination of volatile compounds in Shanxi aged vinegar and its analytical characterization by aroma wheel. Journal of Food Science and Technology, *53*, 2016, pp. 171–183. DOI: 10.1007/s13197-015-2035-5.
19. Butkhup, L. – Jeenphakdee, M. – Jorjong, S. – Samappito, S. – Samappito, W. – Chowtivannakul, S.: HS-SPME-GC-MS analysis of volatile aromatic compounds in alcohol related beverages made with mulberry fruits. Food Science and Biotechnology, *20*, 2011, pp. 1021–1032. DOI: 10.1007/s10068-011-0140-4.
20. Bianchi, F. – Careri, M. – Mangia, A. – Musci, M.: Retention indices in the analysis of food aroma volatile compounds in temperature-programmed gas chromatography: Database creation and evaluation of precision and robustness. Journal of Separation Science, *30*, 2007, pp. 563–572. DOI: 10.1002/jssc.200600393.
21. Samappito, S. – Butkhup, L.: Effect of skin contact treatments on the aroma profile and chemical components of mulberry (*Morus alba* Linn.) wines. African Journal of Food Science, *4*, 2010, pp. 52–61. ISSN: 1996-0794. <http://www.academicjournals.org/journal/AJFS/article-full-text-pdf/0B169F021644>
22. Li, C. – Li, W. – Chen, X. – Feng, M. – Rui, X. – Jiang, M. – Dong, M.: Microbiological, physicochemical and rheological properties of fermented soymilk produced with exopolysaccharide (EPS) producing lactic acid bacteria strains. LWT – Food Science and Technology, *57*, 2014, pp. 477–485. DOI: 10.1016/j.lwt.2014.02.025.
23. Sadler, G. D. – Murphy, P. A.: pH and titratable acidity. In: Nielsen, S. (Ed.): Food analysis. 4th edition. New York, Springer US, 2010, pp. 219–238. ISBN: 9781441914774. DOI: 10.1007/978-1-4419-1478-1_13.
24. Fratianni, F. – Pepe, S. – Cardinale, F. – Granese, T. – Cozzolino, A. – Coppola, R. – Nazzaro, F.: *Eruca sativa* might influence the growth, survival under simulated gastrointestinal conditions and some biological features of *Lactobacillus acidophilus, Lactobacillus plantarum* and *Lactobacillus rhamnosus* strains. International Journal of Molecular Sciences, *15*, 2014, pp. 17790–17805. DOI: 10.3390/ijms151017790.
25. Argyri, A. A. – Nisiotou, A. A. – Mallouchos, A. – Panagou, E. Z. – Tassou, C. C.: Performance of two potential probiotic *Lactobacillus* strains from the olive microbiota as starters in the fermentation of heat shocked green olives. International Journal of Food Microbiology, *171*, 2014, pp. 68–76. DOI: 10.1016/j.ijfoodmicro.2013.11.003.
26. Hosseinzadeh, R. – Khorsandi, K. – Hemmaty, S.: Study of the effect of surfactants on extraction and determination of polyphenolic compounds and antioxidant capacity of fruits extracts. PLOS One, *8*, 2013, Article e57353. DOI: 10.1371/journal.pone.0057353.
27. González, L. – Sacristán, N. – Arenas, R. – Fresno, J. M. – Tornadijo, M. E.: Enzymatic activity of lactic acid bacteria (with antimicrobial properties) isolated from a traditional Spanish cheese. Food Microbiology, *27*, 2010, pp. 592–597. DOI: 10.1016/j.fm.2010.01.004.
28. Benincasa, C. – Muccilli, S. – Amenta, M. – Perri, E. – Romeo, F. V.: Phenolic trend and hygienic quality of green table olives fermented with *Lactobacillus plantarum* starter culture. Food Chemistry, *186*, 2015, pp. 271–276. DOI: 10.1016/j.foodchem.2015.02.010.
29. Tabasco, R. – Sánchez-Patán, F. – Monagas, M. –

Bartolomé, B. – Moreno-Arribas, M. V. – Peláez, C. – Requena, T.: Effect of grape polyphenols on lactic acid bacteria and bifidobacteria growth: Resistance and metabolism. Food Microbiology, *28*, 2011, pp. 1345–1352. DOI: 10.1016/j.fm.2011.06.005.
30. Svensson, L. – Sekwati-Monang, B. – Lutz, D. L. – Schieber, A. – Gänzle, M. G.: Phenolic acids and flavonoids in nonfermented and fermented red sorghum (*Sorghum bicolor* (L.) Moench). Journal of Agricultural and Food Chemistry, *58*, 2010, pp. 9214–9220. DOI: 10.1021/jf101504v.
31. Landete, J. M. – Curiel, J. A. – Rodríguez, H. – de las Rivas, B. – Muñoz, R.: Aryl glycosidases from *Lactobacillus plantarum* increase antioxidant activity of phenolic compounds. Journal of Functional Foods, *7*, 2014, pp. 322–329. DOI: 10.1016/j.jff.2014.01.028.
32. Rivera-Espinoza, Y. – Gallardo-Navarro, Y.: Non-dairy probiotic products. Food Microbiology, *27*, 2010, pp. 1–11. DOI: 10.1016/j.fm.2008.06.008.
33. Bleve, G. – Tufariello, M. – Durante, M. – Perbellini, E. – Ramires, F. A. – Grieco, F. – Cappello, M. S. – De Domenico, S. – Mita, G. – Tasioula-Margari, M. – Logrieco, A. F.: Physico-chemical and microbiological characterization of spontaneous fermentation of Cellina di Nardò and Leccino table olives. Frontiers in Microbiology, *5*, 2014, Article 570. DOI: 10.3389/fmicb.2014.00570.
34. Eduardo, I. – Chietera, G. – Bassi, D. – Rossini, L. – Vecchietti, A.: Identification of key odour volatile compounds in the essential oil of nine peach accessions. Journal of the Science of Food and Agriculture, *90*, 2010, pp. 1146–1154. DOI: 10.1002/jsfa.3932.
35. Añón, A. – López, J. F. – Hernando, D. – Orriols, I. – Revilla, E. – Losada, M. M.: Effect of five enological practices and of the general phenolic composition on fermentation-related aroma compounds in Mencia young red wines. Food Chemistry, *148*, 2014, pp. 268–275. DOI: 10.1016/j.foodchem.2013.10.056.
36. de Lerma, N. L. – Peinado, R.: Use of two osmoethanol tolerant yeast strain to ferment must from Tempranillo dried grapes: Effect on wine composition. International Journal of Food Microbiology, *145*, 2011, pp. 342–348. DOI: 10.1016/j.ijfoodmicro.2010.12.004.
37. Genovese, A. – Lamorte, S. A. – Gambuti, A. – Moio, L.: Aroma of *Aglianico* and *Uva di Troia* grapes by aromatic series. Food Research International, *53*, 2013, pp. 15–23. DOI: 10.1016/j.foodres.2013.03.051.
38. Vilanova, M. – Escudero, A. – Grańa, M. – Cacho, J.: Volatile composition and sensory properties of North West Spain white wines. Food Research International, *54*, 2013, pp. 562–568. DOI: 10.1016/j.foodres.2013.07.036.

Received 25 May 2017; 1st revised 16 July 2017; accepted 4 August 2017; published online 7 October 2017.