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Research article

Production of red beetroot juice by different methods: Kinetics of microbial growth, sugar consumption, and acid production

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ABSTRACT

As a fermentation method, the utilisation of starter culture is a common practice in industrial manufacturing, although spontaneous methods have been employed since ancient times. The objective of this study was to investigate the effect of different production methods on red beetroot juice (RBJ). For this purpose, as a starter culture, the probiotic *Lactibasillus paracasei* (*Lc. paracasei*) was inoculated into the RBJ samples after pasteurization. Also, the growth of cells, acid production, and substrate utilisation were monitored throughout the fermentation process of RBJ under two different methods of fermentation.

The samples produced by the addition of *Lc. paracasei* demonstrated a slightly lower decrease in pH values in comparison to the samples obtained by the spontaneous method. The concentration of lactic acid (LA) and acetic acid (AA) at the end of fermentation reveals that *Lc. paracasei* exhibits a greater capacity for both LA and AA generation compared to the spontaneous method. The ratios of LA and AA molar concentrations of RBJ were determined to be 1.7 and 3.6 for the samples produced by adding *Lc. paracasei* and the spontaneous method, respectively. The samples produced by adding *Lc. paracasei* exhibited a greater consumption of sucrose. Both fermentation methods provide LAB counts exceeding 8 log CFU/mL at the end of fermentation.

Time demonstrated a significant correlation with LA and AA in the method by adding *Lc. paracasei* ($r = 0.942$ and 0.745), respectively ($p < 0.01$). In both methods, it was demonstrated that while sucrose content decreased during the fermentation period, fructose and glucose content remained constant ($p < 0.05$).

1. Introduction

Fermented foods have garnered significant attention due to their notable preservation properties, as well as the several health advantages they provide [1]. Fermantation can be provided by spontaneously and also by adding starter culture [2,3]. Fermented foods, which are derived from the activity of indigenous microorganisms that exist in the natural environment, are usually known as traditionally/spontaneously fermented foods. Spontaneous fermentations, which refer to processes beginning without the utilisation of a starter inoculum, have been employed in the field of food preservation for thousands of years and have been comprehensively understood through a process of experimentation and refinement, potentially spanning thousands of years [1,4,5]. In developing

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countries, a significant proportion of small-scale fermentations, as well as certain industrial operations like sauerkraut fermentations, continue to be carried out through spontaneous methods [5]. In modern times, producers continue to prepare several of these traditional foods as a form of artistic expression, drawing from the knowledge they have acquired through trial and error over successive generations.

The technology employed is frequently applied based on scientific proof without an in-depth understanding of the fundamental principles of the fermentation process and the necessary standards to guarantee the quality and safety of the final product [6]. Furthermore, spontaneous fermentation leads to less predictability in the process, resulting in limited or slow fermentations, which sometimes have a negative impact on the quality of the final product [7,8].

New-generation beverages are produced through a controlled fermentation process carried out by selected bacterial strains that can grow in the human digestive system and show probiotic properties, especially *Lactobacillus*, *Bifidobacterium,* and *Enterococcus* genera. The use of probiotic beverages leads to a notable rise in the population of lactic acid bacteria (LAB) within the colonic region. LAB has the capacity to decrease the growth of certain bacteria, including *Escherichia coli*, *Salmonella* sp., *Staphylococcus aureus,* and *Enterococci* which are commonly present in fermented food products [9]. The protective mechanism observed in fermented products can be attributed to the existence of organic acids, including lactic acid (LA) and acetic acid (AA), that are biosynthesized by microorganisms throughout the process of fermentation. In addition to organic acids, certain species have the ability to generate hydrogen peroxide (H_2O_2), diacetyl, and bacteriocins. These compounds have a significant role in antibacterial action, as well as contribute to nutritional competition. The maintenance of a low redox potential characteristic of lactic acid fermentation and the lower pH of the medium show a protective effect for vegetable juice [7]. The variation in organic acid production during fermentation is dependent upon the predominant microorganisms in the fermentation environment. An obligatory homofermentative bacterium primarily synthesizes lactic acid as its principal metabolic byproduct, whereas bacteria classified under the facultative heterofermentative or heterofermentative category generate other substances such as organic acids [10]. Organic acids can serve as significant secondary carbon sources for several microorganisms that grow in the process of food fermentation [11].

As a plant-based beverage, red beetroot is an excellent environment for the development of probiotics due to the carbohydrates it contains. Red beetroot (*Beta vulgaris* L.), a plant within the family *Amaranthaceae*, was found to possess a sugar content approximately half that of other subspecies of sugar beet, specifically *Beta vulgaris* subsp. *vulgaris altissima* [12]. This feature allows red beetroot to be farmed for culinary products (pickles, salads, and vegetable juices) as opposed to sugar manufacturing. In comparison to other vegetables, beetroot is characterised by its predominant sugar content, which is sucrose [13]. Two bioactive substances, betalains and polyphenols, are the main culprits for the red beetroot's total antioxidant capacity [14]. Studies on LAB demonstrate good growth in the production of fermented red beetroot juice [15–19] as well as various vegetable juices [20–23].

Recent developments in microbiology have provided biological foundations for fermentation, ultimately leading to the industrialization of certain fermented foods and beverages through fermentation by starter cultures [24–26]. The potential utilisation of starter cultures may be appealing to small-scale processors if they perceive various advantages, including cost reduction (e.g. energy consumption), decreased fermentation durations, enhanced shelf-life, improved process control, enhanced sensory attributes (such as taste, aroma, visual appearance, texture, and consistency), improved safety features (e.g. reduced risk of diarrhoea and detoxification of cassava), and simplified preparation procedures for the end product [27,28]. Nevertheless, these scientific advancements demonstrate that these "successful" approaches frequently miss some quality attributes attained through traditional methods. For example, some investigators have shown that the production of wine of superior quality is still accomplished by traditional methods that depend on spontaneous fermentation [29,30]. This study aimed to compare the fermentation method used to the produce of red beetroot juice (RBJ), a fermented beverage. A comparative analysis was conducted to examine the fermentation method employed in the manufacturing of RBJ. For this purpose, two methods have been selected: i) the spontaneous method; ii) the method by adding starter culture. In order to enhance the health benefits of the fermented beverage, the probiotic *Lacticaseibacillus paracasei (Lc. paracasei*)was inoculated into the medium. The study focused on monitoring the growth of microorganisms during fermentation, their utilisation of substrates, and the generation of products. This is crucial for accurately identifying the behaviour of microorganisms involved in fermentation. There is a need for scientific investigations to uncover the positive and negative effects of both production method on fermented foods. The behaviour of microorganisms, fermentation time and end products exhibit variations depend upon the employed methodology. In this scenario, it impacts the flavour, sensory characteristics, and nutritional value of the end product. This study serves as a valuable resource for determining the fermentation method used in the manufacturing of RBJ. Previous research has primarily concentrated on examining the viability of various probiotic strains in RBJ. However, no studies have been found that investigate the effect of different production methods on RBJ.

2. Material and method

2.1. Material

The beetroot, garlic, water, bay leaves and salt used to prepare the RBJ samples were provided from local markets. *Lacticaseibacillus paracasei* (*Lc. paracasei*) 431 probiotic bacterial cultures used in the study were supplied from CHR-HANSEN (Horsholm, Denmark).

2.2. Method

2.2.1. Preparation of vegetable juice mixture and pasteurization

After being peeled, cleaned, and cut into slices, red beetroots were put through a juice extractor (Philips, HR1861) and then mixed

with the appropriate amounts of vegetable juice (62.5 %), water (37.5 %), salt (2 %), garlic (1.25 %), and bay leaf (a little piece). Our research team conducted preliminary investigations to determine the ingredients and their respective amounts in the recipe, with the goal of producing a delectable beverage. Thus, the vegetable juice mixture to be used in both methods was prepared.

In the method by adding *Lc. paracasei*, first the vegetable juice mixture was pasteurized, then pre-developed probiotic *Lc. paracasei* was inoculated into the mixture. Our team established the parameters of pasteurization temperature, time, and incubation temperature in this case by an earlier optimization study [31]. The optimum pasteurization conditions and incubation temperatures that maximized the total betalain content, LAB, and overall acceptance levels of the RBJ samples in this study were reported 60 °C for 22 min and 31 °C, respectively. These parameters were employed in the method by adding *Lc. paracasei*. In Section 2.2.2, the growth conditions and methods for *Lc. paracasei* are explained.

In the spontaneous method, the obtained vegetable juice mixture was mixed with yoghurt juice (25 %) and permitted to undergo fermentation at ambient temperature (24–25 °C) without any heat treatment. In both procedures, a distinct fermentation setup was constructed for each sampling time.

2.2.2. Preparation of cultures for the method by adding Lc. paracasei

Strains were kept at a temperature of −20 °C until use. Before the process of fermentation, the strains were cultivated in MRS broth medium and inoculated in a shaking incubator (ISS-3075, Jeio Tech Lab Companion) at 37 °C, 250 rpm for one night (ISS-3075, Jeio Tech Lab Companion). After the incubation period, a volume of suspension was inoculated into tubes which contains 10 mL of previously sterilized RBJ. After a further night of incubation under the same conditions, 2 % of the suspension was inoculated into RBJ vegetable juice mixture. In this case, the amount of bacteria that has been introduced was estimated to be around 6–7 log CFU/mL.

2.2.3. pH analysis

During fermentation, the pH of the RBJ samples was measured by pH metre (Hanna HI 1221, Czeck).

2.2.4. Organic acid analysis

The evaluation of time-dependent organic acid contents of the samples during the fermentation period was used to conducted using high performance liquid chromatography (HPLC) (Shimadzu Prominence Series, Kyoto, Japan) according the procedures reported by Ref. [32] with some modifications. For good separation in HPLC, samples centrifuged at 15000 rpm, 15 min (Sigma 2-16 PK, Germany), diluted four times with purified water and subsequently filtered using a membrane filter with a pore size of 0.45 μm, respectively. HPLC conditions used in the study; specifically, a volume of 20 μL of each filtered RBJ sample was utilized. Chromatographic separation was conducted using a 5 μm Inertsil ODS-3 column with dimensions of 250 × 4.6 mm and a particle size of 5 μm. The column was equipped with a Photodiode array (PDA) detector, which monitored absorbance at a wavelength of 210 nm. The column temperature was maintained at 30 °C during the analysis. The mobile phase comprised of a 0.005 N solution of sulphuric acid, which was eluted isocratically at a flow rate of 1 mL/min.

The identification of organic acids in the samples was accomplished through a comparative analysis of the retention time spectra of the compounds in the column, supplemented by the addition of standard substances to the samples.

2.2.5. Sugar analysis

The investigation involved conducting analyses on the time-dependent quantities of glucose, fructose, and sucrose during the fermentation process [33].

To achieve the most effective separation in HPLC, the samples were subjected to centrifugation at a speed of 15000 rpm for a duration of 15 min (Sigma 2-16 PK, Germany). Subsequently, the centrifuged samples were diluted fourfold using purified water, followed by filtration through a 0.45 μm membrane filter. Finally, a volume of 20 μL from each of the filtered samples were injected into the HPLC system which consisted of an Inertsil NH_2 column measuring 250 × 4.6 mm with a particle size of 5 μm. The HPLC system was equipped with a Diode Array Detector (DAD) detector and operated at a temperature of 40 °C. The quantification of sugars in the samples was achieved by comparing the retention time spectra of the compounds in the column and supplementing the samples with appropriate standards.

2.2.6. Microbiological analysis

The growth of Total yeast and mold (TYM), Total mezophilic and aerobic bacteria (TMAB) and total LAB in the RBJ samples was carried out following the methodology described by Szutowska et al. [22], with some modifications. Sterile saline water (0.085 % w/v) was used to prepare serial dilutions of RBJ samples, and the spread plate method was employed for microbiological cultivation in triplicate. The viable cell counts of the RBJ samples were determined by counting the TYM, TMAB, and LAB colonies on plates that were incubated under certain circumstances. The TYM colonies were incubated on PDA at a temperature of 30 °C for a duration of 7 days. The TMAB colonies were incubated on PCA at a temperature of 37 °C for a duration of 2 days. Lastly, the LAB colonies were incubated on MRS agar at a temperature of 37 °C for a duration of 2 days. The results were reported in the form of Log CFU/mL.

2.2.7. Statistical analysis

Three repetitions of all experiments were conducted. Results are presented as the mean values ± standard deviation (SD) of three repetitions.

The effect of fermentation time on the chemical and microbiological properties of RBJ samples produced by each different method was investigated by employing a one-way analysis of variance (ANOVA) test with a confidence level of 95 %. Tukey post-hoc tests were

conducted subsequent to the identification of a statistically significantin MINITAB 20 (State College, PA). The correlation and regression analyses were conducted using SPSS Statistics for Windows (version 22.0, Armonk, NY: IBM Corp.).

3. Results and discussions

Examples of RBJ samples were produced in two separate ways as the method by adding *Lc. paracasei* and by the spontaneous method.

3.1. pH and organic acid analysis

The pH levels of the samples exhibit a decline with time in both method, as shown in Fig. 1 ($p < 0.05$). The fermentation process was completed when the pH levels of the control samples reached approximately 4 or lower. Fermentation was observed for about 44 h in the method by adding Lc. paracasei and 96 h in the spontaneous method. At the conclusion of the specified times, the pH values of the samples were found to be 3.83 and 4.00, respectively. It was observed that the spontaneous method exhibits a longer duration for the pH value of the samples to approximate 4, compared to the method adding by Lc. paracasei (Fig. 1). This is an accepted situation for spontaneous fermentation. The first phase of a spontaneous fermentation process requires a considerable duration (24–48 h) and carries a substantial probability of failure. During the initial stage, known as the lag phase of microbial development, microorganisms and the surrounding environment gradually multiply and compete for nutrients to generate metabolites. However, by employing methods that involve the application of starter cultures, it is possible to reduce the duration of fermentation [28]. According to Ref. [34], *Lc. paracasei* 431 is classified as a facultative heterofermentative species of LAB, has the ability to generate either LA or AA. In this regard, the pH values of the samples produced by the adding of *Lc. paracasei* exhibited slightly lower decrease compared to the samples produced by the spontaneous method.

In both methods, LA is the main organic acid found in the environment, with AA being the next most prevalent. As a result of the both fermentation method, the LA and AA amounts of RBJ increased depending on the time ($p < 0.05$). At 0 and 96 h in the spontaneous method, LA concentrations were 519.30 mg/L and 1024.30 mg/L, respectively (Fig. 1). The AA concentrations of the samples were 42.46 mg/L and 191.33 mg/L at 0 and 96 h, respectively. In the spontaneous method, LA and AA could be measured during the 0th hour because yoghurt water was added to the fermentation media. In the method by adding *Lc. paracasei,* LA and AA could not be detected in the samples at the 0th hour, while 851.10 mg/L and 327.76 mg/L were detected, respectively, at the 44th hour (Fig. 1). The analysis of LA and AA concentrations at the initiation and conclusion of fermentation reveals that *Lc. paracasei* exhibits a greater

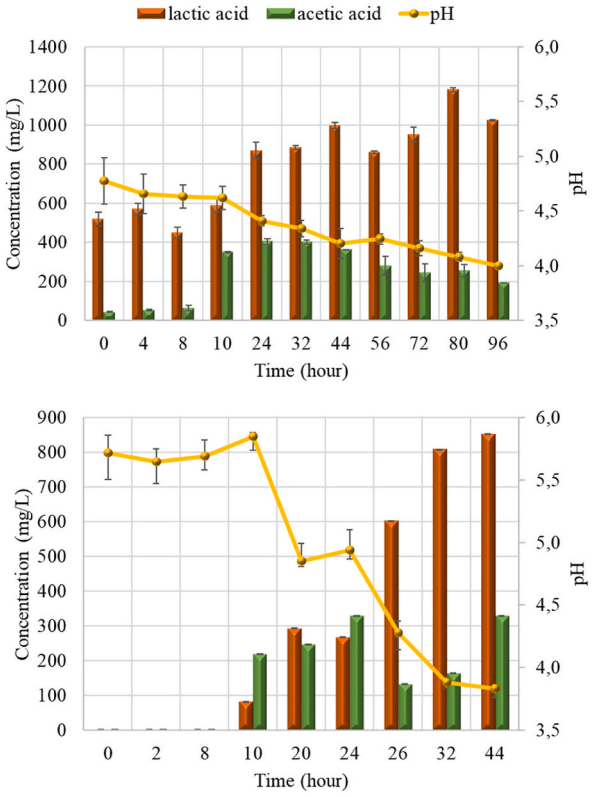

Fig. 1. The acid concentration of RBJ samples thorughout fermentation period (A: spontenous method, B: adding by *Lc. paracasaei*).

capacity for both LA and AA generation.

A significant decrease in the pH level of the samples produced by the spontaneous method appears after the 10th hour, accompanied by a parallel increase in the acid concentration (Fig. 1). It was seen that the quantity of organic acid had a positive correlation with the reduction in pH levels of the samples produced by adding *Lc. paracasei* after 10 h. The datas provide evidence supporting the correlation between pH levels and the concentration of organic acids.

At the end of fermentation, the ratios of LA and AA molar concentrations of RBJ produced by the method by adding *Lc. paracasei* and spontaneous method were determined as 1.7 and 3.6, respectively. In the samples produced by adding *Lc. paracasei* produced higher amount of LA and AA, results in a lower ratio of LA to AA due to its facultative heterofermentative behaviour. In fermented pineapple juice beverages produced by *Lc. plantarum* 299 V, *L. acidophilus* La5, and *B. lactis* Bb-12, Truong et al. (2019) [35] found that the LA/AA ratio was 10.05, 4.93, and 0.27 at the end of 24th hour of the fermentation, respectively. In a related study, Mai et al. (2018) [36] employed the *L. acidophilus* La5, *B. lactis* Bb-12, and *Lc. casei* strains to produce fermented apricot juice and declared that the products' LA/AA ratios were 2.67, 4.42, and 2.98 at the end of fermentation. The findings of our investigation align with Mai et al. (2018) in which we utilized the same strain for fermentation, and with Hing (2020) [36,37] which produces RBJ spontaneously. As the study results support, the production of organic acids can be regulated by both the food media, which plays a crucial role infor the concentration and type of sugar, and the type of bacteria that will be used them for metabolism [38]. LA commonly provides a better taste to fermented beverages than acetic acid as it has a milder flavor [39].

3.2. Sugar analysis

Sucrose, fructose, and glucose have been detected in RBJ samples produced by both fermentation methods (Fig. 2). As Table 1 shows that the concentrations of sucrose, fructose, and glucose in the RBJ samples produced by the spontaneous method were measured as 8038 mg/L, 3802 mg/L, and 65.66 mg/L, respectively, at the beginning of fermentation (0th hour). At the end of fermentation (96th hour) the concentrations were found to be 7373 mg/L, 3764 mg/L, and 73.76 mg/L, respectively (Table 1). The concentrations of sucrose, fructose, and glucose in the samples at the beginning of fermentation (0th hour) were measured to be 14490 mg/L, 3495 mg/L, and 233.57 mg/L, respectively, in the method by adding *Lc. paracasei.* At the conclusion of the 44th hour of fermentation, the concentrations were determined to be 10080 mg/L, 2484 mg/L, and 250.44 mg/L, respectively. Fig. 2 shows that the sucrose RBJ samples decreased because of the degradation of disaccharides by LAB during fermentation, and that RBJ can be a good substrate for LAB activities. Higher consumption of sucrose was detected in the samples produced by the method addition of *Lc. paracasei.* However, in the samples of RBJ produced by both methods, there was no significant difference observed in the concentrations of glucose and fructose in the fermentation media ($p < 0.05$). Depending on the rates at which monosaccharides are consumed and the breakdown of polysaccharides and disaccharides into monosaccharides, the concentration of monosaccharides may increase or remain stable during fermentation [40]. In prior studies that investigated the sugar composition of RBJ found the same sugars in the

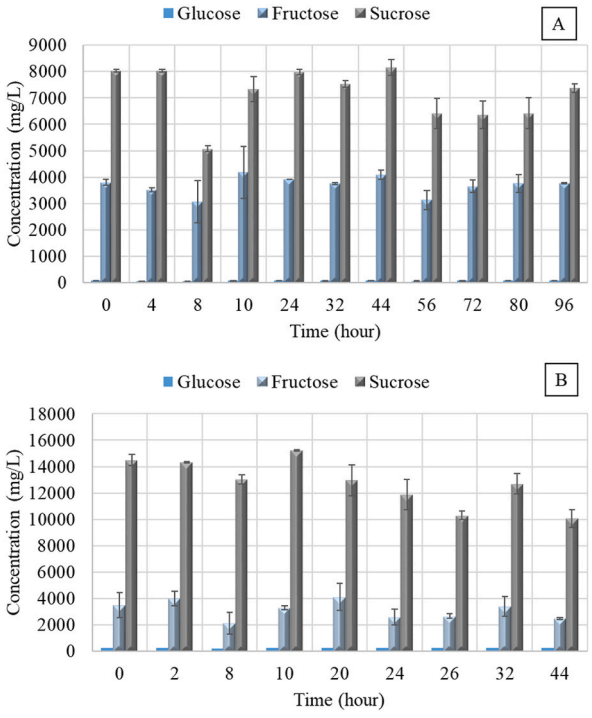

Fig. 2. The sugar concentration of RBJ samples thorughout fermentation period (A: spontenous method, B: adding by *Lc. paracasaei*).

Table 1
Changes in the concentration of acid (LA, AA) and sugar (glucose, fructose and sucrose) during the fermentation period of RBJ samples produced by two different methods.

Method	Time (h)	LA[b] (mg/L)	AA (mg/L)	Glucose (mg/L)	Fructose (mg/L)	Sucrose (mg/L)
Spontaneous	0	519.30 ± 34.60 d	42.46 ± 2.16 d	65.66 ± 5.56	3802.10 ± 79.40	8038.00 ± 38 a
	4	570.10 ± 30.20 d	50.75 ± 3.31 d	49.2 ± 16.8	3508.70 ± 55.6	8038.00 ± 38 a
	8	449.20 ± 28.10 d	62.70 ± 15.20 d	38.83 ± 6.00	3062 ± 561	5075 ± 75.30 c
	10	589.00 ± 35.50 d	346.84 ± 3.72 ab	61.69 ± 7.95	4178.00 ± 698.00	7331.60 ± 331 ab
	24	870.50 ± 42.20 c	403.30 ± 15.60 a	64.24 ± 5.63	3920.70 ± 0.32	7983.20 ± 71.50 a
	32	883.78 ± 9.95 bc	401.70 ± 10.40 a	60.89 ± 9.56	3757.00 ± 28.40	7543.70 ± 87.90 ab
	44	997.70 ± 14.80 bc	360.63 ± 1.37 ab	73.32 ± 0.67	4085.00 ± 125.00	8169.00 ± 212.00 a
	56	860.44 ± 6.10 c	280.10 ± 45.70 abc	59.10 ± 15.40	3136.00 ± 257.00	6405.90 ± 405 bc
	72	950.70 ± 37.60 bc	244.00 ± 46.20 bc	62.69 ± 0.99	3648.00 ± 166.00	6370 ± 370 bc
	80	1179.60 ± 10.30 a	254.60 ± 30.10 bc	62.88 ± 5.97	3761.00 ± 239.00	6414 ± 414 bc
	96	1024.30 ± 2.85 b	191.33 ± 1.55 c	73.76 ± 2.60	3764.50 ± 10.60	7373.00 ± 109.00 ab
Lc. paracasei	0	N.D d[a]	N.D d	233.57 ± 3.32	3495.00 ± 955.00	14490.00 ± 417.00 a
	2	N.D d	N.D d	231.48 ± 8.83	3992 ± 570	14339 ± 62.2 a
	8	N.D d	N.D d	215.82 ± 6.17	2134 ± 818	13029 ± 339 ab
	10	81.30 ± 11.00 d	218.43 ± 7.62 b	259.48 ± 6.13	3297.00 ± 148.00	15213.00 ± 52.70 a
	20	292.73 ± 8.15 c	29.9 ± 10.20 d	256.60 ± 26.40	4108 ± 1020	12961 ± 1165 ab
	24	267.30 ± 40.10 c	328.10 ± 30.20 a	232.39 ± 3.67	2616.00 ± 603.00	11878.00 ± 1145.00 ab
	26	601.30 ± 42.10 b	131.49 ± 2.26 c	237.98 ± 1.24	2666 ± 187	10390 ± 339 b
	32	807.10 ± 22.90 a	162.80 ± 27.20 bc	259.29 ± 2.50	3390.00 ± 758.00	12702.00 ± 765.00 ab
	44	851.10 ± 25.00 a	327.76 ± 1.48 a	250.44 ± 3.99	2484.00 ± 65.60	10080.00 ± 675.00 b

Values mean ± SD; n = 3.
*letters indicate statistical differences within the columns of each method ($p < 0.05$).
[a] N.D: Not detected.
[b] LA: lactic acid, AA: acetic acid, RBJ: red beetroot juice.

fermentatioın enviroment [41,42]. *Lc. casei* and *Lc. paracasei* strains, which are facultative heterofermentative LAB, have the potential to metabolise carbohydrates by either the glycolysis pathway or the pentose phosphate pathway [43,44] resulting in a noticeable decrease in sucrose concentrations over time.

3.3. Microbiological analysis

At the beginning of the fermentation process (Oth hour) the counts of TYM and TMAB of RBJ samples produced by the spontaneous method were determined to be 4.85 log CFU/mL and 4.91 CFU/mL respectively, and were found to be 8,56 log CFU/mL and 8.19 log

Table 2
Changes of microbiological counts (TYM, TMAB and LAB) of RBJ samples during the fermentation period of RBJ samples produced by two different methods.

Method	Time (h)	TYM (log CFU/mL)	TMAB (log CFU/mL)	LAB (log CFU/mL)
Spontaneous	0	4.85 ± 0.12 g	4.91 ± 0.12 h	4.39 ± 0.16 g
	4	5.00 ± 0.09 fg	5.00 ± 0.09 gh	4.58 ± 0.15 fg
	8	5.13 ± 0.07 fg	5.13 ± 0.06 fg	5.18 ± 0.02 e
	10	5.22 ± 0.06 f	5.25 ± 0.02 f	4.86 ± 0.13 ef
	24	6.69 ± 0.04 e	6.62 ± 0.03 e	6.74 ± 0.01 d
	32	7.38 ± 0.02 d	7.23 ± 0.02 d	7.36 ± 0.01 c
	44	8.31 ± 0.02 c	8.34 ± 0.02 c	8.42 ± 0.02 b
	56	8.45 ± 0.03 bc	8.27 ± 0.02 c	8.45 ± 0.04 b
	72	8.91 ± 0.05 a	8.75 ± 0.06 a	8.88 ± 0.07 a
	80	8.67 ± 0.02 ab	8.56 ± 0.02 b	8.73 ± 0.02 ab
	96	8.56 ± 0.03 bc	8.19 ± 0.04 c	8.49 ± 0.06 ab
Lc. paracasei	0	3.52 ± 0.12 f	6.62 ± 0.10 d	6.95 ± 0.01 b
	2	3.62 ± 0.02 ef	6.64 ± 0.07 d	6.96 ± 0.01 b
	8	4.17 ± 0.09 de	7.14 ± 0.09 cd	7.48 ± 0.42 ab
	10	4.58 ± 0.02 d	7.67 ± 0.04 bc	7.65 ± 0.05 ab
	20	7.84 ± 0.10 c	8.06 ± 1.18 ab	7.89 ± 0.12 ab
	24	7.69 ± 0.15 c	7.83 ± 0.10 abc	8.20 ± 0.56 ab
	26	8.00 ± 0.19 bc	8.14 ± 0.18 ab	8.46 ± 0.50 a
	32	8.75 ± 0.22 a	8.62 ± 0.21 a	8.67 ± 0.21 a
	44	8.59 ± 0.05 ab	8.36 ± 0.32 ab	8.59 ± 0.06 a

Values mean ± SD; n = 3.
* letters indicate statistical differences within the columns of each method ($p < 0.05$), LAB: total lactic acid bacteria count, TYM: total yeast and mold count, TMAB: total aerobic mesophilicbacteria count.

CFU/mL, respectively, at the end of the fermentation process (96th hour) (Table 2). The average TYM and TMAB counts of RBJ samples produced by adding *Lc. paracasei* were found to be 3.52 log CFU/mL and 8.59 log CFU/mL, and 6.62 log CFU/mL and 8.36 log CFU/mL, respectively, over the 0th and 44th hours of fermentation (Table 2). In the method by adding *Lc. paracasei*, results of TYM and TMAB values approximately 1.3 and 1.5 log lower for RBJ samples at the beginning of fermentation than the other method. Samples are pasteurized at a low temperature before being inoculated in this method, which is responsible for the lower microbial load. The initial microbial load was likewise higher in the spontaneous method since yoghurt water was added to the fermentation media. Both the counts of TYM and TMAB of samples are close to equal at the end of the fermentation. The key element in this situation is that PBJ provides excellent conditions for the development of microorganisms. The obtained counts of TYM and TMAB are similar to studies carried out on both production of fruit and vegetable beverages spontaneously [20,23,45] and controlled [46] fermentation.

At 0 and 96 h, the average LAB count of RBJ samples obtained by the spontaneous approach was found to be 4.39 log CFU/mL and 8.49 log CFU/mL, respectively (Table 2). The average count of LAB was determined 6.95 log CFU/mL at 0 h and 8.59 log CFU/mL at 44 h in RBJ samples produved by adding *Lc. paracasei*. Additionally, in a probiotic product, probiotic microorganisms should be at the optimum level of 7–8 log CFU/mL, with at least 6 log CFU/mL [47].

3.4. Correlation analysis

The relationship of the measured parameters in the RBJ samples produced by both methods was examined by Pearson correlation. The relationships found to be significant with the Pearson correlation were revealed by regression analysis. Using the data obtained, the two methods used in the production of RBJ were compared with each other.

Table 3 provides the Pearson correlation coefficients that illustrate the correlations between microorganisms growth, produced acid, and sugar utilized in RBJ samples produced by the spontaneous method. LA observed in the fermentation media has a positive and close correlation with LAB, TYM, and TMAB in the medium, with r values of 0.915, 0.923, and 0.923, accordingly ($p < 0.01$). The increases in LAB, TYM, and TMAB counts were 0.007 log CFU/mL, 0.006 log CFU/mL, and 0.007 log CFU/mL, respectively, compared to the one unit increase in LA in the fermentation medium (Fig. 3A). The relationship between LA and AA detected in the fermentation medium were found to be significant at the $p < 0.01$ level, the relationship were positive and r value was 0.556 (Table 3). The r value of 0.453 (Table 3) indicates that the association between the measured LA and glucose, among the sugars, was significant at the 0.05 level. As fermentation progressed, the amount of sucrose was decrease, but this decline was not statistically significant ($p < 0.05$). The increase in glucose levels as a function of time was explained as follows: The amount of glucose utilized by microorganisms is less than the amount of glucose generated by the breakdown of sucrose in the fermentation medium. But according to statistical analysis, the increase was not significant (p>0.05). These datas indicate that in the spontaneous method, microorganisms in the environment may have utilized both sugar and organic acids in the environment as a source of energy. AA produced by LAB during the fermentation of carbohydrates can be used as a natural energy source by other microorganisms in the environment [48,49]. The relationship between time and AA was insignificant ($p < 0.05$), however there was a strong correlation ($p < 0.01$) between the fermentation time and LA in the medium with $r = 0.868$ (Table 3). The amount of LA in the environment increases by 6249 mg/L for every additional unit of time that in the saples of RBJ produced by spontaneous method (Fig. 3B). The fermentation times of the samples are positively and closely correlated with LAB, TYM, and TMAB, with r values of 0.906, 0.920, and 0.905, respectively ($p < 0.01$). According to the results of the regression analysis, an increase of 0.043 log CFU/mL, 0.046 log CFU/mL, and 0.049 log CFU/mL was observed in the counts of LAB, TYM and TMAB, respectively, against an increase of 1 unit in the time as the fermentation progresses. (Fig. 3C).

The correlation between total sugar and LA was significant at the $p < 0.05$ level and the r value was −0.586 in RBJ samples which were fermented with *Lc. paracasei* (Table 4). The relationship between LA and sucrose was significant at the $p < 0.01$ level and the r value was −0.703. A one-unit increase in LA resulted in a decrease of 3887 mg/L and 4328 mg/L in sucrose and total sugar, respectively (Fig. 4A). Fermentation time had a strong correlation with LA, AA, and sucrose, with r values of 0.942, 0.745, and −0.769, respectively, and a significant correlation with total sugar, with r values of 0.672 ($p < 0.01$) (Table 4). An increase of one unit in the fermentation time caused the formation of 22.4 and 6.957 mg/L LA and AA, respectively (Fig. 4B). In the fermentation by adding *Lc.*

Table 3
Correlation between fermentation time and the responses of RBJ produced spontenously

	Time	LAB	TYM	TMAB	LA	AA	glucose	fructose	sucrose
LAB	0.906[a]								
TYM	0.920[a]	0.996[a]							
TMAB	0.905[a]	0.993[a]	0.996[a]						
LA	0.868[a]	0.915[a]	0.923[a]	0.923[a]					
AA	0.266	0.504[b]	0.488[b]	0.484[b]	0.556[a]				
glucose	0.388	0.379	0.416	0.406	0.453*	0.336			
fructose	0.011	0.015	0.032	0.041	0.233	0.412	0.333		
sucrose	−0.215	−0.187	−0.137	−0.136	0.044	0.229	0.411	0.491*	
TS	−0.158	−0.135	−0.090	−0.086	0.121	0.327	0.446*	0.738**	0.950**

LAB: total lactic acid bacteria count (Log CFU/mL), TYM: total yeast and mold count (Log CFU/mL), TMAB: total aerobic mesophilicbacteria count (Log CFU/mL), LA: Lactic acid (mg/L), AA: Acetic acid (mg/L), TS: Total sugar (mg/L).

[a] $p < 0.01$.

[b] $p < 0.05$.

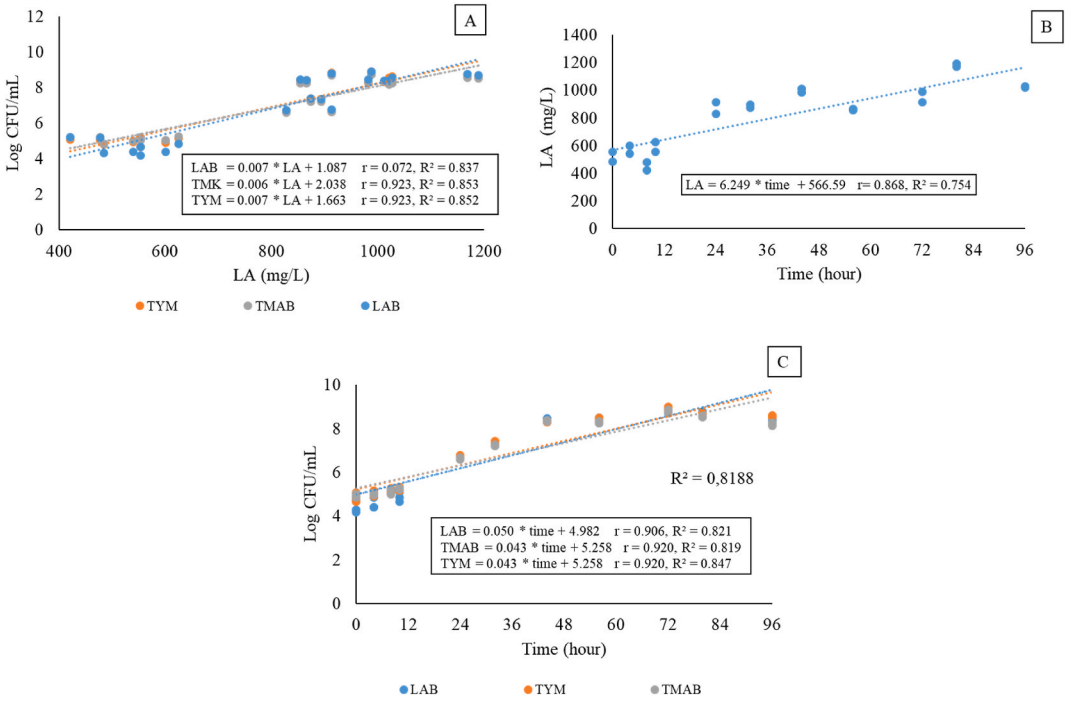

Fig. 3. The relationship between the variables of RBJ samples produced by the spontaneous method.

Table 4
Correlation between fermentation time and the responses of RBJ produced with *Lc. paracasei*.

	Time	LAB	TYM	TMAB	LA	AA	glucose	fructose	sucrose
LAB	0.784[a]								
TYM	0.919[a]	0.716[a]							
TMAB	0.861[a]	0.799[a]	0.799**						
LA	0.942[a]	0.710**	0.970**	0.820[a]					
AA	0.745[a]	0.634[a]	0.509*	0.645[a]	0.548[b]				
glucose	0.387	0.316	0.352	0.400	0.420	0.536[b]			
fructose	−0.258	−0.338	0.177	−0.238	−0.164	−0.061	−0.258		
sucrose	−0.769**	−0.621**	0.758**	−0.523*	−0.703**	−0.441	0.109	0.503*	
TS	−0.672**	−0.592**	0.633**	−0.480*	−0.586*	−0.350	0.278	0.763**	0.942**

LAB: total lactic acid bacteria count (Log CFU/mL), TYM: total yeast and mold count (Log CFU/mL), TMAB: total aerobic mesophilicbacteria count (Log CFU/mL), LA: Lactic acid (mg/L), AA: Acetic acid (mg/L), TS: Total sugar (mg/L).
[a] $p < 0.01$.
[b] $p < 0.05$.

paracasei, sucrose decreased by 101.13 mg/L, while the total sugar amount decreased by 117.95 mg/L (Fig. 4C). Also, there was a positive correlation between sucrose and fructose with a value of $r = 0.503$ ($p < 0.05$) (Table 4).

The degradation products, glucose and fructose, were utilized significantly by microorganisms in the environment during the excessive sucrose breakdown. The correlation between time and glucose was shown to be positive, much like in the spontaneous method, because the glucose released after the breakdown of sucrose is more than what microorganisms desire, but this relationship was statistically insignificant ($p < 0.05$). The concentrations of sugars found in the RBJ samples were sucrose, fructose, and glucose, ranging from high to low, as previously mentioned in the section of sugar analysis.

There was a positive and close correlation between LA found in the fermentation medium of RBJ samples produced by the addition of *Lc. paracasei* and LAB, TYM, and TMAB in the medium with r values of 0.710, 0.970, and 0.820, respectively ($p < 0.01$). The increases in LAB, TYM, and TMAB numbers were 0.002 log CFU/mL, 0.006 log CFU/mL, and 0.002 log CFU/mL, respectively, compared to the one-unit increase in LA in the fermentation medium (Fig. 4D).

The correlation between LA and AA found in the fermentation medium was significant at the 0.05 level, was positive, and had a $r = 0.548$ value (Table 4). In the RBJ samples produced by the addition of *Lc. paracasei*, there was a strong correlation between time and the count of LAB ($r = 0.784$), TYM ($r = 0.919$), and TMAB ($r = 0.861$), and the relationship was positive ($p < 0.01$). By one unit increase throughout the fermentation time, the counts of LAB, TYM, and TMAB increased to 0.043 log CFU/mL, 0.144 log CFU/mL, and 0.045 log CFU/mL, respectively (Fig. 4E).

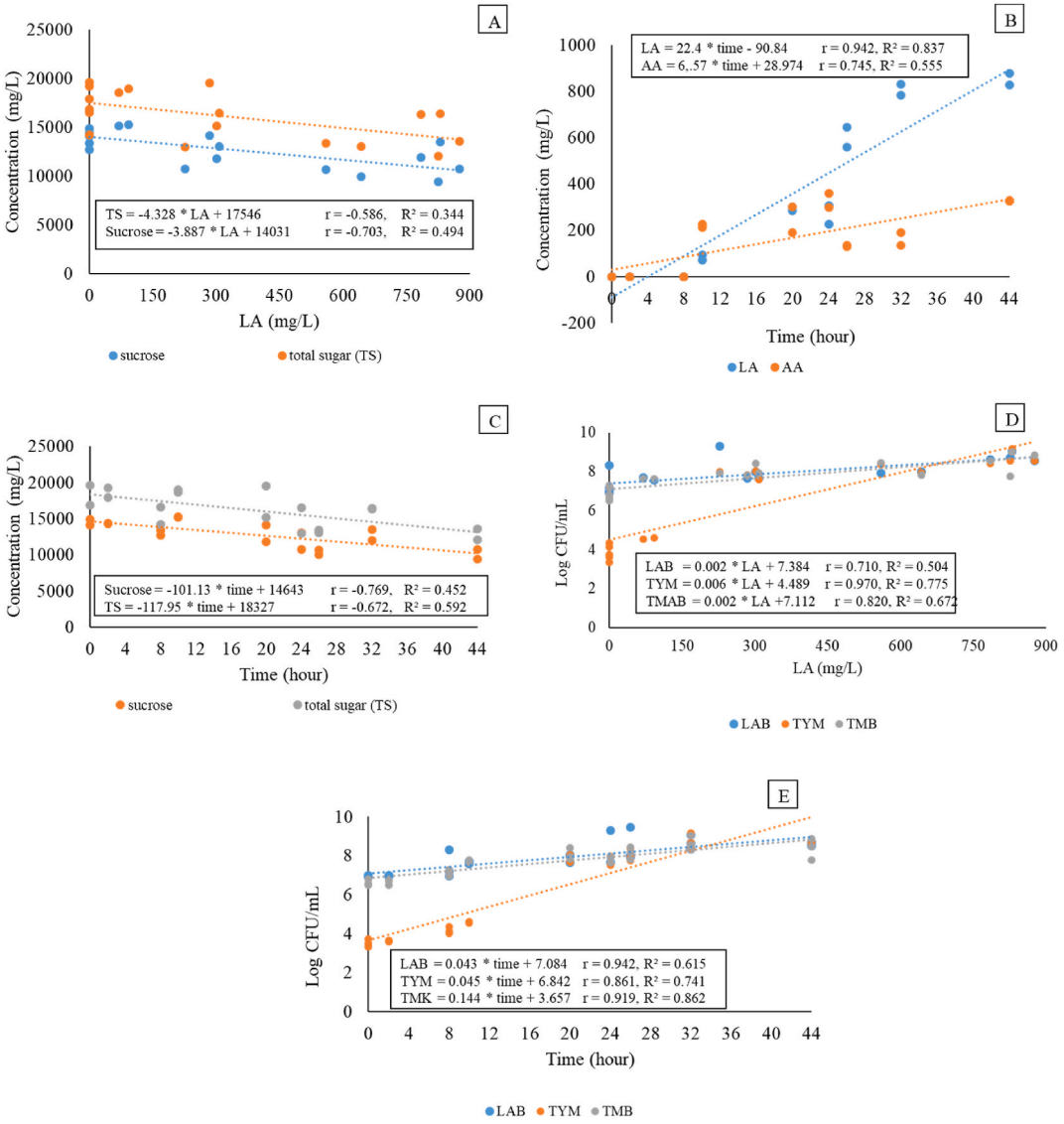

Fig. 4. The relationship between the variables of RBJ samples produced by adding *Lc. paracasei*.

When conducting a comparison between the two methods, it was observed that the correlation between time and LAB growth was stronger (r = 0.906), while the correlation between LA production was slightly weaker (r = 0.868) than the other method. Furthermore, there was no significant correlation between time and AA production (r = 0.266). Additionally, no significant correlation was found between glucose (r = 0.388), fructose (0.011), and sucrose (r = −0.215). Due to the larger variability of dominating microorganisms in the environment, we lack information regarding homofermentative or heterofermentative strains. As previously stated, the observation that the generation of AA exhibits a gradual decline over time, along with the limited consumption of sugars in the surrounding environment, implies that bacteria possess the capability to utilise environmental acids as substrates. However the samples obtained by adding *Lc. paracasei* exhibited a significant correlation between time and AA production (r = 0.745) as well as sucrose utilisation (r = −0.769). In the context of controlled fermentation, it is possible to visualise the behaviour of a starting culture.

4. Conclusion

While there was a stronger correlation observed between time and LAB count in spontaneous method, the correlation between time and TMAB was also shown to be stronger compared to the other method. Both methods result in nearly the same total LAB, TMAB, and TYM counts at the end of fermentation. Nevertheless, under the controlled experimental conditions, the process of fermentation exhibited a significantly faster rate, resulting in a lower pH value for the final product. Although both LA and AA exhibited larger concentrations and sucrose in the medium reduced over time, this decrease was not observed in the other method. In the controlled

method, the behaviour of the dominating microorganisms in the environment can be easily defined due to its known characteristics. However, in the spontaneous method, the behaviour of the microorganisms in the environment is more challenging to precisely define because they are mixed. The composition and quantity of microorganisms in the environment are contingent upon factors such as the specific red beetroot variety employed and the prevailing climatic conditions, exc. Understanding the behaviour of the microorganisms present in this context poses a considerable challenge, as it is dependent upon a multitude of factors. This causes a significant concern in relation to food safety.

In further studies, the investigation of microorganisms diversity and behaviour in fermented beverages derived from vegetables sourced from various locations and subjected to diverse climatic conditions provides potential for understanding the complex nature of the spontaneous fermentation process. Furthermore, understanding the impacts of method comparisons on various organoleptic, sensory, or chemical properties of food would yield favourable outcomes in terms of enhancing consumer awareness and preferences.

Data availability statement

The corresponding author can provide the data supporting the findings of this study upon a reasonable request.

CRediT authorship contribution statement

Sura Melisa Duyar: Writing – original draft, Methodology, Investigation. **Ferda Sari:** Writing – review & editing, Writing – original draft, Investigation, Formal analysis. **Hatice Aybuke Karaoglan:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Hatice Aybuke Karaoglan reports financial support was provided by Tubitak Turkey. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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