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Developing probiotic pumpkin juice by fermentation with commercial probiotic strain *Lactobacillus casei* 431

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Abstract

Pumpkin (*Cucurbita pepo*) showed to be an excellent source of nutrients for the probiotic growth reaching around 10^{10} CFU/ml in just 24 hr. Growth kinetics of the culture was followed during juice fermentation and the Gompertz model was fitted to the data. Approximately 9.6 g/L lactic acid was produced lowering the pH from 6.5 to 3.6 at the end of fermentation. The culture mostly utilized glucose while the fructose was consumed to lesser extent. The survivability test showed that the culture will remain above 10^6 CFU/ml during 13 days of refrigerated storage. Sensory evaluation of the fermented pumpkin juice mixed with other juices showed high acceptance. The principal component analysis revealed three components responsible for the largest portion of the variability within pumpkin juice sensory descriptors. Consensus configuration analysis showed the highest agreement of sweetness, fruity and pleasant smell with samples to which blubbery and fruit juice mix were added.

Practical applications

This study represents an original contribution in the field of nondairy probiotic beverages. The research is a comprehensive approach to product development using thorough statistical analysis of data. This is the first time to our knowledge that *L. casei* 431 is used to ferment vegetable juice.

1 | INTRODUCTION

The importance of the digestive system, represented by complex hormonal system, large number of immune cells, and intrinsic nervous system, to human health is vast. The gut microbiota represented by various microorganisms interacting with the host is important part of this system as microbial imbalance can result in obesity, insulin resistance, and other hallmarks of metabolic syndrome (Wang et al., 2015). The necessity of probiotics intake is present due to the fast and stressful way of living which causes alterations in gut microbiota composition. It happens as a consequence of the major sensitivity of gut microbiota to systemic inflammation, oxidative stress, and increased adiposity (Yoo & Kim, 2016). Hence, the well-known benefits of taking probiotics are becoming widely recognized by the consumers since continuous scientific evidence is pointing to their beneficial effects. As a result, different probiotic products are gaining popularity.

By using modern techniques the mechanisms of the known probiotic effects, like amelioration of type 2 diabetes mellitus symptoms (Salgaco et al., 2019), improvement of intestinal function to immune support (Laws & Kemp, 2019), improving colitis (Hrdý et al., 2020), improving clinical outcomes of inflammatory bowel disease pathogenesis (Sinagra et al., 2020), etc., have been confirmed. As the efficacy of probiotic products is both strain specific and disease specific, Sniffen et al. (2018) have created evidence-base practical guide for choosing the appropriate probiotic(s) with the targeted disease or condition, type of formulation, dose used, and the source (manufacturing quality control and shelf-life).

The most usual way of probiotic intake is either by direct consumption of a pharmaceutically designed probiotic supplement or consumption of probiotic functional food and drinks. Yogurt has been the most popular fermented probiotic drink up to the present time. However, lactose intolerance and the cholesterol content are the two major drawbacks related to probiotic foods based on dairy products. Taking into account the increasing number of vegan consumers as well, the market for nondiary naturally fermented probiotic drinks is open and promising and should be considered.

Botanically, the pumpkin belongs to the family *Cucurbitaceae* and is thought to have originated in Central America. Its internal flesh is golden yellow to orange which comes from the carotenoid pigments such as α- and ß-carotene and lutein. Pumpkin is characterized as a food that has a very low caloric value (100 g provides only 26 kcal) and a good source of Vitamin A but also Vitamins B (folate, riboflavin, niacin, thiamine, and pantothenic acid) and E (alfa-tocopherol) are present (Kim et al., 2012). It includes calcium, potassium, iron, zinc, and phosphorus. Pumpkin was found to exert various effects beneficial to health such as antidiabetic, anticarcinogenic, antioxidant, and antimicrobial potential (Yadav et al., 2010).

Lactobacillus casei along with closely related species *L. paracasei* and *L. rhamnosus* are some of the most studied and applied species as probiotics. Most of their commercial application is in dairy industry producing foods with improved flavor and texture (Hill et al., 2018). However, Cespedes et al. (2013) added two strains of *L. casei* to different commercial nondairy drinks and monitored the cell viability after simulated gastric digestion and storage. They found that commercial probiotic cultures of *L. casei* can be added to commercial fruit juices. There are also many examples of lactic acid fermented vegetables traditionally made in different regions of the word, sauerkraut being the most famous (Karovicova & Kohajdova, 2003). The bacterium *Lactobacillus casei* was mentioned by Swain et al. (2014) together with other lactic acid bacteria as participants in spontaneous fermentation of various substrates in Asia. They point out that many of the lactic acid bacteria are present in the fermentation medium of fruits and vegetables due to the availability of certain specific nutrients, such as vitamins and minerals, and due to their acidic nature.

The single probiotic strain *Lactobacillus casei* 431 (ATCC 55544) is a registered trademark of Chr. Hansen A/S (Hørsholm, Denmark). According to the company information's, the strain was isolated from infant feces and identified as *Lactobacillus paracasei* subsp. *paracasei*. It is designated as Generally Recognised as Safe (GRAS) from the US Food and Drug Administration (FDA) and Qualified Presumption of Safety (QPS) by European Food Safety Authority (EFSA). Many clinical studies were done on safety and survival of this strain as well as on its beneficial effects (Scott-Lutyens & Beeson, 2019). *L. casei* 431 is found to enhance the immune response (Gonzalez et al., 1990), reduce the incidence of upset stomach (Jespersen et al., 2015), reduce the duration of common cold such as running nose, cough, and sore throat (de Vrese et al., 2005). The strain is applied in the industry for enriching fruit juices with probiotics by introducing it before packaging.

The objective of the present research was to study the possibilities of production of functional nondairy drink by pumpkin juice fermentation using commercial probiotic strain, *Lactobacillus casei* 431. Growth kinetic, storage survivability, and sugar consumption were studied. Moreover, the acceptability of the obtained products was tested by sensorial analysis performed by two panels and evaluating the data statistically.

2 | MATERIALS AND METHODS

2.1 | Bacterial strains and culture conditions

The lyophilized commercial probiotic strain, *L. casei* 431 (Chr. Hansen, Denmark), was incubated overnight in semi-anaerobic conditions at 37°C in De Man, Rogosa, and Sharpe (MRS) broth (Merck, Whitehouse station, New Jersey, USA). The produced biomass was then separated by centrifugation (1,398*g*, 5 min at 20°C), washed with peptone water, and used as inoculum for the pumpkin juice fermentation.

2.2 | Substrate preparation

Pumpkin (*Cucurbita pepo*) fruit was purchased from a local supermarket, thoroughly washed, chopped into small pieces, and juiced in a kitchen juicer. The pumpkin juice was filtered through cheese cloth and bleached in water bath at 100°C for 1 min. The native pH was 6.5, while the soluble solids was corrected to 10 °Brix by addition of sterile distilled water.

2.3 | Inoculation and fermentation

The fermentation was carried out in 500 ml Erlenmeyer flasks with 200 ml pumpkin juice inoculated with 200 μl of culture containing minimum cell concentration of 10^7 CFU/ml. The Erlenmeyer flasks were placed on a rotary shaker (1,300 rpm) at 37°C for 48 hr. Samples were taken at specific time intervals for cell count, pH, total titratable acids, and sugar concentration analysis.

2.4 | Cell count

The growth of *Lactobacillus casei* 431 in the fermented products was monitored using the Miles and Misra method (Hedges, 2002). From every sample, a respective dilutions were made with sterile peptone water (0.1% w/v) and three identical drops (5 μl) of each dilution were placed on a MRS agar Petri dish in a previously marked area. The Petri dishes were placed in thermostat to be incubated in a semi-anaerobic environment at 37°C, for 48 hr. After incubation, the colonies were counted, calculated as colony forming units (CFU) per ml.

2.5 | pH and titratable acidity

pH was measured during the fermentation of pumpkin juice using calibrated pH meter Sartorius PB-11, Goettingen, Germany.

Titratable acidity expressed as lactic acid (MW = 90.0 g/mol) was measured at fermentation times: 0, 6.3, 19.0, 32.6, and 48.7 hr in order to calculate the amount of lactic acid generated during the fermentation. Sample of 1 ml fermented juice was inserted in a 200 ml Erlenmeyer flask and diluted with 25 ml of distilled water, phenolphthalein (1% w/v) as indicator was added, and titrated with 0.1 N NaOH until color change.

2.6 | Sugar analysis

High-Performance Liquid Chromatography (Agilent 1200 infinity series, Agilent Technologies, Santa Clara, US) with a Supelcosil™ LC-NH_2 column (25 cm × 4.6 mm I.D. and 5 μm particle dimensions) was used to analyze the concentration of sugars in the sample. Acetonitrile (HPLC grade, Merck KGaA, Darmstadt, Germany) and DI water were used as a mobile phase in ratio of 75:25, with 1 ml/min flow rate and 40°C column and detector (refractive index) temperature. The run time of the analysis was 15 min.

Quantification of the analytes was done using external calibration at five levels. Analytical standards of fructose, glucose, and sucrose (Merck, Darmstadt, Germany) were used. Samples of fermenting pumpkin juice (5 ml) were taken at 0, 5, 8, 25, and 34th hour from the inoculation, centrifuged for 15 min at 503*g* and the supernatant kept in a freezer at −18°C. The samples were defrosted just before the analyses, filtered through 0.45 μm cellulose filter (Econofilter, Agilent Technologies, USA), inserted in a vial, and placed in an autosampler for analysis.

2.7 | Sensory analysis

Sensory analysis by two panels, consumer and expert, was performed on the fermented pumpkin juice as well as on its 50:50 mixtures with commercial fruit juices. The prepared mixtures are shown on Table 1.

For the consumer panel, 60 participants (22–60 age range, 62% women; 48% men) were included in the sensory analyses of the products giving their preferences by grading from 1 (dislike) to 5 (like it a lot) about the color, smell, taste, aroma (perceived by entering the nose posteriorly through the nasopharynx to reach the olfactory receptor via retronasal olfaction), and total acceptance. The expert panel consisted of 20 panelists ranging the intensities (1—not detected to 5—extremely intensive) of the following smell and taste descriptors: (1) Smell—fruity, green, pleasant, milky, rancid, off smell; and (2) Taste—sweet, sour, bitter, milky, rancid, off taste.

The panelists were invited in groups of 10 persons in a specially prepared room with natural light and 21°C–23°C ambient temperature. Each participant in the sensory analyses was previously familiarized with the procedure of degustation and filling the questionnaire. Water was used in between two degustation stages as a medium for eliminating the flavor of the previous sample.

TABLE 1 Prepared mixtures of fermented pumpkin juice with four other commercially available juices

Juices	Pumpkin juice	Apple juice	Blueberry juice	Kiwi and apple juice	Mixed fruit juice
Description	Fermented with *L. casei* 431	Delicious and Idared cultivars, 100% fruit content, no addition of water, sugar, and preservatives	Min. 40% fruit content, water, sugar, and citric acid	Min. 40% fruit content, water, sugar, and citric acid	4% orange juice, 4% carrot juice, 2% lemon juice, sugar, citric acid, vitamins C and E, provitamin A, aroma, aspartame, acesulfame, and water
Mixtures/Abbreviation	PJ	AJ	BJ	KJ	FJ
PJ	+				
PJ + AJ	+	+			
PJ + BJ	+		+		
PJ + KJ	+			+	
PJ + FJ	+				+

Note: The sign "+" marks the juices that were used in the mix.

2.8 | Statistical analysis and kinetic modelling

The presented growth curves are representative results of three fermentations where the data are average values of triplicate measurements of the samples. The presented data for the HPLC analysis are average values of three independent fermentations. Standard deviations are shown as error bars.

For a description of microbial kinetics, modified Gompertz model (Equation 1) was fitted to the colony count data. A nonlinear survival model, the Weibull distribution function (Equation 2) was fitted to the data derived from the strain survival during storage, and used to estimate the specific survival rate (van Boekel, 2009). Using this model, the storage time by which the probiotic juice would retain viable count above 10^6 CFU/ml was calculated.

$$\ln\frac{N}{N_o} = A_s \exp\left[-\exp\left(\frac{\mu_{max}e}{A_s}(\lambda - t) + 1\right)\right] \quad (1)$$

where As is asymptote (max growth cycles ln N_{max}/N_o), μ_{max} is maximal specific growth rate, and λ is the time of the lag phase.

$$\log S(t) = \log\frac{N}{No} = -b \cdot t^n \quad (2)$$

with $b = \frac{1}{2.303} \cdot \left(\frac{1}{\alpha_w}\right)^{\beta}$ and $n = \beta_w$, where α_w and β_w are the two parameters of the distributions; α_w is a scale parameter (a characteristic time) and the β_w is the so-called shape parameter.

The kinetic parameters estimations of both models were calculated by using Excel add-in Analysis ToolPak and a nonlinear least squares program Solver. The variations of these parameters were calculated using Solver-Aid macro (de Levie, 2012).

The obtained data from the sensory evaluation were statistically analyzed using one-way ANOVA with Tukey B post hoc multiple comparisons (SPSS Statistics). The Levene method was used for testing the homogeneity of variances and for those parameters with unequal variances, Welch's ANOVA with Tamhane T2 post hoc analysis was used.

Principal component analysis was used to determine the components of the sensory descriptors evaluated by a professional tasting panel. The method identifies the underlying factors explaining the pattern of correlation within the analyzed set of sensory variables. Its aim was identifying smaller number of factors that explain the most of the variance observed in the analyzed sensory descriptors. Kaiser's criterion or the eigenvalue rule and the Scree test were used to determine the number of components. According to eigenvalue rule, only the components with an eigenvalue of 1.0 were retained for further investigation. The scores of the descriptors were mapped using principal components analysis (PCA) to determine how useful the developed terms were in explaining the diverse characteristics present in each of the pumpkin juice samples.

3 | RESULTS

3.1 | Cell growth and modelling

The prepared inoculum provided about 10^6 CFU/ml and after 24 hr the cells reached their stationary phase attaining nearly 10^{10} CFU/ml. Figure 1 shows the fit of the modified Gompertz model to the experimental data obtained from fermentation of pumpkin juice by *Lactobacillus casei* 431. The estimated kinetic parameters were as follows: specific growth rate 0.27 ± 0.02 hr^{-1}, lag phase 1.21 ± 0.56 hr, and maximum growth 4.48 ± 0.10 log cycles.

It can be noticed that at the beginning of the fermentation, the Gompertz curve is not very well fitted to the data. This can also be seen from the high standard deviation (46% RSD) of the lag phase estimate. Other kinetic models should be tested in order to find the best fitted one and obtain more accurate value of the lag phase. Nevertheless, the specific growth rate and the maximum growth estimations are with low relative standard deviation of 7.4% and 2.2%, respectively.

3.2 | Acidification and sugar consumption during *L. casei* 431 fermentation of pumpkin juice

The pH and the titratable acidity expressed as % lactic acid are shown on Figure 2a. From starting value of 6.5, which did not change in the first 5 hr, the pH was continuously decreasing until reaching 3.6 after 33 hr. Titratable acidity was following this trend and 9.6 g/L lactic acid was produced from the culture during the 33 hr. It can be noticed that the production of lactic acid is closely associated with the cell growth until the onset of stationary phase when the cell growth stopped (around 24 hr) while the lactic acid production continued until the 33 hr. Afterwards, there was no increase in lactic acid or significant decrease in pH till the end of the experiment (49 hr).

The concentrations of glucose, fructose, and sucrose were monitored during the pumpkin juice fermentation with *L. casei* 431 Figure 2b). It was found that glucose was the main carbon and energy source of the culture (8 g/L utilized) besides the fact that sucrose

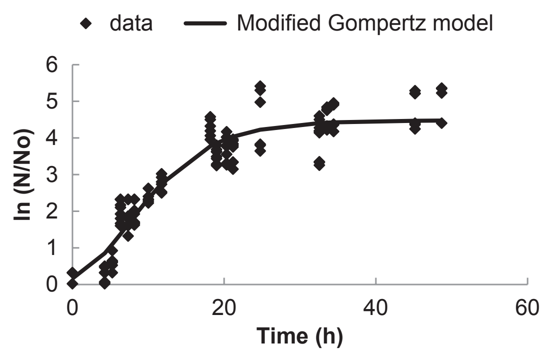

FIGURE 1 Fit of the modified Gompertz model to the growth of *L. casei* 431 in pumpkin juice

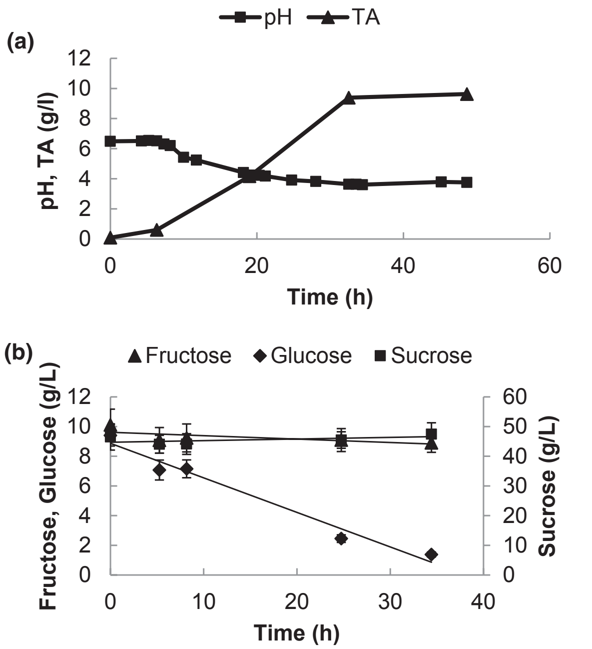

FIGURE 2 pH and titratable acidity expressed as lactic acid (a) and sugar concentration (b) of pumpkin juice during fermentation with *L. casei* 431

was much more abundant in the pumpkin juice (47 g/L). Also, a small fraction of fructose, 1.2 g/L, was also consumed by the culture.

3.3 | Survival of *L. casei* 431 during fermented pumpkin juice storage

The survival rate of the probiotic culture after the fermentation was monitored at refrigeration temperatures (4–7°C). The results presenting the viable count over time are shown in Figure 3. The Weibullian model was fitted to the data for cell survival and the parameters n and b were determined: $n = 0.79 \pm 0.048$ and $b = 0.52 \pm 0.048$. Storage time by which the probiotic juice would retain a viable count of above 10^6 CFU/ml, which is the minimum viable count for probiotics at the time of consumption (*Codex Alimentarius Commission*, Codex Standard 243), was predicted by using the estimated model parameters and the initial cell count before storage. The storage time for the pumpkin juice with an initial cell concentration of 10^{10} CFU/ml was 13.2 ± 0.6 days (inactivation of 10^4 CFU/ml).

3.4 | Sensory analysis

The results of the expert sensory panel grading different smell and taste intensities are presented in Table 2. The data show that regarding the smell, all tested juices were graded as moderately fruity and with pleasant smell (except for PJ). The panelists recognized week sensations of green, rancid, and off-smell in the tested juices while milky smell was almost not detected. Sweet taste was the sensation that was perceived with highest grade (3—moderately) in all mixtures

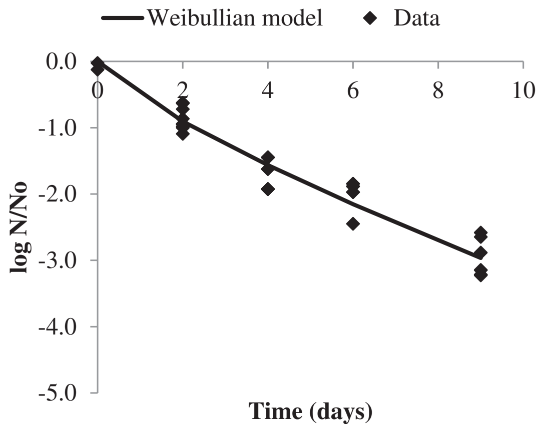

FIGURE 3 *L. casei* 431 count in pumpkin juice during storage at refrigeration temperature

except in PJ. Sour taste was the sensory perception present at a weak intensity in all tested juices, while the other tastes were almost not detected. The pure fermented pumpkin juice was least sweet and with the highest rancid and off-taste intensity compared to all other juices. Also, this juice had least pleasant smell, statistically different from PJ + BJ, and slight bitter taste, which is not significantly different only from PJ + FJ. Overall, this juice had the most unfavorable perceptions. The mix with blueberry juice (PJ + BJ), on the other hand, had the most pleasant and fruity smell, sweet taste, and no detection of rancid and off-taste. The rest of the mixed juices, PJ + AJ, PJ + KJ, and PJ + FJ, had lower values for the smell and taste perceptions, however, not significantly different from the PJ + BJ.

Table 3 shows the results of the consumer sensory panel. The mix with blueberry juice (PJ + BJ) obtained the highest grades while the pure fermented pumpkin juice (PJ) the lowest for all the tested sensory perceptions except for the color. Due to the relatively small mean difference and the high variance, there is no statistical difference between the samples for the smell and color. On the other hand, for the taste, aroma, and the overall acceptance, PJ + BJ had statistically higher grades than the PJ and PJ + KJ. The taste of the mixtures with apple juice (PJ + AJ) and with mixed fruit juice (PJ + FJ) was not statistically different from the PJ + BJ and the PJ, yet concerning the overall acceptance, they were statistically better than PJ. For the aroma preference, PJ + AJ was not statistically different from all other juices.

The sensory attributes describing the smell and the taste of the juices were tested for correlation using the Pearson correlation coefficients (Table S1). Several significant correlations among analyzed variables describing the olfactory and gustatory characteristics of the pumpkin juice samples were confirmed. For example, there was a high positive correlation between off-smell and rancid taste ($r = 0.76$, $p < .001$), between rancid taste and off-taste ($r = 0.75$, $p < .001$), and also between milky smell and milky taste ($r = 0.75$, $p < .001$) which was expected. There was also a high positive correlation between milk taste and sour taste. The highest negative correlation was observed between off-smell and pleasant smell ($r = -0.46$, $p < .001$) and between off-smell and fruity smell ($r = -0.41$, $p < .001$).

TABLE 2 Smell and taste attribute intensities of fermented pumpkin juice mixed with different fruit juices

Sensory descriptors		PJ	PJ + AJ	PJ + BJ	PJ + KJ	PJ + FJ
Smell	Fruity	2.3 ± 1.45^a	2.7 ± 1.42^a	3.4 ± 1.57^a	3.0 ± 1.32^a	3.3 ± 1.08^a
	Green	2.2 ± 1.47^a	2.0 ± 1.43^a	1.5 ± 1.10^a	1.8 ± 1.16^a	1.7 ± 1.14^a
	Pleasant	2.2 ± 1.18^b	$2.9 \pm 1.30^{a,b}$	3.6 ± 1.61^a	$2.8 \pm 1.16^{a,b}$	$3.2 \pm 0.93^{a,b}$
	Milky	1.3 ± 1.03^a	1.3 ± 1.11^a	1.0 ± 0.51^a	0.9 ± 0.37^a	1.3 ± 1.13^a
	Rancid	1.7 ± 1.42^a	1.8 ± 1.44^a	1.3 ± 0.85^a	1.4 ± 1.10^a	1.4 ± 1.23^a
	Off-smell	2.1 ± 1.68^a	1.5 ± 1.36^a	1.5 ± 1.19^a	1.5 ± 1.28^a	1.2 ± 0.88^a
Taste	Sweet	1.9 ± 1.12^b	3.1 ± 1.09^a	3.6 ± 1.32^a	3.0 ± 1.26^a	3.1 ± 1.28^a
	Sour	2.3 ± 1.30^a	1.6 ± 1.28^a	2.2 ± 1.35^a	2.2 ± 1.15^a	2.2 ± 1.32^a
	Bitter	1.6 ± 1.23^a	0.9 ± 0.36^b	0.9 ± 0.37^b	0.9 ± 0.37^b	$1.1 \pm 0.64^{a,b}$
	Milky	1.3 ± 1.03^a	1.0 ± 0.63^a	1.1 ± 0.69^a	0.9 ± 0.45^a	1.1 ± 0.79^a
	Rancid	1.8 ± 1.61^a	$1.1 \pm 0.70^{a,b}$	1.0 ± 0.51^b	$1.2 \pm 0.81^{a,b}$	$1.1 \pm 0.60^{a,b}$
	Off-taste	2.5 ± 1.82^a	1.2 ± 1.04^b	1.0 ± 0.51^b	1.2 ± 0.81^b	1.2 ± 0.77^b

Note: The grades are presented as average values and the corresponding standard deviations. Post hoc tests yielded the statistical difference ($p < .05$) of sensory parameters between juices, shown as different superscript letters after the standard deviation.

Abbreviations: AJ, apple juice; BJ, blueberry juice; FJ, mixed fruit juice; KJ, kiwi and apple juice; PJ, pumpkin juice.

TABLE 3 Consumer preferences for the main sensory descriptors of fermented pumpkin juice mixed with different fruit juices

	PJ	PJ + AJ	PJ + BJ	PJ + KJ	PJ + FJ
Taste	2.8 ± 1.34^b	$3.6 \pm 1.02^{a,b}$	4.2 ± 0.87^a	3.0 ± 1.12^b	$3.6 \pm 0.93^{a,b}$
Smell	2.9 ± 1.39^a	3.5 ± 0.93^a	3.8 ± 1.29^a	3.0 ± 0.92^a	3.2 ± 0.94^a
Color	4.0 ± 1.2^a	4.5 ± 0.81^a	3.7 ± 1.1^a	3.8 ± 0.89^a	4.4 ± 0.97^a
Aroma	2.6 ± 1.4^c	$3.4 \pm 1.2^{a,b,c}$	4.0 ± 1.2^a	$3.0 \pm 0.89^{b,c}$	$3.6 \pm 0.87^{a,b}$
Acceptance	2.3 ± 1.2^c	$3.6 \pm 0.92^{a,b}$	4.3 ± 1.15^a	3.2 ± 1.03^b	$3.6 \pm 1.12^{a,b}$

Note: The grades are presented as average values and the corresponding standard deviations. Post hoc tests yielded the statistical difference ($p < .05$) of sensory parameters between the juices, shown as different superscript letters after the standard deviation.

Abbreviations: AJ, apple juice; BJ, blueberry juice; FJ, mixed fruit juice; KJ, kiwi and apple juice; PJ, pumpkin juice.

The parameters of fruity, sweet, and pleasant were negatively correlated with green, milky, and off-smell as well as with milky, sour, and off-taste. The green smell was positively correlated with sour taste, milky smell, and milky taste.

The principal component analysis showed that about 26.2% of the total variation is explained by the first principal component (PC), 50.3% by the first two principal components, and 68.5% by the first three principal components (Table S2). In other words, 68.5% of the total variance in the 12 considered variables can be condensed into three new variables (PCs). The most important variables for the first PC were milky smell, sour, bitter, and milky taste. The PC2 was characterized by four descriptors: rancid smell and taste and off-taste and off-smell. Finally, sweet, fruity and pleasant smell were important for the PC3 (Table S3).

Figure 4 shows the results of the consensus configuration between sensory descriptors and pumpkin juice samples visualized using PCA. As seen on the map, sample PJ + FJ was stretched in the area of the sensory descriptors fruity and pleasant smell. Sample PJ + BJ was characterized by having sweet aromatics notes. The samples PJ + AJ and PJ + KJ were close to the sweet, and still on the side of the map with rancid smell. On the other side of the PCA map where sample PJ was located no near sensory descriptor was present, however, the closest found were green, sour, milk smell and taste.

4 | DISCUSSION

L. casei 431 have grown very well in the pumpkin juice reaching approximately 4,5 log cycles growth in 24 hr. Therefore, one glass (250 ml) of the fermented pumpkin juice will provide from $6.3.10^{12}$ to $2.5.10^{8}$ (at the end of the storage time, 13 days) viable *L. casei* 431 which is enough for a daily dose of probiotics, from 10^9 to 10^{10} CFU (Sanders et al., 2010). The acidity (pH = 3.6) makes the fermented pumpkin juice relatively safe from bacterial infections. However, fungi are more resistant to low pH values and can deteriorate the product so it should be stored at refrigeration temperatures.

There are few studies regarding fermentation of pumpkin with probiotics. Kohajdová et al. (2006) fermented pumpkin juice by *L. plantarum* strain reaching pH of 3.6 and TA of 1.4% in 72 hr, however,

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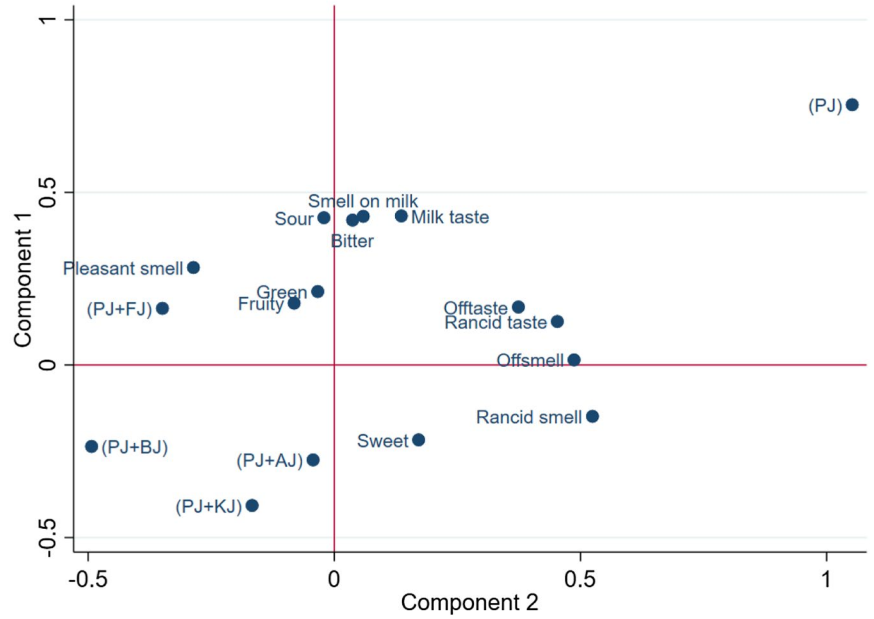

FIGURE 4 Consensus configuration for sensory descriptors and pumpkin juice samples derived from the first and second component of PCA

due to low sensory grades, other vegetable juice (cabbage and courgette) was promoted. In our study, the pH of 3.6 was achieved in 33 hr which is much shorter time and the sensory grades of the fermented juice were corrected by addition of commercial juices. Koh et al. (2018), on the other hand, managed to optimize the fermenting conditions of pumpkin juice with their isolate *Lactobacillus mali*, and obtained sensoricaly acceptable product with high alfa-glucosidase inhibitory activity and very high survival rate of 4 weeks cold storage. The survival rate of *L. casei* 431 in our case was 13 days, hence, further research should be made to extend the storage time, maybe by addition of some prebiotics. Most recently, pumpkin pure fermented with *L. rhamnosus* mixed with pineapple juice was used to create probiotic frozen dessert with good sensorial properties and prolonged storage time (due to the freezing) of 6 months (Szydłowska & Kołożyn-Krajewska, 2019). As seen, probiotic pumpkin products offer wide area of research.

Fermentation of probiotic pineapple (Costa et al., 2013), cashew apple (Pereira et al., 2011), and cantaloupe juice (Fonteles et al., 2012) with *L. casei* NRRL B442 was done developing successful products. Although the growth was not extensive, only around 2 log cycles during 10 to 24 hr, they obtained 42 days storage time at 4°C. In their study they observed decrease in sucrose levels while glucose and fructose were increased which was explained by the acidic hydrolysis of sucrose. Although reaching the same pH value of 3.6 and similar concentration of lactic acid, this was not the case in our study where only the glucose, and to smaller extent, fructose were decreasingly associated with cell consumption.

The production of lactic acid was comparable with other studies on lactic acid fermentation of vegetables (Kohajdová et al., 2006; Lavinia et al., 2012) all reaching between 0.8% and 1.5% of lactic acid. Several studies have targeted increased production of optically pure lactic acid by different strains of LAB by disruption/deletion of the *ldh* gene or by genome shuffling, reaching L-lactic acid production of up to 215 g/L (*Lactobacillus paracasei* 7BL) (Hatti-Kaul et al., 2018). Due to the facultative heterofermentative nature of *L. casei*, hexose sugars are almost exclusively metabolized, under microaerophilic conditions, via Embden-Meyerhof (EM) pathway to lactic acid (Huang et al., 2018). Only under carbon limitation, low growth rates, and change in oxygen concentration, the bacteria will ferment hexoses to lactic acid, acetic acid, ethanol, and formic acid while pentoses will ferment by the phosphoketolase pathway to lactic acid and acetic acid (De Angelis & Gobbetti, 2016).

The lactic acid yield of *L. casei* 431 during pumpkin juice fermentation was 1.1 g/g of consumed glucose. Since the theoretical yield of lactic acid is 1 g/g of consumed sugars, it can be concluded that other carbon source was also consumed in small amounts generating additional lactic acid. It was seen that fructose decreased by 1.2 g, which is comparable with the findings of Mousavi et al. (2011). In their study, while fermenting pomegranate juice with *L. Paracasei*, fructose was also consumed (in smaller extent) together with the glucose. Many bacterial species, including *L. casei*, have the capacity to transport sucrose via an inducible sucrose-specific phosphoenolpyruvate-dependent phosphotransferase system. However, the bacteria will use the glucose with priority as a carbon source, followed by fructose, and after consuming them, will start producing enzymes for sucrose utilization (Araya-Cloutier et al., 2012). This example of carbohydrate consumption was also seen in the study of Chan-Blanco et al. (2003) where the sucrose consumption of *L. casei* while fermenting banana was insignificant, compared to the consumption of glucose and fructose. The authors suggest enzymatic hydrolysis of the substrate before fermentation in order to increase the total carbohydrate consumption, lower the lag phase, and amplify the production of lactic acid.

Sensory analysis yielded several interesting conclusions about the acceptability of tested samples and the descriptors which influence it. This can be very helpful in the process of commercializing this kind of products. The results from the PCA analysis show that trained panel found differences among the pumpkin juice samples on different sensory descriptors. Although the map shows that the sensory descriptors tend to group together, it clearly shows that they can effectively separate pumpkin juice samples. As shown, the pumpkin juice samples allowed panelists to describe specific characteristics that were present in formulated fermented products; the developed descriptors were successfully used to describe pumpkin juice samples differences. It was seen that PJ + BJ and PJ + FJ are positively connected to sweet taste, pleasant, and fruity smell while negatively to green smell and also negatively connected to rancid and off-smell and taste (Figure 4). Taking that these samples obtained the highest grades of preference for aroma and taste in the consumer panel, it can be concluded that these descriptors are the most influential to the acceptance of these samples.

PJ + AJ is not close to any of the descriptors, conversely, this mixture obtained high grades for the taste and smell (similar as to PJ + FJ), during the consumer panel. This can be tentatively explained by the fact that this sample had the highest grades for color which is perceived in advance to the other perceptions and can influence the rest of them.

Sample PJ + KJ was gradated by the consumers as not as good as the PJ + BJ, PJ + FJ, and PJ + AJ but slightly better than PJ. That can be explained by its negative connection to the bitter, sour, milky smell and taste. These preferences can be tentatively defined as neutral in comparison to the negative, like off- and rancid smell and taste, and positive preference, like fruity and pleasant smell and sweet taste. However, in this case still not so desirable by the consumers for this type of products. PJ was positively correlated to the descriptors in PC1 and PC2 and negatively to PC3 which is consistent with the low consumer preferences to its taste and aroma.

5 | CONCLUSION

Probiotic bacterium *Lactobacillus casei* 431 was successfully cultivated in pumpkin juice and so obtaining probiotic nondairy beverage. The culture attained 10^{10} CFU/ml in just 24 hr of growth and sustained its number above 10^{6} CFU/ml for 13.2 ± 0.6 days of refrigerated storage. Gompertz and Weibullian kinetic models were successfully fitted to the data of the growth and the survivability of the culture thus providing aim in further scaling up of the process and trying different growth parameters. Carbohydrate analysis showed that despite high abundance of sucrose, the culture used glucose and a small amount of fructose as carbon and energy source. Lactic acid was the main growth metabolite reaching 9.6 g/L. The best sensory results were obtained when the fermented pumpkin juice was mixed with blueberry juice obtaining overall acceptance grade of 4.3 ± 1.15 (of 5) from the consumer panel. Sweet taste as well as fruity and pleasant smell were the positive perceptions for the juices while milky smell and taste, sour taste and bitter were neutral but still not very desired.

Fermentation of already mixed preparations of pumpkin and blueberry juice in different ratios could be considered as a follow-up of this study in future. Also, studding the relationship between the chemical parameters, such as pH, titratable acidity, sugar concentration, etc., and attributes scores of consumers and expert sensory analysis of taste and acceptance would give further depth to understanding appealing characteristics of nondairy probiotic beverage.

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CONFLICT OF INTEREST

The authors have declared no conflicts of interest for this article.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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