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Physicochemical and structural characterisation of fermented pumpkin purees: a sustainable approach to pulp utilisation

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ABSTRACT

This study focused on pumpkin pulp as a promising raw material for the development of value-added plant-based foods in the context of the sustainable utilization of underutilized biomass. In this context, the influence of blanching, sterilization, and lactic acid fermentation using *Pediococcus pentosaceus* on pumpkin purees from two pumpkin cultivars of different technological relevance, namely, the carotenoid-rich pumpkin variety *Cucurbita moschata* and the oil pumpkin variety *Cucurbita pepo* var. *styriaca*, was studied. Structural, physicochemical, and chemical properties were evaluated. The results showed that the fermentation process resulted in a significant decrease in pH, as the pH values decreased from 6.8 to 4.2 and 5.9 to 4.3 for fermented *Moscata* puree and fermented *Styriaca* puree, respectively. Moreover, the fermentation process affected the particle size distribution and rheological properties of the pumpkin purees. On the other hand, the β-carotene content remained relatively stable during the processing steps, especially in the purees of the variety *Curcubita moschata*, which reached values of up to 31.55 ± 0.08 μg/g f.w. Raman spectroscopy combined with principal component analysis (PCA) enabled clear differentiation between processing treatments and cultivars, explaining 87 % of the variance. The results of this study demonstrate that the thermal processing and controlled lactic acid fermentation of pumpkin purees affect the microstructure and rheology of the pumpkin purees while retaining the β-carotenoid content.

1. Introduction

The valorisation of agro-industrial by-products, including pumpkin pulp and its derived fractions, has gained increasing attention due to the rising demand for sustainable and functional plant-based foods (Gómez-García et al., 2021; Nardone et al., 2024). Pumpkin by-products, such as pulp, seeds, and peel, contain nutritionally relevant compounds, including carotenoids, polyphenols, and dietary fibres, which contribute to health-promoting properties (Gavril et al., 2024; Ratu et al., 2023; Rošul et al., 2022; Villamil et al., 2023).

Pumpkin and squash are cultivated on approximately 3 million hectares worldwide, with a total annual yield of around 27.8 million tons. China dominates global production with a share of approximately 58 %, followed by India (20 %), Ukraine (4 %), and Russia (4 %) as other notable producing countries (Hosen et al., 2021). Among cultivated species, *Cucurbita moschata* ("*Moschata*") is valued for its flavour and carotenoid richness, while *Cucurbita pepo* var. *styriaca* ("*Styriaca*") is grown primarily for seed oil, generating substantial amounts of pulp as an under-utilised by-product. Pumpkins have high nutritional value and relatively low production costs, making them attractive for the food industry. However, their industrial utilisation remains limited, despite the potential for developing novel food products from underutilised pumpkin fractions (Aziz et al., 2023; El Khatib & Muhieddine, 2019). Therefore, both the valorisation of oil pumpkin pulp and the technological utilisation of carotenoid-rich pumpkin pulp represent promising approaches for sustainable food production.

Among the various valorisation techniques, lactic acid fermentation as a mild and green technology is a promising approach for the stability and functionality of plant matrices. Lactic acid bacteria (LAB) are recognized as safe (GRAS), and LAB fermentation is accepted as a simple and successful biotechnological tool for improving the nutritional value, safety, and storage properties of fruit and vegetable products (Wang

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et al., 2023). Fermented vegetable products are increasingly relevant for vegetarian, vegan, and lactose-intolerant consumers (Liu et al., 2024; Sajjad et al., 2020) and can serve as non-dairy carriers of probiotic lactic acid bacteria (LAB) (Višnjevac et al., 2025). Several LAB strains have been shown to ferment pumpkin substrates, including *Lactobacillus mali* K8 (Koh et al., 2018) and *L. sakei / L. plantarum* (Janiszewska-Turak et al., 2024). Despite high costs of heat treatments and equipment investment, fermented pumpkin pulp appears to hold considerable promise as an intermediate product for the development of functional beverages and smoothies, containing lactic acid bacteria, particularly in the context of the growing trend for plant-based alternatives (Garcia et al., 2020). *Pediococcus pentosaceus* is particularly attractive for plant-based fermentations due to its flavour-forming and nutritional effects (Gupta et al., 1998; Jiang et al., 2021), antioxidant and cholesterol-reducing activities (Qi et al., 2021), and its ability to synthesise β-glucans that can improve texture (Velasco et al., 2007).

Thermal treatments such as blanching and sterilisation remain necessary to control enzymatic activity and microbial safety (Cvetković et al., 2025), although they may adversely affect colour and carotenoids (Contador et al., 2014; Janiszewska-Turak et al., 2024).

Combining thermal pre-treatment with fermentation may provide a synergistic strategy for preserving nutritional value and improving technological quality of pumpkin-based matrices. It was hypothesised that the two cultivars would exhibit differential physicochemical, rheological, and structural responses to the applied treatments, owing to differences in their native matrix composition, fibre structure, and carotenoid distribution.

This study investigates the physicochemical, rheological, colour, carotenoid, and structural responses of *Curcubita moschata* and *C. pepo* var. *styriaca* purees to blanching, sterilisation, and fermentation with *P. pentosaceus*, to support the sustainable valorisation of pumpkin pulp into functional, clean-label food ingredients.

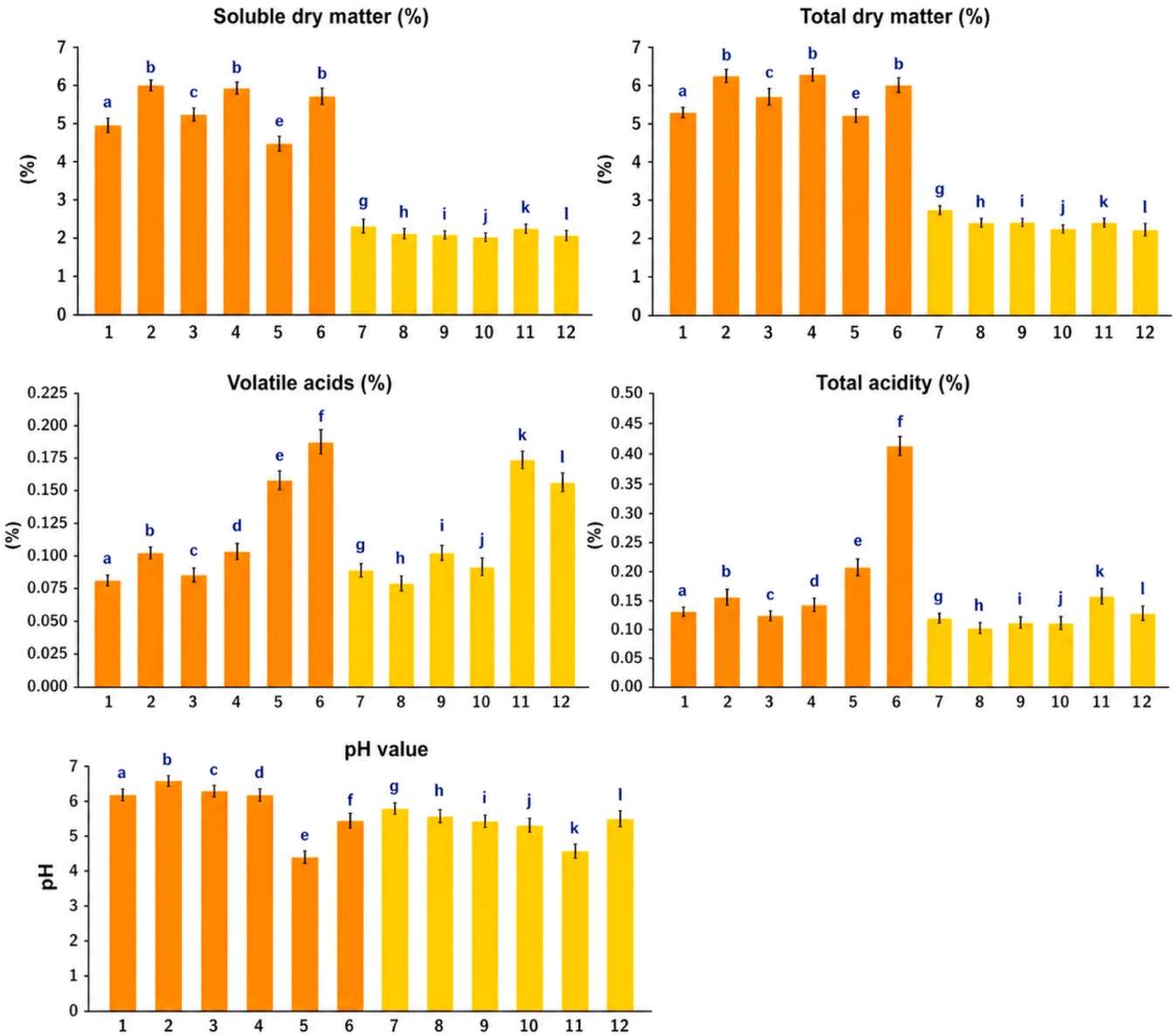


Fig. 1. Basic chemical composition of pumpkin purees during different treatments. All values are expressed as percentage (% w/w) on a fresh weight basis. 1 – Raw *Moscata* puree; 2 – Blanched *Moscata* puree; 3 – Sterilized *Moscata* puree; 4 – Blanched sterilized *Moscata* puree; 5 – Fermented *Moscata* puree; 6 – Blanched fermented *Moscata* puree; 7 – Raw *Styriaca* puree; 8 – Blanched *Styriaca* puree; 9 – Sterilized *Styriaca* puree; 10 – Blanched sterilized *Styriaca* puree; 11 – Fermented *Styriaca* puree; 12 – Blanched fermented *Styriaca* puree. *Moscata* = butternut squash (*C. moschata*), *Styriaca* = oil pumpkin (*C. pepo* var. *styriaca*). Results are presented as mean ± standard deviation ($n = 2$). Bars marked with different letters are significantly different at the $p < 0.05$ level.

2. Materials and methods

2.1. Pumpkin puree preparation, treatment and fermentation

Two pumpkin cultivars were used in the study: butternut squash (*Cucurbita moschata*, 'Moscata') and oil pumpkin (*Cucurbita pepo* var. *styriaca*, 'Styriaca'). Fresh fruits were washed, peeled, deseeded, and cut into approximately 2 cm × 2 cm cubes. Half of the pumpkin cubes were steam-blanched by placing them on a metal mesh above boiling water in a cooker (Coral Electric, MK-1500, Serbia), allowing heat transfer through water steam without direct contact with liquid water. The temperature of the heating medium was maintained below 100 °C. The temperature inside the pumpkin cubes was monitored using a probe thermometer to ensure that the internal temperature reached approximately 70 °C. Blanching was performed for 5 min, to inactivate enzymes and facilitate processing. All samples were then homogenized using a Thermo Mix for 150 s with added water at different ratios: 1:1 for raw *Styriaca*, 1:0.4 for raw *Moscata*, and 1:0.5 for the blanched samples. Different water addition ratios were applied to raw samples (1:1 for '*Styriaca*' and 1:0.4 for '*Moscata*') to compensate for cultivar-dependent differences in native moisture content and tissue firmness, thereby achieving a comparable initial puree consistency prior to further processing. The initial dry matter content (and soluble solids, °Brix) of both cultivars was determined and is reported in Section 3.1 (Fig. 1) to ensure transparency and reproducibility. Although compositional parameters are expressed on a fresh-weight basis, it should be noted that different water additions may influence direct quantitative comparisons between cultivars. Prior to fermentation, all pumpkin purees were sterilized to eliminate background microbiota and ensure controlled fermentation conditions. Sterilization was conducted in sealed glass jars in a steam sterilizer (Raypa Trade, Spain) at 121 °C for 15 min, ensuring uniform heat treatment of the puree before inoculation with *Pediococcus pentosaceus*. *Pediococcus pentosaceus* Mees 1934 (DSMZ 20,336) was maintained on de Man, Rogosa and Sharpe (MRS) agar. A single colony from an MRS agar plate was transferred into MRS broth and incubated at 30 °C for 18–24 h under static conditions. The resulting overnight culture was used to prepare the inoculum for pumpkin puree fermentation. The cell concentration was adjusted to approximately 10^7 CFU/mL, and the puree was inoculated at 2 % (v/v). The bacterial population during fermentation was determined by serial dilution and plate counting on MRS agar and expressed as colony-forming units per gram (CFU g^{-1}). Fermentation took place at 30 °C for 24 h in 750 g glass jars under anaerobic conditions, achieved by tightly sealing the vessels to prevent oxygen entry; no CO_2 chamber or gas flushing was applied.

2.2. Chemical and microbiological analysis

Total dry matter (AOAC 935.34), soluble dry matter (AOAC 932.12), volatile acids (AOAC 964.22), total acidity (AOAC 925.53), expressed as % (w/w) lactic acid equivalents, pH (AOAC 981.12), and dietary fibre (AOAC 985.29) were determined according to the Official Methods of Analysis of AOAC International (20th Edition) (Cunniff & Washington, 1997). The microbiological profile of the samples was determined by SRPS EN ISO 4833-1:2013, Microbiology of the food chain — Horizontal method for the enumeration of microorganisms — Part 1: Colony count at 30 °C by the pour plate technique), *Enterobacteriaceae* (ISO 21528-2:2017), yeasts and moulds (SRPS ISO 21527-1:2008), and lactic acid bacteria (ISO 27205:2010). All compositional parameters are reported as percentages (% w/w) on a fresh weight basis, unless otherwise stated.

2.3. Light microscopy

The particles of pumpkin puree were investigated using a Leica DMLS microscope, and images were recorded using a Leica DC300 digital camera. Image analysis of particles in terms of size distribution was carried out using ImageJ software (Rasband, W.S., National Institutes of Health, USA) after capturing optical micrographs of particles. Image analysis of particles in terms of size distribution gave the following parameters: minimum particle diameter (Min), maximum particle diameter (Max), mean particle diameter (Mean), median particle diameter (Median), projected particle area (μm²), and perimeter (μm). These are not volume-weighted diameters such as D[3,2] or D[4,3] using a laser diffraction technique.

2.4. Rheological measurements

The rheological properties of pumpkin purees were analysed with a Haake MARS rheometer (Thermo Scientific, Karlsruhe, Germany) according to the methodology defined in the paper by Belović, Torbica, Pajić-Lijaković and Mastilović (2017). The flow curves were recorded at shear rates from 0 to 100 s^{-1}, with three ramps (up, constant and down), each lasting for 120 s. Flow curve parameters were calculated by fitting the experimental data for the descending flow curve to the Ostwald-de Waele equation (Eq. (1)):

$$\tau = K\dot{\gamma}^n \quad (1)$$

where τ is the shear stress (Pa), $\dot{\gamma}$ is the shear rate (s^{-1}), K is the flow consistency index (Pa sn), and n is the flow behaviour index. Apparent viscosity was calculated as a mean value at 100 s^{-1}, during constant ramp. The thixotropic behaviour of the samples was determined by measuring the hysteresis loop area (S) between the ascending and descending flow curves.

Dynamic oscillatory measurements were performed using parallel plate sensor system (PP35) with a diameter of 35 mm and a 1 mm gap. Frequency sweeps were measured over a frequency range of 0.1–10 Hz at a stress of 0.1 Pa, which was within the linear viscoelastic region as determined by a stress sweep test at 1 Hz. All measurements were done in triplicates at 25 °C, with equilibration time of 5 min for each measurement, where G′ represents the storage modulus (Pa), reflecting elastic behaviour, and G″ represents the loss modulus (Pa), associated with viscous behaviour.

2.5. Textural measurements

Textural measurements were done with a TA.XT Plus Texture Analyser (Stable Micro Systems, Godalming, UK) equipped with a 5 kg load cell. Back Extrusion Rig (A/BE) was used to measure the consistency of the puree samples using a 45 mm disc and an extension bar. The samples were tempered for 30 min at a room temperature (23 ± 2 °C) in a back extrusion container (50 mm diameter) that was filled to around 65 %. The Texture Exponent Software (TEE32, version 6.1.6.0, Stable Micro Systems, Godalming, UK) sample project YOG1_BEC provided the instrumental parameters, which were modified to establish a trigger force of 5 g and a distance of 20 mm. The firmness was calculated as the maximum force on the positive part of the curve, while the area of the curve up to that point was taken as a measurement of consistency. The negative region of the graph was produced on probe return as a result of the weight of sample that was lifted on the upper surface of the disc on return, i.e. due to back extrusion. The maximum force on the negative part of the curve represented the cohesiveness of the sample. The area of the negative region of the curve indicated the viscosity of the sample. Every measurement was performed in triplicates.

2.6. Colour measurements

The colour of pumpkin purees was measured using a Konica Minolta Chroma Meter CR-400 using the attachment CR-A33f for the measurement of wet materials. The CIE L* (lightness), CIE a* (red-green), and CIE b* (yellow-blue) colour parameters were measured using a D65 light source and an observer angle of 2° in three repetitions.

2.7. Raman instrumentation and multivariate analysis of Raman spectra

Raman microspectroscopy of pumpkin puree samples was performed using an XploRA Raman spectrometer (Horiba Jobin Yvon, France) coupled with an Olympus BX41 microscope (Olympus, Tokyo, Japan). To account for sample inhomogeneity, ten spectra were recorded from different areas of each sample. Raman scattering was excited using a 532 nm laser source equipped with a 1200 lines/mm grating. Spectra were collected with an acquisition time of 5 s and five accumulations using a 10 % neutral density filter to avoid thermal degradation. The spectral resolution was approximately 3 cm^{-1}, and wavelength calibration was verified using the 520.47 cm^{-1} silicon band. Band assignment was carried out based on previously published carotenoid and plant-matrix Raman literature.

Pre-processing included baseline correction using a second-order Savitzky–Golay polynomial (7-point window), smoothing, normalization to the dominant carotenoid band at ~1516 cm^{-1}, and mean-centring. Spectra were pre-treated using Spectragryph 1.2.14.

2.8. β-carotene determination

For the determination of β-carotene, a modified spectrophotometric method described in the work of Luterotti et al. (2013) was used. Approximately 2 ± 0.25 g of pumpkin puree was weighed into a 50 mL centrifuge tube. A mixture of n-hexane, acetone and ethanol (2:1:1, 40 mL) was added, followed by 7.5 mL of water after shaking. The tube was kept for 5 min in an ultrasonic bath at a temperature of 25 °C (Velleman, VTUSC6, Belgium), with ultrasound frequency and power of 35 kHz and 100 W, respectively. After that, the mixture was centrifuged for 5 min at 3000 rpm to separate the upper clear hexane layer (20 ml). The absorbance of clear upper hexane layer was measured at 475 nm for β-carotene determination, using its specific absorption coefficients in hexane of 2049 dL g-1 cm-1. The concentration of β-carotene was calculated according to the following formula (Eq. (2)):

$$c = ((A - A_0))/(\varepsilon \cdot l) \quad (2)$$

where c (%) is the concentration, A is the sample absorbance, A_0 is the absorbance of the blank sample (n-hexane), ε (dL g^{-1} cm^{-1}) is the specific absorption coefficient, l (cm) is the length of light beam path.

2.9. Statistical analysis

All physicochemical measurements were performed in triplicate and expressed as mean ± standard deviation. All physicochemical, particle size, rheological and colour measurements were performed in triplicate ($n = 3$). Fermentation experiments were conducted in duplicate ($n = 2$) using a single batch of pumpkin. Fermentation kinetics and microbiological parameters were evaluated descriptively and were not subjected to inferential statistical analysis. One-way analysis of variance (ANOVA) followed by Bonferroni post hoc test was used to determine significant differences between pumpkin puree samples ($p < 0.001$). Statistical analyses were performed using Statistica 14.0.0.15 software (Tibco Inc., USA, 2020; https://www.tibco.com/products/data-science). Pearson correlation coefficients (r) were calculated to evaluate relationships between selected physicochemical, rheological, and compositional parameters. Raman spectra were pre-processed using Spectragryph 1.2.14 software (http://www.effemm2.de/spectragryph/). Spectral pre-processing included baseline correction and smoothing using a Savitzky–Golay filter (7 points, second-order polynomial), followed by normalization to the most intense band at approximately 1516 cm^{-1}. Principal component analysis (PCA) was applied to the pre-processed Raman spectral dataset using PAST software (Hammer et al., 2001) to explore multivariate relationships among samples and evaluate clustering patterns according to cultivar and processing treatment. Leave-one-out cross-validation was used to assess model robustness.

3. Results and discussion

3.1. Chemical composition

The results show significant variations in the physicochemical properties of different pumpkin puree treatments. Blanched samples generally showed higher soluble and total dry matter compared with raw samples. Fermentation was associated with a slight increase in volatile acid content in several samples, reflecting microbial metabolic activity. This observation aligns with earlier reports on LAB-mediated acidification in pumpkin and vegetable substrates (Janiszewska-Turak et al., 2024; Sajjad et al., 2020). The pH values remained relatively stable across heat treatments, although fermented samples exhibited a marked decrease, reflecting acid production.

Autoclaving affected pH and acidity, while changes in soluble solids were moderate and dependent on cultivar and treatment as noted in some previous research (Al-Ghamdi et al., 2020; Provesi et al., 2011; Santos et al., 2017). This sterilization process slightly reduced volatile acidity, likely due to the inactivation of enzymatic and microbial activities, a trend also observed in other thermally treated vegetable matrices (Cvetković et al., 2025; Contador et al., 2014). Thermal disruption of plant tissues during heat treatment may also lead to the release of intracellular compounds, which can modify the buffering capacity of the puree. These findings highlight the impact of thermal and biological treatments on pumpkin puree composition, which is important for optimizing processing techniques in food technology.

3.2. Fermentation kinetics and microbiological parameters

Table 1 presents the fermentation kinetics of blanched and non-blanched fermented pumpkin purees from the *Styriaca* and *Moscata* varieties. Fermentation kinetics and microbiological counts were evaluated exclusively for the four fermented samples, as these parameters are only biologically meaningful after the inoculation of lactic acid bacteria (LAB). The pH values presented in Fig. 1 represent physicochemical measurements across all processing treatments, while Table 1 focuses specifically on fermentation kinetics of inoculated samples.

During the fermentation process, all samples exhibited a progressive decrease in pH, with the raw purees displaying a more pronounced acidification compared to the blanched ones. The more pronounced acidification observed in raw samples may be related to the greater availability of fermentable substrates and less structural disruption compared with heat-treated tissues, which can influence LAB metabolism. In raw tissue, parenchyma cells maintain intact vacuolar compartments, allowing for quicker diffusion of soluble sugars into the medium and thereby accelerating the acidification kinetics of LAB. Conversely, blanching disrupts membrane integrity, leading to some leaching of sugars into the cooking water. This reduction in fermentable substrates slows LAB metabolism (Filannino et al., 2014). Additionally, blanching partially disrupts tissue integrity, lowers the availability of soluble sugars, and may limit diffusion, resulting in a slower reduction in pH (Choi et al., 2024).

LAB counts increased from approximately 10^5 to 10^8 CFU/g within 24 h, confirming active microbial growth and successful fermentation under the applied processing conditions. The microbial safety and selectivity of the process were further supported by consistently low counts of yeasts and moulds (<100 CFU/g), *Enterobacteriaceae* (<10 CFU/g), and total aerobic mesophilic microorganisms (<10 CFU/g) across all samples.

This approach enables the fermentation behaviour to be interpreted within its appropriate biological context, while structural and chemical changes are traced systematically throughout the full technological pathway.

Table 1
Fermentation and microbiological parameters of pumpkin purees.

Sample	pH 0h	pH 12h	pH 24h	LAB 0 h (CFU/g)	LAB 12 h (CFU/g)	LAB 24 h (CFU/g)	Yeasts & molds (CFU/g)	Total MO count (CFU/g)	*Enterobacteriaceae* (CFU/g)
Raw Moscata puree	6.7 ± 0.1	5.5 ± 0.1	4.2 ± 0.0	$2.10 \pm 0.12 \times 10^5$	$6.50 \pm 0.11 \times 10^6$	$2.00 \pm 0.10 \times 10^8$	<100	<10	<10
Blanched Moscata puree	6.9 ± 0.1	5.8 ± 0.1	5.2 ± 0.0	$1.90 \pm 0.08 \times 10^5$	$5.50 \pm 0.14 \times 10^6$	$1.60 \pm 0.09 \times 10^8$	<100	<10	<10
Raw Styriaca	5.9 ± 0.1	5.3 ± 0.0	4.3 ± 0.0	$2.30 \pm 0.11 \times 10^5$	$7.00 \pm 0.18 \times 10^6$	$2.10 \pm 0.10 \times 10^8$	<100	<10	<10
Blanched Styriaca puree	5.9 ± 0.1	5.7 ± 0.1	5.5 ± 0.0	$1.80 \pm 0.09 \times 10^5$	$4.00 \pm 0.11 \times 10^6$	$8.70 \pm 0.15 \times 10^7$	<100	<10	<10

Values are presented as mean ± SD ($n = 2$) for descriptive purposes only. No inferential statistics were performed; data are provided to illustrate general trends in pH, titratable acidity, and microbial growth during fermentation.

3.3. Particle size

The analysis of particle area, perimeter, and maximum particle size revealed no obvious trends (see Table 2). However, the mean, median, and minimum particle sizes demonstrated a clear trend: oil pumpkin consistently had statistically significantly lower particle sizes compared to butternut squash pumpkin. Specifically, the mean, median, and minimum particle sizes for butternut squash ranged from 143.7 to 214.6 μm, 149.8 to 223.0 μm, and 15.0 to 100.3 μm, respectively. In contrast, the mean, median, and minimum particle sizes for oil pumpkin were in the ranges of 120.9 to 168.3 μm, 124.6 to 172.3 μm, and 5.8 to 6.5 μm, respectively.

Additionally, both pumpkin samples exhibited reduced particle sizes following sterilization, with further reductions observed after fermentation, all with varying levels of statistical significance. The sterilization process likely disrupted the bonds between particles (Torbica et al., 2016), resulting in some of them becoming even looser after the fermentation of polysaccharides through the action of *Pediococcus* enzymes. Microscopic images confirmed the structural differences between the two cultivars and their treatments, showing more pronounced cell wall disruption in the thermally and biologically treated samples (see Supplementary Figure S1).

The observed reduction in particle size after fermentation can be attributed to acid-induced cell wall weakening and lactic acid bacteria (LAB)-associated pectin degradation. Acidification to a pH of approximately 4 promotes pectin deesterification and depolymerisation, which facilitates the breakdown of parenchymal cells and results in a finer, more homogeneous puree matrix. This reduction in particle size after sterilization and fermentation directly contributes to lower viscosity, consistent with the shear-thinning behavior observed in plant purees, where smaller particles reduce inter-particle friction. Acidification to around pH 4 promotes pectin depolymerisation and solubilisation of the middle lamella, which weakens cell adhesion and generates a smoother, less resistant matrix, thereby mechanistically explaining the rheological trends.

The observed reduction in particle size after sterilisation and especially after fermentation suggested substantial matrix loosening and partial depolymerisation of pectin-rich cell wall components. Since rheological behaviour in plant purees is strongly governed by the size, shape and integrity of dispersed particles, these microstructural changes are expected to translate into measurable differences in viscosity, thixotropic and viscoelastic properties. Therefore, the rheological and texture analysis further clarifies how the structural modifications observed here influence flow behaviour and texture.

3.4. Rheological and textural measurements

As shown in Table 3, all pumpkin purees exhibited clear time-dependent shear-thinning behaviour, consistent with previous reports for pumpkin systems (Dutta et al., 2006) and with temperature–particle size interactions described for pumpkin dispersions (Ahmed et al., 2014). Apparent viscosity at 100 s^{-1} ranged from 0.06 Pa·s (fermented blanched *Styriaca*) to 0.58 Pa·s (fermented unblanched *Moscata*), showing statistically significant differences. Within *Styriaca*, only the native unblanched sample differed significantly, while in *Moscata* no differences were found between blanched and unblanched samples before/after sterilisation. Hysteresis loop values followed a different pattern: the highest value occurred in unblanched butternut squash (36, 040 Pa/s), while the lowest was in blanched oil pumpkin (162 Pa/s). Blanched samples consistently showed reduced thixotropy compared to unblanched samples, indicating disruption of the particle–polysaccharide network by thermal treatment (Belović et al., 2016). Flow behaviour indices were closer to 1 in blanched samples of both cultivars, confirming the detrimental effect of combined heating (blanching + sterilisation) on flow structure. In contrast, the lowest flow behaviour indices and highest pseudoplasticity were recorded in unblanched sterilised fermented samples of both cultivars, accompanied by larger hysteresis loop areas than in sterilised-only samples. This

Table 2
Particle size of pumpkin purees samples.

Sample	Area (μm^2)	Mean (μm)	Minimum (μm)	Maximum (μm)	Perimeter (μm)	Median (μm)
1	9559 ± 2995^{ab}	174.9 ± 34.6^{de}	100.30 ± 23.81 g	235.4 ± 22.6^{ab}	354.3 ± 53.5^{abc}	175.2 ± 37.0^{bc}
2	$10{,}972 \pm 4638^{abc}$	214.6 ± 9.4^{f}	84.71 ± 21.25^{fg}	254.2 ± 2.4^{c}	381.6 ± 77.2^{abcd}	223.0 ± 10.7^{d}
3	$10{,}615 \pm 3850^{abc}$	190.8 ± 16.6^{ef}	68.50 ± 24.30^{ef}	246.8 ± 9.5^{abc}	372.4 ± 62.7^{abcd}	196.3 ± 17.3^{c}
4	8437 ± 2905^{a}	185.1 ± 10.4^{de}	45.57 ± 21.57^{cd}	244.9 ± 9.2^{abc}	341.5 ± 58.8^{ab}	194.8 ± 11.4^{c}
5	7431 ± 1811^{a}	143.7 ± 13.5^{ab}	14.96 ± 9.78^{ab}	233.2 ± 13.9^{a}	314.6 ± 38.6^{a}	149.8 ± 14.5^{ab}
6	$11{,}529 \pm 4081^{abc}$	160.9 ± 20.4^{bcd}	27.82 ± 14.44^{bc}	243.6 ± 12.3^{abc}	389.9 ± 68.1^{bcd}	168.2 ± 22.9^{b}
7	$10{,}919 \pm 4142^{abc}$	168.3 ± 21.1^{cde}	60.50 ± 13.46^{de}	243.9 ± 16.1^{abc}	379.9 ± 67.8^{abcd}	171.7 ± 22.9^{bc}
8	$13{,}823 \pm 5192^{c}$	149.4 ± 17.7^{bc}	20.21 ± 15.80^{ab}	235.6 ± 15.6^{ab}	424.1 ± 80.6^{cd}	154.3 ± 19.2^{b}
9	$13{,}630 \pm 2031^{bc}$	147.4 ± 21.6^{bc}	15.29 ± 14.25^{ab}	242.5 ± 11.2^{abc}	425.3 ± 31.5^{d}	152.6 ± 23.1^{b}
10	$10{,}736 \pm 2749^{abc}$	166.9 ± 28.8^{bcde}	26.61 ± 17.68^{bc}	238.6 ± 19.6^{abc}	382.9 ± 49.2^{abcd}	172.3 ± 29.7^{bc}
11	9766 ± 2379^{abc}	166.7 ± 24.0^{bcde}	19.04 ± 14.98^{ab}	251.4 ± 8.1^{bc}	361.3 ± 44.5^{abcd}	171.5 ± 25.3^{bc}
12	9988 ± 3753^{abc}	120.9 ± 16.2^{a}	5.75 ± 8.54^{a}	231.2 ± 22.1^{a}	362.2 ± 64.6^{abcd}	124.6 ± 17.6^{a}

Sample codes correspond to the treatments defined in Fig. 1. Results are presented as mean ± standard deviation ($n = 30$). Results in a column marked with different letters are significantly different at the $p < 0.05$ level.

Table 3
Results of pumpkin purees rheological measurements.

Sample	Apparent viscosity at 100 s^{-1} (Pa s)	Hysteresis loop area, *S* (Pa/s)	Consistency index, *K* (Pa s^n)	Flow behaviour index, *n* (-)	Coefficient of correlation, *r*
1	0.46 ± 0.03^{b}	$36{,}040 \pm 4879^{a}$	15.54 ± 6.00^{bc}	0.28 ± 0.10^{abcd}	0.85
2	0.44 ± 0.00^{b}	921 ± 154^{bc}	8.77 ± 1.90^{cde}	0.35 ± 0.05^{ab}	0.95
3	0.30 ± 0.06^{c}	2823 ± 547^{bc}	17.47 ± 1.47^{bc}	0.11 ± 0.03^{d}	0.89
4	0.30 ± 0.02^{c}	1652 ± 39^{bc}	11.82 ± 0.52^{bcd}	0.19 ± 0.03^{bcd}	0.87
5	0.58 ± 0.05^{a}	6521 ± 1216^{bc}	31.11 ± 5.60^{a}	0.13 ± 0.02^{d}	0.89
6	0.09 ± 0.01^{d}	638 ± 72^{c}	2.27 ± 0.04^{de}	0.27 ± 0.00^{abcd}	0.79
7	0.40 ± 0.01^{bc}	7451 ± 3051^{b}	20.91 ± 0.06^{b}	0.14 ± 0.01^{cd}	0.75
8	0.17 ± 0.04^{d}	162 ± 15^{c}	2.24 ± 0.60^{de}	0.43 ± 0.01^{a}	0.99
9	0.08 ± 0.03^{d}	434 ± 68^{c}	3.11 ± 0.72^{de}	0.18 ± 0.06^{bcd}	0.84
10	0.11 ± 0.02^{d}	285 ± 119^{c}	2.32 ± 0.99^{de}	0.33 ± 0.06^{abc}	1.00
11	0.07 ± 0.01^{d}	614 ± 40^{c}	2.50 ± 0.78^{de}	0.21 ± 0.07^{bcd}	0.95
12	0.06 ± 0.00^{d}	163 ± 54^{c}	0.73 ± 0.05^{e}	0.45 ± 0.01^{a}	1.00

Sample codes correspond to the treatments defined in Fig. 1. Results are presented as mean ± standard deviation ($n = 3$). Results in a column marked with different letters are significantly different at the $p < 0.05$ level.

behaviour is consistent with possible LAB-driven production of exopolysaccharides (EPS) (Ilić et al., 2024), although this should be confirmed in future work. However, as EPS production was not directly quantified in the present study, this interpretation should be considered indicative rather than conclusive and warrants further confirmation through targeted EPS analyses. The results could suggest that the composition of butternut squash provides a more favourable substrate for *Pediococcus* spp. EPS synthesis. Flow curves and oscillatory measurements further demonstrated that fermentation substantially affected viscoelastic behaviour. The increase in viscosity and G′ after fermentation likely reflects matrix restructuring and potential EPS formation. Although EPS were not directly quantified, the rheological signature, characterized by higher G′ and increased consistency, is in line with LAB-mediated thickening reported for *Pediococcus* species (Ilić et al., 2024; Velasco et al., 2007).

Texture measurements are presented in Table 4 and show trends somewhat similar to those observed in rheological measurements conducted at high shear rates. This similarity is not unexpected, given the comparable forces at play in both tests. However, the differences between the butternut squash and oil pumpkin samples were less pronounced in texture measurements than in rheological measurements.

The analysis included raw pumpkin puree, *Styriaca* fermented pumpkin puree, and blanched pumpkin puree. The *Styriaca* fermented samples exhibited significantly reduced parameters across the board. In contrast, the fermented raw pumpkin puree, *Moscata* fermented puree, and blanched *Moscata* fermented puree samples demonstrated higher firmness, consistency, cohesiveness, and viscosity index compared to the sterilized samples. This may indicate the potential presence of extracellular polysaccharides (EPSs) (Ilić et al., 2024).

Thermal treatments likely induced partial solubilisation of pectin and weakened the middle lamella, a well-documented effect of heating on plant tissues (Qi et al., 2000). Such structural softening reduces particle cohesion, which explains the lower viscosity observed in thermally treated samples, particularly in *C. pepo* var. *styriaca*, whose inherently weaker parenchyma structure makes it more susceptible to heat-induced breakdown.

Table 4
Textural parameters.

Sample	Firmness (g)	Consistency ($g \cdot s^{-1}$)	Cohesiveness (g)	Index of Viscosity ($g \cdot s^{-1}$)
1	698.8 ± 80.8^{c}	10838 ± 636^{e}	-222.2 ± 24.4^{ab}	-393.6 ± 74.5^{a}
2	78.3 ± 3.8^{ab}	1873 ± 133^{ab}	-61.3 ± 4.4^{cd}	-147.9 ± 8.4^{bcd}
3	206.7 ± 59.1^{ab}	2055 ± 177^{c}	-83.9 ± 38.7^{c}	-38.7 ± 15.8^{bcd}
4	86.7 ± 16.8^{ab}	1803 ± 395^{ab}	-52.7 ± 17.4^{cd}	-111.5 ± 45.5^{cde}
5	774.5 ± 186.6^{c}	7361 ± 1070^{d}	-279.7 ± 61.2^{a}	-254.0 ± 105.5^{ab}
6	179.7 ± 28.5^{ab}	3031 ± 81^{bc}	-106.1 ± 8.3^{c}	-188.8 ± 8.7^{bcd}
7	666.2 ± 65.4^{c}	8763 ± 409^{d}	-265.4 ± 58.9^{a}	-226.3 ± 19.9^{bc}
8	101.6 ± 11.9^{ab}	1711 ± 99^{ab}	-57.6 ± 9.6^{cd}	-91.0 ± 16.2^{cde}
9	249.7 ± 33.4^{b}	4234 ± 730^{c}	-137.0 ± 37.3^{bc}	-165.2 ± 71.1^{bcd}
10	125.7 ± 8.7^{ab}	2077 ± 123^{b}	-61.3 ± 7.5^{cd}	-80.6 ± 28.4^{de}
11	64.8 ± 32.9^{ab}	572 ± 83^{a}	-10.6 ± 7.7^{d}	-2.6 ± 8.9^{e}
12	49.9 ± 6.9^{a}	623 ± 32^{a}	-8.3 ± 0.9^{d}	-1.9 ± 3.9^{e}

Sample codes correspond to the treatments defined in Fig. 1. Results are presented as mean ± standard deviation ($n = 5$). Results in a column marked with different letters are significantly different at the $p < 0.05$ level. The negative values reported for cohesiveness and viscosity index originate from the negative area/force recorded during the probe return phase in the back-extrusion test.

3.5. Colour and β-carotene content

Colour and β-carotene content varied notably by cultivar and treatment (Table 5). *C. moschata* purees showed lighter (higher L*) and more vivid red-yellow hues (a*, b*), along with significantly higher β-carotene levels (24.5–31.6 μg/g f.w.), especially in raw fermented sample 5. In contrast, *C. pepo* var. *styriaca* samples exhibited duller colours and low carotenoid content (<2.2 μg/g f.w.), consistent with its seed-focused breeding. Fermentation appeared to enhance carotenoid retention, likely by improving release and stability (Mapelli-Brahm et al., 2020), while sterilization may have facilitated extractability (Ouyang et al., 2022). Although similar treatments were applied, the impact on *Styriaca* was limited by its inherently low carotenoid levels (Azevedo-Meleiro & Rodriguez-Amaya, 2007). Colour parameters a* and b* generally mirrored β-carotene trends, supporting their use as visual indicators of carotenoid content. These results emphasize the relevance of cultivar and processing choice in producing pigmented, nutritionally enhanced

Table 5
Colour parameters and β-carotene content of pumpkin purees.

Sample	L*	a*	b*	β-carotene (μg/g f.w.)
1	47.4 ± 0.5^{b}	12.6 ± 0.7^{c}	47.0 ± 2.1^{a}	24.51 ± 0.09e
2	46.1 ± 0.2^{bc}	12.6 ± 0.2^{c}	44.5 ± 1.2^{a}	29.36 ± 0.10c
3	49.6 ± 0.7^{a}	14.6 ± 0.6^{b}	46.6 ± 1.2^{a}	31.11 ± 0.10b
4	43.8 ± 0.3^{d}	11.6 ± 0.4^{d}	40.6 ± 0.5^{b}	29.19 ± 0.08c
5	50.8 ± 0.5^{a}	16.4 ± 0.5^{a}	41.2 ± 1.2^{b}	31.55 ± 0.08a
6	45.4 ± 0.4^{cd}	13.0 ± 0.8^{c}	39.2 ± 2.2^{b}	26.42 ± 0.03d
7	41.2 ± 1.4^{e}	0.8 ± 0.2^{f}	23.3 ± 0.8^{c}	1.66 ± 0.02g
8	37.3 ± 0.8^{f}	-0.9 ± 0.1^{g}	24.5 ± 0.7^{c}	0.55 ± 0.00i
9	41.9 ± 1.0^{e}	-0.8 ± 0.3^{g}	20.3 ± 1.2^{d}	1.32 ± 0.00h
10	33.7 ± 0.7^{g}	0.3 ± 0.1^{f}	19.0 ± 0.6^{d}	2.16 ± 0.03f
11	41.1 ± 3.5^{e}	1.9 ± 0.7^{e}	23.5 ± 4.3^{c}	1.65 ± 0.00g
12	31.3 ± 0.9^{h}	0.7 ± 0.2^{f}	4.8 ± 0.5^{e}	0.53 ± 0.00i

Sample codes correspond to the treatments defined in Fig. 1. Results are presented as mean ± standard deviation ($n = 3$). Results in a column marked with different letters are significantly different at the $p < 0.05$ level.

pumpkin products.

While instrumental colour and β-carotene assays provide quantitative information about pigment concentration, Raman spectroscopy offers direct molecular insight into carotenoid structure and the surrounding carbohydrate matrix.

3.6. Raman spectroscopy of pumpkin purees

Raman analysis was used to confirm whether the processing induced changes in colour and carotenoid levels corresponded to detectable alterations in vibrational fingerprints associated with chromophores, polysaccharides and cell wall components. The Raman microspectroscopy analysis of pumpkin purees included six *C. moschata* samples (1–6) and five *C. pepo* var. *styriaca* samples (7–11), as shown in Fig. 2. Sample 12 (blanched–fermented *Styriaca*) was excluded due to pronounced pigment loss and extensive cell-wall breakdown, which resulted in a Raman signal below the detection threshold. Across all samples, characteristic carotenoid bands were observed at 1516 cm^{-1}, 1150 cm^{-1}, and 1001 cm^{-1}, corresponding to the $\nu 1$ (C=C), ν_2 (C–C) and $\rho(C–CH_3)$ carotenoid vibrational modes. These peaks, forming the carotenoid "fingerprint region," confirm the presence of conjugated polyene structures typical of pumpkin puree (Bhosale et al., 2004; Pećinar et al., 2019, 2021). Weak additional bands were detected at 1676 cm^{-1}, and in the 300–950 cm^{-1} region, with low-intensity signals near 1446, 1308, and 1184 cm^{-1}.

The bands related to carbohydrates, the most abundant macronutrients in the pumpkin puree appear with a lower intensity in the region 300–950 cm^{-1}. Bands at 359, 489, 534, 640 cm^{-1} and 747, 845 cm^{-1} indicated on C—C or C—O stretching and banding vibration of polygalacturonic (pectic) acid or pectin (Synytsya et al., 2003; Chylińska et al., 2014).

The band at ~1446 cm^{-1} is associated with $\delta(CH_2)$ vibrations and glycosidic structures (Boyaci et al., 2015; da Silva et al., 2008), while the ~950 cm^{-1} band corresponds to polygalacturonic acid (Synytsya et al., 2003). Signals in the 1600–1700 cm^{-1} region may reflect phenolic or protein contributions (Boyaci et al., 2015; Prats Mateu et al., 2016). Comparison of *C. moschata* spectra (samples 1–6) revealed minimal changes in peak positions or intensities across treatments. In contrast, oil-pumpkin (*C. pepo* var. *styriaca*) samples exhibited more pronounced spectral variation after sterilisation, especially in regions associated with glycosidic structures (950, 1446 cm^{-1}), xylans (1308 cm^{-1}) and lignin-related bands (1569, 1676 cm^{-1}), suggesting modifications in cell-wall components induced by processing. The more intense carotenoid signals in *C. moschata* reflect its naturally higher β-carotene content (Table 5), whereas *C. pepo* var. *styriaca* contains more fibrous cell wall components such as cellulose and hemicellulose. These compositional differences not only influence Raman profiles but also affect textural behaviour and fermentation performance, since soluble sugars in *C. moschata* support faster LAB metabolic activity than the more fibrous *Styriaca* matrix.

Spectral changes in the carbohydrate-related region (~1000–1200 cm^{-1}) were consistent with the measured reductions in particle size and the rheological strengthening of fermented samples. This alignment between Raman signals and bulk physical properties confirms that processing induced modifications of the carbohydrate matrix play a key role in defining the structural behaviour of the purees.

3.7. Multivariate analysis with the aim of determining the influence of the treatment effect of native pumpkins purees

Sometimes individual averaged Raman spectra do not show pronounced visual differences between the compared treatments, such as in the case of pumpkin purees (Fig. 2, 1–8). Because subtle spectral differences across treatments may not be fully distinguishable by visual inspection alone, Principal Component Analysis (PCA) was applied to the pre-processed Raman data to highlight multivariate trends associated with thermal processing, fermentation and cultivar-specific composition. Multivariate analysis based on PCA was applied to distinguish between the chemical compositions of purees two pumpkin species in native state (1, 7) and their treatments (2, 3, 4, 5, 6 and 8, 9, 10, 11 respectively). The PC analysis was performed with a total of about 110 Raman spectra. The score plot of PC1 versus PC2 shows a fairly good separation between the samples, with the first (PC1) and second principal components (PC2) describing 87.0 % of the data variance. The mutual projections of the factor scores and their loadings for the first two PCs are shown in Fig. 3.

The PCA score plot (Fig. 3A) showed a clear separation of samples along PC1 and PC2, explaining 87.0 % of the total variance (PC1: 74.2 %, PC2: 12.8 %). PC1 primarily differentiated fermented from non-fermented samples, while PC2 reflected cultivar differences. *C. moschata* samples (1–6) and *C. pepo* var. *styriaca* (7–11) formed two distinct clusters. Raw and blanched samples grouped closely, dominated by carotenoid bands at ~1516 and 1154 cm^{-1}. Fermented samples shifted strongly along PC1, reflecting structural changes associated with lactic acid fermentation, including carotenoid modification and reduction of cell wall polysaccharide signals (~1000–1200 cm^{-1}). The loading plot (Fig. 3B) indicated that PC1 variation was mainly driven by carbohydrate- and lignin-related bands at 1313, 1434, and 1569 cm^{-1}

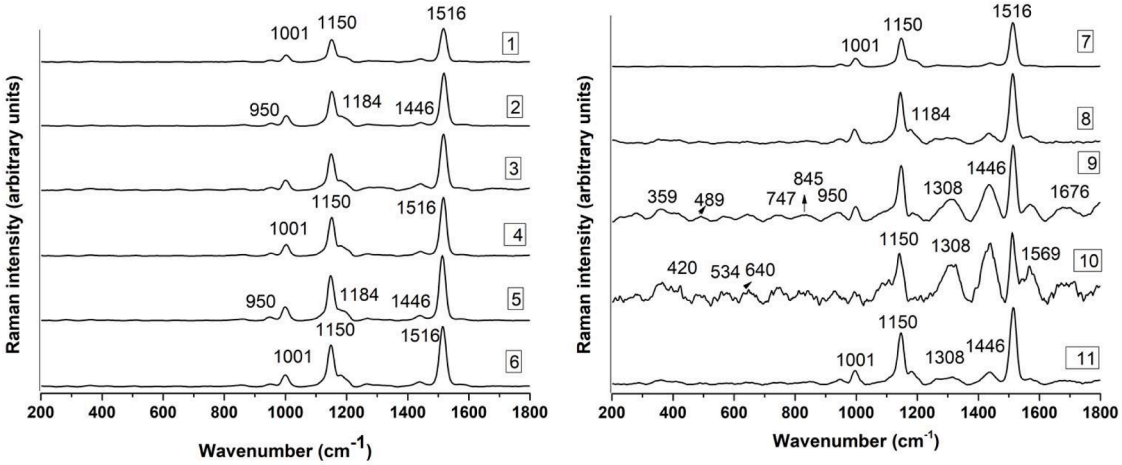


Fig. 2. Averages of normalized Raman spectra of 1 to 11 samples of pumpkin purees recorded from parenchyma cell in the spectral range from 200 to 1800 cm^{-1}. On carotenoids indicated bands at 1516, around 1150 and 1001 cm^{-1}, glucosidic linkage stretches (359, 845, 950, 1446 cm^{-1}), xylan (1308 cm^{-1}) and lignin (1569, 1676 cm^{-1}). Sample codes correspond to the treatments defined in Fig. 1.

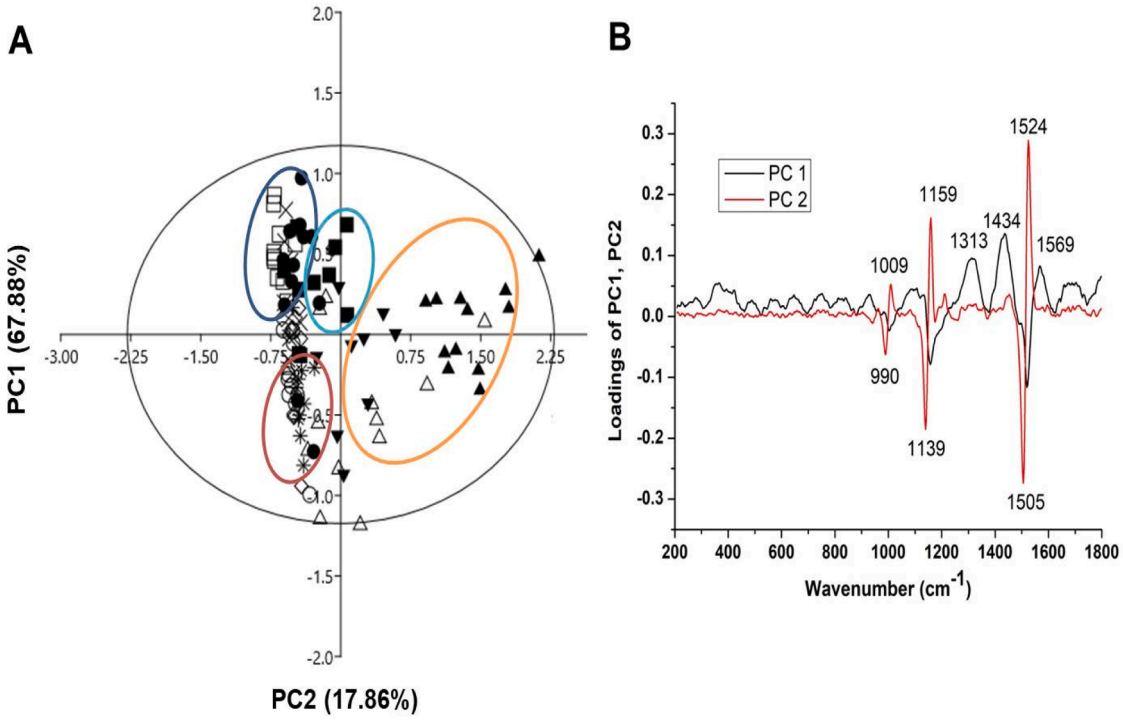


Fig. 3. Principal component analysis (PCA) applied to Raman spectral data of pumpkin purees from two pumpkin species. A) PCA score plot; B) PCA loading plot. Different symbols represent individual samples corresponding to the sample codes defined in Fig. 1 (1–11). Symbol shapes indicate the following samples: 1 – circle, 2 – open square, 3 – closed square, 4 – cross, 5 – open circle, 6 – open diamond, 7 – star, 8 – open triangle, 9 – closed triangle, 10 – inverted open triangle, 11 – inverted closed triangle. Coloured ellipses indicate clustering of samples according to pumpkin cultivar and processing treatment identified by PCA.

(Boyaci et al., 2015; Prats Mateu et al., 2016). PC2 was associated with carotenoid-related peaks at 1524, 1159, and 1009 cm^{-1}, explaining higher carotenoid content in *C. moschata* (Campos et al., 2023; Dhanani et al., 2022; Legner et al., 2021; Pećinar et al., 2021). *C. pepo* var. *styriaca* showed stronger signals at 1505 and 1139 cm^{-1}, related to phenolics and polyene structures, and a band near 990 cm^{-1} indicating higher cellulose/hemicellulose content (Gierlinger et al., 2013). C1 likely reflects processing-related structural modifications (thermal + acidic), while PC2 reflects inherent cultivar differences in carotenoid density and fibre composition. This aligns with the chemical and rheological results, confirming that processing primarily changes matrix structure, whereas cultivar dictates pigment- and fibre-related spectral features. The score plot showed clustering of samples according to cultivar and processing stage, indicating that both factors influenced the overall physicochemical profile. The corresponding loadings indicated that β-carotene, colour parameters and acidity contributed most strongly to the observed sample separation. These results demonstrate a clear processing-structure-function relationship in pumpkin purees, where thermal pre-treatment initiates cell wall weakening, and fermentation enhances matrix breakdown through acidification, and cultivar-specific composition determines the rheological and structural properties of the final product.

3.8. Correlation analysis

Correlation analysis (Fig. 4) was performed using selected independent physicochemical, rheological, and compositional parameters of pumpkin purees. The colour parameter a* showed a strong positive correlation with b* ($r = 0.94$), indicating coordinated variation of these attributes. β-carotene content was strongly associated with a* ($r = 0.96$) and b* ($r = 0.94$), which is consistent with previous reports suggesting that colour parameters reflect pigment levels (Ouyang et al., 2022). Firmness showed a positive association with consistency ($r = 0.95$), reflecting expected relationships between texture parameters. The

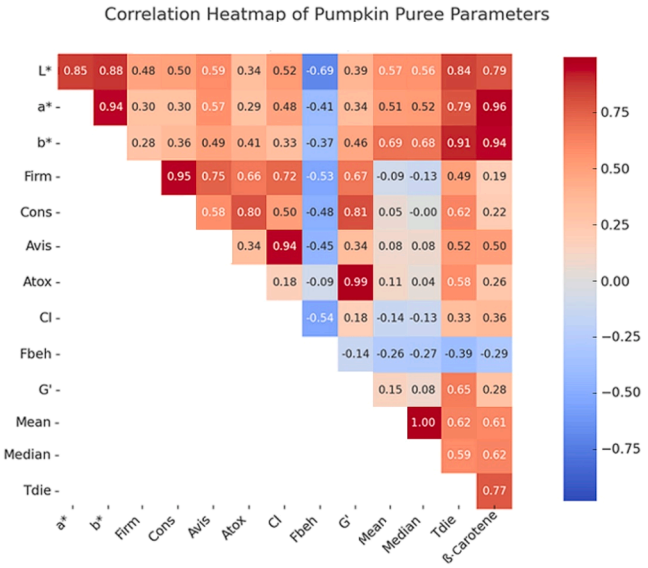


Fig. 4. Pearson correlation matrix between parameters of pumpkin purees. L* – lightness; a*- red/green coordinate; b*- yellow/blue coordinate; Firm - firmness (g); Cons - consistency ($g \cdot s^{-1}$); Avis - apparent viscosity (Pa·s); Atox - torque at 100 s^{-1} (g·cm); CI - consistency index; Fbeh - flow behaviour index; G′ - storage modulus (Pa); Mean - mean particle size (μm); Median - median particle size (μm); Tdie - total dietary fibre (%); β-carotene - beta-carotene content (μg/g dry matter).

associations observed between total dietary fibre and rheological properties are in agreement with previous reports describing the role of fibre in structuring plant-based matrices (Gupta et al., 1998; Jiang et al., 2021). Overall, the correlation analysis highlights coordinated variation among physicochemical and compositional attributes relevant to

functional quality. Integrating these datasets demonstrates a coherent treatment-composition-structure pathway: thermal processing initiates cell wall weakening, fermentation enhances biochemical transformations through acidification and enzymatic activity, and the cultivar's intrinsic carotenoid–fibre balance influences the final rheological and spectral response.

A limitation of the present study is the use of a single pumpkin batch and a single lactic acid bacterial strain *Pediococcus pentosaceus*, which restricts the generalizability of the findings. Nevertheless, this controlled experimental design enabled a clear assessment of the effects of processing treatments (blanching, sterilisation, and lactic acid fermentation) and cultivar-specific differences between oil pumpkin (*Cucurbita pepo* var. *styriaca*) and butternut squash (*Cucurbita moschata*). The results provide new insights into how thermal processing combined with fermentation influences physicochemical properties, particle size distribution, rheological behaviour and carotenoid retention in pumpkin purees. Future research should investigate additional cultivars and microbial strains to further clarify the variability of fermentation responses in pumpkin-based systems.

4. Conclusion

This work has shown that blanching, sterilization, and lactic fermentation cause a cultivar-dependent set of physicochemical changes in pumpkin purees. *Cucurbita moschata* showed a higher retention of β -carotene and a higher colouring intensity than *Cucurbita pepo* var. *Styriaca. Cucurbita pepo* var. *Styriaca* showed a higher degree of rheological changes. Fermentation with *P. pentosaceus* increased acidity and modified viscoelastic behaviour, while reducing particle size due to acid-induced cell wall weakening. Raman spectroscopy with PCA was used successfully to extract and verify the differences in the chemical constitution of different cultivars and stages of processing. Nutritional analysis of raw fermented pumpkin purees indicated maximum β-carotene retention, making it clear that fermentation without blanching is more efficient regarding β-carotene conservation and extraction. On the contrary, blanched and fermented pumpkin demonstrated higher structural homogeneity and resistance to rheological and processing instabilities. In light of obtained results, it can be concluded that depending on the processing conditions and goals of use, either raw fermentation or blanching and fermentation should be considered for the production of nutrition-based or texture-based products, respectively. Although the study was conducted at laboratory scale, the experimental results will contribute to the mechanism of optimization of heat treatment and fermentation of pumpkin puree for vegetarian food purposes. The next step of research will feature the above characteristics of the food product.

Ethical statement

This study did not involve human participants, animal experiments, or the use of any genetically modified organisms. All experimental procedures were performed exclusively on plant materials (pumpkin purees) and microorganisms generally regarded as safe (GRAS), following standard laboratory and food-processing protocols. Therefore, no ethical approval was required for this research.

CRediT authorship contribution statement

Biljana Cvetković: Writing – review & editing, Writing – original draft, Supervision, Project administration, Conceptualization. **Miona Belović:** Writing – original draft, Methodology, Formal analysis. **Ilinka Pećinar:** Writing – original draft, Investigation, Formal analysis. **Aleksandra Bajić:** Writing – review & editing, Resources, Formal analysis. **Lato Pezo:** Validation, Software, Data curation. **Ana Varga:** Methodology, Formal analysis. **Christian Philipp:** Resources, Methodology, Investigation, Funding acquisition. **Reinhard Eder:** Supervision, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors 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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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.afres.2026.102135.

Data availability

Data will be made available on request.

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