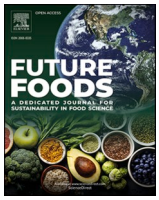
Future Foods 10 (2024) 100502

Contents lists available at ScienceDirect

Future Foods

journal homepage: www.elsevier.com/locate/fufo



Dextran-enriched pea-based ingredient from a combined enzymatic and fermentative bioprocessing. Design of an innovative plant-based spread.

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ARTICLE INFO

Keywords:
Pea flour
LAB Fermentation
Dextran, Defatted durum wheat germ
Plant-based spreads

ABSTRACT

In this study a plant-based spread was developed using dextran-enriched ingredients derived from pea flours, supplemented with defatted durum wheat germ and almond flour. Optimization of fermentation with *Leuconostoc pseudomesenteroides* DSM 20193, both with and without enzymatic hydrolysis, aimed to enhance exopolysaccharide production and the nutritional value of pea flours. Best results were achieved through enzymatic hydrolysis with Veron PS protease followed by fermentation at 25°C, resulting in elevated dextran levels and increased peptides and total free amino acid concentration in green and yellow pea-based ingredients. The yellow pea-based ingredient was selected for the final plant-based spread formulation, blended at 35% w/w, with 45% w/w defatted durum wheat germ, and 20% w/w almond flour. The resultant spread exhibited elastic and solid-like characteristics like milk-based spreadable cheese and yogurt, boasting 'high protein' (12.49 g/100g) and 'high fiber' (11.01 g/100g) designations. It maintained chemical, biochemical, and microbiological stability over a 10-day shelf-life under refrigerated conditions. Sensory evaluation confirmed the acceptability of the plant-based spread (PBS), highlighting a well-balanced aroma and a grainy, adhesive texture. This research underscores the potential of an integrated approach utilizing food-grade enzymes and fermentation for the *in-situ* production of dextran to create innovative, clean label, and plant-based foods.

1. Introduction

Feeding a growing global population, mitigating climate change, reducing pollution, managing waste, preserving biodiversity, and addressing public health issues are among the challenges of the agri-food sector is facing (Mishra et al., 2023). Addressing these issues requires dietary changes, as animal products account for 83% of global agricultural land use and contribute 56-58% of emissions while providing only 37% of protein and 18% of calories (Poore and Nemecek, 2018). Numerous studies highlighted the sustainability benefits of plant-based proteins over their animal-based counterparts (Alexander et al., 2017; Poore and Nemecek, 2018). Amidst this backdrop, a paradigm shift in food design is imperative, entailing increased use of plant-based raw materials, repurposing agri-food by-products, using local sourcing, and incorporating high-nutritional-value ingredients (Brennan, 2024). Dairy products play a crucial role in our daily dietary intake, and both research and industry are actively working to address the increasing demand for plant-based alternatives to dairy (Plamada et al., 2023). Spreadable or soft cream cheese is a type of processed ingredient that is versatile for use in the fast-food industry and is globally popular, both as a spread and as an ingredient in many cold recipes (Mefleh et al., 2022). Indeed, spread (e.g., butter and cheese spreads) is a type of condiment that can be used while eating bread, toast, biscuits, and other similar foods. Spreads owe their technological properties (e.g., spreadability) to the continuous protein network (casein) and stabilized fat globules (Mefleh et al., 2022). Despite their technical benefits,

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https://doi.org/10.1016/j.fufo.2024.100502
Received 2 July 2024; Received in revised form 25 August 2024; Accepted 10 November 2024
Available online 12 November 2024

saturated and trans fats from meat and dairy have been associated with numerous health problems, including cardiovascular disease, high cholesterol, diabetes, and increased cancer risk (Lichtenstein, 2014). Consequently, over the years, researchers have explored plant-based ingredients and formulations to replace animal fats, alter fatty acid compositions, and incorporate other health-promoting substances in dairy substitutes (Plamada et al., 2023).

Legumes, particularly peas, have gained attention due to their potential role in this transition phase (Ma et al., 2018). However, challenges such as consumer acceptance, presence of antinutritional factors, and technological properties (e.g., pulse proteins do not form a continuous protein network) hinder their widespread use in the food industry (Tuccillo et al., 2022; Millar et al., 2019; Konieczny et al., 2020). Cost-effective and sustainable biotechnologies, such as bioprocessing with food-grade enzymes (Mookerjee and Tanaka, 2023) and lactic acid bacteria (LAB) fermentation, have shown success in mitigating technological and nutritional issues associated with pea as well as other legume flours (Gobbetti et al., 2019; Senanayake et al., 2023). Plant-based proteins do not naturally form a continuous protein network, so the dairy-free industry typically uses non-protein ingredients like modified or unmodified starches, gums, and hydrocolloids to fulfill structural requirements and stabilize fat globules with starch (Grasso et al., 2021; Ningtyas, 2023).

A promising strategy involves enhancing legume flour properties by integrating polysaccharides, either natural (e.g., guar gum) or chemically modified (e.g., hydroxypropyl methylcellulose). Yet, this approach has limited interest due to its inability to provide nutritional improvements and align with the growing demand for "clean label" products since they must be declared (Papagianni et al., 2024). Certain LAB species, belonging to *Weissella, Leuconostoc*, and the former *Lactobacillus* genus, can produce dextran, a natural polysaccharide, through fermentation (Kajala et al., 2015). Dextran, approved for food use in Europe since 2001 (European Commission, 2001), offers advantages such as masking off-flavors, mimicking gluten functionality, altering consistency, and exhibiting prebiotic effects (Korcz and Varga, 2021; Lynch et al., 2018; Shuai et al., 2023; Kothari et al., 2015). Moreover, being synthesized *in-situ* during fermentation, dextran must not be declared on the label (Galle and Arendt, 2014).

Dextran, synthesized by LAB through the action of extracellular dextransucrase (DSR), is a homopolysaccharide with at least 50% α-1,6 linked glucose as the backbone and varying percentages of α-1,4, α-1,3 and α-1,2 branched linkages (Monsan et al., 2001). Nevertheless, its structure is mainly dependent on the type of DSR present in the bacterial strains, and on growth conditions such as sucrose amount, acidity and temperature (Dols et al., 1998).

Among LAB, *Leuconostoc pseudomesenteroides*, listed in the EU's Qualified Presumption of Safety (QPS), has been employed for *in situ* dextran synthesis, enhancing techno-functional and sensory aspects of legume-based ingredients (lentil, fava bean, chickpea, pea) used in fortifying foods like bread, pasta, and snacks (Perri et al., 2021, Galli et al., 2021, Wang et al., 2019, Shuai et al., 2023).

Although *in-situ* dextran-enriched ingredients offer well-documented technological and nutritional advantages, their use in innovative food products remains underexplored. In this study, dextran synthetized *in-situ* by *L. pseudomesenteroides* during pea fermentation was used as fat replacer in an innovative plant-based spreadable cream. Moreover, aiming at further improve the nutritional (e.g., free amino acids and peptides) features and positively affect the microbial stability of the pea-based mixture through the release of potentially bioactive peptides with antifungal activity (Rizzello et al., 2017), enzymatic hydrolysis by using commercial food-grade protease Veron PS, was combined with the fermentation.

To contribute to sustainable food production while reaching a balanced nutritional composition the dextran-enriched field pea flour was combined with defatted durum wheat germ (DWG), the second largest by-product of the wheat milling process, that contains over 30% dietary fiber and between 10-30% protein, along with beneficial bioactive compounds such as tocopherols and phytosterols (Sun et al., 2015), and local almond flour, as a rich source of healthy fats, proteins, fibers, vitamins (especially vitamin E), and minerals such as magnesium and calcium (Burbano and Correa, 2024). The plant-based spread was characterized and thoroughly evaluated over a 10-day refrigerated storage period to determine its microbial, technological, and sensory stability.

2. Material and methods

2.1. Raw materials

The raw materials used to produce the fermented ingredients were: green field pea flour (Var. Faquir, seed company Apsovsementi, Voghera, Italy) and yellow field pea flour (Var. Astronaute, Società Produttori Sementi, Argelato, Italy), cultivated in 2022-2023 growing season side by side in the same field in North Italy, ground to wholemeal using a laboratory centrifugal mill equipped with a 500 μm sieve (Model ZM-200, Retsch, Haan, Germany) and supplied by the Department of Agricultural, Forest and Food Sciences, University of Turin (Turin, Italy) having, respectively, the following gross chemical compositions: moisture, 8.70% and 9.50%; protein, 23.7% and 22.9% of dry matter (d.m.); fat, 2.4% and 2.1% of d.m.; dietary fiber, 15.89% and 16.04% of d.m.; carbohydrates, 49.56% and 49.68% of d.m. The protease Veron PS (227 UHb/g) was obtained from AB Enzymes GmbH (Darmstadt, Germany), and sucrose (99%) from Sigma-Aldrich (Saint Louis, USA). The ingredients used to refine the formulation of the plant-based spread included defatted and dry-fractionated durum wheat germ (DWG) and peeled almond flour. DWG, sourced from Molino Casillo (Corato, Italy) and certified to meet mycotoxin thresholds (aflatoxins, zearalenone, deoxynivalenol, ochratoxin A and fumonisin) as set by Reg. UE 915/2023, contained 11% moisture, 31.3% protein of d.m, 0.6% fat of d.m, 28% dietary fiber of d.m, and 29% carbohydrates of d.m. To reduce phytates, the DWG was mixed with tap water in ratio 1:2 and cooked for 7 minutes at 1600 kW with continuous stirring until it reached a moisture content of 50%. The moisture was measured by a thermobalance (Radwag Mac 110/NP, Poland). The peeled almond flour (Local shop, Apulia, Italy), with a moisture content of 9%, 24% protein of d.m, 50% fat of d.m, 5.8% dietary fiber of d.m, and 12% carbohydrates of d.m, and the premium xanthan gum (FoodBites) were purchased from local retailers.

2.2. Bioprocessing: synthesis of dextran and enzymatic treatment

Mixtures based on green- and yellow-pea flours with a solid:liquid ratio of 25:75 (%) were prepared (GP and YP). Both GP and YP mixtures were fermented for 24 h at 30°C after the addition of 5% sucrose (w/w) (with or without prior enzymatic treatment) with the exopolysaccharides (EPS)-producing LAB *Leuc. pseudomesenteroides* (DSM 20193), belonging to Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures (DSMZ, Germany). Cells were harvested via centrifugation (10,000 × g, 10 min, 4°C), washed in 50 mM phosphate buffer, pH 7.0, and suspended in tap water prior to inoculation at a cell density (final in the mixture) of 7 Log cfu/g. Veron PS (AB Enzymes GmbH, Darmstadt, Germany) was used at 1% (w/w) of the protein content. Four mixtures were prepared: (i) GP_F (fermented green pea), a mixture based on green pea flour fermented with *Leuc. pseudomesenteroides* DSM 20193; (ii) YP_F (fermented yellow pea), a mixture based on yellow pea flour fermented with *Leuc. pseudomesenteroides* DSM 20193; (iii) GP_{VF} (Veron fermented green pea), a mixture based on green pea flour treated with Veron PS and fermented with *Leuc. pseudomesenteroides* DSM 20193; (iv) YP_{VF} (Veron fermented yellow pea), a mixture based on yellow pea flour treated with Veron PS and fermented with *Leuc. pseudomesenteroides* DSM 20193. Three different fermentation and enzymatic treatment temperatures of 20, 25, and 30°C were tested. In

addition, control samples corresponding to EPS negative samples (without sucrose addition) were obtained following the same protocols without the addition of sucrose (Xu et al., 2017). Supplementary Table 1 summarizes the ingredients and technology parameters of the mixtures used in this study.

2.3. Characterization of fermented pea flour ingredients

2.3.1. Pro-technological properties and biochemical characterization

The growth and acidification capacity of the starter was monitored. LAB cell density before and after the fermentation was determined on De Man, Rogosa and Sharpe (MRS) agar medium (Oxoid, Basingstoke, Hampshire, UK) supplemented with cycloheximide (0.1 g/L). The plates were incubated at 30°C for 48 hours. The pH was determined using a pH meter (model 507, Crison, Italy) and the total titratable acidity (TTA) was determined as the volume (mL) of 0.1M NaOH required to bring a suspension of 10 g of mixture in 90 mL sterile water to pH 8.4. The concentration of sugars (sucrose, glucose, and fructose) and organic acids (lactic and acetic) before and after the fermentation were determined using the K-SUFRG, K-DLATE, and K-ACET kits (Megazyme International Ireland Limited, Bray, Ireland) according to the manufacturer's instructions. The proteolytic activity of the starter was monitored by determining the concentration of peptides and total free amino acids (TFAA) using the O-phthalaldehyde and cadmium-ninhydrin spectrophotometric assays, respectively (Church et al., 1985; Doi et al., 1981).

2.3.2. Viscosity and dextran content

To preliminary assess the presumptive *in-situ* production of EPS (dextran) before and after fermentation, 50g of each mixture was brought to a temperature of 20°C, and the viscosity was determined using the MYR viscometer, type VR3000, model L (Viscotech Hispania S. L., Spain). Samples with the highest viscosity increase as compared to prior fermentation were selected and subjected to dextran quantification, according to the enzymatic assisted method based on dextranase and transglucosidase activity as reported by Katina et al. (2009).

2.3.3. Rheological properties

Based on the above screening, two fermented pea flour ingredients (GP_{VF} and YP_{VF}) were selected for further evaluation of rheological properties. The rheology analysis was performed using a rotational rheometer (HAAKE MARS iQ Air, Thermo Fisher Scientific, Waltham, MA, USA) equipped with a heat exchanger (MTMC-iQ (MARS iQ Air), Thermo Fisher Scientific, Waltham, MA, USA) and parallel plate geometry (P35/Ti-02180932). All determinations were carried out at 25 °C. Measurements were repeated on samples after storage at 5 °C for 24 hours. The flow behavior of each sample was evaluated through a shear rate ramp test, increasing the shear speed from 1 to 100 1/s at 25 °C. The viscosity data (η, expressed in Pa*s) were fitted to the Ostwald-de Waele (power law) model, according to the following equation:

$$\eta = \frac{K * \gamma}{n}$$

where: η = viscosity (Pa*s), K = consistency index (Pa*sn); ɣ = shear rate (1/s) and n = flow behavior index.

The behavior of samples as a function of frequency was determined using the oscillatory frequency sweep, according to the method described by Krystyjan et al. (2015) with some modifications. The frequency ranged from 0.1 to 10 Hz at 1% strain (which falls within the linear viscoelastic regime) (De Angelis et al., 2024). The storage modulus (G') and loss modulus (G") were recorded as a function of frequency. Furthermore, the module data were also fitted to the Power law model (Nilsson et al., 2023). The measurements were carried out in triplicate.

2.3.4. Determination of volatile compounds

The analysis of volatile compounds from the samples was conducted using an Agilent 6850 gas chromatograph (GC) coupled with an Agilent 5975 mass spectrometer (MS) (Agilent Technologies Inc., Santa Clara, CA, USA). The extraction followed the HS-SPME method as detailed by Caponio et al. (2024). A 75 µm CAR/PDMS-coated SPME fiber was used to extract the volatiles in the headspace at 40°C for 50 minutes. Desorption of the fiber occurred in the GC injection port for 6 minutes in split-less mode at 230°C for 3.5 minutes. Separation of the volatile compounds was achieved using an HP-Innowax capillary column (60 m × 0.25 mm ID × 0.25 µm film thickness) with the following settings: inlet temperature of 250°C, flow rate of 1.5 mL/min, and helium carrier gas at 30 kPa. The oven temperature was initially set at 35°C for 5 minutes, then increased by 5°C per minute to 50°C, held for 5 minutes, followed by an increase of 5.5°C per minute to 210°C, and held for 5 minutes. The mass spectrometer conditions included an interface temperature of 230°C, a source temperature of 230°C, ionization energy of 70 eV, and a scan range of 33–260 amu. The internal standard used for peak normalization was 1-propanol. This analysis was repeated three times.

2.4. Production, characterization, and shelf-life monitoring of the plant-based spread

2.4.1. Formulation and preparation

Based on a preliminary screening, one dextran-enriched plant-based ingredient (YP_{VF}) was selected and mixed (35% w/w) with DWG (45% w/w) and peeled almond flour (20% w/w) to obtain a plant-based spread (PBS). The spread was formulated to reach the nutritional claims "rich in fiber" and "rich in protein" according to EC Regulation 1924/2006. To evaluate the rheological fitness of the EPS as natural thickener produced *in-situ* as compared to those of the commercial xanthan (produced by *Xanthomonas campestris*), a mixture of enzymatically treated and fermented yellow pea flour without sucrose, DWG (45% w/w), peeled almond flour (20% w/w) (35% w/w), and 0.5% (w/w) xanthan was used as a control sample (cPBS) for the solely rheological properties. Aliquots of 200 g PBS were placed in glass jars, pasteurized at 72°C for 15 minutes in a water bath (MPM Instruments), cooled, and stored at 4°C for 10 days. Samples before (t0), during (t3 and t6), and after (t10) storage were used for characterization.

2.4.2. Technological characterization

The chromatic coordinates were measured using a CM-600d colorimeter (Konica Minolta, Tokyo, Japan) paired with Spectramagic NX software (Konica Minolta, Tokyo, Japan). The parameters assessed were lightness (L*), redness (a*), and yellowness (b*). The measurements adhered to the CIE (International Commission on Illumination) Lab* scale, under a D65 illuminant. The total color difference (ΔE) for comparing the colorimetric discrepancies between the samples was calculated using the following formula:

$$\Delta E * ab = \sqrt{(\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2}$$

where ΔL, Δa and Δb are the differences for L*, a* and b* values between sample and reference. The mean values were used as references, and the ΔE scale for evaluating the results was: 0–2.0 = unnoticeable difference; 2.0–3.5 = difference noticeable by an experienced observer; >3.5 = clear difference (Brochard et al., 2021). Texture properties, including hardness and brittleness, were analyzed using a 3-point bending test based on the method of Pasqualone et al. (2019) with some modifications. A texture analyzer (Z1.0 TN, Zwick GmbH & Co., Ulm, Germany) equipped with a 1 KN load cell was employed. The distance between the support bars was set at 4 cm, with a probe speed of 5 mm/min. Hardness was defined as the force (N) needed to break the sample, while brittleness was the distance (mm) the blade traveled before the sample broke. Each sample was tested in six replicates. Data

processing was performed using TestXPertII software version 3.41 (Zwick Roell, Ulm, Germany). Rheological analysis was conducted as detailed in section 2.3.3.

2.4.3. Chemical and biochemical characterization

Analysis of biochemical parameters (pH, TTA, organic acids, peptides, TFAA, and volatile compounds) were performed according to the methods described in paragraphs 2.3.1 and 2.3.4. Also, the proximate composition of PBS was determined before (t0) and after 10 days of storage. Protein (total nitrogen x 6.25) and moisture were determined using the UNI CEN ISO/TS 16634-2:2016 (Dumas method) and 712:2010 methods, respectively, while ash was measured using the ISO 2171:2007 method. Total, soluble, and insoluble dietary fiber and fatty acids were determined using the *Association of Official Analytical Chemists* (AOAC) methods 985.29, 993.19:1996, and 996.06, respectively. Total lipids were measured according to the method prescribed by DM 23-07-1994 SO No. 4 G.U. Available carbohydrates were calculated on a dry basis as follows: [100 - (protein + lipids + ash + total dietary fiber)].

2.4.4. Microbiological characterization and aflatoxins monitoring

Plate Count Agar (PCA, Oxoid) was used to determine the cell density of mesophilic aerobic bacteria after incubation at 30°C for 48 hours. Yeast cell density was enumerated on Sabouraud Dextrose Agar (SDA, Oxoid) at 25°C for 48 hours. Moulds were enumerated on Potato Dextrose Agar (PDA, Oxoid) after incubation at 25°C for 5 days. Total *Enterobacteriaceae* were enumerated on Red Bile Glucose Agar (VRBGA, Oxoid) after incubation at 37°C for 24 hours. while presumptive LAB were determined as described in paragraph 2.3.1. The presence of aflatoxins B1, B2, G1, and G2 was monitored using the L-MI067 rev.0 2020 method, accredited by the Italian certification body Accredia. The measurements were conducted with the SCIEX model 5500 + HPLC-MS/MS (AB Sciex LLC, Framingham, MA, USA) and included the use of an isotopically labeled internal standard.

2.4.5. Sensory evaluation

Sensory analysis of bread was performed by a trained panel group composed of ten assessors (five females and five males, aged between 26 and 28 years) with proven skills and previous experiences in sensory evaluation of milk-alternatives products coordinated by a panel leader. All panel members had neither food allergies nor intolerances and were regular consumers of plant-based diary alternatives. Two pre-test sessions were conducted to establish the list of descriptors and their corresponding intensity range (Faccia et al., 2019; Natrella et al., 2020). Panelists were informed to the study's objectives following ethical standards of the laboratory of the Food Science and Technology unit of the Department of Plant, Soil, and Food Sciences of the University of Bari, Italy. No formal ethics committee or documentation process was involved. However, the privacy rights of participants have been observed, the informed consent was obtained for the sensory test experimentation. Moreover, participants were informed that they could withdraw from the study at any time. Sensory evaluations were carried out following the independent method of the "Sensory analysis - Methodology - Flavour Profile" methods (ISO 6564-1985) with some modification. The intensity of appearance (color, uniformity, lightness) and odor, taste-olfactory sensations (toasted, sourdough, legume, almond, cereal), taste (sweet, salty, sour, bitter), and textural attributes perceived in mouth (homogeneity, adhesiveness, solubility, granularity, elasticity, creaminess), were measured using a fully labelled 5-point category scale using twenty-five sensory descriptors with scores ranging from 1 (indicating a minimal sensation or absence of attributes) to 5 (indicating a strong sensation) (Egypto et al., 2013).

2.5. Statistical analysis

All data are presented as means ± SD. Statistical significance between values was assessed at $p < 0.05$ using analysis of variance (ANOVA), followed by Tukey's honest significant difference (HSD) test, Fisher least significant difference (LSD) test, and Student's t-test for multiple comparisons. The statistical analyses were conducted using Minitab Statistical Software (Minitab Inc., State College, PA, USA).

3. Results and discussion

3.1. Pea flour-based ingredient

3.1.1. Synthesis of dextran and enzymatic treatment

To maximize the EPS production in pea mixtures (GP and YP) and favor the release of peptides and FAA two process options were considered: (i) fermentation with *Leuc. pseudomesenteroides* DSM 20193 of pea mixtures enriched with sucrose able to promote the *in-situ* EPS production (Ripari, 2019) (GP_F and YP_F), and (ii) combination of enzymatic hydrolysis using a commercial protease (VERON PS) and fermentation with *Leuc. pseudomesenteroides* DSM 20193 (GP_{VF} and YP_{VF}). The choice of pea flour reflects the food industry's shift toward plant-based proteins as replacements for animal sources, driven by growing consumer demand for sustainable and healthier products (Sridhar et al., 2023). Field peas (*Pisum sativum L. var. arvense*) are favored due to their high protein content, nutritional value, broad availability, and low cost (Lam et al., 2018). *Leuc. pseudomesenteroides* DSM20193 was selected for its ability to synthesize significant amounts of dextran in various food substrates (Galli et al., 2021; Koirala et al., 2021). The intense proteolysis due to the enzymes (commercial and endogenous) and microbial activities allowed to improve the nutritional (higher content of peptides and TFAA) profile of the ingredients (Supplementary Table 2). Enzymatic hydrolysis has widely been used to release bioactive peptides from pea flour for specific applications (e.g., antihypertensive, antifungal activities) (Aluko et al., 2015; Rizzello et al., 2017), and several studies have shown that biological acidification induced by LAB can activate endogenous proteases, leading to primary proteolysis and peptidase activities, ultimately releasing amino acids (Gänzle et al., 2008). Dextran synthesis is driven by the enzyme dextransucrase with temperature playing a crucial role in its activity (Werning et al., 2012), thus, experiments were conducted at three different temperatures: 20, 25, and 30°C. The selection of the best-performing process was based on the higher presumptive EPS concentration and nutritional properties by means of viscosity, TFAA and peptides concentrations, respectively as reported in Supplementary Table 2. Dextran production during fermentation leads to increased mixture viscosity, serving as an early indicator of EPS production (Katina et al., 2009; Immonen et al., 2020; Wang et al., 2019, 2020; Xu et al., 2017). Overall, the combination of enzymatic hydrolysis and fermentation (GP_{VF} and YP_{VF}) led to higher content of TFAA (up to 43.9%), and peptides (up to 29 %) as compared to the sole fermentation (GP_F and YP_F) regardless of the temperature. Nevertheless, the role of the temperature was significant in the presumptive EPS production (viscosity). Indeed, the viscosity of samples treated at 30°C was *circa* 50% lower than that of samples fermented at 20 and 25°C (Supplementary Table 2). Lower temperatures might increase DSR protein turnover, potentially boosting dextran production in some mesophilic LAB strains, such as those in the *Leuconostoc* genus, where dextransucrase activity is more robust at sub-optimal temperatures between 23 and 25°C (Cortezi et al., 2005; Besrour-Aouam et al., 2019). Also, GP_{VF} and YP_{VF} had higher viscosity when compared to their counterparts fermented at the same temperature without enzymatic treatment (GP_F and YP_F), possibly due to elevated dextran concentrations and the combined effect of protease treatment and subsequent fermentation (Supplementary Table 2). This aligns with the findings of Kravchenko et al. (2024), who showed that protease treatment of pea flour improved protein solubility at pH levels ≤ 5, enhancing emulsification capacity and thereby increasing viscosity.

A significant difference was found between in terms of viscosity and

concentration of TFAA and peptides (*circa* 10%) being higher when 25°C was used as process temperature (Supplementary Table 2). According to the above considerations the combination of enzymatic hydrolysis and fermentation at 25°C was selected as the best performing process.

3.1.2. Biochemical characterization and dextran content

Table 1 shows the biochemical and microbiological characteristics of the selected pea-based ingredients (GP_{VF} and YP_V) and their corresponding EPS-negative controls (cGP_{VF} and cYP_V), including viscosity and dextran concentrations. No significant differences ($p<0.05$) were observed in key metrics monitored during fermentation among samples. Presumptive LAB grew approximately 2 log cycles, pH decreased about 2 units, TTA increased from 8 to 10 mL of NaOH, and an average fermentation quotient (molar ratio of lactic acid to acetic acid) of 4.25 was achieved. Similar trends were observed in studies using *Leuc. pseudomesenteroides* as starter for pea flour and other legumes such as fava beans and lentils (Shuai et al., 2023; Xu et al., 2017; Perri et al., 2021).

Green (GP_{VF}) and yellow (YP_{VF}) pea flour formulations were characterized by viscosity of 5.96 and 7.50 Pa*s, respectively corresponding to a dextran concentration of 6.06g/100g and 4.89 g/100g of dry matter, respectively (Table 1). These data represent approximately 60% and 50% of the theoretical limit for dextran production given the added sucrose content (20% w/w of dry matter or 5% w/w of mixture weight), suggesting a significant dextran synthesis (Kothari et al., 2015). Dextran concentrations of 0.80 g/100g and 0.66 g/100g were even detected in EPS-negative controls, cGP_{VF} and cYP_{VF}, respectively. These data might be attributable to dextransucrase secreted into the mixture with a minimum number and quantity of related contaminating enzymes and forming high molecular weight soluble dextran using endogenous sucrose (Xu et al., 2019), which was found in GP and YP at 141 mg/100g and 124 mg/100g, respectively (Table 1). During dextran formation, dextransucrase cleaves the glycosidic bond in sucrose, releasing fructose while adding D-glucopyranosyl residues to a growing dextran polymer (Werning et al., 2012), thus leading to higher fructose content. Indeed, fructose was higher in GP_{VF} and YP_{VF} (770-780 mg/100g), compared to the EPS-negative controls cGP_{VF} and cYP_{VF} (170-180 mg), which is consistent with the increased dextran production (Table 1). Also, the combined effects of enzymatic treatment and fermentation led to a significant release of peptides and TFAA having concentrations 23% - 32% and 2- 3-times higher than the corresponding untreated sample (YP and GP).

3.1.3. Rheological properties

The viscoelastic properties of YP_{VF} and GP_{VF} were investigated through rheological analysis, including viscosity measurement by *shear ramp* test and oscillatory frequency analysis. Fig. 1 shows the viscosity data suggesting that due to viscosity decreased with increasing shear rate, the two samples were characterized by shear-thinning (pseudoplastic) behavior (Fig. 1A). No significant differences were observed in the viscosity of the two samples, as evidenced by their similar trends with *shear rate*. To assess the viscoelastic properties of YP_{VF} and GP_{VF} within a nondestructive strain range, a frequency sweep analysis was conducted to simulate behavior across short (high frequency) and long (low frequency) time scales (Ramli et al., 2022). The results, shown in Fig. 1, revealed that the storage modulus (G') consistently exceeded the loss modulus (G'') in all samples within the frequency range of 0.1 to 10 Hz. Both moduli exhibited frequency dependence, showing a moderate increase at higher frequencies (Fig. 1B). Furthermore, G' and G'' exhibited parallel behavior throughout the analysis, without a clear tendency to intersect, emphasizing the solid-like characteristics across the entire frequency range (Steffe, 1996). This suggests a dynamic balance between the formation and breakdown of intermolecular interactions, which contributes to the structure stability (Lorenzo et al., 2013). These findings indicate that the samples demonstrate solid behavior characterized by elastic deformation (Nilsson et al., 2023). Fig. 1B shows lower frequency sweep values for the GP_{VF} than YP_{VF}, although the trend within the 0.1-10 Hz frequency range remains similar.

3.1.4. Volatile compounds profile

Samples were also analyzed for their volatile compound profiles, resulting in the identification and quantification of 14 volatile compounds grouped into classes such as alcohols, esters, aldehydes, ketones, and acids (data not shown). Overall, GP_{VF} was characterized by a more complex volatile profile in terms of both number and relative abundance of compounds compared to YP_{VF}. Higher content in alcohols, i.e., 1-hexanol (0.70 *vs* 0.02 μg/g), 1-octen-3-ol (0.41 *vs* 0.01 μg/g), 1-hexanol,2-ethyl (0.32 *vs* 0.01 μg/g) were detected. These alcohols are likely generated through the enzymatic oxidation of legume lipids, with 1-hexanol and 2-ethyl being dominant in peas and related to the typical herbaceous and woody aroma (Azarnia et al., 2011). Furthermore, differences in the ester class were observed between GP_{VF} and YP_{VF}. Butanoic acid, 3-methyl, ethyl ester, known for its strong, pungent, fruity odor, and bitter taste (Azarnia, et al., 2011) was found only in the GP_{VF}. Also, aldehydes including hexanal, and 2-heptenal, were found in

Table 1
Biochemical and microbiological characterization, viscosity and dextran concentration of selected enzymatically treated and fermented pea flour mixtures (GP_{VF} and GP_{VF}). Mixtures prior to the enzymatic treatment and fermentation (GP and YP) and enzymatically treated and fermented pea flour mixtures without the addition of sucrose (cGP_{VF} and cYP_{VF}) are also included.

	pH	TTA (ml NaOH)	Lactic acid (mmol/Kg)	Acetic acid (mmol/Kg)	pLAB (Log_{10} cfu/g)	Peptides (g/Kg)	TFAA (mg/Kg)	Sucrose (mg/100g)	Glucose (mg/100g)	Fructose (mg/100g)	Viscosity (Pa*s)	Dextran (g/100g) d.w
GP*	6.60 ± 0.191^{a}	2.21± 0.035^{d}	0.19 ± 0.01^{c}	0.44 ± 0.014^{e}	-	15.41± 0.312^{e}	722 ± 15.88^{d}	141.18 ± 5.11^{a}	287.62 ± 14.12^{a}	339.64 ± 12.57^{b}	n.d	-
YP*	6.62 ± 0.218^{a}	2.02 ± 0.098^{d}	0.21 ± 0.01^{c}	0.56 ± 0.016^{e}	-	13.50 ± 0.226^{f}	814 ± 32.28^{c}	124.63 ± 6.17^{b}	263.80 ± 10.55^{a}	350.62 ± 11.22^{b}	n.d	-
GP_{VF}	4.41 ± 0.180^{b}	12.00 ± 0.238^{a}	65.68 ± 1.77^{a}	13.57± 0.312^{b}	9.18 ± 0.331^{a}	22.73 ± 0.404^{b}	2628 ± 101.35^{a}	3.89 ± 0.146^{d}	12.96 ± 0.313^{b}	760.75 ± 32.09^{a}	5.96 ± 0.087^{b}	6.06 ± 0.123^{a}
cGP_{VF}	4.45 ± 0.166^{b}	12.47 ± 0.477^{a}	52.37 ± 2.01^{b}	15.40 ± 0.389^{a}	8.97 ± 0.224^{a}	23.81 ± 0.373^{a}	2873 ± 124.3^{a}	8.28 ± 0.313^{c}	10.32 ± 0.401^{d}	179.61 ± 17.11 ^{c}	n.d	0.80 ± 0.018^{c}
YP_{VF}	4.41 ± 0.137^{b}	11.30± 0.237^{b}	52.24 ± 1.51^{b}	11.00 ± 0.275^{d}	9.37 ± 0.333^{a}	19.02 ± 0.299 ^{c}	2227 ± 77.95^{b}	2.92 ± 0.193^{e}	9.60 ± 0.318^{d}	778.60 ± 26.65^{a}	7.50 ± 0.093^{a}	4.89 ± 0.098^{b}
cYP_{VF}	4.48 ± 0.122^{b}	10.33± 0.501^{c}	49.23 ± 1.85^{b}	12.03± 0.300^{c}	9.01 ± 0.112^{a}	18.33 ± 0.204^{d}	2190 ± 55.25^{b}	3.21 ± 0.155^{e}	11.81 ± 0.400^{c}	161.12 ± 15.44^{c}	n.d	0.66 ± 0.015^{d}

pLAB, Presumptive Lactic Acid Bacteria; Different lowercase letters within the same column indicate significant differences ($p < 0.05$, one-way ANOVA, and Tukey's HSD test). Ingredients and technology parameters of the mixtures used in this study are summarized in Supplementary Table 1. *GP and YP serve as controls. These mixtures were analysed soon after the mixing and were not subjected to any process (nor enzymatic hydrolysis or fermentation).

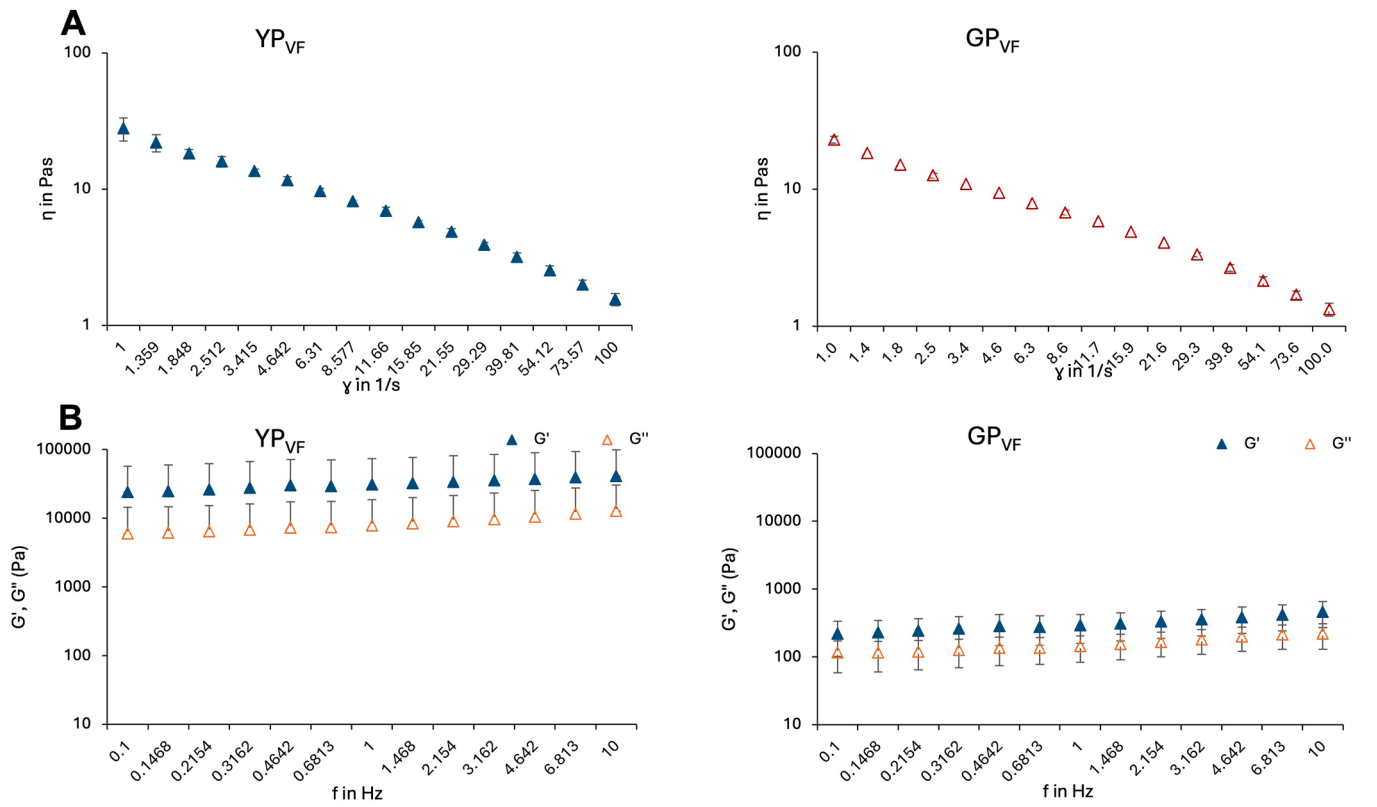

Fig. 1. *Shear ramp* test and oscillatory frequency analysis of green pea- (GP_{VF}) and yellow pea-based (YP_{VF}) ingredients GP_{VF} and YP_{VF}. Ingredients and technology parameters of the mixtures used in this study are summarized in Supplementary Table 1.

higher concentrations in GP_{VF} (0.25 and 0.28 μg/g, respectively) than in YP_{VF} (0.25 and 0.02 μg/g, respectively). These might be product of pea lipid oxidation, with 2-heptenal derived from the oxidation of linoleic acid, and octanal from the oxidation of oleic acid (Mefleh et al., 2022; Shahidi and Hossain, 2022). The ketone class also included a compound resulting from lipid oxidation such as 5-hepten-2-one,6-methyl, with different characteristics that could impact the pea flavor (Oomah and Liang, 2007). YP_{VF} and GP_{VF} contained a low concentration of 5-hepten-2-one,6-methyl of about 0.04 μg/g. Conversely, acid concentrations – which included low molecular weight volatile compounds resulting from legume metabolism or storage-related processes – were higher in GP_{VF} than in YP_{VF}. In summary, differences in the concentration of these lipid oxidation products of legumes may contribute to the distinct sensory profiles of spreadable creams. Consequently, the YP_{VF} sample was selected for formulating a plant-based spreadable cream.

3.2. *Plant-based spread characterization and shelf-life evaluation*

3.2.3. *Chemical and biochemical properties*

Ensuring consumers have access to safe, affordable, and nutritious food is a priority for the food industry. Achieving this goal requires a comprehensive strategy that addresses various aspects, including minimizing losses throughout the agri-food supply chain, enhancing nutritional profiles, and adhering to safety standards to maintain food quality over time (Augustin et al., 2016). Previous studies have highlighted the nutritional profile of DWG, characterized by highly nutritious protein material with a balanced amino acid composition, closely resembling the FAO/WHO model for good amino acid balance (Petrovic et al., 2017). The incorporation of DWG and its fractions into food formulation significantly increased protein and fiber content in products such as biscuits and bread (Arshad et al., 2007; Longo et al., 2024; Perri et al., 2022). Being a by-product of wheat germ oil extraction using n-hexane as a solvent (Xu et al., 2016) DWG is naturally low in fats, whereas, almond flour is rich in monounsaturated fatty acids (MUFA), which contribute to beneficial health effects associated with reduced risk of cardiovascular disease, lower blood cholesterol levels, and improved endothelial function (Richardson et al., 2009). In response to the above requirements and according to the nutritional potentialities of these ingredients the new vegan PBS involved blending 35% w/w of selected YP_{VF}, 45% w/w of DWG, and 20% w/w of almond flour. PBS was subjected to a mild pasteurization process (72°C for 15) to minimize health hazards from pathogenic micro-organisms in low acid foods (PBS, 5.91) while preserving its properties. This exceeds the conventional duration for liquid foods, aiming to uniformly heat the product core and inactivate the enzymes responsible for food quality degradation (Chaikham, 2014; Terefe et al., 2014).

Following the Regulation (EC) No 1924/2006, PBS achieved the 'high protein' and 'high fiber' claims, with 12.49 g/100g protein (approximately 38.5% of the total energetic value) and 11.01 g/100g of total dietary fiber. Specifically, DWG accounted for about 67% of the total protein content, while almond flour and YP_{VF} contributed for about 22% and 10%, respectively. In terms of fiber content, DWG provided 83% of the total fiber, with almond flour and YP_{VF} contributing nearly equally to the remaining content (*circa* 8% and 9%, respectively). Therefore, the inclusion of almond flour at 20% w/w in the final recipe allowed the PBS to achieve a final fat content of 8.10 g/100g, of which approximately 85% were unsaturated fatty acids, thus meeting the criteria for a 'high in unsaturated fat' claim (Table 2). Although pea flour is rich in fiber and protein, adding 35% w/w YP_{VF} to the final formulation had the least effect on the proximate composition of the PBS. This is due to the high-water concentration of YP_{VF} required for higher EPS production (Kaditzky and Vogel, 2008). Additional biochemical parameters were monitored, including peptides and total free amino acids, which reached 21 g/kg and 1249-1563 mg/kg, respectively, along with the total titratable acidity of approximately 6.5 mL of NaOH. The biochemical features (data not shown) as well as the

Table 2
Proximate composition of the vegan spreadable cream plant-based spread (PBS) before (t0) and after (t10) ten days of storage at 4°C. PBS was made of 35% w/w of selected YP_{VF}, 45% w/w of defatted durum wheat germ (DWG), and 20% w/w of almond flour.

Parameters	PBS-t_0	PBS-t_{10}
Moisture (g/100g)	53.80 ± 1.99[a]	53.97 ± 1.24[a]
Carbohydrate (g/100g)	11.87 ± 0.95[a]	12.02 ± 0.62[a]
Protein (g/100g)	12.49 ± 0.38[a]	12.87 ± 0.5[a]
Total dietary fiber (g/100g)	11.01 ± 2.84[a]	9.31 ± 1.27[a]
Fat (g/100g)	8.10 ± 0.97[a]	7.19 ± 1.51[a]
Saturated fats (g/100g)	1.21 ± 0.10[a]	1.07 ± 0.09[a]
Unsaturated fats (g/100g)	6.89 ± 0.34[a]	6.12 ± 0.42[a]
Salt (g/100g)	0.013 ± 0.001[a]	0.012 ± 0.001[a]
Ash (g/100g)	3.21 ± 0.11[a]	3.34 ± 0.14[a]
Energy (Kcal)	198 ± 3[a]	195 ± 4[a]

Data are expressed as mean values ± standard deviation (SD). Different lowercase letters in the same row indicate significant differences between different time points ($p < 0.05$, one-way ANOVA, and Tukey's HSD test). Ingredients and technology parameters of the mixture YP_{VF} used are summarized in Supplementary Table 1.

proximate composition (Table 2) remained stable, showing no significant variations until the tenth day of storage.

3.2.2. Rheological properties

The rheological characteristics of PBS and cPBS were monitored throughout the storage under refrigerate conditions. Overall, the results (Supplementary Fig. 1) show no significant ($p<0.05$) difference among PBS and the control sample containing xanthan (cPBS). The commercial additive was used at the 0.5% representing the manufacturer-recommended amount to achieve an adequate viscosity and guarantee stability of solution (Abdolmaleki et al., 2020). Similarly, dextran concentration in PBS was *circa* 0.5% w/w and based on rheology, *in-situ* produced EPS can represent a valuable clean-label option to substitute the commercial xanthan and achieve optimal technological performance (Zannini et al., 2014).

Understanding the rheological properties of plant-based spreads is crucial for process design, product development, processing, handling, quality control, and storage (Velez-Ruiz, 2008). Fig. 2 illustrates the apparent viscosity of PBS, showing a shear-thinning behavior where viscosity decreases with increasing shear rate (Bayarri et al., 2012). Additionally, frequency sweep analysis was conducted to assess the viscoelastic properties of PBS across non-destructive deformation ranges, simulating short (high frequency) and long (low frequency) time scales (Ramli et al., 2022). The storage modulus (G′) serves as a measure of the energy stored in the sample during shear, indicating its elastic behavior (Cruz et al., 2013). Fig. 3 displays the storage modulus (G′) of PBS from 0.1 to 10 Hz. The loss modulus (G″) showed a similar trend to G′ but with lower values across all frequencies tested, suggesting an elastic, solid-like behavior consistent with rheological parameters observed in milk-based spreadable cheese and yogurt (Biglarian et al., 2022; Florencia, 2013). This pattern remained stable across different time points (t0, t3, t6, t10), indicating minimal variation over time.

3.2.3. Texture profile and color analysis

The main obstacle in producing plant-based spreads of high quality lies in achieving desired textural and spreadability characteristics, which significantly influence the product's stability and shelf-life (Dian Widya Ningtyas, 2023). Several studies have noted that using EPS-producing strains as starter cultures of spreadable cheeses improves their rheology by enhancing texture without the need for gums or stabilizers (Florencia, 2013). Moreover, dry-fractionated proteins (from DWG) can exert several techno-functional properties, such as gelling ability (De Angelis et al., 2024). Overall, the ingredients in the spread formulation allowed us to obtain a good textural stability, since no significant differences ($p<0.05$) in terms of hardness were observed between t0 and t10 (Table 3). This indicates that in 10 days of refrigerated storage PBS maintains its structural profile characteristics unchanged, in line with the results obtained by the rheological analysis.

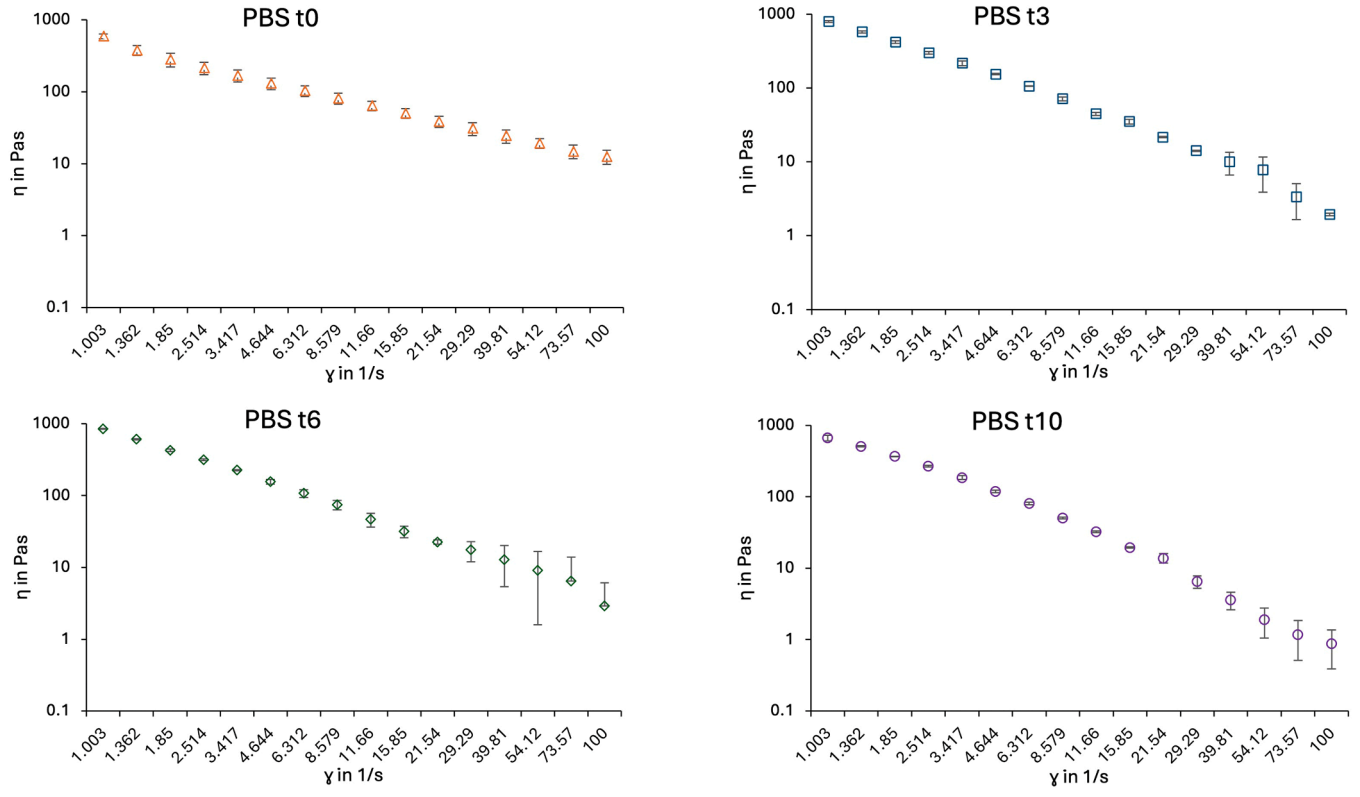

Fig. 2. Shear rate at different time points of plant-based spread (PBS) before (t0), during (t3 and t6) and after (t10) the 10-days storage at 4°C.

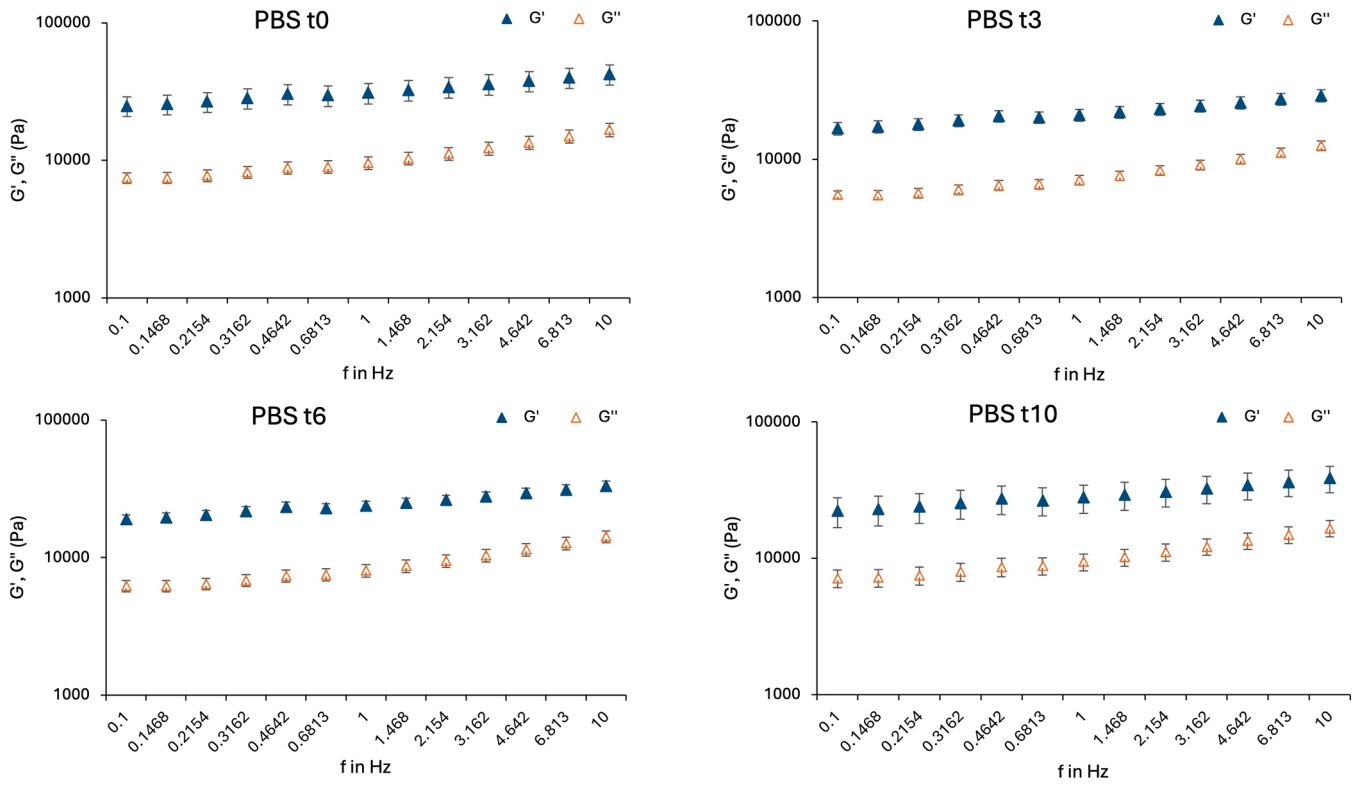

Fig. 3. Viscosity results plotted against storage modulus (G') and loss modulus (G'') as a function of linear frequency (Hz) for plant-based spread (PBS) before (t0), during (t3 and t6) and after (t10) the 10-days storage at 4°C.

Table 3
Colorimetric indices and textural parameters of plant-based spread (PBS) made of 35% w/w of selected YP_{VF}, 45% w/w of defatted durum wheat germ (DWG), and 20% w/w of almond flour.

Storage (days)	Hardness	L*	a*	b*
0	10.19±0.09 ^{c}	55.41±0.13 ^{b}	4.39±0.13	24.26±0.19 ^{c}
3	12.19±0.21 ^{b}	55.18±0.1 ^{b}	4.83±0.25	26.05±0.31 ^{b}
6	15.33±0.32 ^{a}	55.34±0.07 ^{b}	4.77±0.39	26.39±0.08 ^{ab}
10	9.84±0.17 ^{c}	55.9±0.06 ^{a}	4.92±0.02	26.86±0.17 ^{a}

Data are expressed as mean values ± standard deviation (SD). Different lowercase letters in the column indicate significant differences between different time points ($p < 0.05$, one-way ANOVA, and Tukey's HSD test). Abbreviations: a*, redness; b*, yellowness; L*, lightness. Ingredients and technology parameters of the mixture YP_{VF} used are summarized in Supplementary Table 1.

In addition, temperature variations (refrigeration and room temperature) can affect the viscoelasticity and spreadability of spreadable creams (Breidinger and Steffe, 2001; Glibowski et al., 2008; Zulkurnain et al., 2008).

The brightness (L*) and yellow color (b*) indices exhibited nearly identical values across the initial times (T_0, T_3, and T_6), then tended to significantly increase at the final time, t10. The rise in the b* index at the final storage times could be attributed to the formation of free oil in the spreadable cream, as liquid fat tends to separate from the cream and accumulate as oil droplets (Chatziantoniou et al., 2015). However, in accordance with previous studies (Weiss et al., 2018), no significant difference was observed in the a* index across different storage times (Table 3).

3.2.4. *Microbiological stability*

The microbial stability of PBS was assessed by monitoring the cell densities of total presumptive lactic acid bacteria, total mesophilic bacteria, yeasts, molds, and *Enterobacteriaceae* to evaluate the efficacy of the thermal treatment on PBS safety. Results show that pasteurization at 72°C effectively inhibited molds and *Enterobacteriaceae,* which were not detected at t0 (day of production) and throughout the monitoring period until t10 (last day of monitoring). However, yeast, LAB, and total mesophilic bacteria were not completely inhibited, with stable densities of 3.15 ± 0.04, 3.44 ± 0.06, and 4.12 ± 0.03 Log10 cfu/g, respectively, during storage (Supplementary Table 3). According to guidelines for microbial quality in ready-to-eat foods (Gilbert et al., 2000), microbial counts ≤ 4 Log10 cfu/g indicate satisfactory microbiological quality. Furthermore, the complete absence of mycotoxins was observed, ensuring additional safety.

3.2.5. *Volatile profile*

The relative abundance of VOC in PBS before (t_0) and after 10 days storage (t_{10}) is shown in Table 4. The volatile compounds identified and quantified in the samples were grouped into the classes of alcohols, esters, aldehydes, acids, and terpenes with higher abundance and diversity belonging to the former class. Overall, pea-based ingredients, also because accounting for the 35% (w/w) of the formulation, affected only in part the PBS volatile profile being more complex (22 *vs* 14). Moreover, although found in YP_{VF} chetones were not part of the PBS volatile profile. Ethanol, 1-Butanol,3-methyl-, and 1-Hexanol were the most abundant compounds in PBS (t_0) and those subjected to the highest increase during storage (Table 4). 1-Hexanol is a dominant alcohol in peas, contributing to the herbaceous, woody, fragrant, mild, sweet, green fruity odor, and an aromatic flavor (Azarnia et al., 2011). Also, flanked by 1-Butanol,3-methyl, belongs to the main volatile compounds of raw almond (Valdés García et al., 2021). The highest content of ethanol at the end of the storage might be explained by the content of yeast which, although not growing at refrigerated temperature, remains metabolically active (Fleet, 2011). Also, metabolically active yeast could explain the increase of hexanal (aldehyde) and 1-hexanol (alcohol) through the degradation of the flour amino acids via the Ehrlich pathway (Birch et al., 2014). Nevertheless, concentration of alcohol and aldehyde can

Table 4
Volatile compounds of plant-based spread (PBS) made of 35% w/w of selected YP_{VF}, 45% w/w of defatted durum wheat germ (DWG), and 20% w/w of almond flour.

Volatile compounds	PBS-t0	PBS-t10
Alcohols		
Ethanol	1.32±0.01 [b]	7.43±0.73 [a]
1-Propanol, 2 methyl-	0.01±0.00	0.10±0.05
2-Pentanol	0.01±0.00	n.d
1-Butanol,3-methyl-	0.25±0.02 [b]	0.89±0.00 [a]
1-Pentanol	0.08±0.01	0.10±0.14
1-Hexanol	0.44±0.00 [b]	1.51±0.11 [a]
3-Hexen-1-ol	0.01±0.00	0.03±0.00
1-Octen-3-ol	0.01±0.00	0.04±0.00
Benzyl alcohol	0.06±0.00 [b]	0.26±0.00 [a]
Phenylethyl Alcohol	0.02±0.00 [b]	0.07±0.00 [a]
1-Penten-3-ol	n.d	0.02±0.00
2-Buten-1 ol, 3-methyl-	n.d	0.06±0.00
Esters		
Ethyl Acetate	0.04±0.01	n.d
Butanoic acid, 3-methyl-, ethyl ester	0.01±0.00	0.03±0.00
Butanoic acid, 2 methyl-, ethyl ester	n.d	0.01±0.00
Hexanoic acid, ethyl ester	n.d	0.1±0.00
Aldehydes		
Benzaldehyde	0.02±0.00	0.05±0.00
Hexanal	n.d	0.06±0.01
Acids		
Acetic Acid	0.17±0.01 [b]	2.10±0.03 [a]
Butanoic acid	n.d	0.07±0.01
Hexanoic acid	n.d	0.04±0.00
Terpene		
a-Pinene	0.13±0.00 [b]	0.31±0.01 [a]

Data are expressed as mean values ± standard deviation (SD). Different lowercase letters in the same row indicate significant differences between different time points ($p < 0.05$, Student t-test). Abbreviations: PBS, plant-based spread; n.d., not detected. PBS-t0, plant-based spread prior storage at 4°C; PBS-t10, plant-based spread after 10 days-storage at 4°C. Ingredients and technology parameters of the mixture YP_{VF} used are summarized in Supplementary Table 1.

increase during storage due to the oxidation of the lipid fraction (Azarnia et al., 2011). Additionally, the terpene α-pinene, commonly associated with pine and turpentine scents and found in essential oils, increased significantly in during storage possibly due to carotenoid degradation catalyzed by lipo-oxygenase or hydroperoxides (Jakobsen et al., 1998). Acetic acid, most probably deriving from hetero-fermentative lactic acid bacteria fermentation, is the only acid found in PBS and subjected to increase during storage.

3.3. Sensory evaluation

The sensory attributes of PBS are visually represented in a spider chart (Fig. 4). The analysis covered (i) appearance such as color, uniformity, and brightness; (ii) odor, and (iii) taste-olfactory elements including toasted, sourdough, legume, almond, and cereal; (iv) the taste analysis encompassed flavors like sweet, salty, sour, and bitter; (v) textural attributes like homogeneity, adhesiveness, solubility, granularity, elasticity, and creaminess. The odor analysis revealed the highest score for sourdough, likely attributed to the fermented pea mixture used in the cream's production. The taste analysis indicated elevated levels of sour and bitter, while taste-olfactory sensations were most pronounced for cereal and sourdough elements. Regarding texture, PBS exhibited higher adhesiveness and granularity compared to solubility, elasticity, and creaminess. The taste-olfactory and sensory characteristics of a product can be influenced not only by the raw materials but also by microbial activity, heat treatments, fat oxidation, proteolysis, and other enzymatic activities (Mefleh et al., 2022). Overall, the sensory profile of PBS was found to align with the typical aromas of a vegetable spread (Fan et al., 2023). Furthermore, sensory evaluation results confirmed that the typical unpleasant taste associated with legumes was masked by hints of cereals and sourdough, likely due to microbial action. The sample was perceived sweeter rather than saltier. Additionally, the inclusion of defatted wheat germ and almond flour imparted a grainy texture to the final product, which could likely be improved by reducing the particle size of the added raw materials.

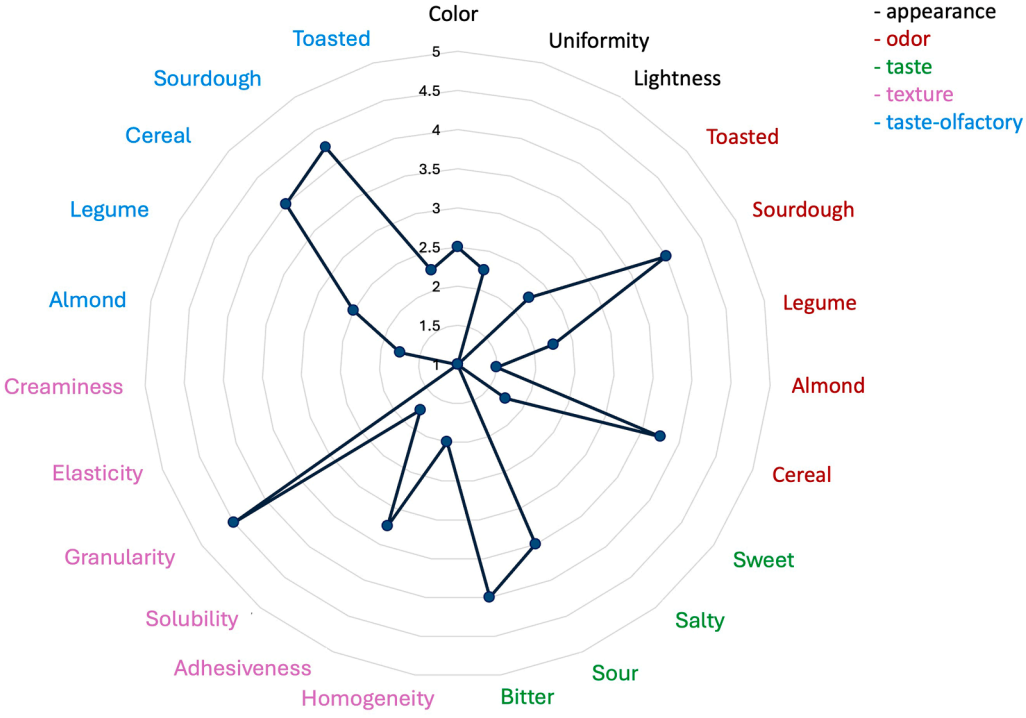

Fig. 4. Spider chart of sensory analysis of plant-based spread (PBS) made of 35% w/w of selected YP_{VF}, 45% w/w of defatted durum wheat germ (DWG), and 20% w/w of almond flour.

3.4. Conclusion

The research aimed to optimize a biotechnological process for creating a pea flour ingredient and evaluating its potential for making a plant-based spreadable cream, addressing challenges in the agri-food sector by promoting sustainable alternatives to animal products. Combination of *Leuc. pseudomesenteroides* fermentation and protease treatment led to enhanced technological, functional, and sensory qualities of pea flour. Moreover, the *in-situ* dextran synthesis ensured the proper viscosity to produce a plant-based spread (PBS) with high protein and fiber content, along with a balanced flavor and stable texture eliminating the need for additional structuring ingredients. Here, a clean-label PBS as a sustainable and nutritious alternative to milk-based spreadable cheese has been proposed to meet the rising demand of consumers for healthier plant-based food options. Incorporating defatted durum wheat germ, almond flour, and field pea wholegrain flour in the final PBS formulation elevated its nutritional value and highlighted the effectiveness of the developed method in overcoming long-standing technological and sensory issues associated with utilizing agri-food by-products or unconventional raw materials in food manufacturing. Overall, this research represents a significant advancement in innovating plant-based foods, addressing both dietary needs and environmental sustainability concerns. Further explorations are necessary to facilitate industrial-scale production validation and ensure long-term market acceptance, which are essential for the widespread adoption of PBS-like products.

CRediT authorship contribution statement

Giuseppe Perri: Writing – original draft, Validation, Supervision, Methodology, Formal analysis, Data curation. **Graziana Difonzo:** Writing – original draft, Formal analysis, Data curation. **Yaqin Wang:** . **Michela Verni:** Validation, Formal analysis, Data curation. **Giusy Rita Caponio:** Writing – original draft, Formal analysis, Data curation. **Rossana Coda:** Writing – original draft, Validation. **Massimo Blandino:** Writing – original draft, Validation. **Erica Pontonio:** Writing – review & editing, Writing – original draft, Supervision, Resources, 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.

Ethical statement - studies in humans and animals

The privacy rights of human subjects have been observed and informed consent was obtained for sensory analysis.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.fufo.2024.100502.

Data availability

No data was used for the research described in the article.

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