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Novel functional plant-based yoghurt designed and analysed using conventional and AI techniques

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ABSTRACT

This research uses supercritical water extraction to extract bioactive compounds from orange peel and the edible mushroom *Suillus granulatus*. The goal was to create a new functional almond yoghurt enriched with these extracts while determining its nutritional, antioxidant, and sensory properties. Moreover, the goal was also to determine the accuracy and reliability of digital sensor analysis using a combination of several software programs. The precise fermentation process was successfully employed during the laboratory production of almond yoghurt enriched with extracts from orange peel and *S. granulatus*. Subcritical water extraction, known as "green extraction," has proven to be an excellent method for extracting valuable components from food waste, such as orange peel. The three types of almond yoghurt produced exhibited remarkably high protein content (ranging from 13.51% to 14.88%) and fat content (between 9.13% and 9.39%), significantly exceeding the protein and fat levels found in traditional yoghurt made from cow's milk. Among the samples, the yoghurt enriched with orange peel extract exhibited the highest antioxidant activity in both tests conducted ($P < 0.05$) compared to the other samples. Additionally, the yogurt enriched with orange peel (Sample 2) showed significantly greater facial expressions, including lip press (10.70), lip suck (2.26), surprise (3.57), and joy (8.71), in comparison to Samples 1 and 3 ($P < 0.05$). This production process will be adapted for industrial-scale production, integrating digitalisation and emphasising the importance of precise fermentation to ensure the production of high-quality, functional plant-based substitutes for dairy products.

Keywords: precision fermentation, smart sensory evaluation, novel functional yoghurt, subcritical extraction, green extraction methods

INTRODUCTION

The rising health concerns linked to dairy consumption have led to a surge in the popularity of plant-based milk alternatives. The global market for these products is expected to reach USD 2.89 billion by 2026, making them an affordable option, especially in regions with limited access to animal milk (Aydar et al., 2020). To meet the increasing demand for non-dairy options, innovative plant-based (PB) foods and beverages have been developed. Traditionally, dairy products have been a key source of essential nutrients, but individuals with conditions such as high cholesterol, lactose intolerance, malabsorption, or milk protein allergies may benefit from plant-based alternatives. Additionally, greater consumer awareness regarding health, environmental sustainability, and the rise of vegetarianism are contributing to the growing preference for PB products (Montemurro et al., 2021).

Various plant sources, including coconut, rice, almonds, peanuts, quinoa, and sesame, are naturally free of lactose and serve as key ingredients in plant-based beverages, catering to those allergic to or intolerant of cow's milk (Sethi et al., 2016). However, these alternatives often come with high costs (Yigit, 2020) and technical challenges related to production and storage. Many suffer from issues with texture and taste (Gorlov et al., 2019) and may not always match the nutritional value of dairy products. Despite these limitations, they contain bioactive components with potential health benefits, making them appealing to health-conscious consumers (Mousel and Tang, 2016).

Subcritical water extraction (SWE), also known as superheated water extraction or hot water extraction under pressure, is an eco-friendly alternative that is gaining popularity over traditional extraction methods. The appeal of water as a solvent in extraction processes lies in its ability to change its physical and chemical properties with temperature variations, making it a versatile and adaptable option (Stojanova et al., 2024; Stojanova et al., 2024a).

Microbial fermentation is widely used to produce fermented foods and food ingredients. This process is typically employed to enhance sensory qualities, nutrition, and preservation in food production (Tangyu et al., 2019). Fermented yoghurt is consumed for its probiotic benefits, which aid digestion, improve gut microbiota, and modulate the immune system (Mahfudh et al., 2021). However, plant-based yoghurts often have inferior sensory attributes, particularly in texture and flavor, due to the absence of lactose and fat found in cow's milk.

Precision fermentation offers a solution by utilising cost-effective and abundant substrates to produce food ingredients. This method involves genetic modifications that optimise the metabolic pathways of microorganisms, enabling the creation of high-quality fermented foods. By leveraging traditional food-fermenting microbes as hosts, precision fermentation can enhance the sensory characteristics of plant-based products. As a result, more research institutions, startups, and established food companies are adopting this technique to develop food ingredients, nutrients, and fermented products more economically and sustainably (Hilgendorf et al., 2024).

This research aimed to extract bioactive compounds from orange peel and the edible mushroom *Suillus granulatus* using supercritical water extraction. Additionally, it seeks to develop a novel functional almond-based yoghurt incorporating precision fermentation, enriched with these extracts, while assessing its nutritional composition, antioxidant properties, and sensory attributes.

MATERIAL AND METHODS

Probiotic starter culture for precision fermentation

Freeze-dried and modified cultures of *Streptococcus thermophilus*, *Lactobacillus acidophilus*, and *Bifidobacterium* sp. were used (Cultech, UK). Before experimental use, cultures were propagated in 10 mL of M17 broth (Neogen, Michigan, United States) for *Streptococcus*; MRS broth (Man, Rogosa, Sharpe broth (De Man et al., 1960)) for *Lactobacillus*; and modified MRS broth for *Bifidobacterium* and incubated at 37 °C for 24h without agitation in microaerophilic conditions. Modified MRS broth consisted of MRS broth with 1% sucrose, supplemented with 0.05% L-cysteine hydrochloride, 0.0005% hemin, and 0.00005% vitamin K (Mehaya et al., 2023). A subculture was prepared in 10 mL of fresh MRS and M17 broths, which were then incubated at 37 °C for 24 hours. For the inoculation of almond milk, the cells were harvested by centrifugation at 6,000 × g for 5 minutes at 4 °C. The cells were washed twice with one-fourth-strength Ringer's solution (Ringer solution Tablets, 96724-100TAB, Merck, Darmstadt, Germany) and then resuspended in the same solution to achieve a final population of approximately 7 log CFU/mL in the almond milk.

Production of plant-based yoghurt

200 g of almonds were washed and soaked in 200 mL of water for 12 hours. After soaking, the almonds were blended with the gradual addition of water until reaching

a total of 1 L. The blended mixture was then transferred to a 1 L glass bottle, and a probiotic starter culture was added at a concentration of 7 log CFU/mL. The bottle was placed in a thermostat under controlled conditions to ferment for 24 hours at a temperature of 40 °C, aiming for a pH value of 4.5. Following fermentation, the yoghurt was cooled to 8 °C and then homogenised using an electric mixer. After the basic production was complete, two types of extracts were added to the yoghurt (4 g extract per sample), resulting in three different types of yoghurt:

- Sample 1: functional almond yoghurt produced by precise fermentation;
- Sample 2: functional almond yoghurt produced by precise fermentation enriched with orange peel extract;
- Sample 3: functional almond yoghurt produced by precise fermentation enriched with *S. granulatus* mushroom extract.

Preparation of the orange (Citrus sinensis) peel and S. granulatus mushroom

The orange peels, consisting of the albedo and flavedo, were air-dried at room temperature (approximately 24 °C) in a dark environment until they reached a constant weight. Once dried, the orange peels were ground using a laboratory blender and stored in a glass jar at room temperature until extraction. The *S. granulatus* mushrooms were dried in a chamber dryer at a temperature of 40 °C for 6 to 7 hours (Stojanova, 2017).

Subcritical water extraction

Subcritical water extraction (SWE) was conducted using a batch high-pressure extractor from Parr Instrument Company (USA), which has an internal volume of 450 mL and can operate at a maximum pressure of 200 bar and a temperature of 350 °C. The extractor is connected to a temperature controller (Model 4838, Parr Instrument Company, USA). A schematic diagram illustrating the main components of the device is provided in Figure 1.

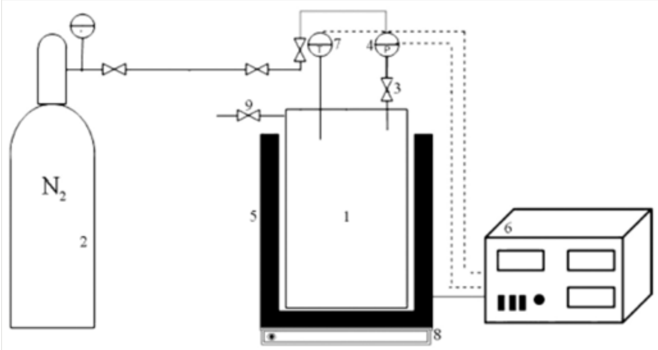

Figure 1. Schematic representation of the apparatus for extraction with subcritical water: (1) extractor; (2) nitrogen bottle; (3) gas inlet valve; (4) pressure gauge; (5) electric heating pad; (6) digital controller; (7) temperature probe; (8) magnetic stirrer; (9) gas outlet valve (Gavarić, 2020)

A total of 10 g of *C. sinensis* peel and 10 g of *S. granulatus* powder were separately mixed with 100 mL of distilled water in an extractor (1). The working pressure was achieved by introducing nitrogen from the bottle (2) through the valve (3) and was measured with a manometer (4). Nitrogen was applied to achieve the working pressure to prevent potential oxidation that can occur at elevated temperatures in the presence of oxygen from the air. The extractor was heated with a heating pad (5), and the temperature was measured and controlled via the control panel (6), connected to the temperature probe (7). A magnetic stirrer (8) (1000 rpm) was used to accelerate mass and heat transfer, as well as to prevent local overheating on the walls of the extractor. The temperature was 120 °C, the extraction time was 25 min, and the pressure during the extraction was 30 bar. After extraction, the extractor was cooled in an ice water bath to 30 °C, and nitrogen was released through the valve (9). Then the extracts were filtered through filter paper under vacuum, collected in glass vials, and stored at 4 °C until analysis (Gavarić, 2020).

Determination of total phenolic compounds (TPC)

The total phenol content was determined using the method adapted from Stojanova et al. (2021) for microplates. The extracts were diluted to suitable concentrations, and a gallic acid solution was prepared to create a calibration curve, also at appropriate concentrations ranging from 0 to 800 µg/ml. All assays were performed in triplicate and the absorbance was measured spectrophotometrically at 760 nm. The phenol content was calculated on the basis of the calibration curve (concentration-dependent absorbance function) of a standard gallic acid solution. The equivalent gallic acid (GAE) per gram of dry matter was determined using the following formula:

mg eq. GAE/g d.m. = read concentration GAE (µg/ml) / working concentration × 1000

Determination of total flavonoid content (TFC)

The flavonoid content was determined using a modified method from Chang et al. (2002), adapted for microplates. This method relies on the ability of flavonoids and flavonol glycosides to form complexes with metal ions, with the aluminium complex being particularly significant. It is a straightforward technique that involves the formation of a colored complex, which has an absorption maximum at 430 nm. The flavonoid content was calculated based on the calibration curve (concentration-dependent absorption function) of a standard quercetin solution. The equivalent quercetin (QE) per gram of dry matter was determined using the following formula:

mg eq. QE/g d.m. = read concentration QE (µg/ml) / working concentration × 1000

Antioxidant activity

The antioxidant activity of the extract samples was assessed using two methods: the ability to capture free DPPH radicals and the antioxidant activity in the linolenic acid system, utilising the conjugated diene method as described by Stojanova et al. (2021). All measurements were conducted in triplicate.

Preparation of yoghurt samples

The extraction method for yoghurt samples was carried out as described by Demirci et al. (2017). To extract the desired components, 5 g of the yoghurt samples were mixed with 25 mL of diluted methanol (80:20, methanol: distilled water). The mixture was then homogenised using an ultra-turrax homogeniser and centrifuged at 7200 rpm for 10 minutes at 4 °C. After centrifugation, the mixture was filtered using Whatman No. 1 filter paper. The liquid portion obtained from the filtration was stored at 4 °C for subsequent analysis of antioxidant activity.

Total carbohydrate content

The content of total carbohydrates in yoghurt samples was determined by the Feling I and Feling II modified methods (Stojanova, 2017).

Total protein content

The content of total proteins in yogurt samples was determined by calculation, by determining the nitrogen content, using the modified Kjeldahl method (Stojanova, 2017):

% proteins = % N · coefficient 6.25

Total fat content

The crude fat content in the samples was determined using a Soxhlet apparatus (modified by Stojanova, 2017), and diethyl ether was used as an organic solvent for extraction. 2 g of the sample was weighed into cellulose paper bags. After the measurement, the sample was transferred to the extraction part of the Soxhlet apparatus, under which there is an electric water bath. Diethyl ether was added to the apparatus. The solvent vapours go to the cooler, where they condense, and the condensate returns in drops to the bags where the fats are extracted. The extraction lasts about 6–10 hours, during which the diethyl ether passes from the extractor into the flask. After extraction, the bags were dried to a constant mass at a temperature of 105 °C. Drying took 2 hours at first, and then the mass was checked every half hour. The fat content was calculated according to the formula.

Determination of total phenolic compounds (TPC)

The total phenol content was determined according to the procedure outlined in section ‘Determination of total phenolic compounds (TPC)’ in the extracts.

Determination of total flavonoid content (TFC)

The flavonoid content was analysed following the procedure outlined in section 'Determination of total flavonoid content (TFC)' in the extracts.

Antioxidant activity of yoghurt

The antioxidant activity of the yoghurt samples was assessed according to the procedure outlined in the section 'Antioxidant activity' of the extracts.

Sensory evaluation

A total of 30 participants took part in the sensory analysis, consisting of 15 women (50%) and 15 men (50%), all aged between 25 and 60 years. The participants analysed three types of yoghurt, each served in a plastic cup containing approximately 30 g at a temperature of 10 ± 2 °C, following the method outlined by Gupta et al. (2021). The facial expressions of the participants were recorded using a video camera, with PsychoPy installed on a tablet. The recordings were captured in a resolution of 1080 × 720 pixels at 30 frames per second. To maintain consistent lighting, a direct current light source illuminated the room from above during the recordings. The videos were imported into the iMotions Biometric Research Platform 6.2 software (iMotions, 2015) and analysed using the Affectiva facial expression recognition engine. Participants were instructed to position their faces between two bars of an adjustable headrest to ensure that their faces remained centred during the recordings. The participant's task was to imitate the emotions expressed in a series of 60 frontal portrait pictures (20 for each emotion category), as described by Kulke et al. (2020). In parallel, consumers' liking for the plant-based yoghurts was assessed using a nine-point hedonic scale, where 1 indicated "dislike extremely," 5 indicated "neither like nor dislike," and 9 indicated "like extremely". The goal of conventional sensor analysis was to determine the accuracy and reliability of digital sensor analysis through a comparison of results. And, the purpose of both sensory analyses is to determine the quality and acceptability of the new product by the tasters.

Statistical analysis

To identify statistically significant differences in the results, an ANOVA test followed by Tukey's test ($P < 0.05$) and a T-test ($P < 0.05$) was conducted using SPSS version 20 software. Additionally, multivariate data analysis was performed through principal component analysis (PCA) to evaluate the relationships and associations among samples and the variables related to the analysed sensory parameters.

RESULTS AND DISCUSSION

Antioxidant activity of the extracts

The data presented in Table 1 indicate that the extract from *S. granulatus* contains a significantly higher concentration of total phenols (86.01 mg GAE/g d.m.) and flavonoids (42.66 mg QE/g d.m.) compared to the orange peel extract, which supports the strong antioxidant potential of *S. granulatus* in phenolic compounds. However, it is noteworthy that the yield from the orange peel extract (43.61%) is significantly higher ($P < 0.05$) than that of the *S. granulatus* extract, suggesting that while the *S. granulatus* extract is more concentrated in bioactive compounds, the orange peel extract offers a more efficient extraction process in terms of yield.

Table 1. Phenolic and flavonoid content in the tested extracts

Extract	n	Parameter		
		TPC (mg GAE/g d.m.)	TFC (mg QE/g d.m.)	Yield (%)
		x̄ ± SD	x̄ ± SD	x̄ ± SD
Orange peel	3	43.19^{a} ± 0.15	13.93^{a} ± 0.02	43.61^{a} ± 0.07
S. granulatus	3	86.01^{b} ± 0.06	42.66^{b} ± 0.10	37.78^{b} ± 0.13

^{a,b} – values for the different extracts and the same parameter marked with different letters are statistically significantly different, T-test ($P < 0.05$).

Figure 2 further reinforces these findings, as the orange peel extract demonstrates superior antioxidant capacity in capturing free DPPH radicals (83.66%) at the highest concentration of 10 mg/mL, compared to *S. granulatus* extract.

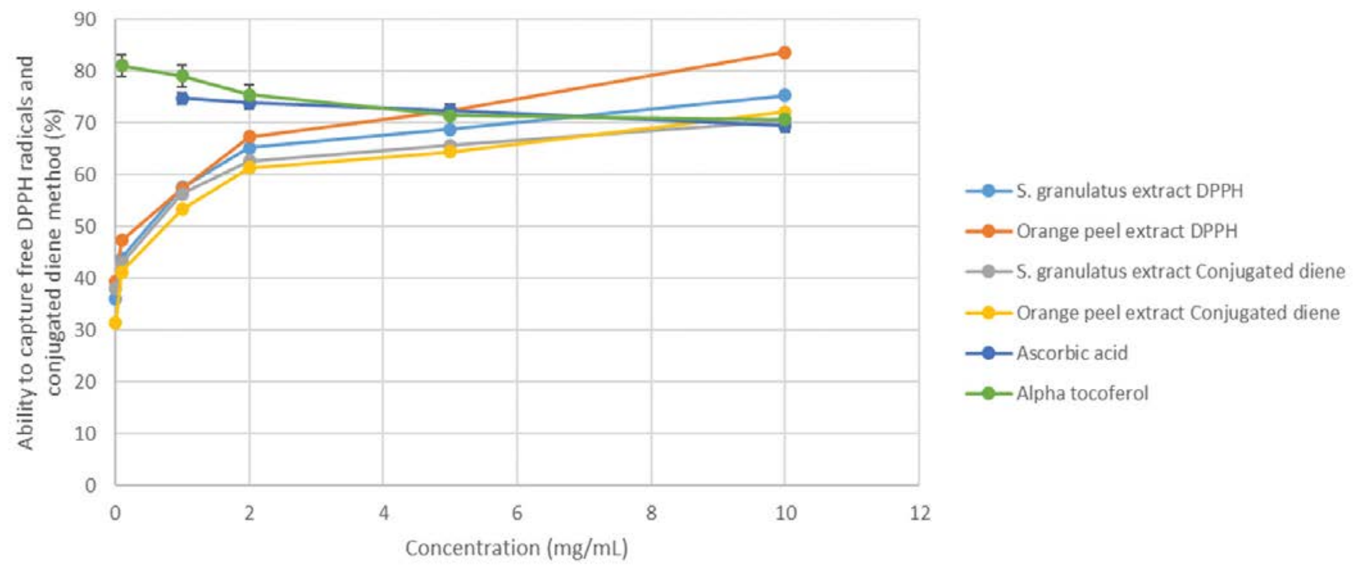

Figure 2. Antioxidant activity of tested extracts (x̄ ± SD)

A similar trend is observed when assessing antioxidant activity via the conjugated diene method, where the orange peel extract exhibits significantly higher antioxidant activity (72.05%). These results indicate that the orange peel extract could be a potent natural antioxidant, comparable to traditional antioxidants such as ascorbic acid and alpha-tocopherol. Importantly, both extracts show competitive antioxidant activity, outperforming the positive controls at higher concentrations, highlighting their potential application in functional food development.

Although citrus peels are not edible, they are a rich source of various bioactive compounds, including phenolic compounds, vitamins, minerals, terpenoids, terpenes, dietary fiber, and polysaccharides. These constituents are associated with several significant biological activities, such as antioxidant, antimicrobial, antidiabetic, and anticarcinogenic effects (Shehata et al., 2021; Brezo-Borjan et al., 2023). The compounds in orange peel possess various beneficial properties, including anti-allergic, anti-ageing, cardioprotective, and neuroprotective effects. Polyphenols, especially flavonoids, represent the most significant class of these bioactive constituents. The nature and concentration of these compounds in extracts can vary due to environmental conditions, the specific variety of the subspecies, and the extraction methods employed (Tomás-Navaro et al., 2014; Brezo-Borjan et al., 2023).

Reis et al. (2014) concluded that the methanolic extract of *S. granulatus* from Serbia exhibited a greater ability to capture free DPPH radicals, with an EC50 value of 0.89 mg/ml, compared to the extract from Portugal, which had an EC50 value of 0.98 mg/mL. In terms of antioxidant activity measured by the conjugated dienes method, the mushroom extract from Serbia had an EC50 value of 0.48 mg/ml, while the mushroom extract from Portugal showed an EC50 value of 0.45 mg/mL. Additionally, Tel et al. (2013) reported that the methanolic extract of *S. granulatus* was able to capture DPPH radicals at a rate of 64.66%, whereas the ethyl acetate extract demonstrated a higher capture rate of 91.52% at a concentration of 400 µg (Stojanova et al., 2021).

Polyphenolic compounds in food are recognised for their high antioxidant capacity. Numerous *in vitro* studies on various food items have demonstrated that their water and alcoholic extracts are rich in phenolic compounds and exhibit significant antioxidant effects (Elmastas et al., 2007). Flavonoids, a type of polyphenolic compound, display antioxidant activity by donating hydrogen, which leads to the formation of a stabilised radical. Additionally, they can chelate transition metal ions. Research shows that flavonoids are equal to or even outperform tocopherols and ascorbic acid in neutralising free radicals in aqueous solutions. Furthermore, flavones have been found to inhibit the proliferation of numerous cancer cell lines (Stojanova et al., 2021).

Chemical composition of the yoghurt

The results presented in Table 2 show that the total carbohydrates, proteins, and fats were almost identical across all three yoghurt samples, with only minimal differences observed, primarily due to the added extracts in the second and third samples. These slight variations serve as confirmation that the over-fermentation process was successfully carried out, resulting in an identical base for all three types of yoghurt. Notably, the almond-based yoghurt, particularly the samples enriched with *S. granulatus* mushroom extract, exhibited protein and fat contents that were significantly higher than those found in traditional cow's milk yoghurt. According to Carić et al. (2000), traditional yoghurt contains approximately 3.90–5.00% protein and 1.25–3.40% fat, whereas the protein and fat levels in the almond-based yoghurt produced in this study were many times higher. This substantial increase in protein and fat content highlights the potential of plant-based yoghurts, especially those enriched with specific extracts, to offer a more nutritionally dense alternative to traditional dairy products.

In terms of bioactive compounds, the second and third yoghurt samples, which were enriched with *S. granulatus* mushroom extract, contained significantly higher ($P < 0.05$) levels of total phenols (59.14 mg GAE/g d.m.) and flavonoids (38.20 mg QE/g d.m.) compared to the first sample. These results align with the findings of Brückner-Gühmann et al. (2019), who suggested that fermentation can alter the structure of plant proteins and lead to enhanced aggregation of bioactive compounds. Similarly, Ogundipe et al. (2021) reported that fermenting plant-based products such as sprouted tiger nut tubers resulted in increased levels of protein and bioactive compounds, including phenols, which are consistent with the elevated phenolic and flavonoid levels observed in the enriched yoghurt samples of this study. This suggests that the fermentation process, in combination with the addition of specific extracts, can significantly increase the concentration of beneficial bioactive compounds in plant-based dairy alternatives.

Plant-based beverages are water-soluble extracts derived from legumes, oilseeds, nuts, cereals, or pseudocereals, and they have a similar appearance and consistency to milk. Currently, there is no widely accepted definition or classification for these beverages, which are often referred to as alternatives or substitutes for milk or dairy, as well as plant-based milk or dairy. Additionally, plant proteins serve as effective carriers for probiotics (Boukid et al., 2023).

The comparison with traditional yoghurt further emphasises the unique nutritional profile of the almond-based yoghurt produced in this study. The enhanced protein and fat contents, along with the elevated levels of phenols and flavonoids, suggest that precision fermentation with bioactive extracts such as *S. granulatus* could offer significant advantages over traditional cow's milk yoghurt. Boukid et al. (2023), Brückner-Gühmann et al. (2019), and Ogundipe et al. (2021) highlighted the role of fermentation in enhancing protein content and improving the nutritional profile of plant-based products,

Table 2. Chemical composition of the almond yoghurt

Yoghurt type	n	Parameter				
		Total carbohydrates (%)	Total proteins (%)	Total fats (%)	TPC (mg GAE/g d.m.)	TFC (mg QE/g d.m.)
		$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD	$\bar{x}$ ± SD
Sample 1	3	4.97^a ± 0.11	13.51^a ± 0.04	9.13^a ± 0.15	2.17^a ± 0.09	0.98^a ± 0.02
Sample 2	3	5.05^a ± 0.03	13.53^a ± 0.16	9.16^a ± 0.10	37.68^b ± 0.05	12.42^b ± 0.02
Sample 3	3	5.41^b ± 0.09	14.88^b ± 0.03	9.39^b ± 0.18	59.14^c ± 0.14	38.20^c ± 0.06

^{a,b,c} – values for the different samples and the same parameter marked with different letters are statistically significantly different, ANOVA, post hoc Tukey's test ($P < 0.05$)

which is consistent with the findings of this study. The incorporation of *Lactobacillus bulgaricus* and *Streptococcus thermophilus* into plant-based yoghurt not only boosts protein and fat levels but also enhances the antioxidant properties of the product, further demonstrating the potential of precision fermentation in developing plant-based alternatives that rival the nutritional qualities of traditional dairy products.

The almond-based yoghurt produced in this study, especially when enriched with *S. granulatus* mushroom extract, offers a substantial improvement in both protein and fat content, as well as antioxidant activity, compared to traditional cow's milk yoghurt. These findings, in line with existing literature, underscore the potential of plant-based yoghurts as a viable, nutritionally enhanced alternative to dairy. The results also highlight the promising role of precision fermentation in elevating the nutritional and functional properties of plant-based yoghurt, paving the way for the development of more nutritious and health-promoting dairy alternatives.

The antioxidant activity of yoghurt

Table 3 highlights the antioxidant potential of all three yoghurt samples, with the orange peel-enriched yoghurt (Sample 2) exhibiting the highest antioxidant activity (83.70% and 75.21%) in the two test methods. These findings align with those shown in Figure 1, where the antioxidant capacity of Sample 2 surpasses that of both Sample 1 and Sample 3 ($P < 0.05$). Given the absence of commercially or experimentally available products comparable to these, the results were benchmarked against the closest available alternatives, providing a valuable point of reference for understanding the functionality of the new yoghurt samples.

Fermentation plays a significant role in enhancing the nutritional value and palatability of plant-based beverages. For instance, Vasilean et al. (2021) showed that the use of starters such as *Lactobacillus* and *Bifidobacterium* improves the mineral content and bioavailability, as well as the antioxidant properties, in beverages made from almonds, soy, chickpeas, and red beans. Furthermore, the study reported that fermentation increased antibacterial activity against pathogens like *Bacillus cereus*, *Escherichia coli*, *Listeria monocytogenes*, and *Staphylococcus aureus*. In addition to this, radical scavenging activity, phenolic content, and the availability of minerals such as calcium and iron were enhanced after fermentation. These results align with the findings in the current study, where fermentation appears to boost the antioxidant activity of the almond-based yoghurt, particularly the enriched sample with orange peel extract.

Table 3. Antioxidant activity of the almond yoghurt

Yoghurt type	n	*In vitro* antioxidant activity	
		Ability to capture DPPH radicals (%)	Conjugated diene method (%)
		$\bar{x}$ ± SD	$\bar{x}$ ± SD
Sample 1	3	49.31^a ± 0.10	37.43^a ± 0.19
Sample 2	3	83.70^b ± 0.16	75.21^b ± 0.03
Sample 3	3	72.06^c ± 0.07	69.74^c ± 0.05

^{a,b,c} – values for the different samples and the same parameter marked with different letters are statistically significantly different, ANOVA, *post hoc* Tukey's test ($P < 0.05$).

Boukid et al. (2023) also reported that fermentation of Chlorella vulgaris and soy extract using *Lactobacillus fermentum* and *Lactobacillus rhamnosus* led to higher polyphenol content and increased dietary antioxidant capacity compared to unfermented soy extract. This is similar to the current study, where the addition of orange peel extract in the yoghurt resulted in a noticeable increase in antioxidant activity, which was measured using both DPPH and ABTS assays. The observed values of 83.70% and 75.21% are likely reflective of enhanced polyphenol and flavonoid levels in the yoghurt enriched with orange peel extract, thus supporting the findings of Boukid et al. (2023).

Sadighbathi et al. (2023) investigated the antioxidant activity of yoghurts fortified with postbiotics, finding that yoghurts containing LB-CW (*Lactobacillus delbrueckii* subsp. *bulgaricus* postbiotic-containing cheese whey) and ST-SM (*Streptococcus thermophilus* postbiotic-containing skim milk) exhibited ABTS values of 51.78% and 51.19%, respectively ($P < 0.05$), on the final day of storage. While these values were lower than the antioxidant activity observed in the yoghurt samples in this study, they still demonstrate significant antioxidant potential. In the current study, the antioxidant activity in the enriched yoghurt was higher, with 83.70% and 75.21% for the DPPH and ABTS assays, respectively, which indicates a potentially stronger antioxidant profile in the orange peel-enriched yoghurt.

Furthermore, Mehaya et al. (2023) found that soymilk fermented with *Saccharomyces boulardii* CNCM I-745 exhibited the highest radical scavenging activity of 6.02 mg TE/g in comparison to *L. plantarum*, which showed 5.86 mg TE/g, and unfermented soymilk at 4.45 mg TE/g. In the same study, total phenolic compounds were most prevalent in *Saccharomyces*-yoghurt, with a concentration of 3.52 GAE mg/g, followed by *Lactobacillus*-yoghurt at 2.29 GAE mg/g, and unfermented soymilk at 2.26 GAE mg/g. These findings highlight a similar trend observed in the current study, where the antioxidant activity and phenolic content were significantly higher in the yoghurt enriched with orange peel extract, showing a strong radical scavenging activity and higher phenolic content than the non-enriched samples. The observed values in the current study are much higher compared to the radical scavenging activities reported by Mehaya et al. (2023), which further supports the conclusion that orange peel extract contributes to a potent increase in antioxidant activity.

The comparison of the antioxidant activity in the almond-based yoghurt enriched with orange peel extract to the results reported by Vasilean et al. (2021), Boukid et al. (2023), Sadighbathi et al. (2023), and Mehaya et al. (2023) demonstrates that the yoghurt in this study possesses superior antioxidant potential. The significantly higher values for both DPPH test and the conjugated diene method, particularly in the sample enriched with orange peel extract, underscore the effectiveness of this natural additive in enhancing the functional properties of plant-based yoghurt.

Sensory evaluation and biometric responses

Sensory evaluation is typically divided into three main categories: discriminative, acceptability, and descriptive tests. Acceptability tests often generate subjective responses, which require a larger sample of consumer evaluations to produce more reliable results, making the process time-consuming (Fuentes et al., 2018).

In recent studies, sensory evaluations involving consumers have incorporated biometric methods. These techniques gather physiological data, such as heart rate, body temperature, and facial expression changes, through personal identification methods. This approach helps assess participants' subconscious reactions to various stimuli, including images, videos, real-life situations, and food and beverages like chocolate and beer (Fuentes et al., 2018; Torrico et al., 2018).

The analysis presented in Table 4 reveals that the yoghurt enriched with orange peel (Sample 2) elicited the highest number of significant facial expressions, such as lip press (10.70), lip suck (2.26), surprise (3.57), and joy (8.71) compared to the other two samples. These responses, which are statistically significant ($P < 0.05$), indicate that the orange peel-enriched yoghurt was more stimulating and enjoyable for the consumers. The presence of expressions like surprise and joy suggests that the product was perceived as pleasant and perhaps surprising in flavour. Additionally, the results show that while consumers appear satisfied with the taste of these new yoghurt variants, they did not seem particularly relaxed during the tasting. Sample 3 had the lowest relaxation score (1.06), followed by Sample 2 (1.15) and Sample 1 (2.47), indicating that consumers were somewhat more tense or alert during the tasting of these new products. This is not unexpected, given the novelty of the flavours, and it suggests that physiological responses to food stimuli (Gupta et al., 2021), such as facial expressions and relaxation, are influenced by the specific characteristics of the product. So, while consumers enjoyed the flavour of the yoghurt, particularly the orange peel variant, they were still more focused or attentive than relaxed, which can be attributed to the unfamiliarity of the product.

Table 4. Facial expression recognition values responses of the yoghurt samples

Type	Parameter	Sample 1	Sample 2	Sample 3
		x̄ ± SD	x̄ ± SD	x̄ ± SD
Facial Expression	Lip Press	$6.35^{a} \pm 1.52$	$10.70^{b} \pm 1.67$	$8.95^{c} \pm 0.29$
	Lip Suck	$1.72^{a} \pm 2.01$	$2.26^{b} \pm 1.15$	$2.21^{b} \pm 1.01$
Head Orientation	Yaw	$-2.45^{a} \pm 1.08$	$-0.61^{b} \pm 1.34$	$-0.58^{b} \pm 0.91$
Emotion	Surprise	$2.63^{a} \pm 1.07$	$3.57^{b} \pm 1.39$	$2.75^{c} \pm 1.60$
	Joy	$5.27^{a} \pm 1.93$	$8.71^{b} \pm 1.31$	$7.04^{c} \pm 1.29$
	Relaxed	$2.47^{a} \pm 1.01$	$1.15^{b} \pm 1.19$	$1.06^{c} \pm 0.99$
Emoji	Smiley	$9.78^{a} \pm 1.06$	$15.31^{b} \pm 1.14$	$18.22^{c} \pm 1.20$
	Stuck Out Tongue	$-3.61^{a} \pm 1.29$	$-3.89^{b} \pm 1.26$	$-3.80^{b} \pm 1.17$
	Smirk	$4.23^{a} \pm 1.03$	$5.07^{b} \pm 0.92$	$5.29^{b} \pm 1.10$

$^{a, b, c}$ – values for the different samples and the same parameter marked with different letters are statistically significantly different, ANOVA, post hoc Tukey's test ($P < 0.05$); (n = 30)

The reliability of Affectiva software has been confirmed through validation studies involving static images and videos, which demonstrated its accurate emotion recognition capabilities. Research by Kulke et al. (2020) found that participants are quicker to produce facial expressions and exhibit stronger muscle activation associated with specific emotions when they see a face expressing the same emotion.

Zhou et al. (2019) found that the probiotic yoghurt containing *Lactobacillus helveticus* H9 received lower scores in flavour and texture. However, the overall sensory quality changes were not significantly different compared to the control samples. Similarly, Mani-López et al. (2014) noted that consumers did not perceive any notable differences in texture or flavour between the control and the probiotic yoghurts, and there were no significant differences in preferences among the different yoghurts.

In a study by Broad et al. (2022), participants revealed that their value-driven decisions in food choices were often outweighed by self-centred considerations and ingrained habits. They identified key factors influencing their decisions as organoleptic qualities, including taste and texture, along with price and the effect on individual health.

Figure 3 further corroborates the consumer preference data, as Sample 2 received the highest ($P < 0.05$) average rating of 8.85, underscoring its overall acceptance. When comparing the results from Table 4 and Figure 3, it is evident that Sample 2 was consistently rated most favorably. This consistency validates the accuracy and reliability of the digital sensory analysis tools employed in this study, including the combination of PsychoPy, iMotions Biometric Research Platform 6.2 software, and the Affectiva facial expression recognition engine.

Finally, the Principal Component Analysis (PCA) biplots presented in Figure 4 provide additional insight into the relationship between facial expressions and consumer responses to the yoghurt samples. PC1, which accounts for 97.77% of the variation, correlates with positive facial expressions such as smirk, surprise, lip suck, lip press, joy, and smiley, suggesting that these expressions are indicative of favorable consumer reactions. In contrast, PC2, which explains 1.81% of the variation, is associated with more negative expressions such as disappointment, disgust, and yawning.

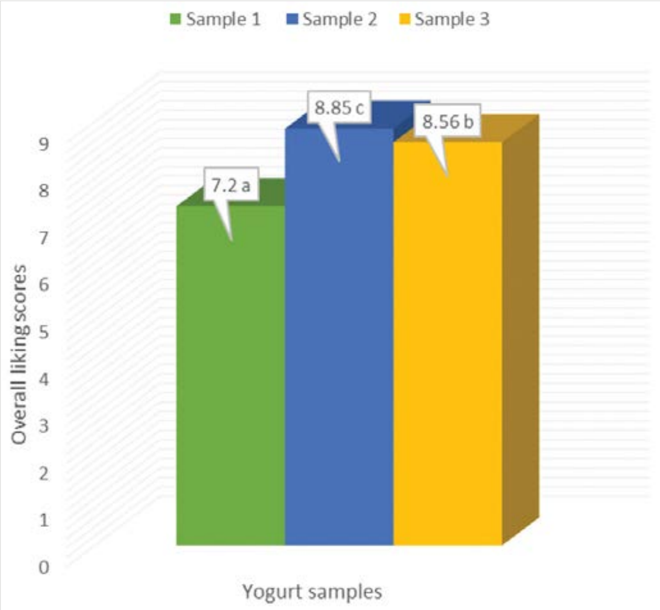

Figure 3. Participant-reported overall liking scores for each yoghurt sample ($\overline{x} \pm SD$)

These findings highlight the potential of PCA in identifying key emotional responses to food stimuli, further enhancing the understanding of consumer preferences.

Gupta et al. (2022) found that head orientation, or yaw, is linked to positive emotions, suggesting that consumers tend to turn their heads to the right when they like a product. This aligns with the findings of Soussignan et al. (2006), which indicated that both adults and infants often tilt their heads to the left when encountering highly bitter foods, signalling rejection of potentially harmful substances. In the same study, the smiley emoji was identified as an ineffective indicator for yoghurt products, as it was associated with negative rather than positive emotions, pointing to a misclassification. This underscores the importance of combining biometric measures with self-reported preferences for gaining more comprehensive insights into consumer acceptance of yoghurt products (Gupta et al., 2022).

A potential limitation of the study is the relatively small sample size of 30 participants used for the sensory evaluation of the tested yoghurt products. While this size may not fully capture the diversity of preferences and

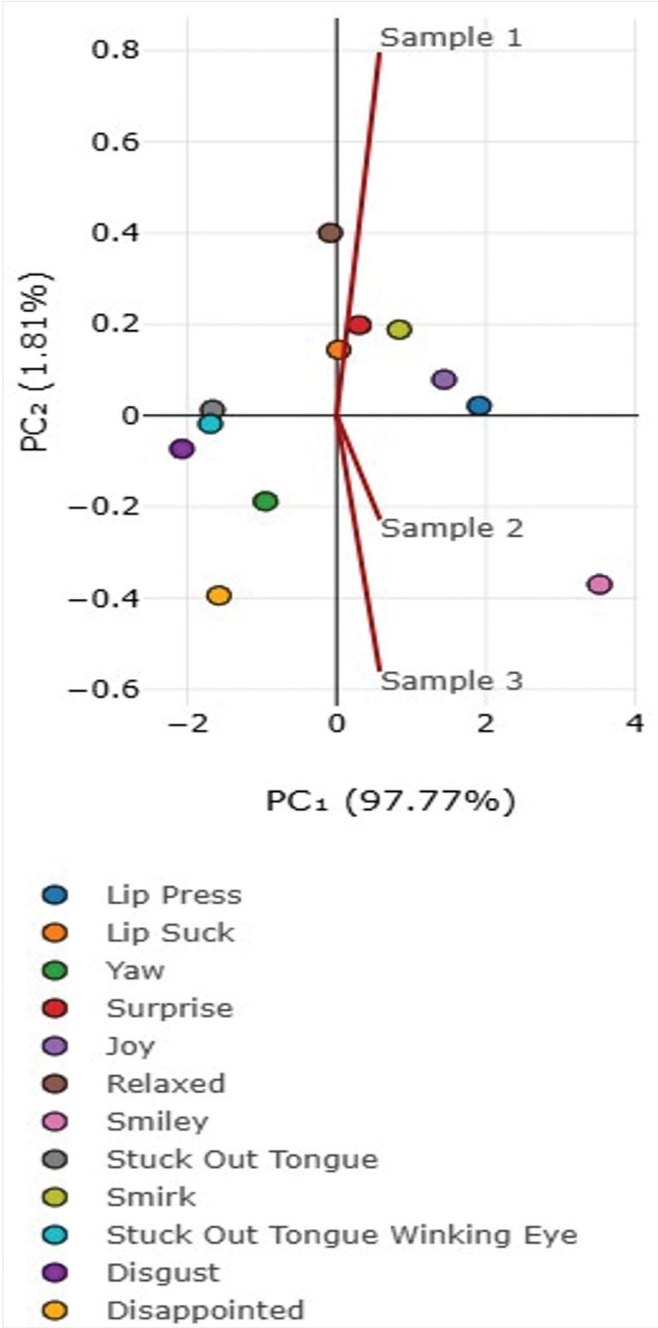

Figure 4. Principal Component Analysis of emotional responses for the three yoghurt samples

physiological responses across a broader population, it still provides valuable insights into consumer reactions, especially in terms of facial expressions, which offer real-time emotional responses. The smaller sample allows for a more focused analysis, making it easier to observe and interpret individual reactions. Despite this limitation, the study offers a useful foundation for understanding consumer perceptions of new yoghurt flavors and could inform product development. A larger, more diverse sample in future studies would help to confirm and expand upon these initial findings, providing a more robust and generalizable understanding of how such products are perceived.

CONCLUSION

Based on the data presented, it can be concluded that precise fermentation was successfully carried out in the laboratory production of almond yoghurt, which was enriched with orange peel extract and *S. granulatus* edible mushroom extract. Subcritical water extraction, a "green extraction" method, proved to be an excellent technique for extracting bioactive components, even from food waste such as orange peel, contributing to the circular economy and food waste management.

The resulting plant-based yoghurt is a completely new product with an exceptionally rich nutritional profile. It contains significantly higher levels of carbohydrates, proteins, fats, total phenols, and flavonoids ($P < 0.05$) compared to conventional yoghurts on the market. Additionally, the yoghurt exhibits very high antioxidant activity (up to 83.70%), qualifying it as a functional food. This represents an important step forward in the exploration of alternative plant-based proteins.

Moreover, the combination of various software algorithms was successfully applied in the sensory analysis of the new product, which was positively received by the tasters. This research paves the way for future studies aimed at further enhancing the basic formulation of almond yoghurt with extracts that could provide additional antimicrobial and cytotoxic potential. Furthermore, the production process, initially designed for experimental purposes, will be adapted for industrial-scale production by integrating digitalisation and focusing on the importance of precise fermentation to ensure the quality of plant-based alternatives to dairy products.

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