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Semi-industrial scale-up of functional biscuits enriched with wine lees: nutritional enhancement and consumer acceptance

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A R T I C L E I N F O

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A B S T R A C T

The increasing consumer demand for healthier and more sustainable food products has driven interest in functional ingredients derived from food by-products. Wine lees, a by-product of winemaking, are rich in polyphenols, anthocyanins, and dietary fiber, offering significant nutritional and functional potential. However, their incorporation into baked products is often limited by sensory challenges. This study aimed to develop semi-industrial scale-up biscuit formulations enriched with wine lees, addressing both nutritional enhancement and consumer acceptance. Three prototype formulations were produced, and the most promising – identified through sensory evaluation – was selected for scale-up (B3s). The selected formulation was further characterized for physicochemical and nutritional properties and evaluated through consumer testing. B3s exhibited a high nutritional profile, meeting the criteria for "high in fiber" in accordance with Regulation (EC) 1924/2006, and demonstrating a low predicted glycemic index according to the International Standards Organization (ISO) criteria. Based on simulated colonic fermentation, B3s also exhibited a slight prebiotic effect supported by the significant increase in total aerobes and bacillus-shaped lactic acid bacteria. The consumer test indicated that the prototype was well received by consumers, with liking for all assessed variables, including color, flavor, aroma, texture, and overall acceptability, scoring above 6 ("liked slightly"). These findings suggest the potential marketability of biscuits enriched with wine lees, as their nutritional and sensory characteristics are positively accepted by consumers.

1. Introduction

In recent years, consumers' food choices have changed, driven by an increasing awareness of the impact of food on both health and the environment. Consequently, the demand for healthier, more sustainable, and functional products has increased. This has driven reformulation strategies that aim to reduce the caloric density, glycemic index and content of added sugars and saturated fats, while enhancing the content of dietary fiber and antioxidant compounds (Mollakhalili-Meybodi et al., 2023; Syahputri & Aprianingsih, 2015; Ungureanu-Iuga et al., 2020). Although baked goods are widely consumed for their convenience and sensory appeal (Siddiqui et al., 2022), they are often nutritionally imbalanced and typically formulated with refined flours that lack bioactive compounds, such as phenolic compounds (Caponio et al., 2022; Iqbal et al., 2021). Biscuits represent a diverse sector of the baked goods industry. They are widely consumed by a wide population because they are easy to consume (ready-to-eat) (Banerjee et al., 2014; Klunklin & Savage, 2018). Furthermore, they are particularly well-suited for the incorporation of fiber and other bioactive compounds. Their simple formulation, low moisture content, and long shelf life make them technologically stable (Adebowale et al., 2012; Tüter et al., 2025).

In line with the principles of the circular economy, increasing attention has been paid to the valorization of agri-food by-products to fill this gap (Caponio et al., 2024a). Within the wine industry, grape pomace and wine lees are two important by-products that are rich in fiber and phenolic compounds, albeit with different compositions. Grape pomace, consisting of skins, seeds, and solid residues from grape pressing, is a widely studied source of fiber and polyphenols (Deng et al., 2011). Wine lees – a residue that is formed at the bottom of wine

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containers, after fermentation, during storage or after treatments, as well as the residue obtained after the filtration or centrifugation of this product (Pérez-Serradilla & de Castro, 2008) – contain higher levels of proteins (approximately 15–30% on a dry matter basis), yeast-derived polysaccharides, and molecules with antioxidant or antimicrobial properties (Barcia et al., 2014; Landeka et al., 2017; Rivas et al., 2021). Incorporating them into food matrices has produced promising technological and nutritional results (Sharma et al., 2015; Troilo et al., 2021). A recent study by our group showed that replacing 10–20% of wheat flour with wine lees produced biscuits that were enriched with fiber and polyphenols and met the specifications for being labelled "source of fiber" or "high in fiber" (Caponio et al., 2024b). However, issues such as increased hardness, darkening of color, bitterness, and astringency may arise negatively affecting consumer acceptance.

The reduction of added sugars in traditionally sugar-rich foods, alongside fiber enrichment, has stimulated the use of alternative sweeteners. Maltitol is one such sweetener and is widely used due to its sucrose-like sweetness, low glycemic index and reduced calorie content (Ding et al., 2019; Kearsley & Deis, 2012; Livesey, 2012; Sahin et al., 2019). However, polyols can affect the key technological attributes of baked goods, such as texture, spread ability, moisture distribution and structural development, requiring adjustments to the formulation to maintain product quality (Sahin et al., 2018; Souza et al., 2022). Recent literature also highlights growing interest in combining maltitol with fiber- or polyphenol-rich flours to improve nutritional properties and glycemic response simultaneously (Di Cairano et al., 2022a; Livesey, 2003; Roze et al., 2021). Notably, interactions between maltitol and specific dietary fiber can cause effect on textural and sensory characteristics, emphasizing the importance of selecting suitable combinations of fiber and sweeteners (Marzec et al., 2025).

Consumer acceptance is a critical factor for the successful introduction of fiber- and polyphenol-enriched foods, especially given the potential sensory disadvantages associated with the incorporation of wine lees such as the perception of bitterness and astringency (Caponio et al., 2024b). Furthermore, despite numerous laboratory-scale studies having been conducted, evidence on the industrial scalability of such formulations remains limited. It is essential to consider process and plant scalability to ensure that the nutritional, technological, and sensory improvements observed on an industrial scale are maintained under real production conditions.

Thus, this study aimed to integrate sustainability, technological innovation, sensory perception, and industrial feasibility. Specifically, the study aimed to develop optimized biscuit formulations produced on a semi-industrial scale and enriched with wine lees. This was achieved by combining (i) nutritional improvement (an increase in fiber and phenolic compounds and a potential reduction in the glycemic index); (ii) the use of alternative sweeteners, such as maltitol; (iii) consumer acceptability, by mitigating the undesirable attributes of bitterness and astringency often associated with polyphenol-rich by-products; and (iv) an evaluation of the technological, nutritional, functional and sensory properties of the new products.

2. Materials and methods

2.1. Wine lees flour preparation

Wine lees used were obtained from different varieties of red grapes of *Vitis Vinifera* L. Puglia such as Primitivo, Negramaro, and Susumaniello and were supplied by the winery Tenuta Viglione located in Santeramo in Colle, Bari (Italy). The moisture of wine lees was reduced to values below 3% by freeze-drying (Buchi Lyovapor L-200, Switzerland). Subsequently the lees were crushed and, to recover the particles with diameters equal or smaller than 300 μm, they were sieved (Giuliani, Turin, Italy) in accordance with previous studies conducted on muffins, biscuits and pizza base enriched with grape pomace and wine lees (Difonzo et al., 2023; Troilo et al., 2022).

2.2. Ingredient optimization

Three biscuit formulations (B1, B2, B3) were developed as prototypes to determine the optimal ingredient balance between technological feasibility, nutritional improvement, and sensory quality. Rather than using a factorial experimental design, the formulation process followed a product development approach. The formulations were therefore progressively modified based on the results of preliminary laboratory tests, the findings of our previous study (Caponio et al., 2024b) and literature on the incorporation of polyphenol-rich by-products (Boff et al., 2022; Kausar et al., 2024; Mildner-Szkudlarz et al., 2013; Theagarajan et al., 2019; Naseem et al., 2024). Preliminary laboratory tests were conducted on the formulations, and the balance of ingredients was monitored in terms of sensory characteristics. The initial formulation (B1) was designed to maximize the incorporation of wine lees while maintaining workability. Because sensory evaluations conducted in our previous study (Caponio et al., 2024b) indicated that high levels of wine lees intensified bitterness, and astringency, the formulations in the present study were modified to enhance sensory balance while maintaining the nutritional benefits of wine lees. Accordingly, the proportion of wine lees was reduced progressively from 11.43% (B1) to 10.61% (B2) and then to 6.10% (B3) (Table 1). Although the reduction in wine lees content between B1 and B2 was small (<1%), preliminary sensory tests indicated that this reduction still reduced bitterness and astringency. Reducing B3 to 6.10% was intended to further improve the sensory quality while maintaining high fiber content. In addition, ingredients known for their flavour-modulating properties, such as cocoa and almonds, were added (Takano et al., 2019). Natural flavours, which are essential for modulating aroma perception and creating a more familiar sensory profile (Gaudette & Pickering, 2013) reducing the impact of wine lees bitterness and astringency, were also incorporated into the formulations. B1 and B2 were added with honey and orange and butter aroma, while B3 was added with almonds (9.18%), almond aroma (0.32%), and maltitol (19.27%). The target level of wine lees in the formulations was adjusted to achieve biscuits with a dietary fiber content higher than 6/100 g, allowing them to be labelled as "high fiber" in accordance with Regulation (EC) 1924/2006. The estimated fiber levels were calculated during the formulation process using the compositional data of the raw materials. Different sweetening systems were also evaluated. In formulations B1 and B2, sucrose was replaced with honey. In formulation B3, honey was replaced with maltitol to classify the biscuit as "with no added sugar". The amount of maltitol used was adjusted based on its relative sweetness compared to sucrose (approximately 0.8–0.9) (Ding et al., 2019; Young & O'Sullivan, 2011) in order to achieve a comparable level of sweetness while maintaining the workability of the dough. Additionally, the effects of modifying the flour

Table 1
Recipes of the biscuits produced in the pilot-plant.

Ingredients (%)	B1	B2	B3
Wheat flour	52.77	26.54	45.89
Wine lees flour	11.43	10.61	6.10
Cocoa	2.63	2.65	3.07
Sodium bicarbonate	1.05	1.06	1.37
Ammonium bicarbonate	0.26	0.53	0.23
Almonds	-	-	9.18
Honey	14.07	14.15	-
Corn starch	-	26.54	-
Maltitol	-	-	19.27
Sunflower oil	15.83	15.92	13.76
Salt	0.52	0.53	0.32
Vanillin aroma	0.17	0.17	0.45
Orange aroma	1.05	1.06	-
Butter aroma	0.17	0.17	-
Almond aroma	-	-	0.32

Abbreviations: B1: first biscuit trial; B2: second biscuit trial; B3: third biscuit trial.

base on dough structure were explored. In B2, part of the wheat flour was replaced with corn flour to modify the dough's viscosity and how much the biscuits spread during baking. As a result, prototype B2 was the lowest in wheat flour (26.54%) but was added with corn starch (26.54%).

2.3. Biscuit preparation

The production technology included the following steps: (i) mixing the dry ingredients, which included wheat flour, wine lees flour, cocoa, almonds, corn starch, maltitol, salt, and vanillin aroma. The dry ingredients were mixed for 1 min at speed 1 using mechanical kneading (Mfitaly, Marano Vicentino, Italy); (ii) the addition of liquid ingredients: firstly, sunflower oil, then orange, butter and almond aroma, and finally, ammonium and sodium bicarbonate; (iii) mechanical kneading process (Mfitaly, Marano Vicentino, Italy) at a speed of 1 for a period of 6 min, followed by a second kneading process at a speed of 2 for an additional 6 min; (iv) rolling and shaping the dough, setting the thickness to 5 mm using circular industrial molds with a diameter of 6 mm (Mfitaly, Marano Vicentino, Italy); (vi) baking in a ventilated electric oven (Mfitaly, Marano Vicentino, Italy) for 16 min at 160 °C.

The prototype with the best performance based on sensory analysis was selected for scale-up and then produced in a semi-industrial plant as described in Section 3.1 (B3s).

2.4. Sensory analysis

The prototypes were assessed by a panel using generic descriptive analysis. The group consisted of nine panelists selected belonging to University of Bari Aldo Moro and Laurieri S.r.l. A preparatory training phase was conducted to familiarize the panel with the product category (biscuits) followed by an evaluation phase. The training lasted one week (three sessions of one hour). A panel discussion was organized to generate the attributes through a tasting of commercial cocoa biscuits. At this purpose, typical sensory attributes of wine lees were selected based on sensory analysis performed on laboratory cookies enriched with wine lees (Caponio et al., 2024b). Consensus was obtained on ten sensory attributes that were then used in the evaluation sessions. The evaluation of three samples of biscuits named B1, B2, and B3 was carried out in three replicates under white light. The three replicates were evaluated on different days at the sensory laboratory of the Department of Food Science and Technology (University of Bari) designed according to ISO guidelines (ISO, 8589, 2007). The panelists were provided with one sample of each biscuit (weighing 5 *g*) for a total of three biscuits per tasting session. The administration of the samples was conducted in white plastic dishes, which were coded with the sample abbreviations (B1, B2, and B3). Following each tasting, a one-minute pause was observed, during which the tasters were required to consume water to cleanse their mouths. The order of presentation of the samples was randomized across participants and replicates. This was done to minimize potential interference due to the order of administration and carry-over effects (Macfie et al., 1989). Attributes were evaluated using a 10-point scale in the evaluation sessions. A visual attribute was assessed through the evaluation of the color of the snacks (0 = yellow; 9 = light brown). The odor attributes assessed included overall odor intensity and intensity of the typical odor of biscuits (0 = not perceived; 9 = very intense). The flavor attributes evaluated included sweetness, astringency, bitterness, off-taste intensity (in the mouth) and after-taste intensity using the following rating scale (0 = not perceived; 9 = very intense). Finally, the texture characteristics perceived during the tasting were assessed using the following parameters: hardness (0 = soft; 9 = very hard), and friability (0 = not perceived; 9 = very perceived). Sensory analysis was conducted following the ethical guidelines of the Food Science and Technology Laboratory of the Department of Soil, Plant and Food Science of the University of Bari.

2.5. Color and texture analyses

The one scaled-up semi-industrially produced (B3s) were submitted to instrumental analysis of the color and the texture. The color of the biscuits surfaces was measured using the CM-600d colorimeter (Konica Minolta, Tokyo, Japan) supported by Spectramagic NX software (Konica Minolta, Tokyo, Japan). The color assessment of the biscuit surface is based on consumers' perception of the product, which is influenced by the color of the surface (Clydesdale, 1991; Saadoudi et al., 2024; Uyanwa et al., 2024; Zucco et al., 2011). Therefore, several studies measured the color surface of biscuits (Benanti et al., 2023; Marzec et al., 2025; Serrem et al., 2011). The colorimetric indices measured were Lightness (L*), Redness (a*), and Yellow (b*). The biscuits were tested at three points: two in the distal part and one in the centre. The 3-point bending test was used to evaluate the texture parameters of cookies in accordance with Pasqualone et al. (2019) with some modifications. Hardness and brittleness were measured using a texture analyzer (Z1.0 TN, Zwick Gmbh & Co., Ulm, Germany) equipped with a load cell of 1 KN A distance between the bars of 4 cm and a probe speed of 5 mm/min were set. The downward movement of the probe was stopped when the biscuit was broken. The hardness (N) corresponded to the strength necessary to break the biscuit and brittleness (mm) is the distance crossed by the blade before biscuits broke (mm). The results were analyzed using the software TestXPertII version 3.41 (Zwick Roell, Ulm, Germany). The cutting test was carried out as described in Squeo et al. (2023) with some modification. The sample was placed on the base to be cut by a 50 mm-long blade on its short side, positioned 25 mm from the sample's surface. The blade descended at a speed of 1 mm/s until the sample was fully cut. The cutting stress (*i.e.*, the maximum force applied to the surface until complete cutting, N/mm^2) and the deformation at the cut (mm) were measured.

2.6. Proximate composition

Moisture of the scaled-up prototype (B3s) were determined using a thermobalance (Ladwag MAC 110/NP, Radwag, Wagi Elektroniczne, Poland) and pH-meter (inoLab pH 720; WTW, Weilheim, Germany) respectively. The official AOAC methods 979.09, 923.0 (2023), were used to determine the protein content (total nitrogen × 6.25) and ash, respectively. The lipid content was determined by the AOAC method 920.85 (2023) after Soxhlet extraction (SER 148 extraction system, Velp Scientifica srl, Usmate Velate, Italy) using ethyl ether (Merck KGaA, Darmstadt, Germany) as extraction solvent. The total dietary fiber of biscuit was determined by the enzymatic-gravimetric procedure according to the AOAC method 985.29 (2023). Total carbohydrates (%) were calculated by difference as follows: 100 - (moisture %, protein %, lipids %, ash %, total dietary fiber %).

2.7. Total phenol content and antioxidant activity

The extraction of polyphenols from the scaled-up prototype (B3s) was carried out as described by Difonzo et al. (2023); Troilo et al. (2022) with some modifications. An amount of 10 mL of a methanol/water mixture (80:20 v/v) was added to 3 g of sample. Subsequently samples were stirring for 10 min, sonicated (Ultrasonic cleaner CP104, EIA, Arezzo) at 50 Hz for 15 min at room temperature and stirred for 30 min. Finally, samples were centrifugated at 12,000 *g* for 15 min at 4 °C (Thermo Scientific Massachusetts, USA).

Afterward, the pellets underwent two additional extractions, and the resulting supernatants were recovered and filtered through nylon filters (pore diameter 0.45 μm, Sigma, Ireland) into vials. This process was repeated three times in total. The Folin-Ciocalteu method was used to determine the total polyphenol content as reported by Difonzo et al. (2017). To 20 μL of the sample, within the cuvettes, 980 μL of deionized water and 100 μL of Folin-Ciocalteu reagent were added. After 3 min of incubation, 800 μL of Na_2CO_3 to 7.5% were added and stored at room temperature in the dark for 60 min. Absorbance was read at 720 nm using Cary 60 spectrophotometer (Agilent, Cernusco, Milan). Results were expressed as mg of gallic acid equivalents (GAE)/g of biscuits. The antioxidant activity of extracts was evaluated by ABTS (2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) and DPPH (2,2-diphenil-1-picrylhydrazyl) tests as reported by Noviello et al. (2022). Spectrophotometric readings were performed using a Cary 60 spectrophotometer (Agilent, Cernusco, Milan).

The ABTS test was conducted by placing within cuvettes suitable for spectrophotometry 50 μL of sample and 950 μL of final solution of ABTS. After 8 min of incubation in the dark, the absorbance was read at 734 nm. The DPPH test involves the preparation of a 0.08 mm DPPH solution and subsequently, in the spectrophotometry cuvettes, 50 μL of sample was added to 950 μL of DPPH solution. After 30 min of incubation in the dark, the absorbance was read at 517 nm. The results of ABTS and DPPH tests were expressed as μmol Trolox equivalents (TE)/g of biscuits.

2.8. *In vitro starch hydrolysis*

In vitro starch hydrolysis of the scaled-up prototype (B3s) was determined according to Caponio et al. (2024a) simulating the *in vivo* digestion of starch. Briefly, 1 g of samples were subjected to *in vitro* enzymatic digestion to simulate starch hydrolysis. During this process, starch was progressively hydrolyzed and the released glucose was measured using the D-Glucose Assay Kit (GOPOD Format) (Megazyme, Wicklow, Ireland). Starch was calculated as glucose (mg) × 0.9 (the conversion factor). Simulated digests were dialyzed (cut-off of the membrane: 12,400 Da) for 16 h. Aliquots of dialysate, containing free glucose, and partially hydrolyzed starch were sampled after 3 h and at the end of the digestion, after 16 h. These were further treated with amyloglucosidase. Then, free glucose was determined using the above-mentioned enzyme-based kit and finally converted into hydrolyzed (digested) starch in the biscuits. Control white wheat bread was used as the control to estimate the hydrolysis index (HI = 100). The predicted glycemic index (pGI) was calculated using the equation pGI = 0.549 × HI + 39.71 as described by Caponio et al. (2022).

2.9. *Simulated colonic fermentation*

Before proceeding with the colonic fermentation *in vitro*, retentates from dialysis were used to constitute the fecal media. By following previous standardized procedures (Vacca et al., 2021), K_2HPO_4 2 g/L, $C_2H_3NaO_2$ 5 g/L, $C_6H_{17}N_3O_7$ 2 g/L, $MgSO_4$ 0.2 g/L, $MnSO_4$ 0.05 g/L, glucose 2 g/L, inulin 4 g/L, fructo-oligosaccharides 4 g/L, Tween 80 polysorbate 1 mL/L were homogenized in distilled-water before to be sterilized (121 °C for 15 min), while retentates were cold-sterilized with filters (0.22 μm). Into the final fecal medium formulations, biscuits were present at the 2.5% (w/v). The fecal slurry was obtained as previously detailed (Vacca et al., 2023). Fresh feces were provided by a healthy volunteer who had no antibiotics or probiotics treatments in the last 3 months before sample delivery. After collection in sterile tubes filled until 4/5 of the total volume, feces were processed within 1 h from sampling in bags with filter (250 μm) added with distilled water (in a final ratio of 32% w/v), homogenized in a lab stomacher for 3 min, centrifuged (8000 × *g*, 20 min, 4 °C). Pellets were recovered while supernatants were replaced by an equal volume of fecal medium to constitute the fecal batch. Fecal batches were incubated anaerobically at 37 °C, for 42 h, under gentle stirring conditions (150 rpm).

2.10. *Viable fecal microbiota profiling*

At the end of the fecal batch incubation time, viable fecal microbiota was profiled by plate-counts. After serial 10-fold dilutions in sterile saline (NaCl 0.9%) solution, to inspect the viable microbiota, Plate Count Agar (PCA), Wilkins-Chalgren anaerobe agar (WCAn), de Man, Rogosa and Sharpe (MRS) agar, M17 agar, Violet Red Bile Glucose Agar (VRBGA), and modified Bifidobacterium agar (mBifA) were used as culture media for total aerobes microbial (TAMC), total anaerobes microbial (TANMC), lactic acid bacteria (LAB – bacilli), LAB (cocci), Enterobacteriaceae, and fecal *Bifidobacterium* counts, respectively. Exception made for mBifA, which was purchased by Becton Dickinson GmbH (Heidelberg; Germany), all other media were purchased by Oxoid Ltd. (Basingstoke, Hampshire, England). WCAn and mBifA were anaerobically incubated, while other media were incubated aerobically. Time and temperature of incubation followed those defined by the related manufacturers.

2.11. *Consumer test*

Liking for the scaled-up prototype (B3s) was measured with 170 consumers to evaluate if incorporating wine lees at a percentage of 6.1% in a biscuit without added extra sugar could meet consumer acceptability in terms of color, smell, texture, taste and overall pleasantness. Prior to participation, all subjects were required to sign an informed consent form in accordance with ethical research standards. In addition, they were provided with a list of ingredients used, in compliance with transparency and allergen awareness guidelines. Liking was measured with a hedonic scale ranging from “dislike extremely” to “like extremely” for color, odor, texture, taste and overall evaluation (Peryam & Pilgrim, 1957). The test was administered to a convenience sample of students, teachers, and university staff at the University of Bari Aldo Moro (Pagliarini, 2021; Porretta, 2000). The gender distribution of survey participants was characterized with a slight predominance of female respondents (54%) over male respondents (46%), likely reflecting the higher proportion of women in Italy (Istat, 2022).

2.12. *Statistical analysis*

Sensory data were analyzed through a two-way ANOVA model, with panelists (as random factor), samples, and their interaction as factors, followed by Tukey test for multiple comparisons. Texture instrumental data were analyzed applying a one-way ANOVA (factor: sample) followed by Tukey's multiple comparison test. Consumer liking data were analyzed to test the effect of gender (men, women) and age group (18-over 50) through two-tailed Student's *t*-test and a one-way ANOVA model. The results of proximate composition, total phenolic compounds, antioxidant activity, color, texture analysis, pGI, viable fecal microbiota profiling were expressed as the mean ± standard deviation (SD). The statistical analysis was performed using Minitab 17 (Minitab Inc., State College, PA, USA), GraphPad Prism 10.3.1 (Boston, MA 02,110) statistical software and PanelCheck and Xlstat (Lumivero) for the sensory analysis.

3. Results and discussion

3.1. *Sensory analysis*

To select the best formulation for further scaling-up, a sensory evaluation was conducted. Table 2 reports the results of the sensory analysis of semi-industrial experimental biscuits. A significant effect of product was found on all the attributes ($p < 0.0001$), with the exception of overall odor and after-taste intensity (Table 2). Although the difference in wine lees concentration between B1 and B2 was only 0.8%, the high pigment content of the wine lees probably explains the significant differences in the color intensity. The darker brown color is due to the presence of anthocyanins in wine lees, which impart a characteristic purple hue. Similar findings have been reported by Bianchi et al. (2023) and Caponio et al. (2024b), in studies on biscuits and muffins enriched with wine lees flour.

No significant difference among the formulations was found in overall odor intensity (that was evaluated around 7), while B2 was characterized by a higher typical odor of biscuit compared to B3,

Table 2
Sensory analysis of the biscuits.

Attributes (intensity)	B1	B2	B3	p-value
Color (yellow/light brown)	8.89 ± 0.19 [a]	8.00 ± 0.33 [b]	6.44 ± 0.38 [c]	<0.0001
Overall odor	7.33 ± 0.88	7.44 ± 0.69	7.56 ± 0.51	0.561
Typical smell of biscuits	6.56 ± 0.77 [ab]	6.33 ± 0.58 [b]	6.89 ± 0.51 [a]	<0.0001
Sweetness	3.11 ± 0.19 [c]	4.22 ± 0.51 [a]	3.67 ± 0.67 [b]	<0.0001
Astringency	3.11± 0.51 [a]	3.22 ± 0.51 [a]	2.44 ± 0.19 [b]	<0.0001
Bitterness	2.11 ± 0.19 [b]	3.11 ± 0.19 [a]	2.06 ± 0.20 [b]	<0.0001
Off-taste	1.89 ± 0.19 [a]	0 [b]	0 [b]	<0.0001
After-taste	4.67 ± 0.33	4.67 ± 0.33	5.00 ± 0.58	0.138
Friability	3.67 ± 0.33 [c]	5.67 ± 0.33 [a]	5.00 ± 0.58 [b]	<0.0001

Data are represented as means ± SD. Different letters indicate a significant difference ($p \leq 0.05$) among samples by one-way ANOVA followed by Tukey's multiple comparison test. Abbreviations: B1: first biscuit trial B2: second biscuit trial; B3: third biscuit trial.

probably for its butter aroma. B2 was found to be sweeter than B3, that was in turn sweeter than B1. B2 was also found to be more bitter than the other two and more astringent than B3, probably due to the higher presence of wine lees, in line with previous studies that showed that oenological by-products, including wine lees and grape pomace, increase the astringency and bitterness perception in products such as muffins, biscuits, and ice cream (Bianchi et al., 2023; Caponio et al., 2024b; Tsevdou et al., 2019). Off-taste intensity was noted exclusively in B1 with a recorded score of 1.89 ± 0.19, probably due to the higher amount of wine lees in this formulation. Similar results were also observed by Bianchi et al. (2023) and Caponio et al. (2024b) adding wine lees in muffin and biscuits, respectively. This may have impacted its evaluation: in fact, while both B1 and B2 have honey, vanillin and orange aroma in the same proportion, B2 was found to be sweeter. Panelists also reported higher friability in B2, followed by B3 and B1.

Based on the results of the sensory evaluation, formulation B3 was selected for scale-up production. This formulation demonstrated the lowest perceived bitterness and astringency, as well as an acceptable level of sweetness – attributable to the use of maltitol and the balanced composition of the ingredients – and desirable friability characteristics.

3.2. Optimization and scalability of the biscuit production

Scaling up from laboratory to semi-industrial manufacturing is a complex process that requires careful management of ingredients, equipment, and production timelines to maintain product quality. The transition of wine lees-enriched biscuits from small-scale production to an industrial setting presented several challenges, including recipe reformulation, adaptation to large-scale production systems, and optimization of operational parameters (Fig. 1).

In laboratory-scale production, small ingredient quantities allow for greater flexibility in preparation. However, at a semi-industrial scale, larger dough volumes were required to ensure compatibility with production equipment. Additionally, unlike the laboratory-scale process (Caponio et al., 2024b), which did not include ingredients to enhance sensory properties, the semi-industrial-scale formulations incorporated cocoa, flavorings, and dried fruit to improve sensory characteristics.

The ingredients used to produce the scaled-up biscuits were as follows: wheat flour (45.89 g), wine lees flour (6.10 g), cocoa (3.07 g), sodium bicarbonate (1.37 g), ammonium bicarbonate (0.23 g), almonds (9.18 g), maltitol (19.27 g), sunflower oil (13.76 g), salt (0.32 g), vanillin aroma (0.45 g), almond aroma (0.32 g).

Scaling up production necessitated the use of specialized equipment and machinery, requiring adjustments to operating conditions compared to artisanal laboratory production. In particular, mixing large-scale doughs requires consistent homogeneity to prevent lumps or ingredient separation. To achieve this, a professional mixer with adjustable speed was used, kneading the dough for six minutes at speed 1 to ensure uniformity, followed by six minutes at speed 2 to optimize structure.

During the rolling stage, an industrial roll mill replaced the domestic roller used in the laboratory. Additionally, the biscuit thickness was increased by 1 mm (total 5 mm) compared to the 4 mm laboratory formulation to improve structural integrity. The forming phase was also adapted, using professional circular molds to achieve a uniform shape. Finally, the baking process was transferred to an industrial oven while maintaining the same time and temperature settings. The final

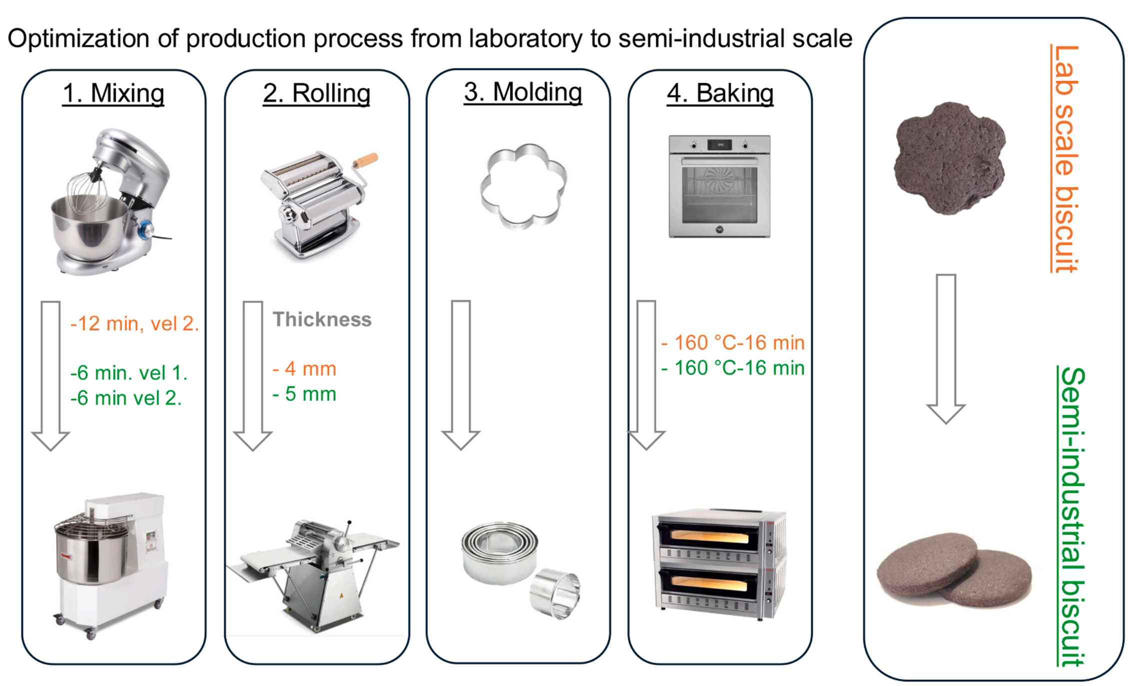

Fig. 1. Optimization of production process from laboratory to semi-industrial scale.

production yield was approximately 90%.

Overall, the scale-up process confirmed the technological feasibility of producing wine lees flour-enriched biscuits under semi-industrial conditions. The incorporation of new ingredients enhanced the biscuits' sensory profile, while optimized production parameters ensured scalability. However, achieving a balance between innovation and industrial feasibility was essential, considering the impact of new ingredients on dough workability, baking, and final product structure. These optimizations resulted in a biscuit that retains the functional benefits of wine lees and is more appealing and suitable for large-scale production. The effectiveness of the scale-up process was evaluated based on the sensory attributes and textural characteristics of the biscuits, which served as indicators of product quality and process suitability.

3.3. Chemical and nutritional characterization of selected biscuits

The results of proximate composition, antioxidant activity, and total phenol content of the selected sample (B3s) are shown in Table 3. The moisture content was 6.06 g/100 g, slightly higher than the typical humidity of biscuits, which should be <5% (Manley, 2000). The water activity of the biscuits was 0.47, which are generally ≤ 0.6, according to Arepally et al. (2023), Schmidt et al. (2020) and Sławińska et al. (2024). This ensures a mold-free shelf life of several months (Manley, 2000). The protein content of 9.00 g/100 g was similar to findings by Caponio et al. (2024b) in biscuits fortified with wine lees flour, where the protein increase compared to the control was attributed to the addition of wine lees flour (Delgado de la Torre et al., 2015). The lipid content was equal to 14.31 g/100 g. Of note, the ingredient formulation was modulated to reach the nutritional claim "high fiber content" in accordance with Regulation (EC) 1924/2006 (European Commission, 2006), as the fiber content in B3s was 9.35 g/100 g. The ashes content, of about 2.64 g/100 g, revealed the high mineral content of biscuits (Maner et al., 2017; Sancho-Galán et al., 2020). The antioxidant activity, measured by the ABTS and DPPH assays, was 15.21 μmol TE/g and 7.90 μmol TE/g respectively. Additionally, the total phenolic content observed was 1.46 mg GAE/g. Notably, the phenolic compounds and the antioxidant activity were primarily provided by wine lees and cocoa, both of which represent an excellent source of phenolic compounds with antioxidant activity (Kongor et al., 2016; Sharma et al., 2023). Polyphenols are widely recognized for their beneficial effects on human health, including their protective effects of polyphenols against chronic diseases and metabolic disorders (Ganesan & Xu, 2017; Grosso et al., 2017; Li et al., 2022; Soares et al., 2017; Zhang et al., 2023). They exhibit several biological properties including anti-inflammatory, antiviral, anticancer, antihypertensive, antimicrobial, antiaging effects, as well the prevention of low-density lipoproteins oxidation Cabrera-Bañegil et al., 2017; Tedesco et al., 2000). These health benefits are largely attributed to their ability to modulate gene expression, support lipidic metabolism, and positively modulate gut microbiota compositions, particularly in case of polyphenol derived from grapes (Lippolis et al., 2023; Martínez-Huélamo et al., 2017). Additionally, polyphenols exhibit strong antioxidant and anti-inflammatory properties by neutralizing reactive oxygen species, chelating metal ions, and inhibiting the oxidation of essential biomolecules such as DNA, LDL, and proteins, thus protecting cells from oxidative stress (Rudrapal et al., 2022). Overall, incorporating wine lees significantly improved the phenolic profile of the final product, suggesting its potential as a functional ingredient in baked goods. Notably, the results suggest that polyphenolic compounds and antioxidant activity are preserved even during large-scale industrial production. This highlights the technological feasibility of incorporating wine lees into industrial biscuit formulations without substantially compromising their bioactive properties.

Table 3
Proximal composition, antioxidant activity, total phenol content, HI, and pGI of the biscuit made with maltitol and wine lees flour (B3s).

Parameters	B3s
Moisture (%)	6.06 ± 0.15
a_w	0.47 ± 0.02
Protein (%)	9.00 ± 0.15
Lipid (%)	14.31 ± 0.38
Carbohydrates (%)	58.64 ± 0.08
Fiber (%)	9.35 ± 0.05
Ash (%)	2.64 ± 0.20
ABTS (μmol TE/g)	15.21 ± 0.30
DPPH (μmol TE/g)	7.90 ± 1.16
TPC (mg GAE/g)	1.46 ± 0.05
HI (%)	22.65±3.59
pGI (%)	52.15±1.97

Data are represented as means ± SD of three lots of biscuits.
Abbreviations: ABTS, 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid); DPPH, 2,2-diphenil-1-picrylhydrazyl; HI, hydrolysis index; pGI, predicted glycemic index; TPC, total phenol content.

The hydrolysis index (HI) and the predicted glycemic index (pGI) were calculated by simulating the process of digestion through the gastrointestinal tract. The HI was 22.65 ± 3.59, and pGI, calculated from the HI, was 52.15 ± 1.97% (Table 3). The inclusion of wine lees flour as an ingredient significantly may contribute to reducing HI and pGI values, due to the beneficial effect of the fibers, which slow down digestion and absorption and decrease the insulin response (Reynolds et al., 2020; Riccardi et al., 2008). Additionally, fibers can interfere with digestive enzymes, reducing the rate at which carbohydrates are broken down into simple sugars, which are quickly absorbed by the intestine. According to the International Standards Organization (ISO), which classifies foods into high-GI (GI ≥70), medium-GI (GI 56–69), and low-GI (GI ≤55) categories (Standard ISO, 26642:2010), the produced biscuits had pGI values that classify them as low-GI. Furthermore, compared to the results of our recent study (Caponio et al., 2024b), the formulation developed in the present study exhibited a lower glycemic index with a higher fiber content.

3.4. Viable fecal microbiota profiling after simulated colonic fermentation

The viable fecal microbiota profiling conducted after 42 h of simulated colonic fermentation revealed significant changes in bacterial densities following exposure to innovative wine lees flour-containing biscuits (Fig. 2). This investigation was prompted by previous studies highlighting the multifaceted roles of polyphenols in modulating gut microbiota composition and activity (Rodríguez-Daza et al., 2021; Wang

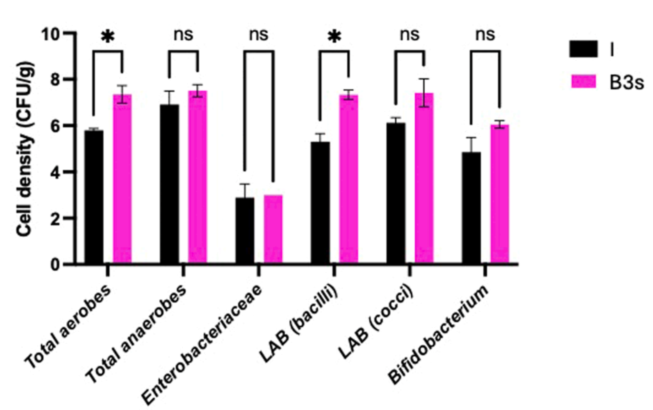

Fig. 2. Viable bacterial counts (Log10 CFU/g) observed in digested B3s biscuits under simulated colonic conditions. The I-sample represents the cell density used as the initial inoculum. On stacked bars, the asterisk (*) represents a significant difference ($p < 0.05$; multiple t-test) compared to the inoculum cell density, while ns means not significant.

et al., 2022). These compounds can act as prebiotics, promoting the growth of beneficial bacteria, such as *Bifidobacterium* and *Lactobacillus*, enhancing short-chain fatty acid (SCFA) production, and inhibiting pathogenic bacterial proliferation (Rodríguez-Daza et al., 2021; Wang et al., 2022).

In the present study, the fermentation substrates were derived from *in vitro* starch hydrolysis, resulting in lower sugar bioavailability compared to conventional fecal media enriched with carbohydrate derivatives. This approach allowed for a more targeted evaluation of the prebiotic potential of polyphenols, independent of other undigested carbohydrates. Despite the limited carbohydrate content, 42 h of fermentation resulted in a significant increase in total aerobes and bacillus-shaped lactic acid bacteria (LAB). Although not statistically significant, slight increases were also observed for coccus-shaped LAB and *Bifidobacterium*, consistent with previous findings that reported enhanced LAB and *Bifidobacterium* growth in the presence of ellagic acid and glycosylated ellagic acid substrates (Li et al., 2015).

These results align with established evidence regarding the metabolic fate of polyphenols, which are not absorbed in the upper gastrointestinal tract but instead reach the colon largely intact, where they undergo microbial degradation into bioactive metabolites with potential health benefits (Nemzer et al., 2025; Wang et al., 2022). The antimicrobial activity observed in the present study further supports the proposed prebiotic role of wine lees flour-based biscuits. Notably, the inhibitory effect on *Enterobacteriaceae* proliferation aligns with findings from quercetin-based exposure in murine models, where a reduction in *Escherichia coli* and *Proteobacteria* abundance was reported (Ju et al., 2018). Although *Enterobacteriaceae* belong to the core gut microbiota since early life (Vacca et al., 2022) and, therefore, these cannot be completely depleted, the observed bacteriostatic effect is particularly relevant given the association between *Enterobacteriaceae* overgrowth and various gastrointestinal and systemic disorders (Khorsand et al., 2022; Sharabi & Rezaie, 2024).

Moreover, the current findings are consistent with previous research on wine lees flour-based biscuits (Caponio et al., 2024b), where the highest polyphenol concentration (F20 formulation) exhibited a suppressive effect on *Enterobacteriaceae* cell density, while lower wine lees flour concentrations (previous F0 and F10 formulations) were associated with increased *Enterobacteriaceae* density. This outcome highlights the concentration-dependent nature of polyphenol–microbiota interactions, as polyphenols can either inhibit or stimulate LAB and *Bifidobacterium* growth depending on their concentration and composition (Gwiazdowska et al., 2015; Wang et al., 2022).

Overall, the results from the *in vitro* fermentation experiments suggest that B3s consumption could provide promising health benefits by promoting beneficial bacterial growth while limiting the proliferation of potentially harmful bacteria.

3.5. Color and texture analysis

Table 4 presents the results of color analysis of the scale-up biscuit (B3s), expressed in terms of lightness L*, redness a*, yellowness b* with indices equal to 22.77, 2.70, and 4.05, respectively. The observed data were lower comparing to the values collected by Di Cairano et al. (2022b) and Caponio et al. (2024b) in biscuit prepared with maltitol and wine lees flour, respectively. This discrepancy is likely attributed to differences in ingredient composition and formulation.

Table 4
Colorimetric and texture index of biscuits made with maltitol and wine lees flour.

Parameters	B3s
Colorimetric indices	
L*	27.77 ± 0.64
a*	2.70 ± 0.21
b*	4.05 ± 0.40
Texture indices	
Hardness (N)	40.73 ± 0.32
Brittleness (mm)	0.94 ± 0.01

Data are represented as means ± SD of three lots of biscuits.

Textural parameters, including hardness and brittleness, were evaluated using texture profile analysis (TPA), a fundamental method for assessing the quality of baked goods (Cauvain, 2016). The hardness of the biscuits was measured at 40.73 N (Table 4), which is comparable to the findings of Sahin et al. (2019) and Roze et al. (2021), who reported a hardness of 29.00 N for biscuits formulated with maltitol and sorbitol as sugar substitutes.

3.6. Consumer test

Consumer test is an essential tool in new product development, as it provides valuable insights into consumer acceptability and preferences (Yang & Lee, 2018). The literature suggests that for functional biscuits to be accepted, they should primarily satisfy sensory attributes such as taste, texture, aroma, aftertaste, and overall acceptability (Bora et al., 2019; Zarroug et al., 2021). Fig. 3 presents a box plot illustrating the distribution of the acceptability ratings for the scaled-up biscuit (B3s), focusing on four key sensory attributes, color, odor, taste, and texture, and overall liking. All attributes show values between 6.5 and 7.5, suggesting an overall favorable perception by consumers. Among these, liking of color and odor exhibit relatively narrow interquartile ranges and/or limited whisker extension, indicating consistent ratings and lower variability among panelists. These results suggest that the liking for visual and olfactory characteristics of the product was relatively homogeneous across consumers. By contrast, liking for taste and especially texture display greater variability, as indicated by wider interquartile ranges and/or the presence of lower extreme values (down to approximately 2 in the case of texture).

Mean overall liking and liking for color, taste and texture was on average between 6.62 and 6.76 (color=6.62, taste=6.74, odor=6.78, texture=6.76, overall=6.64), falling between “like slightly” and “like moderately” on the 9-point hedonic scale. This indicates that the biscuit is on average accepted.

Color is a key parameter in determining food acceptability, as it is associated with freshness, ripeness, variety, and desirability (Iqbal et al., 2010; McCaig, 2002; Pallottino et al., 2010). The use of maltitol as a sugar substitute and wine lees may have resulted in biscuits perceived as too few sweet. To better align with consumer preferences, a slightly higher level of sweetness may be required, as sweetness remains one of the most globally appreciated flavors, innately preferred over sour and bitter tastes (Kim et al., 2017). These findings align with those reported by Davidov-Pardo et al. (2012) and Roze et al. (2021) in studies on biscuits incorporating grape marc and maltitol, respectively. Adding different aromas that enhance sweetness (*e.g.* vanilla) may also give positive results through odor-taste integration. The average liking score for odor suggests that the wine lees did not negatively impact the typical aroma of the biscuits, likely due to the addition of almonds, flavorings, and cocoa, which helped mask any undesirable odors from the wine lees. These findings offer practical guidance for optimizing wine lees-enriched bakery products, showing that strategic ingredient selection can improve consumer acceptance while preserving health benefits. However, the greater variability in taste and texture scores suggests the need for further refinement to ensure consistency and broader appeal.

Fig. 4 illustrates the age distribution of participants in the consumer test. Respondents (54% females) were aged 18–24 years (36%), 25–30 years (39%), 31–over 50 years (25%). The consumer test was conducted at the University of Bari Aldo Moro, which explains the higher representation of respondents aged 18–30 years.

No significant differences were observed in liking for the specific dimensions and overall, between female and male participants, except for odor that was more appreciated by females (p = 0.0174). Overall, liking, and liking for odor and taste, exhibited significant differences

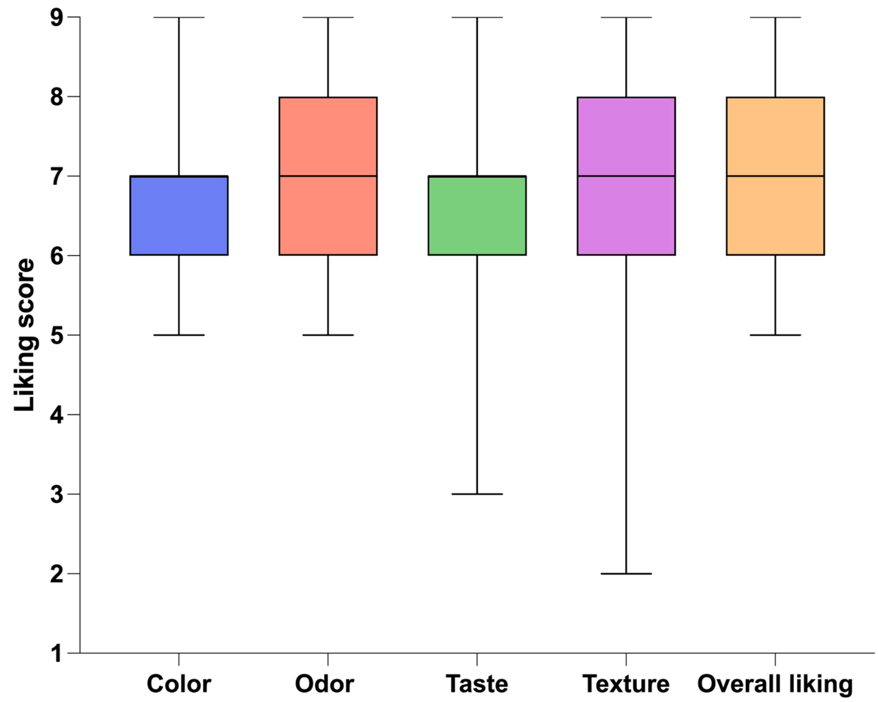

Fig. 3. Box plot of distribution of liking score for specific dimensions of the tested product.

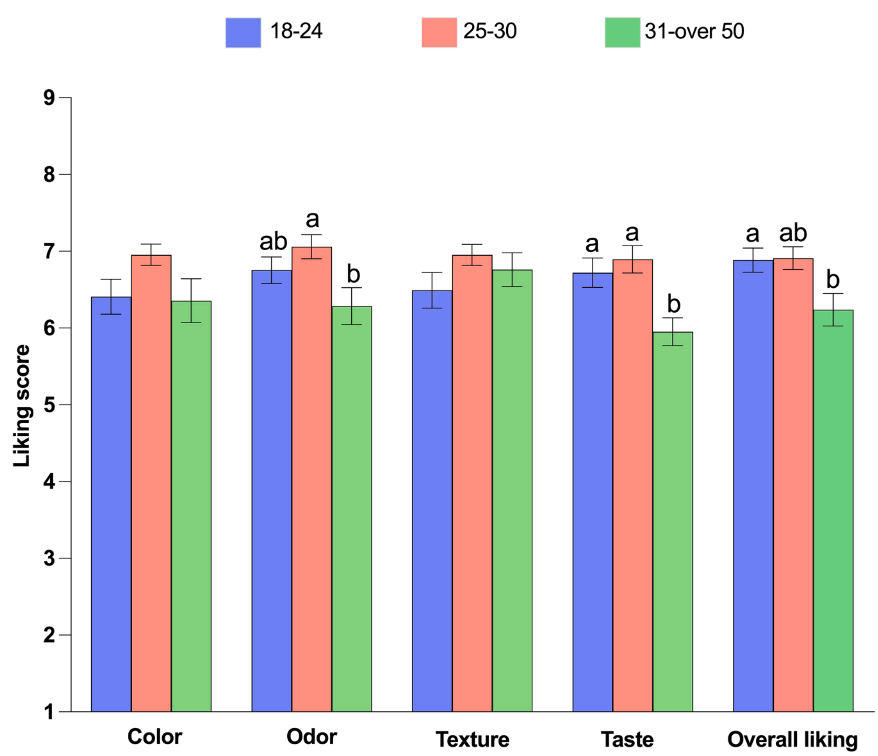

Fig. 4. Mean liking ratings by age groups (18–24, 25–30, over 31). Data are represented as means ± SEM. Different letters indicate a significant difference ($p \leq 0.05$) according to Tukey's multiple comparison test.

between age groups ($p = 0.0153$, $p = 0.0170$, $p = 0.0026$, respectively). In particular, the group aged over 31 expressed lower overall liking, and lower liking for the odor and taste compared to the other age groups (Fig. 4). This finding aligns with existing literature, which highlights age as a key demographic factor influencing consumer behavior (Cooil et al., 2007; Fisher & Dubé, 2005; Lambert-Pandraud & Laurent, 2010; Meyers-Levy, 1988; Meyers-Levy & Maheswaran, 1991; Reynolds et al., 1977). Respondents aged 18–30 years reported the highest liking for the biscuits.

Notably, previous research has identified a consumer segment known as "emerging young adults," typically aged 18–30 years, who are particularly receptive to innovative and functional foods (Arnett & Wittmann, 2014; Carrillo et al., 2013; Jezewska-Zychowicz, 2009; Tuorila et al., 2001). This tendency may be attributed to their heightened awareness of health and physical well-being (Nelson et al., 2008). Moreover, younger consumers, being more familiar with this category of products, are likely already accustomed to flavors that differ from conventional ones, making them more inclined to appreciate such innovations. The higher appreciation observed among individuals aged 18–30 years may also be linked to their greater sensitivity and awareness of environmental sustainability. In fact, several studies have reported a higher acceptance of sustainable food products among younger consumers compared to older ones (Berghoef & Dodds, 2011; Brécard et al., 2009; Cholette et al., 2013; Finisterra do Paço & Barata Raposo, 2010; Yu & Porth, 2013; Zhu et al., 2013). The environmental consciousness of younger generations may stem from their awareness of the potential negative impacts on the planet (Wiernik et al., 2013). Additionally, Perito et al. (2019) identified a positive correlation between sustainable consumption and a high willingness to accept such products. Therefore, the 19–30 age group represents a promising target market for innovative functional biscuits (Markovina et al., 2011).

4. Conclusions

The present study successfully optimized a high-fiber, low-glycemic-index biscuit formulation enriched with wine lees and assessed its feasibility on a semi-industrial scale. A sensory evaluation showed that consumers positively accepted the B3s formulation, giving it scores between 6 and 7 (liked slightly"/"liked moderately) for all attributes, including color, flavor, odor, texture, and overall pleasantness. Demographic analysis revealed that individuals aged 18–30 were more inclined to accept the innovative functional biscuits, with no significant differences by gender. This may be attributed to their heightened awareness of environmental issues and greater familiarity with such products. Consumer research suggests that clearly labelling functional ingredients and their associated health benefits could enhance acceptance further (Baker et al., 2022; González-Díaz et al., 2020; Lusk & Marette, 2010; Markosyan et al., 2009; Verneau et al., 2019). Marketing strategies also play a crucial role in introducing innovative products, as demonstrated by the organic food market in Europe, where public and private initiatives have been key drivers of consumer adoption (Haas et al., 2010). Similar approaches could help increase acceptance among older consumers, who tend to be more cautious about innovative formulations. Overall, the results demonstrate that incorporating wine lees into maltitol-sweetened cookies is a viable strategy for producing products that are both nutritionally enhanced and accepted by consumers, while supporting the principles of the circular economy and industrial scalability.

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Ethical statement

For the sensory evaluation, no human ethics committee or formal documentation process is available, but the appropriate protocols for protecting the rights and privacy of all participants were utilized during the execution of the research. Participants gave informed consent via the statement "I am aware that my responses are confidential, and I agree to participate in this survey" where an affirmative reply was required to enter the survey. They were able to withdraw from the survey at any time without giving a reason. The products tested were safe for consumption."

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CRediT authorship contribution statement

Giusy Rita Caponio: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Roberta Miolla:** Writing – review & editing, Investigation, Formal analysis. **Mirco Vacca:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Maria De Angelis:** Writing – review & editing, Funding acquisition. **Sara Spinelli:** Writing – review & editing, Writing – original draft, Methodology, Data curation. **Graziana Difonzo:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare no conflicts of interest.

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Data availability

Data will be made available on request.

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