

Formulation-dependent Effects of Hemp Seed By-products on Batter Properties, Structure, and Check-All-That-Apply -derived Consumer Response of Gluten-free Muffins

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ABSTRACT

The valorisation of hemp seed oil by-products offers a promising strategy to enhance the nutritional and technological quality of gluten-free baked goods. This study evaluated the incorporation of hemp seed cake flour (HSCF) and hemp seed protein concentrate (HSPC) at 15%, 20%, and 30% substitution levels in gluten-free muffins, assessing effects on batter properties, muffin structure, composition, colour, texture, and sensory perception. Compared with the control, HSCF slightly increased batter texture parameters and, at 30%, reduced density (0.886 vs 0.927 g/mL) while increasing volume (90.6 vs 80.9 mL) and specific volume (2.3 vs 2.0 mL/g). HSPC produced stronger increases in batter texture parameters and density (0.962–1.028 g/mL), yielding the lowest volume (77.0 mL) and specific volume (1.9 mL/g) at 30% due to matrix strengthening. Fortification progressively improved nutritional quality: protein rose from 5.4 g/100 g (control) to 9.1 g/100 g in 30% HSPC muffins, and fibre increased from 0.2 to 5.2 g/100 g in 30% HSCF samples, enabling “source of fibre” labelling at 20–30% HSCF. Colour shifted towards darker, duller reddish brown hues, with ΔE up to 21.84 in 20% HSPC crust and 32.50 in 30% HSCF crumb. Muffin texture remained largely unaffected at $\leq 20\%$ substitution. Sensory analysis revealed a gradual transition from cake-like to hemp-characteristic profiles, with bitterness, herbaceous notes, dark colour, dryness, grittiness, and crumbliness driving disliking. Overall, moderate fortification with hemp by-products provides an optimal balance of nutritional enhancement, structural performance, and sensory quality, supporting their potential for sustainable gluten-free product development.

1. Introduction

The production of hemp (*Cannabis sativa* subsp. *sativa*) seed oil has expanded markedly over the past decade, driven by its growing use across food, cosmetic, nutraceutical, and pharmaceutical sectors and by rising consumer awareness of its health-promoting properties (Future Market Insight, 2026). Cold pressing generates large amounts of by-products - mainly hemp seed cake, which can represent up to 85% of the seed - along with meal, protein concentrates, and hulls (Burton et al., 2022). Hemp seed by-products exhibit considerable potential as protein-rich food ingredients of high biological value, owing to their notable levels of arginine and essential amino acids - often exceeding those of soybean - and their suitability for individuals with food allergies

or coeliac disease (Xu et al., 2021). Moreover, hemp seed cake provides minerals, bioactive compounds, and polyunsaturated fatty acids with an optimal $\omega 6/\omega 3$ ratio for human metabolism, and, when derived from unhulled seeds, it is also a valuable source of crude fibre.

Gluten-free (GF) foods are defined as products formulated without gluten-containing ingredients or processed to contain < 20 ppm gluten (U.S. FDA; EC No 41/2009). They are essential for individuals with coeliac disease, an autoimmune inflammatory condition affecting the upper small intestine (Green & Cellier, 2007), for whom a lifelong GF diet remains the only effective treatment.

Commercial GF cereal products often show poor nutritional and sensory quality, being typically high in fat and carbohydrates and, unless fortified, low in essential nutrients. The absence of gluten also leads

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to weak structure that compromises sensory attributes (Rai et al., 2018; Kumar, 2018; Moreno et al., 2014; Matos et al., 2015). To overcome these issues, various technological approaches have been investigated, such as the use of alternative structuring ingredients or flour modification through heat treatment or extrusion, to better mimic gluten's viscoelasticity and enhance product quality (Marston et al., 2016; Das & Bhattacharya, 2019; Hussein et al., 2023; Hussein et al., 2025; Mostafa, et al., 2025).

Within this framework, the valorisation of hemp seed oil by-products represents a promising approach for enhancing the nutritional and technological performance of GF baked goods. This study examines the use of hemp seed cake flour (HSCF) and hemp seed protein concentrate (HSPC) in GF muffins, testing different rice-flour substitution levels to identify the optimal inclusion rate that enhances nutritional, textural, and sensory attributes while preserving consumer acceptability.

To date, these hemp-derived by-products have been applied as functional ingredients in GF bread (Korus et al., 2017) and crackers (Radočaj et al., 2014), but their use in GF sponge cake-type products has not yet been explored. Cake-like systems differ markedly from bread matrices in terms of structure formation, aeration mechanisms, and sensitivity to ingredient interactions. Indeed, while GF bread relies on hydrocolloid-starch to retain gas, GF cake systems are governed by an aerated emulsion/foam structure and limited water availability (Wilderjans et al., 2013). Consequently, their sensitivity to the addition of plant fibres and proteins is different and mainly dictated by distinct physico-chemical mechanisms, such as batter aeration, foam stability, and restricted starch gelatinisation, rather than structural matrix disruption (Du et al., 2025). This work therefore addresses a clear gap by evaluating, for the first time, the behaviour of two compositionally distinct hemp by-products, namely high-fibre HSCF and high-protein HSPC, in a sponge-cake system, while integrating batter properties, physico-chemical and nutritional attributes, along with consumer CATA profiling. From a sustainability standpoint, this work promotes the reintroduction of abundant hemp-processing by-products into the food chain, providing nutritious, low-cost ingredients for GF formulations and aligning with the food industry's interest in environmentally sustainable products with good nutritional value, flavour, and functionality.

2. Materials and methods

2.1. Raw Materials

Rice flour, sunflower oil, sucrose, eggs, UHT full-fat milk and baking powder (labelled composition: disodium diphosphate, sodium hydrogen carbonate, stabilisers E470a) were purchased at a local market in Messina, Italy. HSCF and HSPC were provided by Soc. Coop. Molino Crisafulli r.l., (Caltagirone, Sicily, Italy) together with certification of compliance with Italian legislation on tetrahydrocannabinol (THC) content (Republic of Italy 2019). HSCF was obtained by milling the seed cake of *Cannabis sativa* L. cv. Futura 75 after cold-press oil extraction, whereas HSPC was prepared by dry fractionation of HSCF.

2.2. Muffin Formulations and Preparation

Muffins were prepared following a modified version of the formulation described by Shevkani & Singh (2014). The base recipe consisted of 100 g rice flour (RF), 50 g sunflower oil, 60 g sucrose, 50 g eggs, 50 g milk, and 3.3 g baking powder. Fortified formulations were obtained by replacing rice flour with 15%, 20%, or 30% (w/w) of either HSCF or HSPC. Substitution levels were established through preliminary sensory screening and were consistent with literature reporting reduced acceptability at higher hemp inclusion (Mocanu et al., 2025; Du et al., 2025).

Dry ingredients (RF and, when applicable, HSCF or HSPC, sucrose, and baking powder) were mixed for 1 min using a professional planetary

mixer (Girmi IM30, Girmi S.p.A., Omegna (VB), Italy), operating at 800 rpm with the beater attachment. Liquid ingredients (eggs, milk, and oil) were then added and mixing continued for 5 min. Fresh whole eggs were incorporated directly into the batter, with no whisking or other pre-treatment applied. Approximately 60 g of batter was sampled for analytical determinations. The remaining batter was portioned into a 9-cup muffin pan (26.5 cm × 26.5 cm), with 45 g of batter accurately weighed into each cup, and baked in a preheated convection oven (BG91IX2, Smeg S.p.A Instruments, Guastalla (RE), Italy) at 180°C for 20 min. Muffins were baked on the middle rack, with the pan centred in the oven to ensure uniform airflow and heat distribution. After baking, muffins were cooled to room temperature for 20 min and stored in sealed glass jars until analysis, which was performed on the same day. Three independent batches were produced for each formulation on separate days. For each batch, the base recipe was scaled up by 1.5× to obtain sufficient batter for nine muffins. Unless otherwise specified, all analyses were performed in triplicate.

2.3. Batter pH and Density

For pH measurement, 10 g of batter was diluted to 100 mL with distilled water, homogenized using a stomacher (Stomacher 400 Circulator; Seward Ltd, Worthing, UK), and filtered (Whatman No. 1 filter paper). The pH of the filtered solution was measured at 20°C using a calibrated pH meter (HI90023CW, Hanna Instruments Italia s.r.l., Villafranca Padovana (PD), Italy). Additionally, the intrinsic pH of RF, HSCF and HSPC was determined by measuring a 10% (w/v) aqueous suspension of each flour under the same conditions mentioned above.

Batter density was determined as described by Majzoobi et al. (2016). A 25 mL graduated cylinder was filled with distilled water and was weighed using a four-decimal-digit balance (ATX224R, Shimadzu, Milan, Italy); afterwards, the water was removed, and the cylinder was filled with batter and weighed again. Tare correction and the actual density of water at 20 °C were applied, ensuring that the reported values represent true density (g/mL). Batter density was calculated according to Equation 1:

$$\text{Batter density} = \frac{\text{weight of batter}}{\text{weight of distilled water}} \quad (1)$$

2.4. Muffin Physical and Physico-chemical Characterization

The physical and physico-chemical characterization included the determination of moisture content, baking loss, volume, specific volume, diameter, and height. Moisture content was determined by drying 10 g of the sample in a forced-draft oven at 110 ± 5 °C until constant weight. This temperature was selected to ensure the complete removal of water bound by the fibre- and protein-rich hemp matrices while avoiding thermal degradation. Baking loss was calculated as the difference between batter weight before baking and muffin weight after cooling for 1 h at room temperature. Muffin volume was measured by the rapeseed displacement method according to AACC Method No. 10–05.01 (AACC 2010), and specific volume (mL/g) was obtained by dividing the measured volume by the corresponding muffin weight. Dimensional attributes (diameter and height) were recorded using a digital calliper (RS Pro 150 mm, RS Pro, Sesto San Giovanni, Italy). All measurements were performed in six independent replicates.

2.5. Colour Analysis

Colour measurements were performed on batter, crust, and crumb using a Minolta CR 300 tristimulus colourimeter (Konica Minolta Business Solutions Italia Spa, Milan, Italy) in the CIELab colour system. L^* (scale: 0 to 100), a^* (scale: -50 to +50) and b^* (scale: -50 to +50) parameters were determined using D65 illuminant and 10° observer. For batter, 15 measurements were taken per sample. For crust and crumb,

measurements were performed in triplicate on 3 muffins per batch, yielding a total of 27 determinations for each formulation (3 muffins $\times$ 3 batches $\times$ 3 replicates).

L^* , a^* , and b^* values were used to calculate chroma (C^*), hue angle (H°) according to the following equations:

$$\text{Chroma } (C^*) = \sqrt{a^{*2} + b^{*2}} \quad (2)$$

$$\text{Hue angle } (H^\circ) = \arctan2(b^*, a^*) \quad (3)$$

Total colour differences (ΔE_{Lab}) between control and fortified samples were calculated according to Equation 4:

$$\Delta E_{\text{Lab}} = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (4)$$

where ΔL^* = brightness difference, Δa^* = redness difference, and Δb^* = yellowness difference.

Interpretation thresholds followed Mokrzycki et al. (2011).

2.6. Back-extrusion test

Batter texture was evaluated using a TA.XT Plus Texture Analyzer (Stable Micro Systems Ltd., Godalming, UK). A 36 mm compression platen (P/36) probe (Stable Micro Systems Ltd., Godalming, UK) was used on 50 g of batter sample with the following parameters: test speed 5.00 mm/s; distance 40 mm; trigger force 5.0 g; acquisition rate 400 pps. Firmness, consistency, cohesiveness, and viscosity index were recorded. Seven replicates were performed per sample.

2.7. Texture profile analysis

Texture profile analysis (TPA) was performed using a TA.XT Plus Texture Analyzer equipped with a 5 kg load cell and a 50 mm compression platen (P/50) probe (Stable Micro Systems Ltd., Godalming, UK), following Martínez-Cervera et al. (2011) with modifications. Muffin tops were removed by cutting horizontally at 1.5 cm from the base, and samples underwent double compression with the following settings: pre-test speed 3.00 mm/s; test speed 1.00 mm/s; post-test speed 5.00 mm/s; distance 5 mm; trigger force 5 g; 5 s interval between cycles; acquisition rate 400 pps. Analyses were performed on the same day the muffins were baked. Seven replicates were performed per sample. Exponent software 6.1.4.0 (Stable Micro Systems Ltd., Godalming, UK) was used for data acquisition and curve integration. The test results were expressed as hardness, cohesiveness, gumminess, chewiness, and springiness.

2.8. Sensory evaluation

The sensory analysis of GF muffins, including control and fortified samples, focused on consumer acceptability, consumption intent, and the Check-All-That-Apply (CATA) test. Sensory evaluation was conducted with 24 untrained panellists (14 females, 10 males; aged 22–50 years), all regular consumers of GF bakery products. All participants, voluntarily recruited, signed an informed consent form in accordance with the principles of the Declaration of Helsinki before the beginning of the tasting sessions.

Sessions were held in eight individual sensory booths at a controlled temperature of 24 °C under white light at the Aroma and Sensory Laboratory (Department of Veterinary Sciences, University of Messina), in accordance with ISO 8589 (2007) standards.

Each group evaluated control and fortified muffins in triplicate across three sessions. Samples were served on white plates, coded with random three-digit numbers, and presented in a balanced monadic random order. Mineral water at room temperature was provided for palate cleansing.

For the CATA test, a list of 21 descriptors covering appearance, odour, flavour, taste, and texture—both at the touch and in the

mouth—was created, along with a definition for each descriptor (Table 1). These descriptors were established based on literature data on GF sponge cake and hemp-enriched bakery products. They took into account both sensory defects and desirable sensory features of muffins. The descriptors were presented in a randomised, subject-balanced order. Assessors received a preliminary explanation of each descriptor and the corresponding list of definitions to ensure consistent interpretation across the panel and were instructed to check all descriptors perceived in each sample.

Acceptability was assessed using a 9-point hedonic scale (1 = dislike extremely, 2 = dislike very much, 3 = dislike moderately, 4 = dislike slightly, 5 = neither like nor dislike, 6 = like slightly, 7 = like moderately, 8 = like very much, and 9 = like extremely) for appearance, odour, flavour, taste, aftertaste, and texture. A composite-liking score was calculated as the mean of the individual hedonic attributes, providing an integrated indicator of overall acceptability. Consumption intent was evaluated with a yes/no question: “Would you consider consuming this product?” (Merlino et al., 2022). Acceptability and consumption intent were collected immediately after the CATA task for each sample.

2.9. Statistical analysis

Means and standard errors were calculated for each formulation using Excel 2010 (Microsoft, Milan, Italy). The effects of the fortifying ingredient (FI), the fortification level (FL), and their interaction (FI $\times$ FL) were evaluated through a two-way ANOVA based on a 2 $\times$ 3

Table 1

- List of attributes and their definition selected for CATA test sensory analysis of the control and HSCF- or HSPC-fortified gluten-free muffins.

Attributes	Definitions
Appearance	
Dark surface	Brown colour of the crust
Sponginess	Amount and size of gas cells that make the crumb appear either light and open or dense and compact
Texture to the touch	
Springiness	Quickness of returning to the original shape after gentle pressure on the crumb with a finger.
Stickiness	Stickiness of the crust when touched with a finger
Odour	
Sponge cake odour	Typical odour of sponge cake/muffin when freshly baked and cut in half.
Hemp odour	Typical odour of hemp seed cake flour
Herbaceous odour	Typical odour of raw vegetables and freshly cut grass.
Taste	
Sweet taste	Degree of perceived sweetness
Bitter taste	Degree of perceived bitterness
Astringency	Dry, puckering and slightly rough sensation in the mouth, typically caused by unripe fruits or certain teas.
Bitter aftertaste	Lasting sensation of bitterness that remains in the mouth after eating.
Flavour	
Sponge cake flavour	Typical flavour of sponge cake/muffin when freshly baked and cut in half.
Hemp flavour	Typical flavour of hemp seed cake flour
Herbaceous flavour	Typical flavour of raw vegetables and freshly cut grass.
Texture in the mouth	
Grittiness	Sensation of tiny, coarse particles in the texture of the muffin felt while chewing a piece of it
Dryness	Lack of moistness
Moistness	Moistness felt when chewing a piece of muffin
Softness	Low resistance to deformation while compressing the muffin between the molar teeth
Hardness	High resistance to deformation while compressing the muffin between the molar teeth
Crumbliness	Tendency to break down easily into small, irregular particles
Cohesiveness	Force necessary to crumble a piece of muffin into the palate with the tongue

factorial design, using XLSTAT 2014 (Addinsoft, Paris, France). The control (100% RF) was excluded from the factorial model and was treated as a separate common reference. Tukey's post hoc test ($p < 0.05$) was applied for multiple comparisons among fortified treatments, and each fortified formulation was additionally compared with the control using Dunnett's multiple-comparison test in a separate analysis. CATA data were processed using R 4.4.0 (R Foundation for Statistical Computing, Vienna, Austria). Cochran's Q test was applied to identify significant differences among samples for each descriptor, followed by pairwise McNemar tests when appropriate. Citation frequencies were computed for all descriptors, and a Correspondence Analysis (CA) was performed on the contingency table (samples $\times$ attribute frequencies). Penalty analysis was also performed to evaluate the relationship between CATA attributes and composite-liking score without relying on an ideal product reference (Meyners et al., 2013; Ares et al., 2014).

3. Results and Discussion

3.1. Batter properties

Batter pH, evaluated under a 2×3 factorial structure for the fortified samples, was significantly affected by FI, and the interaction FI $\times$ FL, whereas FL alone did not show a significant effect (Table 1S, Figure 1). HSCF-fortified batters exhibited higher pH values than HSPC samples, and within each ingredient group, pH remained unaffected by the substitution level. The control sample, evaluated independently and compared with the fortified samples using Dunnett's test, showed the highest pH values (Figure 1). These pH trends mirror the experimentally determined pH values of the raw materials, i.e., RF (pH = 6.630), HSCF (pH = 6.265), and HSPC (pH = 6.100). The lower pH of HSCF and HSPC is consistent with their known composition, as hemp seed proteins are reported to contain substantial amounts of glutamic and aspartic acids (Shen et al., 2021; Absi et al., 2023), which, together with organic acids (e.g., citric, oxalic) and free fatty acids (Alonso-Esteban et al., 2022; Merlino et al., 2022), could contribute to the observed reduction in batter pH.

The batter density of the fortified samples was influenced by the type and the percentage of the by-products (HSCF or HSPC) used, as well as their interaction (Table 1S, Figure 1). According to the Tukey test, the 30% HSPC formulation exhibited the highest density, whereas 30%

HSCF showed the lowest value. Within each ingredient group, the 15% and 20% formulations did not differ from one another, and HSPC consistently produced denser batters than HSCF. When compared with the control, all HSPC formulations showed a statistically significant increase in batter density, with the highest value observed at the highest inclusion level. In contrast, HSCF did not affect batter density at 15% and 20%, while the 30% formulation resulted in a significant decrease, indicating greater air incorporation and retention during mixing.

These trends may relate to the different chemical compositions of the two by-products. HSCF contains 46% total fibre, with a high proportion of insoluble fractions, whereas HSPC is characterised by a high protein content (40%). A plausible explanation is that fibrous particles may promote bubble nucleation and provide Pickering-type stabilisation at the air-liquid interface, thereby increasing the amount of entrapped gas. Conversely, the higher protein content of HSPC may contribute to a more cohesive and continuous matrix that reduces air incorporation. Although not directly demonstrated here, these interpretations align with mechanisms described in the literature: insoluble particles may contribute to Pickering-type stabilisation of air-water interfaces, whereas protein-rich systems generally form more cohesive interfacial films that limit gas entrapment (Ma et al., 2025). Literature findings show that the effect of fibre on batter density strongly depends on the physico-chemical nature of the fibre source. For instance, Gómez et al. (2010) reported an increase in batter density with larger fibre particles in layer cakes, a trend opposite to that observed in the present study. This discrepancy likely reflects the distinct structural characteristics of HSCF, whose insoluble, porous particles may promote air incorporation and Pickering-type stabilization, rather than acting as dense, water-binding fibre fragments. This interpretation is consistent with Majzoobi et al. (2016) who reported that carrot pomace powder (a high-fibre by-product) with larger particle sizes decreased batter density due to its more porous, open structure. High-protein ingredients, on the other hand, have been shown to impair air retention and increase batter density (Gomes et al., 2015), in agreement with the effect observed for HSPC.

The incorporation of HSCF and HSPC, together with the FL and their interaction (FI $\times$ FL), significantly affected batter colour (Table 1S, Figure 2). Within the fortified batters, statistically significant differences were detected between HSPC and HSCF samples at the same corresponding inclusion levels. At 20% and 30%, HSPC significantly reduced

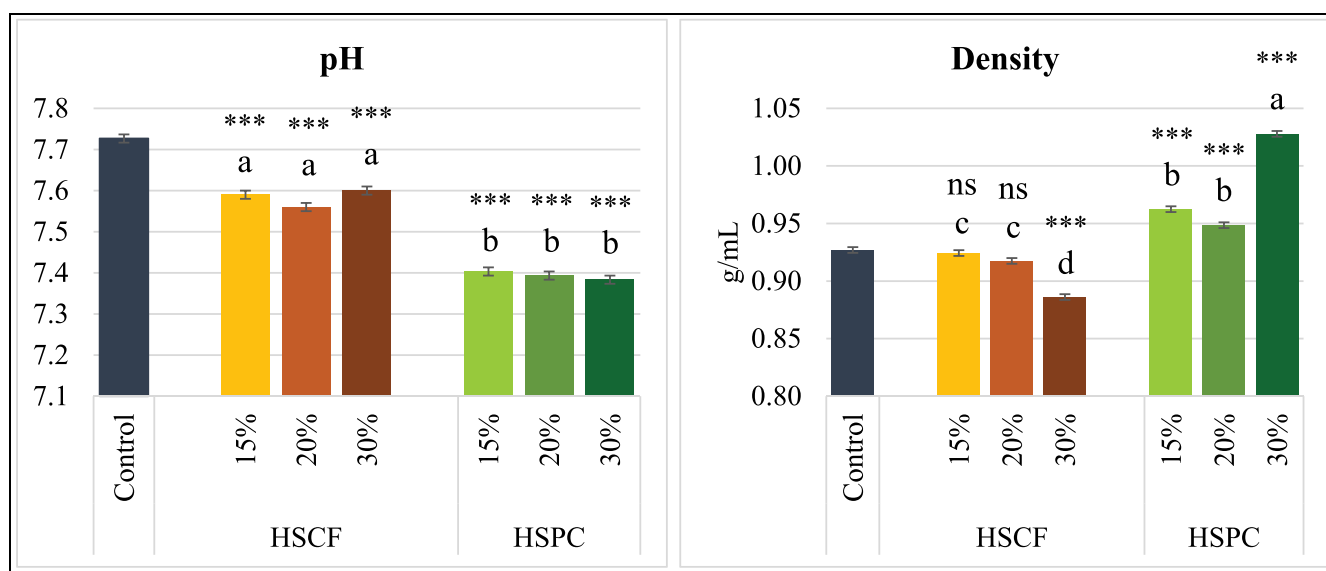


Figure 1. – pH and density (g/mL) values of batter samples as affected by the interaction between fortifying ingredient (HSCF, HSPC) and fortification level (15%, 20%, 30%). Values are means of nine replicates ($n = 9$) $\pm$ SE represented by the vertical bars. The control (C) was excluded from the 2×3 factorial ANOVA and is shown as a separate reference. Different letters indicate significant differences among fortified formulations according to Tukey's test ($p < 0.05$). Asterisks indicate significant differences versus the control according to Dunnett's test (ns = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

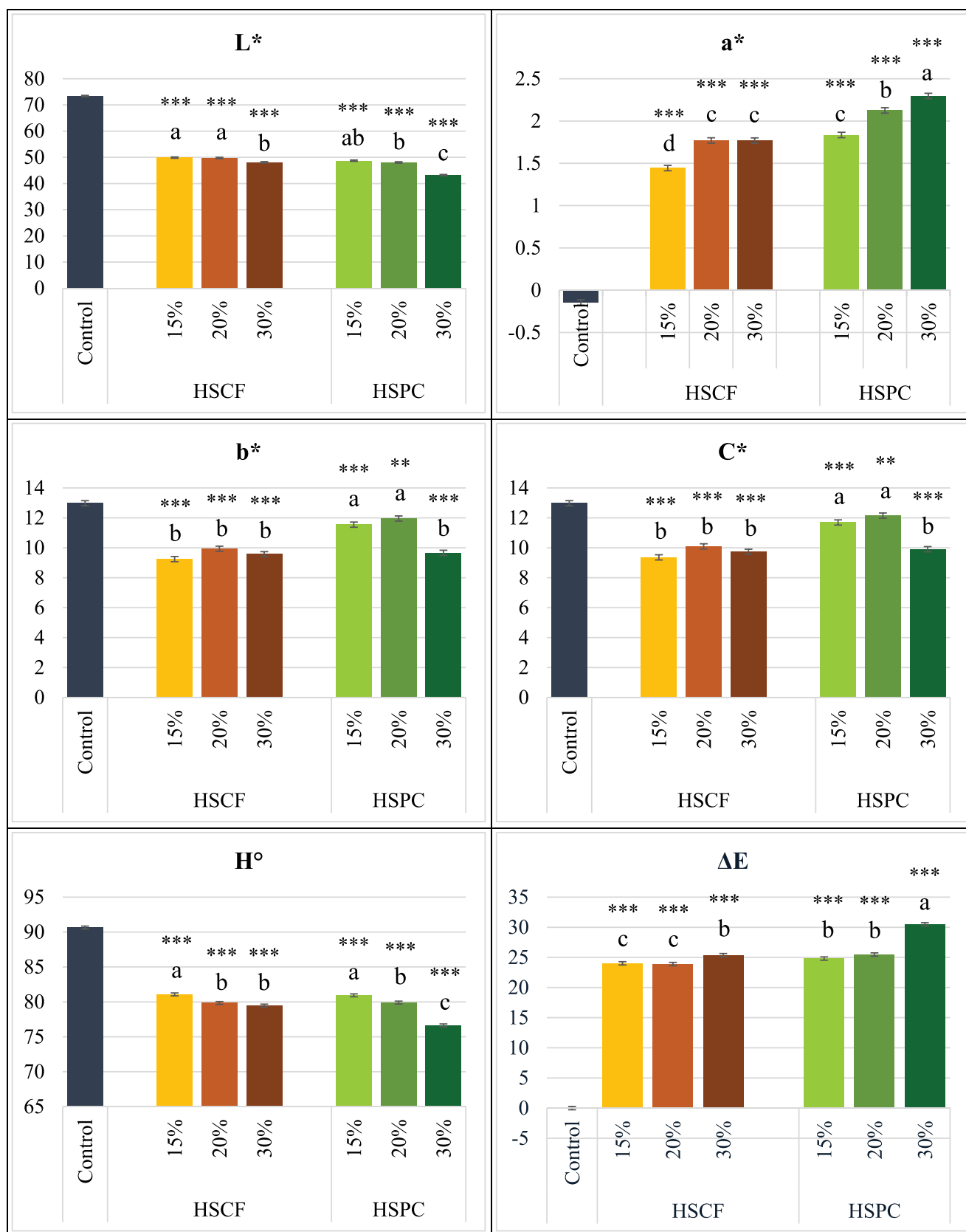


Figure 2. - Colour characteristics of batter samples as affected by the interaction between fortifying ingredient (HSCF, HSPC) and fortification level (15%, 20%, 30%). Values are means of forty-five replicates ($n = 45$) $\pm$ SE represented by the vertical bars. The control (C) was excluded from the 2×3 factorial ANOVA and is shown as a separate reference. Different letters indicate significant differences among fortified formulations according to Tukey's test ($p < 0.05$). Asterisks indicate significant differences versus the control according to Dunnett's test (ns = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

L*, resulting in darker batters. HSPC also consistently exhibited higher a* values across all substitution levels, indicating a systematically more pronounced red component. Likewise, b* and C* were significantly higher in 15% and 20% HSPC batters. H° was significantly lower in HSPC than in HSCF only at the 30% inclusion level, whereas no significant differences were observed at 15% and 20%. Finally, ΔE was consistently higher in HSPC batters across all matched inclusion levels. When considering the comparison with the control, Dunnett's test indicated that all fortified batters differed significantly from the control across every colour parameter (L*, a*, b*, C*, and H°). The control batter was markedly lighter, showing the highest L* value (73.4), whereas fortified samples ranged from 49.9 to 43.2. In addition, the control exhibited negative a* values, in contrast with the consistently positive and significantly higher a* values observed in all fortified formulations. The control also retained higher b* and C* values and a greater H°. In particular, H° decreased from 90.65° in the control to 79.47° - 81.07° in most fortified samples, with a further decrease to 76.64° in the HSPC 30% formulation, indicating a shift from yellow towards orange-brown/reddish tones, consistent with higher a* and lower b* values. Additionally, control batters showed a C* value of 12.96, whereas fortified samples ranged from 9.36 to 12.15, indicating a shift towards duller, less vivid tones. Total colour difference (ΔE) values further confirmed the strong visual impact of hemp ingredients, with fortified batter showing ΔE values far exceeding the perceptible difference threshold (ΔE > 5). The greatest deviation was observed in HSPC samples at the highest substitution levels, reaching a ΔE of 30.48 at 30% HSPC. These modifications, driven by reduced lightness, increased redness, and decreased yellowness relative to the control, primarily reflect the intrinsic pigmentation of hemp-derived ingredients. Similar darkening effects were previously reported in wheat doughs enriched with hemp press cake or grit, even at low substitution levels (1–5%) (Wiedemair et al., 2022).

The texture properties of the batter samples, such as firmness, consistency, cohesiveness, and viscosity index, are reported in Table 2S and Figure 3. Firmness represents the maximum force required for the probe to penetrate the muffin batter, with higher values indicating a firmer and more resistant batter. The positive area under the force–time curve corresponds to consistency, and larger areas are associated with denser or more viscous batters. Due to the intrinsic stickiness of cake batter, removal of the probe after compression generates a negative region in the extrusion curve. The maximum negative force is defined as cohesiveness, while the negative area under this force corresponds to the viscosity index. In general, batters with higher stickiness tend to exhibit greater cohesiveness (in absolute magnitudes) and larger negative areas (viscosity index). Textural properties of the batters were significantly affected by FI, FL, and FI × FL interaction. Batters from the 20% and 30% HSPC formulations exhibited higher firmness, consistency, and cohesiveness than the corresponding HSCF samples, whereas no significant differences were detected between the 15% HSCF and 15% HSPC batters. In addition, firmness values of HSCF-fortified samples did not differ significantly across substitution levels. Regarding the viscosity index, all HSCF-fortified batters differed from their HSPC counterparts:

HSCF samples showed a lower viscosity index (in absolute magnitude) at 15% inclusion, but higher values at 20% and 30%. When comparing with the control, Dunnett's test indicated that partial replacement of rice flour with HSCF or HSPC significantly increased firmness, consistency, and, in absolute magnitude, viscosity index, and cohesiveness. Hydration-related functional properties of HSCF and HSPC, previously characterised in our laboratory, provide additional context for interpreting batter behaviour. HSCF and HSPC exhibited markedly different water-interaction profiles: HSCF showed higher Water Holding Capacity (128.6%) and Oil Absorption Capacity (95.9%), whereas HSPC displayed greater Water Absorption Index (197.1%) and Solubility Index (109%). These differences indicate that HSCF, rich in insoluble fibre, binds water physically without contributing to network cohesion, while HSPC, characterised by higher protein content and solubility, competes more effectively with rice starch for hydration and participates in protein–protein and protein–starch interactions. Such competition for water is particularly relevant in batters enriched with HSPC, where extensive protein hydration may limit starch granule swelling during gelatinisation and contribute to the increases observed in batter density and instrumental texture parameters (firmness, consistency, cohesiveness, viscosity index) across substitution levels. In contrast, HSCF binds water mainly through physical entrapment rather than competition with starch, and its insoluble, porous fibres favour greater air incorporation, yielding a more open batter structure and attenuated increases in instrumental texture parameters. These observations are consistent with previous findings on GF batters enriched with plant-based ingredients, where higher fibre and protein contents promote hydrogen bonding with water and starch, reducing free water and limiting system mobility, thereby increasing firmness, viscosity and consistency (Majzoubi et al., 2016; Burbano et al., 2022; Xia et al., 2014). Adequate batter consistency and viscosity are necessary to retain the air bubbles formed during mixing and the CO₂ produced by the leavening agent during baking, achieving the desired muffin volume. Low-consistency batters allow air bubbles to rise and escape, resulting in low-volume cakes, whereas excessively high viscosity may retain bubbles but restrict their expansion during baking (Gómez et al., 2010; Turkut et al., 2016; Burbano et al., 2022; Gualarte et al., 2012).

3.2. Muffin properties

3.2.1. Nutritional profile

The theoretical nutritional composition of control and fortified muffins, calculated from the labelled composition of the flours and the ingredient proportions in the recipe, is reported in Table 2. These data clarify the contribution of hemp ingredients to the nutritional profile of gluten-free formulations. As expected from the intrinsic composition of HSCF and HSPC in comparison with that of RF (Table 2), fortification with hemp seed by-products progressively increased the protein content of the muffins, with enhancements spanning from 19% at 15% HSCF formulation to 68% at 30% HSPC substitution. This improvement is particularly relevant for gluten-free products, which are typically characterised by low protein levels due to the reliance on refined cereal

Table 2

- Labelled flour composition and calculated nutritional composition of control and fortified muffins.

Nutrients (g/100g)	Rice flour	HSCF	HSPC	Control	15% HSCF_Muffin	20% HSCF_Muffin	30% HSCF_Muffin	15% HSPC_Muffin	20% HSPC_Muffin	30% HSPC_Muffin
Protein	7.0	26.0	40.0	5.4	6.4	6.8	7.5	7.2	7.8	9.1
Fat	0.5	9.6	7.4	20.7	21.2	21.3	21.8	21.1	21.1	21.6
Carbohydrates	82	5.1	10.7	52.8	48.5	47.1	44.5	48.9	47.3	45.3
of which sugar	0.5	1.8	1.4	23.0	23.0	23.1	23.2	23.0	22.9	23.3
Fibre	0.5	46.0	24.0	0.2	2.7	3.5	5.2	1.5	1.9	2.8
NaCl	0.10	0.05	0.05	0.06	0.06	0.06	0.05	0.06	0.06	0.06
Energy (Kcal/100g)	361	211	269	419	410	408	404	414	410	413

Nutritional data of control and fortified muffins are theoretical calculations; therefore, no statistical analysis is applicable.

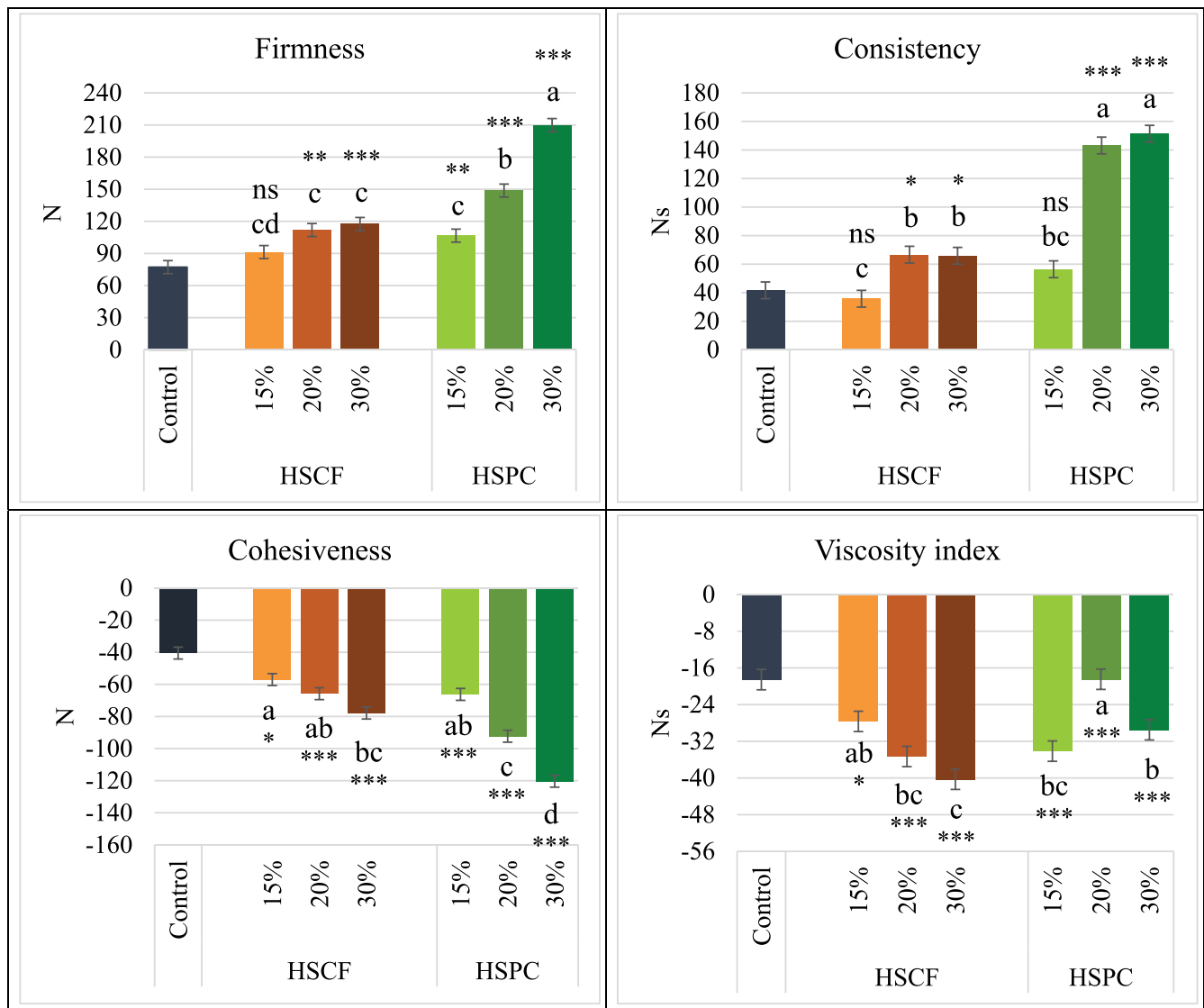


Figure 3. - Textural properties of batter samples as affected by the interaction between fortifying ingredient (HSCF, HSPC) and fortification level (15%, 20%, 30%). Values are means of twenty-one replicates ($n = 21$) $\pm$ SE represented by the vertical bars. The control (C) was excluded from the 2×3 factorial ANOVA and is shown as a separate reference. Different letters indicate significant differences among fortified formulations according to Tukey's test ($p < 0.05$). Asterisks indicate significant differences versus the control according to Dunnett's test (ns = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

flours.

Hemp fortification also contributed to a gradual reduction in carbohydrate content and a marked increase in dietary fibre, especially in HSCF formulations, reflecting the high fibre content of the hemp seed cake fraction. Even at the lowest inclusion level (15%), both HSPC and HSCF markedly enhanced fibre content, resulting in approximately 7.5- and 13.5-fold increases over the control for 15% HSPC and HSCF, respectively. When used at 20% and 30% substitution levels, HSCF enabled the formulation of muffins meeting the legal requirements (Eu Regulation No 1924/2006) to be designated as a "source of fibre", supporting the development of nutritionally improved gluten-free baked goods. Despite these compositional changes, the energy value of fortified muffins remained comparable to the control, indicating that the nutritional enhancement did not result in a caloric penalty. Overall, the incorporation of HSCF and HSPC allowed the formulation of gluten-free muffins with higher protein density and improved fibre content, also enabling fibre-related nutritional claims, thus addressing two of the main nutritional limitations of rice-based gluten-free products. These results confirm that hemp seed by-products represent effective fortifying ingredients capable of enhancing the nutritional quality of gluten-free

bakery items while supporting sustainable ingredient valorisation.

3.2.2. Physical Characteristics and Baking Performance

The incorporation of HSCF or HSPC into GF rice muffins produced statistically significant modifications in geometry (height and diameter), volume, and specific volume. Muffin height and diameter were affected by FI and FI $\times$ FL interaction, whereas volume varied significantly with FL and FI $\times$ FL interaction. In contrast, specific volume was significantly influenced exclusively by the FI $\times$ FL interaction (Table 3S). Among fortified muffins, 20% and 30% HSPC exhibited the greatest height values, whereas no statistically significant differences were observed among the remaining formulations. In addition, all fortified muffins showed significantly higher height values than the control (Figure 4). Moreover, HSCF-fortified muffins had significantly larger diameters than the control, whereas all HSPC muffins displayed smaller diameters; within each ingredient group, fortification level did not produce significant differences. These trends were reflected in volume and specific volume: 30% HSCF-fortified muffins, together with 15% and 20% HSPC muffins, showed higher volume and specific volume than the control, whereas 30% HSPC sample exhibited the lowest volume and specific

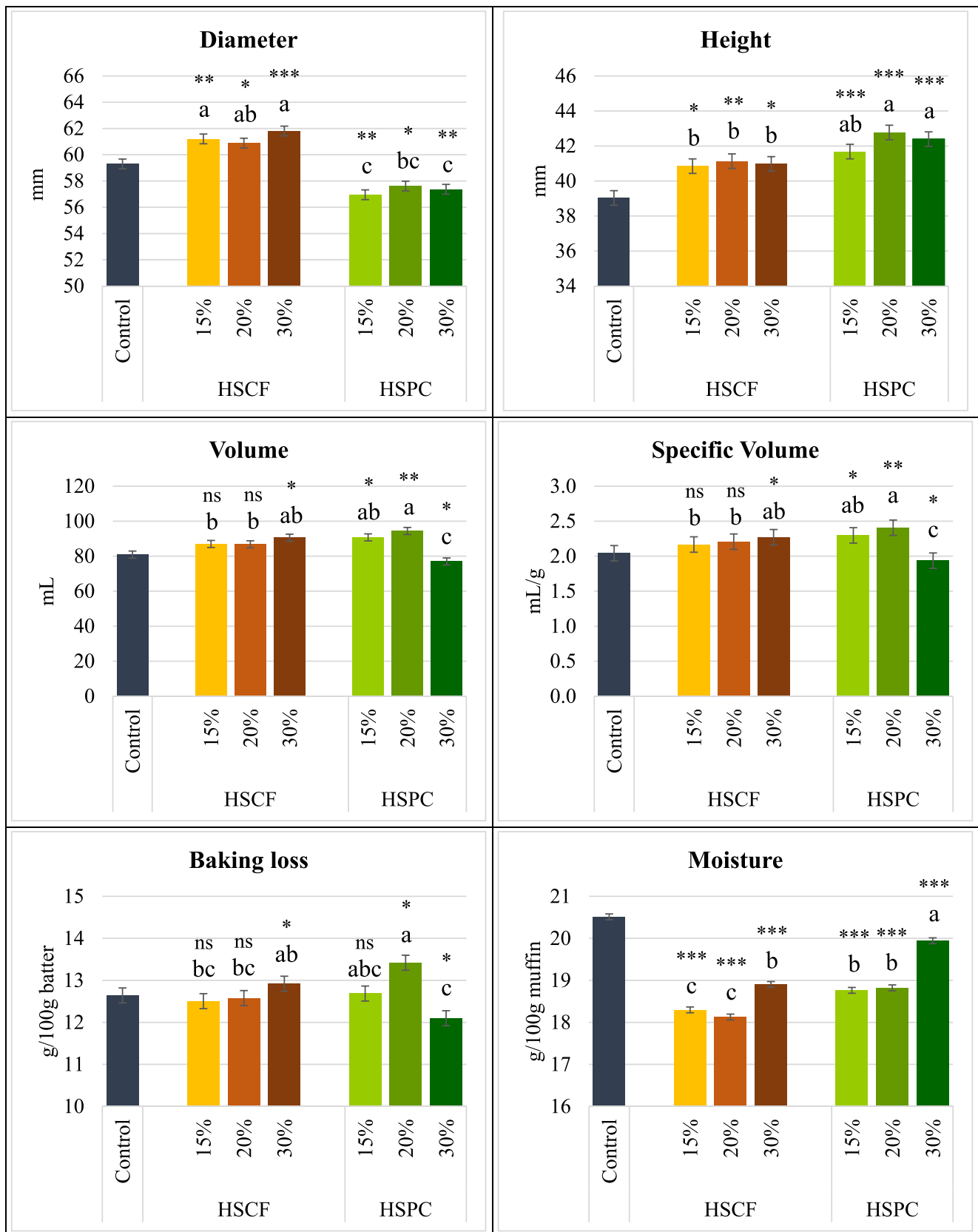


Figure 4. - Physico-chemical characteristics of muffin samples as affected by the interaction between fortifying ingredient (HSCF, HSPC) and fortification level (15%, 20%, 30%). Values are means of eighteen replicates ($n = 18$) $\pm$ SE represented by the vertical bars. Different letters indicate significant differences among fortified formulations according to Tukey's test ($p < 0.05$). Asterisks indicate significant differences versus the control according to Dunnett's test (ns = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

volume. At the highest HSPC level, the relatively high muffin height was accompanied by reduced lateral expansion, resulting in lower overall volume and specific volume. The observed differences align with the texture profile of the batters (Table 2S, Figure 3). The high firmness and consistency of the 30% HSPC batter likely limited lateral expansion during baking, reducing final volume. Conversely, the lower density of the 30% HSCF batter suggests greater air incorporation, which may have favoured gas retention and expansion during baking, resulting in characterised higher final volume. These findings agree with previous studies reporting that fortification generally increases muffin volume (Majzoobi et al., 2016), although excessive fibre levels (>24%) may reverse this trend (Gómez et al., 2010). In this study, HSCF-fortified muffins reached a fibre content of about 5.2% at the highest substitution level, placing them within the fibre range associated with volume enhancement. Similarly, high-protein fortificants have been shown to affect height and specific volume in a dose-dependent manner: improvements are typically observed at low levels of protein addition (Shevkani & Singh, 2014; Gularte et al., 2012; Ozgolet et al., 2024), whereas high protein levels tend to reduce these parameters (Jeong et al., 2021). In the present study, HSPC-fortified muffins exhibited higher specific volume than the control at 15% and 20% substitution levels, but values dropped below the control at 30% HSPC. This pattern may reflect water competition between hemp proteins and rice starch. At intermediate inclusion levels, the balance between protein hydration and starch water availability seems compatible with improved gas retention and structural setting, whereas at higher levels, greater protein hydration could reduce starch gelatinisation, helping explain the lower expansion observed.

Moisture content was significantly affected by FI, FL, and FI $\times$ FL interaction. Baking loss did not differ significantly as a function of the ingredient alone, but it varied significantly in response to FL and FI $\times$ FL interaction (Table 3S). Among fortified samples, HSCF muffins had lower moisture content than HSPC muffins, and within each ingredient group, the highest value was observed at the highest fortification level (Figure 4). A similar pattern was observed for baking loss in HSCF-fortified muffins, whereas HSPC samples showed maximum baking loss at 20% and minimum at 30%. Compared with the control, fortified samples exhibited lower moisture content, reflecting a reduced amount of free water in the matrix due to the water-binding capacity of hemp by-products. A similar trend has been observed by Mocanu et al. (2025) in wheat and whole-wheat muffins fortified with hemp seed cake flour, as well as in GF muffins enriched with various ingredients (Jeong et al., 2021; Majzoobi et al., 2016; Matos et al., 2015). The differences between HSPC and HSCF may reflect how water interacts with the crumb structure. In HSPC muffins, the comparatively more cohesive matrix appears compatible with higher water retention after baking, whereas the more open and porous structure associated with HSCF formulations could favour water evaporation during baking, resulting in lower moisture values.

Regarding baking loss, no statistically significant differences were detected with respect to the control, except for 30% HSCF and 20% HSPC, which showed higher values, and 30% HSPC, which was lower than the control. Previous studies have generally reported that fortification does not significantly affect muffin baking loss and may even reduce it in some cases, depending on matrix structure and specific volume (Ozgolet et al., 2024; Gulsunoglu-Konuskan et al., 2025; Matos et al., 2015; Bala et al., 2019). In our study, the insoluble, porous fibres of HSCF may favour vapour escape, contributing to the higher baking loss, whereas the more cohesive and less porous crumb structure of 30% HSPC limits water evaporation and results in higher residual moisture and lower baking loss at 30%. Additionally, the trend observed in baking loss mirrors the trend in volume and specific volume, suggesting that differences in specific volume likely contributed to the observed pattern, as baking loss - driven mainly by water evaporation - tends to be greater in samples with higher volume and specific volume.

3.2.3. Colour of crust and crumb

The colour parameters of crust and crumb showed that the incorporation of hemp by-products markedly modified the chromatic profiles of GF muffins (Tables 4S, Figures 5A and 5B). In the crust, all parameters were significantly affected by FI, FL, and FI $\times$ FL interaction, except for L^* , which did not vary significantly with FL, and a^* , which did not vary significantly with FI. Examination of the FI $\times$ FL interaction (Figure 5A) revealed that both HSCF and HSPC caused a significant decrease in L^* , a^* , and b^* values, together with a C^* reduction, indicating darker and less saturated crusts. These decreases tended to become more marked at higher fortification levels, although not all levels differed significantly, and were more pronounced in HSPC-fortified muffins than in those enriched with HSCF. Additionally, HSPC caused a stronger shift towards red hues (lower H°) than HSCF. Moreover, fortified samples exhibited statistically lower values for all colour parameters (L^* , a^* , b^* , C^* , H°) than the control, except for the hue values of HSCF-fortified muffins, which did not significantly differ from the control. Finally, the ΔE values (approximately 12.3–21.8) were consistently high, especially for HSPC muffins, confirming that hemp by-products impart a clearly perceptible colour difference relative to the control formulation.

Fortification also influenced the crumb colour profile, with all parameters affected by FI, FL, and FI $\times$ FL interaction, except for H° , which did not vary significantly with FI (Table 4S). Looking at the FI $\times$ FL interaction results (Figure 5B), within each ingredient group, L^* , b^* , C^* , and H° values generally decreased with increasing fortification level, whereas the opposite trend was observed for the a^* parameter. In addition, no differences were observed between HSCF and HSPC samples at the same fortification level for L^* , whereas for a^* , b^* , C^* , and H° parameters, HSPC samples tended to exhibit higher values than HSCF samples. When comparing the fortified samples with the control (Figure 5B), the control showed the highest L^* and the lowest a^* values; regarding the b^* values, HSCF fortification reduced this parameter, with a marked decrease at the higher level, whereas HSPC increased b^* at 15–20% substitution, with 30% HSPC comparable to the control. The same trend was observed for chroma values, indicating lower saturation in HSCF muffins and higher saturation in muffins fortified with 15% and 20% HSPC. As in the crust, ΔE values - always above 12.5 - confirmed that all fortified samples were visually distinct from the control, with differences increasing with fortification level and being more marked in HSCF than in HSPC samples. The chromatic modifications observed in both crust and crumb can be attributed to a combination of thermal reactions and the intrinsic pigmentation of the hemp-derived ingredients. During baking, the crust is exposed to high temperatures, where Maillard reactions and caramelisation dominate colour formation. The incorporation of hemp by-products - particularly HSPC, which provides a higher availability of amino groups - likely enhanced Maillard browning, promoting the formation of dark brown melanoids and resulting in crusts that were darker, less saturated, and more reddish than the control. Similar trends have been reported for muffins fortified with protein-rich ingredients, such as soy protein isolates, which intensify Maillard-driven browning and reduce lightness (Majzoobi et al., 2016), supporting the behaviour observed in the present study. In the crumb, where thermal gradients are milder, colour differences arise primarily from the inherent dark, brown-greenish pigmentation of HSCF and HSPC. These ingredients are rich in phenolic compounds, fibre-associated pigments, and protein-bound chromophores that naturally reduce lightness and modify hue. Comparable findings have been reported for bakery products enriched with dark-coloured plant materials, such as grape pomace (Tolve et al., 2021), grape seed flour (Yalcin et al., 2022), carob flour (Voučko et al., 2026), oilseed cake flours (Kaur et al., 2022), or hemp seed derivatives (Korus et al., 2017; Mikulec et al., 2019; Mocanu et al., 2025), which consistently decrease L^* and increase a^* or b^* depending on pigment composition, confirming that plant by-products rich in pigments and phenolic compounds can markedly modulate the chromatic profile of bakery systems, fully in line with the present findings.

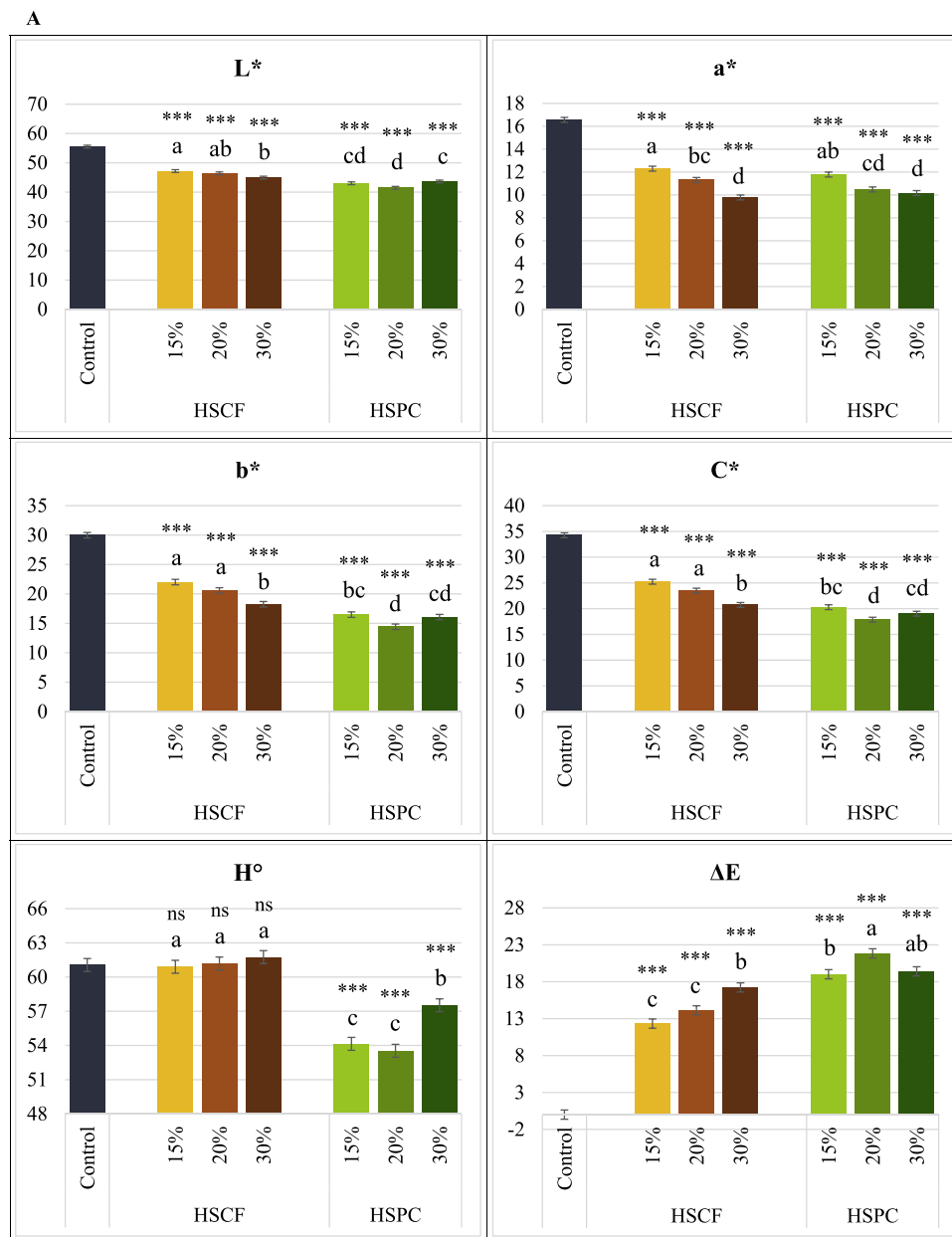


Figure 5A. - Colour characteristics of crust of muffin samples as affected by the interaction between fortifying ingredient (HSCF, HSPC) and fortification level (15%, 20%, 30%). Values are means of twenty-seven replicates ($n = 27$) $\pm$ SE represented by the vertical bars. The control (C) was excluded from the 2×3 factorial ANOVA and is shown as a separate reference. Different letters indicate significant differences among fortified formulations according to Tukey's test ($p < 0.05$). Asterisks indicate significant differences versus the control according to Dunnett's test (ns = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

3.2.4. TPA

The TPA parameters of control and fortified gluten-free muffins are reported in Table 5S and Figure 6. Significant differences were observed for hardness, gumminess, and chewiness, based on both FI and FL, as well as their interaction. Conversely, springiness and cohesiveness were not affected by either the type of hemp by-product or the substitution level or their interaction.

Among fortified samples, only 30% HSPC significantly differed from the other formulations in hardness, gumminess, and chewiness, exhibiting the highest values. In contrast, 20% HSPC showed the lowest value for hardness. At the 30% substitution level, both HSCF and HSPC showed significantly higher hardness, gumminess, and chewiness than the control. However, the effect was markedly more pronounced for HSPC. These textural parameters are closely related to the mechanical effort required during mastication: hardness measures the force required

to compress the muffin; gumminess represents the energy required to disintegrate the product to a state ready for swallowing, combining hardness and cohesiveness, whereas chewiness reflects the time and effort needed to chew the muffin to a swallowable state, combining gumminess and springiness. Higher hardness and chewiness are typically associated with denser, less aerated crumbs, which consumers often perceive as less fresh and less pleasant, while increased gumminess can generate a pasty mouthfeel that reduces overall acceptability.

The increases in hardness, gumminess and chewiness at higher substitution levels suggest a strengthening of the crumb structure associated with hemp ingredients. HSCF increased these parameters to a lesser extent, likely due to the physical water-binding properties of its insoluble fibres. The effects of HSCF addition are consistent with the high fibre content of this by-product, which at elevated levels can limit water availability and promote a denser crumb structure. Similar trends

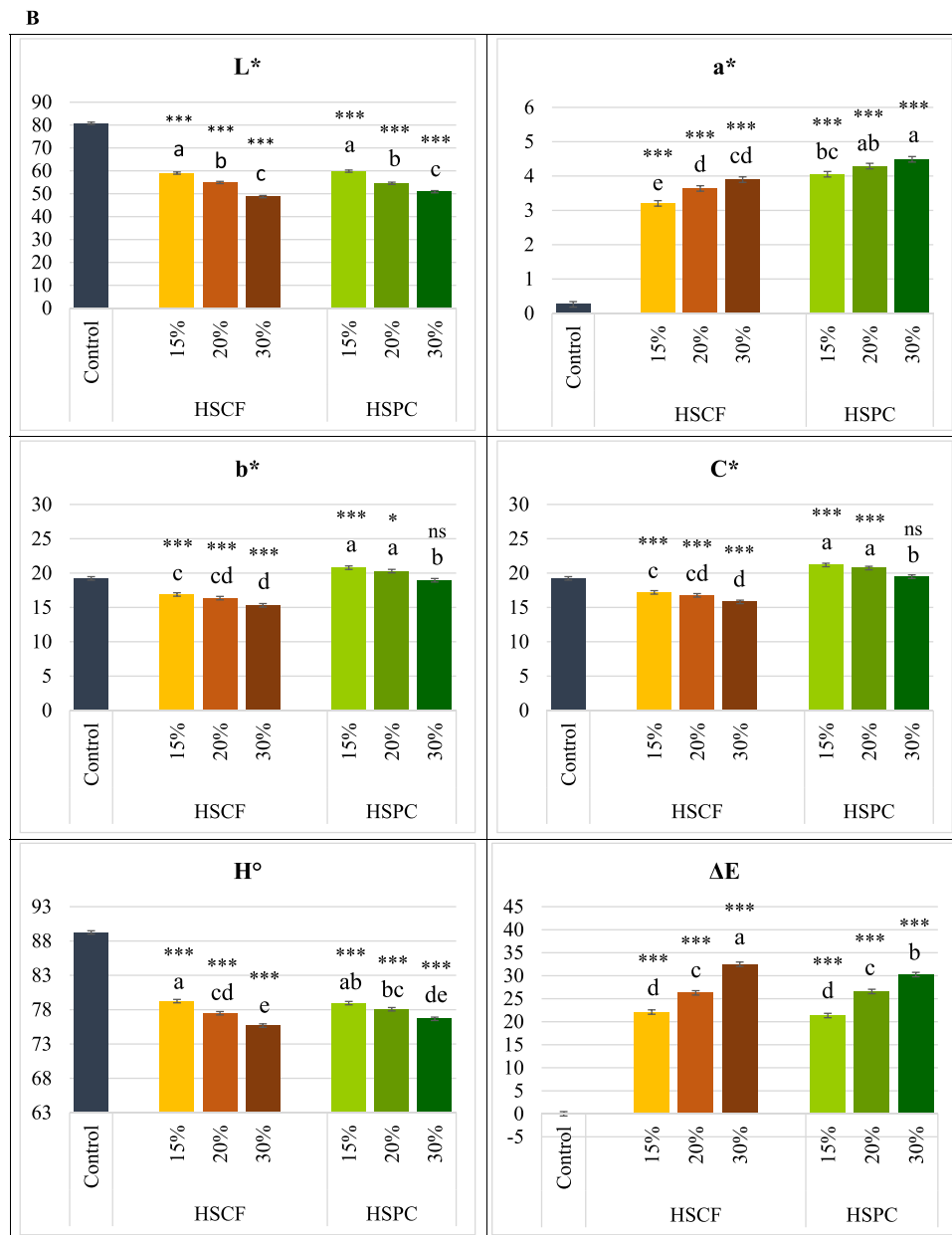


Figure 5B. – Colour characteristics of crumb of muffin samples as affected by the interaction between fortifying ingredient (HSCF, HSPC) and fortification level (15%, 20%, 30%). Values are means of twenty-seven replicates ($n = 27$) $\pm$ SE represented by the vertical bars. The control (C) was excluded from the 2×3 factorial ANOVA and is shown as a separate reference. Different letters indicate significant differences among fortified formulations according to Tukey's test ($p < 0.05$). Asterisks indicate significant differences versus the control according to Dunnett's test (ns = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

in hardness, chewiness, springiness, and cohesiveness have been reported for muffins fortified with high-fibre ingredients, such as cauliflower by-products (Tukassar et al., 2023) and artichoke leaves (Gulsunoglu-Konuskan et al., 2025), although some fibre sources may also affect springiness and cohesiveness (Majzoobi et al., 2016).

The addition of HSPC produced a more pronounced effect: muffins formulated with 30% HSPC exhibited the highest hardness, gumminess and chewiness values overall. This behaviour may reflect how the higher protein content of HSPC influences water interactions within the matrix. Greater protein hydration could reduce the amount of free water available for starch swelling and gelatinisation, a condition typically associated with firmer and less extensible crumbs and consistent with the higher hardness, gumminess and chewiness observed at 30% HSPC. Conversely, the insoluble fibres in HSCF bind water physically but are less likely to compete with starch for hydration, which could contribute

to the comparatively lower textural values. As discussed previously, the high protein level in the 30% HSPC formulation also impaired batter aeration and increased density, further contributing to the marked rise in hardness. The parallel increase in gumminess and chewiness reflects the greater mechanical effort required to break down and swallow these more compact structures. Comparable trends have been reported for gluten-free muffins enriched with protein isolates or high-protein flours, such as mungbean, cowpea, and chickpea flour (Jeong et al., 2021; Gularte et al., 2012; Shevkani & Singh, 2014). Kaur et al. (2022) similarly reported increased hardness and gumminess in muffins enriched with oilseed cake flours, supporting the link between fibre/protein enrichment and a more compact crumb structure.

Neither HSCF nor HSPC significantly affected springiness or cohesiveness. The literature on the impact of fibre- or protein-rich ingredients on these parameters is mixed, with some studies reporting

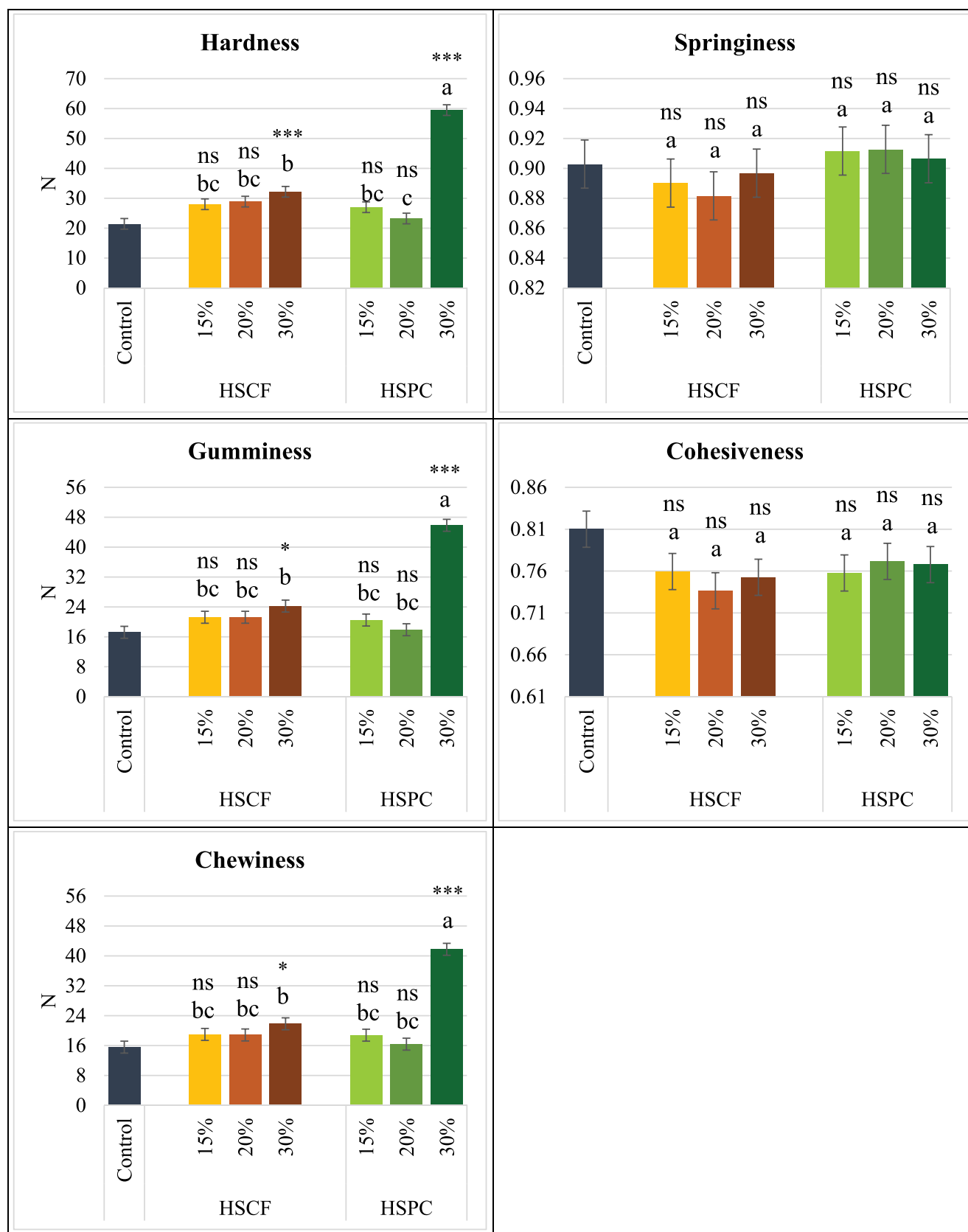


Figure 6. - Textural properties of muffin samples as affected by the interaction between fortifying ingredient (HSCF, HSPC) and fortification level (15%, 20%, 30%). Values are means of twenty-one replicates ($n = 21$) $\pm$ SE represented by the vertical bars. The control (C) was excluded from the 2×3 factorial ANOVA and is shown as a separate reference. Different letters indicate significant differences among fortified formulations according to Tukey's test ($p < 0.05$). Asterisks indicate significant differences versus the control according to Dunnett's test (ns = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

reductions (Majzoobi et al., 2016; Jeong et al., 2021) and others reporting improvements (Gularte et al., 2012). Springiness is associated with a fresh, elastic crumb structure, whereas cohesiveness is inversely related to crumbliness and relates to the energy required to chew food pieces (Jeong et al., 2021). The absence of detrimental effects on these attributes indicates that incorporating hemp by-products did not compromise these key quality traits, which are relevant to consumer perception of product freshness and structural integrity.

3.2.5. Sensory features and consumer acceptability

The sensory impact of hemp-derived ingredients in cake formulations was assessed using the CATA method, conducted as an exploratory sensory evaluation using a convenience sample of 24 consumers. Cochran's Q test identified 16 out of 21 descriptors as significantly discriminating across samples ($p < 0.05$) (Table 6S), confirming the suitability of the CATA list for capturing formulation-driven sensory differences. Significant effects were observed for dark surface, sponginess, hemp odour, sponge cake odour, herbaceous odour, bitter aftertaste, hemp flavour, sponge cake flavour, herbaceous flavour, dryness, moistness, softness, hardness, crumbliness, cohesiveness, and grittiness. Among these, the most discriminating descriptors were herbaceous odour, bitter aftertaste, dark surface, and hemp odour, indicating that colour changes, plant-related aroma notes, and persistent bitterness were the sensory features most affected by hemp ingredient incorporation. Conversely, springiness and stickiness did not show significant discriminative power, in agreement with TPA results, suggesting that these attributes were not markedly influenced by hemp enrichment. Similarly, sweet taste, bitter taste, and astringency did not differ significantly among formulations. This trend aligns with findings by Silva-Paz et al. (2025), who reported that CATA tends to discriminate samples primarily on textural and aroma-related attributes rather than on basic taste characteristics.

Post hoc McNemar tests further revealed that 30% HSPC and 30%

HSCF differed significantly from both Control and 15% substitution levels for most hemp-related descriptors, confirming that higher incorporation levels intensify the perception of plant-derived notes (Table 7S).

The heatmap of CATA frequencies (Figure 7) confirmed these differences and provided a clear visual representation of the sensory shift induced by hemp by-product incorporation. The control sample was mainly associated with conventional bakery-related attributes, such as sponge cake odour, sponge cake flavour, sponginess, cohesiveness, and hardness, reflecting a typical cake-like sensory identity. In contrast, hemp-enriched samples were more frequently described by softness, dark surface, hemp odour and flavour, herbaceous odour and bitter aftertaste, particularly at the highest inclusion levels. This indicates that increasing the amount of hemp-derived ingredients progressively moved the sensory profile away from the traditional sponge-cake character towards a darker, more plant-like and hemp-characterised profile. This trend is consistent with previous studies on hemp-enriched bakery products, where higher substitution levels increased green/herbaceous aromas, bitterness, and astringency, often attributed to phenolic compounds and chlorophyll derivatives (Pojić et al., 2014; Mikulec et al., 2019). The comparison between the two hemp by-products also suggested different sensory effects. Samples containing HSCF were more strongly associated with texture-related limitations, particularly dryness and grittiness. This behaviour is consistent with the higher fibre content of HSCF, which can disrupt crumb structure, reduce moisture retention, and intensify coarse mouthfeel perception. Similar increases in dryness and grittiness have been reported in fibre-rich formulations, where the elevated water-binding capacity and larger particle size of hemp by-products limit water availability and contribute to a rougher oral texture (Wiedemair et al., 2022; Sciacca et al., 2023). Conversely, HSPC-containing samples, especially at higher inclusion levels, were more closely related to moistness, springiness, dark surface, and hemp/herbaceous sensory notes. Therefore, HSPC appeared to contribute to

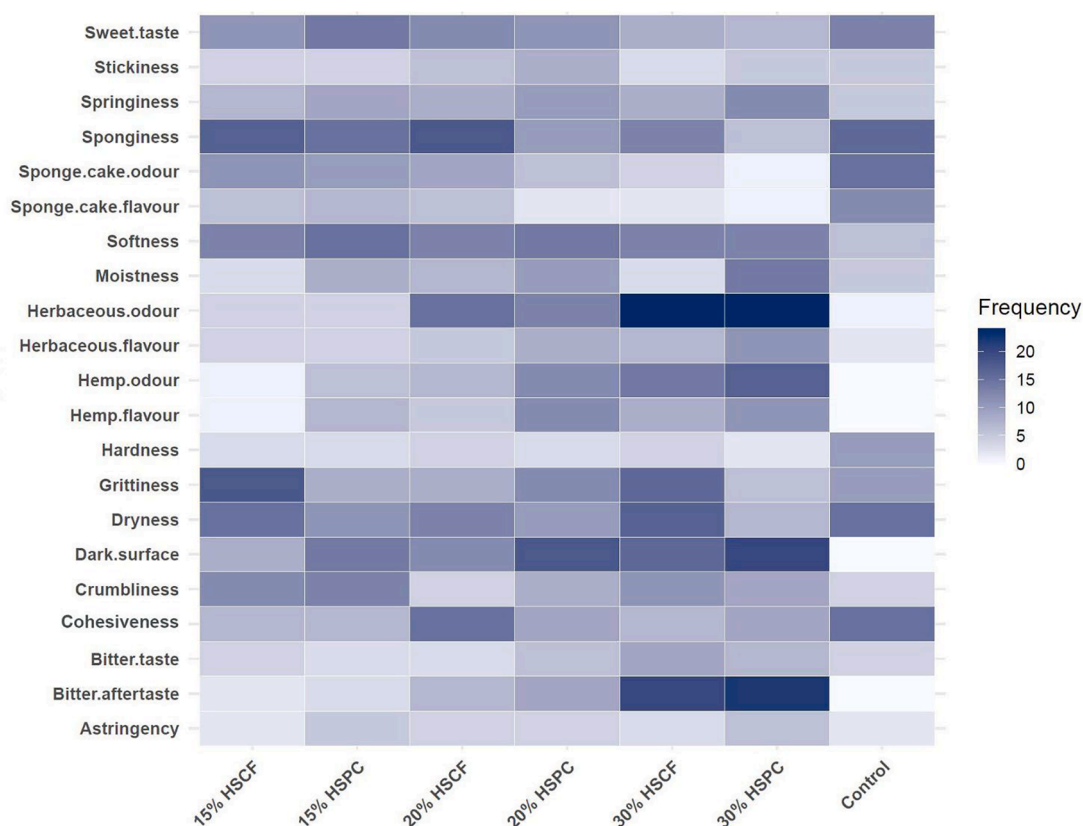


Figure 7. – Heatmap of CATA descriptor frequency.

a moister and more elastic perception, while simultaneously enhancing colour intensity and plant-related sensory characteristics.

Correspondence Analysis (CA), well-suited for contingency tables derived from categorical sensory data, provided an additional overview of the relationships between samples and sensory descriptors by enabling the simultaneous visualization of sample-attribute associations. The first two dimensions explained 82.7% of the total inertia (Figure 8), with Dim1 accounting for 71.2% and Dim2 for 11.5%. Dim1 represented the main gradient of sensory variation, clearly separating the control sample from the hemp-enriched formulations. The control was positioned on the positive side of Dim 1, close to cake-related descriptors, such as sponge cake odour, and sponge cake flavour. In contrast, samples fortified with 30% HSCF, 20% HSPC, and 30% HSPC were located on the negative side of Dim1, near dark surface, hemp odour, hemp flavour, herbaceous odour/flavour, and bitter taste and aftertaste. Intermediate formulations (15% HSCF, 20% HSCF, 15% HSPC) were positioned closer to the control but showed an additional separation along Dim2 driven by texture and mouthfeel attributes, such as stickiness, softness, dryness, grittiness, and crumbliness. Overall, the sensory configuration revealed a gradual shift from a conventional cake-like profile towards a hemp-driven identity, accompanied by additional textural and mouthfeel divergences that further differentiated the intermediate and highly fortified formulations.

Two-way ANOVA, applied only to fortified samples, considering FI (HSCF vs HSPC), FL (15%, 20%, 30%), and their interaction, showed that acceptability attributes such as, flavour, taste, aftertaste, texture, and the composite liking score, were not significantly affected by formulation factors (Table 8S). When subsequently comparing fortified samples with the control, these attributes also did not differ significantly (Figure 9). In contrast, odour and appearance exhibited statistically significant effects. In particular, for odour, both FL and the FI $\times$ FL interaction contributed to differences among fortified samples: the 20%

HSPC formulation achieved the highest odour score, whereas 30% HSPC showed the lowest. With the exception of 20% HSPC, all fortified samples presented odour scores below that of the control. On the other hand, the FI $\times$ FL interaction significantly affected appearance scores, with 20% HSCF higher than 20% HSPC but similar ($p > 0.05$) to the other formulations. Furthermore, all fortified formulations, except 20% HSCF, differed from the control, which exhibited the highest appearance score. These findings indicate a moderate formulation effect on consumer liking. Differences in hedonic responses were less pronounced than those observed in the CATA sensory description. This indicates that consumers perceived differences in specific sensory attributes - such as colour, texture, and flavour notes - without these differences necessarily translating into marked changes in overall liking. This pattern is frequently reported in consumer studies, where descriptive methods reveal clearer product separation than liking scores (Marques et al., 2022). This discrepancy likely depends on the sensory methodologies applied. Indeed, attribute-based methods such as CATA are inherently more sensitive in detecting subtle differences among samples, whereas hedonic evaluations - reflecting a global and subjective consumer response - tend to be less discriminative and may mask small sensory variations. In our case, the limited size of the exploratory consumer panel may have contributed to this discrepancy, as smaller panels often detect attribute-level differences more readily than differences in overall acceptability. Moreover, consumers of functional gluten-free products may tolerate noticeable sensory modifications when these are associated with perceived nutritional or health benefits, resulting in relatively stable liking scores despite distinct sensory profiles.

The PCA derived from the penalty analysis (Figure 10) provides a deeper understanding of how specific sensory deviations contributed to hedonic penalties across formulations. The first two dimensions explained 77.2% of the total variance (Dim 1=59.0%, Dim 2=18.2%). The control was positioned in the region associated with typical sponge

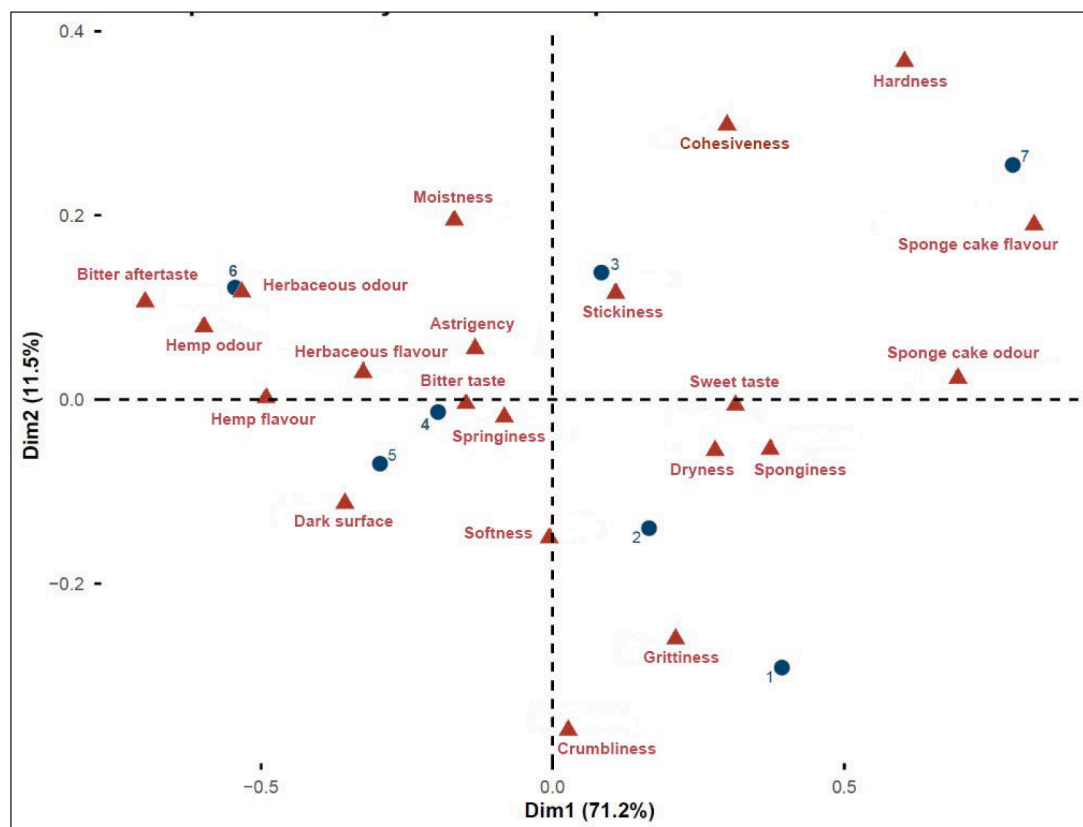


Figure 8. - Correspondence Analysis (CA) biplot of CATA frequencies. Samples are coded as: 1=15%HSCF_Muffin; 2=15%HSPC_Muffin; 3=20%HSCF_Muffin; 4=20%HSPC_Muffin; 5=30%HSCF_Muffin; 6=30%HSPC_Muffin; 7=Control.

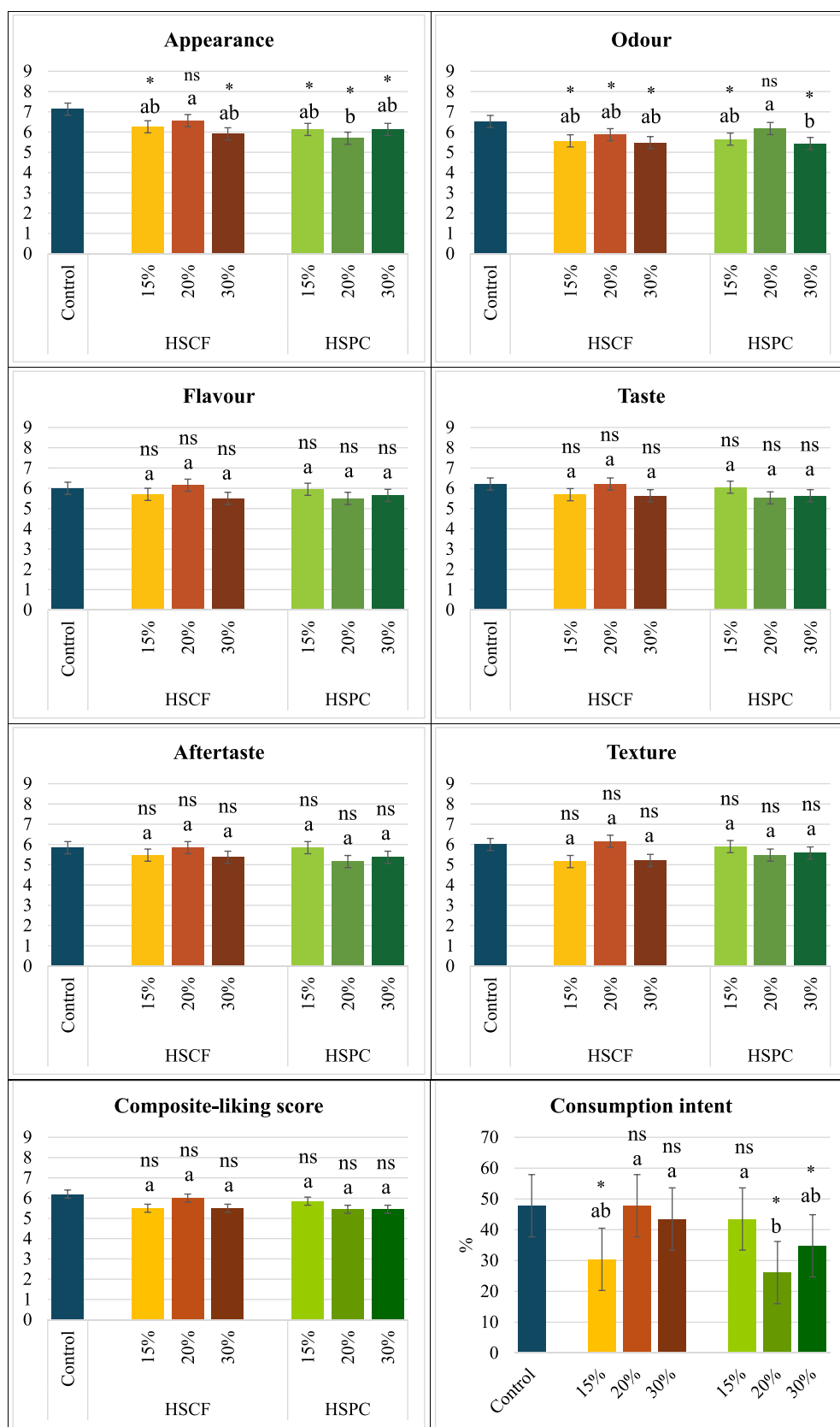


Figure 9. - Hedonic attributes and consumption intent of muffin samples as affected by the interaction between fortifying ingredient (HSCF, HSPC) and fortification level (15%, 20%, 30%). Values are means of twenty-four replicates ($n = 24$) $\pm$ SE represented by the vertical bars. The control (C) was excluded from the 2×3 factorial ANOVA and is shown as a separate reference. Different letters indicate significant differences among fortified formulations according to Tukey's test ($p < 0.05$). Asterisks indicate significant differences versus the control according to Dunnett's test (ns = not significant; * = $p < 0.05$; ** = $p < 0.01$; *** = $p < 0.001$).

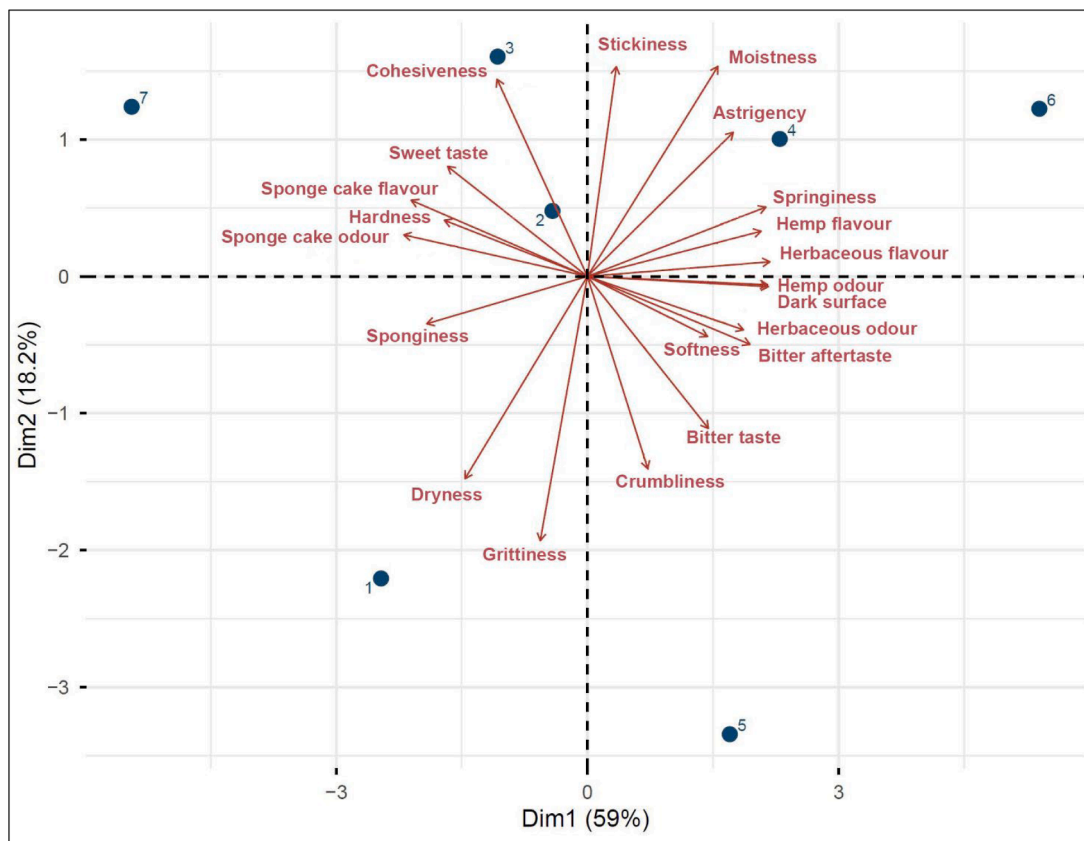


Figure 10. - PCA biplot of Penalty Analysis. Samples are coded as: 1=15%HSCF_Muffin; 2=15%HSPC_Muffin; 3=20%HSCF_Muffin; 4=20%HSPC_Muffin; 5=30% HSCF_Muffin; 6=30%HSPC_Muffin; 7=Control.

cake attributes, including sponge cake flavour, sweet taste, sponginess, and cohesiveness, confirming these descriptors as key drivers of consumer liking. Samples incorporating hemp ingredients progressively shifted away from this area, indicating increasing sensory deviations perceived as penalising.

Formulations containing 15% HSCF and 30% HSCF were located towards vectors associated with dryness, grittiness, crumbliness, and bitter taste. This pattern is consistent with the higher fibre content of HSCF, which increases water absorption and introduces coarse particles, thereby impairing crumb structure and mouthfeel. The 30% HSCF sample, located further from the origin than the 15% HSCF formulation, showed the strongest association with these penalising attributes, reflecting the cumulative impact of fibre-driven textural defects.

Samples containing higher levels of HSPC ($\geq 20\%$) were positioned farther from the control clustered near descriptors linked to hemp-derived sensory notes, such as hemp odour, hemp flavour, herbaceous odour, astringency, and dark surface, indicating that flavour- and colour-related deviations were the primary drivers of hedonic penalties for protein-rich formulations.

Conversely, samples containing 15% HSPC and 20% HSCF did not behave uniformly within their respective ingredient groups. Instead, both clustered in the same quadrant as the control and remained relatively close to the origin, indicating only minor deviations from the reference sensory profile. This trend suggests that these formulations did not introduce strong sensory defects that would drive substantial liking penalties. The 15% HSPC sample was closest to the origin, implying that a 15% inclusion of HSPC preserved a balanced sensory profile, with limited expression of hemp-related notes. This behaviour is consistent with previous studies reporting that moderate levels of hemp protein can be incorporated without markedly altering flavour or colour attributes.

Similarly, the sample containing 20% HSCF remained in the control quadrant and remained relatively close to the origin. This behaviour is

consistent with its distinct physico-chemical and structural properties. Specifically, the 20% HSCF batter exhibited a higher viscosity index than the 15% formulation, which translated into greater air entrapment, resulting in high values of height, volume, and specific volume in the baked product. The increased aeration produced a lighter and more open crumb structure, effectively diluting the perception of fibre-driven defects.

Overall, and in line with consumption intent, the PCA indicates that low levels of HSPC and moderate levels of HSCF can be incorporated without substantially compromising the sensory profile, whereas higher levels introduce ingredient-specific deviations that negatively affect consumer acceptance. This differentiated behaviour underscores the importance of tailoring fortification strategies to the functional properties of each hemp ingredient and supports the feasibility of moderate enrichment as a means to enhance nutritional value while maintaining sensory appeal.

4. Conclusion

This study demonstrates that hemp seed oil by-products can be effectively incorporated into GF muffin formulations, although their impact strongly depends on ingredient type and substitution level. HSCF was associated with lower batter density and greater muffin volume, particularly at higher inclusion levels, whereas HSPC strengthened the batter matrix but reduced expansion when used at 30%. Beyond these technological effects, fortification also enhanced the nutritional profile of the muffins: both HSCF and HSPC increased protein content, while HSCF additionally provided a substantial rise in dietary fibre, even at moderate inclusion levels, without altering the overall energy value. Additionally, HSCF at 20–30% yielded muffins meeting the EU Regulation No 1924/2006 criteria for the “source of fibre” claim. These improvements address key nutritional limitations of rice-based GF

products and support the development of more balanced formulations. Both ingredients markedly affected colour and progressively shifted the sensory profile from a conventional sponge-cake character towards darker, herbaceous, and hemp-related attributes. From a sensory perspective, excessive fortification increased penalising sensory attributes such as bitterness, aftertaste, dryness, grittiness, and crumbliness. Overall, a substitution level of 15–20% represented the most suitable range, balancing technological performance, nutritional enhancement, consumer acceptability, and sustainability. These findings support the use of hemp seed by-products as functional ingredients for the development of nutritionally improved and sustainable GF bakery products.

Given the exploratory nature of the CATA test and the limited panel size, the sensory results should be interpreted as indicative trends rather than definitive conclusions at the population level. Future studies will involve larger and more diverse consumer groups to better understand how specific sensory perceptions and the perceived nutritional and functional qualities of the fortified muffins jointly influence overall acceptability.

Shelf-life and staling behaviour represent additional aspects that warrant investigation in future studies, as formulation-dependent differences may emerge over storage, particularly in high-protein and high-fibre gluten-free systems.

Figure 5A.

Figure 5B.

Ethical statement

This study involved sensory evaluation of GF muffins by adult volunteer consumers. No vulnerable participants were recruited. All participants were informed about the study purpose, procedures, product ingredients/allergens, confidentiality of responses, and their right to withdraw at any time. Written informed consent was obtained before participation. The activity involved only sensory evaluation of food samples and did not include clinical procedures, invasive interventions, administration of substances beyond normal dietary exposure, testing of health claims, or experimentation aimed at assessing physiological effects. Therefore, in line with the European Commission guidance on “Ethics Review and Food-Related Research” and in accordance with the World Medical Association Declaration of Helsinki, ethical committee approval was not required, and the study was not submitted for ethics committee evaluation.

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CRediT authorship contribution statement

Maria Merlino: Writing – review & editing, Writing – original draft, Visualization, Validation, Project administration, Methodology, Funding acquisition, Conceptualization. **Anthea Miller:** Investigation, Formal analysis, Data curation. **Mattea Maria Antonietta Lauceri:** Investigation, Formal analysis, Data curation. **Iolanda Cilela:** Investigation, Formal analysis, Data curation. **Antonella Verzera:** Supervision, Conceptualization. **Concetta Condurso:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Resources, Project administration, Methodology, Conceptualization. **Amalia Piscopo:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence

the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.afres.2026.102621](https://doi.org/10.1016/j.afres.2026.102621).

Data availability

Data will be made available on request.

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