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FRESH ELDERBERRY OR SPINACH PURÉES AS DUAL NATURAL COLOUR AND EARLY TEXTURE MODULATORS IN SORGHUM GLUTEN-FREE BREAD

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KEYWORDS:

acceptability, clean-label, consumer test, principal component analysis, texture profile analysis

ABSTRACT

Gluten-free breads typically exhibit rapid firming and limited natural color diversity, which issues are usually addressed through multiple additives. This study evaluated the properties of adding fresh elderberry (*Sambucus nigra*) and spinach (*Spinacia oleracea*) purées (15–35%, flour basis) as multifunctional ingredients for sorghum (*Sorghum oryzoidum*) gluten-free bread. Nine bread recipes were analysed for their instrumental texture, crumb CIELAB color, sensory profile, and consumer acceptability at 6 h and 24 h post-baking. At 6 h, bread with added spinach puree at 35% achieved wheat-like softness, while bread with added elderberry puree increased crumb firmness. After 24 h, all breads firmed, but bread with added 35% of spinach puree remained substantially softer than the sorghum reference sample. Elderberry purée shifted crumb color towards red-violet hues, spinach puree did it towards green-yellow hues, while the variants of bread with added spinach showed the lowest 24 h color change ($\Delta E^*ab < 3.5$). Consumer scores were consistently high (≥ 7.0 on day 1), with 35% elderberry-enriched bread which retained the highest liking rating after 24 h. These findings demonstrate that fresh plant purées can expand natural colours diversity and attenuate early firming in gluten-free bread, supporting their application as clean-label alternatives to the conventional additives.

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Научная статья

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ПЮРЕ ИЗ СВЕЖИХ ЯГОД БУЗИНЫ ИЛИ ПЮРЕ ИЗ ШПИНАТА КАК ДВОЙНОЙ НАТУРАЛЬНЫЙ КРАСИТЕЛЬ И МОДУЛЯТОРЫ КОНСИСТЕНЦИИ СОРГОВОГО БЕЗГЛЮТЕНОВОГО ХЛЕБА НА РАННИХ СРОКАХ ПОСЛЕ ВЫПЕЧКИ

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КЛЮЧЕВЫЕ СЛОВА: АННОТАЦИЯ

приемлемость, природные ингредиенты, подпадающие под определение «чистая этикетка», потребительская оценка, анализ основных компонентов, анализ профиля консистенции

Безглютеновый хлеб обычно быстро черствеет и имеет ограниченное разнообразие природных цветов, что, как правило, исправляется многочисленными добавками. В этой статье оцениваются свойства пюре из свежих ягод бузины (*Sambucus nigra*) и шпината (*Spinacia oleracea*) (15–35% от количества муки), примененных в качестве многофункциональных ингредиентов для безглютенового хлеба, выпеченного из сорго (*Sorghum oryzoidum*). Был проведен анализ девяти рецептов, где инструментально оценивалась консистенция, цвет мякиша по шкале цвета CIELAB, профиль органолептических свойств и уровень потребительской приемлемости через 6 и 24 часа после выпечки. Через 6 часов хлеб с добавлением шпината в количестве 35% достиг уровня мягкости, сравнимого с пшеничным хлебом, в то время как добавленная бузина повысила черствость мякиша. Через 24 часа все виды хлеба зачерствели, но хлеб, испеченный с добавлением шпината в количестве 35%, оставался значительно мягче, нежели контрольный образец, выпеченный из сорго. Пюре из ягод бузины изменило цвет хлебного мякиша в сторону красно-фиолетовых оттенков, пюре из шпината — в сторону зелено-желтого оттенка, при этом варианты рецепта с добавленным шпинатом показали наименьшее изменение цвета через 24 часа ($\Delta E^*ab < 3,5$). Потребительские оценки были неизменно высокими ($\geq 7,0$ в первый день), при этом хлеб с добавлением 35% бузины сохранил наивысшую оценку и через 24 часа. Эти результаты показывают, что применение пюре из свежих растительных материалов способно расширить естественное разнообразие цвета и уменьшить преждевременное черствение безглютенового хлеба, что подтверждает целесообразность их применения в качестве альтернативы традиционным добавкам, подпадающим под определение «чистая этикетка».

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1. Introduction

The gluten-free (GF) bread market has been expanding steadily, driven by the diagnostics of celiac disease and non-celiac gluten sensitivity, as well as by voluntary adoption of “clean” foods or perceived healthier diets among non-celiac consumers [1,2]. Yet GF breads still lag behind wheat analogues in three persistent quality domains: crumb openness, early (first 24 h) firmness kinetics and naturally appealing color diversity, which together influence repurchase behavior [3–6]. Current technological optimization commonly relies on cumulative “packages” of hydrocolloids (xanthan, psyllium), refined starches, emulsifiers and enzyme systems; for visual differentiation, added colorants or processed extracts are frequently used [3,7–10]. While effective for volume or softness stabilization, this multi-additive reliance can lengthen ingredient lists and erode clean-label positioning, increasingly valued in free-from-artificial ingredients products [11,12]. These pressures motivate interest in whole, recognizable plant ingredients able to contribute both technical & functional (hydration, matrix support) and sensory (natural color) parameters.

Sorghum flour is a relevant GF platform due to its gluten absence, starch functionality and phenolic potential, though unaided it can yield compact crumb and limited elasticity [13,14]. The local hybrid *Sorghum oryzoidum* (“soryz”) — featuring pale kernels, neutral taste, and a moderate protein content (approximately 10%) — adds specific advantages: (i) a light, neutral crumb matrix that accentuates pigment contributions from added plant purées; (ii) valorization of an underutilized regional resource; (iii) distribution of compatible proteins fractions (albumins, globulins and glutelins) with forming a stable matrix in the presence of hydrocolloids [15,16].

Elderberry (*Sambucus nigra*) fruits, abundant and under-exploited locally, supply anthocyanins (cyanidin derivatives, including acylated forms), phenolics and simple sugars with intense red–violet dyeing capacity [17]. Using fresh integral purée rather than extracts or powders retains fibers, pectins and organic acids that may manage water distribution and engage in weak hydrogen bonding or aromatic stacking with polysaccharides/hydrocolloids — mechanisms proposed (not directly confirmed here) to influence early crumb microstructure [18,19].

Spinach (*Spinacia oleracea*) purée contributes chlorophylls, carotenoids (lutein, β -carotene), predominantly insoluble fibers (plus a soluble fraction), vitamins and moderate phenolic levels [20], providing a green — yellow hues palette complementary to the red — violet elderberry spectrum without using of any synthetic colorants. Cellular inclusions and fibers may dissipate localized mechanical stress during baking/cooling, this way potentially supporting acceptable chewiness without increasing perceived density [21].

Literature gaps still persist: many GF enrichment studies use pigment extracts or dried powders, or examine a single botanical kind while processing texture or color in isolation [22]. Integrated evidence is scarce for two fresh, unpasteurized plant purées with complementary pigment spectra — under-utilized *S. nigra* and accessible *S. oleracea* — applied at graded inclusion levels (15–35%) in a neutral *S. oryzoidum* matrix with

concurrent evaluation of early instrumentally tested texture (6 h/24 h TPA), measuring of colour by CIELAB (including Δ over 24 h), descriptive sensory profile and consumer acceptability within the initial freshness window.

The objective of this study was to determine whether fresh elderberry and spinach purées, used at 15–35% inclusion levels, can simultaneously improve early crumb texture and broaden natural color diversity in sorghum-based gluten-free bread. Novel contributions include: (a) dual valorization of a spontaneous local resource and a widely accessible horticultural species as unpasteurized whole purées; (b) establishment of a bidirectional natural color platform (red–violet vs. green — yellow) within a neutral sorghum matrix; (c) integrated multidimensional (texture — color — sensory) assessment focused on the early staling window; and (d) orientation toward reducing reliance on added colorants and auxiliary functional ingredients consistent with clean-label objectives.

2. Materials and methods

The study compared nine bread recipes: one wheat-based bread (WB) used as reference sample, two gluten-free control sample formulations based on sorghum flour [S-GFB and S-GFB+CS], and six gluten-free variants enriched with graded levels (15, 25, 35% w/w of flour basis) of *S. nigra* or *S. oleracea* purée. All bread samples were prepared at laboratory scale in the Department of Food and Nutrition at the Technical University of Moldova under identical processing conditions, as described in Section 2.2.

Instrumental evaluations (Texture Profile Analysis and CIELab color measurement) and sensory assessments were performed at 6.0 ± 0.5 h (after complete cooling to 23 ± 1 °C) and 24.0 ± 0.5 h post-baking. The fruits of *S. nigra* were manually harvested at full ripeness (September 2024) from wild stands in Florești district (Republic of Moldova). After removal of the pedicels, the fruits were rinsed with potable water, drained, and homogenized using a laboratory blender, then passed through a 0.5 mm mesh stainless-steel sieve to remove the seeds. Fresh leaves of *S. oleracea* were purchased in September 2024 from the local growers at the central agricultural market. After rejecting damaged leaves, they were washed, drained, and blended with potable water at a 4:1 ratio (leaf mass: water, w/w) to obtain a smooth, particle-free purée. The purées were used immediately after preparation, unpasteurized, without freezing or storage longer than 1 h.

2.1. Sample formulation

The detailed compositions of all samples (wheat-based reference sample, gluten-free control samples based on sorghum flour, and purée-fortified gluten-free variants) are presented in the Table 1, expressed both as grams per batch and baker’s percentage (values in parentheses; flour basis = 100 g total cereal solids). The nine formulations comprised WB (wheat-based reference sample), S-GFB (sorghum-based gluten-free control sample), S-GFB+CS (sorghum-based gluten-free dilution control samples with corn starch), and six purée-fortified variants prepared with

Table 1. Formulation of control and gluten-free breads enriched with *S. nigra* or *S. oleracea* purée: absolute amounts per batch (g) and baker’s percentages (g (%), values in parentheses = baker’s % w/w relative to 100 g total cereal solids)

Таблица 1. Рецепт контрольного образца хлеба и безглютенового хлеба, обогащенного пюре из *S. nigra* или *S. oleracea*: абсолютное количество добавок на партию (г) и пекарский процент (г (%), значения в скобках = пекарский процент в весовом выражении относительно 100 г общего содержания сухих зерновых веществ)

Ingredients	WB	S-GFB	S-GFB+CS	S-GFB+SN 15%	S-GFB+SN 25%	S-GFB+SN 35%	S-GFB+SO 15%	S-GFB+SO 25%	S-GFB+SO 35%
Water	120 (60%)	200 (100%)	200 (100%)	220 (110%)	200 (100%)	200 (100%)	190 (95%)	180 (90%)	170 (85%)
Sour cream (20% fat)	70 (35%)	70 (35%)	70 (35%)	70 (35%)	70 (35%)	70 (35%)	70 (35%)	70 (35%)	70 (35%)
<i>S. nigra</i> purée	0 (0%)	0 (0%)	0 (0%)	30 (15%)	50 (25%)	70 (35%)	0 (0%)	0 (0%)	0 (0%)
<i>S. oleracea</i> purée	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	30 (15%)	50 (25%)	70 (35%)
Sugar	10 (5%)	10 (5%)	10 (5%)	10 (5%)	10 (5%)	10 (5%)	10 (5%)	10 (5%)	10 (5%)
Dry yeast	4 (2%)	4 (2%)	4 (2%)	4 (2%)	4 (2%)	4 (2%)	4 (2%)	4 (2%)	4 (2%)
Salt	3 (1.5%)	3 (1.5%)	3 (1.5%)	3 (1.5%)	3 (1.5%)	3 (1.5%)	3 (1.5%)	3 (1.5%)	3 (1.5%)
Xanthan gum	0 (0%)	4 (2%)	4 (2%)	4 (2%)	4 (2%)	4 (2%)	4 (2%)	4 (2%)	4 (2%)
Psyllium powder	0 (0%)	4 (2%)	4 (2%)	4 (2%)	4 (2%)	4 (2%)	4 (2%)	4 (2%)	4 (2%)
Baking powder	0 (0%)	3 (1.5%)	3 (1.5%)	3 (1.5%)	3 (1.5%)	3 (1.5%)	3 (1.5%)	3 (1.5%)	3 (1.5%)
Wheat flour	200 (100%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Sorghum flour	0 (0%)	200 (100%)	100 (50%)	100 (50%)	100 (50%)	100 (50%)	100 (50%)	100 (50%)	100 (50%)
Corn starch	0 (0%)	0 (0%)	100 (50%)	100 (50%)	100 (50%)	100 (50%)	100 (50%)	100 (50%)	100 (50%)
Total (g)	407	498	498	548	548	568	518	528	538

Legend: WB = wheat-based bread (reference sample); S-GFB = sorghum-based gluten-free bread (gluten-free control sample); S-GFB+CS = sorghum-based gluten-free bread with added corn starch (dilution degree control sample); SN15, SN25, SN35 = S-GFB+CS formulations containing 15, 25, and 35% *S. nigra* purée, respectively; SO15, SO25, SO35 = S-GFB+CS formulations containing 15, 25, and 35% *S. oleracea* purée, respectively. Percentages are expressed on flour basis.

graded levels (15, 25, and 35 %, w/w of flour basis) of *S. nigra* (SN15, SN25, SN35) or *S. oleracea* (SO15, SO25, SO35).

To ensure process consistency, the amount of added water was adjusted according to volume of purée adding and moisture contribution in order to maintain comparable dough consistency across formulations. Accordingly, added water varied inversely with added purée volume, particularly for the higher-moisture *S. oleracea* purée (in this recipe the water volume was accordingly reduced to maintain target dough consistency). The ingredients common to all recipes (2 % dry yeast, 5 % sugar, 1.5 % salt, 2 % xanthan gum, 2 % psyllium powder, 1.5 % baking powder; baker's %) remained unchanged. Each recipe was prepared in triplicate, with one batch per day over three consecutive days, each batch representing an independent experimental unit. Ingredients were weighed using a precision scales (model XE PLUS-600R, CAS Corporation, China).

2.2. Technological process

The dry phase (flour, starch, salt, sugar, yeast, hydrocolloids, baking powder) and the liquid phase (water, sour cream, vegetable purée) were homogenized separately, then combined and mixed for 2.0 min at speed 3 (medium) using an Electrolux Speedy Mixer (model SPEEDY250, 0.25 kW, Electrolux, Italy), maintaining a total solids-to-water ratio of 1:0.85. The final dough (batter) temperature was $27.0 \pm 1.0^\circ\text{C}$.

The resulting batter (approximately 450 g per loaf) was poured into non-stick metal loaf pans (internal dimensions $20 \times 10 \times 8$ cm; length \times width \times height) to rest for 15–20 minutes at $20\text{--}22^\circ\text{C}$ (bench rest/pre-fermentation). The main fermentation was carried out for 70–80 minutes (until visible volume increased by approximately 80–90%) in a UNOX Rossela TM proofer (XF193-B, UNOX S.p.A., Italy) at $30\text{--}35^\circ\text{C}$ and 70–85% relative humidity.

Baking was performed in a UNOX Rossela convection oven (model XFT193, UNOX S.p.A., Italy) in two stages: 30 minutes at 175°C followed by 30 minutes at 180°C , without steam injection. The loaves were considered fully baked when the internal crumb temperature reached $96.0 \pm 1.0^\circ\text{C}$, then the loaves were cooled on the racks to $23\text{--}25^\circ\text{C}$ (approximately 90 minutes) before analytical and sensory measurements.

2.3. Instrumental analysis

Texture (TPA). Crumb texture profile analysis was performed using a TA.HD Plus C texture analyser (Stable Micro Systems, UK). Crumb cubes ($20 \times 20 \times 20$ mm), taken from the central area of three independent loaves (three cubes per loaf), were tested at $23 \pm 1^\circ\text{C}$ ($n=9$). Each sample underwent double compression to 50% deformation using a cylindrical probe P/36R (\varnothing 36 mm), with a test speed of 1 mm/s for all phases (pre-test, test, and post-test). The following parameters were obtained from the force-time curves: hardness, adhesiveness, springiness, gumminess, and chewiness. Cohesiveness was used only as an intermediate factor in these calculations and therefore is not tabulated. Measurements were performed at 6 ± 0.5 h (day 1, after complete cooling) and 24 ± 0.5 h (day 2) after baking. Results are presented as mean \pm standard deviation.

Colour (CIELab). The L^* , a^* , and b^* parameters of the crumb were recorded using a CR-400 colorimeter (Konica Minolta, Japan), with D65 illuminant and 8 mm aperture. The instrument was calibrated before each session using the manufacturer-supplied white calibration plate (BC-10). Two 10-mm crumb slices were taken from each of three independent loaves (3×2), giving $n=6$ colour readings per formulation per time point. The total colour difference between day 1 and day 2 was calculated following the standard ISO 11664-4:2019 [23]:

$$\Delta E_{ab}^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}, \quad (1)$$

where ΔL^* , Δa^* , and Δb^* are the differences between the corresponding mean values at the two time points. Perceptibility thresholds adopted for ΔE_{ab}^* were: ≤ 1 imperceptible; 1–3.5 slightly perceptible; 3.5–6 clearly visible; > 6 highly noticeable.

2.4. Sensory analysis

Quantitative Descriptive Analysis (QDA). Descriptive evaluation was conducted in accordance with the standard ISO 13299:2016 [24]. Testing booths, lighting conditions (D65, 1000 lx), and serving procedures complied with ISO 8589:2007 [25]. The panel consisted of 12 trained assessors (6 women, 6 men; aged 20–50), selected based on triangle tests (selection threshold $\geq 80\%$ correct identifications) and repeatability verification (non-significant within-one-assessor variance, $p > 0.05$). Training was carried out in four 90-minute sessions using reference samples to define three texture attributes: bite firmness, springiness, and chewiness. The intensity of each attribute was rated using an 11-point category scale (0 = “very low”, 10 = “very high”) with discrete integer scores and anchored descriptors at 0, 5, 10.

Cubic crumb samples ($20 \times 20 \times 20$ mm) were presented in randomized monadic order, coded with three-digit numbers, at $23 \pm 1^\circ\text{C}$ under D65 lighting (1000 lx). Each sample was evaluated at two time points (6 ± 0.5 h and 24 ± 0.5 h post-baking; day 1 and day 2) to assess early texture evolution (one evaluation per an assessor per formulation per time point; $n=12$ replicates per formulation per time point). Water and unsalted crackers were provided as palate cleansers; a 60–90 s interval was introduced between trying the samples.

Hedonic consumer test. Acceptability was assessed in accordance with ISO 11136:2020 [26,27]. The study involved 100 untrained consumers (38 following a gluten-free diet for ≥ 6 months and 62 without dietary restrictions), aged 20–60 years, recruited voluntarily based on written informed consent, with the possibility to withdraw at any time. Samples (25 g slices, coded with three-digit numbers) were served at $23 \pm 1^\circ\text{C}$ in individual booths. Each participant received five samples per session, presented in a Williams Latin balanced design using an incomplete balanced block plan across two sessions (day 1 and day 2). Appearance, oral texture, taste, and overall acceptability were rated on a 9-point hedonic scale (1 = dislike extremely; 9 = like extremely).

The study was approved by the Ethics Committee of the Technical University of Moldova. The study protocol complied with the Declaration of Helsinki and received ethics clearance as stated in the Institutional Review Board Statement.

2.5. Statistical data analysis

Raw data sets were verified and recorded in Microsoft Excel 365 (Microsoft Corporation, Redmond, WA, USA), and then statistically analyzed using GraphPad Prism 10.5 (GraphPad Software LLC, San Diego, CA, USA). For each instrumental and sensory variable, the mean and standard deviation (mean \pm SD) were calculated; the significance level was set at $\alpha=0.05$ (two-tailed criterion). Normality of residuals was checked by Shapiro-Wilk test and homogeneity of variances by Levene's test; where assumptions were not violated, parametric tests were retained. Instrumental texture and colour data were evaluated using two-way ANOVA with factors “Formulation” (9 levels) and “Time” (day 1 vs day 2) plus their interaction. QDA attributes (bite firmness, springiness, chewiness) were analyzed similarly. Hedonic attributes were analyzed by two-way ANOVA. Planned polynomial contrasts (linear, quadratic) were applied to graded purée levels (15–25–35%). Correlations between textural, chromatic, and sensory parameters were estimated using Pearson's correlation coefficient; the resulting matrices were represented as heatmaps (non-significant cells were left uncoloured, $p > 0.05$). Integration of instrumental and sensory variables was performed by Principal Component Analysis (PCA) on standardized data (mean = 0, SD = 1). The first two principal components explained 73% of the total variance on day 1 and 67% on day 2, consistent with the results reported. Loadings with absolute value ≥ 0.60 were considered salient.

Results

3.1. Texture indices

Two-way ANOVA revealed significant main effects of formulation and storage time factors, as well as a significant formulation \times storage time factors interaction, for hardness, adhesiveness, gumminess, and chewiness ($p < 0.0001$ for all). At 6 h post-baking (day 1), crumb hardness ranged from 578.9 ± 3.1 g in the wheat-based reference sample (WB) to $1,487.0 \pm 2.5$ g in the S-GFB+SN (25%) formulation. Among gluten-free (GF) samples, the S-GFB+SO (35%) variant exhibited the lowest hardness (608.9 ± 2.6 g), although it remained significantly higher than WB ($p < 0.05$). Relative to WB, hardness was approximately 144% higher in the sorghum control sample (S-GFB) and 157% higher in S-GFB+SN (25%). Gumminess and chewiness followed the same general pattern as hardness, with the highest values consistently observed for S-GFB+SN (25%), whereas the lowest values were recorded for WB or S-GFB+SO (35%), depending on the additive and storage time (Table 2).

After 24 h of storage (day 2), hardness increased in all samples, with absolute increases ranging from 488.0 g in WB to $1,129.5$ g in S-GFB+SN (25%). The relative ranking of formulations remained unchanged, with S-GFB+SN (25%) remaining the firmest and S-GFB+SO (35%) as the softest GF variant. On the day 2, S-GFB+SO (35%) showed a hardness of $1,098.1 \pm 3.6$ g, corresponding to approximately 56% lower hardness than the sorghum control sample. Gumminess and chewiness also increased after storage and largely mirrored the hardness pattern across formulations, being consistent with progressive crumb firming during post-baking storage.

3.2. Color parameters

Two-way ANOVA revealed significant main effects of formulation and storage time, as well as a significant formulation \times storage time interaction, for crumb L^* , a^* , and b^* coordinates ($p < 0.0001$ for all effects, except

Table 2. Instrumental Texture Profile Analysis (TPA) of control and gluten-free breads fortified with *S. nigra* or *S. oleracea* purée (hardness, adhesiveness, gumminess, chewiness at 6 h (Day 1) and 24 h (Day 2) post-baking)

Таблица 2. Инструментальный анализ профиля консистенции (TPA) контрольного образца хлеба и безглютенового хлеба, обогащенного пюре из *S. nigra* или *S. oleracea* (черствость, липкость, клейкость, разжевываемость через 6 часов (день 1) и 24 часа (день 2) после выпечки)

Sample	Hardness (g) Day 1	Hardness (g) Day 2	Adhesiveness (g-s) Day 1	Adhesiveness (g-s) Day 2	Gumminess (g) Day 1	Gumminess (g) Day 2	Chewiness (g) Day 1	Chewiness (g) Day 2
WB	578.9±3.1 ^a	1066.9±4.1 ^a	-507.6±4.0 ^a	-1143.0±5.0 ^b	303.3±2.8 ^a	643.3±4.7 ^b	345.9±3.0 ^b	600.6±5.0 ^b
S-GFB	1410.9±2.1 ^h	2480.0±5.0 ^h	-1089.0±3.0 ^h	-2751.1±5.4 ⁱ	559.4±4.6 ^e	961.6±5.9 ^g	724.8±4.8 ^g	867.0±5.3 ^f
S-GFB+CS	797.6±4.9 ^f	1435.7±4.3 ^f	-724.8±3.9 ^e	-1292.0±4.6 ^f	427.5±4.1 ^c	832.7±4.5 ^f	470.4±3.8 ^e	832.7±4.5 ^e
S-GFB+SN15	741.1±4.5 ^d	1339.0±4.1 ^d	-735.5±3.6 ^f	-1205.0±4.8 ^d	422.7±3.3 ^c	776.6±4.3 ^d	425.9±2.9 ^c	776.6±4.3 ^c
S-GFB+SN25	1487.0±2.5 ⁱ	2616.5±2.5 ⁱ	-1338.0±3.4 ⁱ	-2643.0±3.1 ^h	862.5±4.2 ^g	1918.0±3.6 ⁱ	862.5±4.2 ^h	1766.0±3.8 ^h
S-GFB+SN35	730.0±2.5 ^c	1281.5±2.5 ^c	-657.0±4.1 ^c	-1067.2±3.9 ^a	423.4±4.0 ^c	717.6±4.1 ^c	423.4±4.0 ^c	597.4±3.9 ^b
S-GFB+SO15	984.0±2.2 ^g	1771.0±3.1 ^g	-885.6±4.0 ^g	-1594.0±4.4 ^g	570.7±4.3 ^f	1027.0±4.6 ^h	570.7±4.3 ^f	1027.0±4.6 ^g
S-GFB+SO25	774.0±2.6 ^e	1393.0±2.9 ^e	-696.6±4.2 ^d	-1254.3±4.2 ^e	448.9±4.0 ^d	807.9±4.1 ^e	448.9±4.0 ^d	807.9±4.1 ^d
S-GFB+SO35	608.9±2.6 ^b	1098.1±3.6 ^b	-537.5±4.1 ^b	-1161.2±4.8 ^c	378.3±3.7 ^b	539.1±4.4 ^a	333.9±3.5 ^a	570.0±4.4 ^a

Legend: WB=wheat-based bread (reference sample); S-GFB=sorghum-based gluten-free bread; CS=corn starch; SN=*S. nigra* purée; SO=*S. oleracea* purée; 15, 25, 35=purée inclusion level (% flour basis).

Note: Values are mean±SD (n=9; 3 loaves×3 central crumb cubes). For each textural parameter, means within the same column bearing different superscript letters differ significantly among formulations at p<0.05 according to Tukey's multiple-comparisons test following two-way ANOVA. Negative adhesiveness values denote detachment work.

storage time for *b*^{*}, p=0.0004). At 6 h post-baking (day 1), crumb lightness (*L*^{*}) ranged from 53.87±0.13 for S-GFB+SN (25%) to 78.00±0.14 for S-GFB+CS, compared with 64.96±0.14 for WB and 75.32±0.33 for S-GFB (Table 3).

The addition of elderberry purée decreased *L*^{*} values and shifted *a*^{*} coordinates toward positive values (red-violet hues), whereas spinach purée increased *b*^{*} values (yellow tones) and shifted *a*^{*} toward more negative values (greenish hues). On the day 1, all formulations differed significantly in *L*^{*}. For *a*^{*}, S-GFB and S-GFB+CS were statistically comparable, whereas for *b*^{*}, S-GFB+SN (25%) and S-GFB+SN (35%) did not differ significantly.

After 24 h of storage (day 2), formulation-dependent changes were observed in crumb colour coordinates and total color difference. WB showed the highest increase in lightness ($\Delta L^* = 8.07 \pm 0.26$) and the largest total color difference ($\Delta Eab = 8.32 \pm 0.10$), indicating a highly noticeable crumb color change during storage. In contrast, S-GFB presented a slight decrease in lightness ($\Delta L^* = -1.50 \pm 0.22$) and a much smaller total colour difference ($\Delta Eab = 2.22 \pm 0.18$). Among elderberry-enriched bread variants, the largest variation occurred in S-GFB+SN (25%) ($\Delta L^* = 6.13 \pm 0.28$; $\Delta Eab = 6.45 \pm 0.17$), whereas S-GFB+SN (35%) showed the greatest colour stability ($\Delta Eab = 0.50 \pm 0.33$). Spinach-enriched bread formulations showed lower total colour differences, with ΔEab values ranging from 1.22 to 3.17, corresponding to slightly perceptible changes. On the day 2, all formulations continued to differ significantly in *L*. For *a*^{*}, WB and S-GFB+CS were statistically comparable, whereas for *b*^{*}, S-GFB+SN (25%) and S-GFB+SN (35%) again did not differ significantly. Overall, storage induced formulation-dependent changes in crumb colour. Compared with WB and S-GFB+SN (25%), spinach-containing breads generally exhibited lower total colour differences during storage, although the highest colour stability was observed for S-GFB+SN (35%).

Table 3. CIELAB crumb color parameters at 6 h (Day 1) and 24 h (Day 2) and total color difference (ΔE^*ab) in control and gluten-free breads fortified with *S. nigra* or *S. oleracea* purée

Таблица 3. Параметры цвета хлебного мякиша по шкале цвета CIELAB через 6 часов (день 1) и 24 часа (день 2), а также общая разница в цвете (ΔE^*ab) в контрольном образце хлеба и безглютеновом хлебе, обогащенном пюре из *S. nigra* или *S. oleracea*

Sample	<i>L</i> [*] D1	<i>a</i> [*] D1	<i>b</i> [*] D1	<i>L</i> [*] D2	<i>a</i> [*] D2	<i>b</i> [*] D2	ΔL^*	Δa^*	Δb^*	ΔE^*ab
WB	64.96±0.14 ^e	-1.71±0.48 ^d	17.88±0.37 ^f	73.03±0.35 ^f	-1.77±0.21 ^d	19.90±0.32 ^f	8.07±0.26	-0.06±0.18	2.02±0.23	8.32±0.10
S-GFB	75.32±0.33 ^h	-0.80±0.44 ^e	15.68±0.39 ^e	73.82±0.31 ^g	-0.73±0.34 ^e	17.31±0.45 ^e	-1.50±0.22	0.07±0.31	1.63±0.16	2.22±0.18
S-GFB+CS	78.00±0.14 ⁱ	-1.00±0.30 ^e	14.00±0.49 ^d	76.21±0.38 ^h	-2.02±0.17 ^d	15.09±0.19 ^d	-1.79±0.48	-1.02±0.12	1.09±0.34	2.33±0.38
S-GFB+SN15	45.00±0.40 ^c	11.00±0.48 ^f	7.00±0.18 ^c	47.80±0.20 ^c	11.31±0.32 ^f	7.69±0.48 ^c	2.80±0.47	0.31±0.36	0.69±0.22	2.90±0.21
S-GFB+SN25	33.87±0.13 ^a	14.81±0.16 ^h	5.14±0.42 ^a	40.00±0.26 ^b	13.00±0.22 ^g	6.00±0.26 ^a	6.13±0.28	-1.81±0.16	0.86±0.18	6.45±0.17
S-GFB+SN35	37.51±0.16 ^b	13.90±0.43 ^g	5.47±0.42 ^a	38.00±0.10 ^a	14.00±0.21 ^h	5.50±0.25 ^a	0.49±0.15	0.10±0.15	0.03±0.26	0.50±0.33
S-GFB+SO15	70.00±0.18 ^g	-3.00±0.19 ^c	25.00±0.21 ^g	71.00±0.27 ^e	-2.80±0.33 ^c	22.00±0.26 ^g	1.00±0.41	0.20±0.42	-3.00±0.29	3.17±0.19
S-GFB+SO25	68.00±0.32 ^f	-4.50±0.10 ^b	28.00±0.41 ^h	69.00±0.49 ^d	-4.80±0.23 ^b	27.00±0.40 ^h	1.00±0.44	-0.30±0.11	-1.00±0.15	1.45±0.12
S-GFB+SO35	66.57±0.40 ^d	-6.07±0.19 ^a	31.56±0.20 ⁱ	67.74±0.14 ^c	-6.42±0.49 ^a	31.53±0.44 ⁱ	1.17±0.33	-0.35±0.36	-0.03±0.35	1.22±0.39

Legend: WB=wheat-based bread (reference sample); S-GFB=sorghum-based gluten-free bread; S-GFB+CS=sorghum GF bread with added corn starch; SN15/SN25/SN35=S-GFB with 15/25/35% *S. nigra* purée; SO15/SO25/SO35=S-GFB with 15/25/35% *S. oleracea* purée (percentages on flour basis).

Note: Values are mean±SD (n=6 colour readings per formulation per time point; 3 loaves×2 slices). For *L*^{*}, *a*^{*}, and *b*^{*} coordinates, means within the same column bearing different superscript letters differ significantly among formulations at p<0.05 according to Tukey's multiple-comparisons test following two-way ANOVA. ΔL^* , Δa^* , Δb^* , and ΔE^*ab denote day 2 – day 1 differences. Perceptibility thresholds (ΔE^*ab): <1 imperceptible; 1–3.5 slightly perceptible; 3.5–6 clearly visible; >6 highly noticeable.

Table 4. Quantitative Descriptive Analysis (QDA) texture attributes for the bread samples
Таблица 4. Количественный описательный анализ (КОА) параметров консистенции образцов хлеба

Sample	Bite firmness Day 1	Bite firmness Day 2	Springiness Day 1	Springiness Day 2	Chewiness Day 1	Chewiness Day 2
WB	4.50±0.30 ^{de}	6.80±0.60 ^b	5.60±0.55 ^{ab}	4.90±0.40 ^{bc}	5.00±0.50 ^{ab}	7.10±0.70 ^{ab}
S-GFB	5.40±0.45 ^{bc}	7.30±0.65 ^a	5.80±0.50 ^{ac}	5.10±0.45 ^b	5.40±0.55 ^{abc}	7.90±0.65 ^{bc}
S-GFB+CS	4.70±0.55 ^{cde}	6.40±0.55 ^c	5.70±0.45 ^{ab}	5.80±0.55 ^a	5.10±0.70 ^{ab}	7.30±0.60 ^{ab}
S-GFB+SN 15%	4.80±0.40 ^{cd}	6.20±0.50 ^{cd}	5.90±0.50 ^a	4.70±0.45 ^{bc}	5.60±0.65 ^{bc}	7.80±0.60 ^{bc}
S-GFB+SN 25%	5.20±0.50 ^{bc}	7.40±0.65 ^a	5.90±0.55 ^a	4.70±0.65 ^{bc}	5.70±0.55 ^c	7.90±0.60 ^{bc}
S-GFB+SN 35%	4.30±0.45 ^e	5.90±0.55 ^d	5.50±0.60 ^{ab}	4.80±0.45 ^{bc}	6.10±0.65 ^d	6.90±0.60 ^a
S-GFB+SO 15%	4.30±0.50 ^e	6.00±0.60 ^d	5.30±0.45 ^b	4.90±0.45 ^{bc}	5.40±0.55 ^{abc}	7.40±0.55 ^{ab}
S-GFB+SO 25%	4.50±0.55 ^{de}	6.10±0.55 ^{cd}	5.70±0.50 ^{ab}	5.10±0.50 ^{bc}	5.00±0.60 ^a	7.00±0.65 ^a
S-GFB+SO 35%	5.80±0.50 ^a	7.60±0.70 ^a	5.40±0.55 ^{bc}	4.60±0.50 ^c	5.60±0.60 ^{bc}	8.20±0.65 ^c

Legend: WB = wheat-based bread (reference sample); S-GFB = sorghum-based gluten-free bread; CS = corn starch; SN = *S. nigra* purée; SO = *S. oleracea* purée; numerals (15, 25, 35) = % purée (flour basis).

Note: Values are mean ± SD ($n = 12$; one evaluation per assessor per formulation per time point). Within each column and for each sensory attribute, means bearing different superscript letters differ significantly among formulations at $p < 0.05$ according to Tukey’s multiple-comparisons test following two-way ANOVA.

3.4. Hedonic test – overall acceptability

Two-way ANOVA revealed significant effects of formulation, storage time, and formulation × time factors interaction on overall hedonic acceptability ($p = 0.0007$ for interaction; $p < 0.0001$ for formulation and time). On the day 1, all gluten-free breads achieved mean scores ≥ 7.0 , comparable to or slightly higher than the wheat control (WB: 6.70 ± 0.70). The highest scores were observed in S-GFB+SN (15%) (7.40 ± 0.70) and S-GFB+SN (35%) (7.50 ± 0.72), whereas WB remained among the lowest-rated samples. Although numerical differences among formulations did not exceed 0.80 points, Tukey’s test indicated significant differences between selected pairs of the samples (Table 5).

Table 5. Overall hedonic acceptability of the bread samples (9-point scale: 1 = dislike extremely; 9 = like extremely). Values are mean ± SD ($n = 100$ consumer)

Таблица 5. Общая гедонистическая приемлемость образцов хлеба (по 9-балльной шкале, где: 1 = крайне не нравится; 9 = крайне нравится). Значения представлены как среднее ± стандартное отклонение ($n = 100$ потребителей)

Sample	Day 1	Day 2
WB	6.70±0.70 ^{ab}	5.90±0.80 ^a
S-GFB	7.00±0.75 ^{abc}	6.20±0.85 ^{ab}
S-GFB+CS	7.10±0.78 ^{bc}	6.10±0.88 ^{ab}
S-GFB+SN 15%	7.40±0.70 ^c	6.00±0.90 ^{ab}
S-GFB+SN 25%	7.20±0.74 ^{bc}	6.20±0.88 ^{ab}
S-GFB+SN 35%	7.50±0.72 ^c	6.80±0.85 ^c
S-GFB+SO 15%	7.20±0.76 ^{bc}	6.40±0.88 ^b
S-GFB+SO 25%	7.30±0.75 ^{bc}	6.50±0.90 ^{bc}
S-GFB+SO 35%	7.10±0.79 ^{bc}	6.30±0.92 ^b

Legend: WB = wheat-based bread (reference sample); S-GFB = sorghum-based gluten-free bread; CS = corn starch; SN = *S. nigra* purée; SO = *S. oleracea* purée; numerals (15, 25, 35) = % purée (flour basis).

Note: Values are mean ± SD ($n = 100$ individual ratings per sample per day). Within each column, means bearing different superscript letters differ significantly among formulations at $p < 0.05$ according to Tukey’s multiple-comparisons test following two-way ANOVA. Hedonic scores were recorded on a 9-point scale (1 = dislike extremely; 9 = like extremely).

On the day 2, overall acceptability decreased in all samples by 0.70 to 1.40 points. The smallest reduction was recorded for S-GFB+SN (35%) (final score 6.80 ± 0.85), which remained the highest-rated variant, whereas WB showed the lowest value (5.90 ± 0.80). Despite this decline, all gluten-free formulations retained scores at or above 6.0, remaining within the acceptable hedonic range.

3.5. Correlation between instrumental parameters and sensory perception

Day 1. Pearson correlation coefficients (formulation means, $n = 9$) indicated a compact instrumental texture cluster: hardness, gumminess, and instrumental chewiness were strongly inter-correlated ($r = 0.90 - 0.99$, $p \leq 0.001$), while adhesiveness correlated inversely with these parameters ($r = -0.96$ to -0.99 , $p < 0.001$). Bite firmness (QDA) showed a weak, non-significant association with hardness ($r = 0.31$, $p > 0.05$). Overall acceptability showed a non-significant trend with chewiness (QDA) ($r = 0.63$, $p = 0.07$) and no association with springiness ($r = 0.06$, $p > 0.05$). Only the high inter-correlations within instrumental parameters and their inverse relation with adhesiveness were statistically robust (Figure 1).

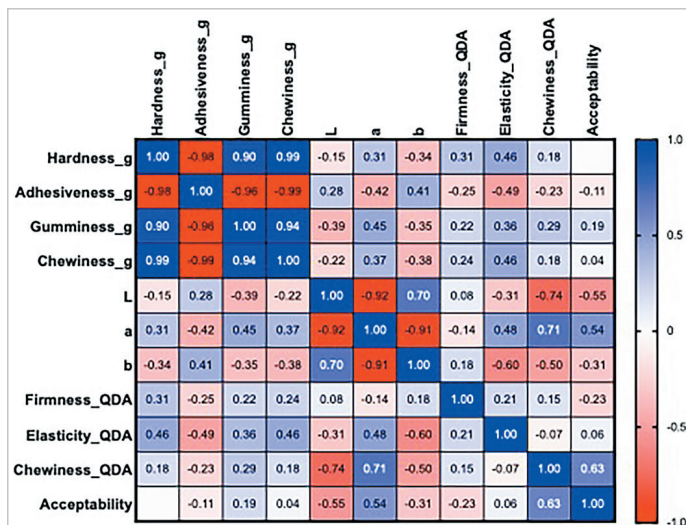


Figure 1. Pearson correlation matrix of instrumental texture, crumb colour, sensory texture attributes, and overall acceptability of bread samples on the day 1

Legend: Pearson correlation matrix of instrumental texture parameters (hardness, adhesiveness, gumminess, instrumental chewiness), crumb colour coordinates (L^* , a^* , b^*), sensory texture attributes [bite firmness, springiness, and chewiness assessed by Quantitative Descriptive Analysis (QDA)], and overall hedonic acceptability of bread samples on the day 1. Cell values represent Pearson correlation coefficients (r) calculated using formulation means ($n = 9$). Blue indicates positive correlations and red indicates negative correlations; color intensity is proportional to the absolute value of the correlation coefficient ($|r|$). White cells denote non-significant correlations ($p > 0.05$)

Рисунок 1. Матрица корреляций Пирсона инструментально измеренных характеристик консистенции, цвета мякиши, органолептических характеристик консистенции хлеба и общей приемлемости образцов хлеба в 1-й день

Day 2. After 24 h, the instrumental texture cluster persisted, with gumminess and instrumental chewiness remaining extremely inter-correlated ($r = 0.99$), while hardness retained a strong but attenuated association with gumminess and instrumental chewiness ($r = 0.81 - 0.84$). Adhesiveness continued to correlate inversely very strongly with hardness ($r = -0.98$), though its inverse links to gumminess and instrumental chewiness weakened ($r = -0.73$ to -0.76). Bite firmness (QDA) showed only a weak, non-significant correlation with hardness ($r = 0.39$), indicating that storage did not increase alignment between perceived and instrumental firmness. Overall acceptability showed weak, non-significant negative associations with bite firmness and chewiness (QDA) ($r = -0.41$ to -0.43). This pattern may indicate a tendency toward better acceptance of formulations retaining lower perceived firmness and chewiness after 24 h, but the associations were not statistically significant. Correlations with colour coordinates remained weak or absent (Figure 2).

PCA. On Day 1, PC1 (48%) represented a firmness-adhesiveness axis (positive loadings ≥ 0.60 for hardness, gumminess, instrumental chewiness, bite firmness (QDA); strong negative for adhesiveness), while PC2 (25%) was mainly associated with variation in a^* , overall acceptability, and springiness (QDA) (Figure 3a).

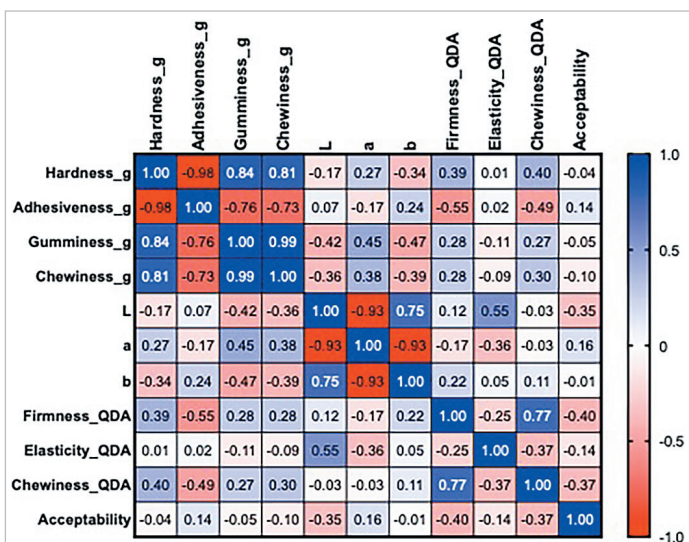


Figure 2. Pearson correlation matrix of instrumental texture, crumb colour, sensory texture attributes, and overall acceptability of bread samples on the day 2

Legend: Pearson correlation matrix of instrumental texture parameters (hardness, adhesiveness, gumminess, instrumental chewiness), crumb colour coordinates (L^* , a^* , b^*), sensory texture attributes [bite firmness, springiness, and chewiness assessed by Quantitative Descriptive Analysis (QDA)], and overall hedonic acceptability of bread samples on the day 2. Cell values represent Pearson correlation coefficients (r) calculated using formulation means ($n=9$). Blue indicates positive correlations and red indicates negative correlations; color intensity is proportional to the absolute value of the correlation coefficient ($|r|$). White cells denote non-significant correlations ($p>0.05$).

Рисунок 2. Матрица корреляций Пирсона инструментально измеренных характеристик консистенции, цвета мякиша, органолептических характеристик консистенции хлеба и общей приемлемости образцов хлеба во 2-й день

On the day 2, the first two components explained 67% of variance (PC1=41%, PC2=26%). PC1 remained dominated by the instrumental axis (gumminess and instrumental chewiness highest; hardness moderate; adhesiveness negative), with bite firmness (QDA) loading modestly, indicating limited convergence of instrumental and perceived firmness after storage. PC2 captured residual sensory-color variation; overall acceptability was positioned away from the high-chewiness/high-firmness region, in line with its weak negative associations with sensory firmness and chewiness (Figure 3b).

4. Discussions

This study shows that the partial replacement of the solid phase in sorghum-based gluten-free bread with fresh integral *S. nigra* and *S. oleracea* purées enables the simultaneous achievement of clear chromatic differentiation and the maintenance of consumer acceptability after 24 h in the gluten-free bread formulations. The principal contribution lies in demonstrating that minimally processed, unpasteurised plant purées can act as multifunctional clean-label ingredients while modulating instrumental texture in a formulation-dependent manner [11,28].

4.1. Texture and color: Integrated interpretation

The strong clustering of instrumental parameters (hardness, gumminess, chewiness) and their inverse correlation with adhesiveness confirms the mechanical interdependence already reported in other hydrocolloid-rich gluten-free matrices [3]. The inclusion of purées did not disrupt this structure; rather, it modulated the magnitude of textural responses in a formulation-dependent manner, with several purée-containing breads showing reduced firmness relative to the entirely sorghum control sample. This attenuation is consistent with mechanisms previously reported for vegetable or fibre ingredients, namely water retention mediated by dietary fibre and partial dilution of the starch-protein fraction [7,29].

From a chromatic perspective, *S. nigra* shifted the crumb towards positive a^* coordinates (red-violet hues), while *S. oleracea* induced shifts towards more negative a^* and higher b^* values (green-yellow tones), thus generating two distinct visual identities without deterioration of hedonic scores [7,29]. The relatively low ΔE^* values observed in spinach variants suggest greater short-term pigment stability, whereas the marked increase in lightness observed in the WB control and in S-GFB+SN (25%) indicates more pronounced colour evolution during storage. Such changes likely reflect moisture redistribution and starch realignment processes,

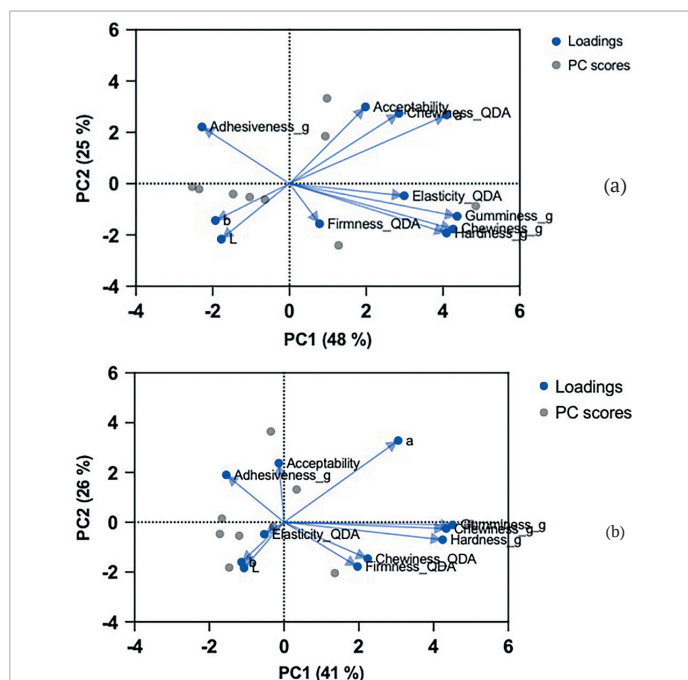


Figure 3. PCA biplots of instrumental, sensory, colour, and hedonic variables

Legend: (a) Day 1; (b) Day 2. Arrows indicate variable loadings and points indicate the scores of the nine bread formulations. Variables included instrumental texture parameters, sensory QDA attributes, crumb colour coordinates (L^* , a^* , b^*), and overall hedonic acceptability. Variance explained by PC1/PC2 was 48%/25% for the day 1 and 41%/26% for the day 2.

Рисунок 3. Двойные диаграммы переменных инструментальных, органолептических, цветových и гедонических значений анализа основного компонента (PCA)

which phenomena are described in early staling studies of gluten-free and composite breads [7,30]

4.2. Correlation with sensory perception and acceptability

The weak correspondence between perceived bite firmness and instrumental resistance parameters indicates that oral perception of structure is influenced by additional factors not fully captured by TPA, such as pore distribution, local moisture, bolus formation, and hydrocolloid-water interactions. This pattern is consistent with previous findings in gluten-free breads [31].

Overall acceptability scores remained high on the day 1 and decreased moderately after 24 h, a trend consistent with early staling mechanisms involving moisture redistribution and starch retrogradation. Despite the increase in instrumental firmness during storage, consumer ratings for all gluten-free formulations remained at or above the acceptance threshold, suggesting that these early texture changes were not sufficient to impair liking within the evaluated time frame. Among the formulations, the 35% elderberry-enriched bread variant combined pronounced visual differentiation with the highest hedonic score after 24 h, indicating good short-term consumer liking retention. In contrast, spinach purée-enriched bread contributed to colour diversification with only limited reductions in acceptability, suggesting that consumers tolerated natural chromatic shifts as long as overall sensory quality remained within an acceptable range [21,32].

4.3. Limitations and future directions

This study has several limitations. First, the temporal window was restricted to 24 h, which does not cover the full staling kinetics of gluten-free bread [30]. Second, the correlation analysis was based on a limited number of formulation means ($n=9$), thus reducing statistical power and requiring cautious interpretation of moderate coefficients [33]. Third, no rheological or microstructural data (e.g., dynamic oscillatory tests, imaging) were collected, and no water-state analyses (DSC or low-field NMR) were performed, so the proposed mechanisms remain inferential only [34,35]. Fourth, the use of unpasteurised purées did not allow assessment of microbiological stability or long-term pigment retention [36,37]. Finally, post-baking nutritional parameters, such as dietary fibre content and residual anthocyanins or carotenoids, were not measured [38].

Future studies should extend the observation period beyond 24 h, ideally to 48–72 h, to track firmness and color dynamics trajectories together with starch retrogradation kinetics [30,39]. Additional analyses should include rheology and quantitative imaging (X-ray micro-CT, SEM,

digital image analysis) to better relate crumb microstructure to textural evolution [34,35], as well as DSC and low-field NMR to assess water redistribution. Chromatographic monitoring (HPLC-DAD, LC-MS) of anthocyanins, chlorophylls, and carotenoids would further clarify pigment stability [36,38]. Moreover, validation using a dedicated celiac consumer panel, alongside a general consumer cohort, would strengthen the translational relevance of the sensory findings [31,40].

5. Conclusion

Fresh whole *S. nigra* and *S. oleracea* purées added to the recipes demonstrated potential as multifunctional clean-label ingredients in sorghum-based gluten-free bread. Their inclusion provided clear bidi-

rectional chromatic differentiation (red-violet vs. green-yellow) while maintaining consumer acceptability within 24 h after the breads baking. The bread with added 35 % of elderberry puree achieved the most favourable balance between distinctive color and hedonic retention, whereas spinach-containing bread formulations contributed to visual diversification without compromising consumer liking.

The partial decoupling observed between instrumental TPA metrics and perceived bite firmness highlights the need for future studies with inclusion of microstructural and rheological analyses. Overall, these findings support the potential use of fresh, unpasteurised plant purées as a dual strategy for natural color enrichment and short-term textural modulation in gluten-free bread.

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