



Production of protein-enriched bread through the incorporation of the black soldier fly (*Hermetia illucens*) larvae

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Abstract

The aim of this study was to produce protein-enriched bread using the black soldier fly larvae (*Hermetia illucens*) powder with the addition of ascorbic acid and mono- and diglycerides. In total, 11 bread formulations were produced using fixed amounts of wheat flour (100%), salt (2%), yeast (3%), black soldier fly larvae powder (15%), and variable amounts of mono- and diglycerides (0–0.8%), ascorbic acid (0–200 ppm), and water (50–70%), according to a 2³ factorial design. Black soldier fly larvae powder showed high protein (42%, dry weight) and lipid levels (29%, d.w.). Enriched breads were darker and exhibited enhanced nutritional quality, with a 48% increase in the protein content, a 21 times increase in the lipid content, and a 1.5 times increase in the ash content. Samples with 60% and 70% water had comparable or better values for the parameters analyzed (baking loss, specific volume, crumb structure, and hardness) when compared with a control bread. The incorporation of 15% of black soldier fly larvae powder in wheat breads improved its nutritional quality while maintaining acceptable technological parameters when used in combination with the mono- and diglycerides and the ascorbic acid.

Keywords: edible insects; response surface; additive; texture; factorial design.

Practical application: The study developed represents a practical application of the black soldier fly powder in the production of wheat breads, showing the percentage of wheat flour substitution, formulation, and additives used, along with the impact of the edible insect on the bread's quality, indicating, in the end, the formulations that provided the best products. These results are useful for professionals and researchers trying to develop and commercialize such type of product.

1 INTRODUCTION

The global population is projected to hit 9.7 billion by 2050 (United Nations Department of Economic and Social Affairs, 2022), fueling increased demand for food and proteins, driven by income growth and dietary shifts toward dairy and meat products (FAO, 2018). However, conventional food production methods have severe environmental repercussions like soil degradation, biodiversity loss, and increased greenhouse gas emissions (IRENA & FAO, 2021), necessitating the search for alternative food sources. Edible insects, notably black soldier fly larvae (BSFL), offer a promising solution. The BSFL is rich in protein, lipids, fiber, and essential nutrients (Ferdousi et al., 2022; Gao et al., 2019), with lower environmental impacts compared to traditional protein sources (Miglietta et al., 2015; Oninx & De Boer, 2012). Despite their nutritional benefits, reluctance persists in Western societies due to cultural perceptions (Poortvliet et al., 2019). Incorporating insects into processed foods could mitigate this aversion while enhancing nutritional and sensory qualities (Sun-Waterhouse et al., 2016). Various insect-based products like bread (Da Rosa Machado & Thys, 2019; De Oliveira et al., 2017), pasta (Duda et al., 2019),

and sausages (Scholliers et al., 2020) have been developed to address this challenge, with bread being the most common product for this due to the low cost and wide consumption worldwide. However, incorporating insect powder into bread can affect gluten formation and quality (Defloor et al., 1993), requiring the use of additives like oxidants, emulsifiers, and enzymes. Therefore, this study aimed to produce insect protein-enriched bread using BSFL powder with the additives ascorbic acid and mono- and diglycerides, employing a factorial design of experiments. This research underscores the potential of insect-based foods to address global food security challenges while emphasizing the importance of understanding processing techniques to optimize product development.

2 MATERIALS AND METHODS

2.1 Chemical reactants and ingredients

Wheat flour (Panfácil, Brazil), dried yeast (Fleischmann, United States), and salt (Cisne, Brazil) were purchased from local stores in Porto Alegre (Brazil). Ascorbic acid and mono- and diglycerides were donated by the company Vallens (Farrroupilha,

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Brazil). Calcium propionate was purchased from Adicel, Brazil. Dehydrated BSFL (*H. illucens*) was purchased from Agrin Criação e Comércio de Insetos (Avaré, Brazil). Chemical reagents were purchased from the manufacturers Dinâmica Química (Brazil), Êxodo Científica (Brazil), Labsynth (Brazil), and Neon Reagentes Analíticos (Brazil).

2.2 BSFL powder

Dehydrated BSFL were ground in a Wiley mill (Thomas Scientific, Model N02, United States), packed in plastic bags, and kept frozen at -10°C until used.

2.3 BSFL powder characterization

The BSFL powder chemical composition was analyzed according to the AOAC official methods (Latimer & AOAC International, 2016). Crude protein content was assessed by the Kjeldahl method (991.20) multiplying by a 6.25 factor. Non-protein nitrogen (NPN) was determined as described by DeVries et al. (2017) with slight modifications; briefly, 10 g of BSFL powder was mixed with 20 g of trichloroacetic acid, filtered, and analyzed by the Kjeldahl method. Fat content was determined using petroleum ether in Soxhlet extractor equipment (920.39). Moisture content was quantified by the thermogravimetric method (925.10). Ash content was determined in a muffle at 550°C (900.02). Carbohydrates were calculated by difference.

To correct protein levels taking into account the NPN, true protein was determined by Equation 1:

$$\text{True protein} = (\text{Crude protein nitrogen} - \text{NPN}) \times 6.25 \quad (1)$$

Water- and oil-holding capacities of BSFL powder were determined as described by Kabirullah and Wills (1982) and expressed as grams of water or oil per gram of powder.

2.4 Microbiological analysis

The following microbiological analyses were carried out: total aerobic colony count (08:2015) and yeasts and molds (21:2015) according to the APHA methods (apud Salfinger & Tortorello, 2015). *Salmonella* spp. (ISO 6579-1:2017) and coagulase-positive staphylococci (ISO 6888-1:1999/Amd 1:2003) were analyzed by ISO methods (apud Latimer & AOAC International, 2016). Enterobacteriaceae (presumptive) (AOAC 2003.1:2016) and *Escherichia coli* (AOAC 991.14:2016) were counted using 3M™ Petrifilm™ plates according to AOAC (apud Salfinger & Tortorello, 2015).

2.5 Breadmaking process and experimental design and data analysis

Notably, 11 insect bread formulations were produced, each containing 15% BSFL powder based on wheat flour. These formulations included varying amounts of mono- and diglycerides, ascorbic acid, and water. Additionally, a wheat control (W), a wheat control with additives (WA), a whole wheat control (WW), and a whole wheat control with additives (WWA) were also included in the study. Samples B1–B11 represent the insect breads and were developed according to a full factorial design (three factors, two levels, and three replicates in the center point) to study the effect of the amount of water, mono- and diglycerides, and ascorbic acid in the bread quality parameters. Table 1 displays the bread formulations.

Breads were produced by mixing and kneading all ingredients in a breadmaker machine (Britânia, Multipane, Brazil). The dough was divided into four parts, each weighing 180 g, rounded in a spherical shape, passed two times through a cylinder (Arke, LEV-30, Brazil), and then the dough was rolled, forming the shape of a loaf of bread, placed into metallic baking tins, and then into a proofer (Venâncio, Crescepão, Brazil) at 30°C for 1 h. The doughs were baked in an electric oven (Tedesco, FTT 150E, Brazil) for 15 min at 180°C. Then the breads

Table 1. Control and insect bread formulations (% of wheat flour weight).

Sample	Wheat flour	Whole wheat flour	BSFL powder	Salt	Yeast	Mono-diglycerides	Ascorbic acid*	Water	Calcium propionate
W	100	0	0	2	3	0	0	60	0.2
WA	100	0	0	2	3	0.8	100	60	0.2
WW	60	40	0	2	3	0	0	56.41	0.2
WWA	60	40	0	2	3	0.8	100	56.41	0.2
B1	100	0	15	2	3	0	0	50	0.2
B2	100	0	15	2	3	0.8	0	50	0.2
B3	100	0	15	2	3	0	200	50	0.2
B4	100	0	15	2	3	0.8	200	50	0.2
B5	100	0	15	2	3	0	0	70	0.2
B6	100	0	15	2	3	0.8	0	70	0.2
B7	100	0	15	2	3	0	200	70	0.2
B8	100	0	15	2	3	0.8	200	70	0.2
B9	100	0	15	2	3	0.4	100	60	0.2
B10	100	0	15	2	3	0.4	100	60	0.2
B11	100	0	15	2	3	0.4	100	60	0.2

BSFL: Black soldier fly larvae; W: wheat control; WA: wheat control with additives; WW: whole wheat control; WWA: whole wheat control with additives; B1–B11: BSFL breads with varying amounts of mono- and diglycerides, ascorbic acid, and water; *Values expressed in parts per million (ppm).

were removed from the pans and placed into a metal grid for cooling for 1 h.

The specific volume of all samples was determined, and then one bread of each formulation was randomly selected for image analysis, color determination, and texture profile analysis. The rest three breads from each formulation were vacuum sealed in plastic bags and stored at room temperature for texture analysis 7, 14, and 21 days after baking.

Statistical analyses were carried out using Statistica v12.0 (StatSoft Europe, Germany). The results were expressed as mean \pm standard deviation. One-way analysis of variance (ANOVA) and Tukey's test with a significance level of $p < 0.05$ were used to compare different samples. Response surfaces of each variable versus factors (mono- and diglycerides, ascorbic acid, and water) were generated.

2.6 Bread quality evaluation

Protein, lipid, and ash content were determined according to the same methods used for the BSFL powder. Moisture was determined through a moisture analyzer (BEL Engineering, Italy). The only difference between the insect bread formulations was the amount of ascorbic acid, mono- and diglycerides, and water; thus, their dry basis composition should be very similar; therefore, only the B9 sample was selected for chemical composition assessment.

The specific volume was measured using the rapeseed displacement method (method 10-05.01) (AACC, 2019) and expressed as specific volume (cm^3g^{-1}). The baking loss was determined as the difference between the weight of the dough before baking (W_{BF}) and after baking (W_{AB}) (Equation 2):

$$\text{Baking loss (\%)} = \frac{W_{BF} - W_{AB}}{W_{AB}} \times 100 \quad (2)$$

The internal structure of the crumb was analyzed using ImageJ version 1.53 (National Institutes of Health, USA) and the Otsu algorithm (Gonzales-Barron & Butler, 2006). Each sample was divided into four slices, and images of the slices were captured. The software's results were used to calculate the mean cell area (in mm^2), porosity (%), cells larger than 5 mm^2 (%), and cell density (in $\text{cells}\cdot\text{cm}^{-2}$). The crumb of the two central slices from each sample was subjected to texture profile analysis using a texture analyzer (Stable Micro Systems, TA-TX Plus, UK) with a 50 kg load cell and a 36-mm cylindrical aluminum probe. The test parameters were: pre-test speed: $3.0 \text{ mm}\cdot\text{s}^{-1}$; test speed: $2.0 \text{ mm}\cdot\text{s}^{-1}$; post-test speed: $2.0 \text{ mm}\cdot\text{s}^{-1}$ and compression to 50%. From the test results, hardness (g) and cohesiveness were determined (Marc, 2015).

The color of the crust and crumb of the breads and of the BSFL powder was determined by a chroma meter (Minolta, CR400, Japan) using the CIE $L^*a^*b^*$ color space. The difference between all the samples and W (ΔE_1) was determined. In addition, the difference between the samples in relation to WA (ΔE_2), the difference between the insect breads and WW (ΔE_3), and the difference between the insect breads and WWA (ΔE_4) were determined according to Equation 3 (Delgado-Nieblas et al., 2012):

$$\Delta E = \sqrt{(L_{\text{sample}}^* - L_{\text{control}}^*)^2 + (a_{\text{sample}}^* - a_{\text{control}}^*)^2 + (b_{\text{sample}}^* - b_{\text{control}}^*)^2} \quad (3)$$

3 RESULTS AND DISCUSSION

3.1 BSFL powder characterization

Table 2 presents the chemical composition of the BSFL powder. Results are similar to the ones reported in other studies involving this species (González et al., 2019; Mintah et al., 2020; Spranghers et al., 2017). Variations in the insect's chemical composition are expected, even when considering the same species, due to several factors related to its rearing such as feed, humidity, temperature, and photoperiod (Finke & Oonincx, 2014). Regardless of that, the BSFL exhibits high levels of protein and fat.

The water and oil holding capacities of BSFL powder ($1.58 \pm 0.05 \text{ g}_{\text{water}}/\text{g}_{\text{powder}}$ and $1.04 \pm 0.07 \text{ g}_{\text{oil}}/\text{g}_{\text{powder}}$) were 22.5% higher and 60.8% lower, respectively, than those of *Tenebrio molitor* ($1.29 \text{ g}_{\text{water}}/\text{g}_{\text{powder}}$ and $1.71 \text{ g}_{\text{oil}}/\text{g}_{\text{powder}}$) and considerably lower than those of *Acheta domesticus* ($2.16 \text{ g}_{\text{water}}/\text{g}_{\text{powder}}$ and $2.16 \text{ g}_{\text{oil}}/\text{g}_{\text{powder}}$) (Zielińska, 2022; Zielińska et al., 2018). When compared to lentil ($1.33 \text{ g}_{\text{water}}/\text{g}_{\text{powder}}$ and $0.93 \text{ g}_{\text{oil}}/\text{g}_{\text{powder}}$), which is often used to enrich baked products, the water and oil holding of BSFL powder are slightly higher (Du et al., 2014). These results are associated with the difference in the protein and fat content of the ingredients (*T. molitor*, *A. domesticus*, and lentil exhibited protein content, $\text{g}/100 \text{ g}$ (d.w.), of 52.35, 70.04, and 31.13 and fat content, $\text{g}/100 \text{ g}$, of 24.7, 20.00, and 1.26, respectively) (Zielińska et al., 2015).

3.2 Microbiological analysis

The microbial counts for BSFL powder met the European Union criteria on the safety of edible insects for most assays, except for yeasts and molds (Table 3). Da Rosa Machado and Thys (2019) and De Oliveira et al. (2017) found lower counts for yeast and molds for the cinereous cockroach (*Nauphoeta cinerea*) and cricket, respectively, both reared in Brazil. However, their counts also exceeded the 100 CFU/g limit, suggesting a need for improved manufacturing practices in insect rearing in Brazil and the establishment of national regulations.

Table 2. BSFL powder chemical composition (g/100 g).

Basis	Moisture	Crude protein	NPN	True protein	Lipid	Ash	Carbohydrates*
Wet basis	4.71 \pm 0.04	46.33 \pm 0.18	0.94 \pm 0.02	40.46 \pm 0.18	27.38 \pm 0.31	6.47 \pm 0.04	15.10
Dry basis	4.71 \pm 0.04	48.62 \pm 0.19	0.99 \pm 0.02	42.46 \pm 0.19	28.74 \pm 0.33	6.79 \pm 0.04	15.85

*Determined by difference; NPN: Non-protein nitrogen.

3.3 Bread quality parameters

3.3.1 Chemical composition

Compositions of the enriched and control breads are presented in Table 4. The inclusion of the insect powder resulted in an increase of 48 and 27% in protein content when compared to W and WW, respectively. De Oliveira et al. (2017) found similar results in replacing 10% of wheat flour with cinereous cockroach (*N. cinerea*) powder in breads. Besides protein, lipid content was also higher in the BSFL bread, showing a 20.6 and 9.3 times increase in comparison to W and WW, respectively. While ash exhibited 1.5- and 1.3-fold increments compared with the wheat and whole wheat breads content. These results were expected since the BSFL powder protein, lipid, and ash content are higher than wheat and whole wheat flour.

According to EU regulation no 1924/2006 (Regulation (EC) No 1924/2006 of the European Parliament and of the Council of 20 December 2006 on Nutrition and Health Claims Made on Foods (Directorate-General for Health and Food Safety, 2006), products where at least 20% of its total calories are provided by proteins can use the nutritional claim “high in protein.” The fiber content of the BSFL breads was not analyzed, so it is not possible to determine its exact caloric value; nonetheless, it is possible to estimate the bread’s maximum caloric value assuming that there is no fiber, that is, all carbohydrates are digestible. By doing this, it can be seen that, regardless of the fiber content, BSFL breads could be labeled “high in protein” since 21.1% of its energy value is provided by protein.

3.3.2 Specific volume, baking loss, and crumb structure

Table 5 shows baking loss, specific volume, and image analysis, while Figure 1 displays response surfaces for specific volume, baking loss, and pores > 5 mm². However, response surfaces for mean cell area, porosity, and cell density exhibited R² values below 0.80 and therefore were excluded (Supplementary Table 1). Regarding baking loss, samples with higher water content (B5, B6, B7, and B8) had higher values, while samples B9, B10, and B11 showed similar results to those of the controls, indicating that insect proteins might not absorb water as effectively as gluten proteins, or else, the baking loss of these three samples would be lower. Baking loss seemed to be mainly influenced by water content rather than the presence of insect powder, as shown in Figure 1a, where higher water content led to increased baking loss.

Samples with higher water content (B5, B6, B7, and B8) displayed higher specific volumes, with increases of 27.1, 28.5, 27.9, and 35.3% compared to W, and 24.9, 25.5, 24.9, and 32.1% compared to WWA. Water content significantly influenced volume, as shown in Figure 1b, with higher water leading to increased hydration of gluten proteins and more free water, which acts as a plasticizer (Parenti et al., 2021).

With respect to B9, B10, and B11, only B11 presented a higher volume than that of control breads, while B10 exhibited better values than the WW and WWA. González et al. (2019) found that the inclusion of *H. illucens* and *T. molitor* powder in wheat bread resulted in lower volume. However, this did not happen in the current study. These results may be attributed to the higher fat content in the BSFL breads. According to Pareyt et al. (2011), lipids help in incorporating air during mixing,

Table 3. BSFL powder microbiological analysis results and the parameters established by the European regulation for the insect species authorized for human consumption.

Microbiological criteria	BSFL powder	EU Regulation 2022/169 (European Commission, 2022)
Total mesophilic aerobic colony count (CFU/g)	1.0 × 10 ⁵	≤ 10 ⁵
Enterobacteriaceae (presumptive) (CFU/g)	< 10*	≤ 100
<i>Escherichia coli</i> (CFU/g)	< 10*	≤ 50
<i>Salmonella</i> spp	N.D. in 25 g	N.D. in 25 g
Coagulase staphylococci (CFU/g)	< 100*	≤ 100
Yeasts and molds (CFU/g)	1.0 × 10 ³ *	≤ 100

BSFL: Black soldier fly larvae; *Estimated value; N.D.: Not detected.

Table 4. Nutritional composition of wheat, whole wheat, and insect breads (on a dry and wet basis).

Sample	Moisture	Protein	NPN	True Protein	Lipid	Ash	Carbohydrates*
g/100 g							
Wet basis							
Wheat control	42.74	8.12 ± 0.11 ^c	–	–	0.13 ± 0.09 ^b	1.69 ± 0.13 ^b	47.32
Whole wheat control	42.90	9.42 ± 0.12 ^b	–	–	0.29 ± 0.04 ^b	1.87 ± 0.07 ^b	45.52
BSFL bread	41.81	13.19 ± 0.18 ^a	0.19 ± 0.00	12.01 ± 0.18	2.75 ± 0.31 ^a	2.53 ± 0.07 ^a	39.71
Dry basis							
Wheat control	74.64	14.19 ± 0.19 ^c	–	–	0.23 ± 0.16 ^b	2.95 ± 0.23 ^b	82.64
Whole wheat control	75.13	16.45 ± 0.22 ^b	–	–	0.51 ± 0.08 ^b	3.28 ± 0.13 ^b	79.72
BSFL bread	71.85	22.67 ± 0.30 ^a	0.32 ± 0.01	20.64 ± 0.30	4.73 ± 0.53 ^a	4.36 ± 0.11 ^a	68.25

*Carbohydrates calculated by difference; NPN: non-protein nitrogen; BSFL: black soldier fly larvae; Different letters within the same column represent significant differences ($p < 0.05$).

stabilizing gas cells during fermentation, and allowing for greater expansion of cells during baking, resulting in a higher volume.

Furthermore, the presence of mono- and diglycerides showed a positive effect on the specific volumes (Figure 1b). Although this type of emulsifier is not known for increasing volume, Garzón et al. (2018) investigated the effect of distilled monoglycerides on wheat bread aeration and found that it increased maximum dough volume during proofing, along with cell density in baked samples, which could result in greater volume.

In terms of crumb structure, WA had the smallest cell area, while WWA had the largest. Most samples, excluding WA, WW, WWA, and B8, showed similar values. For pores > 5 mm², samples with lower water content (WWA, B1, B2, B3, and B4) had the lowest values, with no significant differences among them. Samples with 70% water content (B5, B7, and B8) had the highest values. WWA and B5 had the highest porosity, followed by the other BSFL breads, which, except for B9, showed similar values. Excluding B9, all insect-containing breads had higher porosity than regular wheat bread (W). The lowest cellular density was in sample W, although among BSFL breads, only B1, B2, B9, and B10 were significantly higher.

Water content appears to have a greater effect on the percentage of pores > 5 mm² (Figure 1c). Increasing water content causes the dough viscosity to decrease, which allows greater expansion for the gas cells; however, this expansion tends to be irregular, leading to a less uniform crumb, with larger cell areas, and therefore a greater percentage of pores > 5 mm². The lower viscosity also increases the gas cell's mobility, which in turn favors coalescence of the bubbles, resulting in higher cell areas as well as lower porosity and cell density.

Ascorbic acid strengthens the gluten network, allowing gas cells to expand without rupturing (Cauvain, 2015). This results in smaller and more uniform crumb cells (Zghal et al., 2001).

These effects can be seen when comparing W to WA: the inclusion of ascorbic acid led to smaller means for cell area, a percentage of pores > 5 mm², and higher means for porosity and cell density. However, its effects on pores > 5 mm² were not observed consistently across insect-containing breads (Table 5). This discrepancy could be due to water having a strong effect on these parameters, outshining the ascorbic acid effect, or limitations in the analysis method using ImageJ software. Similar findings were reported by Franco et al. (2022) in whole wheat breads. The authors suggested that the presence of the bran, which has a dark color, could potentially cause optical effects. The dark color of BSFL powder (Section 3.3.4) might similarly affect image processing, impairing the interpretation of results.

3.3.3 Texture profile analysis

On day 0, B5, B6, B7, and B8 showed the lowest hardness (Table 6). There were 35.6, 16.5, 43.8, and 56.0% lower than the W control and 34.7, 15.3, 43.0, and 55.4% lower than the WW control, respectively. This trend continued to the 21st day, as these four samples remained the softest breads. Samples B9, B10, and B11 exhibited comparative values to the W, WA, and WW controls on day 0, and samples B10 and B11 showed lower means compared to all control breads from day 7 to the last day.

Water content had the highest effect on softening the insect breads (Figure 1d), as free water acts as a plasticizing agent in doughs. Also, the lower amount of water in samples B1–B4 probably is not sufficient to hydrate the gluten proteins and form a proper network, resulting in harder breads. Ascorbic acid also reduced hardness in the breads (Figure 1a), it oxidizes sulfhydryl groups forming disulfide bonds between gluten proteins, strengthening the network, which improves gas retention in the dough, contributing to a softer texture (Cauvain, 2015).

The most significant increase in bread hardness occurred within the initial 7 days, with breads becoming 198% harder on

Table 5. Baking loss, specific volume, and image analysis of the wheat, whole wheat, and insect breads.

Sample	Baking loss (%)	Specific volume (cm ³ .g ⁻¹)	Mean cell area (mm ²)	Pores > 5 mm ² (%)	Porosity (%)	Cell density (cell.cm ⁻³)
W (0; 0; 60)	9.3 ± 0.7 ^{cd}	2.62 ± 0.05 ^{cd}	1.9 ± 0.1 ^{bcd}	6.79 ± 1.44 ^{cd}	32.94 ± 5.16 ^e	17.33 ± 2.07 ^d
WA (0.4; 100; 60)	10.0 ± 0.9 ^{cd}	2.69 ± 0.10 ^c	1.4 ± 0.1 ^d	5.62 ± 0.59 ^{cd}	37.97 ± 2.12 ^{de}	27.49 ± 1.83 ^a
WW (0; 0; 56.41)	7.9 ± 0.7 ^{efg}	2.39 ± 0.12 ^{de}	1.8 ± 0.6 ^{cd}	6.44 ± 0.83 ^{cd}	37.27 ± 5.19 ^{de}	21.43 ± 3.56 ^{bcd}
WWA (0.4; 100; 56.41)	8.6 ± 0.4 ^{de}	2.30 ± 0.10 ^{ef}	2.9 ± 0.6 ^a	2.98 ± 0.48 ^e	55.76 ± 3.77 ^a	19.61 ± 2.43 ^{bcd}
B1 (0; 0; 50)	6.4 ± 0.4 ^h	1.77 ± 0.19 ^g	1.9 ± 0.1 ^{bcd}	4.38 ± 0.17 ^{de}	46.90 ± 0.04 ^b	24.70 ± 1.60 ^{ab}
B2 (0.8; 0; 50)	7.1 ± 0.3 ^{gh}	2.33 ± 0.15 ^{ef}	2.0 ± 0.2 ^{bcd}	4.41 ± 0.90 ^{de}	46.56 ± 1.74 ^b	23.80 ± 1.51 ^{abc}
B3 (0; 200; 50)	7.3 ± 0.6 ^{fgh}	2.07 ± 0.03 ^f	2.3 ± 0.0 ^{abc}	4.80 ± 1.09 ^{de}	48.13 ± 1.05 ^b	21.20 ± 0.51 ^{bcd}
B4 (0.8; 200; 50)	8.0 ± 0.4 ^{efg}	2.23 ± 0.09 ^{ef}	2.0 ± 0.2 ^{bcd}	5.02 ± 0.72 ^{cde}	44.57 ± 2.16 ^{bc}	22.68 ± 2.89 ^{abcd}
B5 (0; 0; 70)	12.4 ± 0.4 ^b	3.34 ± 0.16 ^a	2.3 ± 0.2 ^{abc}	9.44 ± 0.30 ^{ab}	44.99 ± 0.95 ^a	20.15 ± 1.89 ^{bcd}
B6 (0.8; 0; 70)	12.1 ± 0.7 ^b	3.37 ± 0.12 ^a	2.1 ± 0.2 ^{bcd}	7.36 ± 0.34 ^{bc}	45.41 ± 0.98 ^b	21.94 ± 1.55 ^{abcd}
B7 (0; 200; 70)	14.6 ± 0.3 ^a	3.36 ± 0.16 ^a	2.3 ± 0.1 ^{abc}	9.97 ± 1.34 ^a	41.89 ± 1.58 ^{bcd}	18.37 ± 0.92 ^{cd}
B8 (0.8; 200; 70)	12.7 ± 0.4 ^b	3.55 ± 0.10 ^a	2.6 ± 0.2 ^{ab}	9.29 ± 0.93 ^{ab}	44.31 ± 0.51 ^{bcd}	17.34 ± 0.74 ^d
B9 (0.4; 100; 60)	8.6 ± 0.4 ^{def}	2.63 ± 0.02 ^{cd}	1.6 ± 0.1 ^{cd}	6.60 ± 0.74 ^{cd}	37.50 ± 2.09 ^{cde}	24.02 ± 2.30 ^{abc}
B10 (0.4; 100; 60)	9.4 ± 0.3 ^{cd}	2.89 ± 0.11 ^{bc}	1.8 ± 0.2 ^{bcd}	6.43 ± 1.07 ^{cd}	43.28 ± 0.14 ^{bcd}	23.60 ± 3.17 ^{abc}
B11 (0.4; 100; 60)	10.3 ± 0.3 ^c	3.04 ± 0.10 ^b	2.0 ± 0.2 ^{bcd}	6.77 ± 0.59 ^{cd}	43.12 ± 1.59 ^{bcd}	22.15 ± 1.62 ^{abcd}

W: wheat control; WA: wheat control with additives; WW: whole wheat control; WWA: whole wheat control with additives; B1–B11: black soldier fly larvae breads; Values in parentheses represent the concentration of mono- and diglycerides, ascorbic acid, and water. Different letters within the same column represent significant differences ($p < 0.05$).

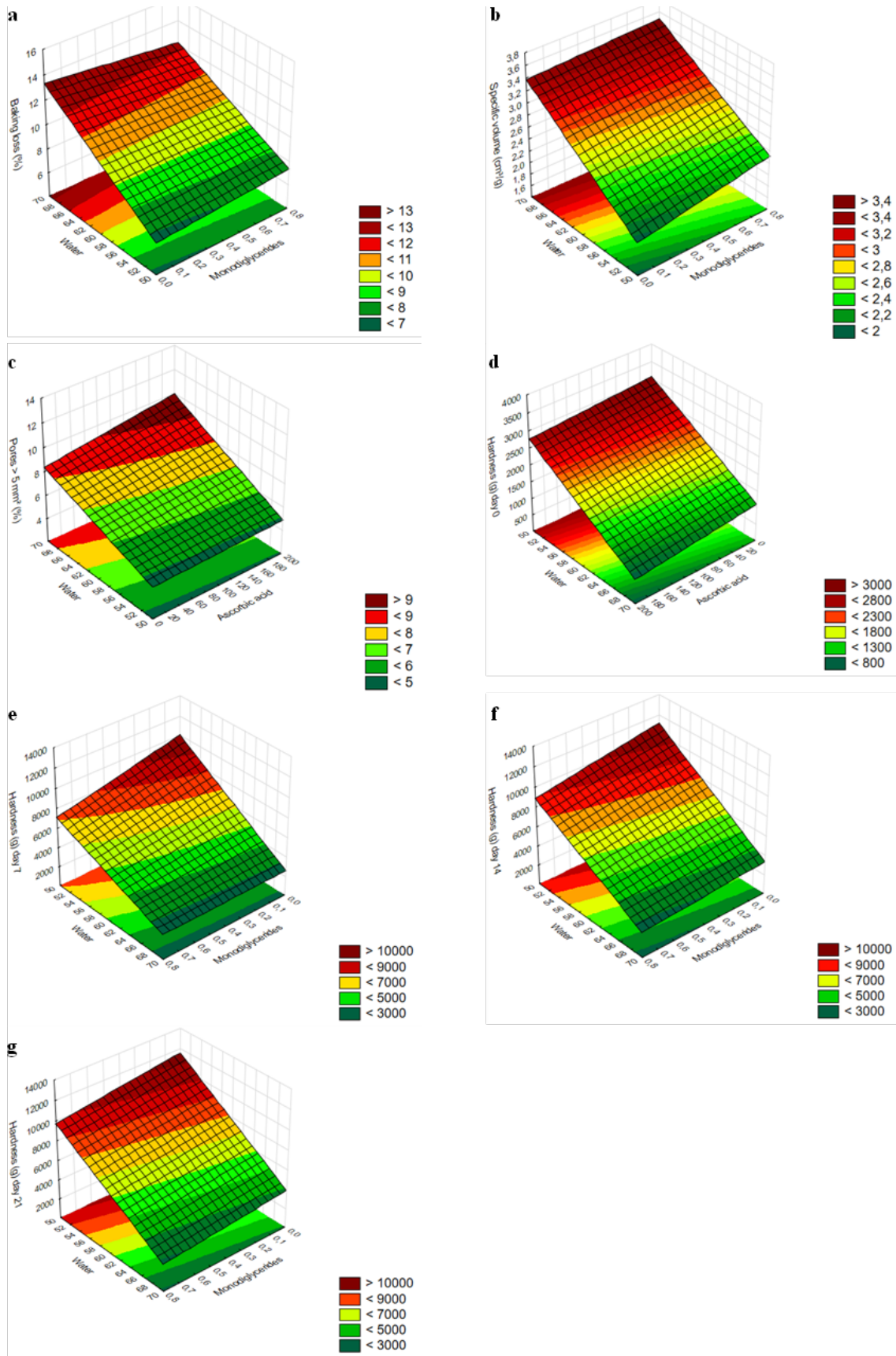


Figure 1. Response surfaces of (a) baking loss (%) for mono- and diglycerides x water; (b) specific volume (cm³.g⁻¹) for mono- and diglycerides x water; (c) pores > 5 mm² for ascorbic acid x water; (d) hardness in day 0 for ascorbic acid x water; (e) hardness in day 7 for mono- and diglycerides x water; (f) hardness in day 14 for mono- and diglycerides x water; (g) hardness day 21 for mono- and diglycerides x water.

Table 6. Hardness of the samples over time and the increase in hardness after 14 and 21 days of the fabrication.

Sample	0	7	14	21	Increase in 14 days (%)	Increase in 21 days (%)
W (0; 0; 60)	1,599.9 ± 1.3 ^{d EF}	4,430.7 ± 4.0 ^{c DE}	6,041.6 ± 295.1 ^{b FG}	7,040.2 ± 237.1 ^{a C}	277.6 ± 18.4 ^{BCD}	340.0 ± 14.8 ^A
WA (0.4; 100; 60)	1,716.2 ± 170.6 ^{c EF}	5,873.7 ± 76.7 ^{b CD}	7,443.9 ± 168.7 ^{a EF}	7,535.0 ± 328.8 ^{a C}	333.7 ± 9.8 ^{AB}	339.1 ± 19.2 ^A
WW (0; 0; 56.41)	1,578.4 ± 190.1 ^{d EF}	4,921.7 ± 267.2 ^{c EF}	7,895.1 ± 310.2 ^{a DEF}	6,397.2 ± 202.9 ^{b C}	400.2 ± 19.7 ^A	305.3 ± 12.9 ^A
WWA (0.4; 100; 56.41)	2,436.2 ± 105.7 ^{c D}	7,969.6 ± 355.6 ^{b B}	10,194.0 ± 967.4 ^{a ABC}	10,219.0 ± 311.5 ^{a B}	318.1 ± 32.7 ^{ABC}	319.5 ± 13.3 ^A
B1 (0; 0; 50)	3,376.0 ± 43.7 ^{b A}	10,779.0 ± 1160.3 ^{a A}	10,584.1 ± 354.1 ^{a AB}	12,412.7 ± 289.9 ^{a A}	213.5 ± 10.5 ^{CDE}	267.7 ± 8.6 ^{AB}
B2 (0.8; 0; 50)	2,905.2 ± 19.8 ^{c BC}	7,398.7 ± 303.1 ^{b BC}	9,853.4 ± 163.5 ^{ab BCD}	10,017.7 ± 1181.4 ^{a B}	239.2 ± 5.6 ^{BCDE}	244.8 ± 40.7 ^{AB}
B3 (0; 200; 50)	3,045.6 ± 172.1 ^{b AB}	10,181.6 ± 220.8 ^{a A}	12,270.5 ± 975.4 ^{a A}	11,204.8 ± 440.2 ^{a AB}	302.9 ± 32.0 ^{ABCD}	267.9 ± 14.5 ^{AB}
B4 (0.8; 200; 50)	2,617.5 ± 116.0 ^{c CD}	7,593.1 ± 441.0 ^{b B}	8,419.7 ± 267.3 ^{b CDE}	9,959.2 ± 516.1 ^{a B}	221.7 ± 10.2 ^{BCDE}	280.5 ± 19.7 ^A
B5 (0; 0; 70)	1,030.2 ± 12.2 ^{b GH}	3,409.5 ± 12.8 ^{a EFG}	4,300.5 ± 795.7 ^{a GHI}	4,164.1 ± 39.8 ^{a DEF}	317.4 ± 77.2 ^{ABC}	304.2 ± 3.9 ^A
B6 (0.8; 0; 70)	1,336.2 ± 12.7 ^{b FG}	3,589.2 ± 272.2 ^{a EFG}	3,348.2 ± 17.4 ^{a HI}	3,466.5 ± 243.9 ^{a F}	150.6 ± 1.3 ^E	159.4 ± 18.3 ^B
B7 (0; 200; 70)	899.0 ± 104.3 ^{c H}	2,561.2 ± 129.9 ^{b FG}	2,920.4 ± 398.9 ^{ab HI}	4,046.4 ± 421.0 ^{a EF}	224.9 ± 44.4 ^{BCDE}	350.1 ± 46.8 ^A
B8 (0.8; 200; 70)	704.7 ± 6.3 ^{b H}	2,285.0 ± 256.6 ^{a G}	2,307.7 ± 215.9 ^{a I}	2,634.0 ± 242.8 ^{a F}	227.5 ± 30.6 ^{BCDE}	273.8 ± 34.5 ^A
B9 (0.4; 100; 60)	1,798.4 ± 27.9 ^{c E}	4,989.7 ± 274.1 ^{b DE}	6,201.2 ± 245.4 ^{ab FG}	7,083.2 ± 812.5 ^{a C}	244.8 ± 13.6 ^{BCDE}	293.9 ± 45.2 ^A
B10 (0.4; 100; 60)	1,643.8 ± 96.5 ^{b EF}	4,387.6 ± 568.7 ^{a DE}	4,865.7 ± 479.1 ^{a GH}	6,206.0 ± 959.1 ^{a CD}	196.0 ± 29.1 ^{DE}	277.5 ± 58.3 ^A
B11 (0.4; 100; 60)	1,634.6 ± 59.3 ^{c EF}	4,177.8 ± 569.0 ^{b EF}	6,384.8 ± 88.0 ^{a EFG}	5,702.4 ± 706.9 ^{ab CDE}	290.6 ± 5.4 ^{ABCD}	248.9 ± 43.2 ^{AB}

W: wheat control; WA: wheat control with additives; WW: whole wheat control; WWA: whole wheat control with additives; B1–B11: black soldier fly larvae breads. Values in parentheses represent the concentration of mono- and diglycerides, ascorbic acid, and water; Different capital letters within the same column and different lowercase letters within the same row represent significant differences ($p < 0.05$).

average. B6 displayed the lowest increase over the first 14 days, while B10 also exhibited low hardening. Figures 1e, 1f, and 1g shows that the mono- and diglycerides reduced hardness on days 7, 14, and 21, thus aiding in maintaining softness in treated samples. These emulsifiers interact with amylose in wheat flour, delaying retrogradation, a process strongly linked to bread staling (Cauvain, 2015). Additionally, emulsifiers can coat starch granules, decreasing gelatinization and retrogradation, further contributing to bread softness (Purhagen et al., 2011).

Breads B9, B10, and B11 exhibited hardness similar to those of WA throughout the observed period. This similarity is noteworthy because the only distinction between these three insect-containing breads and WA was the inclusion of BSFL powder, suggesting that both insect proteins and fat played a role in reducing staling. Proteins may mitigate staling by diluting starch (Gray & Bemiller, 2003). Additionally, the high-fat content in insects could act as a shortening agent, known for its anti-staling effects through mechanisms such as coating gas cells, preventing moisture migration in the crumb, or interacting with BSFL and wheat flour lipids (Pareyt et al., 2011).

Cohesiveness is related to crumb integrity in bread and significantly influences its shelf life. Sample W showed the highest cohesiveness on day 0 (Supplementary Table 2). Regarding the BSFL breads, there is no clear tendency on how the difference in the formulations, i.e., water, mono- and diglycerides, and ascorbic acid, affected cohesiveness, although Franco et al. (2022) observed a slight increase in cohesiveness in whole wheat breads treated with ascorbic acid in comparison with untreated samples. Onyango et al. (2015) also found that ascorbic acid inclusion in wheat and wheat–maize breads affected cohesiveness, increasing it slightly, but this increase was not sufficient to compensate for the impact of wheat flour substitution by maize (10–30%), which was far greater, greatly reducing cohesiveness.

The inclusion of BSFL powder did seem to have an impact on cohesiveness, as on day 0, samples B2, B3, B7, B10, and B11 were less cohesive than the W control, while on day 7, samples B2, B3, B4, and B10 were less cohesive than W. Nonetheless, on days 14 and 21 all samples presented similar cohesiveness. Kowalski et al. (2022) produced breads enriched with buffalo worm (*Alphitobius diaperinus* P.), cricket (*Acheta domesticus* L.), and mealworm (*T. molitor*) and found a significant reduction in cohesiveness from 20% of replacement in wheat flour basis.

The observed results likely stem from disruptions in the gluten network. Gluten-free, non-starch, and high-fiber ingredients can negatively impact breadmaking by diluting gluten and impeding network formation (Villarino et al., 2016). Additionally, studies indicate that moisture content influences cohesiveness, with higher moisture levels generally leading to greater cohesiveness (Sugiura et al., 2017; Yamauchi et al., 2014). While water content in formulations and the presence of mono- and diglycerides likely affect cohesiveness, the validity of these relationships cannot be confirmed due to low R^2 values in the response surfaces (Supplementary Table 1).

3.3.4 Color

Color coordinates and images of the samples are presented in Supplementary Table 3. All BSFL breads presented lower luminosity than the W, WA, and WW controls, ranging from 48.00 to 60.78. Also, the results for a^* indicate that the color of the insect breads tends toward red, while the wheat controls' (W and WA) color tends toward green. BSFL powder had a dark color ($L = 21.47 \pm 1.04$) set toward red ($a^* = 1.62 \pm 0.23$) and yellow ($b^* = 7.53 \pm 0.72$). In general, the insect breads appear to reflect BSFL powder color, showing lower luminosity and higher a^* in comparison with the wheat breads.

All insect breads showed significant global color variation with the breads made with wheat or whole wheat; the greatest

difference was observed in relation to W ($\Delta E1$). Global variation was considerably lower for WWA color comparison ($\Delta E2$), which is positive, as this type of product, i.e., whole wheat breads, is already known by consumers and generally associated with healthiness.

4 CONCLUSIONS

Incorporation of BSFL powder enhanced the breads' nutritional composition, allowing a "high i nutrition" claim, without compromising most properties when the minimum amount of water to develop gluten was used. Samples from the center points (0.4% mono- and diglycerides, 100 ppm ascorbic acid, 60% water) showed properties comparable to those of the controls and therefore achieved the most satisfactory results. Besides, the inclusion of ascorbic acid and mono- and diglycerides had a positive effect on the quality of the enriched breads.

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