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Valorization of *Humulus lupulus* L. Byproducts in Cake Formulations

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ABSTRACT: Hop cultivation generates a significant amount of underutilized biomass, such as leaves and stems, as it primarily focuses on harvesting hop flowers (or cones). These byproducts may have nutritional potential. In this context, the chemical and proximate compositions of hop production byproducts were analyzed, and their incorporation into 15 cake formulations was studied according to the Box-Behnken experimental design. The results indicated that hop byproducts have a promising nutritional profile, due to the high concentrations of total fiber in the stem and leaf flour cakes (4.7% and 8.4% w/w, respectively), having high antioxidant activity, measured by DPPH and ABTS assays (46.4 and 21.1 mg/100 g, respectively) and total phenolic content (78.4 and 41.8 mg/100 g, respectively). Based on the results of the present study, the use of leaf and stem flour in bakery products shows potential for developing items with improved nutritional value and enhanced antioxidant capacity.

KEYWORDS: *hops, proximate composition, residue, Box-Behnken, circular economy*

INTRODUCTION

Food waste has been increasing over the past decade due to large-scale agricultural commodity production, resulting in organic waste that can be converted into food with high nutritional value.[1,2] Thus, aiming to meet the Sustainable Development Goals (SDGs), which include reducing food waste generated through small to large-scale agricultural production, as well as advancing innovative technologies, with the goal of adopting a linear food model for greater sustainability.[1] In this context, these actions advance responsible consumption and production by turning waste into value-added food ingredients.

In this scenario, residual materials from agricultural products, such as grape pomace (*Vitis vinifera*), retain up to 70% of bioactive compounds, including polyphenols, in addition to dietary fiber and unsaturated lipids, making them of great interest to the food industry, particularly for the production of pasta and bakery products.[3,4] Other sources, such as carrot pomace (*Daucus carota*) and olive pomace (*Olea europaea*), are sources of carotenoids, vitamins, minerals, phenolic content, antioxidants, and antimicrobial effects in the production of biscuits and other bakery products.[5−7] These innovations are ideal for addressing problems such as food shortages caused by waste, the search for more affordable food production methods, and the balance of supply and demand for foods rich in micro and macro nutrients.[2]

Hops (*Humulus lupulus* L.) are plants whose flowers, which are also known as cones, represent between 25 and 44.5% of the dry matter (DM) of their total biomass, and they are harvested to produce pellets that are added to beer.[8−13] This addition contributes to the beer's microbiological stability, flavor, and bitterness through the isomerization reaction of α-acids.[8−13] Furthermore, hops possess antimicrobial and antioxidant properties.[8−13] The remaining 63.8 to 79.2% (DM) of the plant biomass, consisting primarily of leaves (25 to 39%, DM) and stems (30.2 to 40.2%, DM), amounts to 10 to 15 tons per hectare annually and is typically discarded without being repurposed, contributing significantly to the global agricultural waste problem.[8−13]

According to the Food and Agriculture Organization (FAO), approximately 1.3 billion tons of agricultural residues are lost or wasted annually.[14] This highlights the importance of exploring alternatives for valorizing agricultural byproducts, such as hop leaves and stems, by leveraging their nutritional properties for the food industry and promoting a circular economy. This is especially important considering the increasing global production of hops and the resulting waste.[8,11,14,15] Furthermore, it can contribute to reducing food insecurity by developing new food products with essential nutritional characteristics for human consumption, as approximately 28.9% of the global population experienced moderate or severe food insecurity in 2023.[8,11,14,15]

The proximate composition of hop byproducts (leaf and stem) is still little explored in the literature, as they are generally treated as a single material, which makes it difficult to determine their real composition. When their proximate compositions are evaluated individually, they are generally used to promote animal feed and evaluate certain components

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(e.g., crude fiber, lipids, and protein), thereby lacking important information to assess their real application in human food.[16−20] Concerning this single material, it presents an important composition, in which it is constituted of dry matter (84.5 to 99.1%, w/w), ash (5.57 to 29.3%, w/w), protein (16.43 to 30.01%, w/w), crude fiber (8.16 to 46.75%, w/w), lipids (0.65 to 9.7%, w/w), sugars (4.97 to 7.87%, w/w), reducing sugars (2.05 to 5.74%, w/w), calcium (1.73 to 9.50%, w/w), potassium (1.28 to 2.66%, w/w), magnesium (0.44 to 0.86%, w/w), phosphorus (0.12 to 0.57%, w/w), iron (0.04 to 1.44%, w/w), manganese (0.03 to 0.38%, w/w), and zinc (0.002 to 0.01%, w/w), which can generally vary due to the variety, cultivation and edaphoclimatic factors.[16−22]

However, this has been changing over the past few years, with the literature evaluating these byproducts individually and from several perspectives: the leaf as a source of bioactive compounds,[11,23−25] the stem as a source of natural fiber,[8,26−28] and both as functional composites for sustainable food applications.[29−31]

Hop leaves have a distinct chemical composition characterized by a diverse range of phenolic compounds, including rutin, kaempferol-O-rutinoside, quinic acids (such as 3-Caffaoyl-quinic, 5-Caffaoylquinic, and Coumaroylquinic acid III), and derivatives of dihexose and hexose-pentose, as well as α- and β-acids. Despite being traditionally overlooked in beer production, hop leaves are increasingly recognized for their potential in various health and food industry applications.[11,15]

Hop stalks contain significant amounts of cellulose, lignin, starch, and pectins (such as galacturonan, rhamnann, and arabinan), which contribute to fiber cohesion. This composition indicates potential for diverse industrial applications, including the production of natural fibers and composite materials. In the food industry, these fibers can enrich products with low levels of insoluble dietary fiber, offering health benefits such as intestinal regularity and increased satiety. As a sustainable solution, this approach supports food security by transforming agricultural byproducts into functional ingredients, expanding access to nutritious foods, strengthening food systems, and promoting the circular economy.[8,17,32]

Food products such as cakes, cookies, and bread are consumed daily by the world's population.[33] However, these products are generally low in minerals and bioactive compounds, in addition to having a high glycemic index.[33,34] Therefore, the use of functional ingredients in products with low nutritional value has been increasing to reduce this deficiency by replacing wheat or incorporating them to enhance their nutritional value.[33,34] However, this implementation should be studied, as it may have negative effects on sensory properties, as wheat provides the gluten and structure to these products.[33]

Therefore, this study aimed to determine the chemical and proximate composition of hop byproducts (leaves and stems), optimize the optimal level of wheat flour replacement using a Box-Behnken experimental design, and then evaluate the proximate chemical, physical, and microbiological properties of cakes fortified with these alternative flours. The hypothesis of this work was to develop a baked good with higher nutritional values, including fiber, bioactive compounds, and minerals, while maintaining sensory acceptability through the incorporation of hop leaf and stem flours. This study aims to contribute to the achievement of the United Nations' second Sustainable Development Goal, which focuses on ending hunger, improving nutrition, and promoting sustainable food production and agriculture.

MATERIALS AND METHODS

Sample Preparation. Hop waste of the Comet variety was sourced from Fartura/SP, Brazil. The byproducts were initially dried in an oven (Tecnal, model TE-0851, Piracicaba, Brazil) with air circulation at 60 °C for 24 h, subsequently milled using a knife mill (Tecnal, model TE-631/4, Piracicaba, Brazil), and standardized to a 20-mesh particle size (with an aperture of 850 μm). There was no degreasing step for the byproducts before they were incorporated into the formulations. The materials used to make the cakes, including wheat flour, eggs, sugar, sunflower oil, whole milk, and yeast, were sourced in Piracicaba/SP, Brazil, from local markets.

Experimental Design. A Box-Behnken design was performed in triplicate, using the response surface methodology (RSM) to optimize the cake formulations incorporated with hop stems and leaves, and a proportional mixture of leaf and stem (50% and 50% (w/w), respectively) in cake formulations. The experimental design consisted of three factors and three levels, totaling 15 formulations, with the inclusion of three central points, evaluating the independent variables used for the planning response: the concentrations of leaf flour (0−20%), stem flour (0−20%), and flour with the mixture of leaf and stem (0−20%). For the dependent variables, the parameters of hardness, resilience, and chewiness were evaluated, as described in the Textural Profile Analysis. Since sensory feedback is fundamental to consumer acceptance and an important quality attribute, the use of Textural Profile Analysis provides important instrumental parameters for assessing the acceptability of incorporating hop residues into the cake structure.

Production of Cakes. The cakes were prepared with chicken egg (21 g), oil (22 g), sugar (50 g), milk (33 g), baking powder (2 g), wheat flour (25 to 45 g), leaf flour (0 to 10 g), stem flour (0 to 10 g), and mixed flour (0 to 10 g).

Wheat, leaf, stem, and/or mixed flour were used to prepare the cakes. The whole milk, previously beaten chicken eggs, sunflower oil, sugar, and baking powder were added and then mixed manually (5 min) until a homogeneous dough was obtained. For the baking stage, the cakes were placed in a rectangular nonstick cupcake pan measuring 25 cm × 26.5 cm with 12 cavities, each with a diameter of 6.5 cm and a height of 3.0 cm, in a conventional electric oven (Consul, model CFO4NAR, Joinville, Brazil). The oven was preheated to 180 °C with a power of 1,500 W. The cakes were baked for 30 min and then cooled to room temperature and stored in polyethylene plastic.

Textural Profile Analysis (TPA). The texture characteristics of the cake samples were obtained using a TA.XTplus Texture Analyzer (Godalming, United Kingdom) with a P/100 cylindrical probe, on which the cakes were placed. For TPA, 50% compression conditions, a pretest speed of 2.0 mm/s, a test speed of 1.0 mm/s, and a post-test speed of 10.0 mm/s were used, as determined using Exponent 6.2.2.0 software (Stable Micro Systems). The texture parameters recorded were: hardness, which is the maximum force during compression, indicating firmness; elasticity, which is the cake's ability to return to its original shape after deformation; cohesiveness, which reflects the internal integrity of the product; resilience, which measures the speed of recovery after initial deformation; gumminess, which represents the structural density formed during cooking; and chewiness, which is the force required to fragment the food during chewing.[35,36]

Analysis of Proximate Composition and Microbiological. The best formulations, 11 and 14 according to the Box-Behnken design, had their ash, moisture, lipids, proteins (conversion factor used was 6.25),[37] total dietary fiber (insoluble and soluble),[38] available carbohydrates, and energy value[39,40] content analyzed. Total sugar contents were quantified using the Somogyi-Nelson assay, with glucose as the analytical standard and spectrophotometric measurements at 540 nm.[41] Total coliforms and *Escherichia coli* were determined as proposed by Salfinger and Tortorello,[42] coagulase-positive *Staphylococcus* per Anvisa,[43] molds and yeasts as described by

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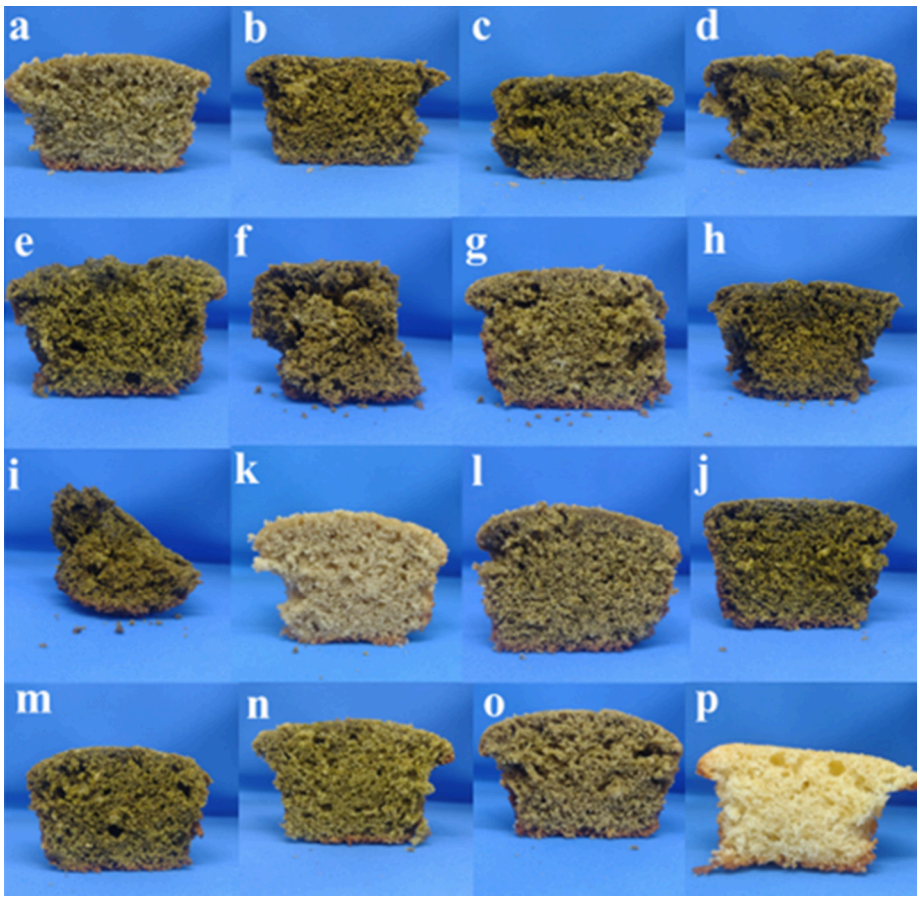

Figure 1. Cross-section of elaborate cakes, according to planning. Formulations 1- 15 (a − o) and (p) control cake

Collins et al.,[44] presumptive *Bacillus cereus* per Agriculture and Consumer Protection,[45] and *Salmonella* spp. in accordance with the European Commission[46] and Anvisa.[47]

Quantification of Phenolic Compounds and Antioxidants. *Dry Extract Preparation.* Methanol extracts were obtained by adding 1.00 g of cakes and 0.700 g of residues into 50 mL Falcon type tubes, followed by adding 25 mL of methanol. The mixtures were vortexed (1900 rpm, SCIENTIFIC INDUSTRIES, model Vortex-Genie 2, Bohemia, USA) and centrifuged (4000 rpm, QUIMIS, model Q222TM2, Diadema, Brazil) for 20 min each.[48]

Total Phenolic Compounds. The Folin-Ciocalteu method[49] was used to determine the total phenolic content in the residues and cake formulations. Absorbance was measured at 770 nm using a spectrophotometer (AGILENT, model Cary 60, Santa Clara, USA). The procedure involved adding 600 μL of the extract to 3000 μL of 10% (v/v) Folin-Ciocalteu reagent. After a 5 min reaction, 2250 μL of 4% (w/v) sodium carbonate solution was added. The mixture was stored at room temperature, protected from light, for 40 min before spectrophotometric measurements. A Gallic acid standard solution was used to construct a calibration curve for determining the total phenolic content.

Antioxidants. In the antioxidant analysis, free radical assays of DPPH (2,2-diphenyl-1-picrylhydrazyl) and ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) were performed. The DPPH analysis was performed according to the methods described by the authors Brand-Williams and Al-Duais.[50,51] A reaction mixture was prepared containing 1320 μL of the methanolic sample extract and 2680 μL of a 15 mmol/L DPPH solution in ethanol. After homogenization, the solution was kept in the dark at 25 °C for 45 min, and then the absorbance was read at 522 nm using a spectrophotometer (AGILENT, model Cary 60, Santa Clara, USA). A stock solution of Trolox standard (4 mmol L^{-1}) was used to construct the calibration curve.

The ABTS test was performed according to the method described in the work of Nenadis.[52] Thirty μL of the extract was pipetted into a 15 mL Falcon type tube, and 3.0 mL of the ABTS radical cation solution was added. After homogenization, the solution was incubated in the dark for 6 min, and then the absorbance was measured at 734 nm using a spectrophotometer (AGILENT, model Cary 60, Santa Clara, USA). A calibration curve was constructed using a 20 mmol L^{-1} Trolox standard solution.

Alpha Acids Analysis. The concentration of alpha acids in all samples was measured by lead conductance titration according to the European Brewery Convention (EBC) method 7.4.[53] For this purpose, a conductivity module 856 with a 5-ring conductivity measuring cell with a constant of 0.7 cm^{-1}, a rod stirrer 802, a dosing device Dosino 800, a 20 mL dosing unit, and a Titrando module 905, all from Metrohm (Herisau, Switzerland), were used. All devices were controlled by the tiamo v2.5 software (Metrohm). The extraction of alpha acids consisted of adding 3.0 g of the sample, ground and sifted, with 30 mL of toluene and shaking for 6 min at 200 rpm (QUIMIS, model Q225M, Diadema, Brazil) with subsequent vacuum filtration and storing in a dark bottle protected from light until the time of titration.

For the titration, 10 mL of the extract and 40 mL of methanol were added to a 100 mL beaker and stored in the equipment, with agitation and immersion of the doser and conductivity meter. Finally, the titration with the lead acetate solution (20 g/L), which was properly standardized with the sulfuric acid solution (0.05 mol/L), started until obtaining the result of the concentration of alpha acids expressed as LCV (lead conductance value) in g/g of sample, which was graphically monitored indicating the volume of lead acetate that corresponds to the equivalence.

Mineral Determination. A 2.0 g portion of each sample was accurately weighed and subjected to dry ashing in a muffle furnace at 550 °C for 4 h, or until a consistent white ash was obtained, indicating

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Table 1. Responses from the Analysis of Hardness, Elasticity, Cohesiveness, Resilience, Gumminess, and Chewiness of the Box-Behnken Experimental Design in the Cake Formulations

Experiments		Values decoded								
Formulation number	Order ofthe test	Leaf flour (%, w/w)	Stem flour (%, w/w)	Mixture of leaf/ stem (%, w/w)	Hardness (*N*)	Springiness	Cohesiveness	Resilience	Gumminess (*N*)	Chewiness (N.mm)
1	12	0	0	10	38.0	0.799	0.509	0.203	1,970.3	1,574.3
2	3	10	10	10	48.0	0.767	0.418	0.149	2,047.4	1,570.6
3	5	10	10	10	42.9	0.692	0.373	0.132	1,628.0	1,126.6
4	7	10	10	10	47.2	0.719	0.376	0.134	1,810.8	1,301.1
5	6	10	0	20	44.4	0.703	0.381	0.141	1,721.8	1,210.5
6	9	10	20	20	57.2	0.534	0.278	0.092	1,618.7	864.04
7	11	10	20	0	45.1	0.734	0.395	0.147	1,816.3	1,333.1
8	15	20	10	20	43.6	0.544	0.260	0.086	1,155.7	629.13
9	13	20	20	10	42.7	0.544	0.251	0.082	1,090.5	593.16
10	14	20	0	10	52.5	0.779	0.422	0.157	2,257.0	1,758.6
11	2	0	10	0	33.8	0.823	0.512	0.207	1,764.7	1,452.1
12	4	0	10	20	37.6	0.736	0.402	0.149	1,540.4	1,133.9
13	1	20	10	0	52.4	0.774	0.423	0.161	2,262.2	1,751.4
14	10	10	0	0	30.4	0.810	0.525	0.209	1,624.7	1,315.7
15	8	0	20	10	35.8	0.724	0.369	0.135	1,349.6	976.90
Control cake[a]		-	-	-	19.2	0.798	0.535	0.211	1,048.2	836.75

[a]The control formulation (without the addition of any residue flour) was prepared to compare the values obtained in the Box-Behnken experimental design.

complete mineralization. Subsequently, the ash was resuspended in 1.0 mL of concentrated nitric acid and 40 mL of 1.9 mol/L of hydrochloric acid, and then adjusted to a final volume of 50 mL with deionized water.[37] Phosphorus (P) content was determined using ammonium vanadomolybdate reagent and readings at 420 nm in a spectrophotometer (AGILENT, model Cary 60, Santa Clara, USA).[37] Sodium (Na, 589 nm) and potassium (K, 766 nm) contents were determined using a flame photometer (DIGIMED, model DM-64−5E, São Paulo, Brazil) according to the Adolfo Lutz Method.[37] The contents of iron (Fe, 248.3 nm), calcium (Ca, 422.7 nm), manganese (Mn, 279.5 nm), magnesium (Mg, 285.2 nm), and zinc (Zn, 213.9 nm) were determined by flame atomic absorption spectroscopy (VARIAN, model FS230).[37,54]

Statistical Analysis. A Box-Behnken experimental design was performed using Statistica software (version 14), and the results were recorded using Exponent software (version 6). The data were then submitted to the ANOVA and Tukey tests at a 95% confidence level (α = 0.05) using OriginPro 2024 (Student version).

The limits of detection (LOD) and quantification (LOQ) were estimated using the following equations: $LOD = 3.3 \times s/a$; $LOQ = 10 \times s/a$, where s is determined from the sum of squared residuals and is a the slope of the calibration curve.[55]

RESULTS AND DISCUSSION

Analytical Parameters. The concentration ranges, the equations of the analytical curves, coefficients of determination (R^2), LOD, and LOQ of each analysis are shown in the Table S in the Supporting Information.

Box-Behnken Experimental Design. The Box-Behnken Design (BBD) presents several advantages in obtaining results, such as the evaluation of the simultaneous interaction between variables, which can provide the best optimized condition for the analysis; it increases the accuracy of the analysis, as it can capture nonlinear relationships within the planning, ensuring the reliability of the results; and it reduces analysis time.[56,57] Additionally, it is possible to use statistical tools for the best analytical responses of optimizations, such as the response surface methodology (RSM). For the Box-Behnken design, it was necessary to perform 15 different formulations (Figure 1a-o), in which residues were added, as well as a formulation of a cake without residues for comparative purposes (Figure 1p).

To evaluate the optimal level of replacement of wheat flour by the flours obtained (leaf, stalk and mixture (50% (m/m) leaf and 50% (m/m) stalk)), the Box-Behnken design (Table 1) was performed randomly and generated contour plots referring to hardness (Figures 2a-c), springiness (Figures 2d-f), cohesiveness (Figures 2g-i), resilience (Figures 2j-l), gumminess (Figures 2m-o), and chewiness (Figures 2p-r). None of the models showed a lack of fit, having p-value > α (hardness: 0.11 > 0.05; springiness: 0.42 > 0.05; cohesiveness: 0.78 > 0.05; resilience: 0.75 > 0.05; gumminess: 0.24 > 0.05; chewiness: 0.36 > 0.05).

Analysis of the contour plot indicated that hardness (Figure 2a-c), which represents compressive strength, was highly influenced by the amount of residue used to replace wheat flour. The highest hardness values were obtained mainly when the amount of hop leaf residue was used. On the other hand, springiness (Figure 2d-f), cohesiveness (Figure 2g-i), and resilience (Figure 2j-l), which represent elastic recovery, resistance to internal disintegration, and recovery from deformation, respectively, had the highest observed values with the lowest residue concentrations, regardless of the composition of the residue, indicating a decreasing trend in these properties with higher levels of residue substitution. Gumminess (Figure 2m-o) and chewiness (Figure 2p-r), which represent energy to disintegrate dense foods and masticatory effort, respectively, were higher mainly when the concentration of leaf residue was higher, demonstrating that using hop leaf will increase the energy required to break the structures formed during the product cooking and chewing process.[35,36,58−62]

These changes in the hardness levels of the control cake compared to the cakes with residue, as observed in the contour graphs, were likely due to the presence of dietary fibers from byproducts, as they can modify the physical structure of food, promoting structural resistance in food products. Hop byproducts have a high content of insoluble fiber (Table 2), because this fiber competes with gluten proteins for water,

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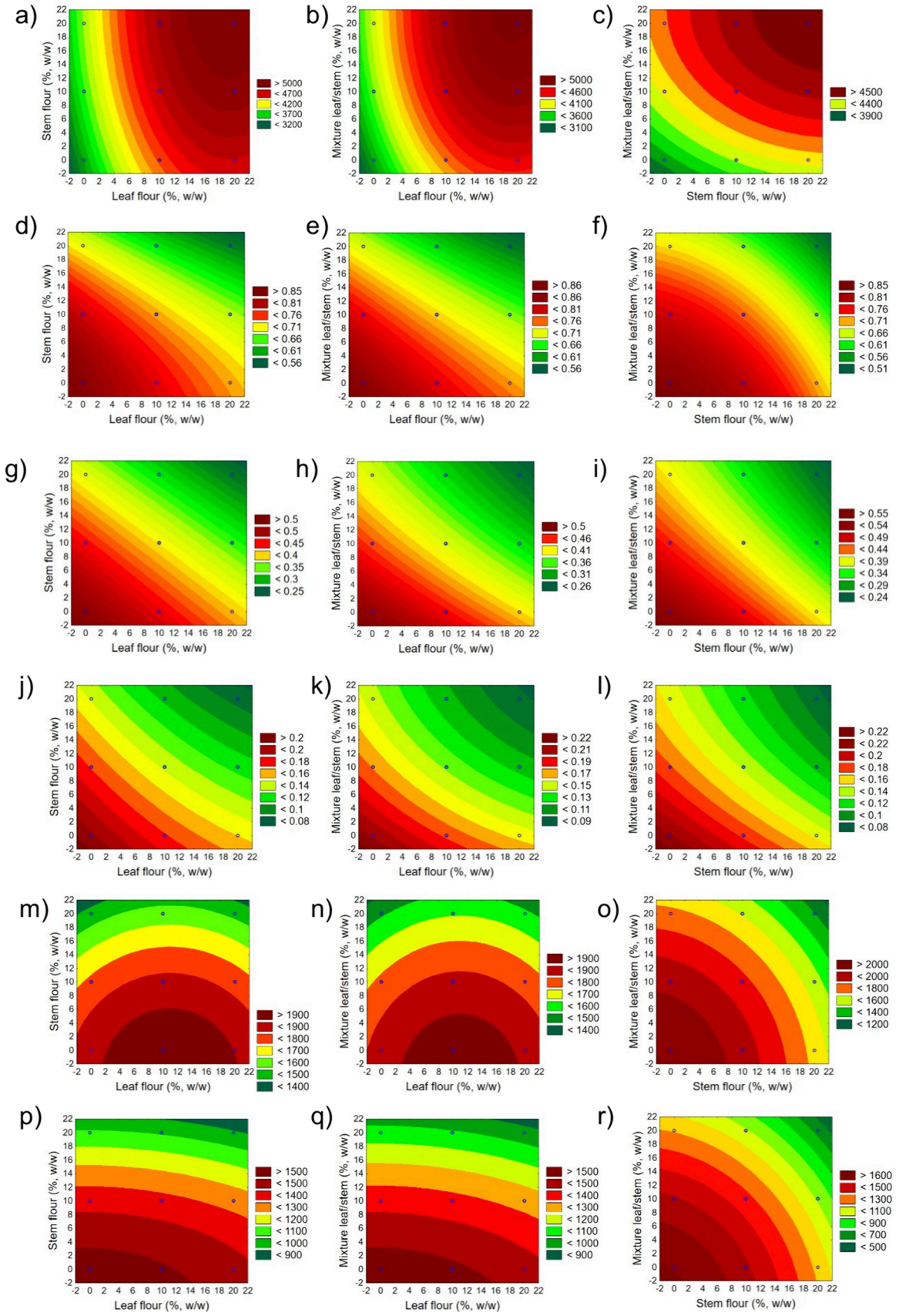

Figure 2. Contour plots for hardness (a-c), springiness (d-f), cohesiveness (g-i), resilience (j-l), gumminess (m-o), and chewiness (p-r).

impairing hydration. This process is known as the "gluten dilution" effect, which makes it difficult to form a cohesive and elastic protein network, thereby negatively impacting the product's rigidity. Furthermore, insoluble fibers physically

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Table 2. Results Obtained for the Proximate Composition Analyses of the Evaluated Cake Samples[a]

Analysis		Leaf flour	Stem flour	Control cake	Leaf cake	Stem cake
Dry matter	%, w/w	93.87 ± 0.07[a]	93.08 ± 0.02[a]	83.2 ± 0.8[A]	84.2 ± 0.9[A]	85.0 ± 2.0[A]
Moisture		6.13 ± 0.07[a]	6.92 ± 0.02[b]	16.8 ± 0.8[A]	15.8 ± 0.9[A]	14.9 ± 2.0[A]
Ash		12.95 ± 0.02[a]	6.5 ± 0.1[b]	2.31 ± 0.03[A]	2.33 ± 0.03[A]	2.62 ± 0.01[B]
Lipid		5.1 ± 0.2[a]	0.9 ± 0.1[b]	17.2 ± 0.6[A]	20.2 ± 0.1[B]	19.5 ± 0.5[B]
Protein		14.9 ± 0.1[a]	7.0 ± 0.5[b]	3.9 ± 0.5[A]	3.9 ± 0.7[A]	3.7 ± 0.3[A]
Total dietary fiber		48.6 ± 0.3[a]	72.5 ± 0.1[b]	2.4 ± 0.2[A]	4.7 ± 0.3[B]	8.4 ± 0.2[B]
Insoluble dietary fiber		43.7 ± 0.3[a]	68.0 ± 0.2[b]	1.9 ± 0.2[A]	3.2 ± 0.5[B]	7.0 ± 0.1[B]
Soluble dietary fiber		4.8 ± 0.5[a]	4.54 ± 0.03[a]	0.4 ± 0.1[A]	1.5 ± 0.2[B]	1.4 ± 0.1[B]
Carbohydrate		12.4 ± 1.0[a]	6.48 ± 0.09[b]	57.4 ± 1.0[A]	53.2 ± 1.4[A]	50,88 ± 1.9[A]
Total sugars		4.7 ± 0.1[a]	5.5 ± 0.5[a]	6.3 ± 0.2[A]	6.4 ± 0.2[A]	6.5 ± 0.2[A]
Energy value (kcal 100 g^{-1})		164.4 ± 1.8[a]	70.6 ± 2.9[b]	394.4 ± 7.2[A]	412.1 ± 2.3[B]	419.7 ± 8.8[B]
Total coliforms (MPN/g)		NA	NA	<3.0	<3.0	<3.0
Escherichia coli (MPN/g)		NA	NA	<3.0	<3.0	<3.0
Coagulase-positive *staphylococci* (CFU/g)		NA	NA	<10	<10	160
Molds and yeasts (CFU/g)		NA	NA	<100	<100	<100
Presumptive Bacillus cereus (CFU/g)		NA	NA	<10	<10	500
Salmonella spp. (presence/absence)		NA	NA	Absent	Absent	Absent

[a]Means followed by the same letter, lowercase (comparison between leaf and stem flour) and uppercase (comparison between the bakery products evaluated) in the line do not differ from each other, according to the Anova and Tukey test, at a significance level of 95% (α = 0.05). NA = No answer.

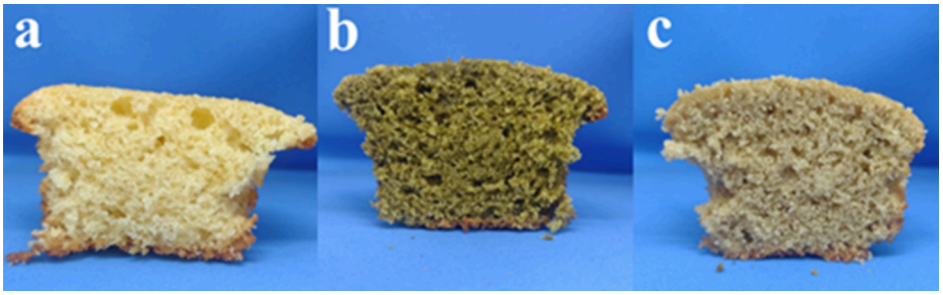

Figure 3. Cross-section of elaborate cakes, according to planning. (a) control cake, (b) leaf flour 10% (w/w), and (c) stem flour 10% (w/w).

interfere with the protein network, causing the fragmentation of its continuous structure and affecting the interaction between the dough components.[35,59,63−67]

Considering the results obtained in the planning and aiming to achieve results similar to the control cake (Table 2), with low hardness and good gumminess and chewiness, formulations 11 (replacement only with 10% stalk residue, w/w) and 14 (replacement only with 10% leaf residue, w/w) were the best options. The high hardness values of the leaf and stem cakes are expected to increase with ingredients that have high fiber concentrations, as they can compete for available water in the batter, making the interior of the cake denser.[65] Additionally, the literature has indicated that the replacement of wheat flour should be between 5 and 10%, a safe and effective way to obtain a product of good structural quality. Formulation 1, which consists of 10% of the mixture (50% leaf and 50% stem), was not evaluated in subsequent studies, as its values for chewiness, gumminess, and hardness were higher compared to formulations 11 and 14, and in some parameters, exceeded the values recommended in the literature. This demonstrates that hop byproducts should be used individually rather than in combination.[64,65,67−70]

Visual Analysis of Formulations. From the formulations with the best results (formulations 11 and 14), the aeration of the cakes was qualitatively observed compared to the control cake (Figure 3a-c). They still present structural stability. These observations are subtle and descriptive only; eggs and chemical leavening were included at the same concentrations in all formulations.

The control cake (Figure 3a) had a more alveolar structure. The cake with stem flour (Figure 3c) had more alveoli than the cake with leaf flour (Figure 3b), resulting in a more aerated structure, while the leaf flour gave a denser and more compact appearance. Formulation 11 (Figure 3c) and the control cake (Figure 3a) had the most efficient aeration process, an indication of quality for the consumer.[71]

Some authors report that the aeration process is associated with the cake manufacturing process, including the use of chemical yeast,[72] as well as the protein content present in the formulation, which comes from eggs or other ingredients, and is fundamental for the final quality and structure of the cake.[72−74] Additionally, eggs can deepen the color of the cake due to the presence of carotenoids in egg yolks. Eggs also contribute to foam formation and protein coagulation during the baking process. It is through this foam formation that air bubbles and the stabilization of the cakes are incorporated.[74,75]

During dough preparation, the gluten network is formed by the hydration of the proteins in the wheat flour, providing cohesion, elasticity, and extensibility to the dough.[75,76] Due to the incorporation of stem and leaf flour, the amount of wheat flour was reduced, consequently impacting the formation of the gluten network, preventing the capture of carbon dioxide (CO_2), which occurs during the action of the chemical

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Table 3. Results Obtained for the Analysis of Bioactive Compounds and Antioxidant Activity of the Evaluated Samples, on a Dry Basis[a]

Analysis	Leaf flour	Stem flour	Control cake	Leaf cake	Stem cake
Total phenolic compounds (mg gallic acid/100 g)	1744.1 ± 85.0^{a}	601.15 ± 9.6^{b}	28.803 ± 3.0^{A}	78.422 ± 6.7^{B}	41.833 ± 6.5^{A}
Antioxidant activity DPPH (mmol Trolox/100 g)	8.7 ± 0.2^{a}	4.4 ± 0.1^{b}	0.03 ± 0.003^{A}	0.19 ± 0.01^{B}	0.08 ± 0.01^{B}
Antioxidant activity ABTS (μmol Trolox/100 g)	7.0 ± 0.2^{a}	4.1 ± 0.6^{b}	0.34 ± 0.1^{A}	0.64 ± 0.03^{B}	0.26 ± 0.1^{A}
Alpha acid content (g/g)	6.4 ± 2.9^{a}	16 ± 1.7^{b}	4.7 ± 1.8^{A}	8.8 ± 2.0^{B}	11 ± 1.2^{B}

[a]Means followed by the same letter, lowercase (comparison between leaf and stem flour) and uppercase (comparison between the bakery products evaluated) in the line do not differ from each other, according to the Anova and Tukey test, at a significance level of 95% (α = 0.05).

Table 4. Results Obtained for the Analysis of the Mineral Composition of the Evaluated Samples on a Dry Basis[a]

Analysis	Leaf flour	Stem flour	Control cake	Leaf cake	Stem cake
			mg/100 g		
Phosphorus	6.55 ± 0.41^{a}	4.46 ± 0.07^{b}	7.23 ± 0.41^{A}	8.16 ± 0.14^{B}	10.4 ± 0.12^{B}
Potassium	1715.5 ± 21.4^{a}	1737.7 ± 22.8^{a}	82.17 ± 2.4^{A}	121.38 ± 3.0^{B}	97.53 ± 1.2^{B}
Iron	37 ± 2.1^{a}	17 ± 1.3^{b}	2.6 ± 1.0^{A}	3.9 ± 1.6^{A}	2.5 ± 0.4^{A}
Calcium	11198.1 ± 848.1^{a}	3359.3 ± 328.2^{b}	2028.2 ± 129.2^{A}	1996.9 ± 82.7^{A}	2308.9 ± 73.5^{B}
Manganese	15 ± 0.6^{a}	8.3 ± 0.1^{b}	0.32 ± 0.02^{A}	0.75 ± 0.03^{B}	0.31 ± 0.02^{A}
Magnesium	9149.2 ± 140.2^{a}	2930.7 ± 120.1^{b}	227.59 ± 13.1^{A}	320.16 ± 82.6^{A}	79.16 ± 10.2^{B}
Zinc	2.4 ± 0.1^{a}	1.3 ± 0.1^{b}	0.62 ± 0.03^{A}	0.49 ± 0.02^{B}	0.41 ± 0.01^{B}
Sodium	18.2 ± 0.5^{a}	17.4 ± 2.8^{a}	254.6 ± 8.2^{A}	200.3 ± 2.4^{B}	280.9 ± 1.2^{B}

[a]Means followed by the same letter, lowercase (comparison between leaf and stem flour) and uppercase (comparison between the bakery products evaluated) in the line do not differ from each other, according to the Anova and Tukey test, at a significance level of 95% (α = 0.05).

leavening agent at the "oven-spring" stage, affecting the structure of the cake.

The impact was more evident in the cake with leaf flour, which presented less aeration due to the reduction in the gluten network.[76] Cakes fortified with residues showed smaller alveolar developments than the control cake, likely due to the lower formation of the gluten network resulting from substitution; however, they still exhibit structural stability.

In addition, plant materials (such as hop residues) may contain certain types of proteins that may contribute to structural stability. Sedlar et al. (2020) conducted a study using broccoli leaves to evaluate the proteins present in them,[77] showing that the proteins studied exhibited functional properties, such as emulsifying ability and stability, and may influence the stability of the dough during cooking, similar to the properties of gluten.[72,73]

Chemical Analysis. The results obtained for the chemical analyses of proximate composition (Table 2), bioactive compounds, and antioxidant activity (Table 3), and minerals (Table 4), in the different samples, may differ from the literature, as the levels were related to some factors, such as the variety, cultivation, and edaphoclimatic factors.[16−19,21,22,32]

Proximate Composition Analysis. The proximate composition analyses of the byproducts from hop production, specifically the flours obtained from the leaves and stems (Table 2), were conducted to assess their potential application in human food products. For the soluble dietary fiber and total sugar contents in the comparisons between the leaf and stem flours, there was no significant difference at the 95% confidence level (α = 0.05). In the comparison of the cake without added flour with the cakes containing 10% (w/w) leaf flour and stem flour, the dry matter, moisture, protein, and total sugar contents (Table 2) did not differ significantly at the 95% confidence level (α = 0.05).

When evaluating the contents of dry matter, moisture, ash, lipids, proteins, carbohydrates, and energy value in the flour obtained from the residues (leaf and stem), significant differences and higher contents were observed for leaf flour, except for moisture. This behavior is expected when comparing the proximate composition analysis of these parameters in plants of the *Cannabaceae* family, to which *Humulus lupulus* belongs.[78−81] This demonstrates that leaf flour can be evaluated for the development of food products with lower concentrations of these compounds, thereby promoting the fortification of these new products that align with the demand for more nutritious and sustainable formulations.

When evaluating the formulations for these components (ash, lipids, proteins, carbohydrates, and energy value), it was observed that, in relation to the ash content, the stem flour cake exhibited a significant difference compared to the control cake. This difference suggests that the final mineral composition of the cakes depends not only on the ash content from the flours used but also on the mineral contribution of the other ingredients in the formulation, particularly the intrinsic mineral composition of each processed flour. In the case of lipids, the control cake presented a lower content, while the leaf and stem cakes did not differ statistically. This difference between the cakes is due to the characteristics of each flour, as commercial wheat flour contains lipid levels equivalent to 1.4 g/100 g according to the Brazilian Food Composition Table.[82] In contrast, stem and leaf flour have higher levels, even with the addition of oil in formulation, which is the main contributor to lipid levels. These significant differences exist between the cakes due to the initial levels of the three flours used. As for proteins, there were no significant differences between the cakes. Regarding carbohydrates, the control cake showed no significant difference compared to the other cakes. This result reflects the secondary contribution of leaf and stem flour to the lipid, protein, and carbohydrate content of the formulations, as the main ingredients, such as oil, egg, milk, and sugar, predominate in supplying lipids, proteins, and carbohydrates. However, the addition of residue flour resulted in a statistically significant change in lipid content and a decrease in carbohydrate content. For the energy

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value, the control cake and the cake with leaf flour showed statistically equal energy values, as did the cakes with leaf and stem flour. However, the energy values of the enriched cakes remained close to those of the control, due to the balance between the other components of the formulation, such as lipids, protein, and carbohydrates.

Soluble fiber levels did not show significant differences in the residues. However, there were significant differences in the cakes compared to the control cake at the 95% confidence level (α = 0.05), demonstrating an increase in nutritional value. Soluble fibers do not undergo digestion in the small intestine but are fermented by the intestinal microbiota in the colon for energy production. During this process, there is an increase in the absorption of vitamins and minerals, in addition to controlling blood glucose levels.[83,84] Insoluble fiber is not digested by the colon, favoring intestinal transit by increasing fecal weight, reducing glucose absorption, and helping to balance the pH in the intestines.[83]

In the evaluation of insoluble and total dietary fiber contents, the results showed that there were no statistical differences between the leaf and stem cake at the 95% confidence level. However, when compared to the control cake, they differ statistically (p < 0.05), as the leaf and stem cakes have higher fiber contents, demonstrating that the implementation of both residues favors the product's insoluble and total dietary fiber contents. Furthermore, when evaluating the results for total and insoluble fiber in the flours, it was noted that they presented statistical differences at the 95% confidence level. This is due to the significant amounts of cellulose, lignin, starch, and pectins (such as galacturonan, rhamnanan, and arabinan), which contribute to the cohesion of the fiber and are found in greater quantities in the stem.[8,32] Reddy and Yang (2009) evaluated the fiber composition in hop stems, obtaining a crude fiber value similar to this work, with levels of 84% (w/w).[26] Fiber levels may change due to plant maturity and variety, and the determination method may also impact final results.[8,26]

The microbiological analyses of the cakes with leaf and stem flour are presented in Table 3. The results showed that the three formulations have microbiological quality, as they do not pose a risk to human health, since they comply with the legislation according to Normative Instruction No. 161 of 2022 and Resolution No. 724 of 2022.[43,47]

Analysis of Bioactive Compounds and Antioxidant Activity. In the analysis of the phenolic and antioxidant compound content, Folin-Ciocalteu, DPPH (2,2-diphenyl-1-picrylhydrazyl), and ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid) free radical assays were performed in triplicate. The results obtained for the evaluation of bioactive compounds and antioxidant activity of flour and products are summarized in Table 3.

The high concentrations of phenolic compounds in the flours (Table 3), particularly in the leaf flour (1870.4 ± 85.0 mg gallic acid/100 g), indicate a high antioxidant potential. These compounds help neutralize free radicals, preventing cell damage and premature aging.[85] Some studies have quantified the total phenolic content in hop leaves. In those studies, the average levels found were similar (500 to 14,000 mg gallic acid/100 g) to the results present in this study.[11,13,23] However, significant differences at the 95% confidence level (α = 0.05) were observed when evaluating the total phenolic content obtained in the residues, with the highest levels found in the leaf flour. This behavior is expected as generally in plant materials, the concentration of phenolic compounds is higher in leaves, followed by flowers, and finally in the stem.[86]

When evaluating the total phenolic content of the cakes, only the leaf cake showed a significant difference at the 95% confidence level (α = 0.05) compared to the control cake. This result is expected, reflecting the contribution of leaf flour to the cake's incorporation. Although the stem flour presented high amounts of phenolic content, its contribution was limited during the production of the product. Furthermore, a considerable fraction of phenolic compounds is found after baking, which is due to the thermostability of some hop polyphenols, such as xanthohumol, that are resistant to manipulation at high heating temperatures.[87]

Regarding the antioxidant activity, leaf flour showed greater antioxidant capacity compared to stem flour in both tests (DPPH and ABTS). This suggests higher antioxidant potential in the flour obtained from the leaves, which can be attributed to the greater presence of bioactive compounds, such as flavonoids and phenolic acids. This indicates that leaf flour should be used when the objective is to fortify food products with bioactive compounds that present antioxidant capacity. Other authors have studied the antioxidant capacity in hop leaves, obtaining levels of 1.2 to 3.2 mmol Trolox/100 g for the DPPH test and 26.7 μmol Trolox/100 g for the ABTS test.[11,23,88] These values were lower than those presented in this work, likely because the antioxidant capacity depends on factors such as hop variety, sample preparation process, and extraction method, which were similar to those used for the quantification of total phenolic content.

The antioxidant levels for the DPPH test showed significant differences at the 95% confidence level (α = 0.05) between the leaf and stem cakes, and the control, with the highest levels found in the leaf flour. For the ABTS test, significant differences at the 95% confidence level (α = 0.05) were obtained in the leaf cake compared to the control cake, with the highest levels also found in the leaf flour. This information shows that implementing these residues increased antioxidant potential.

Alpha-acids are essential compounds for the beer industry because they add bitterness.[9] Since the byproduct is rich in this compound, analyses were carried out to quantify alpha acid in the flours and cakes. The stem flour showed a significant difference at the 95% confidence level (α = 0.05), with higher levels of alpha-acids in contrast to the leaf flour. This scenario is believed to be due to the higher amounts of beta-acids in the leaves compared to alpha-acids, as reported by some authors.[13] The authors Calvert et al. (2025) found a range of 0.3 g/100 g of alpha-acids in the leaves of three varieties, with lower concentrations compared to those reported in this study. However, the authors used HPLC-DAD for the quantification of this compound.[13] Furthermore, the samples analyzed were not grown in Brazil, as climate change is a significant factor in the discussion.[15] The cakes also showed significant differences at the 95% confidence level. Although the cake contained alpha-acids, no residue was incorporated into its composition. It is believed that phenolics from other sources, such as eggs, oil, and wheat flour itself, may interfere with the quantification of these analytes. Thus, they contribute to the alpha-acid levels, as evidenced by the considerable increase in levels observed in the leaf and stem cakes. The authors de Oliveira Sartori et al. (2022) developed a new method for quantifying alpha-acids using HPLC-UV. During the work, they identified that flavanone and dihydroflavonol molecules may interfere with

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the quantification of bitter acids in hops. This lack of specificity in the analysis is a problem for quantification, since the control cake has high levels of total phenolic content, which can present a significant interference in the food matrix.[89]

Mineral Composition. Analysis of the mineral composition data of leaf and stem flours and cakes made with these flours indicates a considerable enrichment in essential minerals compared to the control cake (Table 4).

For potassium and sodium contents in the comparisons between leaf and stem flours, there was no significant difference at the 95% confidence level (α = 0.05). The levels of phosphorus, iron, calcium, manganese, magnesium, and zinc were significantly higher in leaf flour at the 95% confidence level (α = 0.05). The minerals found most commonly in plant materials showed concentrations 1.5 to 3.3 times higher in leaf meals than in stem meals, including iron, calcium, manganese, and magnesium, while the others were found in lower concentrations. This behavior is expected, since leaves have higher amounts of iron, manganese, and magnesium than other parts of the plant material.[90] Iron plays a fundamental role in plant material, as it helps with electron transfers during enzymatic reactions. Calcium helps in the development of plant membranes.[90] The minerals manganese and magnesium are directly linked to respiration and photosynthesis. Magnesium, in particular, is an essential component for the synthesis of DNA and RNA in plants.[90] However, although the iron and magnesium contents increased in the cake made with leaf flour, the differences were not statistically significant at the 95% confidence level (α = 0.05), indicating a limitation in the incorporation of this residue. Other minerals, such as phosphorus, potassium, zinc, and sodium, showed a significant difference at the 95% confidence level (α = 0.05), with higher levels compared to the control cake.

Evaluating the cake with stem flour, the iron content showed no significant difference at the 95% confidence level (α = 0.05). For the other minerals, the values found were significant at the 95% confidence level (α = 0.05). However, the results for magnesium and zinc were lower than those of the control cake.

The presence of minerals in large quantities in flours and cakes is a positive aspect, as these nutrients perform several important functions in the body, such as regulating blood pressure (potassium), forming bones and teeth (calcium and phosphorus), producing energy (magnesium), and protecting against free radicals (manganese), growth hormones (zinc), and preventing anemia (iron).[91−94] The incorporation of these flours into food products can contribute to increased dietary intake of essential minerals. In the samples evaluated, especially the leaf residue, it presented higher levels of iron, manganese, and magnesium than the stem flour. Therefore, leaf flour can be considered a source of iron and rich in manganese and magnesium, according to the limits defined by IN No. 75/2020 (≥10% and ≥ 20% of the daily reference value for "source of" and "rich in," respectively).[40]

In summary, the addition of hop leaf and stem flour to food products has proven to be an effective initiative for developing baked goods with higher nutritional value and antioxidant potential. However, the results suggest that leaf flour may be a better alternative than stem flour for incorporation into these kinds of food products, due to its nutritional profile, antioxidant potential, and mineral content. Therefore, it is necessary to assess the sensory acceptability of these products for the consumer in the future, which was not the focus of this article.

Investigations into the incorporation of *Humulus lupulus* L. byproducts in food products have shown that the plant's leaves and stems, typically discarded as waste, possess a substantial nutritional profile. Rich in dietary fiber (primarily insoluble), minerals, and phenolic compounds, these byproducts present promising potential for fortifying a wide range of food products, including baked goods. Box-Behnken experimental design and texture analysis allowed us to find the optimal incorporation level of *Humulus lupulus* leaves and stems into cakes, which is 10% (w/w). The findings demonstrate that such substitutions not only enhance the nutritional value of the final product but also contribute to reducing agricultural waste, thereby supporting the United Nations' Sustainable Development Goals and promoting a circular economy. Although a full life cycle assessment (LCA) has not been conducted, implementing this residue could reduce the demand for conventional wheat flour, once again favoring the circular economy and the Sustainable Development Goals. Thus, the results obtained in this study are similar to or comparable to those found in the literature on the use of beet and olive pomace in bakery products, which increase the levels of dietary fiber and bioactive compounds in their composition.[7,95,96]

Despite the promising results, there are some sensory limitations, such as the bitterness from hops. Furthermore, the seasonality of the raw material must be considered, as some plant varieties behave differently in different seasons, requiring strict controls to standardize this byproduct. Additionally, this raw material contains tannins that can form complexes with proteins, reducing its bioavailability. Therefore, future studies require evaluating the long-term stability of this product to ensure not only the preservation of these nutrients but also sensory stability. Furthermore, for marketing purposes, sensory evaluation among consumers is required, as textural profile analysis tests do not guarantee consumer acceptability.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsfoodscitech.5c00533.

Analytical parameters used for the determinations (analyte, linear range, analytical curve equation, coefficient of determination (R^2), limit of detection (LOD), limit of quantitation (LOQ)) (PDF)

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