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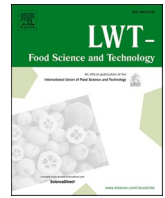
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## Design of a baked good using food ingredients recovered from agro-industrial by-products of fruits

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### ABSTRACT

In the last few years, healthy foods have increasingly attracted consumers' interest, leading to an increase in sales worldwide. Using by-products from the agroindustry to produce healthy/fortified foods is a promising approach since peels and seeds of fruits have significant amounts of bioactive compounds. In this sense, this work evaluated the possibility to add fractions recovered from residues of orange, lime, and peach palm in a food product. First, the proximate, ultimate, and chemical composition of the residues was determined to identify the main substances that could be valorized. Then, the selected high-value-added molecules extracted from fruit residues were used to formulate a high-fiber brownie. A Box-Behnken experimental design was used to determine if fat replacement, flour replacement, and the addition of encapsulated extracts influenced the food product's color, texture, and humidity which were determined from the analysis of the texture profile of the samples. It was possible to identify with the help of an electronic tongue a formulation with similar properties to a commercial brownie but with enhanced functional properties due to the novel ingredients added, which could potentially improve consumers' health.

**Abbreviations:** ABS, Absorbance; AA, Antioxidant Activity; CAGR, Compound Annual Growth Rate; DRV, Daily Reference Value; EE, Encapsulated Extract; HPLC, High-Performance Liquid Chromatography; LR, Lime Residues; NREL, National Renewable Energy Laboratory; NCS, Non-centrifugal Sugarcane; OR, Orange Residues; PR, Peach Palm Fruit Residues; TPA, Texture Profile Analysis; TPC, Total Phenolic Content; TTC, Total Tannin Content; TEAC, Trolox Equivalent Antioxidant Capacity; VS, Volatile Solids.

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## 1. Introduction

In the last few years, “healthy foods” or “foods with added value” obtained from alternative sources have been at the forefront of the food industry. According to Euromonitor’s database, fortified/functional foods reached 274 billion USD in sales worldwide. In Colombia, fortified/functional packaged food is projected to increase at a 3% CAGR to reach 678.37 million USD in sales in 2025 (Passport, 2021). According to these data, it is clear that there is an increased interest in introducing new healthy ingredients to foods to obtain products that could help improve the population’s quality of life.

Colombia produced in 2020, 368 ktms of orange (with a harvested area of around 23.5 thousand hectares, kha), 248.5 ktms of lime (19.2 kha harvested), and 47.6 ktms of peach palm fruit (9.1 kha) (Agronet, 2020). The residual fractions from those productive systems (e.g., peels, seeds, and remnant pulp) have been reported to be left behind in ratios up to 60% of the total weight of the fruits (M. Singh et al., 2012). Hence, vast quantities of residues generated not only in the agricultural stage but also downstream along the value chain could be recovered for their use.

It is well known that fruit residues have significant amounts of bioactive compounds (e.g., polyphenols, fatty acids, and amino acids) and structural carbohydrates (e.g., cellulose, hemicellulose, and pectin) (Cruz Reina et al., 2022). These substances are non-essential compounds that have shown positive effects on human health (Gómez et al., 2016), making their valorization through the incorporation into novel food products a fascinating opportunity. Several value-added compounds can be extracted from vegetable biomass either by conventional or alternative sources; however, these compounds still need to be formulated in a technologically-feasible and cost-effectively manner to be valorized into novel food products. The incorporation of fruit fiber in foods and feeds can promote health benefits like, for example, improve gastrointestinal function and help to control cholesterol concentrations in the body (Gómez et al., 2016; Lim et al., 2014). Likewise, bioactive compounds such as carotenoids, flavonoids, and pectin have been used as functional food ingredients of high bioaccessibility with benefits to human health due to their antioxidant properties (Di Vaio et al., 2010). However, using phytochemicals and fibers as ingredients in baked goods has been shown to alter the final properties of the products (e.g., texture, color, flavor) (Manisha et al., 2022). Hence, there is a great potential to extract and produce novel functional food ingredients from alternative resources (like residues of the citric industry) that can be useful for achieving a final product that is both functional, palatable, and that also satisfies consumers’ needs and expectations (Correa et al., 2012).

The present study proposes a route to valorize residual biomass from orange, lime, and peach palm by recovering functional food ingredients that can later be used in the design of food products, particularly in this case, a baked good. The biomass residues were studied regarding their chemical compositions to identify possible substances that could be extracted and included later in the formulation. After that, a Box-Behnken design was implemented to formulate a brownie in which fats and flour were replaced with the non-soluble and soluble fibers obtained from citrus and peach palm fruits. Furthermore, the product was functionalized by incorporating encapsulated extracts with significant antioxidant activities. The results from this work open new research possibilities for biomass valorization by expanding its applications into food engineering, which can be considered a step forward in the efforts for sustainable food production.

## 2. Materials and methods

### 2.1. Sample collection and preparation

The orange and lime residues were obtained from fresh juice shops near Universidad de Los Andes (Bogotá, Colombia). Peach palm fruit residue was acquired from Frudelpa S.A.S. (Cali, Colombia). Residues

were cleaned using a 1000 ppm sodium hypochlorite solution, chopped into pieces of 2 mm using a vegetable cutter (CL 50 Ultra Vegetable Preparation Machine, Robot Coupe), and kept at  $-20\text{ }^{\circ}\text{C}$  in the freezer until used. Samples were prepared for compositional analysis according to the NREL protocols (de la Torre et al., 2017; NREL, 2022). Fruit residues were put in a container and dried in a convection oven (FD 115, Binder) at  $45\text{ }^{\circ}\text{C}$  for 24–48 h. The dried samples were milled in a universal cutting mill (Pulverisette 19, Fritsch) ensuring a particle size smaller than 1 mm.

### 2.2. Proximate and ultimate analysis

Moisture and total solids were determined according to the ASTM E1756 method. The volatile matter was determined at  $950\text{ }^{\circ}\text{C}$  (ASTM 1755) and ash at  $575\text{ }^{\circ}\text{C}$  (ASTM E872). The ultimate analysis (i.e., contents of C, N, H, S, and O by difference) was determined according to the ASTM D5373-16 method. The calorific value was determined according to the ASTM E711-06 method.

### 2.3. Compositional analysis

Compositional analysis of LR, OR, and PR was determined according to NREL protocols (de la Torre et al., 2017; NREL, 2022). Previously prepared samples were placed in a thermobalance (XM-60, Precisa) at  $105\text{ }^{\circ}\text{C}$  until constant weight to determine moisture. For ash content, samples of each fruit residue were dried in a muffle furnace (Type F62700, Barnstead International) up to  $575\text{ }^{\circ}\text{C}$  using the ramp program specified by the protocol. The Kjeldahl Nitrogen content was followed to determine protein content using a Nitrogen to protein conversion factor of 6.25 (Sáez-Plaza et al., 2013).

Extractive content was determined by Soxhlet extraction using 10 g of the prepared sample. The sequential extraction was performed using 190 mL of HPLC grade water for 24 h (4–5 siphon cycles per hour) and 190 mL of ethanol 96% (v/v) for another 24 h (6–10 siphon cycles per hour). Solvents were removed with a rotary evaporator at  $40\text{ }^{\circ}\text{C}$  (R-114 Rotary Vap System, Buchi) connected to a vacuum source. The extractive content was determined gravimetrically (NREL, 2022).

The determination of cellulose, hemicellulose, and lignin was performed by using 300 mg of the extractive-free sample to perform a sequential acid hydrolysis with sulfuric acid, first at a low temperature ( $30\text{ }^{\circ}\text{C}$ ) in a water bath with a high concentration of acid (72% w/v) and then at a higher temperature ( $121\text{ }^{\circ}\text{C}$ ) in an autoclave (SX-700, Tomy) with a diluted concentration of acid ( $\sim 4\%$  w/v) (NREL, 2022). Filtering crucibles were used to recover the solid fraction, which were later used to determine lignin content in a muffle furnace (Type F62700 Furnace, Barnstead International) at  $575\text{ }^{\circ}\text{C}$ . Liquid phase samples were collected for HPLC quantification of sugars (Glucose, xylose, and arabinose) using a Biorad Aminex HPX-87 P column with an injection volume of 20  $\mu\text{L}$ , HPLC grade water as the mobile phase, a flow rate of 0.6 mL/min, a temperature range of  $80\text{--}85\text{ }^{\circ}\text{C}$  and a run time of 35 min. Sugar recovery standards were included and treated the same way as the extractive-free samples to include a correction factor for all sugars lost in hydrolysis.

Pectin was determined using a colorimetric method. A mix of 140-proof ethanol and 0.5% w/v EDTA was used to remove sugars from the sample. Subsequently, acidity was modified with 1 M sodium hydroxide to a pH of 11.5, then 0.25 M acetic acid was used to achieve pH levels between 5 and 5.5. Pectinase (*Aspergillus niger* 1.0 U/mg) was added, and the mixture was stirred for 1 h. Vacuum filtration was used to recover the liquid, which was later diluted to 250 mL with distilled water. An aliquot of 2 mL of this liquid was diluted again to 100 mL for galacturonic acid measurements (Barazarte et al., 2010). Each sample was treated with 98% w/v sulfuric acid at  $5\text{ }^{\circ}\text{C}$  and cooled in an ice bath until reaching  $3\text{ }^{\circ}\text{C}$ . The sample was then heated to  $90\text{ }^{\circ}\text{C}$  in a water bath (BS-11 Heating Bath, Lab Companion) and a solution of 0.15% w/v of carbazole was used for the colorimetric reaction. Galacturonic acid was determined after 25 min of reaction in a UV-VIS spectrophotometer

(T80+, PG instruments) at 530 nm.

## 2.4. Preliminary phytochemical qualitative identification

A preliminary phytochemical screening was conducted through qualitative tests to identify compounds such as phenolic, tannins, flavonoids, and others. An extract of each fruit was made by dissolving 10 g of the dried sample in 40 mL of ethanol (96% v/v) and sonicated in an ultrasonic bath (Bransonic CPXH 3800, Branson) for 10 min. The solution was filtered, and the liquid was recovered. Aliquots of 2 mL of these extracts were tested for color change as described by (Cruz Reina et al., 2022).

## 2.5. Total phenolic content (TPC), total tannin content (TTC), and trolox equivalent antioxidant capacity (TEAC)

Prepared samples (25 g) were mixed with 100 mL of ethanol (96% v/v) in a 1:1 proportion with water. Ethanol was used to favor the recovery of carotenoids and polyphenols like naringin, which are not water-soluble (Li et al., 2022; Zhang et al., 2015). The flasks were placed in an ultrasonic bath (Bransonic CPXH 3800, Branson) for 30 min at 40 °C and 40 kHz (Khan et al., 2010). The extracts were filtered and stored at -20 °C.

Total phenol quantification was performed through a modified version of the Folin-Ciocalteu method (Oh et al., 2014). A Gallic acid calibration curve in a range between 25 and 500 mg/L was used. Samples of 0.2 mL were mixed with 4.8 mL of distilled water and 0.5 mL of the Folin-Ciocalteu's (Sigma-Aldrich) reagent (2 N). After 2 min in the dark, 1.5 mL of sodium carbonate at 20% (w/v) (PanReac-AppliChem ITW) was added and incubated at room temperature for 2 h in the dark. Absorbance was measured using a UV-Vis spectrophotometer (T80+, PG instruments) at 765 nm and water as the blank. Moreover, tannin content was determined using the same procedure for TPC using a wavelength of 700 nm and a calibration curve with tannic acid ranging from 5 mg/L to 200 mg/L.

Trolox Equivalent Antioxidant Capacity (TEAC) was determined using the DPPH assay using the method of Brand-Williams with some modifications (Brand-Williams et al., 1995). First, a stock solution of the DPPH Free Radical solution (ChemCruz) was made using 0.24 mg of DPPH and 10 mL of absolute ethanol 99.8% (v/v) (PanReac-AppliChem ITW Reagents). The mixture was then diluted with ethanol (~60 mL) until an absorbance of  $1.1 \pm 0.02$  at 517 nm was achieved using a UV-Vis spectrophotometer (T80+, PG instruments). Then, 150  $\mu$ L of each sample were left to react with 2850  $\mu$ L of the working solution for 30 min in the dark. Absorbance was measured at 517 nm. A standard curve was made using Trolox solutions in a range of 75–800  $\mu$ M. Equation (1) was used to calculate the percentage of antioxidant activity considering the absorbance of each sample and the absorbance of the stock DPPH solution.

$$\%AA = 100 - \left[ \frac{ABS_{sample} \times 100}{ABS_{stock}} \right] \quad (\text{Eq. 1})$$

## 2.6. Encapsulation of extracts

Encapsulation was done using maltodextrin (Cimpa S.A.S) to protect the phytochemical compounds present in the extracts from the high temperatures of baking. Each ethanol extract was mixed with maltodextrin (25% w/v) previously heated at 40 °C in a 1:2 vol Proportion between the extract to the encapsulating agent. Samples were homogenized using a magnetic stirrer (Hei-Tec, Heidolph Instruments) at 1400 rpm for 30 min. Then, solutions were fed to the spray dryer (Mini Spray Dryer B-290, Buchi), operated at an inlet temperature of 150 °C. The air flow, rate of feeding, and atomization pressure were 600 L/h, 10 mL/min, and 20 psi, respectively (Robert et al., 2010). From each powder, 2 g were diluted in 100 mL of water to perform the TEAC test (Section 2.5).

Equation (2) describes the encapsulation efficiency (EE).

$$\%EE = \frac{\%AC_{\text{encapsulated powder}}}{\%AC_{\text{Ethanol extract}}} \times 100 \quad (\text{Eq. 2})$$

## 2.7. Product design

### 2.7.1. Survey

To figure out what type of baked food product was to be made, a virtual survey was carried out throughout the last two weeks of August 2021. The survey was anonymous to prevent bias from the consumers and the experimenters. The protocol of the sensory analysis was approved by the Ethics Committee of the Engineering Faculty of Universidad de Los Andes. The survey is available at: <https://forms.gle/ezU4GEzuN7P1fGH6A>. Using the Google's survey tool, participants were asked to disclose their age group, employment status, gender, and socioeconomic status. They were asked about their food-buying habits and how often they consume baked goods. Finally, they were asked which product they would prefer to consume, between a vanilla cake, a cookie, a muffin, or a brownie, if it were a high-fiber, low-fat baked good based on by-products of fruits.

### 2.7.2. Product design

A control recipe was established as a benchmark to formulate the baked product. The control recipe contained (w/w): butter without salt (Colanta) 26%, organic NCS (La Gloria) 31%, wheat flour (Haz de Oros) 14%, cocoa powder (Corona) 7%, eggs (Santa Anita) 19%, vanilla extract (Levapan) 1.5%, baking powder (Royal) 1%, and Salt (Refisal) 0.5%. All ingredients were measured, and then butter was melted at low heat in a small pan with the help of an electric stove. When the butter was melted, non-centrifugal cane sugar was added and mixed with a hand whisk. Once the sugar was included, eggs and vanilla extract were added. The remaining ingredients were sieved together, added to the batter, and hand-whisked until homogenized. After the batter was prepared, a silicone mold was greased, and brownies were baked at 180 °C for 10 min in a gas conveyor oven (Impringer I, Lincoln). Finally, the brownies were left to cool at room temperature, unmolded, and kept in sealed bags inside a desiccator until analyzed.

The incorporation of novel ingredients (recovered from LR, OR, and PR) into the baked product was done through a Box-Behnken experimental design in duplicate (see supplementary information, Tables S-1) to evaluate the effect of incorporating them into the recipe: i) Pectin from citrus residues (butter/fat replacement), ii) PR flour (wheat flour replacement), and iii) Encapsulated extracts of OR, LR, and PR. The response variables evaluated were the brownies' color, texture, and humidity.

Pectin was used as a fat replacement. According to the literature, pectin fat replacement should be around 20% (w/w) (Wafaa et al., 2011). Therefore, the levels chosen for the pectin content of the formulation were 10% (w/w), 20% (w/w), and 30% (w/w) of fat replacement. On the other hand, for flour replacement, levels were chosen based on the National Resolution from the Colombian Health Ministry, "Resolución 810 de 2021" (Minsalud, 2021), which states that food with high fiber content must contribute more than 20% of the DRV (Daily Reference Value) per serving, and food with good fiber content must contribute at least with 10% of the DRV per serving. Considering the above, the selected levels for the PR flour were 10%, 20%, and 30% of the DRV, which corresponds to a wheat flour replacement of 27% (w/w), 53% (w/w), and 80% (w/w), respectively. Finally, encapsulated extracts from each fruit residue were studied. It was established that each brownie would have the amount of extract necessary to achieve the same antioxidant capacity as an average kiwi of 100 g (Wang et al., 2018), a well-known fruit for its antioxidant properties. The quantities of encapsulated extracts added to the formulation were 1.3% (w/w) of PR, 1.7% (w/w) of OR, and 2.3% (w/w) of LR.

**Table 1**  
Characterization of the fruit residues and their extracts.

Proximate analysis*			
Parameter % (d. w.)	OR	LR	PR
Ash (950 °C)	3.36 ± 0.29 <sup>a</sup>	4.44 ± 0.08 <sup>b</sup>	3.68 ± 0.13 <sup>a</sup>
Volatile Solids (VS)	96.64 ± 0.29 <sup>a</sup>	95.56 ± 0.08 <sup>b</sup>	96.32 ± 0.13 <sup>a</sup>
Calorific value (kJ/kg)	16021.49	15179.48	17614.80
Ultimate analysis*			
Parameter % (d. w.)	OR	LR	PR
Nitrogen	0.80 ± 0.05 <sup>b</sup>	0.72 ± 0.05 <sup>b</sup>	0.96 ± 0.05 <sup>a</sup>
Carbon	38.10 ± 0.05 <sup>b</sup>	38.10 ± 0.05 <sup>b</sup>	40.10 ± 0.05 <sup>a</sup>
Sulfur	0.16 ± 0.05 <sup>b</sup>	0.19 ± 0.05 <sup>b</sup>	0.44 ± 0.05 <sup>a</sup>
Hydrogen	5.15 ± 0.05 <sup>b</sup>	4.78 ± 0.05 <sup>c</sup>	5.41 ± 0.05 <sup>a</sup>
Ash (575 °C)	4.10 ± 0.05 <sup>b</sup>	4.90 ± 0.05 <sup>a</sup>	5.00 ± 0.05 <sup>a</sup>
Oxygen	51.69 ± 0.05 <sup>a</sup>	51.31 ± 0.05 <sup>b</sup>	48.09 ± 0.05 <sup>c</sup>
Compositional analysis*			
Parameter % (d. w.)	OR	LR	PR
Ash	3.36 ± 0.29 <sup>b</sup>	4.44 ± 0.08 <sup>a</sup>	3.68 ± 0.13 <sup>b</sup>
Protein	6.56 ± 0.05 <sup>c</sup>	6.75 ± 0.05 <sup>b</sup>	7.38 ± 0.05 <sup>a</sup>
Extractives	22.23 ± 0.54 <sup>a</sup>	19.47 ± 0.17 <sup>c</sup>	20.44 ± 0.04 <sup>b</sup>
Lignin	1.03 ± 0.08 <sup>c</sup>	3.50 ± 0.32 <sup>a</sup>	2.05 ± 0.18 <sup>b</sup>
Hemicellulose	27.81 ± 0.10 <sup>a</sup>	25.24 ± 0.24 <sup>b</sup>	26.62 ± 1.14 <sup>a,b</sup>
Cellulose	18.63 ± 0.52 <sup>b</sup>	13.93 ± 0.08 <sup>c</sup>	42.52 ± 0.66 <sup>a</sup>
Pectin	22.95 ± 0.80 <sup>b</sup>	27.57 ± 0.47 <sup>a</sup>	ND
Total	102.56 ± 2.39	100.90 ± 1.40	102.69 ± 2.19
Phytochemical screening**			
Phytochemical compound	OR	LR	PR
Alkaloids	-	-	+
Carotenoids	+	+	+
Steroids	-	-	+
Tannins	+	+	+
Flavonoids	+	+	-
Leucoanthocyanidins	+	-	-
Saponins	-	-	+
Naphthoquinones and anthraquinones	-	-	-
Phenolic content, tannin content, and antioxidant capacity of the extracts*			
Parameter	OR	LR	PR
TPC (mg GAE/100g)	150.31±6.26 <sup>a</sup>	158.15±5.53 <sup>a</sup>	1.85±0.87 <sup>b</sup>
TTC (mg TAE/100g)	148.38±3.21 <sup>b</sup>	166.62±7.43 <sup>a</sup>	10.77±1.91 <sup>c</sup>
TEAC (mg TE/100g)	28.96±0.72 <sup>b</sup>	46.36±0.63 <sup>a</sup>	6.14±0.38 <sup>c</sup>
%AA	33.64±0.79 <sup>b</sup>	52.76±0.69 <sup>a</sup>	8.57±0.42 <sup>c</sup>
%AA after encapsulation	4.45 ± 0.51 <sup>a,b</sup>	5.59 ± 0.71 <sup>a</sup>	4.50 ± 0.19 <sup>b</sup>

### 2.7.3. Color, texture, and humidity

Each product was first put through a colorimetric analysis using a colorimeter (CR-20, Konica Minolta) under conditions of the standard illuminant D65 and 10° observer. Calibration was carried out using a standard tile provided by the equipment manufacturer. The averaging tool included with the equipment was used when the sample had a non-uniform color. Data were obtained using the CIELAB or CEL L\* a × b\* system, representing the color in three dimensions. The first dimension, lightness (L\*), represents the grayscale, the second dimension (a\*) represents the red/green axis, and the third dimension is the yellow/blue axis. Equation (3) and Equation (4) were used to calculate the chroma (C<sub>ab</sub>\*) and the hue angle (h<sub>ab</sub>\*), respectively.

$$C_{ab}^* = (a^2 + b^2)^{1/2} \quad (\text{Eq. 3})$$

$$h_{ab}^* = \arctan \frac{b^*}{a^*} \quad (\text{Eq. 4})$$

Afterward, texture analysis was performed in a texture analyzer (TA. HD plus C, Stable Micro Systems) with a cylinder probe. The TPA (Texture Profile Analysis) was carried out to measure hardness (the strength used to compress any food with the molars or between the tongue and the palate), adhesiveness (the work required to remove the food from a surface like the teeth or the palate), and cohesiveness (the force keeping the food particles united). These parameters were obtained as described by (Tai et al., 2014). Finally, the product's humidity

was analyzed by using 2 g of sample in a moisture analyzer (XM-60, Precisa) set to 105 °C.

### 2.7.4. Electronic tongue analysis

From the Box-Behnken Experimental design, the two best formulations (i.e., the most similar to the control recipe) were analyzed using a Taste Sensing System (TS-5000Z, Insent). Fifty grams of each baked food product were mixed in a food processor for 1 min. Then, 200 mL of 40 °C water was poured into the food processor and mixed with the sample for 1 min to extract taste substances. The solution was centrifuged (Sorvall Legend XTR centrifuge, Thermo Scientific) for 10 min at 3000 rpm and 20 °C. Finally, the water phase was recovered, and the pH was adjusted to 4.5 with HCl (1% w/w). The analysis was carried out using the food and beverages model where the equipment sensors measure sourness, bitterness, astringency, saltiness, richness, umami, aftertaste from acidic bitterness (Aftertaste-B), and aftertaste from astringency (Aftertaste-A).

## 2.8. Statistical analysis

Data (e.g., biomass composition, extract properties, and brownie properties) were analyzed using a One-way Analysis of Variance (ANOVA). The statistical software Minitab 19® was used for data analysis, while Statistica 12® was used for visualization. The significance level of the differences between means was determined using a Tukey test ( $p < 0.05$ ). Related assumptions were carefully validated,



such as equal variances (Bartlett's Test) and normal distribution (Anderson-Darling test).

### 3. Results and discussion

#### 3.1. Characterization of fruit residues and their extracts

##### 3.1.1. Proximate and ultimate analysis

The ash content and calorific value of the fruit residues (Table 1) were similar to those found in the literature (Siles et al., 2016; Volpe et al., 2015). Ash content is related to the minerals present and may vary depending on the soil and the crop type. The high VS (Volatile Solids) content found (>95.5%) showed the potential of the residues to be used for energy production. For example, food processing residues can be used for anaerobic digestion due to their high methane yield per kilogram of VS (Oosterkamp, 2020).

The main elements present in the residues (Table 1) included oxygen, hydrogen, and carbon. This distribution was expected as the organic part of biomass mainly consists of hemicellulose, cellulose, and lignin, which contain these elements in different molecular proportions. Similar values for the ultimate analysis of these residues have been reported in the literature with calorific values around 17,000 kJ/kg and ash contents ~4–5% (Siles et al., 2016; Volpe et al., 2015), with slight differences that could be associated with plant varieties and the proportions of peel, bagasse, and seeds. PR has shown the highest nitrogen content, which could be related to the presence of protein in this biomass. Nonetheless, similar C/N, C/H, and C/O values were observed for all three biomasses. Studies have found empirical relations between C/N, C/H, C/O, and calorific values as significant parameters for defining fuel quality (Ozyuguran et al., 2018). Even though those relations are not studied in the present work, they demonstrate other possibilities for valorizing fruit residues as biofuels.

##### 3.1.2. Compositional analysis

The compositional analysis (Table 1) included the quantification of ashes, protein, extractives, and structural carbohydrates. After sample preparation, the total solids content was  $92.5 \pm 0.11\%$  for OR,  $92.4 \pm 0.10\%$  for LR, and  $93.85\% \pm 0.13$  for PR. These values correspond to a moisture content after sample preparation of 7.5% for OR, 7.6% for LR, and 6.15% for PR.

PR presents the highest protein content (~7.4% d. w), being the values obtained in this paper are slightly higher than others reported in the literature (~6.4% d. w) (Lachos-Perez et al., 2018). Moreover, the protein contents of OR and LR (~6.6% d. w), are in the same order of magnitude as those reported for other citrus residues (~6.9% d. w) (Lachos-Perez et al., 2018). PR could be considered as a biomass of relevance to recover and design a high-protein product.

For the extractives, the contents found in OR and LR (~20% d. w) were in the range (17%–38% d. w) reported by other authors for citrus residues (de la Torre et al., 2017; Lachos-Perez et al., 2018). Possible differences could be related to the juice production process. The remnant juice left behind in the residues has been reported to contain glucose, fructose, and organic acids (de la Torre et al., 2017). Phenolic compounds have been found in the ethanol extractives of citrus peels (M'hiri et al., 2015). Essential oils have also been found in the ethanol extracts produced from citrus residues, mainly limonene (Di Vaio et al., 2010). Nonetheless, the amount of these bioactive compounds can vary according to the proportions of peels and seeds, and remnant pulp. Similarly, PR residues have been reported to contain soluble sugars like fructose, glucose, and sucrose and phenolic compounds like caffeic acid, coumaric acid, and chlorogenic acid (Giombelli et al., 2020). PR contains carotenes, mainly  $\beta$ -carotene, and the lipidic fraction contains fatty acids and tocopherols (Rojas-Garbanzo et al., 2011). All extracts have been reported to contain sugars that can be used as carbon source for fermentation processes (Giombelli et al., 2020), and substances with relevant antioxidant activities.

It is possible to see in Table 1 that the cellulose content in PR is considerably higher (~42.5% d. w), around 2–4 fold, than the one found in citrus residues (~14–18.5% d. w). Similar values have been reported in the literature for citrus residues (~18.6% d. w) (de la Torre et al., 2017; Senit et al., 2019). Besides, hemicellulose and lignin contents are in the same order of magnitude for all residues. It is evident that the lignin content of OR, LR, and PR is low (1–3%) compared to the one found in other vegetable biomasses like sugarcane bagasse and straw, which present lignin contents higher than 20% (Szczerbowski et al., 2014). The high fiber content present in the residues studied in this work opens up opportunities for their utilization in different applications. For example, cellulose and hemicellulose can be used to produce C5 and C6 sugars, organic acids, and concentrates (Dávila et al., 2017). The fibers could be used as a raw material for designing food products. Processed PR flour has been, for example, used in the formulation of cereals and baked goods since it contains high levels of starch-free polysaccharides that could contribute to colon health (Bolanho et al., 2015).

Regarding pectin content, this fraction was not detected in PR. It appears that PR are characterized by its low pectin content since other authors have found values ~2.5% d. w. In PR (Bolanho et al., 2015). Citrus residues showed a significant amount of pectin in their composition, with 21.8% for OR and 27.6% for LR. These results are congruent with other studies, which found a pectin content of ~18.7% in orange waste (de la Torre et al., 2017), and 28.6% in lime peel (Gómez et al., 2016). The obtained data gives promising results for the extraction of pectin from citrus residues and its use in product design, mainly because orange peels, due to their high pectin content, have been widely used as an industrial source of pectin with high extraction yields (Suliman et al., 2013). Pectin is considered one of the most recommended and safe additives without an acceptable daily intake limit, which could be used as a texturizer, emulsifier, gelling agent, and even as a fat or sugar replacer (Suliman et al., 2013). Specifically for baked foods, pectin has been used as a fat replacer to reduce the caloric content of these products (Lim et al., 2014). Marmalades produced with lime and orange pectin have been highly accepted by specialized panelists (Suliman et al., 2013), and pectin from these fruits has shown the growth of bacteria with a probiotic effect (Gómez et al., 2016).

##### 3.1.3. Phytochemical screening

It was observed that both OR and LR contain carotenoids, tannins, and flavonoids (Table 1), substances that have been reported to be present in citrus residues (M'hiri et al., 2015). OR extracts also contained leucoanthocyanidins (flavan-3,4-diols) (Zhao et al., 2020). In the case of PR, alkaloids, carotenoids, sterols, and tannins were found. These results suggest that using the phytochemicals from OR, LR, and PR in the formulation of food products could be beneficial due to the anticarcinogenic and anti-inflammatory properties attributed to flavonoids and carotenes (Zhao et al., 2020).

##### 3.1.4. TPC, TTC, and TEAC

The results for the phenolic and tannin content of the extracts are also shown in Table 1. Both citrus residues showed similar behavior with concentrations ~150–160 mg GAE/100 g, and are in the same range as other literature reports that used ultrasound extraction (121–200 mg GAE/100 g) (Khan et al., 2010). In future works, different extraction methods could be explored to enhance the recovery of polyphenols. It is known that citrus residues contain some flavanones like hesperidin, naringenin, narirutin, and naringin (Zhao et al., 2020). In contrast, PR presents lower phenolic content than citrus residues, which could be related to its pretreatment. Likewise, the tannin content of citrus residues (150–165 mg TAE/100 g) resulted in higher values than PR (~11 mg TAE/100 g). These results are consistent with those found in the literature, with values between 47 and 135.4 mg TAE/100 g for citrus residues (Jeong et al., 2004). The total tannin content of PR has not been usually reported by other authors, which could be related to its low content. The results shown in Table 1 demonstrate that OR and LR could

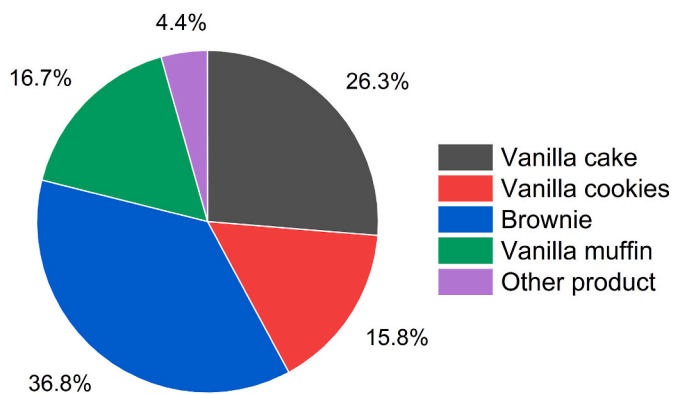


Fig. 1. Preference survey results (Total responses: 209).

contribute to the product’s functional benefits. More studies would be useful to identify and quantify the individual polyphenols present.

In terms of antioxidant capacity, it is possible to see that LR has the highest TEAC and AA% (~46.4 TE/100 g and ~52.8% AA), followed by OR (~29 TE/100 g and ~33.6% AA) and PR (~6.1 TE/100 g and ~8.6%

AA). Literature has shown similar antioxidant capacity values for citrus residues (~53 mg TE/100 g) (B. Singh et al., 2020) and PR (~8 mg TE/100 g) (Rojas-Garbanzo et al., 2011). With this in mind, each extract was encapsulated through spray drying. The highest percentage for encapsulated extract was obtained for PR with 52.7% (4.50 ± 0.19 % AA), followed by LR with 29.2% (5.59 ± 0.71 %AA), and OR 10.3% (4.45 ± 0.51 %AA). Future studies evaluating in detail the spray drying could be performed to further enhance the percentages of encapsulated extract.

### 3.2. Formulation of the baked food product

#### 3.2.1. Selection of the product

The consumer preference survey had 209 responses. The studied demographic was comprised mainly of young people between 18 and 30 years old who are either studying or employed. Additionally, 65.5% of the people surveyed were women, 34% were men, and 0.5% were non-binary. Furthermore, 68.9% of the poll respondents said that they are interested in enhancing their health through food, 61.7% said they pay close attention to the food they consume, and 58.9% said they closely regard the ingredients and nutritional information of the food they buy. Moreover, 35.4% said they regularly work out, only 8.6% of the poll said

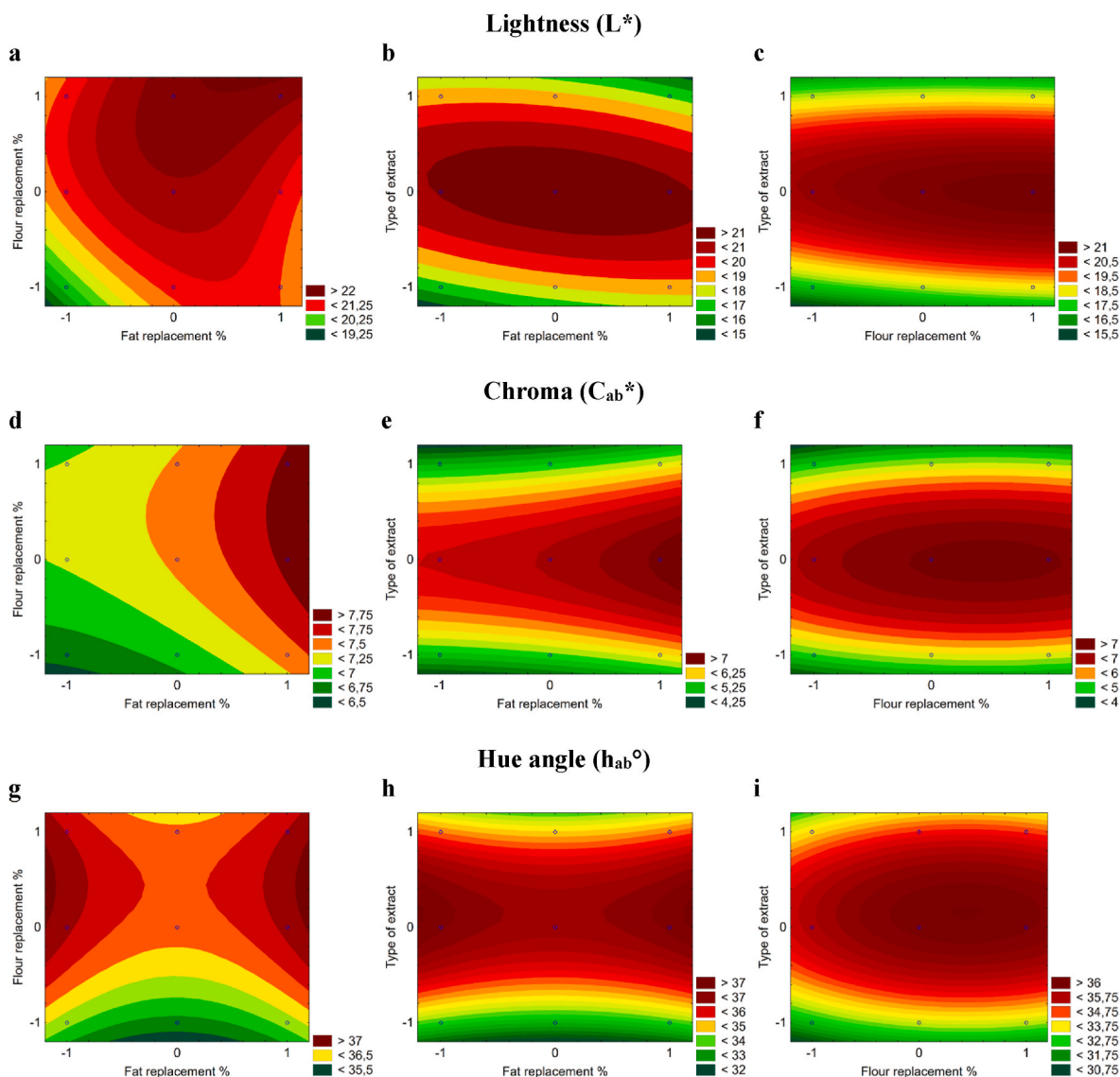


Fig. 2. Contour plots for lightness, chroma, and hue angle.

**Table 2**  
Results from the Box-Behnken experimental design.

Formulation /Experiment	Fat R.	Flour R.	TE	Lightness (L*)	Chroma (C <sub>ab</sub> *)	Hue angle (h <sub>ab</sub> °)	Hardness (N)	Adhesiveness (kg·m <sup>2</sup> /s <sup>2</sup> )	Cohesiveness	Humidity (%)
Control				14.48 ± 0.74 <sup>a</sup>	3.95 ± 0.19 <sup>a</sup>	32.47 ± 2.12 <sup>a</sup>	15.13 ± 0.01 <sup>a</sup>	-0.037 ± 0.001 <sup>a</sup>	-0.020 ± 0.028 <sup>a</sup>	10.50 ± 0.31 <sup>a</sup>
1	-1	-1	0	19.88 ± 2.38 <sup>b</sup>	7.20 ± 0.36 <sup>b</sup>	36.92 ± 1.01 <sup>a</sup>	31.11 ± 0.78 <sup>b</sup>	0.000 ± 0.000 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	6.45 ± 0.52 <sup>b</sup>
2	1	-1	0	21.00 ± 0.57 <sup>b</sup>	6.70 ± 0.63 <sup>a</sup>	34.95 ± 0.52 <sup>a</sup>	65.53 ± 0.56 <sup>b</sup>	-0.205 ± 0.290 <sup>a</sup>	-0.003 ± 0.005 <sup>a</sup>	8.59 ± 0.23 <sup>a</sup>
3	-1	1	0	21.12 ± 0.31 <sup>b</sup>	7.14 ± 0.85 <sup>a</sup>	36.88 ± 0.64 <sup>a</sup>	56.20 ± 4.86 <sup>b</sup>	-0.605 ± 0.856 <sup>a</sup>	-0.013 ± 0.018 <sup>a</sup>	8.31 ± 0.21 <sup>a</sup>
4	1	1	0	21.86 ± 0.15 <sup>b</sup>	8.29 ± 0.03 <sup>a</sup>	37.45 ± 0.03 <sup>a</sup>	66.02 ± 1.05 <sup>b</sup>	-2.520 ± 0.311 <sup>a</sup>	-0.045 ± 0.007 <sup>a</sup>	7.52 ± 0.34 <sup>b</sup>
5	-1	0	-1	16.55 ± 1.63 <sup>a</sup>	4.54 ± 1.43 <sup>a</sup>	32.38 ± 0.37 <sup>a</sup>	22.55 ± 0.59 <sup>a</sup>	-0.015 ± 0.007 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	7.06 ± 0.33 <sup>b</sup>
6	1	0	-1	18.53 ± 2.64 <sup>a</sup>	6.47 ± 0.92 <sup>a</sup>	34.99 ± 0.68 <sup>a</sup>	17.33 ± 0.71 <sup>a</sup>	-0.553 ± 0.166 <sup>a</sup>	-0.033 ± 0.011 <sup>a</sup>	11.37 ± 0.34 <sup>a</sup>
7	-1	0	1	18.53 ± 0.19 <sup>a</sup>	5.43 ± 0.29 <sup>a</sup>	35.83 ± 3.43 <sup>a</sup>	22.44 ± 2.81 <sup>a</sup>	-0.023 ± 0.019 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	7.94 ± 1.97 <sup>b</sup>
8	1	0	1	16.88 ± 0.53 <sup>a</sup>	5.42 ± 1.61 <sup>a</sup>	34.72 ± 0.94 <sup>a</sup>	30.05 ± 1.77 <sup>a</sup>	-0.188 ± 0.059 <sup>a</sup>	-0.007 ± 0.005 <sup>a</sup>	11.69 ± 0.22 <sup>a</sup>
9	0	-1	-1	17.15 ± 0.28 <sup>a</sup>	5.49 ± 1.67 <sup>a</sup>	32.99 ± 3.18 <sup>a</sup>	20.16 ± 1.52 <sup>a</sup>	-0.125 ± 0.163 <sup>a</sup>	-0.005 ± 0.007 <sup>a</sup>	8.51 ± 0.61 <sup>a</sup>
10	0	1	-1	18.49 ± 1.19 <sup>a</sup>	4.97 ± 1.18 <sup>a</sup>	32.6 ± 0.64 <sup>a</sup>	22.43 ± 3.94 <sup>a</sup>	-0.237 ± 0.222 <sup>a</sup>	-0.013 ± 0.011 <sup>a</sup>	9.15 ± 0.86 <sup>a</sup>
11	0	-1	1	18.00 ± 0.28 <sup>a</sup>	4.36 ± 0.56 <sup>a</sup>	33.45 ± 3.64 <sup>a</sup>	25.17 ± 1.67 <sup>a</sup>	-0.015 ± 0.021 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	8.75 ± 0.49 <sup>a</sup>
12	0	1	1	18.26 ± 0.98 <sup>a</sup>	5.63 ± 1.32 <sup>a</sup>	34.99 ± 0.56 <sup>a</sup>	29.48 ± 0.15 <sup>a</sup>	-0.117 ± 0.009 <sup>a</sup>	-0.002 ± 0.002 <sup>a</sup>	9.06 ± 0.34 <sup>a</sup>
13	0	0	0	21.19 ± 0.08 <sup>b</sup>	6.91 ± 1.34 <sup>a</sup>	35.74 ± 1.39 <sup>a</sup>	66.23 ± 0.63 <sup>b</sup>	-0.005 ± 0.007 <sup>a</sup>	0.000 ± 0.000 <sup>a</sup>	8.56 ± 0.45 <sup>a</sup>
14	0	0	0	22.67 ± 1.37 <sup>b</sup>	7.72 ± 0.98 <sup>a</sup>	36.96 ± 0.90 <sup>a</sup>	49.89 ± 12.40 <sup>b</sup>	-2.448 ± 0.016 <sup>a</sup>	-0.052 ± 0.012 <sup>a</sup>	6.37 ± 0.18 <sup>b</sup>
15	0	0	0	21.23 ± 1.59 <sup>b</sup>	7.27 ± 1.25 <sup>a</sup>	37.15 ± 0.90 <sup>a</sup>	57.74 ± 4.82 <sup>b</sup>	-1.668 ± 0.930 <sup>a</sup>	-0.035 ± 0.028 <sup>a</sup>	6.94 ± 0.62 <sup>b</sup>

Values with the letter "a" indicate are not significantly different from the control. Values with the letter "b" are significantly different from the control. R: Replacement %. TE: Type of extract.

they would not buy food with artificial additives, and just 1% said they do not consume meat. When asked about the frequency with which they consume baked goods, 47.8% said they did it once a week and 21.1% every day. The preference survey results can be seen in Fig. 1, in which 36.8% of the participants said they would prefer brownies. With these data in mind, the baked food product was selected.

### 3.2.2. Color analysis

First, it was observed that lightness is influenced by the type of extract and has a slight influence by the percentage of pectin included in the product (Fig. 2a, b, and 2c). Other authors have reported that the presence of pectin in small amounts resulted in lower values of lightness (Olaya Vañó, 2019). It is possible to infer that a formulation using OR extracts and low amounts of pectin and PR flour could result in lightness values similar to the control (~14.48 ± 0.74). Table 2 shows that the lightness of formulations 5–12 is not significantly different from the control (Uncoded variables are shown in Tables S–1). Regarding to chroma (i.e., sample saturation), only the type of extract had a significant effect on the results (Fig. 2d, e, and 2f), which means that the use of either OR PR extracts would favor values closer to those of the control. Nonetheless, using low amounts of PR flour would help to obtain low chroma values, which has also been reported in the literature (Olaya Vañó, 2019). Similarly, hue is highly influenced by the type of extract (Fig. 2g, h, and 2i); however, neither of the evaluated conditions was significantly different compared to the control (Table 2). Overall, it appears that the use of LR extracts in the formulations could negatively affect the color of the brownies. The models and the Pareto charts for the color analysis can be found in Figure S-1.

### 3.2.3. TPA and humidity

It is possible to see in Fig. 3a, b, and 3c that the hardness of the

brownies is highly influenced by the amount of pectin used as fat replacement and the type of extract. The lowest hardness value was obtained with the lowest level of both fat replacement and flour replacement, and also when using either OR PR for the encapsulated extract. It has been reported that an increment in the pectin content of the formulations could increase hardness (Lim et al., 2014). Fats are mainly responsible for the fluffiness of baked goods by interacting with gluten networks (Conforti et al., 1997). The replacement of fat with pectin hinders this effect causing a reduction of product volume and resulting in a more compact structure after baking (Colla et al., 2018; Lim et al., 2014). With this in mind, to obtain similar results to the control (~15.1), the hardness of the evaluated samples should be the lowest possible, which was observed for formulations 5 to 12.

Adhesiveness (Fig. 3d, e, and 3f) was influenced by the replaced percentage of fat and flour. Adhesive samples had more negative values (Table 2) since adhesiveness is the work required to remove a food product from any surface in kg·m<sup>2</sup>/s<sup>2</sup>. None of the measured samples differed from the control. However, formulations 5, 7, and 11 had closer values (~-0.037). The best results for adhesiveness were obtained using 27% flour replacement and 20% fat replacement (Formulation 11). Furthermore, it was observed that the fat replacement and the type of extract influence cohesiveness (Fig. 3g, h, and 3i). The contour plots show that the optimum region is located at the lowest levels of all three experimental design factors, considering the control's cohesiveness (~-0.020). There were no significant differences between the formulations and the control.

Humidity (Fig. 3j and k, and 3L) was affected by the amount of pectin used as fat replacement and the type of extract. This makes sense since pectin is highly hydrophilic. These results are consistent with other authors who also reported that a high pectin content in baked products resulted in a product with high moisture (Correa et al., 2012; Jinxin



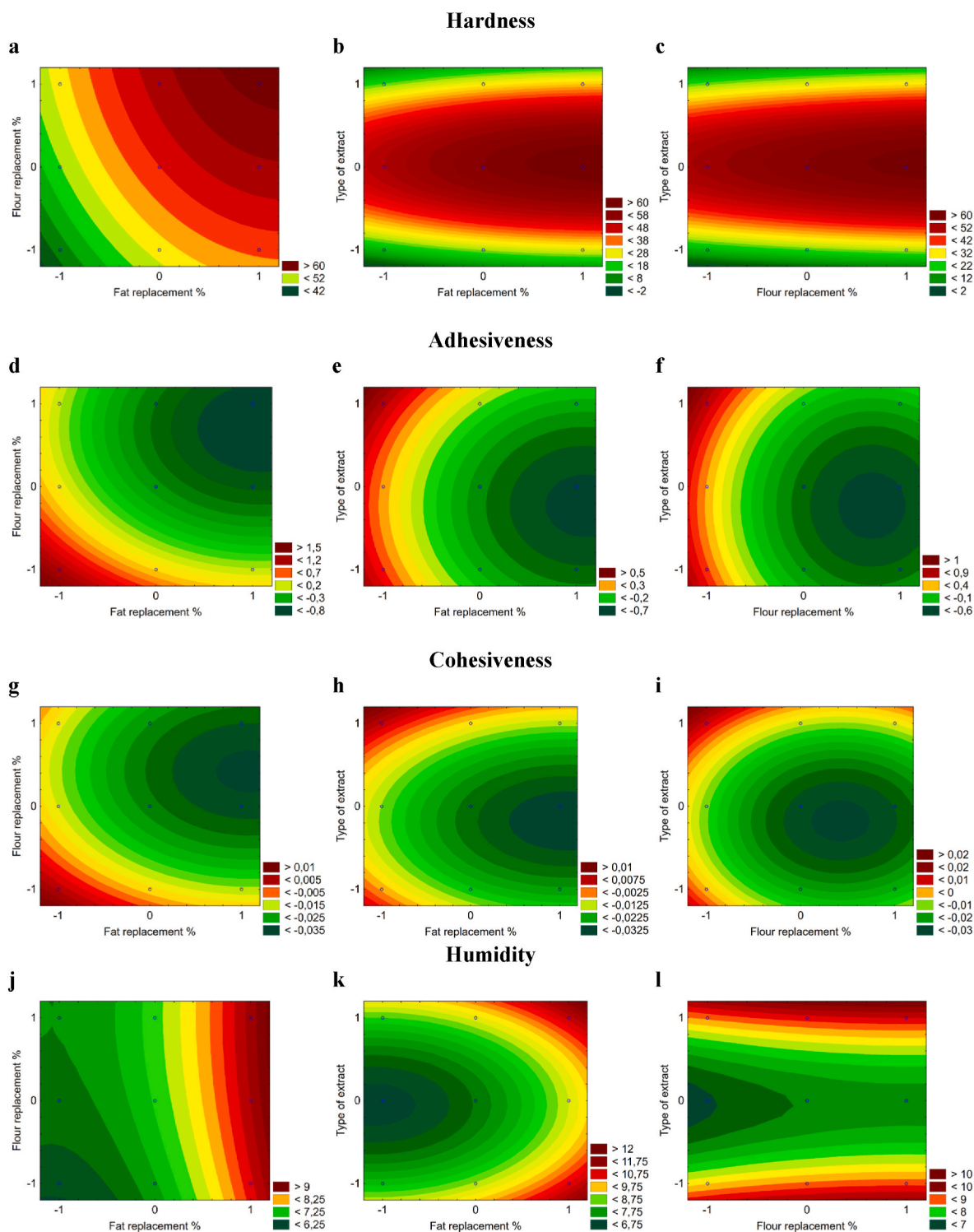


Fig. 3. Contour plots for hardness, adhesiveness, cohesiveness, and humidity.

et al., 2018). A fat replacement between 20 and 30% would result in a humidity of around 8–11%. Formulations 8 to 12 (Table 2) had a humidity similar to the one observed for the control ( $10.50 \pm 0.31\%$ ). Regarding the type of extract, using LR causes a decrease in humidity; using either PR or extracts would be preferable to obtain a baked product with similar properties to the control.

Between the evaluated formulations and their results in terms of color, texture, and humidity, two were selected, which had a behavior closer to the one observed for the control (Table 2): i) Formulation #8

with 30% of fat replacement, 53% of flour replacement, and PR encapsulated extract, and ii) Formulation #9 with 20% of fat replacement, 27% of flour replacement, and OR encapsulated extract.

### 3.2.4. Electronic tongue analysis

The two selected formulations and the control were tested to obtain the taste profile shown in Fig. 4. The first parameter was sourness, a crucial element in flavor profile, which is related to the presence of organic acids in the sample. In this regard, formulation #8 presented a

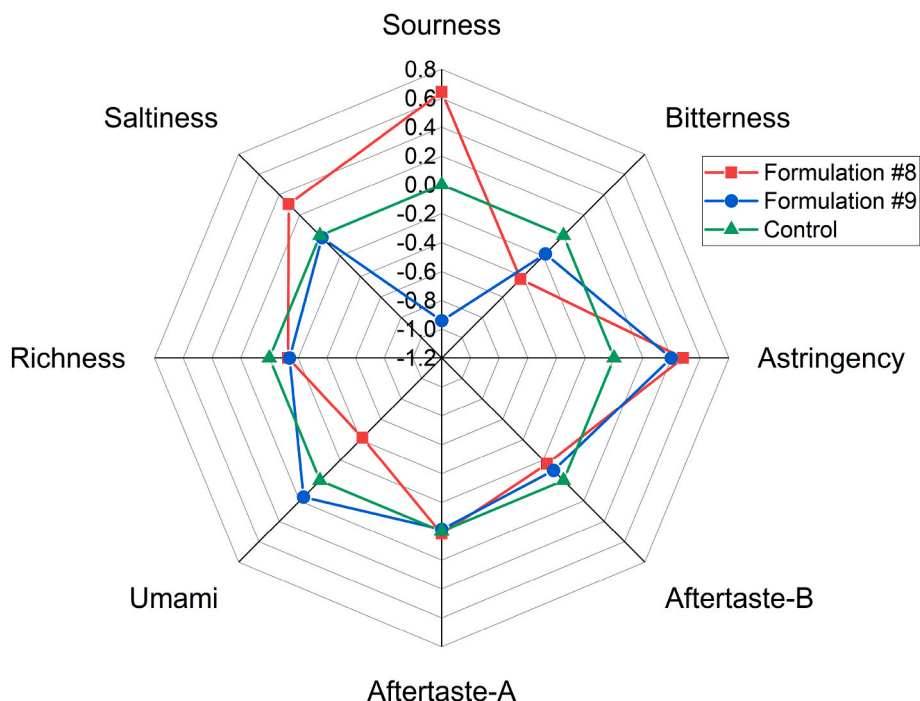


Fig. 4. Electronic tongue results.

value closer to the control than formulation #9. Nonetheless, it would be preferable to use formulation #9 since it would be less sour, a characteristic that a consumer would expect for a brownie. The bitterness values observed in Fig. 4 favor formulation #9, closer to the control than formulation #8. Bitterness is crucial since it plays a role in preventing the intake of poisonous materials, usually related to a sense of distastefulness in food. Similar values for astringency were obtained for formulations #8 and #9, both higher than the value measured for the control. Astringency is related to phenolic compounds like those present in the encapsulated extracts and causes “complex sensations due to shrinking, drawing or puckering of the epithelium as a result of exposure to substances such as alums or tannins” (Bajec & Pickering, 2008). However, the presence of terpenes and carotenoids could also cause the astringency of the formulations. It would be desirable to remove essential oils from the residues using selective extraction techniques. This way, it would be possible to encapsulate separately the polyphenols that enhance the baked product.

The values obtained for aftertaste-A, aftertaste-B, and richness were similar to the control. It is a good indicator that both formulations had the same aftertaste from acidic bitterness and astringency since consumers would have the same experience they could have with a regular brownie. In Fig. 4, values for umami or “savoriness” for formulation #9 were closer to the control. This meaty flavor could alter the final taste of the product with just minor variations in the formulation (Yamaguchi, 1998). The brownie from formulation #9 had a similar taste related to the presence of glutamates and nucleotides compared to the control, which is convenient for consumers. The value obtained for saltiness in formulation #9 was closer to the control, meaning that formulation #8 could be perceived as too salty. This taste is most associated with sodium chloride, a major ingredient in the food industry used to help mask bitter notes and enhance flavor intensity (Batenburg & van der Velden, 2011).

The formulation that presents the slightest differences with the control brownie, not only in color, texture, and humidity but also in terms of flavor profile, was formulation #9. This formulation demonstrated the potential of a 20% fat replacement with pectin, 27% wheat flour replacement with PR flour, and the addition of OR encapsulated extract in the baked product. It is important to note that both selected formulations presented a very similar behavior to the control in terms of

flavor, considering that the differences are less than one unit for all the sensors. Further studies, including a human taste panel, would be able to enhance the formulation of the brownie even more.

#### 4. Conclusions

This work demonstrated the use of relevant fractions from fruit residues as food substitutes for formulating a food product, a baked good. A thorough compositional analysis identified the main substances that can be recovered and included in the product such as phenolic compounds, pectin, and non-soluble fiber. A Box-Behnken experimental design allowed us to identify that the type of extract and the amount of pectin used as a fat replacement in the brownies influenced most product properties such as color, texture, and humidity. From the evaluated conditions, it was possible to identify two promising recipes that had an overall behavior similar to a control brownie. One of these formulations had a similar flavor to the control, which contained 20% fat replacement with pectin, 27% wheat flour replacement with PR flour, and OR encapsulated extract. This study reveals the great potential of using fruit residues in the food industry to enhance their functional properties and design healthier products sustainably.

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#### CRediT authorship contribution statement

**D.D. Durán-Aranguren:** Writing – original draft, Conceptualization, Methodology, Investigation, Validation, Visualization, Formal analysis, Data curation. **L.F. Muñoz-Daza:** Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **L.J. Castillo-Hurtado:** Writing – original draft, Methodology, Investigation, Formal analysis,

Data curation. **J.A. Posada:** Writing – review & editing, Supervision. **S. I. Mussatto:** Writing – review & editing, Supervision. **R. Sierra:** Writing – review & editing, Supervision. **M. Hernández-Carrión:** Writing – review & editing, Supervision.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data availability

Data will be made available on request.

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None.

### Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.lwt.2023.115174>.

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