

# Development and characterization of functional cookies enriched with spray dried strawberry particles: Sensorial, textural, physicochemical properties, antioxidant capacity and bioaccessibility assay of phenolic compounds

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## ABSTRACT

Today's food consumers seek functionality in the products they consume. In this context, the market offers foods enriched with vitamins, minerals, antioxidants, polyphenols, etc. However, it is essential to determine the bioaccessibility of these compounds to understand the amount available for absorption by the body. The aim of this study was to determine the antioxidant capacity and total phenolic content bioaccessibility in a cookie formulated with spray dried strawberry particles (SP) after *in vitro* digestion. SP was produced by spray drying at 160 °C with the addition of a carrier agents (Arabic gum and maltodextrin) at a concentration of 22 % w/v in the feed solution. Two types of cookies were formulated: one without spray dried strawberry particles (F1) and one with spray dried strawberry particles as a partial replacement (38 %) for wheat flour (F2). Moisture, firmness, antioxidant capacity, and total phenolic content were analyzed; bioaccessibility was quantified using the *in vitro* gastrointestinal digestion method. The moisture content for both formulations ranged between 7 % and 8 %, while the firmness was 22.73 N for F1 and 64.27 N for F2. The antioxidant capacity in SP, F1, and F2 was 3.14, 0.62, and 1.66 (mg Trolox Equivalent/g sample); after digestion, the antioxidant capacity was increased in 165.92 %, 108.06 %, and 128.92 % for SP, F1, and F2, respectively. The total phenolic content (mg Gallic Acid Equivalent/g sample) was 11.15 for SP, 0.94 for F1, and 1.45 for F2, with respective bioaccessibility values of 86.19 %, 78.72 %, and 80 %. In conclusion, it was possible to obtain a functional cookie by adding spray dried strawberry particles, with antioxidant capacity and total phenolic content bioaccessibility values that indicate their controlled release after digestion, thus potentially contributing to the achievement of third Sustainable Development Goal.

## Practical application

Food development as part of achieving the Sustainable Development Goals (SDG) is becoming increasingly important. The production of foods rich in bioactive compounds, vitamins, and minerals plays a role in ensuring or helping to meet the nutritional requirements of vulnerable populations in developing countries. Malnourished pregnant women in poor communities are at a highest risk of death. Therefore, this project

proposes a scalable and transferable solution for society by designing, formulating, functionally characterizing, and analyzing the antioxidant capacity and the total phenolic content during *in vitro* gastrointestinal in cookies enriched with strawberry powder produced via spray drying, thereby contributing to the achievement of SDG 3. However, the functional properties of the product correspond to preliminary tests at a pilot scale. In order to achieve a true societal impact, it is necessary to carry out an industrial scaling-up of the product, as well as additional

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nutritional studies.

## 1. Introduction

Fruits and vegetables constitute a fundamental component of human nutrition, providing an essential source of micronutrients required for the optimal physiological functioning of the body (Waleed Fouad Abo-batta, 2021). Adequate consumption of these foods can be associated with a lower incidence of Chronic non-communicable diseases (NCDs) such as obesity, cancer, neurological, and cardiovascular diseases (Campos et al., 2023). In particular, the regular consumption of berries—such as strawberries, blackberries, blueberries, and raspberries—has been associated with notable health-promoting effects. These include the prevention of cardiovascular and inflammatory diseases, as well as protective mechanisms that mitigate the risk of various forms of cancer. Such benefits are largely attributed to their rich composition of bioactive compounds, encompassing vitamins, minerals, aromatic constituents, and polyphenols (Gündeşli et al., 2019).

Strawberries are a source of antioxidants and phenolic compounds (Bebek Markovinović et al., 2022; Bhat & Stammering, 2015), in addition to containing vitamins, carotenoids, and organic acids (Hossain et al., 2016; Ruiz-Rodríguez et al., 2011; Wang et al., 2019). Beyond their nutritional value strawberries are also affordable, commercially significant, and widely consumed, either fresh or processed (Giampieri et al., 2012). Products derived from strawberries include candies (Ali et al., 2021), beverages (Zhao et al., 2021), dairy drinks (Mizuta et al., 2025), jams (Prisacaru et al., 2025), and powdered products (Gong et al., 2018; Leyva-Porras et al., 2021; Sadowska et al., 2020).

Spray drying is a technique that transforms liquid food matrices directly into powdered products through the application of heat and pressure (Osorio-Arias et al., 2020). This method constitutes an efficient alternative to other dehydration processes, such as freeze-drying and convective drying, owing to its relatively low operational costs and its capacity to encapsulate sensitive bioactive compounds—including nutrients, pigments, and flavoring agents—thereby improving their stability and preservation (Shishir & Chen, 2017). The encapsulation of bioactive compounds, such as colorants, antioxidants, phenolic compounds, vitamins, and minerals, plays a crucial role in enhancing their stability and functionality; this technological approach provides effective protection against environmental factors such as light, heat, and oxygen, which can compromise their chemical integrity; additionally, encapsulation minimizes undesirable interactions with other food components during industrial processing and enables a controlled release of these compounds throughout the gastrointestinal digestion process (Đorđević et al., 2016; de Souza Simões et al., 2017). Accordingly, spray drying has been employed by researchers to produce powdered forms of various food products, including blackberry (Vega-Castro et al., 2025), date (Arumugham et al., 2023), and watermelon, orange and mango (Ankeli et al., 2025). The technique has also been utilized for the encapsulation of diverse bioactive and functional compounds, such as vitamin A (Ribeiro et al., 2020), sodium selenite (Grenha et al., 2022), flavoring agents (Pellicer et al., 2019), and strawberry powders fortified with zinc and folic acid (Henao-González et al., 2024).

On the other hand, bakery products are daily consumed and in high quantities due to their low cost, easy accessibility, and sensory acceptance (Nikolaou et al., 2022). Among these products, there are cookies, which provide nutrients such as carbohydrates, proteins, dietary fibers, B-group vitamins, and minerals (Gherghina et al., 2015), and they are an easy vehicle for the incorporation of bioactive compounds. Despite the high consumption of cookies, consumers nowadays prioritize products that fulfill their nutritional, functional, and health requirements (Nikolaou et al., 2022). In this regard, there is a growing interest in developing food products incorporating raw materials with a high content of bioactive compounds, such as antioxidants and phenolic compounds; among these, fruit powders stand out due to their ability to

be easily incorporated into formulations and processing stages during industrial production (Shishir & Chen, 2017). Researchers have reported using flaxseed (Ganorkar & Jain, 2014), soy, cassava, and peach (Cervejeira Bolanho et al., 2014), chestnut shell extract (Pinto et al., 2023), grape pomace (Theagarajan et al., 2019), saffron extract (Bhat et al., 2018), blueberry by-products (Perez et al., 2018), and pumpkin seeds (Weldeyohanis Gebremariam et al., 2024) to make functional cookies.

In addition to the nutritional, functional, textural, sensory, and shelf-life properties that a food may possess, it is now essential to determine the bioaccessibility of the micronutrients present in food during the digestion process. In this regard, bioaccessibility can be defined as the percentage of a micronutrient released from a solid matrix that is potentially available for absorption by the human body after digestion (Pinto et al., 2023). Various authors have determined the bioaccessibility of different compounds in food matrices. In this regard, Pollini et al. (2022) determined that the bioaccessibility of phenolic compounds from freeze-dried apple pomace was 42 %. Liu et al. (2022) found that the bioaccessibility of folate in 12 commercial bread samples varied between 60 % and 120 %. Pinto et al. (2023) reported bioaccessibility values for total phenolic content during the oral, gastric, and intestinal stages at 33 %, 56 %, and 93 %, respectively, for cookies made with chestnut shell (Pinto et al., 2023).

The aim of this study was to evaluate the effect of *in vitro* gastrointestinal digestion on the antioxidant capacity and bioaccessibility percentage of total phenolic content in cookies enriched with spray dried strawberry particles. As a result, this can be translated into a potential contribution to the achievement of the third Sustainable Development Goal (SDG): "Ensure healthy lives and promote well-being for all at all ages" (WHO, 2024), provided that governmental policies are in place to support the industrial scaling and distribution of this type of food, allowing vulnerable communities to effectively access and consume them.

## 2. Materials and methods

### 2.1. Chemicals and reagents

The following materials were used: maltodextrin DE: 18–20 from LLC Interstarch Ukraine and Arabic gum from Alimentos Madretierra. Additionally, Tween 80 from Bell Chem International S.A. was used as a surfactant. To prepare the cookies, the ingredients included butter from KumeY®, baking soda and sugar from Lider®, eggs from Coliumo®, and wheat flour from Mont Blanc®, all of which were purchased from a local market in Valparaíso, Chile.

The following reagents were used: gallic acid from Sigma Life Science Lot. SLBC5726V, acetone from J.T. Baker Lot. M49C58, sulfuric acid 95–97 % from Merck Lot. K50346233 827, methanol from J.T. Baker Lot. C39W64, anhydrous sodium carbonate from GA Lot. IB-1703171, Folin-Ciocalteu's phenol reagent from Merck Lot. HC15894101, 2,2-diphenyl-1-picrylhydrazyl (DPPH) from Aldrich Chemistry, 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) from Aldrich Chemistry.

For the *in vitro* digestion process, the following reagents were used: potassium chloride (KCl) from Riedel de Haën-Arquimed S.A., monopotassium phosphate (KH<sub>2</sub>PO<sub>4</sub>) from Winkler, sodium bicarbonate (NaHCO<sub>3</sub>) from Merck, sodium chloride (NaCl) from GA, magnesium chloride hexahydrate (MgCl<sub>2</sub>(H<sub>2</sub>O)<sub>6</sub>), ammonium carbonate ((NH<sub>4</sub>)<sub>2</sub>CO<sub>3</sub>) from Merck, hydrochloric acid (HCl) from Fermont, calcium chloride dihydrate (CaCl<sub>2</sub>(H<sub>2</sub>O)<sub>2</sub>) from GA, sodium hydroxide (NaOH) from GA, and porcine bile extract (Sigma Aldrich).  $\alpha$ -amylase from porcine pancreas type VI-B  $\geq 5$  U/mg (Sigma Aldrich), pancreatin 8xUSP from porcine pancreas which contains proteolytic, lipolytic and amylolytic activity (Sigma Aldrich), and pepsin  $\geq 25$  U/mg (Sigma Aldrich) were used.

## 2.2. Spray dried strawberry particles production

To obtain the spray dried strawberry particles, fresh strawberries of the *Sabrina* cultivar were collected from the municipality of La Unión, located in the eastern region of the Department of Antioquia, Colombia, at coordinates 5°58'22"N 75°21'40"W, at an average altitude of 2500 m. a.s.l and an average temperature of 17°C. The methodology given by Henao-Gonzalez et al. (2024) was followed to obtain the pulp and design the strawberry dispersion, these methods are briefly described below: Strawberry pulp was then extracted using a fruit pulper (Estructuras y Montajes, Colombia) with a standard sieve No.16, featuring an 1180-micron opening (Blamis Laboratory Supplies, Colombia). A 500 mL strawberry dispersion was then formulated, containing 300 g of strawberry pulp, 5 % Tween 80, 16 % maltodextrin, and 6 % Arabic gum; the latter two were employed as carrier agents in order to minimize stickiness issues in the final product, thereby improving the shelf life and stability of the powder (Shishir & Chen, 2017). All components were homogenized at 14,000 rpm in 3 cycles of 3 min each, using an Ultra-Turrax MDT-G25 homogenizer (Kinematica, Switzerland). Spray dried strawberry particles was produced using a Lab Spray Dryer TP-S15 (Toption Instrument Co., Ltd., China) adapting the methodologies described by Gong et al. (2018) and Leyva-Porras et al. (2021). The conditions of the spray drying process were the drying air temperature was 160°C and the outlet temperature was 88°C and the inlet airflow was 5.5 m<sup>3</sup>/min and the dispersion feeding flow rate was 1.26 mL/min.

## 2.3. Cookie formulation

González Pérez (2007) provided a standard formulation, which was followed to make the cookies on a laboratory scale. Two types of cookies were prepared: a control cookie made without spray dried strawberry particles (formula 1) 160 g of wheat flour, 30 g of sugar, 30 g of butter, 1 g of baking soda, and 64 g of egg; and the second cookie with a partial substitution of wheat flour (38 %) by spray dried strawberry particles (formula 2): 100 g of wheat flour, 30 g of sugar, 30 g of butter, 1 g of baking soda, 64 g of egg and 60 g of spray dried strawberry particles.

The cookie preparation methodology was adapted from Ganorkar and Jain (2014) and Wani et al. (2015). Firstly, the butter was manually whipped until it creamed, then the sugar, baking soda, and egg were added while beating the mixture until it became homogeneous. Once the mixture was uniform, the wheat flour and/or spray dried strawberry particles were added, and the mixture was beaten again until a consistent dough was formed. The dough was then wrapped in plastic film in

cylindrical rolls 3 cm in diameter and refrigerated at 4°C for 10 min. Finally, we cut and baked 0.5 cm thick cookies at 200°C for 10 min in an oven (Thomas elektrogerate model TH-42N01, Germany). Every formulation was made in triplicate, producing approximately ten cookies weighing 20 g each, which is comparable to the weight of store-bought cookies. Fig. 1 shows a photographic summary of the cookie-making process.

## 2.4. Physicochemical characterization of cookie formulations

### 2.4.1. Proximal composition and water activity analysis

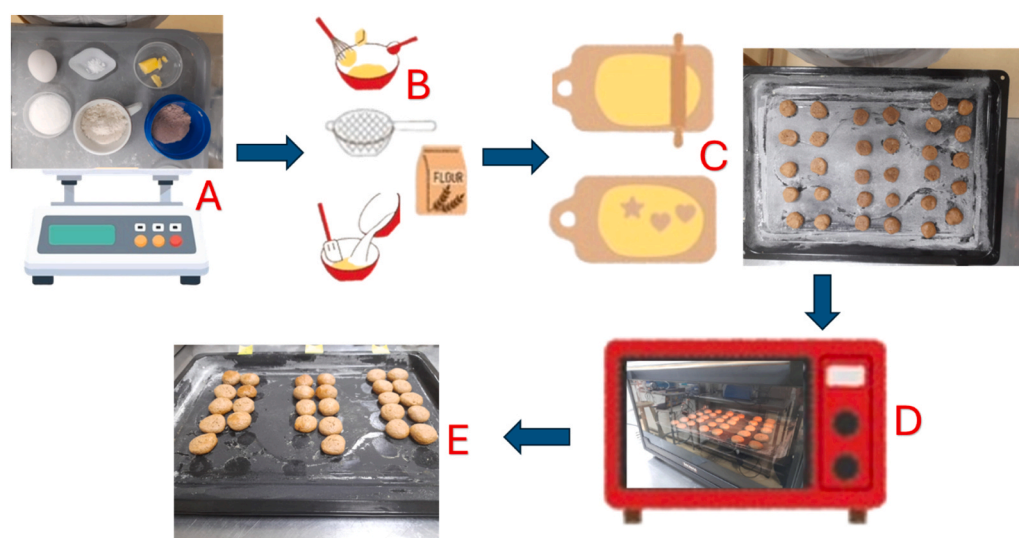
The proximal composition of the cookies was determined by measuring moisture and fat content following the Chilean standards NCh841:2018 and NCh3547:2018 (Instituto Nacional de Normalización, 2018), respectively. Protein, ash, and total dietary fiber content were analyzed according to AOAC (1997) 928.08, 923.03 y 928.29. Water activity (aw) was determined using a ch 8303 device (Rotronic, Switzerland), following the methodology described by Beuchat and Mann (2015).

### 2.4.2. Texture analysis

The hardness of the cookies was determined in Newtons (N) following the methodologies proposed by Cervejeira Bolanho et al. (2014) and Ganorkar and Jain (2014) using a Brookfield CT3 texture analyzer (Massachusetts, US). The test was conducted through a triple beam compression test, using an activation load of 0.07 N, a pre-test speed of 1 mm/s, a test speed of 3 mm/s, and 16 mm of distance. The sample was placed on two support beams separated by 2 cm, and a third beam connected to the movable part of the texture analyzer was lowered until the cookie fractured. Measurements were performed in triplicate.

## 2.5. Sensory analysis of the cookies

A sensory analysis of the two cookie formulations was conducted according to NTC 3932-ISO 11035 (Icontec, 1996). The test was an analytical evaluation based on a sensory profile test by multidimensional scaling (Severiano Pérez, 2019). The test was performed by five trained panelists aged between 25 and 60 years, who evaluated descriptors related to aroma, taste, appearance, texture, and overall quality. The intensity of the descriptors was rated on a scale from 0 to 5, where 0 indicates absence of the parameter, and 5 represents intense presence. Overall quality was rated from 1 to 3, indicating low to high quality, respectively. The significant differences between the results of



**Fig. 1.** Laboratory-scale cookie preparation process. Legend: A. Weighing of ingredients; B. Dough preparation; C. Kneading and shaping; C. Baking process; D. Final product.

both cookie formulations (F1 and F2) were assessed using an independent *t*-test with a 95 % confidence level. However, conducting a single study with only five trained panelists is a limitation acceptable only for pilot-scale product development. For industrial-scale development, more robust sensory tests would be required, involving a larger number of panelists and including consumer evaluations.

## 2.6. In-vitro gastrointestinal digestion

An *in-vitro* static gastrointestinal digestion simulation was performed on the spray dried strawberry particles and the two cookie formulations following the INFOGEST 2.0 protocol (Brodtkorb et al., 2019) and the methodology proposed by Minekus et al. (2014). Simulated digestive fluids: simulated salivary fluid (SSF), simulated gastric fluid (SGF), and simulated intestinal fluid (SIF) were prepared by adding electrolyte stock solutions. The stock solutions of electrolytes for simulated digestive fluids were made in 400 mL of distilled water according to the methodology proposed by Minekus et al. (2014).

The reduction of particle size in the samples is a crucial factor for initiating *in-vitro* gastrointestinal digestion. In general, particle sizes below 2 mm are accepted; therefore, the use of a food chopper allows the particle size to be standardized to this level and enables adequate homogenization of the food bolus (Minekus et al., 2014).

For the procedure, 50 mL centrifuge tubes were used, preparing three tubes for each sample (spray dried strawberry particles and cookie formulations 1 and 2). The cookies were ground using a Thomas Elektrogeräte TH-8735i food processor (Deutschland) before digestion.

### 2.6.1. Oral digestion

For oral digestion, 5 g of each sample was weighed in each tube (Minekus et al., 2014; Zhang et al., 2022; Nuñez et al., 2025) followed by the addition of 3.5 mL of SSF electrolyte stock solution, 0.5 mL of  $\alpha$ -amylase solution (1500 U/mL), 25  $\mu$ L of  $\text{CaCl}_2$  (0.3 M), and 975  $\mu$ L of distilled water to achieve a 1:1 (w/v) ratio. Each tube was incubated at pH 7.0 in a water bath at 37°C for 2 min with constant stirring at 200 rpm. After this, one of the tubes with 10 mL of the resulting oral bolus was taken for each sample, centrifuged at 10,000 rpm for 10 min, the supernatant was collected and stored frozen (-20°C) for later analysis of antioxidant capacity and total phenolic content, while the remaining tubes proceeded to the gastric stage. For the spray dried strawberry particles, sampling was not performed during the oral stage, as it is a powder product that does not require chewing for digestion initiation; therefore, sampling for analysis was done during the gastric stage (Minekus et al., 2014).

### 2.6.2. Gastric digestion

For gastric digestion, each tube containing 10 mL of the oral bolus was mixed with 7.5 mL of preheated SGF electrolyte stock solution (37°C), 1.6 mL of porcine pepsin solution (2000 U/mL), 5  $\mu$ L of  $\text{CaCl}_2$  (0.3 M), 1 M HCl to adjust the pH to 3.0, and distilled water to achieve a 1:1 (v/v) ratio, according to INFOGEST 2.0 protocol. The mixture was incubated in a water bath at 37°C for 2 h with constant stirring at 200 rpm. At the end, one of the tubes containing the resulting gastric chyme was taken for each sample, centrifuged at 10,000 rpm for 10 min, the supernatant was collected and stored frozen (-20°C) for later analysis of antioxidant capacity and total phenolic content. The remaining tubes proceeded to the intestinal stage.

### 2.6.3. Intestinal digestion

For intestinal digestion, each tube containing 20 mL of gastric chyme was mixed with 11 mL of preheated SIF electrolyte stock solution (37°C), 5 mL of pancreatin solution (100 U/mL) based on the trypsin activity, 2.5 mL of bile, 40  $\mu$ L of  $\text{CaCl}_2$  (0.3 M), 1 M NaOH to adjust the pH to 7.0, and distilled water to achieve a 1:1 (v/v) ratio, according to INFOGEST 2.0 protocol. The mixture was incubated in a water bath at 37°C for 2 h with constant stirring at 200 rpm. At the end, the tubes

containing the digestion products were centrifuged at 10,000 rpm for 10 min, the supernatant was collected and stored frozen (-20°C) for later analysis of antioxidant capacity and total phenolic content.

Finally, bioaccessibility was calculated using Eq. 1, comparing the total phenolic content and antioxidant capacity in the sample with the content in the gastrointestinal fluids (oral, gastric, and intestinal stages) (Pollini et al., 2022).

$$BAC = \frac{E}{A} \times 100 \quad (1)$$

Where: BAC: Bioaccessibility (%). E: Total phenolic content and antioxidant capacity in the gastrointestinal fluids at the end of each stage. A: Total phenolic content and antioxidant capacity in the initial sample.

## 2.7. Quantification of antioxidant capacity and total phenolic content

### 2.7.1. Preparation of extracts

For the determination of antioxidant capacity and total phenolic content, extracts of undigested samples (spray dried strawberry particles and cookies), digestion products and digestion blanks were prepared. A total of 0.5 g of spray dried strawberry particles and cookies, and 5 g of digestion products were placed in centrifuge tubes and then mixed with 8 mL of acidified methanol-water (50:50, v/v, pH 2). The mixture was vortexed (FINEPCR, Model: FINEVORTEX, Korea) for 1 min and then subjected to ultrasonic treatment (Elma Ultrasonic Steam, Singen, Germany) at  $25 \pm 0.1^\circ\text{C}$  for 15 min, followed by centrifugation at 5000 rpm for 15 min in a centrifuge (HERMLE Labortechnik Z323K, Germany). The supernatant was recovered; the residue was then treated with 8 mL of acetone-water (70:30), repeating the previous steps (shaking, ultrasonic treatment, centrifugation, and supernatant recovery). The recovered supernatants were combined and transferred to a 25 mL volumetric flask, diluted with distilled water to the mark. The extracts were stored at -20 °C. This method was slightly modified from that described by Contreras-Calderón et al. (2011).

### 2.7.2. Antioxidant capacity

Antioxidant capacity were determined in each stage of *in-vitro* digestion using the DPPH method, following the procedure described by Hoyos-Arbeláez et al. (2018) with minor modifications. For the calibration curve, aqueous solutions of Trolox with concentrations ranging from 0 to 500  $\mu$ M were used. An aliquot of 200  $\mu$ L of the sample extract or Trolox standard was mixed with 1000  $\mu$ L of methanol and 400  $\mu$ L of DPPH solution (0.5 mM) and incubated in the dark at room temperature for 30 min. Absorbance was measured at 515 nm using a spectrophotometer (Spectronic Genesys 5, Thermo Scientific, USA). Results were expressed as  $\mu$ mol of Trolox Equivalents (TEs) per gram of sample ( $\mu$ mol of TEs/g).

### 2.7.3. Total phenolic content (TPC)

Total phenolic content was determined using the Folin-Ciocalteu assay, following the methodology described by Contreras-Calderón et al. (2011). The calibration curves were constructed as detailed by Henao-González et al. (2024). Results were expressed as milligrams of Gallic Acid Equivalents (GAEs) per gram of sample (mg of GAE/g).

### 2.7.4. Experimental Design of cookie formulations and simulated digestion

Data from the characterization of the two cookie formulations were analyzed using a completely randomized experimental design, with the independent variable being the type of cookie formulation: F1 and F2. Response variables included density, aw, moisture, fat, protein, ash, fiber, and hardness. Data was analyzed using an ANOVA table, with a 95 % significance level, the ANOVA table is used to determine whether the independent variable significantly affected the response variables ( $p < 0.05$ ) (Gutiérrez-Pulido and Vara-Salazar, 2012). In turn, LSD-Fisher test at the 95 % confidence level was applied to determine



significant differences between the treatments (F1 and F2). To determine the correct error distribution of the measured data, the assumption of normality was verified, which aims to confirm that all data fit a normal distribution; for this purpose, the Shapiro–Wilk test was performed ( $p > 0.05$ ). In addition, to assess whether all the data fitted the mathematical model of the design (Eq. 2), the Kolmogorov–Smirnov test was applied with a 95 % significance level.

$$Y_{ij} = \mu + \tau_i \varepsilon_{ij} \quad (2)$$

Where:  $\mu$  is the scale parameter common to all treatments, designated as the *overall mean*.  $\tau_i$  represents a parameter that quantifies the effect of the treatment.  $\varepsilon_{ij}$  represents the error associated with the measurement  $Y_{ij}$ .

For the analysis of simulated digestion data across the three food matrices studied: spray dried strawberry particle sand the two cookie formulations (F1 and F2); data were analyzed using a completely randomized experimental design. The independent variable was the type of digestion phase, with 4 levels: no digestion (ND), oral digestion (OD), gastric digestion (GD), and intestinal digestion (ID). Response variables included total phenolic content and antioxidant capacity. Data was analyzed using an ANOVA table, with a 95 % significance level, to determine if different stages of digestion had a significant influence on the studied variables. Differences between food with and without digestion were assessed using multiple range tests by the LSD-Fisher method at 95 %.

### 3. Results

#### 3.1. Effect of spray dried strawberry particles addition on the proximal composition of cookies

Table 1 presents the results of the proximal composition of the two cookie formulations developed in this study (F1: Control Cookie; F2: Spray dried strawberry particles replacement Cookie). It can be observed that the water activity (aw) values for F1 and F2 are 0.636 and 0.577, respectively. These values fall within the aw range of 0.5–0.8, which is favorable for the shelf life of cookies, as in this range reduces the likelihood of microbial proliferation (Cervejeira Bolanho et al., 2014). The lower aw observed in F2 can be attributed to the maltodextrin (MD) and gum arabic (GA) present in the spray dried strawberry particles, due to their high molecular weights (MD > 1800 g/mol and GA > 380 kDa) (Kingwater et al., 2015). In general, all proximal parameters evaluated for the two cookie formulations showed significant differences ( $p < 0.05$ ), as detailed in Table 1.

The moisture content in F1 and F2 was 7.28 % and 8.81 %, respectively, while the protein content was 10.2 % and 8.63 % (Table 1). The lower moisture content in F1 may be due to its higher protein content, which results in greater water retention and lower aw (Rodríguez Carbajo, 2014). Conversely, the reduction in protein content in F2 is associated with the decreased amount of wheat flour in its formulation. Regarding moisture content, the values shown in Table 1 are consistent with those reported by Lee and Ko (2009), who found that cookies

formulated with spray dried strawberry particles had higher moisture content compared to those with only wheat flour.

The higher ash content in F2 (Table 1) is attributed to the presence of various minerals (iron, magnesium, phosphorus, etc.) in strawberries, which are retained in the final product (Giampieri et al., 2012). This effect was also reported by Ngo et al. (2024) for sweet cookies with 10 % - 40 % replacement of wheat flour with jackfruit peel powder. The lower fiber and fat content in F2 is related to the lower amounts of fiber and fat in strawberries (2 % and 0.3 %, respectively) compared to wheat (2.7 % and 2 %) (Giampieri et al., 2012; Surco Almendras & Alvarado Kirigin, 2010).

Table 2 presents the lack-of-fit test values for water activity, moisture, fat, protein, ash, and total dietary fiber of the studied cookies (F1 and F2). Consequently, the p-value of the Kolmogorov–Smirnov test, being greater than 0.05, indicates that the experimental data fits the mathematical model of the proposed design. Similarly, Fig. 2a–f exhibit the symmetrical graphs for the normality test, showing that the data in Table 1 corresponds to a normal distribution and hence align with the mathematical model of a free random experiment design.

#### 3.2. Effect of spray dried strawberry particles addition on cookie texture

Analysis of variance showed significant differences ( $p < 0.05$ ) in the hardness parameters between the two cookie formulations. The hardness values for F1 and F2 were  $22.73 \pm 1.55$  N and  $64.27 \pm 2.51$  N, respectively (Fig. 3). This behavior may be due to the partial substitution of wheat flour and the maltodextrin contributed by the spray dried strawberry particles in the formulation, as maltodextrin gels/gelatinizes in the presence of water, resulting in increased viscosity in the dough and, consequently, higher hardness values of the cookies (Nadian et al., 2021).

The results regarding the texture of cookies with and without spray dried strawberry particles addition are like those found by Cervejeira Bolanho et al. (2014), who reported hardness values ranging from 18 N to 62.5 N for various cookie formulations made with wheat flour and partial replacements with soy, peach, and cassava flours. This study also highlights that the formulation with only wheat flour has a lower hardness value compared to formulations with replacements of other flours, which is consistent with our findings. Similarly, Blanco Canalis et al. (2020) observed that the hardness of sweet cookies increased from 21.8 N to 36.3 N when peach puree was incorporated into the formulation.

#### 3.3. Effect of spray dried strawberry particles addition on the sensory quality of the cookies

Fig. 4 shows the multidimensional sensory profile for the two cookie formulations (F1 and F2). Regarding taste, the most important descriptors and their ratings were: cereal ( $2.1 \pm 0.2$  and  $0.7 \pm 0.2$ ), sweet ( $1.2 \pm 0.2$  and  $2.2 \pm 0.2$ ), fresh ( $3.4 \pm 0.2$  and  $4.0 \pm 0.0$ ), fruity (0.0

**Table 1**  
Proximal composition of cookie formulations.

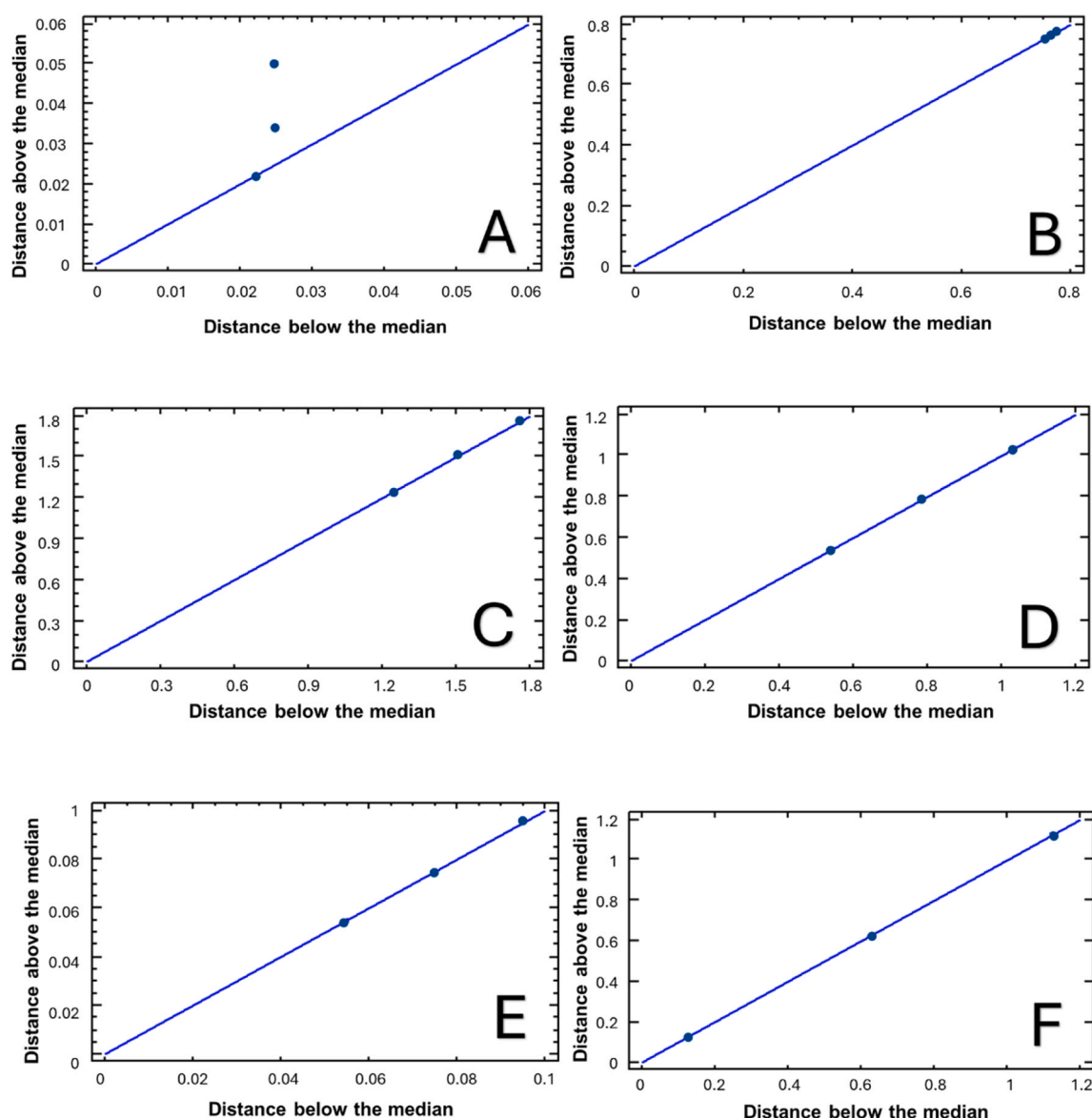
| Parameter                     | Formula 1          | Formula 2          |
|-------------------------------|--------------------|--------------------|
| Water activity                | $0.64 \pm 0.01^a$  | $0.58 \pm 0.00^b$  |
| Moisture (g/100 g)            | $7.28 \pm 0.01^a$  | $8.81 \pm 0.01^b$  |
| Fat (g/100 g)                 | $13.00 \pm 0.25^a$ | $10.00 \pm 0.25^b$ |
| Protein (g/100 g)             | $10.2 \pm 0.25^a$  | $8.63 \pm 0.25^b$  |
| Ashes (g/100 g)               | $1.02 \pm 0.02^a$  | $1.17 \pm 0.02^b$  |
| Total dietary fiber (g/100 g) | $4.53 \pm 0.50^a$  | $3.28 \pm 0.50^b$  |

Legend: The measurements were carried out in triplicate, the different letters (a, b) on the same line denote significant differences between the samples ( $p < 0.05$ ).

**Table 2**  
P-values for normality test and lack of fit test of the different response variables.

| Variable            | Shapiro-Wilk test p-value | Kolmogorov- Smirnov test p-value |
|---------------------|---------------------------|----------------------------------|
| Water activity      | 0.098                     | 0.677                            |
| Moisture            | 0.004                     | 0.586                            |
| Fat                 | 0.052                     | 0.757                            |
| Protein             | 0.273                     | 0.918                            |
| Ashes               | 0.166                     | 0.869                            |
| Total dietary fiber | 0.987                     | 0.999                            |

Legend: P-values greater than 0.05 from the Shapiro-Wilk test indicate that the variable meets the normality assumption. P-values greater than 0.05 from the Kolmogorov–Smirnov test indicate that the variable fits the mathematical model of the experimental design.



**Fig. 2.** Symmetry graphs of the normality test, for the different response variables. Legend: A: Water activity. B: Moisture. C: Fat. D: Protein. E: Ash. F: Total dietary fiber.

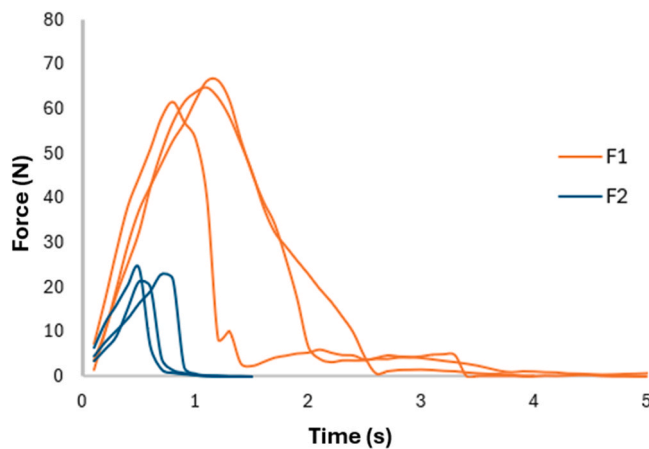
$\pm 0.0$  and  $0.5 \pm 0.1$ ), and baked ( $2.0 \pm 0.0$  and  $2.6 \pm 0.2$ ) for F1 and F2, respectively. Significant differences ( $p < 0.05$ ) were observed between the two formulations. For aroma, the most prominent attributes and their respective ratings for F1 and F2 were cereal ( $2.0 \pm 0.21$  and  $0.5 \pm 0.0$ ), sweet ( $1.2 \pm 0.3$  and  $2.4 \pm 0.2$ ), fresh ( $3.5 \pm 0.1$  and  $4.0 \pm 0.0$ ), fruity ( $0.0 \pm 0.1$  and  $0.9 \pm 0.3$ ), and baked ( $2.0 \pm 0.0$  and  $2.5 \pm 0.0$ ), again showing significant differences ( $p < 0.05$ ).

The predominance of cereal aroma and taste in F1 can be attributed to its higher wheat flour content (Hernández et al., 2015), while the higher ratings for sweet, fresh, and fruity aromas and tastes in F2 can be explained by the contribution of total soluble solids, citric acid, esters, aldehydes, alcohols, terpenes, and furanones from the spray dried strawberry particles (Kafkas et al., 2005; Pérez De Camacaro et al., 2017).

In terms of texture, the main descriptors and ratings for F1 and F2 were: fracturable ( $2.8 \pm 0.3$  and  $1.9 \pm 0.4$ ), chewy ( $2.7 \pm 0.3$  and  $2.5 \pm 0.1$ ), and dry ( $3.5 \pm 0.0$  and  $3.0 \pm 0.0$ ), with significant differences ( $p < 0.05$ ) between both formulations. The higher dry texture rating in F1 may be due to its lower moisture content, while the gluten in wheat

flour contributes to a higher chewiness rating in F1, which has a higher wheat flour content (Rincón Montiel, 2017). Additionally, the higher fat content in F1 (Table 1) improves the aeration of the cookie, increasing its fracturable texture score. Meanwhile, the lower fracturable texture in F2 may improve its storage and transport stability (Díaz S. & Hernández G., 2012).

Concerning overall cookie quality, the analysis of variance revealed significant differences ( $p < 0.05$ ) between the two formulations. F1 received a rating of  $2.0 \pm 0.0$ , while F2 was rated  $2.5 \pm 0.1$ , with F2 containing spray dried strawberry particles showing better overall quality. The differences observed in the descriptors for aroma, taste, and texture, as well as the improvement in overall quality, indicate that spray dried strawberry particles influenced the sensory characteristics of the cookie. This effect has also been reported by other authors. For instance, Blanco Canalis et al. (2020) found that adding peach puree to a sweet cookie formulation significantly affected perceived sweetness, while Indrianingsih et al. (2024) found that adding pumpkin and cassava flour influenced sensory parameters such as color, taste, and texture, leading to increased general acceptance.



**Fig. 3.** Textural properties of cookies. Legend: The different lines of the same color represent triplicate analyses. Orange line corresponds to F1: Control cookie. Blue line corresponds to F2: Spray dried strawberry particles replacement Cookie.

### 3.4. Evaluation of total phenolic content bioaccessibility and antioxidant capacity

Table 3 shows the antioxidant capacity and total phenolic content (TPC) of the products, both before and after digestion, for the spray dried strawberry particles and the two cookie formulations (F1 and F2). Table 4 also displays the percentages of bioaccessibility after each stage of digestion.

The TPC of the spray dried strawberry particles, control cookie (F1), and strawberry powder cookie (F2) were 11.15, 0.94, and 1.45 mg GAE/g sample, while the antioxidant capacity was 3.14, 0.62, and 1.45 mg TE/g sample, respectively (Table 3). Total phenolic content and antioxidant capacity values for spray dried strawberry particles have been reported by Leyva-Porras et al. (2021), who found total phenolic content ranging from 5.9 to 10.8 mg GAE/g sample and antioxidant capacity, using the DPPH method, from 48.95 to 81.50  $\mu\text{mol TE/g}$  sample. Differences between reported values and those of this study can be attributed to the method employed, the concentration of drying agents, drying temperature, as well as the strawberry variety and ripeness (Pérez De Camacaro et al., 2017; Shishir & Chen, 2017).

The total phenolic content in F1 is due to contributions from wheat flour (4.6 – 5.0 mg GAE/g) (Yu et al., 2013) and melanoidins formed by Maillard reactions during the baking process (Blanco Canalis et al., 2020). Meanwhile, the antioxidant capacity is attributed to the wheat flour (1.16 – 1.55  $\mu\text{mol TE/g}$ ) (Yu et al., 2013). The lower antioxidant capacity and total phenolic content in the cookies compared to wheat flour may result from oxidative degradation during baking (Shishir & Chen, 2017). The increase in total phenolic content and antioxidant capacity in F2 compared to F1 is due to the incorporation of spray dried strawberry particles, a finding also observed by Ngo et al. (2024), who reported increased total phenolic content and antioxidant capacity in sweet cookies when jackfruit peel powder was added.

After the oral phase, the TPC in F1 and F2 were 0.59 and 0.67 mg Gallic Acid/g sample, while the antioxidant capacity was 0.26 and 0.97  $\mu\text{mol TE/g}$  sample, respectively (Table 3). These values indicate partial release of TPC and antioxidant compounds by the end of the oral phase, likely due to the brief contact with the enzymatic system (Lucas-González et al., 2018; Pinto et al., 2023).

At the end of the gastric phase, TPC values were 5.46, 0.68, and 0.76 mg Gallic Acid/g sample, and antioxidant capacity values were 1.87, 0.28, and 1.02  $\mu\text{mol TE/g}$  sample for the spray dried strawberry particles, F1, and F2, respectively (Table 3). The increase in TPC and antioxidant capacity in the gastric phase compared to the oral phase may be due to better digestion under acidic pH and prolonged contact with

gastric enzymes, allowing greater release of bioactive compounds from the food matrix (Blanco Canalis et al., 2020; Lucini Mas et al., 2022; Pinto et al., 2023).

Upon completing the intestinal phase, TPC values were 9.61, 0.74, and 1.16 mg Gallic Acid/g sample, while antioxidant capacity was 5.21, 0.67, and 2.14  $\mu\text{mol TE/g}$  sample for the spray dried strawberry particles, F1, and F2, respectively (Table 3). The increase in total phenolic content and antioxidant capacity in the intestinal phase compared to the oral and gastric phases can be explained by the action of pancreatin and bile salts at neutral pH, which facilitate the release of total phenolic content bound to the cookie matrix under prolonged digestion (Blanco Canalis et al., 2020; Lucini Mas et al., 2022; Pinto et al., 2023). Significant differences ( $p < 0.05$ ) were observed for TPC and antioxidant capacity throughout the three digestion phases (oral, gastric, and intestinal) compared to undigested samples (Table 3).

The behavior of TPC and antioxidant capacity throughout the three digestion phases corresponds to the results of Blanco Canalis et al. (2020), who reported a reduction in TPC from 194.07 to 56.40 mg GAE/100 g sample after the oral phase, an increase to 148.90 mg GAE/100 g sample after the gastric phase and a final value of 88.61 mg GAE/100 g sample after the intestinal phase in cookies enriched with peach pulp. Pinto et al. (2023) similarly reported that the total phenolic content (TPC) and antioxidant capacity, evaluated using the DPPH method, were 164 mg GAE/100 g sample and 67 mg TE/100 g sample, respectively, in cookies enhanced with chestnut shell extract. After simulating the digestive process, TPC values of 54, 92, and 153 mg GAE/100 g sample, and antioxidant capacities of 40, 50, and 161 mg TE/100 g sample, were observed for the oral, gastric, and intestinal phases, respectively.

Using the values of TPC and antioxidant capacity from each digestion stage and applying Eq. 1, bioaccessibility percentages for the three analyzed products were calculated. After digestion, the bioaccessibility of TPC was 86 %, 79 %, and 80 %, while the antioxidant capacity increased in 166 %, 108 %, and 129 % for spray dried strawberry particles, F1, and F2, respectively (Table 4). These results align with those reported by Pinto et al. (2023), who observed bioaccessibility percentage of 93 % for total phenolic content and an increase of 240 % for antioxidant capacity after digestion of cookies enriched with chestnut shell extracts.

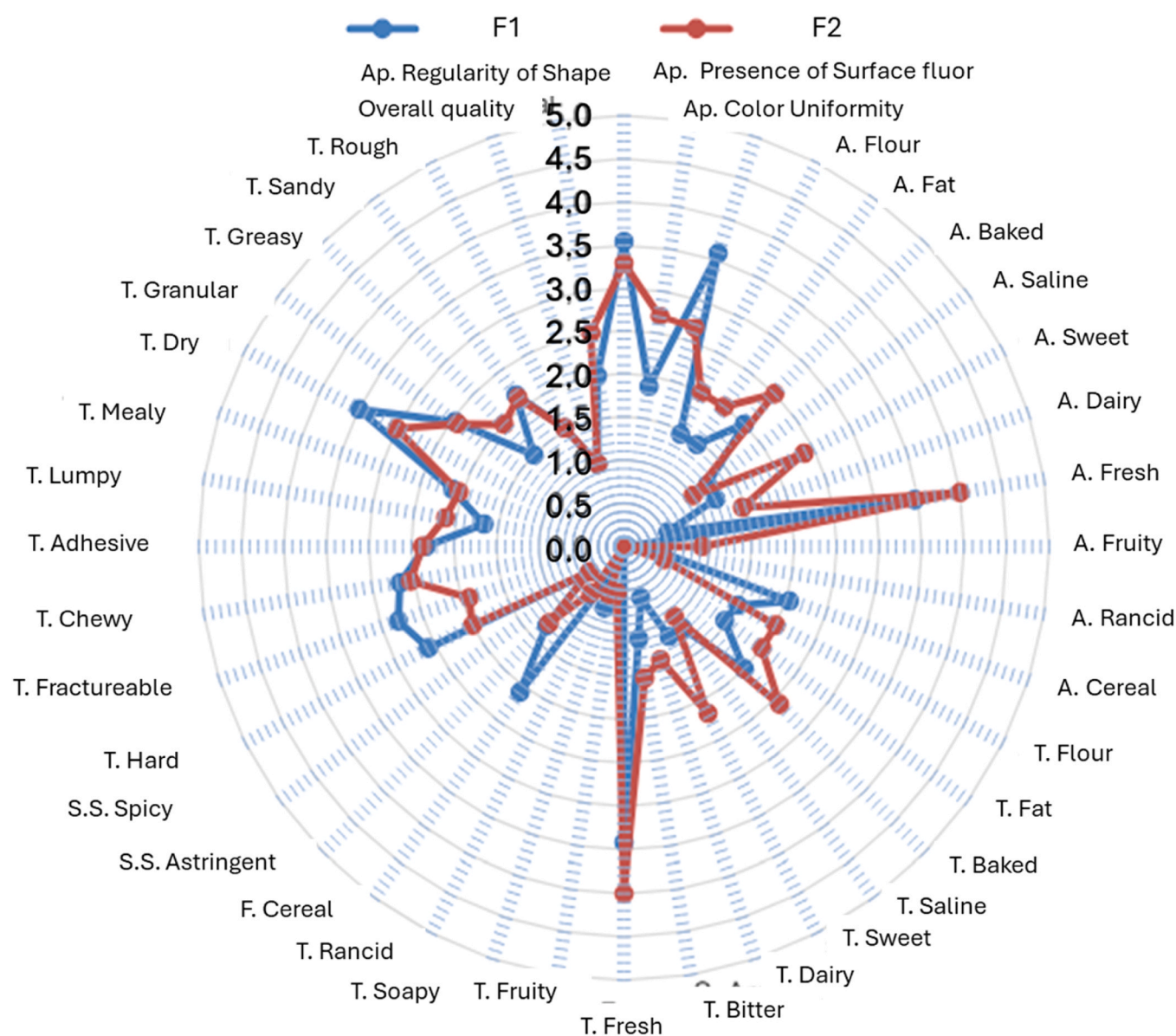
In general, the total phenolic content and antioxidant capacity during the three digestion stages for the products analyzed in this study, as well as those reported in the literature, followed the pattern: oral stage < gastric stage < intestinal stage. This suggests a controlled release of bioactive compounds throughout the digestion stages, ensuring the highest bioaccessibility of these compounds in the intestinal stage, which is expected since this is where absorption of bioactive compounds occurs.

## 4. Conclusions

It was possible to develop and characterize a cookie with the addition of spray dried strawberry particles, featuring an appropriate proximal composition, consistent with this type of product, retention of bioactive compounds derived from the raw materials, and a sensory quality acceptable to consumers; this was done because the results obtained are consistent with those reported in the literature for the development of cookies with partial substitution of wheat flour. After *in vitro* digestion, the total phenolic content bioaccessibility percentages were 86 % and 80 %, while antioxidant capacity exhibited an increase of 166 % and 129 % for the spray dried strawberry particles and the cookie formulated with spray dried strawberry particles, respectively. This indicates that the bioactive compounds present in the spray dried strawberry particles were retained in the cookies and were predominantly released at the final stage of digestion, potentially contributing to the fulfillment of the Sustainable Development Goal 3 (Good Health and Well-being).

However, future studies should include a comparative analysis of





**Fig. 4.** Sensory profile by multidimensional scaling for the two cookie formulations. Legend: A: Aroma. T: Taste. Ap: Appearance. T: Texture. S.S: Somatosensory sensation. F1: Control cookie. F2: Spray dried strawberry particles replacement cookie.

**Table 3**

Total phenolic content and antioxidant capacity of cookies and spray dried strawberry particles in the different stages of simulated *in vitro* digestion.

| Product           | Parameter       | Undigested product        | simulated <i>in vitro</i> digestion |                          |                          |
|-------------------|-----------------|---------------------------|-------------------------------------|--------------------------|--------------------------|
|                   |                 |                           | Oral digestion                      | Gastric digestión        | Intestinal digestión     |
| Strawberry powder | TPC (mg GAE/g)  | 11.15 ± 0.23 <sup>a</sup> |                                     | 5.46 ± 0.21 <sup>c</sup> | 9.61 ± 0.17 <sup>d</sup> |
|                   | Aox (μmol TE/g) | 3.14 ± 0.22 <sup>a</sup>  |                                     | 1.87 ± 0.11 <sup>c</sup> | 5.21 ± 0.36 <sup>d</sup> |
| F1                | TPC (mg GAE/g)  | 0.94 ± 0.04 <sup>d</sup>  | 0.59 ± 0.02 <sup>c</sup>            | 0.68 ± 0.03 <sup>b</sup> | 0.74 ± 0.03 <sup>a</sup> |
|                   | Aox (μmol TE/g) | 0.62 ± 0.02 <sup>d</sup>  | 0.26 ± 0.01 <sup>c</sup>            | 0.28 ± 0.01 <sup>b</sup> | 0.67 ± 0.01 <sup>a</sup> |
| F2                | TPC (mg GAE/g)  | 1.45 ± 0.06 <sup>a</sup>  | 0.67 ± 0.03 <sup>d</sup>            | 0.76 ± 0.03 <sup>c</sup> | 1.16 ± 0.05 <sup>b</sup> |
|                   | Aox (μmol TE/g) | 1.66 ± 0.07 <sup>c</sup>  | 0.97 ± 0.04 <sup>d</sup>            | 1.02 ± 0.02 <sup>d</sup> | 2.14 ± 0.04 <sup>b</sup> |

Legend: F1: Cookie formula 1 (Control cookie). F2: Cookie formula 2 (Spray dried strawberry particles replacement cookie). GAE: Gallic acid equivalent. TE: Trolox equivalent. g: sample gram. BAC: Bioaccessibility. TPC: Total Phenolic Content. Aox: Antioxidant capacity. The different letters (a, b, c, d) in the same row denote significant differences ( $p < 0.05$ ) in TPC and Aox at different digestion stages for each sample. The different letters (a, b, c, d) in the same column denote significant differences ( $p < 0.05$ ) in F1 and F2 for Aox and TPC at different digestion stages for each sample.

antioxidant capacity using methods such as ABTS, FRAP, and ORAC, which will provide a broader assessment of the antioxidant potential of the analyzed samples. Likewise, it is essential that future research focuses on the determination and characterization of the various types of

individual polyphenols present in food matrices through advanced chromatographic techniques, such as HPLC or LC-MS, thereby improving our understanding of their behavior and release throughout the stages of simulated *in vitro* digestion.



**Table 4**

Percentage of bioaccessibility, total phenolic content and antioxidant capacity for cookies and spray dried strawberry particles in the different stages of simulated *in vitro* digestion.

| Product           | Parameter   | Simulated <i>in vitro</i> digestion |                               |                                |
|-------------------|-------------|-------------------------------------|-------------------------------|--------------------------------|
|                   |             | Oral digestion                      | Gastric digestion             | Intestinal digestion           |
| Strawberry powder | Aox (%)     |                                     | 59.55<br>± 11.28 <sup>b</sup> | 165.92<br>± 23.09 <sup>c</sup> |
|                   | BAC TPC (%) |                                     | 48.97<br>± 2.90 <sup>b</sup>  | 86.19 ± 3.30 <sup>c</sup>      |
| F1                | Aox (%)     | 41.94<br>± 2.97 <sup>c</sup>        | 45.16<br>± 3.07 <sup>b</sup>  | 108.06 ± 5.10 <sup>a</sup>     |
|                   | BAC TPC (%) | 62.77<br>± 4.80 <sup>c</sup>        | 72.34<br>± 6.27 <sup>b</sup>  | 78.72 ± 6.54 <sup>a</sup>      |
| F2                | Aox (%)     | 58.43<br>± 4.87 <sup>a</sup>        | 61.45<br>± 3.80 <sup>c</sup>  | 128.92 ± 7.85 <sup>b</sup>     |
|                   | BAC TPC (%) | 46.21<br>± 3.99 <sup>a</sup>        | 52.41<br>± 4.24 <sup>c</sup>  | 80.00 ± 6.76 <sup>c</sup>      |

Legend: F1: Cookie formula 1 (Control cookie). F2: Cookie formula 2 (Spray dried strawberry particles replacement cookie). BAC: Bioaccessibility. TPC: Total phenolic content. Aox: Antioxidant capacity. The different letters (a, b, c, d) in the same row denote significant differences ( $p < 0.05$ ) in BAC TPC (%) and Aox (%) at different digestion stages for each sample. The different letters (a, b, c, d) in the same column denote significant differences ( $p < 0.05$ ) in F1 and F2 for Aox (%) and BAC TPC (%) at different digestion stages for each sample.

#### CRediT authorship contribution statement

**Vega-Castro Oscar:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Funding acquisition, Data curation, Conceptualization. **Ramírez-Bustos Cristian:** Writing – review & editing, Software, Resources, Project administration, Funding acquisition, Conceptualization. **Henao-González Daniel:** Writing – review & editing, Writing – original draft, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Almonacid Sergio:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Funding acquisition, Conceptualization. **Núñez-Andrade Helena:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Conceptualization.

#### Ethical statement

This sensory evaluation study was conducted by the ethical guidelines established by Colombian legislation for scientific research. All participants of the sensory panel were thoroughly informed about the objectives and procedures of the study and provided their **verbal consent prior to participation**. Colombian Law 1581 of 2012 on protecting personal data guaranteed the confidentiality of participants' personal information and respected their rights. The research complied with relevant Colombian technical standards, including NTC 3929 for sensory evaluation of food. In addition, the ethical principles of the Declaration of Helsinki and the Council for International Organizations of Medical Sciences (CIOMS) recommendations for research involving human subjects were applied. The study was conducted in accordance with the ethical criteria established in the 1975 Declaration of Helsinki and its subsequent modifications.

The University of Antioquia's expert sensory analysis laboratory handled the sensory analysis **without the need for an ethics committee**. The University of Antioquia employs trained, competent, and optimally healthy judges for food analysis. The objective of the Specialized Laboratory for Sensory Analysis at the University of Antioquia is to conduct sensory evaluations of raw materials, in-process foods, and finished products, contingent upon their adherence to the microbiological standards mandated by Colombian regulations, relative to the specific product under assessment. The cookies complied with all

microbiological standards outlined in section 4.2.2 of Colombian Technical Standard NTC 1241/2007, which pertains specifically to cookies. Consequently, when the microbiological analyses of the cookies were provided and verified that they fulfilled the specified criteria, the panelists started their evaluation of the samples.

#### Declaration of Competing Interest

All authors declare that we have no conflicts of interest and that the manuscript “**Development and Characterization of Functional Cookies Enriched with Spray Dried Strawberry Particles; Sensorial, Textural, Physicochemical Properties, Antioxidant Capacity and Bioaccessibility assay of Phenolic Compounds**” has only been submitted to this Journal. If you have any questions, please do not hesitate to contact me.

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