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CHICKEN MEAT COATED WITH POLYPHENOL-ADDED HYDROCOLLOID FILMS

Paola Bartolucci
Faculty of Veterinary Sciences UNR, Casilda, Santa Fe, Argentina

Germán Báez
LIDEA (Mixed Research Laboratory for Food Development and Evaluation), Faculty of Biochemical and Pharmaceutical Sciences UNR, Rosario, Santa Fe, Argentina

Virginia Giordanengo
LIDEA (Mixed Research Laboratory for Food Development and Evaluation), Faculty of Biochemical and Pharmaceutical Sciences UNR, Rosario, Santa Fe, Argentina

Romina Berino
LIDEA (Mixed Research Laboratory for Food Development and Evaluation), Faculty of Biochemical and Pharmaceutical Sciences UNR, Rosario, Santa Fe, Argentina

Emilce Llopart
IQUIR (Instituto de Química Rosario), CONICET, Rosario, Santa Fe, Argentina
Área Alimentos y Sociedad, Facultad de Cs. Bioquímicas y Farmacéuticas UNR, Rosario, Santa Fe, Argentina

Pablo Busti
LIDEA (Mixed Research Laboratory for Food Development and Evaluation), Faculty of Biochemical and Pharmaceutical Sciences UNR, Rosario, Santa Fe, Argentina

Abstract: Edible films (CP) were prepared based on sodium alginate (AS) dissolved in chicken broth (CP) reduced in fat and sodium. As an antioxidant additive, polyphenols (PF) extracted from *Malus domestica* apple peels (Red Delicious variety), previously stressed for 14 days to increase their PF content, were incorporated. The CPs were obtained by heat treatment and applied as coatings on shredded chicken snacks. Additionally, the films were characterized by physicochemical and mechanical analysis to evaluate their structure and quality. Finally, a sensory evaluation of the baked snacks was performed. This study establishes the basis for developing healthier alternatives to traditional chicken skin, reducing harmful components such as fats (including cholesterol) and potentially carcinogenic compounds, without compromising flavor intensity.

INTRODUCTION

Edible coatings have historically been used to preserve moisture and extend the shelf life of foods. Currently, CPs are presented as a sustainable alternative to replace coatings of animal origin in cooked products, simultaneously reducing the content of harmful compounds such as cholesterol and sodium. These films, formulated based on hydrocolloids (alginates, pectins, chitosan), with broths or natural juices, not only improve the barrier properties of foods, but can also enrich their nutritional and sensory profile (Baez *et al.*, 2017; Cazón *et al.*, 2017; Falguera *et al.*, 2011). Among the hydrocolloids, alginates stand out, polysaccharides composed of β-D-manuronic and α-L-guluronic acids, whose molecular structure confers adjustable mechanical properties (elasticity, rigidity) (Ron *et al.*, 2013). Their low cost and versatility has positioned them as key additives in ice cream, sausages and sauces, where they act as thickeners and protectors against environmental factors such as UV radiation (Jayakody *et al.*, 2022; Pereira *et al.*, 2013). On the other hand, pectin -abundant in apples and traditionally used in jams- forms gels whose solubility depends on its degree of esterification (Venkatanagaraju *et al.*, 2020; Vázquez-Chávez and Zarazúa-Sánchez, 2023). A recent advance in this field is heat-resistant CPs (Costa *et al.*, 2018), which can be complemented with bioactive compounds such as FPs. These plant metabolites, recognized for their antioxidant, anti-inflammatory and cardioprotective properties (Zhang *et al.*, 2021; Rana *et al.*, 2022), represent an opportunity for the development of functional foods. In particular, Red Delicious apple peel -a variety widely cultivated in Argentina- contains high concentrations of FP, and its use could add value to the 8% of the productive discard (Pavicich *et al.*, 2020). It should be noted that aqueous extraction of these compounds guarantees their food safety by avoiding solvent residues (Bustos-Hipólito *et al.*, 2012). The evaluation of these CPs requires sensory tests and suitable panelists (evaluators trained in descriptive tests and consumers in hedonic tests) for the validity of the results (Barton *et al.*, 2020; IRAM 20001, 2012). This study seeks to enhance the applications of PCs as functional coatings, integrating their mechanical, nutritional and sensory properties in an innovative approach.

MATERIALS AND METHODS

REAGENTS

Analytical grade reagents were used, including Folin-Ciocalteau reagent, gallic acid (GA) and ABTS (Sigma-Aldrich A1888, Saint Louis, USA). The AS (Breaking Lab, Buenos Aires, Argentina) and all the ingredients used in the work were food grade. Stock solutions of AS at 1% w/V were prepared for use in the formulations.

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STRESS TREATMENT IN APPLES

Red Delicious apples (Alto Valle de Río Negro) were purchased in June. They were divided into three lots of seven units each, washed with water to remove waxes, drained and dried. Two lots were stressed for 7 and 14 days, simulating February harvest conditions (light 16 h/day, 6500K, 26°C ± 2, atmospheric oxygen), using a thermostat stove and a light timer. Samples were analyzed at 0 (M0), 7 (M7) and 14 (M14) days. It was not extended to 21 days because previous experiences (Llopart et al., 2023) showed no significant differences with respect to 14 days.

EXTRACTION OF POLYPHENOLS (PF)

Aqueous extraction was performed, 1:5 ratio (peel: distilled water), agitation at 70°C for 30 min. with a shaker (Boeco OSD-20, Germany). Filtration and lyophilization: Filtration with Whatman N°1 paper, lyophilized (Liotop L101, Brazil) at -40°C (24 h). T 25°C and 50 µmHg vacuum. Samples were stored at -20°C for quantitative analysis by RP-HPLC, total PF (TPC) and antioxidant activity (AO).

FP ANALYSIS

Determination of total polyphenols (TPC)

The Folin-Ciocalteau method (Singleton *et al.*, 1999) was applied, obtaining a standard curve: Gallic acid (0-500 µg/mL). 40 µL sample + 2000 µL Folin reagent (1:10) + 1000 µL Na_2CO_3 7.5% was incubated at 40°C (10 min) and absorbance was measured at 765 nm (Jasco V-550, Tokyo, Japan). The results were expressed in gallic acid equivalents (GAE g/100g dry sample) measurements were performed in quadruplicate.

Antioxidant activity (ABTS+)

To estimate the AO, the ABTS+ cation radical inhibition method was used (Cian *et al.*, 2011), expressed as µmol trolox equivalent antioxidant capacity (TEAC)/g dry sample, then the reaction was carried out between ABTS+ and the reducing agent, which can be the sample or the standard (Re *et al.*, 1999). Absorbance was measured at 734 nm, 6 min later, using a plate reader (Biotek, Epoch2). The assays were performed in quadruplicate.

Quantitative analysis of FP by RP-HPLC

The levels of FP in apple peel were analyzed by RP-HPLC. Chromatographic analysis was performed at M0 and M14 with the highest TPC value. For this purpose, an Agilent HPLC 1260 Infinity II quaternary system (Agilent Technology, Santa Clara, CA, USA) was used. The system was equipped with a photodiode array detector (PDA) and a vial sampler. A Poroshell 120 EC-C18 column (4.6x100 mm, 2.7 µm) and a Poroshell 120 EC-C18 precolumn were used for the separation of FP compounds. RP-HPLC setup and FP analysis were performed according to a previously described methodology (Jakobek *et al.*, 2016, Jakobek *et al.*, 2020). Each FP present in the different samples was identified by comparison of retention times and spectral peaks (200-600 nm) with authentic FP standards. Chlorogenic acid (Cha), (+)-catechin (Ca), (-)-epicatechin (ECa), quercetin-3-O-glucoside (QGlu) and quercetin (Q) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Procyanidin B1 (PB1), procyanidin B2 (PB2), cyanidin-3-O-galactoside chloride (CGa), quercetin-3-O-galactoside (QGa), quercetin-3-O-rhamnoside (QRh) and floretin-2'-O-glucoside (PhGlu) were obtained from Extrasynthese (Genay, France). The identified FPs were quantified using calibration curves of authentic standards. For the tentative identification of quercetin-3-O-xyloside (QXy), a quercetin calibration curve was used. M0 and M14 extracts were injected to obtain the FP concentration. All samples were analyzed in duplicate. The relative percent change (CR%) in percent increase when relating M14 and M0 of each FP subclass according to equation 1

CR% = [(M14) PFS/(M0) PFS].100 (1)

where (M14) PFS and (M0) PFS correspond to the average content in mg/kg of each PF subclass in the samples.

ELABORATION OF EDIBLE FILMS

Chicken stock (CP)

Initial preparation: 2 kg of chicken carcasses were baked for 40 min. They were boiled in a *mirepoix* (250 g carrot, 250 g onion, 150 g celery, 150 g leek) for 6 h. The *broth* was refrigerated for 24 h to crystallize fats. The broth was refrigerated for 24 h to crystallize fats. The solid fat layer was removed and filtered through cloth. To clarify, egg whites were added and heated slowly to boiling, holding 30 min for the fat to adhere to the whites. It was refrigerated for a further 24 h, the supernatant of egg whites with fat was removed and filtered again. For storage, the resulting liquid was fractionated into 100 mL containers and frozen at -20°C until use. Adapted from Baez *et al.*, 2017.

CP composition was determined according to Association of Official Agricultural Chemists International methods (AOAC 2002): moisture (method 925.10); protein (method 920.87, factor 6.25); fat (method 932.06); ash (method 923.03); glucose concentration in CP by the methodology proposed by Trinder (1969). Total carbohydrates were determined by difference and calcium content by atomic absorption spectroscopy (Unicam 969).

Alginate films (PCP and PCP+PF)

Films of 1% w/V sodium alginate (AS) in chicken broth (CP) were prepared by mixing until homogenization and molding in polypropylene containers (22x17 cm). Drying was carried out in an oven at 50±4°C for 6 h (Baez *et al.*, 2017). Chicken broth films (PCP) were thus obtained. For polyphenol-enriched films (PCP+PF), 341.6 mg of lyophilized PF was added per 100 mL of CP, dosage based on previous studies by Ma and Chen, 2020. All films were demolded by controlled wetting and stored under vacuum (Erlich 34 cm Beth vacuum. Buenos Aires. Argentina) under dark conditions until use (Baez *et al.*, 2017).

CHARACTERIZATION OF FILMS

Thicknes

The thickness of the films (PAS, PCP and PCP+PF samples) was measured with a high precision digital micrometer (Schwyz™, Switzerland), following the protocol of Chen *et al.* (2009). To ensure representative results, 10 measurements were taken in different areas of each film and the average of the values obtained was calculated.

Mechanical properties

The mechanical properties of PAS, PCP and PCP+PF films were evaluated using a texturometer (Mecmesin Multitest 2.5-d, Mecmesin Ltd., UK), equipped with a 100 N load cell. The stretching to failure of 7x60 mm film strips (n=3) with an initial spacing of 30 mm and crosshead speed of 0.05 mm/s was analyzed, where tensile strength (TS = maximum load/cross-sectional area), elasticity (E = % strain) and elastic modulus (EM = initial stress-strain slope) were determined, parameters that respectively represent the maximum strength, elongation capacity and stiffness of the materials (Báez *et al.,* 2017; Soazo *et al.*, 2015; Da Silva *et al.*, 2009).

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Color

The color analysis was performed by determining the parameters L* (lightness), a* (red-green) and b* (yellow-blue) using digital photographs (Canon EOS REBEL T6, Tokyo, Japan) with manual settings (f/8.0, 1/200s, ISO 400, maximum resolution in RAW format), taken in a special illumination box on a white matte background, using an IT8 card for calibration and ICC profile generation with Lprof software, whose values were processed in Photoshop to obtain the color averages (L*, a*, b*) from the image histogram following the methodology of Yam and Papadakis (2004), performing all measurements in quintuplicate with methodological adaptations based on Mendoza and Aguilera (2004) and Soazo *et al.* (2015).

Viscosity

Viscosity measurements as a function of velocity gradient ($\dot{\gamma}$) were performed for CP, CP plus 0.3416 g of PF (CP+PF) and AS 1% as blank; a rheometer (Rheometer TA Discovery HR-30, USA) was used for this purpose.

The geometry used was concentric cylinders for 25 mL of sample at 25°C and a flow velocity of 1.00000 e $^{-6}$ rad/s (*Flow velocity limit*).

EVALUATION OF SANDWICH COOKING CONDITIONS

The effects of different baking conditions on the FP content in PC films were evaluated. The FP concentration cannot be measured in the CP or in the PCs, due to the presence of interfering substances, therefore, aqueous solutions of the extracted FPs were used, loading Petri dishes with the same volume and concentration with which the PCs were made for the snacks. The response surface methodology was used taking as variables the cooking temperature (T) (150, 180 and 210 ºC) and the cooking time (t) (1, 2 and 3 min) and was carried out according to a 3^2 type experimental design, with 3 central points, resulting in 11 experiments with a triplicate of the central point. In response, the TPC content was evaluated by the Folin-Ciocalteau method, discarding those samples that decreased the PF concentration by more than 17 %. With the rest of the samples, a sensory analysis was carried out by an internal panel of five experts to evaluate the best cooking condition of the snacks and the one that allowed obtaining a sensorially pleasant product and with the highest amount of FP to elaborate the CPs was selected.

PREPARATION OF SNACKS

The skinless chicken breast pieces were baked in an oven at 180 °C for 20 min, ground in a food processor with sunflower oil (1:6 w/V), and cylinders (7x1.6 cm) were made from this mixture and coated with the PCP and PCP+PF films.

SENSORY ANALYSIS

Fifty-one untrained evaluators (UNR) were selected (65% women, 35% men; average age: 35 years), as they were chicken consumers and had no medical or dietary conditions affecting their sensory perception. The tests were carried out in individual white booths, with good lighting and adequate temperature, without noise or foreign odors that could interfere with the analysis and allowing a good concentration of the evaluators (IRAM 20003, 2012). The attributes analyzed were: odor, appearance, color, flavor, chewiness, general acceptability (Stone *et al.*, 2020). A 9-point hedonic scale was applied (IRAM 20002, 2012), being 9: I like it very much, 8: I like it very much, 7: I like it quite a lot, 6: I like it a little, 5: I neither like nor dislike it, 4: I dislike it a little, 3: I dislike it quite a lot, 2: I dislike it very much and 1: I dislike it very much (Stone *et al.*, 2020; Curia *et al.*, 2001). The last questions were about which sample they liked the most and their likelihood of purchase, to be indicated on a 9-point hedonic scale (9: extremely likely to 1: extremely unlikely) (Gębski *et al.*, 2019).

ANALYSIS

One-way ANOVA plus Tukey's test (Statgraphics Plus 3.0). Significance: $p < 0.05$. Data expressed as mean ± SD.

RESULTS AND DISCUSSION

POLYPHENOL CONTENT AND ANTIOXIDANT ACTIVITY

Figure 1 shows the FP content of the apple peels studied, representing TPC vs. days of environmental stress. Different letters above the columns indicate significant differences ($p < 0.05$). On the other hand, the color bars gray, show the AO vs. days of environmental stress. The AO results are expressed as µmol TEAC /g dry sample and the error bars indicate standard deviations.

Exposure to environmental stress generated a significant increase in TPC and AO in apple peels (Figure 1). The highest TPC value was reached at 14 days of stress, which represented a 39% increase over unstressed (M0) apple peels. Following the same trend, the highest AO value was also reached at 14 days of stress (112% increase). It can be concluded that the applied environmental stress was an effective treatment to increase the FP content and antioxidant properties of apple peels.

QUANTIFICATION OF PHENOLIC COMPOUNDS

The results obtained are shown in Table 1

Identification proposal	M0	M14	CR%
	average mg/kg	average mg/kg	
Cha	11.57± 0.38^{a}	35.82± 0.95^{b}	309.6
PB1	28.90± 0.45^{a}	58.95± 0.85^{b}	203.9
Ca	58.42± 3.85^{a}	116.77± 4.65^{b}	199.8
PB2	55.57± 1.88^{a}	134.47± 4.45^{b}	241.9
ECa	42.25± 1.20^{a}	111.71± 2.58^{b}	264.4
GGa	166.25± 9.30^{a}	668.22± 19.05^{b}	401.9
QGa	115.00± 3.68^{a}	278.72± 7.65^{b}	242.3
QGl	76.97± 4.71^{a}	210.17± 10.35^{b}	273.0
QX	42.42± 2.25^{a}	124.15± 3.93^{b}	292.7
QRh	58.75± 2.55^{a}	181.33± 7.68^{b}	308.6
PhGlu	153.00± 4.35^{a}	371.,47± 6.53^{b}	242.8

Table 1. Identification of phenolic compounds soluble in water

Results are expressed as x ± SD. Different letters in a row indicate significant differences ($p < 0.05$) between samples.

Abbreviations: Cha, chlorogenic acid; PB1, procyanidin B1; Ca, (+)-catechin; PB2, procyanidin B2; ECa, (-)-epicatechin; CGa, cyanidin-3-O-galactoside chloride; QGa, quercetin-3-O-galactoside; QGlu, quercetin--3-O-glucoside; Q, quercetin; QXy, quercetin-3-O-xyloside. QRh, quercetin-3-O-glucoside; QGlu, quercetin-3-O-galactoside; Q, quercetin-3-O-glucoside. and PhGlu, floretin-2′-O-glucoside.

PRODUCTION OF EDIBLE FILMS

Proximal composition of chicken broth

Parameters	Results (x ± SD) (g/100g)
Water	96.8 ± 2.7
Ash	0.500 ± 0.015
Grease	0.0300 ± 0.0009
Protein	2.0 ± 0.3
Carbohydrates	0.60 ± 0.03
Glucose	0.126 ± 0.003
Calcium	0,0130 ± 0,0004

Table 2. Composition of the CP.

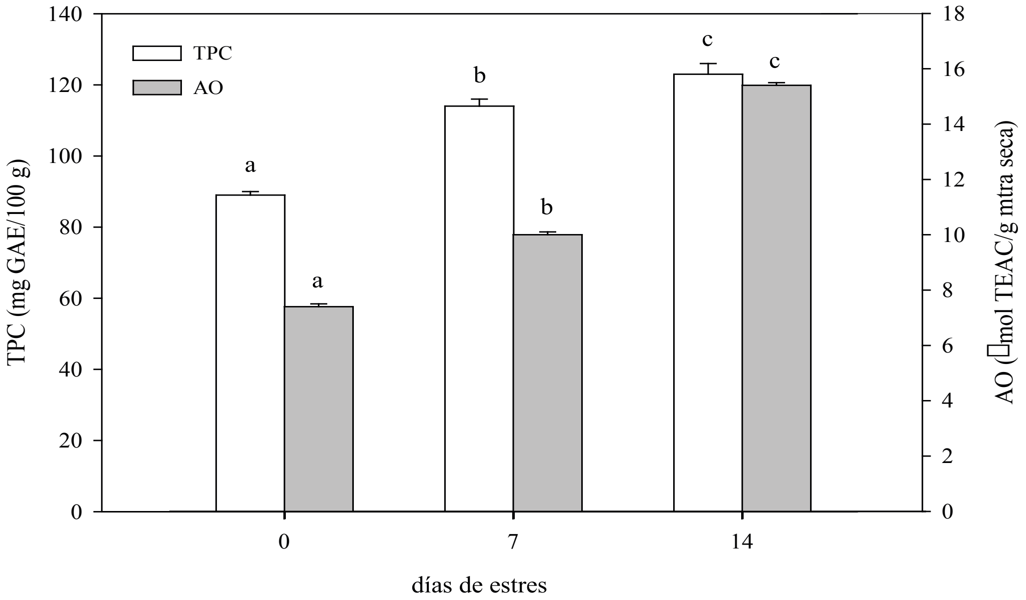

Figure 1. TPC and AO content vs. days of stress. Different letters above the columns indicate significant differences ($p < 0.05$).

Mechanical properties of PAS, PCP and PCP+PF.				
	TS (MPa)	E (%)	MS (MPa)	Thickness (mm)
PAS	35.64± 0.33[c]	14.81± 0.07[a]	8.45± 0.53[c]	0.087± 0.011[a]
PCP	14.26± 1.86[b]	38.51± 0.23[b]	1.20± 0.03[b]	0.198± 0.038[b]
PCP+PF	6.57± 0.13[a]	53.01± 0.02[c]	0.23± 0.02[a]	0.198± 0.027[b]

Different letters above the rows indicate significant differences ($p < 0.05$).

Lyophilized polyphenols

As a result of freeze-drying, an amber-colored solid was obtained, which was then used to add to the PCP films.

FILM PROPERTIES

Mechanical properties and thickness

From the data in Table 3, it is observed that the PAS membrane is rigid and inelastic, requiring greater force to rupture. In contrast, PCP and PCP+PF are more elastic due to CP, which contains plasticizers such as carbohydrates and fats (Table 2). The addition of PF (apple peel, rich in pectin and sugars) (Pérez Espitia *et al.*, 2014), improves the properties of PCP+PF, resulting in higher E%, lower TS and lower MS, as confirmed by the viscosity data (Figure 2).

The results showed that no significant differences ($p > 0.05$) were observed between PCP and PCP+PF samples, in contrast to PAS, the thicknesses obtained for both films allowed both to be easily demoldable and manageable for subsequent coating of meat pieces.

Color

The color of freeze-dried apple peels is due to browning reactions (enzymatic, non-enzymatic/Maillard) and PF oxidation. CP also exhibits browning due to its ingredients and Maillard reaction (Table 2) (Baez *et al.*, 2017). PCP and PCP+PF films showed significant differences ($p < 0.05$) in L^*, a^* and b^* coordinates, indicating changes in their visual appearance. The addition of FP darkened the color of PCP+PF compared to PCP.

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	PAS	PCP	PCP+PF
L*	-	61.78 ± 2.08[b]	51.75 ± 1.05[a]
a*	-	6.07 ± 0.31[b]	3.86 ± 0.18[a]
b*	-	48.97 ± 0.55[b]	45.79 ± 0.12[a]

Table 4. Color of the films.

Different letters above the columns indicate significant differences ($p < 0.05$).

PAS gave no color, the films were transparent.

3.4.3. Viscosity

The results shown in Figure 2, show for PC and PC+PF, a much more marked pseudoplastic fluid behavior than for AS, the aggregates trapping liquid phase disintegrate and release this phase as the velocity gradient ($\dot{\gamma}$) increases, this can be related to the presence of CP and pectin. The shape of the curves complies with the power law (Ostwald-de Waele), $\eta = k\,\dot{\gamma}^{\,n-1}$ for $n < 1$ with k being the consistency index and n the flow rate. The PC+PF curve gave a higher k than PC, due to the presence of pectin (Isopencu *et al.*, 2021).

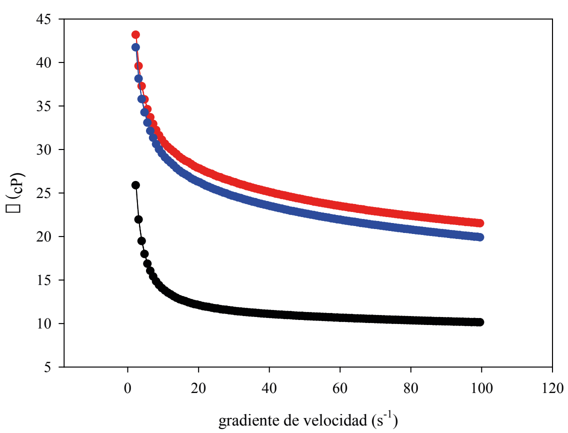
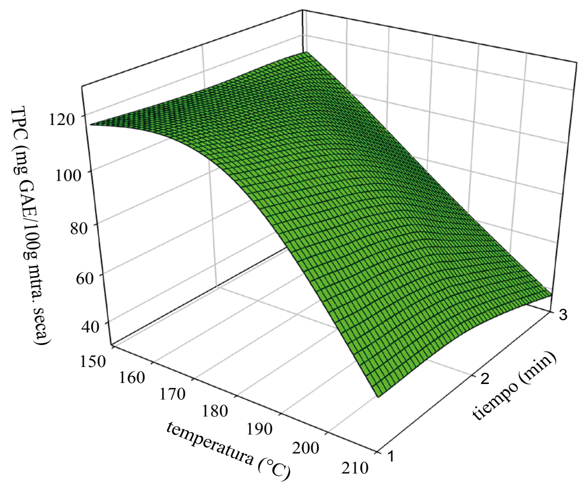


Figure 2. Viscosity as a function of velocity gradient ($\dot{\gamma}$) ● AS, ● CP, ● CP+PF.

Firing conditions - Response surface

A significant effect of T and t on FP content was observed (Figure 3), with the reduction trends being 210 > 180 > 150 °C and 3 > 2 > 1 min and varying TPC content between 117.07 ± 1.98 and 37.42 ± 1.78 TEAC /g dry sample (for 150 °C - 1 min and 210 °C - 3 min, respectively). Thus, the T of 210 °C at 3 t and 180 °C at 3 min were discarded for generating TPC reductions greater than 17 %. On the other hand, the panel of experts discarded the conditions 150 °C at 3 t and 180 °C at 1 min, because the snacks did not reach an adequate T for consumption. Thus, it was determined that the cooking condition to perform the sensory evaluation of the PCP and PCP+PF coated snacks was baking at 180 °C for 2 min. Considering the reduction of FP under this condition, 475 mg of freeze-dried FP were incorporated per snack to cover the recommended daily dose of 396 mg/day



Surface response for TPC as a function of temperature and firing time.

Sensory analysis

The evaluators showed frequent consumption of chicken: 61.3% consumed it 1 to 3 times per week, 32.3% consumed it 4 to 6 times, and only 3.2% consumed it daily or once a week. Regarding skin consumption, 35.5% always included it, 32.3% avoided it, and 32.2% alternated both options.

Figure 4 presents the acceptability scores for each attribute evaluated. The data do not include standard deviations (ranging from 0.9 to 1.5) due to the intrinsic variability of hedonic tests, where individual perception generates dispersion (Parmigiani *et al.*, 2025). The snack with PCP+PF obtained the highest scores in all attributes except chewiness -possible effect of pectin, which increases film elasticity (Table 3)-.

Although color variations were observed in the films (Table 4), these were not noticeable in the baked snacks. The PCP+PF treatment excelled in key attributes for consumers: odor, coating appearance and flavor (Koubaa *et al.*, 2021). This was reflected in 79% of evaluators preferring it, with 72.5% being regular chicken consumers (1-3 times/week) who alternated consumption with/without skin, suggesting its potential as a healthy substitute.

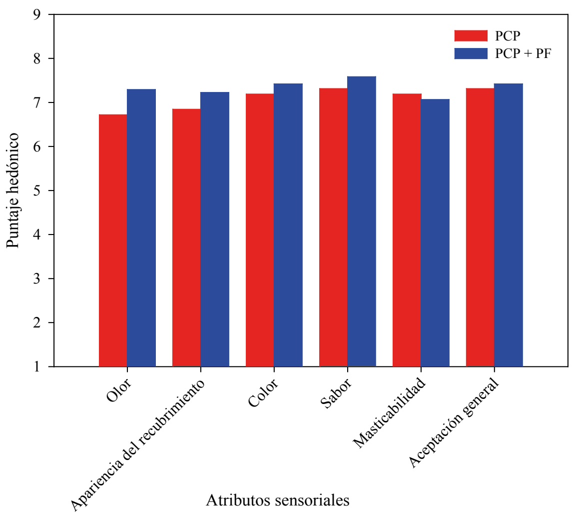


Figure 4. Hedonic evaluation of snacks.

PCP+PF films demonstrated superiority in thickness, elasticity and sensory acceptability, positioning them as promising functional coatings. It should be noted that this evaluation, although it analyzed basic sensory attributes for food innovation, did not cover other multidimensional factors that influence acceptability (sociocultural context, marketing, sustainability, etc.) (Hellwig *et al.*, 2022

CONCLUSIONS

The results demonstrate that time stress in apples significantly increases the FP content in their peels, which can be efficiently obtained by aqueous extraction. This finding supports the use of this by-product to enrich the antioxidant properties of CP.

It should be noted that the incorporation of FP did not compromise the sensory acceptability of meat products, maintaining key attributes such as odor, appearance and flavor, which are determining factors in consumer preference. This positions PCP+PF as a promising alternative to replace traditional coatings in chicken pieces, combining technological benefits and nutritional potential.

However, it is recommended to evaluate the industrial scalability of the process, considering economic and sustainability parameters.

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