ISSN: 2347-467X, Vol. 09, No. (2) 2021, Pg. 521-532

Current Research in Nutrition and Food Science

www.foodandnutritionjournal.org

Strategies to Enhance Propolis Ethanolic Extract's Flavor for its Use as A Natural Preservative in Beef

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Abstract

Propolis is rich in bioactive compounds like phenols and flavonoids, which make it a promising alternative as a natural preservative. However, the use of propolis in the food industry is limited due to its strong, characteristic flavor. To overcome this problem, several strategies such as the combination with other ingredients or propolis microencapsulation have been studied. In this work, the addition of honeydew (no floral honey) to, and the elimination of the ethanol from, the propolis extract solution were used to mitigate this sensorial defect. Thus, the effect of these propolis solutions on beef quality during refrigerated storage were evaluated. Throughout storage, a decrease in the pH and the a*, indicative of microbial growth and oxidation, respectively, was observed in the control samples. Both deterioration processes were slowed down in the treated samples, demonstrating the antioxidant and antibacterial properties of the propolis and the honeydew. What is more, neither ethanol evaporation nor the addition of honeydew impaired the antimicrobial activity of the solutions, with the greatest microbial reductions observed in the former. With this solution, a reduction of 0.77 and 1.45 logarithmic units were observed for the mesophiles and the Enterobacteria, respectively. Finally, the propolis and honeydew solution presented the highest scores in all the sensory attributes analyzed. In conclusion, the strategies applied in this study were effective at enhancing propolis extract flavor whilst maintaining its antimicrobial and antioxidant properties. This could be a promising starting point for a wider use of propolis as a natural preservative in the food industry.

Article History
Received: 14 May 2021
Accepted: 23 June 2021

Keywords
Beef;
Flavor;
Honeydew;
Natural-Preservative;
Propolis.

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Doi: http://dx.doi.org/10.12944/CRNFSJ.9.2.15

Introduction

Fresh meat presents high protein and variable fat content together with high water activity (>0.95) and an acidic pH (around 5.5). These conditions make it an optimal media for microbial growth.[1] The preservation of fresh food with high water content, such as meat, for short periods of time is usually achieved by storage at low temperature.[2] In the case of "meat preparations" the European legislation[3] allows the addition of preservatives like antioxidants and seasonings.

Many bioactive compounds synthesized by plants are recognized as effective antimicrobials against a wide range of microorganisms.[4] As well, it is possible to find other types of natural products, rich in bioactive compounds. One of these products is propolis, an elaborated by bees from natural resinous collected from plants.[5] Several authors have demonstrated propolis to be a strong antimicrobial agent in many foods, such as sausages, beef patties, minced meat, minced *Cyprinus carpio*, poultry and burger meat.[5–13] This antimicrobial activity was attributed to the high phenolic compounds and flavonoids content in propolis.[14–17]

Propolis bactericidal and bacteriostatic effects depend on several influence factors, such as, extract concentration, propolis composition, its geographical and botanical origin and extraction method. Ethanolic extraction method is the most efficient way of taking propolis out of the extract.[12] Nevertheless, it presents disadvantages like a strong residual flavor and the intolerance in sensitive consumers.[18]

In summary, research on propolis indicates its potential to reduce spoilage and extend the shelf-life of some food products. However, propolis' strong flavor is negatively influenced by the ethanol's residual flavor, which may limit its use.[19] Therefore, if propolis is to be used in the food industry, more research is needed in order to mitigate its effects on the organoleptic properties of the treated food.[20] In this sense, we hypothesize that it is possible to soften the strong flavor of the propolis extract whilst maintaining its preservative effects on refrigerated beef. Consequently, the objective of this work was to evaluate the preservative effects of three propolis-extracts solutions on beef during refrigerated storage. The effects on the sensory quality were also evaluated.

Materials and Methods

Raw Materials

In this study, twenty beef fillets (outside flat) were used. The meat was bought in a local market ten days after slaughter, stored overnight in original packaging (vacuum) at 4 ± 1ºC and used one day after purchase. Unpasteurized raw propolis and honeydew (which is commonly known in the apiculture business as a bee's secretion from sources different than flowers) were kindly provided by the Beekeepers Association of Navarre and stored in the dark at 20 ± 2ºC until use. Propolis and honeydew come from northern Spain, a region called "Comunidad Foral de Navarra". Regarding the botanical origin, propolis come from aromatic plants, fruit trees, holm oak and forest and honeydew comes from heather.

Propolis Extract and Treatment Solutions

The raw propolis resin was frozen, grinded and extracted using a 1:3 propolis:ethanol (70%, v/v) ratio, following the method described by Keskin.[21] The obtained Propolis Ethanolic Extract (PEE) was homogenized with water using an Ultraturrax (Model T25 basic IKA, Staufen, Germay) at 11000-13000 rpm to prepare the three treatment solutions (8%, v/v) as follows: PE1 was directly packed into amber flasks after the homogenization. In order to diminish the negative effects of the ethanol flavor on the sensory properties of the meat, PE2 was evaporated for 5 min in a Büchi rotary evaporator R-200 which includes a heating bath (B-490, Flawil, Switzerland) at 38 ± 1ºC. PE3 was prepared by adding honeydew at a concentration of 10% (w/v) to PE1.

Treatment Application

The beef fillets were cut into 10-g rectangular prisms and the fat and connective tissue were removed. For the physicochemical analyses, 24 pieces of meat/treatment were immersed for 10 min in one of the propolis solutions (PE1, PE2 and PE3) described above and untreated meat was used as the control. Two additional batches were prepared: 12 pieces/treatment for microbiological analyses and 27 pieces of 3x3x1 cm per treatment for the sensory analysis. The solutions and the immersion time used in the study were selected from preliminary studies conducted in our laboratory (data not shown).

Packing and Storage

After the immersion in the treatment solutions, the samples for the shelf life studies were packed in polyamide/polyethylene bags (PA20/PE70 TR 300.0, Spain), thermally sealed (SACOPISA SB-400-ECO, Barcelona, Spain) and stored under atmospheric conditions at 4 ± 1ºC for 14 days. For physicochemical analyses, three bags/treatment/evaluation day were used; each bag contained 2 pieces of meat. In addition, three bags/treatment/evaluation day with each bag containing one piece of meat were used for the microbiological analyses.

Analytical Methods

Physicochemical Parameters

The water content, soluble solids, free acidity, electrical conductivity and pH of the honeydew were determined in triplicate according to the official method of honey analysis.[22] The pH of the PEE and the treatment solutions was measured in triplicate using a pH-meter (Sentron Europe B.V., Netherlands) equipped with a penetration probe.

Total Phenols Content

Total phenols content of raw materials and treatment solutions was determined following the Folin-Ciocalteu method.[23] The results were expressed as mg gallic acid equivalent per 100 g (honeydew), mL (PEE) or L solution (PE1, PE2, PE3).

Total Flavonoids Content

The flavonoids content of the raw materials and treatment solutions was determined using the aluminum chloride method described by Meda.[24] The results were expressed as mg quercetin per 100 g (honeydew), mL (PEE) or L solution (PE1, PE2, PE3).

Antioxidant Activity

Antioxidant activity was evaluated by the 2.2-diphenyl-1-picrylhydrazyl (DPPH) assay, following the method described by Bobo-García.[23] The results were expressed as µmol Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent per 100 g (honeydew), mL (PEE) or L solution (PE1, PE2, PE3).

Microbiological Analysis of Meat

Total aerobic mesophiles and Enterobacteria were analyzed on day 0, and after 7, 10 and 14 days of storage. For this purpose, 10 g of beef were homogenized in 90 mL sterile buffered peptone water (Cultimed, Spain) using a Stomacher 400 circulator (Seward, UK) for 120 s at high speed. Serial decimal dilutions of each homogenized sample were made in peptone water. From each dilution, 1-mL aliquots were aseptically pour-plated using the following media and culture conditions: (1) PCA (standard plate count) agar (Cultimed, Spain) incubated at 35 ± 2°C for 48 h for total aerobic mesophiles;[25] and (2) VRBG (Cultimed, Spain) incubated at 35 ± 2°C for 24 h for Enterobacteria.[26] All the samples were analyzed in triplicate and microbial counts were expressed as log (CFU/g) of meat.

Physicochemical Parameters of Meat

All the physicochemical parameters were determined in triplicate on day 0, and after 7, 10 and 14 days of storage. For texture, three subsamples per sample were taken with a cork borer; totalizing 9 measurements per treatment and evaluation date.

The pH of the meat was measured at different sites of each sample with a pH-meter (SENTRON Europe B.V. Netherlands) equipped with a penetration probe.

Color

The L*, a* and b* color coordinates were determined using a spectrophotometer (Minolta CM-2500d, Minolta CO, Japan), the CIELab color space,[27] the illuminant D65 and a 10º observer.

Texture Profile Analyses

Texture Profile Analysis (TPA) was performed at room temperature using a TA.XT2icon texturometer and the Exponent lite V.6.1 software (Stable Micro System LTD., Surrey, UK). From each piece of meat, 3 cylindrical subsamples (14-16 mm diameter x 8-10 mm height) were taken and compressed twice to 50 and 75% deformation with a 35 mm-diameter probe. The following texture parameters were determined: hardness (N): resistance at maximum compression during the first compression cycle and cohesiveness (dimensionless): ratio of positive force during the second to that of the first compression cycle.[28]

Sensory Analysis of Meat

Immediately after the treatments, a sensorial analysis was carried out to evaluate the effect of the propolis solutions (PE1, PE2 and PE3) assayed on the organoleptic quality of the meat. One sample per treatment was evaluated by a sensory panel

(15 female and 12 male) using a hedonic test. The analyses were carried out in individual booths and all the samples were presented coded with random numbers. Immediately before serving, the meat was cooked in a pan with olive oil for 3 minutes on each side.

The appearance (juiciness and general appearance) and global impression were rated from 1 (worst) to 7 (best quality). Color uniformity, from 1 (less uniform) to 7 (uniform). For aroma, the scale ranged from 1 (none) to 7 (full typical aroma). Tenderness was evaluated after the first and second bite with molar teeth, from 1 (least tender) to 7 (most tender). Finally, taste (full taste) was evaluated after eating the sample, from 1 (unpleasant taste) to 7 (pleasant taste). Panelists were asked to indicate if they detected off-flavors or off-odors that might impact their willingness to purchase the product. Scores below 4 in any of the attributes indicated the rejection of the product.

The study was reviewed and approved by the Public University of Navarre and informed consent was obtained from each subject prior to their participation in the study.

Statistical Analysis

Data were subjected to a one-way analysis of variance ($\alpha = 0.05$) and when significant differences were observed, mean treatments were compared using Tukey's test. A discriminant analysis (test of equality of means of Lambda Wilks groups; $p \leq 0.05$) was also performed with the measured parameters to determinate which were useful in differentiating among the treatments applied. All the statistical analyses were performed with the IBM SPSS Statistics 25 software for Windows (IBM Corp., New York, U.S.A.).

Results and Discussion

Characterization of the Raw Materials and Treatment Solutions

The physicochemical attributes of the honeydew were as follows: water content of 12.69%; soluble solids of 84.39 °Bx free acidity of 22.42 meq/Kg honeydew; electric conductivity of 0.33 mS/cm and a pH of 4.10. Regarding the antioxidant activity and the total content of phenols and flavonoids of the honeydew, the values were 153.79 µmol Trolox, 88.46 mg gallic acid and 10.90 mg quercetin equivalents per 100 g of honeydew, respectively. After the ethanolic extraction, the solution had a pH of 5.10 and contained 13.46% of propolis. The propolis ethanolic extract (PEE) had an antioxidant activity of 677.07 µmol Trolox equivalents and contained 134.75 mg gallic acid (total phenols) and 26.55 mg quercetin equivalents (flavonoids) per mL PEE. The antioxidant activity and bioactive compounds content of the propolis used in this study were higher than those previously reported by Palomino;29 Vargas[30] and Vit.[31] The pH, the total phenols and flavonoids content, and the antioxidant activity of the three treatment solutions are listed in Table 1. The lower pH of the added honeydew in PE3 could have caused the pH in this treatment solution to be more acidic. At the same time, the contribution of the honeydew in PE3 is noteworthy, it provoked a significant increase in the antioxidant activity and in the phenolic compound and flavonoid contents of this solution.

Table 1: Characterization of treatment solutions

Treatments	pH	Total phenols†	Flavonoids†	Antioxidant Activity†
PE1	5.20 ± 0.10^{C}	159.58 ± 18.45^{A}	36.64 ± 1.51^{A}	335.16 ± 11.80^{A}
PE2	4.40 ± 0.00^{B}	147.15 ± 13.80^{A}	32.33 ± 3.03^{A}	348.19 ± 10.77^{A}
PE3	4.00 ± 0.06^{A}	167.66 ± 18.12^{A}	43.85 ± 1.70^{B}	435.62 ± 37.58^{B}

†Total phenols, flavonoids and antioxidant activity are expressed as mg gallic acid, mg quercetin and µmol Trolox equivalents per L solution. Values are the mean ± standard deviation (n=3). For each parameter, different letters indicate significant differences ($p < 0.05$) among treatment solutions.

Meat Quality Evaluation during Storage

Microbiological Analysis

All the applied treatments were effective at reducing the total aerobic mesophiles growth on day 7 with reductions of 0.24, 0.77 and 0.19 logarithmic units for PE1, PE2 and PE3, respectively (Figure 1 A). Furthermore, on day 10 the control samples almost reached the limit established in the European normative[32] of 5x10E5- 5x10E6 CFU/g expressed as 5.70 to 6.70 log CFU/g. On day 14 the mesophiles' counts were above 7 log (CFU/g), exceeding the legal limit mentioned. During storage, the counts for this microbial group increased for all the samples. However, the tested solutions were effective in slowing down this increase; mesophiles' counts of PE1-treated meat (6.60 log CFU/g), also complied during the whole storage period within the normative mentioned above. Finally, the samples treated with the PE2 and PE3 solutions exceeded this limit only on day 14.

Our results are in accordance with those of Istrati,[33] who reported a decrease in mesophilic aerobic and lactic acid bacteria until day 14 of storage in vacuum-packed beef marinated with honey solutions prepared at a concentration of 4%. In the same way, Rojo[34] observed an antimicrobial effect of both, floral honey and honeydew against mesophilic aerobic and lactic acid bacteria in beef. The antimicrobial activity of propolis was also demonstrated in other types of meat like chicken breast[9] and *Piaractus brachypomus* fillets.[35]

Enterobacteria are considered to be indicators of potential fecal matter, food borne pathogen contamination and as bioindicators of food hygiene.[32] The propolis and honeydew solutions were also effective in reducing the Enterobacteria speed growth in comparison with the control samples everyday these were tested (Figure 1 B). The greatest reductions (1.45 logarithmic units) were obtained in the PE2-treated samples. Similar results were reported by Mehdizadeh and Langroodi[9] in chicken breasts treated with propolis extract and in *in vitro* studies carried out by Petruzzi.[20] According to these authors, propolis exerts its antibacterial activity by multiple effects, among which the inhibition of cell division and protein synthesis are included. Furthermore, Pobiega[18] and Petruzzi,[20] stated that the antimicrobial action of PEE is related to the propolis chemical composition, the solvent used for extraction and the PEE dose used in the different assays.

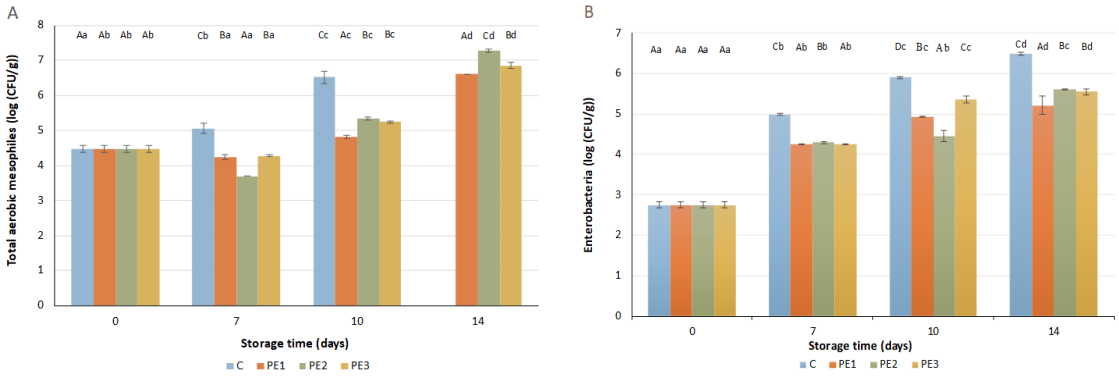

Fig.1: Total aerobic mesophiles (A) and Enterobacteria (B) growth in control (untreated) and treated meat samples (PE1: propolis extract solution with ethanol; PE2: propolis extract solution without ethanol; PE3: propolis extract solution with honeydew) during 14 days of refrigerated storage. Values represent the mean of 3 measurements for each treatment and evaluation date. Error bars represent the confidence interval (95 %) for the mean. For each evaluation date, capital letters indicate significant differences ($p < 0.05$) among treatments; for each treatment, different lowercase letters indicate significant differences ($p < 0.05$) among evaluation dates

pH and Color

Table 2 shows the pH and color evolution of the control and the treated samples during 14 days of refrigerated storage. On day 0, the pH ranged between 5.50 (control) and 5.40 (PE3); these values are similar to those reported by López-Gajardo.[36]

The lower pH of the treated meat compared to the control samples can be explained by the pH of the solutions applied. Regardless of the treatment, the pH decreased significantly during storage, with the greatest decrease observed in the control samples. In all the evaluation dates, the PE3-treated meat presented the lowest, yet insignificant, pH values. Similar results were found in beef meat treated with either floral honey or honeydew,[34] in chicken meat treated with honey[37] and in chicken patties treated with honey and a mixture of spices.[38] The general decrease in the pH during storage can be attributed to the increase in the Enterobacterias' growth throughout the storage period.

Table 2: Changes in pH and color parameters of raw beef (Control: untreated; PE1: propolis extract solution with ethanol; PE2: propolis extract solution without ethanol; PE3: propolis extract solution with honeydew) during 14 days of refrigerated storage

		Treatment			
Parameter	**Storage time (days)**	**Control**	**PE1**	**PE2**	**PE3**
pH	0	5.50 ± 0.00 ^{Bc}	5.45 ± 0.06 ^{ABb}	5.40 ± 0.00 ^{Ab}	5.40 ± 0.00 ^{Ab}
	7	5.35 ± 0.10 ^{ABb}	5.30 ± 0.00 ^{ABa}	5.40 ± 0.08 ^{Bb}	5.23 ± 0.05 ^{Aa}
	10	5.28 ± 0.05 ^{Aab}	5.25 ± 0.58 ^{Aa}	5.33 ± 0.05 ^{Ab}	5.28 ± 0.05 ^{Aab}
	14	5.18 ± 0.05 ^{Aa}	5.20 ± 0.08 ^{Aa}	5.20 ± 0.00 ^{Aa}	5.15 ± 0.13 ^{Aa}
L	0	41.78 ± 1.07 ^{Aa}	46.86 ± 0.71 ^{Ca}	45.90 ± 0.75 ^{BCb}	45.14 ± 0.92 ^{Bab}
	7	45.72 ± 3.79 ^{Ab}	46.00 ± 1.26 ^{Aa}	45.96 ± 1.88 ^{Ab}	45.01 ± 1.81 ^{Aab}
	10	45.15 ± 3.52 ^{Aab}	45.18 ± 1.27 ^{Aa}	42.90 ± 2.44 ^{Aa}	43.80 ± 2.08 ^{Aa}
	14	44.45 ± 2.14 ^{ABab}	45.74 ± 3.28 ^{ABa}	43.10 ± 0.92 ^{Aa}	46.63 ± 2.77 ^{Bb}
a*	0	15.31 ± 1.18 ^{Cc}	11.65 ± 0.05 ^{Bc}	12.38 ± 0.44 ^{Bc}	10.18 ± 0.41 ^{Ab}
	7	8.59 ± 1.04 ^{Aa}	8.02 ± 0.91 ^{Aa}	7.62 ± 0.75 ^{Aab}	8.58 ± 1.39 ^{Aa}
	10	10.45 ± 1.17 ^{Bb}	8.10 ± 0.80 ^{Aa}	7.42 ± 0.58 ^{Aa}	7.88 ± 1.06 ^{Aa}
	14	11.59 ± 1.56 ^{Bb}	10.22 ± 1.73 ^{ABb}	8.62 ± 1.19 ^{Ab}	8.58 ± 1.02 ^{Aa}
b*	0	15.46 ± 0.32 ^{Cb}	14.48 ± 0.86 ^{Ba}	14.93 ± 0.88 ^{BCa}	13.43 ± 0.75 ^{Aa}
	7	14.80 ± 1.08 ^{ABab}	15.53 ± 1.21 ^{Ba}	15.92 ± 0.74 ^{Bb}	13.92 ± 1.35 ^{Aa}
	10	14.74 ± 1.17 ^{Aab}	14.52 ± 0.59 ^{Aa}	16.60 ± 0.80 ^{Bb}	15.67 ± 1.13 ^{ABb}
	14	14.06 ± 0.70 ^{Aa}	14.69 ± 0.77 ^{Aa}	14.23 ± 0.44 ^{Aa}	14.75 ± 1.30 ^{Aab}

Values are the mean ± standard deviation (n=3). For each evaluation date, different capital letters indicate significant differences ($p < 0.05$) among treatments; for each treatment, different lowercase letters indicate significant differences ($p < 0.05$) among evaluation dates.

Table 2 also illustrates the evolution of L, a* and b* color coordinates of the control and treated samples during refrigerated storage. Color is one of the main quality attributes influencing consumers' buying decisions of fresh meat at the point of sale.[39] More specifically, the a* values that represent an index of redness have been used as an indicator of meat stability during storage.[40] In this study, the luminosity ranged between 41.78 and 46.86 and the b* values fluctuated between 13.43 and 16.60, which are in accordance with the results reported by Li[41] for fresh beef. In all the meat samples evaluated, both color attributes remained stable during the storage period, indicating that none of the solutions used caused negative effects on these color parameters. Only slight differences were observed among treatments and between the dates when the samples were tested, but without a clear trend. This could have been influenced by the great variability observed in the superficial color of the raw meat, which in turn, may mask differences among treatments and evaluation dates.

The initial a* value for the control was of 15.31 ± 1.18, which was similar to the values reported by López-Gajardo[36] and Li.[41] After the immersion in the treatment solutions, a significant decrease to 11.65 ± 0.05, 12.38 ± 0.44 and 10.18 ±0.41 was observed

in the PE1, PE2 and PE3-treated meat samples, respectively. This could be caused by the dark brown color of the propolis and the honeydew used to prepare the treatment solutions. During storage, a significant decrease in a*, which could be indicative of oxidation, occurred in all the samples. However, this reduction was more pronounced in the control samples as the solutions containing either propolis or honeydew were effective in mitigating this oxidation process. Several authors 34,36,41,42 reported a decrease in a* value over time. According to these authors, the changes are related to the redox status of the pigment myoglobin, responsible for color evolution in fresh meat.

Texture

Texture is an important quality parameter determining the acceptability of fresh meat.[43] The hardness and cohesiveness values determined using the TPA method are listed in Table 3. This method, measures the meat resistance to compression simulating the force used by the molar teeth during the mastication.[36] On day 0, no significant differences were observed among treatments for any of the texture parameters, indicating that none of the solutions used caused negative effects on the meat samples. The hardness of the meat ranged from 17.33 ± 2.70 to 20.36 ± 9.44 N, which is similar to the hardness reported by Ruiz-de-Huidobro[28] for raw meat. Regarding cohesiveness, the values ranged from 12.17 ± 3.66 to 15.88 ± 4.54, showing that the harder the meat, the lower was its cohesiveness. During storage, all the samples exhibited a similar behavior, with no clear tendency found for texture evolution. This could be attributed to the great variability observed among samples. According to López-Gajardo,[36] this variability in instrumental texture usually found in beef can be attributed to the intrinsic properties of the meat such as the fat content and the fiber distribution in each piece. As it can be seen in Table 3, during storage, there were no significant effects of the solutions on the texture of the meat. Similar results were found in beef meat marinated with fruit vinegar[44] and pork meat marinated with vinegar and honey.[45]

Table 3: Changes in instrumental texture of control and treated raw beef samples during refrigerated storage

		Treatment			
Parameter	**Storage time (days)**	**Control**	**PE1**	**PE2**	**PE3**
Hardness (N)	0	20.36 ± 9.44 ^{Aa}	18.07 ± 3.66 ^{Aa}	17.44 ± 7.73 ^{Aa}	17.33 ± 2.70 ^{Aa}
	7	19.87 ± 3.96 ^{Aa}	21.18 ± 7.27 ^{Aa}	17.41 ± 10.03 ^{Aa}	19.94 ± 7.49 ^{Aa}
	10	15.36 ± 4.31 ^{Aa}	18.17 ± 6.04 ^{Aa}	22.07 ± 6.47 ^{Aa}	20.96 ± 6.16 ^{Aa}
	14	17.14 ± 6.41 ^{Aa}	15.57 ± 4.00 ^{Aa}	17.27 ± 5.85 ^{Aa}	18.42 ± 6.54 ^{Aa}
Cohesiveness (dimensionless)	0	12.17 ± 3.66 ^{Aa}	13.43 ± 2.95 ^{Aa}	15.88 ± 4.54 ^{Aa}	15.03 ± 0.66 ^{Aa}
	7	11.72 ± 2.12 ^{Aa}	15.84 ± 7.12 ^{Aa}	13.90 ± 3.26 ^{Aab}	12.79 ± 2.55 ^{Aab}
	10	13.72 ± 1.48 ^{ABa}	14.44 ± 4.50 ^{Ba}	10.22 ± 1.88 ^{Aa}	12.47 ± 2.15 ^{Aab}
	14	13.08 ± 1.81 ^{Aa}	13.09 ± 3.86 ^{Aa}	12.76 ± 5.08 ^{Aab}	12.64 ± 1.57 ^{Aab}

Values are the mean ± standard deviation (n=9). For each evaluation date, different capital letters indicate significant differences ($p < 0.05$) among treatments; for each treatment, different lowercase letters indicate significant differences ($p < 0.05$) among evaluation dates

Sensory Evaluation

Regardless of the treatment, the scores for the color, aroma, tenderness and appearance were above the acceptance limit of 4, with slight, yet insignificant differences among the treated meat (Table 4). Similarly, the color, aroma and appearance of chicken fillets,[46] minced beef[47,48] and minced *Cyprinus carpio*[11] were not negatively affected by the addition of propolis extract solutions. On the other hand, the scores for both, the flavor and the global impression were significantly lower in the PE1-treated samples, being the former below the acceptance limit. The

presence of ethanol could be the responsible for the unpleasant taste causing the consumer's rejection of these samples. The evaporation of the ethanol in the PE2 solution was found to be effective at reducing the negative effects on the sensory quality of the meat observed in PE1. Propolis has been studied for its antimicrobial and antioxidant properties in diverse formulations, with variable results in the sensory quality. In effect, due to its intense flavor, which can impair the organoleptic properties of the foods, it is usually added in concentrations lower than 1%,[49] microencapsulated[50] or combined with other ingredients that are able to mask its taste.[51] In our study, despite the fact the differences against PE2 are not statistically significant, meat treated with the PE3 solution, containing honeydew, received the higher scores for all the sensory parameters studied with no off-flavors or off-odors described by the panelists for these samples. Moreover, when asked about their intention to purchase the meat, 66.7% of the panelists answered they would be willing to buy the PE3-treated meat whilst only 11.1% and 29.6% would buy the meat treated with the PE1 and PE2 solutions, respectively.

Table 4: Effect of propolis extract solutions (PE1: propolis extract solution with ethanol; PE2: propolis extract solution without ethanol; PE3: propolis extract solution with honeydew) on the sensory quality of beef (on an intensity scale from 1: worst to 7: best quality)

Sensory descriptors	Treatment		
	PE1	PE2	PE3
Appearance	5.63 ± 1.01 ^{A}	5.70 ± 0.95 ^{A}	5.78 ± 1.12 ^{A}
Color	5.70 ± 1.07 ^{A}	5.74 ± 0.90 ^{A}	5.89 ± 1.01 ^{A}
Smell	4.96 ± 1.40 ^{A}	5.41 ± 1.15 ^{A}	5.44 ± 1.34 ^{A}
Texture	4.93 ± 1.14 ^{A}	4.85 ± 1.46 ^{A}	5.59 ± 1.12 ^{A}
Taste	3.96 ± 1.56 ^{A}	5.11 ± 1.34 ^{B}	5.44 ± 1.25 ^{B}
Global impression	4.37 ± 1.11 ^{A}	5.26 ± 0.94 ^{B}	5.65 ± 1.02 ^{B}

Values are the mean ± standard deviation (n=27). Different letters indicate significant differences ($p < 0.05$) among treatments.

Multivariate Analysis

In the discriminant analysis, two functions together, (F1 and F2): (1) and (2), explained 99.7% of the variance. F1 was associated with a positive value of the Enterobacteria counts and a negative value of the color coordinate a*, and explained 97.2% of the variance. F2 explained 2.5% of the variance and was associated with a positive value of the total aerobic mesophiles' counts. Meat samples could be classified according to both, the treatment applied and the storage time (Figure 2). In effect, in figure 2 it is possible to identify five groups: on the top left part (quadrant II) are the samples of day 0 of all the treatments. From day 7 onwards, the antimicrobial and antioxidant effects of the applied solutions became evident, with the PE1, PE2 and PE3-treated samples conforming the second group in the bottom left part (quadrant III) as the control moved to the bottom right (quadrant IV), together with the treated samples of day 10. The fourth group, located in the top right (quadrant I), comprised the samples from day 14 of all the treated meat together with the control of day 10. This confirms that all propolis and honey solutions were effective in retarding microbial growth and preserving the meat from oxidation. Finally, in the last group only the control samples of day 14 were included. The microbial counts of these samples were above the limits of acceptance established in the European normative.[32]

$$F1=1.080E-0.471a^{*} \quad ...(1)$$

$$F2=0.928A \quad ...(2)$$

where; E: Enterobacteria; a*: color parameter (red) and A: total aerobic mesophiles.

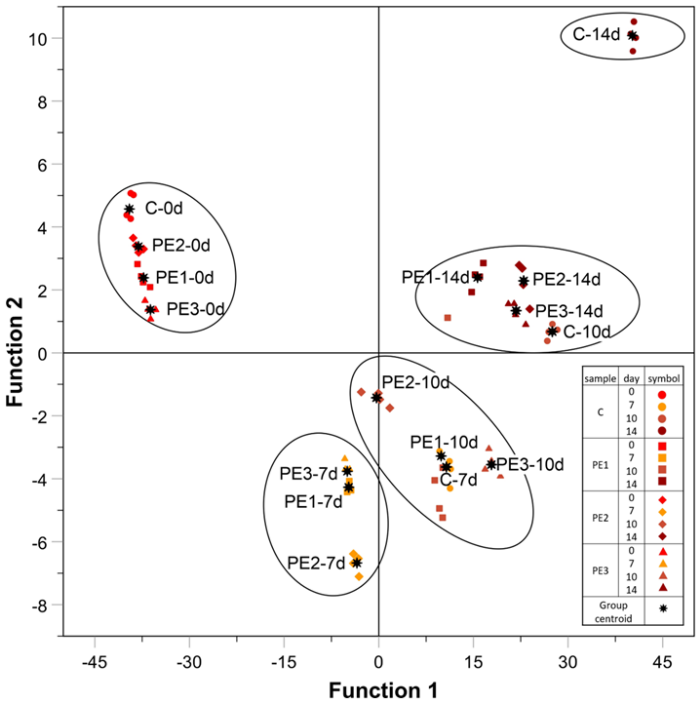

Fig. 2: Discriminant functions for the instrumental parameters and microbiological analysis in treatments: untreated meat samples (C) and meat treated with propolis extract solutions: PE1, PE2, PE3, according to storage time (from 0 to 14 days). (affinities among samples are highlighted by elliptical areas)

Conclusion

In this study, the combination of propolis with honeydew and the evaporation of the ethanol from the propolis ethanolic solution, resulted in promising strategies to mitigate the strong flavor associated to propolis. The solution containing propolis extract (8%) in mixture with honeydew (10%) was well accepted by the sensorial panelists, who expressed their willingness to buy the product. Moreover, this treatment demonstrated antioxidant and antibacterial properties when applied to beef. In effect, the oxidation process along with the Enterobacteria and total aerobic mesophiles growth were delayed with no negative effects on the physicochemical quality of the meat. From these results, further studies including the combination of propolis with different natural products (e.g. essential oils) are suggested with the aim of broadening propolis use as a natural preservative in the food industry.

Author Contributions

C. López-Patiño, C. Arroqui and P. Vírseda designed the experiment. C. López-Patiño, collected test data. C. López-Patiño and S. Horvitz interpreted the results and drafted the original paper. C. Arroqui, S. Horvitz and P. Vírseda, reviewed and revised the paper. C. Arroqui and P. Vírseda were both responsible for project administration.

Acknowledgments

The authors thank the Beekeepers Association of Navarre for providing the propolis and honeydew used in this study. Carmenza López-Patiño acknowledges to the University of Cauca (Colombia) for the financial support for her Ph.D. studies.

Funding

The authors received no financial support for the research of this article.

Conflicts of Interest

The authors declare no conflict of interest.

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