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Enhancing stability and health benefits of goat meat snacks using a linseed oil emulsion with lyophilized Surinam cherry (*Eugenia uniflora* L.) fruit extract

Mejora de la estabilidad y los beneficios para la salud de los snacks de carne de cabra utilizando una emulsión de aceite de lino con extracto de fruta pitanga (*Eugenia uniflora* L.) liofilizado

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RESUMEN

La mejora de la composición lipídica de los productos cárnicos puede llevarse a cabo mediante la incorporación de diferentes tipos de aceites, incrementando el contenido de ácidos grasos poliinsaturados, y el uso de sistemas de emulsiones acuosas para agregar componentes saludables. En este estudio, se prepararon nuggets de carne de cabra utilizando una emulsión de aceite de lino que contenía extracto liofilizado de fruta de pitanga, *Eugenia uniflora* L, como antioxidante. La adición del extracto de fruta mejoró la estabilidad oxidativa de los nuggets durante el almacenamiento en congelación sin afectar el sabor ni la aceptabilidad general. El perfil de ácidos grasos de los nuggets se mantuvo estable durante todo el almacenamiento, y los valores de biodisponibilidad lipídica e índice de degradación de la matriz fueron consistentes con otros productos cárnicos. La calidad microbiológica de los nuggets cumplió con los estándares regulatorios. En general, la incorporación de la emulsión de aceite de lino con el extracto de fruta de pitanga muestra promesa para desarrollar snacks de carne de cabra más saludables y estables, con posibles beneficios para la salud. Se necesita más investigación para respaldar estos hallazgos y determinar la fecha de caducidad durante el almacenamiento.

Palabras clave: nuggets de carne de cabra, antioxidante natural, perfil de ácido grasos, rancidez oxidativa, digestibilidad.

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ABSTRACT

The improvement of the lipid composition of meat products can be carried out by incorporating different types of oils, increasing the content of polyunsaturated fatty acids, and the use of water emulsion systems to add healthy components. In this study, goat meat nuggets were prepared using a linseed oil emulsion containing lyophilized Surinam cherry, *Eugenia uniflora* L, fruit extract as an antioxidant. The addition of the fruit extract improved the oxidative stability of the nuggets during frozen storage without affecting taste or overall acceptability. The fatty acid profile of the nuggets remained stable throughout storage, and lipid bioavailability and matrix degradation index values were consistent with other meat products. The microbiological quality of the nuggets met regulatory standards. Overall, incorporating linseed oil emulsion with Surinam cherry (*Eugenia uniflora L*) fruit extract shows promise in developing healthier and more stable goat meat snacks with potential health benefits. Further research is needed to support these findings and determine the expiration date during storage.

Keywords: goat meat nuggets, natural antioxidants, fatty acids profile, oxidative rancidity, digestibilityy.

INTRODUCTION

Nowadays, consumers perceive meat and meat products as the ideal sources of nutrients with specific functions in the body, leading to an increase in worldwide consumption. However, the consumption of meat has also been associated with negative health consequences due to components such as lipids, salt, and additives (Ruiz-Capillas and Herrero, 2021).

Among meat products, beef burgers are one of the most popular choices consumed by millions of people worldwide. To achieve the desired flavor and texture, approximately 20-30% of fat is added to these products, resulting in a high content of saturated fatty acids (SFAs) from meat and animal fat (Gahruie et al., 2017). Besides beef meat, other non-traditional meats such as lamb, pork, veal, and goat are used in Argentine to formulate meat products. Goat meat has low fat and saturated fatty acid content while being rich in unsaturated fatty acids such as oleic acid, which have been shown to possess hypocholesterolemic properties. Goat meat also contains protein levels comparable to beef, lamb, and veal prepared in a similar manner. Due to its lower fat content and potential health benefits, and nowadays people are including goat meat and its derivatives in their diets (Malekian et al., 2014).

To improve the composition of meat products, various strategies have been implemented, including the incorporation of different types of oils (Jiménez-Colmenero et al., 2013; Martínez et al., 2012; Romero et al., 2019). Water emulsion (O/W) systems are also utilized to add healthy components, such as n3 polyunsaturated fatty acids (PUFA) or antioxidants,

to the lipid phase of the product. When product reformulation results in an increased n3 fatty acid content, the addition of antioxidants becomes necessary as a health-promoting strategy (Navas-Carretero et al., 2015). Furthermore, considering that dietary fat intake has a proven effect on the development of diet-based diseases, the digestibility of lipids in meat should be considered, especially in products where the structure and processing can influence the digestibility of this nutrient (Asensio-Grau et al., 2019).

Limited information is available, particularly regarding reformulated goat products enriched in n3 PUFAs and natural antioxidants. Only the evaluation of aqueous leaf extracts of Pitanga as antioxidants on the stability of beef patties under cold storage has been conducted (Vargas et al., 2016). In a previous study, we developed goat meat snacks by adding an emulsion of flax oil with pitanga (Surinam cherry) (*Eugenia Uniflora* L) fruit extract as an antioxidant and evaluated their quality characteristics during refrigerated storage (Romero et al., 2022). The results indicated that substituting pork fat with the flax oil emulsion increased the content of nutritionally important fatty acids in the snacks, such as C18:2c and C18:3. Moreover, this substitution did not affect the taste or overall acceptability, and tenderness improved. The addition of the fruit extract to the linseed oil emulsion provided better oxidative stability during refrigerated storage. However, the digestibility of food components under simulated gastrointestinal conditions was not evaluated. Therefore, this research aims to assess the quality characteristics and fat digestibility of goat meat nuggets with a linseed oil emulsion containing *Eugenia Uniflora* L fruit extract as an antioxidant during frozen storage.

MATERIALS AND METHODS

Materials

Goat meat, pork back fat, and bovine blood were obtained from different local processors. Linseed oil, mixed spices (including dehydrated garlic, parsley, oregano, chili, ground black pepper, thyme, and bay leaf), and dry breading material (ground corn flakes) were purchased in a local market and used without modifications. *Eugenia Uniflora L.* fruits were harvested from trees located near the National University of Chaco Austral in the Province of Chaco, Argentina. Fatty acid standard methyl esters (Supelco® 37 Components FAME Mixture) and Boron trifluoride/methanol were purchased from Sigma-Aldrich (USA); thiobarbituric acid reactive substances (TBARS) and butilhydroxianisole (BHA) from Merck (Germany); trichloroacetic acid (TCA) from Biopack (Argentine); chloroform and methanol from Cicarelli (Argentine). All other reagents and chemicals used were of analytical grade. The lyophilized extract of *Eugenia Uniflora* (*L*) fruit (LEUE) and linseed oil emulsion with bovine plasma as emulsifier were prepared according to the method described by Romero et al. (2022).

Preparation of goat meat nuggets

The goat meat nuggets were prepared following the procedure described by Romero et al. (2022). Minced goat meat (77.8% w/w) was mixed with linseed oil emulsion (20% w/w), salt (1.2% w/w), and mixed spices (1% w/w) in the processor for 2 min. Three types of nuggets were prepared as follows: nuggets without an antioxidant added to linseed oil emulsion (negative control), nuggets with LEUE (2% w/w) added to linseed oil emulsion (experimental group), and nuggets with butylated hydroxy anisole (BHA, 0.01% w/w) added to linseed oil emulsion (positive control). Model systems were called NC, NLEUE, and NBHA, respectively.

The nuggets were then cooked in a hot air oven at 180 °C for 15 minutes until the internal temperature reached 72 ºC at the thermal centre of the samples, ensuring microbiological safety. The internal temperature was monitored using a puncture thermometer (Testo model 925, Lenzkirch, Germany). After cooking, the nuggets (30 per treatment or 750 g per treatment) were packaged in oxygen-permeable bags (2000 cm^3/m^2 day) using a packing machine (RAPI-VAC S-750 ® SERVIVAC S.R.L., Buenos Aires, Argentina). The samples were stored at -18 °C and analysed at 0, 30, and 60 days. The experiments were replicated twice.

Lipid profile of goat meat nuggets

Extraction and purification of fat were performed according to Bligh & Dyer (1959), using BHA as an antioxidant. The fatty acid methyl esters were prepared according to AOAC Method 969.33 (AOAC INTERNATIONAL, 1998), and quantified with an Agilent Technologies gas chromatograph equipped with a 60 m capillary column Supelco 2340 and FID detector according to Romero et al. (2018).

Quality evaluation of goat meat nuggets during storage

pH and Microbiological Analysis

The pH of the goat meat nuggets was determined using a puncture pH meter (SATIA S.R.L.) on five separate occasions. For the microbiological analysis, ten grams of each sample were aseptically collected and transferred to a stomacher bag containing 90 ml of sterile peptone water for homogenization. The homogenate was then appropriately diluted, and duplicate inoculations were made on plate count agar (PCA). The plates were incubated at 37˚C for 48 hours. The results were expressed as the log number of colony-forming units per gram of sample (log cfu/g).

Lipid oxidation

Lipid oxidation was assessed using the thiobarbituric acid-reactive substances (TBARS) method, as described by Jimenez-Colmenero et al. (2015). Briefly, 5 grams of each sample were homogenized with 35 mL of 75 g/L trichloroacetic acid for 30 seconds using a high-speed mixer blender. The homogenized sample was then centrifuged (Hettich Zentrifugen®, Rotina 380 R, Germany) at 4000 × g for 5 minutes. Subsequently, 5 mL of the supernatant

was mixed with 5 mL of 20 mmol/L thiobarbituric acid. The resulting pink-colored solution was measured at 532 nm using a UV-Vis spectrophotometer (Thermo Scientific UV-Vis, Evolution 600). The TBARS values were expressed as milligrams of malondialdehyde (MDA) per kilogram of fat in the samples, with 1,1,3,3-tetramethoxypropane (TEP) serving as a standard.

Digestibility assays

The in vitro human digestion process employed in this study consisted of three distinct stages: oral, gastric, and intestinal. This static digestion method followed the standardized protocol for in vitro digestion of food and was performed on the day of preparation as well as at 30 and 60 days of frozen storage. The enzymatic composition of the simulated saliva, gastric fluid, and duodenal fluid was freshly prepared daily based on stock solutions, following the protocol outlined by Minekus et al. (2014). The digestion process proceeded as follows:

I. Oral stage: five grams of nuggets were mixed with 5 mL of simulated saliva fluid and stirred for 5 minutes at 37°C.

II. Gastric stage: approximately 12 mL of simulated gastric fluid (pH 3) was added to the mixture and stirring continued for 2 hours at 37°C. Pepsin was added to the simulated gastric fluid according to the specified concentration in the standardized protocol. The pH of the mixture was adjusted to pH 2.8 ± 0.1 using 1N HCl, and the samples were subjected to head-over-heels rotation at 55 rpm for 2 hours at 37°C using a magnetic stirrer with a heating probe.

III. Intestinal stage: immediately after the gastric stage, simulated intestinal fluid (SIF; pH 7) was added to each tube containing the gastric chyme in a 1:1 (v/v) ratio. The pH of the mixture was adjusted to pH 7.0 ± 0.1 using 0.1N NaOH. The samples were then stirred from top to bottom at 55 rpm for an additional 2 hours at 37°C. pH levels were monitored throughout the digestion process and readjusted as needed to maintain constancy.

Matrix degradation index (MDI)

To determine the MDI in all samples following in vitro digestion, the procedure described by Asensio-Grau et al. (2019) was employed, in accordance with the methodology outlined by Lamothe et al. (2012, 2014).

Statistical analysis

Two independent experiments were conducted under identical conditions, and each assay was performed in duplicate or more. The obtained results were analyzed using the Statgraphics Plus for Windows software package. All data were presented as mean ± standard deviation. To assess the differences between formulations, a one-way analysis of variance (ANOVA) was performed with the formulations as the factor. In cases where the

analysis yielded statistical significance at a level of $p < 0.05$, Duncan's multiple range test was utilized to determine the significant differences between the means.

RESULTS AND DISCUSSION

pH and Microbiological Analysis

As the expected, the pH values of the goat meat nuggets during chilling storage showed no significant differences ($p > 0.05$), ranging from 5.27 at the beginning to 5.18 at the end of storage, which could be due to a slow degradation of proteins and liberation of peptides during storage. These findings are consistent with previous studies on meat products incorporating natural antioxidant extracts (Gahruie et al., 2017; De Carvalho et al., 2019).

The processing of meat products is of great importance to ensure hygiene and safety for consumers. In terms of microbiological quality, the total count of mesophilic bacteria in the products remained within the range of 0.92 to 1.21 log CFU/g (Figure 1), which is below the limit established for this type of product (5 log CFU/g) according to the Argentine Food Code for frozen samples. The test conditions, including heating up to 72°C in the thermal centre and freezing storage at -18°C, create limiting conditions for microbial growth. Although, these results indicate that the goat meat nuggets are safe and suitable for consumption, complementary studies should be carried out.

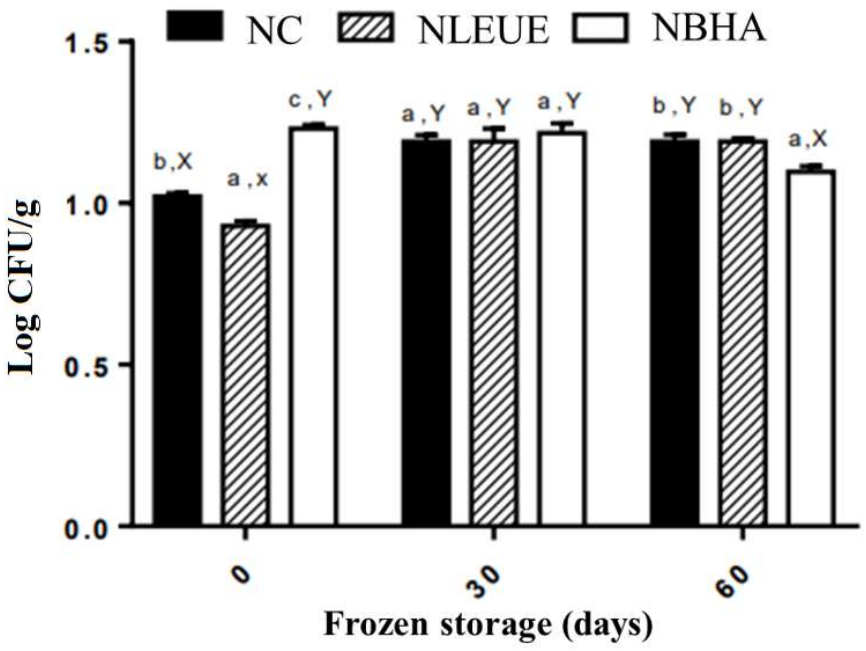

Figure 1. Microbiological count of mesophilic bacteria in goat meat nuggets (NC: nugget control, NLEUE: added with linseed oil emulsion with bovine plasma as emulsifiers and 2% of LEUE. NBHA: nugget with 0.01% of butylated hydroxy anisole). a, b, c Different letters for each day means significative difference ($p<0.05$). X, Y, Z Different letters for each formulation means significative difference ($P<0.05$) between storage days.

Lipid oxidation and fatty acids profile

Lipid oxidation is a critical factor in maintaining the stability of the fatty acid profile, which is of nutritional importance. The results of lipid oxidation in the nuggets are presented in Table 1. It was observed that *Eugenia uniflora* extracts exhibited a similar TBARS value to the synthetic antioxidant (BHA), indicating their effectiveness in preventing lipid oxidation in the nuggets ($p < 0.05$). The antioxidants present in the extract were found to be sufficient in preventing a significant degree of oxidation in the frozen nuggets. This suggests that *Eugenia uniflora* extract (EUE) can serve as a suitable alternative to synthetic antioxidants, contrary to the findings reported by Vargas et al. (2016) for *Eugenia uniflora* aqueous leaf extracts in beef patties under cold storage. Similar observations were made by Aksu, Alinezhad and Erdemir (2015) in beef steaks supplemented with lyophilized *Urtica dioica* L. water extract, and by Alejandre et al. (2019) in a gel emulsion system containing microalgal oil supplemented with blackthorn branch extract (*Prunus spinosa* L.). Additionally, Jagtap et al. (2020) successfully added papaya and oregano leaf extract as natural bioactive antioxidants in a goat meat system. Therefore, it is recommended to conduct assays for a longer period, exceeding 60 days, to determine the expiration date during storage.

Table 1. Oxidative stability of goat meat nuggets fortified with linseed oil emulsion with or without antioxidant

Day	NC	NLEUE	NBHA
0	0.37 ± 0.001 c, XY	0.06 ± 0.001 a, X	0.12 ± 0.030 b, X
30	0.42 ± 0.004 b, X	0.32 ± 0.030 a, X	0.32 ± 0.06 a, X
60	0.91 ± 0.010 c, X	0.39 ± 0.010 b, X	0.33 ± 0.01 a, X

NC refers to nuggets without an antioxidant added to the linseed oil emulsion (negative control).
NLEUE refers to nuggets with *Eugenia uniflora* extract (LEUE) added to the linseed oil emulsion at a concentration of 2% (w/w) (experimental group).
NBHA refers to nuggets with butylated hydroxyanisole (BHA) added to the linseed oil emulsion at a concentration of 0.01% (w/w) (positive control).
Different letters (a, b, c) within each row indicate significant differences ($p < 0.05$).
Different letters (X, Y, Z) within each column indicate significant differences ($p < 0.05$).

At the beginning of the storage period, the fatty acid profile of the nuggets was like that reported in the previous study (Romero et al., 2022). Minor changes were observed in the fatty acid profile, which can be attributed to the natural variation in the raw materials used. This indicates that the emulsions utilized in the products were nutritionally optimal, making

them suitable ingredients for the development of healthier meat products. These findings are consistent with the study conducted by Gómez-Estaca et al. (2019), where they evaluated the combination of healthy oils structured with methylcellulose and beeswax oleogels as substitutes for animal fat in low-fat pork burgers enriched with polyunsaturated fatty acids (Table 2).

Table 2. Fatty acids profile of formulations at the end of frozen storage

Fatty acids	NC	NLEUE	NBHA
(14:0)	0.91 ± 0.007 b	0.76 ± 0.010 a	0.72 ± 0.002 a
(16:0)	10.30 ± 0.005 b	9.77 ± 0.040 a	9.79 ± 0.020 a
(18:0)	12.95 ± 0.010 c	12.03 ± 0.010 a	12.62 ± 0.001 b
(18:1)	20.28 ± 0.005 c	19.17 ± 0.020 b	18.30 ± 0.002 a
(18:2)	14.99 ± 0.020 c	14.01 ± 0.010 b	13.69 ± 0.010 a
(18:3)	39.18 ± 0.060 a	42.25 ± 0.004 c	41.97 ± 0.010 b

NC refers to nuggets without an antioxidant added to the linseed oil emulsion (negative control).
NLEUE refers to nuggets with *Eugenia uniflora* extract (LEUE) added to the linseed oil emulsion at a concentration of 2% (w/w) (experimental group).
NBHA refers to nuggets with butylated hydroxyanisole (BHA) added to the linseed oil emulsion at a concentration of 0.01% (w/w) (positive control).
Different letters (a, b, c) within each row indicate significant differences ($p < 0.05$).

Lipid bioavailability and MDI

Regarding lipid bioavailability, the values ranged from 51% ± 1.41 to 58% ± 7.09 for the three formulations up to day 60, and no significant differences were observed between samples or storage days ($p > 0.05$). These findings suggest that both the formulation variation and the storage time did not have a statistically significant effect ($p > 0.05$). Previous studies have reported that in matrices where protein structures predominate, the release of fat particles is a gradual and slow process (Asensio-Grau et al., 2019; Hur et al., 2009). In such systems, the hydrolysis of lipids is dependent on the extent of proteolysis. Only when the protein structure is disrupted, lipids are released from the matrix, and lipases can initiate hydrolysis, leading to increased lipid release during storage (Dickinson, 2012).

The Matrix Degradation Index (MDI) values at the beginning of storage were 77.39% ± 3.42, 76.24% ± 3.36, and 78.96% ± 3.79 for NC, NLEUE, and NBHA, respectively ($p < 0.05$). At the

end of storage, these values increased to 84.88% ± 5.96, 81.08% ± 6.43, and 87.31% ± 5.08, respectively, but the differences were not statistically significant ($p > 0.05$). Asensio-Grau et al. (2019) found that unstructured meat products exhibited higher MDI values compared to structured products. No other studies evaluating these parameters under similar conditions were found, indicating the need for further research to support these results.

CONCLUSION

The findings of this study demonstrate that the utilization of linseed oil emulsion with bovine plasma as an emulsifier and Lyophilized *Eugenia uniflora* fruit extract (LEUE) holds promise for the production of healthy goat meat snacks. The snacks exhibited comparable oxidative stability to those fortified with the synthetic antioxidant (BHA), indicating the effectiveness of *Eugenia uniflora* as a natural source of bioactive compounds, such as phenolic and flavonoid compounds. Furthermore, the lipid bioavailability and matrix degradation index values were consistent with those observed in other meat products. The microbiological quality of the snacks also met the regulatory standards established for such products. These findings suggest that incorporating linseed oil emulsion with LEUE can contribute to the development of nutritious and safe meat snacks with improved oxidative stability and potential health benefits.

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