

Antioxidant Effect of Bovine Plasma Proteins Modified via Maillard Reaction on n3 Fortified Beef Patties

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Abstract

This work aimed to develop a healthier meat product, by improving nutritional properties related to lipid profile, using a Maillard reaction product (MRP) obtained from recycling blood from local slaughterhouses as antioxidant. Soybean oil was used as partial fat replacer and the lipid profile was modified as a decrease in the n6/n3 ratio (11.59 to 9.50), and atherogenic and thrombogenic index (from 0.42 to 0.27 and 0.94 to 0.56 respectively) was observed. Beef patties were added with two MRP levels (1% w/w and 3% w/w). Patties with no antioxidants were used as control and patties containing 0.01% w/w of butylhydroxyanisole (BHA) were used as reference. Oxidative stability of refrigerated vacuum-packed patties was evaluated during 15 days of storage. Results showed 3% of MRP as the most appropriate concentration to prevent lipid oxidation with inhibition percentages of 75%, and 9 days as the limit time of chilled storage regarding consumers' perceptions.

Keywords

Meat Industry Waste, Natural Occurring Antioxidants, Lipid Oxidation, Healthier Meat Commodities, Functional Meat Products

1. Introduction

Meat is an essential component of a healthy and well-balanced diet, since it is an interesting protein and iron

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source [1]. However, it usually shows high amounts of saturated fat, which have been closely related to some chronic diseases [2]-[4]. As a result of the scientific evidence, health authorities recommend a reduction of cooked meat products intake or directly to avoid certain meat products [5]. In this regard, beef patties are still consumed world-wide despite of its apparent bad nutritional aspect. Therefore, in recent years great efforts have been exerted in order to develop nutritionally improved meat patties using reformulation strategies, e.g. the substitution of pork back fat with vegetable oils, which has been demonstrated to be a good tool to achieve a healthier lipid profile in these products [6] [7], by decreasing saturated fatty acids (SFA) levels and improving certain nutritional indices, as n6/n3 ratio, atherogenic index (AI) and thrombogenic index (TI). The beneficial effect of the partial replacement of animal fat with different vegetable oils has been investigated in numerous meat products such as dry-cured sausages [8], frankfurters [9] and burger patties [10] [11]. Therefore, increasing the polyunsaturated fatty acid (PUFA) content seems to be the most suitable way to improve the lipid fraction of meat products [12]-[14] but this implies a higher susceptibility to oxidative processes, which generate a decrease of the nutritional value of the food, and the formation of potentially toxic compounds [15].

Food industries usually resort to the use of synthetic antioxidants to prevent lipid oxidation, although the scientific evidence of their possible toxic effects and the consumers' preferences for natural products force the meat industry to use naturally occurring antioxidants. In this regard, research works include fruit juices [16], tea extracts [17], residues from the wine industry [18] and different Maillard reaction products such as amino acid-sugar reaction products [19] [20], porcine plasma proteins-sugar reaction products [21] and other protein-aldehydes reaction products [22] [23]. The addition of such substances usually darkens the meat product, limiting its use due to the impact on food colour.

On the other hand, blood is an inevitable by-product of the meat industry representing up to 4% of the live animal weight. Animal blood produced in slaughterhouses represents a problematic waste of the meat industry due to the high volumes generated and its very high pollutant load when discarded directly into the environment [24]. It also has a high protein content (17%), with a reasonably good balance of amino acids, however, the use of blood in meat processing may mean that the final product is dark in colour [25]. Several studies report the antioxidant properties of bovine blood proteins hydrolysates [26]-[28], but there is not enough information about the use of plasma proteins as a source of amino groups for the Maillard reaction, which is known to provide antioxidant compounds. Previous studies of this research group have demonstrated the antioxidant action of a Maillard reaction product (MRP) obtained by reacting bovine plasma proteins and aldehydes when used in model systems [23], although tests on food products were not conducted.

When using a fat replacer, a careful approach is needed in order to achieve the appearance and technological, rheological and sensory properties required in the food product, thus the aim of this work was to improve the fatty acid profile of beef patties through the partial replacement of animal fat by soybean oil, and to study the effect of a MRP antioxidant, obtained from recycling blood from a local slaughterhouse, on the oxidative stability and colour of beef patties subjected to cooking and chilled storage.

2. Material and Methods

2.1. Chemicals

Sodium citrate, potassium ferricyanide, trichloroacetic acid (TCA) and ammonium thiocyanate were purchased from Biopack (Argentina). Hexanal, sodium phosphate, butylhydroxyanisole (BHA), ferrous chloride and thiobarbituric acid (TBA) were obtained from Merck (Germany). Ferrozine, ethylenediaminetetraacetic acid (EDTA), boron trifluoride, standard fatty acid methyl esters (Fame 37) and 1,1,3,3-tetramethoxypropanewere provided by Sigma-Aldrich (Germany). Chloroform, methanol, hydrochloric acid and ethanol were obtained from Cicarelli (Argentina).

2.2. Material

2.2.1. Raw Material

Fresh beef meat, pork back fat and soybean oil were obtained from a local market. Bovine blood was kindly provided by Cooperativa Agropecuaria La Union Ltda (Chaco, Argentina).

2.2.2. Obtaining of Bovine Plasma Proteins (BPP)

Bovine blood was collected from a local slaughterhouse every two months, starting on February and ending at

June 2014. In all cases, the provided blood was mixed with sodium citrate (2.5 w/w) to prevent clotting and refrigerated until use. All collected blood was centrifugated at 2500 g and 2°C during 10 minutes (Hettich Zentrifugen, Rotina 380 R, Germany). The supernatant was refrigerated until use. Protein content was determined by the Biuret method as described by Robson, Goll and Temple [29].

2.2.3. Obtaining of Aldehydes Mixture by Oxidizing Soybean Oil (SO)

Soybean oil (SO) (3.5 g) was oxidized in rotary evaporator (Figmay SRL, Argentina) at 80°C; aldehydes arising were removed by nitrogen flow and collected in Sorensen buffer (pH 7.6). Aldehydes content was determined at 265 nm in a UV-visible Beckman DU 640B, Fullerton, CA, USA spectrophotometer and expressed as mg hexanal/mg soybean oil. Ethanolic solutions of hexanal (1000 to 3000 mg/L) were used as standard to prepare a calibration curve. The end of the process was reached after 6 hour of heating, when absorbance measurements remained constant. The aldehydes content achieved was 0.006 mg hexanal/mg soybean oil.

2.2.4. Preparation of the Maillard Reaction Product (MRP)

MRP was obtained from the reaction between bovine plasma proteins and aldehydes arising from SO oxidation. Plasma proteins were incubated with aldehydes in a ratio 3.5:1. The mixture (pH 7.6) was kept in rotatory evaporator at 80°C for 4 h under moderated agitation (56 rpm). These temperature-time conditions were determined in previous studies [30]. The resultant mixture was lyophilized to obtain the dry product (MRP).

2.2.5. Manufacture of Traditional and n3 Fortified Beef Patties

Five types of beef patties were prepared including a control batch manufactured with pork back fat and no added SO. The ingredients per kg of patty in the basis formulation were as follows: 800 g meat, 200 g pork back fat and 20% sodium chloride. In the formulation of patties manufactured with SO (n3 fortified patties), 50% of the animal fat was replaced with 100 g of SO per kg patty.

The experiments were conducted as follows: the n3 fortified patty formulation was divided into four treatments: 1) control without antioxidant; 2) MRP at 1% w/w; 3) MRP at 3% w/w; and 4) 0.01% w/w of BHA as a reference. Patties were formed in Petri dishes to obtain samples of 100 g each one (9 cm diameter and 1.5 cm thickness). The MRP levels were established in previous studies [22]. According to the limit level allowed, 0.02 g of BHA per g of fat was added [31].

They were then cooked in forced convection oven (TecnoDalvo, SRL, Argentina), at 200°C for 15 minutes, until 72°C internal temperature (indicated by digital stem thermometer TPD 33, Omega Engineering, Inc., Connecticut, USA) was reached. Cooked patties were cooled at room temperature and vacuum-packed (Rapi-Vac S-750) in polypropylene bags and kept at 4°C for further analysis at 0, 8 and 15 days of storage.

Three independent trials were done. Each time three (3) burgers for type of treatment were prepared, for each storage time analyzed.

2.3. Physicochemical Determinations

2.3.1. Fat Extraction

The Bligh and Dyer method [32] was used for fat extraction from meat samples. The extraction procedure was briefly as described by Miranda *et al.* [20]: 4.5 ml of chloroform were added to a 10 g sample followed by the addition of 10 ml of methanol, and 10 µl of an appropriate solution of BHA, and mixed for 3 minutes; then 6.5 ml of distilled water and 4.5 ml chloroform were added. After decantation, the organic phase was isolated, collected in cap tubes and heated in a water bath (50°C) and kept under nitrogenous flow for chloroform evaporation. The lipids obtained were kept at -20°C until analysis.

2.3.2. Fatty Acid Profile

Boron trifluoride/methanolic solution (14%) was used for fatty acid methyl esters preparation according to AOAC N° 969.33 method. Fatty acid composition was determined by using a HP 6850 Gas chromatograph equipped with a Supelco 2340 capillary column (60 m length, 0.25 µm internal diameter) and a FID detector. Standard methyl esters of fatty acids of 99% purity were used (FAME mix). The injector and detector temperature were 240°C; the oven temperature was held at 140°C for 5 min and subsequently increased at 4°C/min to 240°C and kept at 240°C during 20 min. Fatty acid composition of patties was obtained by comparing the rela-

tive retention times of standards and expressed as relative percentage.

2.3.3. Lipid Quality Indices

Data obtained from fatty acid composition values of samples were used to calculate the nutritional ratios as PUFA/MUFA and n6/n3, and the Atherogenic index (AI) and Trombogenic index (TI) according to Zhang *et al.* [33], using Equations (1) and (2) as follows:

$$AI = [(C12:0) + (4 \times C14:0) + (C16:0)] \times [PUFA (n6 \text{ and } n3) + MUFA]^{-1} \quad (1)$$

$$TI = [(C14:0) + (C16:0) + (C18:0)] \times [(0.5 \times MUFA) + (0.5 \times n6) + (3 \times n3) + (n3 \times n6 - 1)]^{-1} \quad (2)$$

2.3.4. Evolution of Oxidation Process

Oxidation process was monitored by measuring Peroxide Value (PV) and Thiobarbituric Acid Reactive Substances (TBARS).

1) Peroxide Value (PV)

The ferric thiocyanate (FTC) method was adapted from the FIL-IDF method 74A:1991. Samples (0.02 g of extracted fat) dissolved in 9.8 ml of methanol: chloroform (70:30) solution and 0.1 ml of 30% (w/v) ammonium thiocyanate were added and mixed. Precisely 5 min after the addition of 0.1 ml of ferrous chloride (3.5% v/v hydrochloric acid solution) to the reaction mixture, the absorbance of the resulting red colour was measured at 501 nm against solvent solution as blank. PV was expressed in terms of mili equivalent of oxygen per kg of sample (mEq O₂/kg sample).

2) Thiobarbituric Acid Reactive Substances (TBARS)

TBARS were determined according to Sinnhuber and Yu [34] with slight modifications. Sample (100 µg of extracted fat) was placed in 10 ml test tubes; then 10 µl of BHA (appropriate ethanolic solution) and 2 ml of TBA solution (20 mM TBA in 15% TCA) were subsequently added. The mixture was vortexed and heated in a water bath (90°C) for 15 minutes. After cooling at room temperature, 2 ml of chloroform were added, followed by centrifugation at 650 g for 15 minutes. The absorbance of supernatant was read at 532 nm against a blank containing 0.1 ml of distilled water and 2 ml of TBA/TCA solution. Malondialdehyde (MDA) standard curve was prepared from different 1,1,3,3-tetramethoxypropane solutions, and TBARS were expressed as mg of MDA/kg dry matter.

3) Antioxidant activity

The antioxidant activity at the end of the assay time was expressed as inhibition percent of peroxidation (IP%) and calculated according to Equation (3) for each indicator referred to a control without antioxidant (blank):

$$IP\% = \left[\frac{(\text{Peroxidation indicator value blank} - \text{Peroxidation indicator value sample})}{\text{Peroxidation indicator value blank}} \right] * 100 \quad (3)$$

The higher the IP, the higher the antioxidant activity.

2.3.5. Hunter L*, a* and b* Values Colour

Colour measurements were performed using an Evolution 600 UV-Vis (ThermoScientific) equipped with an integrated sphere with Visionlite Colour Calc Software (Germany) to obtain CIE L*, a* and b* values for the MRP and all beef patties. The American Meat Science Association Guidelines [35] for colour measurements were followed in order to calculate total colour change (ΔE) over a selected period of time:

$$\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2} \quad (4)$$

2.4. Statistical Analysis

A 4 treatment × 3 storage periods factorial design with three replicates was used. Data analysis was done with the ANOVA function using the software package Statgraphics Plus. Duncan's multiple range test (p < 0.05) was used to determine mean differences between treatment means.

3. Results and Discussion

3.1. Fatty acid Composition of Meat Patties

Consumers are becoming more aware of the relationship between diet and health and this fact has increased their interest in the nutritional value of foods [36]. According to Morales *et al.* [37] healthy aspects are one of the relevant factors affecting consumers to purchase certified beef products. Thus, we focused on the analysis of beef patties fortified with beneficial fatty acid achieved through the modification of the lipid profile of the control (traditional patty). This in order to provide a healthier meat product in terms of indices which take into account different effects of fatty acids on cardiovascular risk, such as PUFA/SFA ratio, n6/n3 ratio, the Index of Atherogenicity (AI) and the Index of Thrombogenicity (TI).

Table 1 compares the most important aspects of the fatty acid profile of SO, TP and FP (cooked products). Fortified meat system contained approximately a 90% more PUFA than the conventional product. A 2-fold increment for α -linolenic acid was observed in modified cooked product (1.74 vs 3.70) as a consequence of the addition of soybean oil in relation to the control (TP). There was a strong increment in linoleic acid content (20.21 vs 35.14). These modifications were obviously reflected in nutritional ratios, the PUFA/SFA ratio increased from 0.62 in product without soybean oil to 1.47 in the modified one, and n6/n3 ratio decreased for fortified meat products (11.6 to 9.5).

According to nutritional recommendations, fats having a low PUFA/SFA (<0.4) and a high n6/n3 (>5) ratio are considered unfavourable since they may induce an increase in cholesterolaemia. However, these two indexes are not totally adequate to evaluate the nutritional value of fats owing to the lack of information regarding MUFA. Therefore, the AI and TI are useful to a better approach to the nutritional evaluation of fats, since they are calculated on the basis of fatty acids with functional effects [38].

The improved lipid profile of beef patties contributed to decrease AI and TI, from 0.41 to 0.26 and 0.94 to 0.56 respectively. Variations in fatty acid profiles were similar to those described by Delgado-Pando, Cofrades, Ruiz-Capillas, and Jiménez-Colmenero [39] using healthier oil combination-in-water emulsions as pork back fat replacers in low-fat frankfurters, with improved PUFA/SFA and n6/n3 ratios compared to the control (1.7 to 0.27 and 9.27 to 0.47 respectively).

3.2. Lipid Oxidation

Data obtained from monitoring lipid oxidation showed that the PV of the sample without antioxidant (A) increased throughout the storage time, whereas 1% of MRP (B) inhibited the formation of peroxides only as from

Table 1. Principal fat components and lipid quality indices of soybean oil and the two types of patties (g/100g fatty acids. Mean \pm standard deviation). TP = traditional patty; SO = soybean oil; FP = fortified patty.

	TP	SO	FP
Σ SFA	35.25 \pm 0.18 ^c	17.29 \pm 0.26 ^a	26.42 \pm 0.18 ^b
Σ MUFA	42.80 \pm 0.22 ^c	23.37 \pm 0.19 ^a	34.73 \pm 0.22 ^b
Σ PUFA	21.95 \pm 0.04 ^a	59.33 \pm 0.45 ^c	38.84 \pm 0.04 ^b
n3	01.74 \pm 0.03 ^b	07.74 \pm 0.07 ^a	03.70 \pm 0.03 ^c
n6	20.21 \pm 0.01 ^a	51.59 \pm 0.43 ^c	35.14 \pm 0.01 ^b
n9	39.96 \pm 0.14 ^b	23.70 \pm 0.38 ^a	32.91 \pm 0.14 ^c
PUFA/SFA	00.62 \pm 0.00 ^a	03.43 \pm 0.05 ^c	01.47 \pm 0.01 ^b
n6/n3	11.59 \pm 0.19 ^c	06.66 \pm 0.14 ^a	09.50 \pm 0.07 ^b
AI	00.42 \pm 0.00 ^c	00.19 \pm 0.00 ^a	00.27 \pm 0.00 ^b
TI	00.94 \pm 0.01 ^c	00.01 \pm 0.00 ^a	00.56 \pm 0.00 ^b

^{a, b, c}Means in a same line followed by a different letter are significantly different ($p < 0.05$).

seven days. PV of the sample with 3% MRP (C) were lower than A and B during all the assayed time. Nevertheless, PV achieved at the end of the storage time for samples with 1% MRP showed no significant difference with samples containing 3% MRP (Figure 1).

Figure 2 shows that the development of TBARS in A samples (0% MRP) also increased throughout the storage, while 3% of MRP inhibited TBARS formation more efficiently than 1%.

Lipid hydroperoxides are the primary oxidation products and, thus, a precursor for further decomposition into the volatile secondary oxidation products causing off-flavours in meat. According to Argentinean Food Code [31], the PVs of edible oils and fats should not exceed 10 mEq O₂/kg, while MDA concentration should not exceed 2 mg/kg since this is the cut value of rancidity perception by consumers as indicated by Karakaya & Simsek [40]. In this regard PV values of n3 fortified beef patties with 3% MRP was kept under the limit level during all the storage time, whereas a higher oxidation in terms of TBARS was reached just at 9 days, which was considered as the maximum acceptable storage time.

Thus, PV and TBARS at 9 days of storage (values obtained from linear regression of the corresponding evolution graphs) were used to estimate the antioxidant activity for each indicator as inhibition percentage of peroxidation (%IP), comparing with the antioxidant effect of the synthetic antioxidant reference.

This can be seen in Figure 3, which shows that the MRP exerted an acceptable inhibition effect on both stages of lipid oxidation, since IP on hydroperoxides formation was higher than 70%, whereas IP on TBARS was higher than 45%, stating that the antioxidant action of the MRP was suitable in the first stages of the peroxidation (IP = 70%), which was highly positive given the high content of PUFAs, with the highest inhibition on secondary oxidation products (IP = 74%).

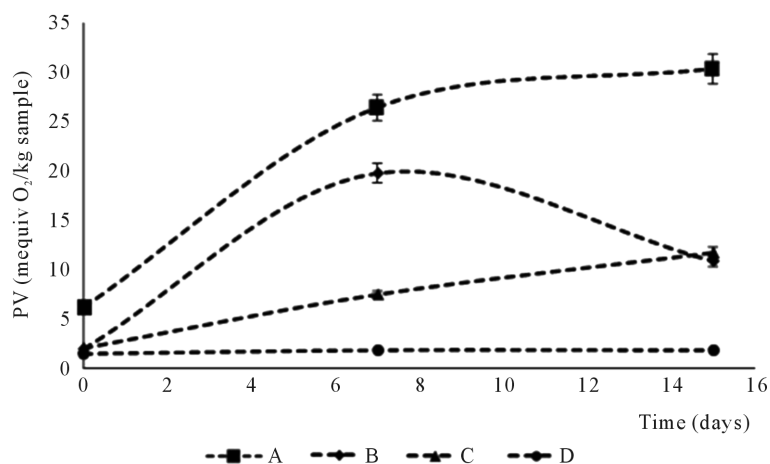


Figure 1. PV evolution during chilling storage of n3 fortified beef patties added with A) 0% MRP; B) 1% MRP; C) 3% MRP and D) 0.01% BHA.

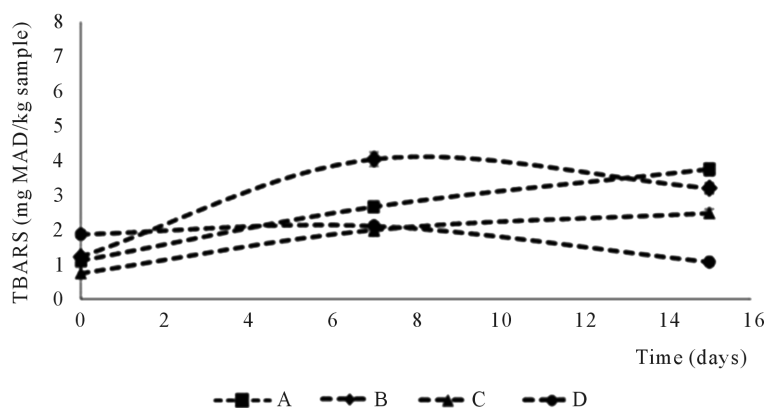


Figure 2. TBARS evolution during chilling storage of n3 fortified beef patties added with A) 0% MRP; B) 1% MRP; C) 3% MRP and D) 0.01% BHA.

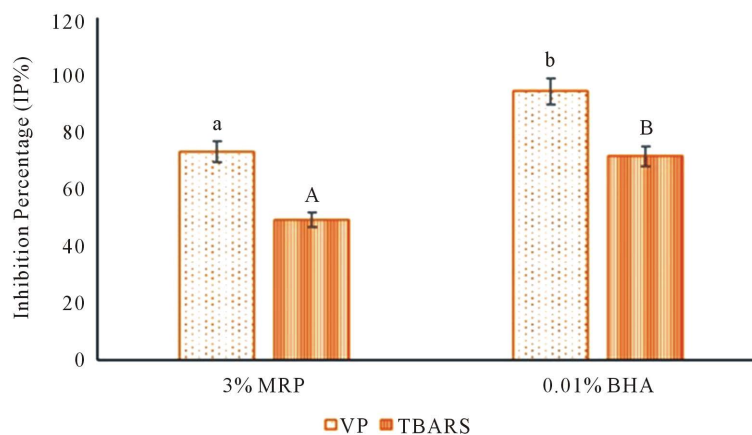


Figure 3. Inhibition percent on hydroperoxides (VP) and TBARS formation achieved in fortified meat patties added with 3% MRP and 0.01% BHA.

Fortified meat formulation is highly susceptible to lipid oxidation since it has a high PUFA content because of the SO added. This formulation was also prepared with salt (2% NaCl) which is known to enhance pro oxidant activity in myofibrillar foods [41]. Moreover, some of the additives with antioxidant activity (for example, nitrites or ascorbic acid) commonly used in cooked meat processing were not included in the formulations so that the real MRP antioxidant effect could be measured. On the other hand, lipid oxidation in muscle foods is inter-related with myoglobin oxidation, and both reactions appear capable of influencing each other [42]. Myoglobin and other heme compounds in red meats act as catalysts resulting in the formation of metmyoglobin and H_2O_2 necessary to induce lipid peroxidation. Previous studies have demonstrated that the MRP under study had 71% of chelating activity [23] in model systems, which was attributed to the presence of hydroxyl and pyrrol groups [43] [44]. It has also been demonstrated that the MRP exhibited a high reducing power, related to the presence of reductones which have the ability to donate hydrogen atoms [45]. These two attributes of the MRP could explain its antioxidant action in the fortified meat assessed, since it acts as metal chelator and at the same time has a great ability to generate a reducing environment.

Two aspects of the antioxidant product must be highlighted: first, in this study the antioxidant effect was evaluated on cooked products subjected to chilled storage, while most of the reports about the effect of different MRPs refers to raw meat [20] [46] or raw meat products [47]-[49], both of them under freezing conditions, or model systems [50] [51]. Secondly, this product can be directly added to the formulation, with no requirement of a previous treatment with the natural antioxidants, such as immersion in a solution of them.

3.3. L^* , a^* and b^* Determination

Considering meat products, colour is the first attribute evaluated by consumers as an indicator of quality and freshness [52] [53]. In this regard, L^* , a^* and b^* determinations were carried for fortified meat patties with and without antioxidants to evaluate changes in the colour of the final product.

The partial replacement of animal fat with soybean oil led to raw patties with higher L^* values (51.90 to 42.94), because small oil globules reflect more light than larger beef fat globules, as indicated by Youseff and Barbut [54].

The addition of the MRP seems to represent an extra protective effect against protein oxidation, since fortified patties with 3% MRP showed the lowest decrease for a^* values compared to the control (28.24 to 28.34). This is concordant with Gañhao and others [55] who correlated the oxidation of beef proteins with the instrumental colour parameters, and different studies which noticed a protection action of MRPs against protein damage [42]. On the other hand, when $\Delta E > 3$ colour differences are obvious for the human eye [56]. In this sense, the colour differences were appreciative by the human eye only at 10°C and after 5 days of storage.

Given the high amounts of MRP added, no colour changes in the final product represented an important advantage in comparison with other frequent food antioxidants, which generally darken meat products. Different studies report this fact, *i.e.* Gibis & Weiss [57] observed a significant decreased of L^* value when grape seed and rosemary extract were added to beef patties, while Kim *et al.* [58] observed the same effect during the sto-

rage of ground beef treated with rosemary extract.

However, this study does not consider the effect of other ingredients commonly used in the industry, suggesting the need to verify what happens in the presence of such substances. On the other hand, the results are limited to meat slaughter provided by the single available slaughterhouse in the area, which should be considered when repeating the study.

4. Conclusions

The manufacture of beef patties with soybean oil as a partial pork back fat replacer leads to products with enhanced nutritional properties as a result of a more favorable fatty acid profile. The addition of MRP clearly provides significant protection against lipid oxidation, being an attractive alternative to synthetic antioxidants for extending the shelf life of processed meat.

These results highlight remarkable technological applications of the MRP as a food ingredient in the design of healthier meat commodities, with the added value of being obtained from bovine blood, which renders a traditional waste of the meat industry. On the other hand, finding new applications for blood components represents an important challenge for scientists. Thus the development of procedures and applications that allow the utilization of animal blood on a larger scale will contribute both to eliminate a sizable pollution hazard and prevent the loss of potentially valuable material. This is particularly true in developing countries, where traditional sources of proteins and irons are either expensive or unavailable.

The accomplishment of further sensory, texture and microbiological analyses should confirm the extent of the impact on particular sensory features and on consumer's acceptance.

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