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Bovine Blood Constituents as Fat Replacers in Ham Pâté

Fabiana Ribeiro Viana[1], Carolina Schaper Bizzotto[1], Disney Ribeiro Dias[2],
Afonso L. Oliveira[3] and Marialice Pinto Coelho Silvestre[1]*

[1]Departamento de Alimentos, Faculdade de Farmácia, UFMG, Av. Olegário Maciel,
2360 – CEP 30180-112, Belo Horizonte, MG, Brazil

[2]Centro Universitário de Lavras – UNILAVRAS, Lavras, MG, Brasil

[3]Departamento de Tecnologia e Inspeção de Produtos de Origem Animal, Escola de Veterinária,
UFMG, Belo Horizonte, MG, Brazil

[3]Departamento de Alimentos, Faculdade de Farmácia, UFMG, Belo Horizonte, MG, Brazil

Summary

Some tests were carried out in this work with the aim of evaluating a partial replacement of fat in the raw batter of ham pâté by using bovine blood constituents, such as globin (GL), plasma (PL) or 1:1 globin and plasma (GP). Plasma was separated from red cells by blood centrifugation, and globin was extracted by the carboxymethylcellulose method. The salt-soluble protein content (SSP) and the binding properties including water holding capacity (WHC) and raw batter stability (RBS) were estimated. The results indicated that among the 3 treatments studied, the use of globin showed to be a little more advantageous for the quality of the raw batter of ham pâté, since its incorporation as fat replacer led to an increase in RBS but no change of SSP was observed.

Key words: bovine blood, fat replacer, ham pâté, salt-soluble proteins, binding properties

Introduction

Fat has an important role in the diet as a source of energy, essential fatty acids and liposoluble vitamins (*1,2*). On the other hand, epidemiological, biological and clinical studies have shown the correlation between dietary fat and risk of cardiological diseases as well as incidence of some types of cancer like colon, breast and prostate. This resulted in some changes of consumer habits, increasing their interest for low-fat food in order to reduce fat ingestion (*1,3–7*). In Brazil, as well as in other South American countries, the consumption of low-fat products is still incipient, but some researches have reported the growing interest in this kind of food, stating that 78 % of inquired consumers have shown to be concerned (*1*).

Generally, emulsified meat products have high fat content. In case of pâtés, this value may reach 32 % (*8*),

which is excessively high, especially considering the saturated fatty acids found in the fat portion. Fortunately, the fat content of pâtés may be drastically reduced by using new technology which employs fat replacers, giving rise to healthier products (*9*). However, according to Colmenero (*10*), the production of meat emulsions with low-fat content depends on some factors such as the amount of fat that may be removed from the system, the nature of the product and the type of processing used. Among the features of meat emulsions that may be changed by fat replacement are salt-soluble proteins and binding properties, responsible for the stability and quality of the raw batter (*10–12*).

The use of proteins as fat replacers in meat products has been suggested by several authors, since it may have some advantages from the nutritional point of

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* Corresponding author; Phone: ++55 31 33 397 633, Fax: ++55 31 33 397 632; E-mail: malice@farmacia.ufmg.hr

view, as well as caloric, when the interest is related to the calorie reduction of diet (5,9,11,13–17).

Several reports in the literature pointed out the important role of blood as potential source of proteins, which has been used in some countries for human nutrition (16,18–25). In Brazil, bovine blood has been used in animal feed, but considering the low cost of this protein source, some works, which started in 1979 by Penteado *et al.* (26) and continued later by our group (27,28), have been done aiming to study functional properties of blood constituents in order to increase their use in food products for human nutrition.

After the successful incorporation of bovine globin in ham pâté as emulsifier (29), the interest of our group turned to the use of this protein and plasma as fat replacer in the same meat emulsion. Thus, the goal of this work was to study the effects of the partial replacement of fat by globin, plasma and a mixture of both, on the salt-soluble proteins and binding properties of the raw batter of ham pâté.

Material and Methods

Obtaining blood constituents

Blood was collected at a slaughterhouse under federal inspection, in flasks containing the anticoagulant (2 mL of a 10 % EDTA solution/100 mL of total blood). The contact between the collecting recipient and the animal skin was avoided. The blood was immediately taken to the laboratory, where it was centrifuged (Jouan centrifuge, Br4i model) at 1000 x *g* for 15 min, in order to separate the red cells (erythrocytes) from the plasma. The red cells were stored under refrigeration (5 °C) until the bovine globin was extracted (maximum of 24 h), and the plasma was kept at –18 °C until use.

Determination of haemoglobin content

The concentration of haemoglobin in the total blood was determined by the cyanomethyl-haemoglobin method (30), using the Drabkin reagent as a diluting solution. A standard commercial haemoglobin was used in a 10 % concentration (HiCN, Labtest, Belo Horizonte, MG, Brazil). The calibration factor (CF) was calculated according to the Equation /1/:

$$CF = \frac{[SH]}{ASH} \qquad /1/$$

in which [SH] is the concentration of the standard haemoglobin and ASH its absorbance at $A_{540\ nm}$.

The concentration of haemoglobin in the sample was expressed in g of haemoglobin per 100 mL of the total blood and calculated by the Equation /2/:

$$[\text{Haemoglobin}] = CF \times A_{\text{sample}} \qquad /2/$$

in which A_{sample} is the value of the sample absorbance at 540 nm.

Determination of the haematocrit value

The measurement of the volume of red cells in the total blood was performed after centrifuging at 1000 x *g* for 30 min (microhaematocrit centrifuge, FANEN, 207 model, Sao Paulo, SP, Brazil) using graduated centrifugal tubes, and the haematocrit value was expressed in percentage of the total volume.

Extraction of bovine globin

The carboxymethylcellulose (CMC) method, described by Auvinen (22), was used to extract bovine globin. Initially, distilled water was added to the erythrocytes, obtained as described above, in order to have a solution containing 13.4 g of haemoglobin/100 mL. After adjusting the pH to 1.5 with a 1.0 M HCl solution, it was heated to 75 °C for 20 min, cooled down to room temperature (25 °C) and then 1.96 g of CMC/100 mL of water was added. After adjusting the pH to 3.06 with a 1.0 M NaOH solution, the mixture was centrifuged and the pH set to 7.0–8.5, with the same alkaline solution. Globin, precipitated and recovered by filtration through filter paper, was weighed, transferred to an ice container and stored at –18 °C until use.

Preparation of the raw batters of ham pâtés

The ingredients for preparing the raw batter of ham pâtés are shown in Table 1. The pork shoulder and belly were purchased in a supermarket (Belo Horizonte City, MG, Brazil), and then ground in a machine using a 1-mm disk. After that, they were stored under refrigeration (5 °C) until use. The seasonings were purchased at the Central Market (Belo Horizonte City, MG, Brazil), and stored in hermetically closed flasks at 25 °C until use.

The scheme for preparing the raw batters of ham pâtés is shown in Fig. 1. A cutter (Sire, model Super cutter, Sao Paulo, SP, Brazil) with maximum capacity of 3 kg was used for preparing 4 types of pâtés in 3 portions of 1.5 kg (3 replications in the same day), which were packed separately in glass flasks. Control sample of pâté (COP) contained 26.2 % of total fat; and the other types in which 38.2 % of belly was replaced by 3 fat replacers: 10 % of globin (GL), 10 % of plasma (PL) and 5 % of globin plus 5 % of plasma. These replacements gave rise to 3 formulations called GLP, PLP and GPP, respectively. The samples were stored under refrigeration (5 °C) until the analyses.

Salt-soluble protein content

The method described by Knipe *et al.* (31) was used to determine the salt-soluble protein content (SSP). Initially, the extraction of SSP was made by using 5 g of raw batter, which was mixed with 50 mL of 0.6 mol/L NaCl solution in a shaker (MS2 Minishaker, IKA, Wilmington, USA) for 15–20 s at 1000 rpm and 25 °C. This mixture was centrifuged (Jouan, BR4i model) at 5000 x *g*, for 15 min at 5 °C. Then, a 200-µL aliquot of the supernatant was used to quantify SSP by the Lowry method (32) modified by Hartree (33). The bovine serum albumin (BSA, Sigma Chemical Co.) was used as standard and the SSP content was expressed in mg of soluble protein per g of sample.

Water holding capacity

The filter press paper method adapted by Zayas and Lin (34) was used to determine the water holding

Table 1. Formulation of ham pâté

Ingredients	Control pâté	Ham pâtés with fat replacers		
	COP[1]	GLP[2]	PLP[2]	GPP[2]
	Mass ratio/%			
Pork meat	37.3	37.3	37.3	37.3
Globin (emulsifier)	3.0	3.0	3.0	3.0
Fat replacers:				
Globin	–	10.0	–	5.0
Plasma	–	–	10.0	5.0
Belly	26.2	16.2	16.2	16.2
Water	30.93	30.93	30.93	30.93
Nitrite	0.5	0.5	0.5	0.5
Erythorbate	0.16	0.16	0.16	0.16
Salt	1.0	1.0	1.0	1.0
Phosphate	0.16	0.16	0.16	0.16
Starch	0.5	0.5	0.5	0.5
Mustard	0.1	0.1	0.1	0.1
Sugar	0.08	0.08	0.08	0.08
Ginger	0.05	0.05	0.05	0.05
Pepper	0.02	0.02	0.02	0.02

[1]COP – Control pâté, containing the belly content (26.2 %)
[2]Ham pâtés with 38.2 % of belly replacement: GLP – 10 % of globin; PLP – 10 % of plasma; GPP – 5 % of globin and 5 % of plasma
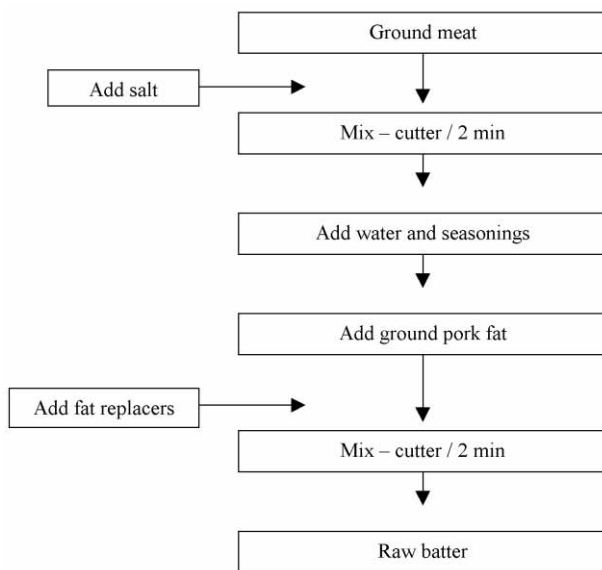

Fig. 1. Main steps for preparing the raw batter of ham pâté

capacity (WHC). A sample of 0.3 g was weighted over a filter paper (Whatman #1), previously stored for 24 h in a desiccator containing a saturated KCl solution. Then, the paper was pressed between two acrylic plates (12 mm thickness) for 20 min. The areas (cm^2) of the pressed meat film (MFA) and those of the spread of juice (SJA) were estimated by the formula used for the circle area (πr^2), and the WHC was calculated by the Equation /3/, according to the calculation described by George *et al.* (*35*):

$$WHC = MFA/SJA$$

Raw batter stability

The raw batter stability (RBS) was determined according to the method described by Ambrosiadis *et al.*

(*36*). After weighting 50 g of raw batter in a 50-mL centrifuge tube, the tubes were submerged in a water bath at 75 °C for 30 min and centrifuged at 400 x *g* for 10 min. Then, the released juice was discarded and the samples were removed, dried with paper towel and re-weighed to determine the liquid loss. The RBS was expressed as the inverse of the mass loss, in percentage of the initial mass.

Statistical analysis

The preparation of pâté was repeated 3 times (3 experiments) for each type of pâté (2 types: standard and lower fat products and 4 formulations) and all the results represent the mean values of triplicates. The analysis of variance, using one factor, was performed to investigate the significant effects ($p<0.05$) of fat replacement over salt-soluble proteins, water holding capacity and raw batter stability. The Duncan test was applied to establish the differences among the average values (*37*). The coefficient of linear correlation was calculated among the averages of the 3 parameters studied (salt-soluble proteins, water holding capacity and raw batter stability).

Results and Discussion

Salt-soluble protein content

Compared to control, in Fig. 2 it can be seen that the incorporation of globin (GLP formulation), as a fat replacer, had no influence on SSP, while that of plasma and globin plus plasma, PLP and GPP formulations, respectively, resulted in an increase of SSP, which was similar in both procedures. Probably incomplete emulsification of proteins took place in the raw batter when the last two formulations were prepared, and consequently a loss of proteins during the processing occurred.
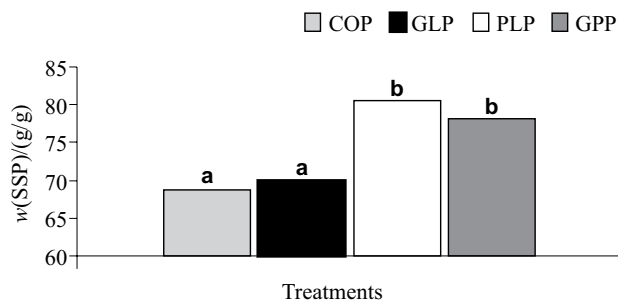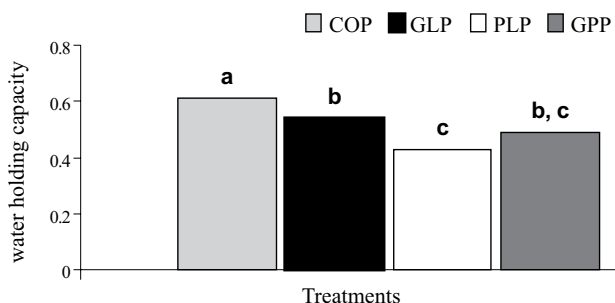
Fig. 2. Effect of fat replacement on the salt-soluble protein mass fraction (SSP), in raw batter of ham pâté. COP – control pâté. Samples with 38.2 % of belly replacement: GLP – 10 % of globin; PLP – 10 % of plasma; GPP – 5 % of globin and 5 % of plasma. Each value represents the mean value of triple determinations. a,b – statistically significant differences (p≤0.05)

No data concerning the influence of fat reduction on SSP of any meat products were found in the literature. However, in an earlier study in our laboratory (*29*), the incorporation of 3 % bovine globin in ham pâté as emulsifying agent produced similar results to those in the present work containing 10 % globin (GLP). This indicates that the addition of this protein was unable to affect the SSP of ham pâté. Also, this fact in the present study shows that one can reduce the fat content of ham pâté, which demonstrates the advantageous utilisation of this protein as fat replacer in emulsified meat products.

Binding properties

First of all, it is worth stating that the formula used for calculating WHC agrees with the calculation described by George *et al.* (*35*), according to which the ratio of meat and fluid area on the filter paper squares was calculated and expressed as the WHC. Also, this formula agrees with the statement of Honikel and Hamm (*38*), who showed that when using the filter paper press method (FPPM), the higher the fluid area the higher is the WHC.

The effect of fat replacement on the binding properties of the raw batter of ham pâté can be observed in Figs. 3 and 4. Considering the use of globin as fat replacer, it may be pointed out that this protein reduced the WHC but, on the other hand, it was able to increase

Fig. 3. Effect of fat replacement on the water holding capacity (WHC) in raw batter of ham pâté. COP – control pâté. Samples with 38.2 % of belly replacement: GLP – 10 % of globin; PLP – 10 % of plasma; GPP – 5 % of globin and 5 % of plasma. Each value represents the mean value of triple determinations. a,b,c – statistically significant differences (p≤0.05)
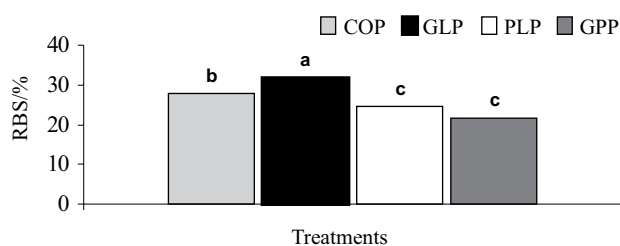
Fig. 4. Effect of fat replacement on the raw batter stability (RBS) of ham pâté: COP – control pâté. Samples with 38.2 % of belly replacement: GLP – 10 % of globin; PLP – 10 % of plasma; GPP – 5 % of globin and 5 % of plasma. Each value represents the mean value of triple determinations. a,b,c – statistically significant differences (p≤0.05)

the RBS compared to the control. The result for RBS could be explained by the fact that globin, an isolated protein with good emulsifying properties (*27*), which was added to the raw batter of pâté in a paste form (low water content), can have an effective participation in the protein network during the emulsifying process, contributing to emulsion stability. This positive effect of globin on the stability of raw batter could also be associated with the SSP content, since the addition of this protein produced no change in this parameter, but increased the RBS value, indicating that most of the added protein took part in the formation of the emulsion and was not lost in a salt-soluble form.

In our previous study where 3 % globin was incorporated into ham pâté as an emulsifier, a similar result was observed for the RBS as the one related here. On the other hand, no change was observed for WHC (*29*). This indicates that the added amount of globin may have a negative influence on WHC of ham pâté, contrary to SSP and RBS.

The use of plasma as a fat replacer (PLP formulation) led to a reduction in both WHC and RBS. This could be related to the fact that plasma is a mixture of proteins and was added to the pâté in a liquid form (high water content). Contrary to globin, plasma had none or little participation in the protein network of the emulsion. This result is in agreement with that pointed out by Carballo *et al.* (*13*), when egg white was incorporated to bologna sausages as a fat replacer.

The incorporation of globin plus plasma (GPP formulation) produced the same result described for the addition of plasma (PLP formulation), which reduced both WHC and RBS. It is worth stating that the results of SSP and WHC for PLP and GPP formulation are in agreement with the statement of Hamm (*39*), in which an increase of SSP represents a higher loss of proteins, which do not participate in the emulsion formation and reduce the capacity of holding water of the product.

Some authors have theoretically discussed the negative effect of fat replacements on binding properties of meat products. According to their statements, whenever the reduction of fat is followed by an increase in water content, as it happens with the incorporation of plasma as a fat replacer, the products exhibit poor binding properties (*10,11,14*). An explanation for this, given by these authors, may be the decrease of the density of the continuous phase in the emulsion due to the reduction

of fat content, a phenomenon that is more prominent when the water content of the product is higher.

The practical data found in the literature regarding the effect of fat replacements on binding properties of meat products are inconsistent. None concerns ham pâté and all of them were performed on sausages using other kind of proteins as fat replacers (*11,14,15,17,40*).

Conclusion

From the presented results the following general conclusions can be made. The use of bovine globin as a fat replacer was barely more advantageous to the quality of ham pâté compared to plasma, isolated or in association with globin, since the former had no influence on SSP but increased the RBS, while the latter increased SSP and RBS.

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Sastojci goveđe krvi kao zamjena za masnoće u pašteti od šunke

Sažetak

U radu su provedeni pokusi kako bi se provjerila djelomična zamjena masnoća u sirovoj smjesi za paštetu od šunke koristeći sastojke goveđe krvi, i to globina (GL), plazme (PL) ili smjese globina i plazme u omjeru 1:1. Plazma je odvojena od eritrocita centrifugiranjem krvi, a globin je izdvojen karboksimetilcelulozom. Određen je udjel u soli topljivih proteina (SSP) i svojstva vezanja, uključujući kapacitet zadržavanja vode (WHC) te stabilnost sirove smjese. Rezultati pokazuju da se korištenjem globina dobiva nešto bolja kakvoća sirove smjese jer se njegovom ugradnjom, kao zamjena za masnoće, postiže veća stabilnost sirove smjese, a ne mijenja udjel proteina topljivih u soli.