



Antioxidant and emulsifying properties of alcalase-hydrolyzed potato proteins in meat emulsions with different fat concentrations

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ABSTRACT

The effect of hydrolyzed potato protein (HPP), a natural antioxidant, on emulsion quality was investigated using a factorial design with two *Fat* (15%, 30%) and two *HPP* (0%, 2.5%) levels, with three replications. The colour of the raw emulsions as well as cooking losses, textural properties and TBARS of cooked frankfurters were measured. Increasing the *Fat* proportion significantly ($P < 0.05$) increased L^* , H_{ab}^0 and decreased a^* , b^* , C_{ab}^* and hardness. Meat emulsions with added *HPP* were darker (lower L^*) than those made without *HPP* and also had lower values of a^* and b^* . The addition of *HPP* (2.5%) significantly ($P < 0.05$) decreased cooking losses and fracture force, and had a significant ($P < 0.05$) inhibitory effect on lipid oxidation in cooked frankfurters. These results suggest that *HPP* has both antioxidant and emulsifying properties which may be of potential use in meat emulsion manufacturing.

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1. Introduction

Frankfurters are non-fermented, emulsion type cooked sausages composed of water, muscle proteins, fat particles, salt and small amounts of non-meat ingredients, where the meat proteins serve as natural emulsifier. In this group of processed meat products, fat and protein concentration and their chemical interactions, especially those occurring during the emulsification process, exert a marked impact on final product quality. According to Barbut (1998), fat stabilization during chopping is due to the formation of a protein film around the fat particles that allows fat to be retained fat inside the protein matrix. During chopping, certain attractive forces contribute to holding the raw materials together, creating a homogeneous matrix structure (Allais, Christophe, Pierre, & Dufour, 2004). Inadequate soluble protein extraction or an inadequate fat to protein, ratio as well as an excessive reduction of fat particles size, could lead to reduced emulsification ability, which might affect fat oxidation.

During the production of frankfurters, ingredients such as lean and adipose tissue are finely minced. This disrupts the integrity

of membranes and exposes the phospholipids to molecular oxygen, oxidative enzymes, heme pigments, metal ions, etc., all of which greatly enhance the development of oxidative reactions during subsequent refrigerated storage. Oxidative reactions destroy some desirable organoleptic, nutritional and technological attributes and decrease the shelf life of processed meat products. Indeed, lipid oxidation is considered the main non-microbiological cause of food deterioration (Eburne & Prentice, 1996) and the phenomenon induces changes in both charge and three-dimensional conformation of proteins (Zamora, Alaiz, & Hidalgo, 1999), which directly affects the emulsifying and water binding properties of proteins.

Several strategies are currently used to reduce lipid oxidation and meat emulsion stability defects. Leaner raw materials or non-meat ingredients such as proteins (Riisom, Sims, & Fioriti, 1980), vegetable oils (Paneras & Bloukas, 1994) or oat fibre (Chan & Carpenter, 1997) are added to meat emulsions. However, the use of leaner meats not only increases the cost of production, but also yields products that are firmer, darker and less succulent and tasty than standard products (Crehan, Hughes, Troy, & Buckley, 2000). Often, lipid oxidation in meat products is controlled or minimized by addition of either synthetic or natural antioxidants. The latter being especially accepted by consumers.

Several studies have shown the antioxidant potential of protein hydrolysates from potato and whey (Peña-Ramos & Xiong, 2003; Wang & Xiong, 2005). Protein hydrolysates may possess

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physicochemical characteristics and bioactivities not found in the original proteins, such as antioxidant activity and greater water-holding capacity (Cumby, Zhong, Nack, & Shahidi, 2008). The generated peptides could inhibit the harmful changes induced by lipid oxidation, due to the presence of certain amino acid residues, such as tyrosine, methionine, histidine, tryptophan and proline, and the peptide sequence. Although these amino acids are capable of chelating pro-oxidative metal ions, at high concentrations they may also act as pro-oxidants (Marcuse, 1960).

In this study, *HPP* previously identified in a radical-scavenging model system (Wang & Xiong, 2005), was selected as potential natural emulsifying and antioxidant agent. Although *HPP* is known to confer nutritional and functional properties, there is little information regarding its antioxidant and emulsifying potential in comminuted meat products. Therefore, the objective of this study was to investigate the antioxidant and emulsifying properties of *HPP* in cooked pork frankfurters containing a wide range of fat concentrations.

2. Material and methods

2.1. Experimental design

The antioxidant and emulsifying effects of *HPP* on meat emulsions were investigated using a completely randomized factorial design with two factors and three replications. Two *HPP* levels (0 – control – and 2.5% w/w) were tested within a range of lipid oxidation and emulsion stability tendencies that were induced using two different levels of fat (15% and 30% w/w; i.e. fat/lean ratio of 0.18 and 0.43, respectively). A total of 12 tests ($N = nab = 3 \times 2 \times 2$) were conducted under this design. Experiments were carried out in the absence of any other emulsifier or antioxidant agent than *HPP* to avoid confounding effects between the antioxidant/emulsifying activities of regular meat emulsion additives and *HPP*. Fresh pork lean and fat from the same meat batch were used in all the experiments to minimize the experimental error. Lean and fat were chopped to an emulsion temperature of 15 °C, using constant processing conditions. The optical properties of the raw emulsions were determined by reflection photometry (CIELAB colour coordinates). A variety of final product quality indices (dependent variables) were determined to establish the degree of lipid oxidation (thiobarbituric acid-reactive substances or *TBARS*) and stability (cooking losses, gel strength) of the cooked meat emulsions.

2.2. Materials

Potato protein concentrate was obtained from AVEBE ba (Veenendam, The Netherlands). The dry protein powder contained 80% protein. Alcalase (E.C. 3.4.21.62; endoproteinase from *Bacillus licheniformis*; density of 1.18 g mL⁻¹) was obtained from Novo Nordisk Biochem Inc. (Franklinton, NC, USA). Thiobarbituric acid (TBA) was purchased from ICN Biomedicals Inc. (Aurora, OH, USA). All other chemicals were purchased from Fisher Scientific (Fair Lawn, NJ, USA) and were of at least reagent grade. Fresh, semi-boneless pork meat shoulders (Boston butts) were purchased from a local meat purveyor.

2.3. Meat samples preparation

Upon arrival, the Boston butts were processed in a cool room at ~6 °C to obtain all the meat samples required to carry out the entire three replications of the experiment using one single batch of meat. Fat and lean tissues were manually separated and the connective tissue rejected. Fat and lean were separately ground with a meat grinder (4146SS, Hobart Corp., Troy, OH, USA) using two

orifice plates (25 and 0.32 mm) in sequence. This procedure provided the fatter and leaner ground tissues needed to achieve the levels of fat required by the experimental design. The chemical composition (fat and moisture content, %) of the ground fat and lean sources were analyzed using a HFT-2000 fat analyzer (Data Support Co., Inc., Encino, CA, USA; accuracy, ±0.5%). The fat concentration was used to calculate mixture proportions of the fat and lean sources to obtain meat samples with the target fat concentration (15% and 30%). Small aliquots of the two different treatment mixtures were prepared and the fat concentration was analyzed as described above to verify that the selected fat levels were attained. After verification, the corresponding amounts of ground fat and lean were weighed to obtain meat samples of 3565 ± 0.01 g with the target fat concentration. Samples were separately vacuum-packed and frozen at –18 °C until used.

2.4. Preparation of potato protein hydrolyzates

Potato protein concentrate was suspended in aqueous solution to obtain a 15% w/v protein concentration, and the pH was adjusted to 8.0 with 1 N NaOH. Protein was hydrolyzed with alcalase for 1 h at 50 °C, using an enzyme/substrate ratio of 0.01. For further details, refer to Wang and Xiong (2005).

2.5. Meat emulsion manufacturing

The day before the experiment, ground lean and fat samples were stored at 4 °C overnight. The pre-weighed amounts of lean and fat, salt, crushed ice, water, and *HPP* solution were mixed according to the target treatment formulation as shown in Table 1. Treatment formulations were adapted from Feng, Xiong, and Mikkelr (2003). Special care was taken to prepare raw material mixtures of 5000 ± 0.01 g with the target fat/lean ratio and *HPP* level in compliance with the experimental design. Raw material mixtures were chopped using a 9 kg capacity bowl chopper (CM-14, Mainca, St. Louis, MO, USA). Knife and bowl speeds of 3000 and 10 rpm, respectively, were used. The mixture was chopped to an emulsion temperature of 15 °C (~9 min). The raw emulsion was immediately split into two homogeneous aliquots. The first aliquot was used to measure pH, and CIELAB coordinates L^* , a^* and b^* , of the raw emulsions. The second aliquot was stuffed into 27 mm diameter frankfurter cellulose casings (Teepak LLC., Danville, IL, USA) using a hand stuffer (The Sausage Maker, Buffalo, NY, USA). Frankfurters were then manually tied into ~12 cm sausages, weighed and cooked for 90 min in an Alkar smokehouse (450 U, Alkar-RapidPak Inc., Lodi, WI, USA) to an internal temperature of 71 °C. The relative humidity of the smokehouse was in the range

Table 1
Formulation of frankfurters used for the different experimental treatments.

Formula	Experimental treatments			
	0% <i>HPP</i>		2.5% <i>HPP</i>	
	15% Fat	30% Fat	15% Fat	30% Fat
Fat/lean ratio	0.177	0.429	0.177	0.429
Raw materials (g)				
Fat + Lean	3565	3565	3565	3565
<i>HPP</i> ^a	0	0	667	667
Water	860	860	193	193
Ice	500	500	500	500
Salt (NaCl) ^b	75	75	75	75
Total	5000	5000	5000	5000

Source of original formulation: Feng et al. (2003).

^a The mass of hydrolyzed potato protein (*HPP*) solution used (see materials and methods section for further details) represents to 2% of the frankfurter formulation.

^b The amount of NaCl used represents 1.5% of the salt content of the raw batter.

65–68%. After cooking, the frankfurters were immediately cooled with cold water for 2 min, packed in polystyrene trays, over-wrapped with oxygen-permeable film ($650 \text{ cm}^3 \text{ m}^{-2} \text{ h}^{-1}$ at 23°C), and stored at 4°C for 7 days to evaluate the inhibitory effect of HPP on lipid oxidation.

2.6. Emulsion temperature control and pH measurement

Temperature control during chopping is crucial for adequate product quality assurance. The bowl chopper was placed in a cool room with a controlled temperature of $\sim 6^\circ \text{C}$. The raw materials were placed in the bowl chopper at 4°C and the mixture temperature was measured during chopping every 30 s using a 15-078G digital thermometer (Traceable[®], Control Company, TX, USA). The chopping end-point was selected when the emulsion reached a maximum temperature of 15°C .

The pH of the emulsion at different chopping times was measured using a pH tester Oakton (Mod. Spear, Eutech Instruments, Malaysia).

2.7. Colour

CIELAB colour coordinates, L^* , a^* , and b^* , were measured 1 h after the emulsion was prepared using a hand held tristimulus Chroma Meter (CR-310 Minolta Camera Co. Ltd., Osaka, Japan), CIE standard “C” illuminant and 0° viewing angle geometry. The measurements were performed in a 10.5 cm diameter plate (Glass Light-projection Tube, CR-A33e). The chroma meter was calibrated prior to testing using a calibration plate (CR-A44; CIE; $Y = 93.80$, $x = 0.3138$, $y = 0.3195$). Lightness, L^* , and chromaticity coordinates a^* (redness/greenness) and b^* (yellowness/blueness) were measured six times per meat emulsion sample and the respective averages were recorded. Coordinates a^* and b^* were used, according to Hunt (1977), to calculate both chroma, C_{ab}^* , and hue, H_{ab}^0 , values as follows:

$$C_{ab}^* = \sqrt{a^{*2} + b^{*2}} \quad (1)$$

$$H_{ab}^0 = \arctan(b^*/a^*) \quad (2)$$

2.8. Cooking losses

Once the chopping process was completed, the cooking losses (C_L) of each emulsion sample were measured in triplicate. Three meat emulsion aliquots of 30 g were stuffed into plastic screw top test tubes. The aliquots were heat treated in a scalding bath (Lauda RM20, Brinkman Instruments Inc., NY, USA) at 70°C for 30 min and then quickly refrigerated in a cool water bath (Lauda Ecoline RE220, Brinkman Instruments Inc.) for 10 min at 5°C . Total C_L (%) of the samples was calculated from the weight of the final cooked emulsion (W_F) and the initial weight (W_0) of the sample before cooking, as follows:

$$C_L = 100(1 - W_F/W_0) \quad (3)$$

In addition, normalized cooking losses (C_{Lnor}) were calculated, taking into account the initial moisture content of the frankfurters.

2.9. Texture profile analysis (TPA)

The influence of HPP and fat concentration on the textural properties of frankfurters was investigated by uniaxial compression tests using an Instron Universal Testing Machine (Model 4301; Instron Corp., Canton, MA, USA) as described by Xiong, Noel, and Moody (1999). All samples were compressed 24 h after cooking using a 100 N load cell at a crosshead speed of 50 mm min^{-1} . Hardness (H) of the sample was measured as the force (N) of the first

compression peak (F_1). The force of the second compression peak was designated as F_2 . The percent reduction in the compression force between the first and second compression peaks was defined as structure “Deformability” (D) and was calculated as D (%) = $100(F_1 - F_2)/F_1$. Cohesiveness (C), as defined by Bourne (1978), was estimated as $(F_2/F_1)^2$ (dimensionless), assuming that peaks of the first and second compression form similar triangles with the baseline. Another set of nine samples was compressed to 20% of its original height ($\varepsilon = 0.8$) to determine the breaking force (F_B) (N).

2.10. Thibarbituric acid-reactive substances (TBARS)

TBARS were measured to evaluate lipid oxidation on days 0, 1, 3 and 7 of storage at 4°C , as described by Wang and Xiong (2005). The TBARS value, expressed as mg of malondialdehyde per kg of sausage sample, was calculated using the following equation:

$$TBARS = 9.48(A_{532}/W_s) \quad (4)$$

where A_{532} was absorbance at 532 nm, W_s was the sausage sample weight (g), and 9.48 was a constant derived from the dilution factor and the molar extinction coefficient ($1.52 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$) of the red TBA reaction product.

2.11. Statistical analysis

The experimental data for the dependent variables studied were processed and analyzed using the Statistical Analysis System (SAS, version 9.1, 2002–2003, SAS Institute, Cary, NC, USA). Pearson’s correlation coefficients between dependent variables were determined using the correlation (CORR) procedure of SAS. Analysis of variance (ANOVA) was performed using general linear model (GLM) of SAS for each dependent variable. Least squares means (LSM) and the significance of treatments were calculated using type IV sum of squares. Fisher’s LSD comparisons were used and LSM were considered to be statistically different when $P < 0.05$.

3. Result and discussion

3.1. Analysis of variance of dependent variables

The experimental design facilitated the study of the antioxidant and emulsifying properties of HPP on meat emulsions containing different ratios of Fat. The main sources of variation in the dependent variables were determined by ANOVA and the R^2 values and F -statistics reported for each dependent variable. The preliminary ANOVA model had as the main effects: Fat concentration “Fat” and hydrolyzed potato protein “HPP”, “Rep” (to quantify the effect of replication) and the interaction “Fat \times HPP”. The replication effect was kept in the final model since it was found to be significant for several dependent variables such as H_{ab}^0 , F_B , and C_L . The ANOVA model was found to be significant ($P < 0.05$) for all the meat emulsion quality metrics studied except for the textural parameters D and C .

3.2. Effect of HPP and Fat concentrations on fresh emulsion colour

Table 2 shows the influence of the main effects, HPP and Fat concentration, on colour attributes of the raw emulsions. As can be observed, L^* , a^* , b^* , C_{ab}^* and H_{ab}^0 were significantly ($P < 0.05$) affected by Fat concentration and HPP addition and by their interaction (a^* , b^* , C_{ab}^*).

LSM values for the CIELAB colour coordinates L^* , a^* , b^* and C_{ab}^* fell by $\sim 5\%$, 41%, 15%, and 24%, respectively, as a result of HPP addition, while the LSM for H_{ab}^0 increased from 77 to 95. In general,

Table 2

Influence of main effects, *HPP* and *Fat* concentrations, on colour of fresh frankfurters as well as on cooking losses, textural attributes and lipid oxidation of cooked frankfurters^A.

Parameters ^B	Main effects			
	<i>HPP</i> ^C (%)		<i>Fat</i> ^D (%)	
	0	2.5	15	30
L^* (CIE Units)	74.2 ^a	70.1 ^b	68.7 ^a	75.6 ^b
a^* (CIE Units)	17.1 ^a	10.8 ^b	16.4 ^a	11.4 ^b
b^* (CIE Units)	13.3 ^a	11.7 ^b	13.4 ^a	11.6 ^b
H_{ab}^0 (CIE Units)	76.8 ^a	95.3 ^b	79.9 ^a	92.1 ^b
C_{ab}^* (CIE Units)	21.7 ^a	16.0 ^b	21.2 ^a	16.3 ^b
C_L (%)	10.5 ^a	8.10 ^b	8.10 ^a	9.61 ^a
C_{Lnor} (%)	13.1 ^a	10.5 ^b	11.4 ^a	11.7 ^a
H (N)	9.09 ^a	10.7 ^a	10.5 ^a	9.27 ^b
D (%)	4.76 ^a	3.99 ^a	4.04 ^a	4.72 ^a
C (dimensionless)	0.913 ^a	0.923 ^a	0.921 ^a	0.909 ^a
F_B (N)	120 ^a	28.7 ^b	78.4 ^a	70.2 ^a
$TBARS_7$ (mg MDA kg ⁻¹ sample) ^E	9.55 ^a	3.19 ^b	6.70 ^a	6.04 ^a

^A $N = 12$; number of observations; LSM with the same letters were not significantly different ($P < 0.05$).

^B L^* , lightness; a^* , redness; b^* , yellowness; H_{ab}^0 , hue angle; C_{ab}^* , chroma; C_L , cooking losses; C_{Lnor} , normalized cooking losses; H , hardness; D , structure deformability; C , cohesiveness; F_B , breaking force; $TBARS_7$, $TBARS$ day 7.

^C LSM value for each *HPP* concentration was based on the average of six trials using two fat concentration levels.

^D LSM value for each *Fat* concentration was based on the average of six trials using two *HPP* concentration levels.

^E LSM values for $TBARS$ day 0 were not included because they were all ≤ 0.6 mg kg⁻¹. LSM values for $TBARS$ days 1 and 3 were not included in the table because they were similar to those for $TBARS_7$ (see text and Fig. 2 for further details).

meat emulsions made with added *HPP* were darker and less red and yellow than those made without *HPP* (Fig. 1; arrow indicating the effect of increasing *HPP* concentration). These changes in colour were mainly attributed to the typical brown/dark appearance of the *HPP* solutions. Several authors have demonstrated that natural pigments in meat emulsion ingredients influence the final colour of frankfurters (Estevez, Ventanas, & Cava, 2005a; Hughes et al., 1998). However, the observed changes in colour might also have been due, at least partially to chemical interaction of *HPP* with myoglobin and fat molecules in the fat–protein interfacial layers. In addition, there was an interaction effect of *HPP* and *Fat* on colour

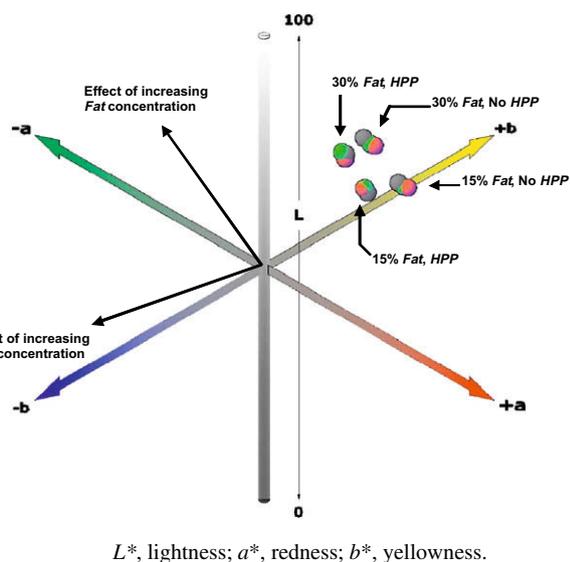


Fig. 1. Effect of hydrolyzed potato protein (*HPP*) and *Fat* concentrations on CIELAB space attributes of fresh meat emulsions.

attributes a^* ($P < 0.05$), b^* ($P < 0.05$), C_{ab}^* ($P < 0.01$). The effect of *HPP* addition on a^* , b^* , C_{ab}^* was mitigated by increased fat concentrations.

Furthermore, considerable changes in light reflection ($P < 0.05$) were observed as a result of the different fat levels in fresh frankfurters (Fig. 1; arrow indicates the effect of increasing *Fat* concentration). Meat emulsions made with 30% fat had lower a^* , b^* and C_{ab}^* values and higher L^* and H_{ab}^0 values than low-fat emulsions (15% fat). Changes in L^* with the fat content were as expected since the increase in the proportion of the whitish fat contributes to the increase in L^* . Logically, the redness values, a^* , were inversely proportional to fat content. Reduced protein content (i.e. increased fat to protein ratio) resulted in dilution of myoglobin and consequently a less intense red colour.

According to Bañón, Díaz, Nieto, Castillo, and Álvarez (2008), high fat concentration emulsions (0.66 fat/lean ratio) show greater light reflection (larger L^*), and less yellowness/redness than low fat concentration emulsions (0.25 fat/lean ratio). Several other authors (Allais et al., 2004; Jo, Lee, & Ahn, 1999) have also found that fat reduction results in darker and redder meat products.

3.3. Effect of *HPP* and *Fat* concentrations on cooking losses

Table 2 shows the influence of the independent variables, *HPP* and *Fat* concentrations, on the C_L of frankfurters. It was found that the addition of *HPP* significantly ($P < 0.05$) decreased C_L by $\sim 20\%$. This result underlines the improved capability of the *HPP*-added emulsions to bind and retain water during cooking, and suggests that *HPP* addition improved the stability of the meat emulsion. The hydrolysis of potato proteins results in the additional release of small peptides and amino acids. Thus, the addition of *HPP* increases the proportion of both polar and charged groups within the meat emulsion matrix, which enhances water–protein interactions, increasing the ability of the gel to retain water molecules. These results agree with those of Wang and Xiong (2005), who showed that the addition of *HPP* reduced the cooking losses of patties. An improvement in the water-holding capacity of meat emulsions as a result of the addition of protein of different origins (e.g., soy, canola, whey) has also been observed by several authors (Cumby et al., 2008; Feng et al., 2003; Peña-Ramos & Xiong, 2003).

Regarding the effect of fat concentration on emulsion stability, Pietrasik (1999) and Serdaroglu (2006) observed a significant increase in cooking losses with reduced fat content in meat emulsions. The observed decrease of emulsion stability with decreasing fat content is consistent with increasing weight losses when the reduction of fat is accompanied by an increase in the proportion of moisture. Note that under these conditions, the lower the fat content in the formulation, the higher and the amount of fluids available. In contrast, other authors such as Foegeding, Laniar, and Hultin (2000) and Allais et al. (2004) have reported greater instability in the emulsion, and hence lower yields, when larger amounts of fat were used.

Similarly to Foegeding et al. (2000) and Allais et al. (2004), a slight increase ($P > 0.05$) of LSM values for C_L from 8.1% to 9.6% with increasing fat concentration were observed. Nevertheless, a proper comparison between C_L values of emulsion samples with different levels of fat would require C_L to be normalized in relative terms to the moisture content of the initial samples, since fatter meat usually has a lower moisture content than lean meat. LSM values for normalized cooking losses, C_{Lnor} , were found to be not significantly different ($\sim 11\%$; $P < 0.05$). These results show that, under our experimental conditions, emulsions with 15% fat did not attain a degree of emulsification/stabilization significantly greater than observed for emulsions with 30% fat. These findings suggested that the amount of soluble protein extracted from the lean meat during the emulsification process was sufficient for the adequate

stabilization of fat particles in the range of fat concentrations studied. Increasing cooking losses may be observed with increasing fat levels involving larger proportions of fat than used in this study, especially if the emulsion is intensively chopped and presents a higher fat to protein ratio. A large fat to protein ratio would generate a much less stable gel matrix, with reduced binding properties, which would probably lead to the greater release of water and fat (Carballo, Mota, Baretto, & Colmenero, 1995).

3.4. Effect of HPP and Fat concentration on texture of frankfurters

The influence of HPP and Fat concentrations, on the textural attributes of frankfurters (hardness, structure deformability, cohesiveness, and breaking strength) is shown in Table 2. The addition of HPP had no significant effect on gel H , D or C . These findings are similar to those of Crehan et al. (2000), who observed that the addition of maltodextrin to frankfurters containing 5% and 12% fat resulted in similar hardness as observed in control products with no added ingredients. However, a dramatic (76%) and significant ($P < 0.0001$) decrease of F_B in samples with 2.5% HPP was observed compared with those without HPP. The addition of HPP apparently led to a weaker and more deformable gel structure; probably the binding between meat particles was decreased due to interference with network of myosin. This agrees with the results found by McCord, Smyth, and ÓNeill (1998) and Feng et al. (2003) who showed that replacing 10% or more of muscle protein by hydrolyzed soy protein significantly reduced the strength of the gel. The present results suggest that adding HPP to frankfurters leads to a greater tendency to fracture.

The textural attributes of the sausages were also found to be affected by fat concentration. Emulsions with the lower fat proportion (15%) were slightly but significantly harder ($P < 0.05$) than those with 30% fat. In agreement with these results, Allais et al. (2004) observed that frankfurters with ~15% fat were harder than frankfurters with ~29% fat. In contrast, Cofrades, Carballo, and Colmenero (1997) reported that high-fat frankfurters are harder than low-fat frankfurters. However, no significant differences in D , C or F_B were observed by these authors between samples with 15% and 30% fat. The discrepancies found in the literature regarding the effect of fat concentration on gel strength (i.e. H) and other textural parameters in sausages can be mainly attributed to differences in the emulsion formulation, especially as regards emulsion fat to protein ratio and moisture content. These differences would strongly modify the rheological and microstructural properties of the matrix formed.

In addition, a significant interaction ($P < 0.01$) was noticed between the percentage of fat and the presence of HPP for parameter F_B , suggesting that the effect of HPP on F_B depends on the fat level. Thus, the decrease in F_B , induced by adding HPP is more noticeable at low fat concentrations, which might be related to the fact that sausages with 15% fat concentration were harder than those with 30% fat.

3.5. Effect of HPP and Fat concentrations on lipid oxidation in frankfurters

The influence of HPP and Fat concentrations on lipid oxidation of frankfurters at day 7 is shown in Table 2. In addition, Fig. 2 shows the antioxidant effect of HPP as a function of the storage time at different Fat levels. According to Estevez, Ventanas, and Cava (2007), TBARS values increased during storage. However, the oxidation trend was clearly diminished by the addition of HPP. LSM values (Table 2) for TBARS₇ were found to be significantly ($P < 0.05$) different for HPP samples compared with emulsions without HPP. LSM values for TBARS₇ fell about threefold, from 9.6 to 3.2 mg MDA per kg, as a result of HPP incorporation.

Irrespective of the fat concentration, significant generation of TBARS was observed in samples without added HPP as early as the first day of storage. The production of TBARS in samples with 2.5% HPP after 7 days was 25% and 45% lower for meat emulsions containing 15% and 30% fat, respectively, compared with emulsions without HPP. This demonstrates, for the first time, the significant ($P < 0.004$) protective role that HPP plays against lipid oxidation in finely comminuted meat emulsions. The results agree with previous findings in beef patties (Wang and Xiong (2005)). In that study, it was shown that the addition of 4% HPP (w/w) reduced TBARS production in cooked beef patties by 50%. Several other protein hydrolysates were also found to inhibit lipid oxidation in meat and/or meat products. Shahidi, Han, and Synowiecky (1995) reported that capelin protein hydrolysate, added at a concentration of 0.5–3.0%, inhibited the formation of TBARS by ~17–60% in a cooked pork model system.

According to Amarowicz (2008), the antioxidant properties of protein hydrolysates typically result from their capability to stabilize or terminate radicals, donate a hydrogen atom, and/or chelate pro-oxidative metal ions. Hydrolysis of the polypeptide into various portions increases the accessibility of amino acids to the solvent, which results in increased radical-scavenging and metals chelating activity. The capability of different protein hydrolysates to chelate metal ions [Fe(II), Cu(II)] depends on the enzymes used in hydrolysis and the degree of hydrolysis of the hydrolysate (Raghavan & Kristinsson, 2008), as each cleavage releases an available carboxylic group. The radical-scavenging activity is due to the hydrogen donor activity of the hydroxyl groups of aromatic amino acids. It has been reported that tryptophan and cysteine residues from potato protein (1.31% and 1.50% in HPP, respectively) may be released in the process of hydrolysis, acting as strong antioxidants (Saito et al., 2003). Several other amino acids, particularly methionine, histidine and lysine, which are also fairly abundant in HPP (2.24, 2.06 and 7.29 respectively), have also been shown to inhibit lipid oxidation in model systems (Marcuse, 1960).

Further, the radical-scavenging activity of potato protein has been claimed to increase dramatically after hydrolysis (Wang & Xiong, 2005). In emulsion models, peptides can act as a physical barrier around fat droplets; short peptides diffuse to the water-oil interface, where they can be adsorbed or loosely bind to the phospholipids' membrane in the liposome – where oxidation takes places – (Kong & Xiong, 2006) to prevent oxidation.

According to Gray and Pearson (1987), rancidity flavour is initially detected in meat products with TBARS values between 0.5 and 2.0 mg MDA kg⁻¹. Hence, assuming this value as a rancidity detection threshold, we can state that in frankfurters containing no HPP, the MDA concentration reaches rancidity detection on about the first storage day (see Fig. 2), while in frankfurters containing 2.5% HPP such a level is not reached until the 4th or 5th

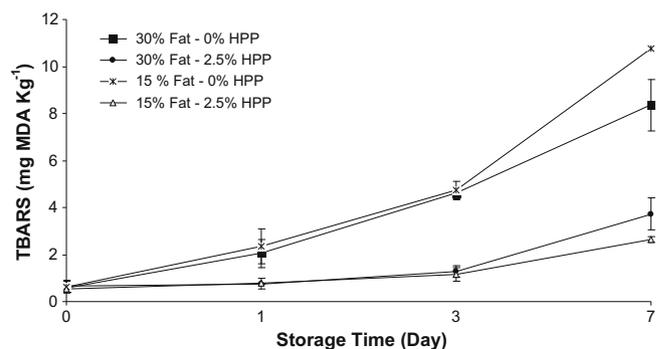


Fig. 2. Evolution of TBARS values during 7 days of refrigerated storage (4 °C) at different Fat concentration levels.

Table 3Pearson's correlation coefficients between colour attributes and meat emulsion quality metrics^A.

	C_L	H	D	C	F_B	$TBARS_0$	$TBARS_7$
L^*	-0.881***	0.743**	0.263 ^{ns}	-0.250 ^{ns}	0.380 ^{ns}	0.194 ^{ns}	0.326 ^{ns}
a^*	0.552 ^{ns}	-0.171 ^{ns}	0.183 ^{ns}	-0.192 ^{ns}	0.813**	-0.143 ^{ns}	0.765**
b^*	0.691*	-0.047 ^{ns}	0.145 ^{ns}	-0.155 ^{ns}	0.695*	-0.159 ^{ns}	0.692*
H_{ab}^0	-0.457 ^{ns}	0.221 ^{ns}	-0.126 ^{ns}	0.135 ^{ns}	-0.824***	0.170 ^{ns}	-0.750**
C_{ab}^*	0.584*	-0.144 ^{ns}	0.183 ^{ns}	-0.192 ^{ns}	0.791**	-0.142 ^{ns}	0.753**

ns: Not significant.

 L^* , lightness; a^* , redness; b^* , yellowness; H_{ab}^0 , hue angle; C_{ab}^* , chroma; C_L , cooking losses, H , hardness; D , structure deformability; C , cohesiveness; F_B , breaking force; $TBARS_0$, $TBARS$ day 0; $TBARS_7$, $TBARS$ day 7. Correlations for $TBARS$ on days 1 and 3 were not included in the table because they were similar to $TBARS_7$.^A $N = 12$.* $P < 0.05$.** $P < 0.01$.*** $P < 0.001$.

day of storage (depending on the fat concentration of the emulsion). Thus, the incorporation of *HPP* in frankfurter formulations clearly decreased their oxidation tendency, improving quality of the final product.

With regard to fat concentration, no significant ($P > 0.05$) differences were observed in LSM values for $TBARS$ after 7 days of storage, which suggests that a higher fat content does not increase the lipid oxidation tendency, at least at the fat levels studied. In contrast, Jo et al. (1999) and Estevez, Ventanas, and Cava (2005b) found a significant and positive correlation between fat content and lipid oxidation. Of the two major lipid fractions of meat (phospholipids and triglycerides), the first contains the highest proportion of unsaturated fatty acids (usually the primary lipid fraction responsible for initiating lipid oxidation). Fat reduction mainly tends to affect the content of triglycerides, while the phospholipid fraction is typically less affected (Monahan et al., 1993). Thus, a similar degree of lipid oxidation is to be expected for emulsions containing 15% and 30% fat (Table 2 and Fig. 2).

3.6. Correlations between quality parameters of frankfurters

Correlations between quality parameters were measured before (colour) and after (C_L , texture and lipid oxidation) cooking. Table 3 shows the Pearson's correlation coefficients and P -values for the CIELAB colour coordinates and the main dependent variables characterizing meat emulsion quality. The colour coordinates were strongly correlated with C_L , F_B , and $TBARS_7$. As can be observed, lightness (L^*) was significantly ($P < 0.001$) and negatively ($R = -0.88$) correlated with C_L . This correlation confirmed previous results by Álvarez, Castillo, Bañón, Garrido, Payne, Nieto (2007), and strongly suggests that the measurement of lightness just before heat treatment of the emulsion might be a predictor of the emulsion's stability during cooking. C_L was also highly correlated ($R = 0.69$; $P < 0.05$) with the colour coordinate b^* , suggesting the separation of liquids was associated with increasing yellowish colour in frankfurters.

On the other hand, the increase in lightness (L^*) was found to be significantly ($P < 0.01$) and negatively correlated with H ($R = -0.74$). These results are contrary to those obtained by Álvarez et al. (2007) and Bañón et al. (2008) who reported that lower L^* values resulted in softer meat emulsion gels. A positive and significant ($P < 0.05$) correlation was found between F_B and both a^* ($R = 0.81$) and b^* ($R = 0.70$), respectively, suggesting that the redder and yellower the frankfurters the stronger the gel. This is to be expected if we consider that the frankfurters were not only softer but also less red and yellow with added *HPP* (Table 2 and Fig. 1). For the same reason, redness and yellowness were also significantly and positively ($P < 0.05$) correlated with $TBARS$ values at day seven ($R = 0.77$ and $R = 0.70$, respectively). In this case, the inhibition of lipid oxidation by *HPP* addition resulted not only in a less red/yel-

low colour but also in the reduction of $TBARS$ values (Table 2 and Fig. 1). The same type of relationship can be observed between colour parameters and C_L (Table 3).

In this study, changes in colour coordinates before cooking were clearly associated with changes in texture, C_L , and lipid oxidation (measured as $TBARS$), which underlines the potential use of colour for monitoring the final quality of frankfurters. Using L^* values and other colour coordinates, such as a^* , b^* and C_{ab}^* , would allow us to predict the degree of emulsion stability as well as its oxidative tendency before cooking. This would improve the industrial control of cooking losses, lipid oxidation and textural properties of meat gels.

4. Conclusions

Meat emulsions containing 2.5% *HPP* were found to be significantly darker and less red and yellow than those made without *HPP*. The addition of *HPP* also significantly decreased cooking losses, fracture force and lipid oxidation in frankfurters. $TBARS$ values after 7 days of storage in these frankfurters were 25% and 45% lower for emulsions containing 15% and 30% fat, respectively, compared with control emulsions. The results show for the first time the antioxidant and stabilizing effects of *HPP* in finely comminuted meat emulsions. The *Fat* concentration had no significant effect on cooking losses or lipid oxidation, although meat emulsions containing the lower *Fat* concentration (15%) had significantly higher a^* and b^* values and lower L^* ; they were also slightly but significantly harder.

It can be concluded that *HPP* is a good alternative to using synthetic antioxidants and emulsifiers in frankfurters, although the textural effect of *HPP* on these products requires further evaluation.

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