LWT - Food Science and Technology 44 (2011) 1435–1442

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Influence of canola-olive oils, rice bran and walnut on functionality and emulsion stability of frankfurters

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ARTICLE INFO

Article history:
Received 23 May 2010
Received in revised form
3 January 2011
Accepted 9 January 2011

Keywords:
Functional ingredients
Fat substitutes
Natural extracts
Emulsification
Lipid oxidation

ABSTRACT

Fat content of frankfurters (20 g/100 g) was replaced with canola and canola-olive oils. Rice bran (RB) and walnut (WE) were added (2.5 g/100 g) to emulsions as macronutrients. Changes in energy values, color, emulsion stability and lipid oxidation of frankfurters during storage were investigated. ANOVA model was highly significant for color parameters and energy values ($P < 0.001$). The canola-olive oil replacement led to a high capacity to hold water and fat exudates in frankfurters, reporting higher emulsion stabilization parameters than regular frankfurters. The addition of RB led to an increase of cooking and fat exudates, indicating high emulsion instability possible due to interactions between RB fiber and fat-protein binders. Walnut addition reported low cooking loss values, and a significant capacity for emulsion stabilization in comparison with regular and RB frankfurters. Lipid oxidation increased from days 0–7 in all frankfurters, declining afterwards until end of storage. TBARS was not influenced by type of emulsions control, but significant ($P < 0.05$) differences were observed in vegetable oil emulsions made with RB; as well as between RB and WE added to either vegetable oil emulsions. These results suggest the use of these natural ingredients as valuable promoters of healthy meat products.

1. Introduction

The consumer demand for healthier foods has led to the development of unsaturated fat replacements and antioxidant enriched emulsion-type meats in recent years. Some frequent diseases in developed societies such as obesity or cardiovascular disease have been associated with an excessive consumption of animal products that are high in saturated fats (O'Neil, 1993). Regrettably, consumers often associate meat with a negative image that meat contains high fat and red meat, in particular, is regarded as cancer-promoting (Ruusunen & Puolanne, 2005). This growing interest for health has led food industries worldwide to make big efforts in the development of novel products with improved functional properties, nutritional value, and product stability. Vegetable oils play an important role during emulsification process, favoring tenderness of meat products (Marquez, Ahmed, West, & Johnson, 1989). Their inclusion as fat substitutes has been related with an important increase of unsaturated fatty acids, and decreasing low density cholesterol. In addition, canola oil helps to increase the level of omega-3 fatty acids of platelet phospholipids, essential for preventing coronary heart disease (Chan et al., 1993), and is a relatively rich source of α-tocopherol (Eskin et al., 1996). Olive oil added to meat products is a good source of linoleic and linolenic acids that helps to increase the nutritional value and reduce the lipid oxidation (Ansorena & Astiasarán, 2004). Moreover, low-fat frankfurters made with vegetable oils are a valuable option for reducing saturated fatty acids, calories, and cholesterol in comparison to regular frankfurters made with pork fat (Paneras & Bloukas, 1994). However, the incorporation of vegetable oils in meat products can play an important role in the deterioration of meat quality through lipid oxidation, especially in the presence of oxygen during mechanical processing such as grinding or chopping, cooking treatments, and addition of salt during the processing procedures (Ahn, Ajuyah, Wolfe, & Sim, 1993). The oxidation of unsaturated lipids leads to rancid odors and flavors, which decreases the quality of meat and meat products. Numerous studies have been carried out on different aspects of lipid oxidation in meat products to improve their oxidative stability. Recently, scientists are utilizing antioxidants to enhance the oxidative stability and thus extend the shelf life of meat products (Lund, Hviid, & Skibsted, 2007). The addition of dietary fiber in meat products is desirable for their nutritional properties but also for their technological improvement and functional properties related with the benefits for

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doi:10.1016/j.lwt.2011.01.006

Table 1
Quantities of ingredients (g) used in the formulation of meat emulsions.

Emulsion	Meat	Backfat	C	O	RB	WE	Salt	Spice	SE	STP	SN	I + W
B	2500	800	–	–	–	–	60	24	2	12	2	600
B + RB	2400	800	–	–	100	–	60	24	2	12	2	600
B + WE	2400	800	–	–	–	100	60	24	2	12	2	600
C	2500	–	800	–	–	–	60	24	2	12	2	600
C + RB	2400	–	800	–	100	–	60	24	2	12	2	600
C + WE	2400	–	800	–	–	100	60	24	2	12	2	600
CO	2500	–	600	200	–	–	60	24	2	12	2	600
CO + RB	2400	–	600	200	100	–	60	24	2	12	2	600
CO + WE	2400	–	600	200	–	100	60	24	2	12	2	600

C, canola oil; O, olive oil; RB, rice bran; WE, walnut paste; B, overall control made with 20 g/100 g backfat; B + RB (20 g/100 g backfat + 2.5 g/100 g RB), B + WE (20 g/100 g backfat + 2.5 g/100 g WE); C, overall control made with 20 g/100 g canola oil; C + RB (20 g/100 g canola + 2.5 g/100 g RB), C + WE (20 g/100 g canola + 2.5 g/100 g WE); CO, overall control made with 15 g/100 g canola and 5 g/100 g olive oils; CO + RB (15 g/100 g canola, 5 g/100 g olive oil + 2.5 g/100 g RB), CO + WE (15 g/100 g canola, 5 g/100 g olive oil + 2.5 g/100 g WE); SE, sodium erythorbate; STP, sodium tripolyphosphate; SN, sodium nitrite; I + W, ice and water (8:7).

human health (National Cancer Institute, 1984). Rice bran is rich in dietary fiber, proteins, minerals, and vitamin B components, and has been frequently used in prepared foods as a potential dietary fiber source (Lee & Moon, 1994), as well as fat substitute in low-fat meat products (Hsu & Chung, 2001). Rice bran proteins have a high water and oil binding capacities and show a good potential for producing stable emulsions under high sugar and salt concentrations (Chandi & Sogi, 2007). Several studies have reported that regular consumption of walnuts is related to the prevention of coronary heart disease (FDA, 2004). Walnut is rich in unsaturated fatty acids than typical vegetable oils. The addition of walnut extracts leads to an improvement in the nutritional profile of frankfurters, in comparison to commercial sausages made with animal fats, and contains several bioactive components that improve the sensory and physicochemical properties (Jiménez-Colmenero, Ayo, & Carballo, 2005).

In this study, fat content of frankfurter-type sausages was substituted with canola or canola-olive oil mixes (20 g/100 g), and 2.5 g/100 g of extracts (rice bran and walnut paste) were added to the sausages as macronutrients. The aim of this study was to investigate the changes in calorie and meat emulsion quality metrics (i.e. color, cooking loss and oxidative stability) of the sausages after substituting fat by vegetable oils and after adding rice bran and walnut paste in the formulation.

2. Materials and methods

2.1. Ingredients

Commercial fresh pork meat and pork backfat were obtained from a local meat purveyor (Smithfield Packing Company Inc., Grayson, KY, USA). Excess fat and connective tissue were trimmed from pork meat. Pork meat and backfat were separately ground twice through 25.4–9.6 mm (meat) and 9.6–3.2 mm (fat) orifice plates with a meat grinder (Model 4146SS; Hobart Corp., Troy, OH, USA), weighted (Metler Toledo, mod. 8140, Worthington, OH, USA), vacuum packed (Sipromac, Mod 600A, St. Germain, Canada) into individual plastic bags, and frozen at −18 °C until product formulation. All the experiments were carried out within two months. Canola and olive oils (Pompeian, Inc., Baltimore, MD, USA), rice bran, (Ener-G Foods, Inc. Seattle, WA. USA) and walnuts were obtained from a local purveyor (Kroger Co., Cincinnati, OH, USA). Walnuts were processed according to method described by Ayo, Carballo, Solas, and Jiménez-Colmenero (2008), including small modifications. Walnut halves were ground in a lab grinder (KitchenAid, Mod. KFP710, St.Joseph, Michigan USA) at 1750 rpm for approximately 1.5 min, until obtain a finely comminuted paste composed by small particles of reduced size. After grinding, the paste was heated at 80 °C for 1 h in an oven (Barnsteadt Thermolyne, model OV19225, Iowa, USA) in order to obtain a refined extract. The paste was then left at room temperature for 15 min, before weigh into plastic bags, which were vacuum sealed and stored at room temperature until use. The rest of the ingredients used during emulsion manufacturing were salt (Sysco Corp. Houston, TX, USA), spice mix blend 125, erythorbic acid (Old Plantation Seasoning, Birmingham AL, USA), sodium tripolyphosphate, (Sigma Chemical, CO. St. Louis, MO, USA) and sodium nitrite (Fisher Scientific. Fair Lawn, NJ, U.S.A.). All the ingredients of the emulsion composition were kept constant in each batch and replicate (2.5 g/100 g).

2.2. Preparation of frankfurters

Three different frankfurter formulations were prepared in a cooler room (6–8 °C) to obtain 4 kg batter (Table 1), containing 20 g/100 g backfat, 20 g/100 g canola oil, and 20 g/100 g canola-olive oils (3:1). The proximate composition (AOAC, 1996) of the trimmed pork meat was; moisture 72.9 g/100 g, fat 5.1 g/100 g, protein 21.1 g/100g and ash 0.9 g/100 g. Rice bran (RB) and walnut paste (WE) were added separately to these emulsions at an addition rate of 2.5 g/100g. Composition of RB and WE are given in Table 2. Overall control emulsions without RB and WE were also prepared in order to obtain a total of nine treatments (3 × 3 factorial design) by replicate (× 2). Before emulsion preparation, frozen meat and backfat were thawed overnight in a refrigerator at 4 °C. Partially thawed pork meat was placed in a silent cutter (Model CM-14, Mainca USA, St. Louis, MO, USA) and homogenized for 1 min. The total amount of ingredients (salt −1.5 g/100 g-, spice mix −0.6 g100/g-, erythorbic acid −0.05 g/100 g-, sodium tripolyphosphate −0.3 g/100 g-, and sodium nitrite −0.05 g/100 g-) were dissolved in water (7 g/100 ml), and kept in refrigeration (4–6 °C) before being added to the homogenized meat. This mixture was then chopped for another 2 min. Partially thawed pork fat or fat replacements (canola −20 g/100 g- or canola oil + olive oil (3:1)), ice (8 g/100 g) and rice bran (2.5 g/100 g) or walnut paste (2.5 g/100 g) were then added and the mixture was chopped for another 3 min. Total mixing time was standardized to 6 min and the final temperature of the meat emulsion was below 12 °C in all cases. The chopping speed of blades and plates were adjusted to minimal and high revolutions for the first min of chopping and the rest of the chopping procedure, respectively. The final pH of the emulsion was measured with a pHtester Oakton® (Mod. Spear, Eutech Instruments,

Table 2
Nutritional composition of rice bran and walnut paste.

	Rice bran (RB)	Walnut paste (WE)
Dietary fiber (g/100g)	28.4	6.7
Carbohydrates (g/100g)	50.7	13.4
Total fat (g/100g)	20.9	66.7
Protein (g/100g)	10.0	16.7
Energy (kcal/100g)	328.4	666.7

Malaysia). Approximately 3000 g of emulsion was transferred into a hand stuffer (The Sausage Maker, Buffalo, NY, USA) and stuffed into 27.0 mm diameter frankfurter cellulose casings (Teepak LLC., Danville, IL, USA). The frankfurters were manually tied into approximately 12 cm links, washed to remove materials on the outside of the casing, and kept at 4–6 °C for 20–30 min before cooking. The links were cooked in an Alkar smokehouse (Model 450U, Alkar-Rapid Pak Inc., Lodi, WI, U.S.A.) with a programmed temperature-time cooking schedule until the internal temperature of frankfurters reached 71 °C. The relative humidity of the smokehouse was controlled between 36 and 68% and the cooking was completed in about 90 min. The cooked products were then showered with cold water for 2 min until a final temperature lower than 10 °C was reached, then casings were removed. Frankfurters were vacuum packed and finally stored in a 4 °C cooler. All analysis, except for oxidative stability, were conducted within 2 d.

2.3. Color measurement of raw emulsions

The color of each sample was measured after the emulsion was prepared using a Spectrocolorimeter Minolta Chromameter II (Mod CR-310, Minolta Camera Co., Osaka, Japan) having a CIE standard 'C' illuminant and 0° viewing angle geometry. The measurements were performed with a 10.5 cm diameter plate (Glass Light-Projection Tube, CR-A33e) and repeated five times in different areas of the surface of each emulsion to record the average color. The chromameter was calibrated using a calibration plate CR-A44 ($Y = 93.80$, $x = 0.3138$, $y = 0.3195$), and the CIELAB color space was selected to measure the L^* value (metric lightness) and chromaticity coordinates a^* (redness/greenness) and b^* (yellowness/blueness).

2.4. Proximate analysis

Chemical composition (%) of the raw and cook meat emulsions was analysed in triplicate for each emulsion production. Fat and moisture was estimated using an HFT-2000 fat-moisture analyzer (DSC -Data Support Co. - Inc., Encino, CA. USA). Protein content was estimated with ~250 mg of sample by Elementar Organic Nitrogen Determinator (Elemntar Hanan, Mod. Vario Max CN, Hanau, Germany). Protein values were calculated by converting the nitrogen content estimated (6.25 × N). Ash content was determined weighing ~3 g of sample in a porcelain crucible and drying ashes in a furnace (Isotemp® Programable Muffle Furnace, Fischer Scientific, Pittsburg, PA, USA) during 22 h at 500 °C. Total calorie value (kcal) was calculated using the Atwater method with the equation below (Watt & Mersil, 1975):

$$kcal = [(F_P + P) + (F_L + L) + (F_C + C)] \quad (1)$$

where kcal is the calorie; F the multiplication factor for each component (F_P: 4.27 for protein, F_L: 9.02 for lipid, F_C: 4.10 for carbohydrate); and P, L, and C are the content of protein, lipid and, carbohydrates (g/100 g), respectively.

2.5. Meat emulsion stability

The emulsion stability was determined by triplicate according to the empirical procedure followed by Hughes, Cofrades, and Troy (1997). Approximately 30 g of raw emulsion was placed in a 30 ml Nalgene® centriguge tube (Nalge Nunc Int. Corp, Rochester, NY, USA) with a screw top, and centrifuged in a refrigerated superspeed centrifuge (Sorvall® RC-5B, Bioresearch Systems. Wilmington, DE, USA) at 3600 rpm for 2 min. The aliquots were heat treated in a refrigerated circulating water bath (Isotemp 3013S, Fischer Scientific, Pittsburg, PA, USA) at 70 °C for 30 min. After thermal treatment and re-centrifugation at 3600 rpm for 5 min, the supernatants were weighed (Metler® Toledo, Mod. AL104, Worthington, OH, USA) and poured into pre-weighed crucibles and dried overnight at 100 °C, then re-weighed to estimate fat exudates. Sample pellets were also removed and weighed. The emulsion stability values were estimated in function of the volumes of total expressible fluid (*TEF*, cooking loss) and the percentage of fat exudated (TEF_{Fat}), using the following equations:

$$TEF(\%) = \frac{(W_t + W_s) - (W_t + W_p)}{W_s} \times 100 \quad (2)$$

$$TEF_{Fat}(\%) = \frac{(W_c + D_s) - (W_c)}{(W_t + W_s) - (W_t + W_p)} \times 100 \quad (3)$$

where, W_t = weight of centrifuge tube; W_s = weight of sample; W_p = weight of pellet; W_c = weight of crucible, and D_s = weight of dried supernatant.

2.6. Weight loss

Weight loss (W_{Loss}) values were determined by calculating the weight difference of three links of frankfurters before and after thermal treatment in an Alkar smokehouse using the following equation:

$$W_{Loss}(\%) = \frac{(W_0 - W_1)}{W_0} \times 100 \quad (4)$$

where, W_0 = weight before thermal treatment, and W_1 = weight after thermal treatment.

2.7. Thiobarbituric acid-reactive substances (TBARS)

The TBARS method was used to evaluate lipid oxidation in meat emulsions formulated with vegetable oils, rice bran and walnut paste, using the procedure described by Sinnhuber and Yu (1977). Two independent trials were conducted with duplicate sample analyses. Samples were taken on days 0, 7, 14 and 21 of storage at 2 °C by cutting a portion from the middle of the frankfurter, and coring a 12.7 mm diameter coin from the center of each sample. The sample was then blended (Waring Commercial, Mod. 51BL31, Torrington, CT, USA) at a low speed setting, for 30 s. Approximately 1 g of minced frankfurter was stuffed into 25 ml screw cap culture tube and mixed with 150 μl of antioxidant solution (butylated hydroxyanisole, propylene glycol, butylated hydroxytoluene, and tween 20). A mixture of 1 g/100 ml thiobarbituric acid (TBA) solution with 0.075 mol equi/L NaOH (1.5 ml) and 2.5 g/100 ml trichloroacetic acid (TCA) solution with 0.036 mol equi/L HCl (8.5 ml) were then added to each tube. Samples were heated at 100 °C in a water bath (Thermo Fisher Scientific, Mod. 2845, Marietta, OH, USA) for 30 min. After heating, aliquots of 5 ml of supernatant were transferred into a 15 × 125 mm glass centrifuge tube, and 5 ml of chloroform was then added. The samples were centrifuged at 1792 g for 10 min, and the supernatant was transferred to another glass centrifuge tube, containing 2 ml of petroleum ether. Samples were then re-centrifuged at 1792 g for another 10 min. The standard curve of TBA was calculated by diluting 0.0220 g of malonaldehyde bis (diethyl acetal) with 1000 mL nanopure water (stock standard). Working standards were made by transferring 5, 10, 15, and 20 mL of the stock standard into 100-mL amber volumetric flask, then diluting to volume with nanopure water. Using 1.0 mL of the working standards, the values of absorbance at 532 nm of these solution concentrations were registered to plot a standard curve. The standard curve was used to calculate the TBARS value, expressed as mg of malondialdehyde (MDA) per kg of sausage

sample. To calculate milligrams of malondialdehyde the following equation was used:

$$MDA(mg) = (Std., mol/L) \times (72.0g/mol) \times (0.001L) \times (1000mg/g) \quad (5)$$

where, *Std.* = standard concentration (mol/L).

Milligrams of MDA *vs* absorbance was plotted to obtain the line equation, which was used to calculate the concentration of MDA for the samples by dividing the sample absorbance by the line equation and the sample weight.

2.8. Statistical analysis

Data were analyzed using the Statistical Analysis System (SAS®, 2002). The analysis of variance (ANOVA) was performed using the general linear model (GLM) procedure of SAS. The least squares means (LSM) and significance of treatments were calculated using type IV sum of squares. LSM were considered to be statistically different when $P < 0.05$.

3. Results and discussion

Dependent variables tested for monitoring the effect of several functional ingredients on the technological properties directly related to the stability and quality of pork meat emulsions were classified as color parameters (L^*, a^* and b^*), energy values (kcal/100 g), and meat emulsion metrics (W_{Loss}, *TEF* and TEF_{Fat}). An ANOVA was conducted to determine the main sources of variation in the dependent variables (Table 3). Replicate (Rep), use of animal backfat or vegetable oils (*Fat-oils*), rice bran and walnut paste (*Extracts*), were selected as main effects in the preliminary ANOVA model. The main interaction "*Fat-oils* × *Extracts*" was also included. Replication effect was slightly significant for lightness and energy values. The ANOVA model was highly significant for all the dependent variables studied, but especially for color parameters and energy values. The presence of fat or vegetable oils in the meat emulsion formulation was found to have a statistically significant ($P < 0.001$) effect on color parameters and energy values of samples, while no significant effect on meat emulsion metrics was found. The main effect *Extracts* was significant ($P < 0.05$) for all the dependent variables studied, showing the highest significance ($P < 0.001$) on color coordinates a^* and b^*, weight loss (W_{Loss}), and energy values of frankfurters. The interaction "*Fat-oils x Extracts*" only had a statistically significant effect on total expressible fluids (*TEF*) of emulsion samples.

Table 3
Analysis of variance and *F* statistics for dependent variables, color, weight loss, cooking losses and energy values[a].

Model			Variation Source			
			Rep	Fat−oils	Extracts	Fat-oils ×Extracts
			(DF = 1)	(DF = 2)	(DF = 2)	(DF = 4)
	R^2	F	F	F	F	F
L*	0.98	74.9***	3.96*	362***	6.06*	ns
a*	0.95	29.4***	ns	118***	26.6***	ns
b*	0.85	8.83***	ns	18.4***	22.0***	ns
W_{Loss}	0.67	3.31*	ns	ns	11.4***	ns
TEF	0.74	4.45**	ns	ns	10.4**	5.27**
TEF_{Fat}	0.69	3.55*	ns	ns	10.4**	ns
kcal	0.95	31.2***	8.87**	112***	32.6***	ns

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, ns not significant.

[a] $N = 18$; *F*, ANOVA *F*-statistic; *DF*, degree of freedom; *DF error* = 8; *DF model* = 9. R^2, determination coefficient.

Table 4
Mean and standard deviation of chemical composition of frankfurters[a].

	Protein (g/100g)	Fat (g/100g)	Moisture (g/100g)	Ash (g/100g)
B	14.73 ± 0.17^{a}	22.71 ± 0.84^{c}	59.59 ± 0.79^{a}	2.93 ± 0.12^{ab}
B + RB	14.43 ± 0.06^{abc}	22.16 ± 0.18^{c}	59.04 ± 0.19^{a}	3.06 ± 0.07^{a}
B + WE	14.62 ± 0.04^{ab}	23.38 ± 0.28^{bc}	58.82 ± 0.35^{a}	2.82 ± 0.03^{ab}
C	13.80 ± 0.01^{bc}	26.05 ± 0.21^{ab}	57.42 ± 0.30^{ab}	2.69 ± 0.10^{b}
C + RB	13.77 ± 0.08^{bc}	26.08 ± 0.11^{ab}	55.90 ± 0.15^{b}	2.94 ± 0.04^{ab}
C + WE	13.64 ± 0.08^{c}	26.99 ± 0.29^{a}	56.12 ± 0.27^{b}	2.88 ± 0.07^{ab}
CO	13.95 ± 0.21^{abc}	26.09 ± 0.05^{ab}	57.17 ± 0.26^{ab}	2.75 ± 0.01^{ab}
CO + RB	13.73 ± 0.02^{c}	26.28 ± 0.73^{ab}	55.72 ± 0.81^{b}	2.96 ± 0.06^{ab}
CO + WE	13.83 ± 0.43^{bc}	27.20 ± 1.24^{a}	55.77 ± 0.78^{b}	2.83 ± 0.02^{ab}
Signification level (*P*)	0.0007	0.0001	0.0001	0.0096

[a] Values in the same column with different superscripts are significantly different ($P < 0.05$). B, emulsion control with backfat (20 g/100 g); C, emulsion control with canola oil (20 g/100 g); CO, emulsion control with canola-olive oils (15-5 g/100 g); RB, rice bran; WE, walnut paste.

3.1. Proximate analysis and energy values of frankfurters

Chemical composition of the frankfurters made with different formulations is shown in Table 4. Emulsions made with pork backfat (20 g/100 g) had the greatest protein (14.7 g/100 g) and moisture (59.6 g/100 g) contents of all emulsions. The greatest amounts of fat (27.2 g/100 g) and ash content (2.96 g/100 g) were found in canola-olive oil emulsions made with WE and RB, respectively. Least square means of energy values in frankfurters made with different fat/oil and extracts (rice bran and walnut paste) are shown in Table 5. Energy values of frankfurters ranged from 284.9 to 300.9 kcal/100 g. The effect of fat/vegetable oil contents on energy values of frankfurters was significant ($P < 0.05$). Regular frankfurters (frankfurters made with pork fat) showed lower energy values (~285 kcal/100 g) than frankfurters made with vegetable oils (~300 kcal/100 g). This condition could be attributed to the significantly ($P < 0.05$) lower fat composition found in pork backfat than vegetable oils (Table 4), as well as the high capacity to exude fat during cooking of regular frankfurters in comparison to frankfurters made with vegetable oils (Table 5). As a result, pork backfat emulsions showed significantly ($P < 0.05$) lower energy values than other frankfurters made with vegetable oils or extracts (RB and WE). Similarly, other authors found reduced values of fat content in beef frankfurters and cooked salamis in comparison with samples containing vegetable oils (Ambrosiadis, Vareltzis, & Georgakis, 1996), as well as decreases of energy values (27−38%) in frankfurters with reduced-fat compositions (Cengiz & Gokoglu, 2005). The addition of rice bran and walnut paste significantly ($P < 0.05$) raised the ash (rice bran) and fat (walnut paste) contents of frankfurters (Table 4). Energy values of frankfurters with added WE were significantly ($P < 0.05$) higher than control emulsions and emulsions made with RB. However, RB frankfurters also showed higher energy values than control emulsions. This increase of energy values in sausages with added fiber

Table 5
Influence of main effects, fat-oil and extracts (rice bran and walnut paste) compositions, on meat emulsion quality metrics[a].

	Fat-vegetable oil			Natural extracts		
	Backfat	Canola	Canola-Olive	No extracts	Rice bran	Walnut
L*	72.93^{a}	78.06^{b}	78.18^{b}	76.21^{a}	76.12^{a}	76.83^{b}
a*	6.87^{a}	5.33^{b}	4.89^{c}	6.16^{a}	5.17^{b}	5.76^{c}
b*	12.71^{a}	11.48^{b}	12.08^{c}	11.57^{a}	12.89^{b}	11.81^{a}
W_{Loss}	8.86^{a}	9.41^{a}	9.04^{a}	9.25^{a}	8.39^{b}	9.68^{a}
TEF	0.64^{a}	0.61^{a}	0.55^{a}	0.67^{a}	0.65^{a}	0.48^{b}
TEF_{Fat}	7.18^{a}	6.73^{ab}	6.31^{b}	5.83^{a}	7.26^{b}	7.12^{b}
kcal	284.95^{a}	299.01^{b}	300.95^{b}	290.50^{a}	294.27^{b}	299.76^{c}

[a] $N = 18$. LSM with same letters were not significantly different ($P < 0.05$); For the definition of dependent variables, see the material and methods section.

ingredients is consistent with other findings reported in meat batters made with added WE (Ayo et al., 2008), and sausages made with added citrus fiber and soy protein concentrate (Cengiz & Gokoglu, 2005). In spite of the increase in energy values, the high fat exudation in emulsions made with RB suggests low oil capacity absorption during chopping process, and consequently, a lower emulsification capacity in comparison to emulsions made with WE. This is in agreement with Chandi and Sogi (2007), who stated that a high oil absorption capacity is essential to keep the emulsification conditions of sausages.

3.2. Meat emulsion stability

Table 5 shows the influence of fat/oil and extract (rice bran and walnut paste) compositions on the average weight loss (W_{Loss}), total expressible fluids (*TEF*), and fat exudates (TEF_{Fat}) on the different meat emulsion groups studied. Weight loss of frankfurters were unaffected ($P > 0.05$) by differences in fat and vegetable oil contents, but a significant effect was detected when extracts (RB and WE) were used. Thus, the addition of rice bran in meat emulsions led to a significant ($P < 0.05$) decrease of frankfurter W_{Loss}. Similar results were obtained by other authors in frankfurters formulated with oat fiber (Hughes et al., 1997) or maltodextrin (Crehan, Hughes, Troy, & Buckley, 2000). As can be observed in Table 5, a high capacity to hold water and fat exudates was obtained in canola-olive oil emulsions, although *TEF* was unaffected ($P > 0.05$) by differences on fat (0.64 ± 0.17) and vegetable oils (~ 0.61–0.55) formulations. These results are consistent with those obtained by Ambrosiadis et al. (1996), which found cooking loss values in batters containing vegetable oils significantly ($P < 0.05$) lower than batter controls containing pork backfat. The *TEF* values (Table 5) for emulsions formulated with RB were found to be not significantly ($P > 0.05$) different compared with emulsions with no extracts, but were significantly ($P < 0.05$) higher than emulsions with added WE. These results were related with a high instability in meat emulsions when RB was used instead of WE. The highest TEF_{Fat} values detected in emulsions made with rice bran (7.26 ± 0.87) suggest possible interactions between RB fiber and fat-protein binders during the emulsification process that allows for the increase of fat exudates during cooking of meat batters. Crehan et al. (2000) found a similar decrease in emulsion stability in frankfurters formulated with maltodextrin, a non-sweet starch hydrolysate fat substitute. These results are in agreement with Choi et al. (2009) who found higher total expressible fluids (low emulsion stability) and fat losses in emulsion made with RB (2%) in comparison with control emulsions. Previously, Choi, Jeong, Choi, Han, Kim, & Lee (2008) reported that addition of RB fiber to meat products improved emulsion stability and rheological properties. Hughes et al. (1997) also found a significant increase of emulsion stability in frankfurters formulated with 5, 12 and 30% fat and supplemented with oat fiber. The lowest *TEF* values (0.48 ± 0.06) were detected in emulsions made with WE, showing a significant ($P < 0.05$) capacity of emulsion stabilization in comparison with regular frankfurters and RB frankfurters. Similar results were obtained by Ayo et al. (2008) in frankfurters made with normal fat and added walnut paste.

3.3. Color

Table 5 shows that color parameters of cooked frankfurters were affected ($P < 0.05$) by differences in fat, vegetable oils, and extracts (rice bran and walnut paste) used in the formulation. Emulsions made with animal fat showed significantly ($P < 0.05$) lower L^* values, and higher a^* and b^* values than emulsions made with vegetable oils. Youssef and Barbut (2009) found similar results of color in meat batters made with canola oil (25%) and beef fat (25%), with increasing protein levels (10–15%). Ambrosiadis et al. (1996) reported similar findings in color composition (increase in lightness and decrease in redness) in frankfurters when pork backfat (19.5%) was replaced at the same proportion with soya-seed oil, sunflower oil, cotton-seed oil, corn-seed oil or palmine. According to Ambrosiadis and Klettner (1981), these differences of color in emulsions made with vegetable oils are probably, as a result of the distribution of oil phase into the matrix of actomyosin during the chopping process, caused by an increase in the surface of the fat particles, which altered the color after cooking. The addition of extracts (RB and WE) to meat emulsions significantly ($P < 0.05$) affect the lightness and color coordinates of the frankfurters. As can be observed in Table 5, RB induced significant ($P < 0.05$) changes of a^* (decrease) and b^* (increase) in meat batters when this ingredient was used. These results are in agreement with those obtained by Choi et al. (2009) who reported that reduced-fat meat batters supplemented with RB fiber had lower lightness and redness values and higher yellowness than control samples. Similar behavior of color coordinates a^* and b^* were also reported after use a similar source of fiber, oat bran (Yilmaz & Daglioglu, 2003) or rye bran (Yilmaz, 2004) as a fat substitute in low-fat sausages. The addition of WE to meat emulsions reported significant ($P < 0.05$) L^* increases and a^* decreases, but no significant changes in b^*. These results are in agreement with those obtained by Jiménez-Colmenero et al. (2003) in raw restructured beef steaks with addition of increasing amounts of WE (5–15%). However, when meat batters were cooked no walnut-induced changes of L^* were detected (Ayo et al., 2008), and an association between the addition of increasing amounts of WE and increments of a^* and b^* values were obtained (Ayo, Carballo, Solas, & Jiménez-Colmenero, 2005).

3.4. Effect of fat-oil composition on lipid oxidation of frankfurters

Fig. 1 shows the formation of lipid oxidation products (mg MDA/kg sample) in meat emulsion controls and emulsions including extracts (rice bran and walnut paste), during refrigerated storage. Both backfat and vegetable oil emulsions were capable of delaying ($P < 0.05$) lipid oxidation (Fig. 1A) after one week in refrigerated conditions. The concentration of MDA significantly increased on day 7 (0.291 mg/kg) in all emulsion controls, but slowly declined afterwards until reaching the lowest overall means (0.075 mg/kg) on day 21 of storage. The quick increase of TBARS values during the first days of store could be related with the production of acid-reactive substances such as hydroperoxides, which occur at the early stages of lipid oxidation (Frankel, 1996). Similar MDA increases were previously reported in cooked pork patties (Peña-Ramos & Xiong, 2003) and pork emulsions made with different fat concentrations (Nieto et al., 2009) stored for up to 7 days at 4 °C. The decrease in detectable TBARS values after 7 days of refrigeration may be attributed to the decomposition of acid-reactive substances into secondary products (Peña-Ramos & Xiong, 2003) or binding of MDA to other components in meat, such as amino groups in proteins during increasing storage time, which has demonstrated a synergistic antioxidant activity (Marcuse, 1960). Recent studies have demonstrated that the common existence of peptide amino acids such as tyrosine, may have an important role in the antioxidative activity in oil-in-water emulsions (Cheng, Xiong, & Chen, 2010). The authors isolated and identified three peptides from potato protein hydrolysate, all of them contained tyrosin residues, capable of binding to secondary reactive substances and showing a strong lipid antioxidant activity. Several other amino acids, particularly methionine, histidine, and lysine have also been shown to inhibit lipid oxidation in model systems (Marcuse, 1960). Pork backfat control samples had the overall lowest MDA mean values (0.160 mg/kg) during chill storage, compared to vegetable oil

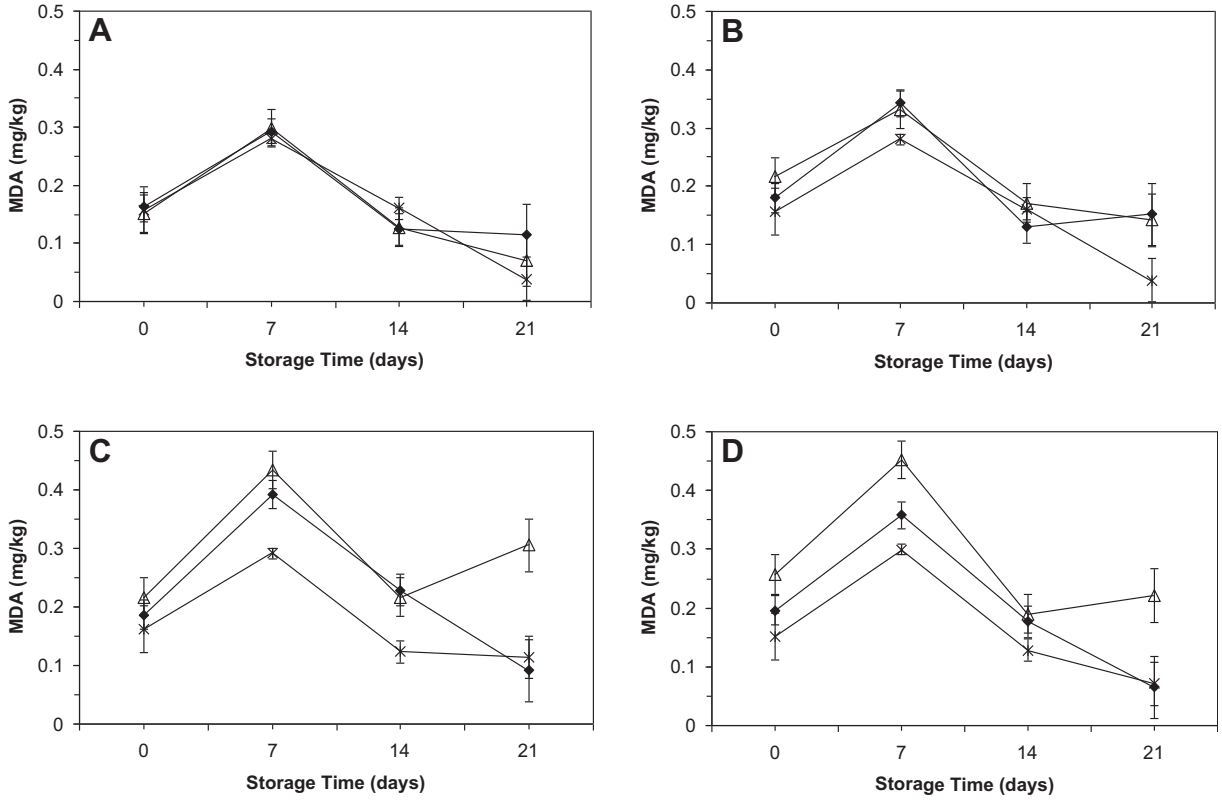

Fig. 1. Secondary lipid oxidation products expressed as mg of malondialdehyde (MDA) per kg of sausage sample during storage for up to 21 days at 2 °C. Fig A:Control emulsions made with pork backfat (*); 20 g/100 g, canola oil (◆); 20 g/100 g, and canola-olive oils (△); 15-5 g/100 g. Fig B: Backfat emulsions (*); 20 g/100 g, with WE (◆); 2.5 g/100 g, or RB (△); 2.5 g/100 g. Fig C: Canola oil emulsions (*); 20 g/100 g, with WE (◆); 2.5 g/100 g, or RB (△); 2.5 g/100 g. Fig D: Canola-olive oil emulsions (*); 15-5 g/100 g, with WE (◆); 2.5 g/100 g, or RB (△); 2.5 g/100 g. WE, walnut paste; RB, rice bran.

control samples (~0.168 mg/kg). This may be due to the lower percentage of unsaturated fatty acids present in pork backfat, compared to vegetable oils. These results were in agreement to Choi et al. (2010) who found higher TBA values for all vegetable oil samples than those for the control containing no added vegetable oil due to the fact that added vegetable oils gave differences in fatty acid composition. Moreover, lipid oxidation was not influenced ($P > 0.05$) by type of fat (Fig. 1A) or addition of extracts, RB and WE (Fig. 1B), and TBA values were within acceptable limits (<1.0 mg/kg) (Yildiz-Turp & Serdaroglu, 2008). This lack of differences between controls suggests that use of these oils replacements could be a valuable option for promoting healthy meat products.

3.5. Effect of rice bran and walnut paste addition on lipid oxidation of frankfurters

Fig. 1 shows that sample controls had the lowest overall MDA means, compared to emulsions made with added extracts, RB and WE (Figs. 1B, C and D). The emulsions made with added RB had the highest TBARS values at the end of refrigerated storage, especially in canola (Fig. 1C) and canola-olive (Fig. 1D) controls, where the overall MDA mean increases were significantly ($P < 0.05$) higher than emulsion controls and emulsions made with WE. Choi et al., (2010) also found the highest TBA values in rice bran supplemented frankfurters. However, the antioxidant capacity of RB emulsions was preserved until day 14 of storage, showing an efficient protection against lipid oxidation. Similar results were obtained by Revilla, Santa-María, Miramontes, Bautista, Martinez, Cremades, Cert & Parrado (2009). The final increase of lipid oxidation in RB emulsions may be attributed to the lipid profile of rice bran, which contains a high percentage of oleic (C18:1) and linoleic (C18:2) acids, all of which are very susceptible to oxidation (Krishna, Hemakumar, & Khatoon, 2006). Furthermore, it is possible that the RB fiber interactions with fat-protein binders during emulsification could also be responsible for furthering or enhancing oxidation by increasing the exposure of lipids to air, light, or heat during processing. The addition of WE (Figs. 1B, C and D) were also able to decrease the formation of TBARS after 1 week of refrigerated storage, contributing to reduced lipid oxidation. At the end of storage (day 21), TBARS reduction observed in vegetable oil emulsions with added WE (~0.078 mg/kg; Figs. 1C, 1D) was not significantly ($P > 0.05$) different in comparison to their respective emulsion controls (0.092 mg/kg; Figs. 1C, D) and backfat emulsions with added WE (0.152 mg/kg; Fig. 1B). However, this TBARS reduction was significantly ($P < 0.05$) lower than those observed in vegetable oil emulsions with added RB (0.263 mg/kg; Figs. 1C, D). Although no data has been reported regarding possible interactions between walnut and muscle proteins and their effects on physicochemical properties of processed meats (Cofrades, Serrano, Ayo, Carballo, & Jiménez -Colmenero, 2008), this TBARS reduction may be due to the peculiar blend of nutrients and phytochemical compounds found in walnuts. In particular, walnuts are especially rich in antioxidant vitamins (tocopherols) and antioxidant substances such as phytosterols and polyphenols. Vitamin E (considered as α-tocopherol) and γ-tocopherol are potent membrane-soluble antioxidants (Minhajuddin, Beg, & Iqbal, 2005), whose antioxidant activity strongly depends on their concentration in foods (Cheftel & Cheftel, 1988). Under this assumption, the high amounts of γ-tocopherol (21.8–26.5 mg/100 g) and α-tocopherol (1.08–4.05 mg/100 g) found in walnuts (Lavedrine, Ravel, Poupard,

& Alary, 1996), could have contributed to reinforce the antioxidant activity observed in frankfurters supplemented with walnut. When compared to storage time, emulsions made with added extracts, RB and WE (Figs. 1B, C and D) showed a similar behavior than their respective controls. As a result, TBARS values of samples showed a high dependence on the day of storage ($P < 0.05$), observing that MDA mean values increased during the first week of storage and decreased for up to 21 days at 2 °C. In general, the results of TBA during 0 and 21 days of storage show that addition of extracts (RB and WE) could retard or maintain oxidation during refrigerated storage when used in regular frankfurters (Fig. 1B), and addition of walnut paste could be useful for preventing lipid oxidation of cooked emulsions, especially when vegetable oils are used instead of animal fats (Fig. 1C, D).

4. Conclusions

Addition of canola or olive oils as fat substitutes, and rice bran or walnut paste as macronutrients in meat emulsions not only offers potential healthy benefits, but also provides an important effect on the quality of meat batters. In spite of the energy values, the use of these oil replacements and extracts (RB and WE) as healthy ingredients in meat products are nutritionally satisfactory. The high fat exudation in rice bran emulsions suggests possible interactions between RB fiber and fat-protein binders during emulsification process that could compromise the cooking stabilization of meat batters. Walnut paste and canola-olive oil emulsions showed a high capacity to hold water and fat exudates and consequently, had the highest emulsion stabilization parameters. The use of vegetable oils did not produce significant changes of lipid oxidation levels in comparison with regular frankfurters. This lack of differences suggests that use of these oil replacements could be a valuable option for promoting healthy meat products. Furthermore, the addition of rice bran and walnut paste could retard or maintain oxidation when used in regular frankfurters and addition of walnut paste could be useful for preventing lipid oxidation of cooked emulsions, especially when vegetable oils are used instead of animal fats.

Acknowledgments

The authors wish to thank the Seneca Foundation (Consejería de Educación y Cultura. CC.AA. Murcia, Spain) for the financial support provided by the research project "Use of functional ingredients as promoters of healthy meat emulsions", and the Animal and Food Science Departments of the University of Kentucky for financial support.

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