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Textural and thermal properties of low-lipid meat emulsions formulated with fish oil and different binders

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ABSTRACT

The effect of the addition of several binders (milk proteins concentrate, whey protein concentrate, thermally treated whey protein concentrate, ovalbumin, hydroxypropylmethylcellulose, methylcellulose, mixtures of κ:ι carrageenans or xanthan-locust bean gums) to low-lipid meat emulsions formulated with fish oil were compared to control formulations with fat or fish oil without any binder added. Process yields were higher than 96 g/100 g, except for formulation with hydroxypropylmethylcellulose. Formulations with hydrocolloids presented less weight lost by centrifugation than both controls. The differences in the amount of non-frozen water explain the results obtained for process yield and liquid released by centrifugation. Significant differences in hardness were found between formulations. Addition of milk proteins concentrate, xanthan-locust bean gums, and mix of carrageenans gave the highest hardness, similar to control formulation with fat. Hardness presented a good correlation with the plateau modulus obtained from frequency sweep curves. The mechanical spectra of cooked batters showed a gel-like behavior. Viscoelastic behavior of the cooked batters was satisfactory modeled using broadened BSW equation to predict the mechanical relaxation spectrum in the linear viscoelastic range. Thermo-rheological curves were related with modulated differential scanning calorimetry results.

1. Introduction

Fats have considerable effects on the binding, rheological and structural properties of meat products (Choi et al., 2009). However meat products with relative high fat content, high in saturated fats and cholesterol have been related to some chronic diseases as cardiovascular diseases and cancer (Siri-Tarino, Sun, Hu, & Krauss, 2010; Gonzalez & Riboli, 2010). Reduction of fat content in frankfurters and substitution of animal fat with vegetable or marine oils should result in a healthier product (Fernández-Martin, López-López, Cofrades, & Jiménez-Colmenero, 2009). However, fat reduction along with increased water content leads to an acceptable texture only when the batter has good water holding capacity. This can be achieved by using fat replacers such as hydrocolloids, vegetable and connective tissue proteins (Cierach, Modzelewska-Kapitula, & Szaciło, 2009).

Long chain n-3 polyunsaturated fatty acids (PUFA) are recognized as essential constituents for normal animal development and growth. Its primary sources are fish and other sea-foods (Zhang, Xiao, Samaraweera, Lee, & Ahn, 2010). Andrés, Zaritzky, and Califano (2009) formulated low-fat chicken sausages with pre-emulsified squid oil that had better fatty acid profile than those formulated with beef tallow, with good stability and quality attributes. The incorporation of fish oil to meat products has been investigated; Muguerza, Ansorena, and Astiasarán (2004), Valencia, Ansorena, and Astiasarán (2006), and Pelser, Linssen, Legger, and Houben (2007) worked on both fermented and cooked sausages; Pennisi Forell, Ranalli, Zaritzky, Andrés, and Califano (2010) and Andrés, Pennisi, Ranalli, Zaritzky, and Califano (2012) found that the presence of high oleic sunflower and deodorized fish oil did not adversely affect the quality of low-fat burgers even after six months of frozen storage. The replacement of fat source involves several changes in textural properties (hardness, chewiness, etc) and rheological behavior of the product. Therefore it would be necessary to add agents to reformulated sausages with fish oil to obtain similar characteristics to the original product containing fat. Biopolymers do not have equivalent functional properties when used in a specific food application. Substitution of one ingredient for another does not produce the same results in different finished product. The reason for a lack of equivalent functionality could be explained by differences in molecular structure, interactions with meat proteins, solution conditions required for functionality (Barbut & Choy, 2007). Data available in literature about different additives behavior in emulsified meat batters were obtained under a broad range of conditions (e.g. meat and lipid sources, proximate

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Table 1
Formulation to prepare 100 g of raw batter of low-fat sausages with beef tallow or squid oil as a lipid source.

Component	Formulation code[a]									
	MPr	WPC	WPCmod	EW	HPMC	MC	Carr	XL	CO	CF
Beef meat	67.09	67.09	67.09	67.09	67.09	67.09	67.09	67.09	68.09	68.09
Water	25	25	25	25	25	25	25	25	25	25
Sodium chloride	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4	1.4
Sodium tripolyphosphate	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Sodium erythorbate	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045	0.045
Sodium nitrite	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015	0.015
White pepper	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Ground nutmeg	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Fish oil	5	5	5	5	5	5	5	5	5	–
Beef tallow	–	–	–	–	–	–	–	–	–	5
Additive according to formulation code[a]	1	1	1	1	1	1	1	1	–	–

[a] Formulation code: milk proteins concentrate (MPr), whey protein concentrate (WPC), modified whey protein concentrate (WPCmod), dry egg white (EW), hydroxypropylmethylcellulose (HPMC), methylcellulose (MC), 2:1 κ:ι-carrageenans (Carr), 2:1 xanthan:locust bean gums (XL), control formulation with fish oil (CO), and control formulation with fat (CF).

composition, NaCl content). It makes difficult to compare, in diverse systems, the effect of each additive on different properties such as process yield, weight lost by centrifugation, color, textural and viscoelastic characteristics. For example, a positive correlation of water holding capacity with carrageenan added to low-fat sausages has been described (Verbeken, Neirinck, Van Der Meeren, & Dewettinck, 2005); conversely, it has also been informed that the addition of carrageenan seems to have no or a very limited effect on the water holding capacity of meat gels (Bernal, Smajda, Smith, & Stanley, 1987; Foegeding & Ramsey, 1987; Barbut & Mittal, 1992). Lurueña-Martínez, Vivar-Quintana, and Revilla (2004) showed that the addition of locust bean/xanthan gum to low-fat frankfurters resulted in an increase in process yield while the addition of methylcellulose increased weight losses with respect to a control (Foegeding & Ramsey, 1986).

We selected several frequently used non-meat proteins and hydrocolloids to compare their effect on low-fat meat sausages with pre-emulsified fish oil. The same amount of additive was incorporated in each case, maintaining the rest of the formulation and processing conditions constant. The additives chosen were: milk proteins, whey protein concentrate, modified whey protein concentrate, ovalbumin, hydroxypropylmethylcellulose, methylcellulose, a mixture of 2κ:1ι carrageenans, and a mixture of 2:1 xanthan-locust bean gums. Two control formulations containing fish oil or solid beef tallow fat without any binder added were included in the study. Our objective was to compare emulsion stability during thermal treatment and textural characteristics of the different cooked products with both control formulations. Temperature and frequency sweep experiments and the mathematical modeling of the results allowed us to characterize the rheological behavior of the systems. The non-frozen water fraction, glass transition temperature, and thermal denaturation of collagen and myofibrillar-proteins were also studied using modulated differential scanning calorimetry.

2. Materials and methods

Low-fat sausages were prepared using fresh lean beef meat (*adductor femoris* and *semimembranosus muscles*) obtained from local processors (pH: 5.48 ± 0.01; total lipids: 1.3 ± 0.17 g/100 g). Meat (9 kg, four muscles) without visible fat and connective tissue was passed through a grinder with a 0.95 cm plate (Meifa 32, Buenos Aires, Argentina). Lots of approximately 500 g were vacuum packed in Cryovac BB4L bags (PO_2: 0.35 $cm^3\ m^{-2}\ day^{-1}\ kPa^{-1}$) at 23 °C, Sealed Air Co., Buenos Aires, Argentina), frozen, and stored at −20 °C until used (no more than three weeks, Ayo, Carballo, & Jiménez-Colmenero, 2005).

As fat sources, commercial beef tallow or deodorized refined fish oil containing 0.1 g of synthetic vitamin E/100 g (24.17 g/100 g *n-3* PUFA, Omega Sur S.A., Mar del Plata, Argentina) was used. As stabilizer or emulsifier agents eight food-grade commercial preparations of non-meat proteins or hydrocolloids were used: milk proteins concentrate by ultrafiltration (MPr, Milkaut, Santa Fé, Argentina), whey protein concentrate (WPC, Lacprodan, Arla Foods Ingredients S.A., Martínez, Argentina), modified whey protein concentrate (WPCmod, Nutrilac, Arla Foods Ingredients S.A., Martínez, Argentina), dry egg white (EW, Tecnovo SA, Entre Ríos, Argentina), hydroxypropylmethylcellulose (HPMC, Droguería Saporiti, Argentina), methylcellulose (MC, Droguería Saporiti, Argentina), a 2:1 κ:ι carrageenans mixture (Carr, Fluka BioChemika, Denmark), and 2:1 xanthan:locust bean gums (XL, Sigma–Aldrich, St. Louis, MO).

Cold distilled water was used in all formulations (4 °C). Analytical grade sodium chloride, sodium nitrite, sodium erythorbate and sodium tripolyphosphate (STPP) were employed. The concentration of sodium nitrite was selected according to the level permitted by the Argentinean Regulations (0.015 g/100 g; CAA, 1996, chap. 6).

2.1. Sausage formulation and processing

Ten different formulations of low-fat sausages were manufactured according to Table 1 in duplicate (two trials each). Eight formulations contained 5 g/100 g of fish oil and 1 g/100 g of either non-meat proteins or hydrocolloids were prepared. Also, two control formulations which did not contain stabilizers or emulsifiers were prepared with 5 g/100 g beef tallow or fish oil (CF and CO, respectively), replacing the additive with ground beef.

Meat packages were thawed (approximately 18 h at 4 °C) before further processing. For each trial, thawed meat was homogenized and grounded in a commercial food processor (Universo, Rowenta, Germany) equipped with a 14 cm blade for 5 min at the highest speed. NaCl and STPP were added to the ground meat and processing continued for 2 min.

Proteins or hydrocolloids were dissolved in cold water and then homogenized with fish oil using a hand-held food processor (Braun, Buenos Aires, Argentina) during 2 min to form a coarse emulsion. The obtained emulsion and the rest of dry ingredients (condiments, $NaNO_2$, sodium erythorbate) were added to the ground meat, processing all ingredients during 5 min afterward. Final temperature of batters varied between 12 and 15 °C. Control formulations with fish oil (CO) sausages were made without proteins or hydrocolloids in an equivalent procedure.

For manufacturing control formulation with fat (CF), dry ingredients were slowly added to the ground meat while processing.

Afterward cold water (25 g/100 g) was incorporated and finally ground beef tallow at room temperature was added.

Batters were immediately stuffed with a vertical piston stuffer (Santini s.n.c., Marostica, Italy) into cellulose casing (22 mm diameter, Farmesa, Buenos Aires, Argentina) and hand-linked to form approximately 8 cm links in length. Sausages were placed in "cook-in" bags (3 or 4 sausages per bag) (Cryovac CN510, Sealed Air Co., Buenos Aires, Argentina) and heat-processed in a temperature-controlled water-bath (Haake L, Haake Buchler Instruments, Karlsruhe, Germany) maintained at 80 °C until a final internal temperature of 74 °C was reached. Temperature was monitored by a type-T (copper-constantan) thermocouple inserted in the center of a link, and connected to a data acquisition system (TESTO175, TESTO AG, Lenzkirch, Germany). Then, samples in the bags were cooled immediately in an ice-water-bath and stored at 4 °C until further analysis.

A portion of raw batter was separated from each trial in order to analyze the non-frozen water fraction and thermal denaturation of collagen and myofibrillar-proteins by modulated differential scanning calorimetry and viscoelastic characteristics of each type of batter by temperature sweeps in a controlled stress rheometer.

For dynamic rheological measurements on cooked batters an alternative heating method was performed (Ferris, Sandoval, Barreiro, Sánchez, & Müller, 2009). Raw batter was placed in containers which consisted of two aluminum flat slabs (0.8 mm thick) assimilated to infinite slab geometry (1.4 mm thickness, 100 mm wide and 120 mm length) secured by bolts and nuts to provide a hermetic seal. They were heated from both sides inside "cook-in" bags (Cryovac CN510, Sealed Air Co., Buenos Aires, Argentina), by immersing the containers in the same 80 °C temperature-controlled-water-bath. Due to the small product thickness and the high thermal conductivity of aluminum, heating of the confined sample took place very fast. The time to reach the processing temperature (74 °C) was measured with a type-T (copper-constantan) thermocouple inserted in the center of the plates. In the same way, boundary effects were also negligible due to the dimensions involved. Samples (1 mm thick and 35 mm diameter) were cut from the slabs to perform the rheological assays with a suitable cork borer, in this way samples fitted exactly the 35 mm parallel plates.

2.2. Physicochemical analysis

Moisture, ash and protein contents of sausages were determined according to the AOAC methods 24.003, 24.009, and 24.027, respectively, $n = 3$/formulation/trial. Fat content was determined on samples previously dried with anhydrous sodium sulfate (SO_4Na_2) by Soxhlet method, using petroleum ether (Bp: 35–60 °C) as extraction solvent (Andrés et al., 2009), ($n = 3$/formulation/trial).

2.3. Process yield

Process yield was determined by weighting the product before and after thermal treatment, and expressed as g/100 g of initial sample weight (Candogan & Kolsarici, 2003; Andrés et al., 2009). Four sausages were measured for each trial.

2.4. Weight loss by centrifugation (WLC)

A modified method was performed to evaluate the stability of the matrices (Eide, Børresen, & Strøm, 1982). Eight cylindrical sausage samples corresponding to each trial were weighted (approximately 0.4 g) and placed in Falcon tubes over glass beads and were centrifuged at 12,000 × g for 5 min at 4 °C. Liquids removed during centrifugations drained through the glass beads and were collected in the bottom of the centrifuge tube (Eide et al., 1982). Weight loss by centrifugation (WLC) was calculated as grams of liquid extracted from 100 g of sausage ($n = 8$/formulation/trial).

2.5. Color

Color was measured at room temperature on the surface of transversally cut slices of sausages using a Chroma Meter CR-400 colorimeter (Minolta Co., Ramsey, New Jersey, USA); CIE-LAB parameters (lightness, L, redness, a^*, and yellowness, b^*) were determined ($n = 5$/formulation/trial). The aperture size was 8 mm and a *C, D65 illuminant was employed.

2.6. Texture profile analysis

Texture Profile Analysis (TPA) was performed on chilled (4 °C) sausages (Bourne, 1978; Brennan & Bourne, 1994). Samples (1.5 cm thick and 1.7 cm diameter) were cut from the center of the links and compressed twice to 30 g/100 g of their original height between flat plates using a TAXT2i Texture Analyzer (Stable Micro Systems, UK) interfaced with a computer, using the software supplied by Texture Technologies Corp. In these experiments the head was operated at 0.5 mm/s.

Hardness (peak force of first compression cycle, N), cohesiveness (ratio of positive areas of second cycle to area of first cycle, J/J, dimensionless), adhesiveness (negative force area of the first byte represented the work necessary to pull the compressing plunger away from the sample, J), chewiness (hardness × cohesiveness × springiness, N), springiness (distance of the detected height of the product on the second compression divided by the original compression distance, mm/mm, dimensionless) and resilience (area during the withdrawal of the first compression divided by the area of the first compression, J/J, dimensionless) were determined ($n = 10$/formulation/trial).

2.7. Thermal analysis by modulated differential scanning calorimetry

Modulated differential scanning calorimetry (MDSC) was performed on a Q100 modulated DSC (TA Instruments, USA). Raw batters were accurately weighted (approximately 12 mg) and sealed in hermetical aluminum pans. An empty sample pan was used as reference. Duplicate samples were annealed in order to allow ice crystallization according to the following protocol: samples were cooled to −50 °C, heated to −25 °C at 5 °C/min, equilibrated at −25 °C for 3 min, cooled to −50 °C at 10 °C/min, and then scanned from −50 °C to 120 °C at a heating rate of 5 °C/min, with a modulation ±1 °C every 60 s ($n = 2$/formulation/trial). After each MDSC run a pinhole was made on the cover of every sample and dried at 105 °C for water content determination (Fernández-Martín, Cofrades, Carballo, & Jiménez-Colmenero, 2002).

Enthalpies (ΔH), expressed as $J/g_{protein}$, associated with the melting of water and protein denaturation were determined by integrating the area under the MDSC curve, using software for thermal analysis (TA Instruments Universal Analysis 2000; TA Instruments, USA), and dividing this area by the weight of the sample. A sigmoidal baseline was used to calculate peak areas and compensate changes in specific heat that occurs when water changes from solid to liquid state (Bourne, 1978). Peak transition temperatures were also determined from MDSC thermograms.

The weight fraction of freezable water was evaluated from the water melting peak (Roos, 1978). Appropriate enthalpy changes integrated from −40 °C to 20 °C were evaluated for meat batters (ΔH_{mb}) and pure water (ΔH_w), and then the frozen water content was calculated as follows:

$$n_w = \frac{\Delta H_{mb}}{\Delta H_w} \quad (1)$$

The non-frozen water fraction relative to the total water content was evaluated as (Anese & Gormley, 1996):

$$x_{\text{non-frozen water}} = \frac{n_{w0} - n_w}{n_{w0}} \quad (2)$$

where n_{w0} is the total water content.

The first derivative of the thermogram was used to determine the temperature at the inflection point which was assigned to the glass transition temperature (T_g) of the sample.

2.8. Rheological analysis

All rheological measurements were performed using a controlled stress rheometer (Haake RS600, Thermoelectron, Germany) provided with a temperature control unit (K-15 Haake, Thermoelectron, Germany). After positioning the sample on the sensor system, it was allowed to rest for 10 min before starting the corresponding measurement. Samples perimeters were covered with a thin film of silicone oil and the measuring system was covered with a special device to prevent evaporation during the measurements ($n = 3$/formulation/trial).

Temperature sweeps. Raw batter was placed between serrated parallel plates (35 mm diameter, 1 mm gap). After equilibration at 25 ± 1 °C, they were sheared at a fixed frequency of 6.28 rad/s with a stress of 5.0 Pa while the temperature was increased to 70 °C at 1 °C/min in small deformation oscillatory mode. Changes in the dynamic storage modulus, G′ (Pa), loss modulus, G″, and phase angle (δ) were monitored continuously throughout the simulated gelling process at 6.28 rad/s (1 Hz) frequency. The stress value used assured that all measurements were performed within the linear viscoelastic range which has been previously determined at 25 °C and 70 °C. The thermo-rheograms presented correspond to mean values of two replicates per formulation and trial, given a total of 4 samples.

Frequency sweeps: Pre-cooked samples (35 mm diameter, 1.4 mm height) were used to study their rheological behavior in oscillatory shear using serrated parallel plates (35 mm diameter, 1.4 mm gap). To determine the limit of linear viscoelastic region, dynamics tests were performed at fixed frequency (6.28 rad/s) and amplitude of the stress σ was stepwise increased. The stress used (5 Pa) was found to be within the linear viscoelastic region of all of the samples measured at 6.28 rad/s. Storage modulus (G′), loss modulus (G″), and tan δ were recorded in the frequency range of 0.0628–62.8 rad/s. Temperature was maintained at 25 °C throughout the experiment.

2.9. Statistical analysis

Analyses of variance were conducted separately on the dependent variables studied considering each formulation as a level in a one-way factorial design. Two complete replicates (trials) per formulation were analyzed to verify reproducibility of the experimental procedure. For simultaneous pairwise comparisons, least significance differences (LSD) test was chosen. Differences in means and *F*-tests were considered significant when $P < 0.05$. All statistical procedures were computed using the SYSTAT software (SYSTAT, Inc., Evanston, IL). Experimental data were reported as mean values.

3. Results and discussion

No significant differences were found between the two replicates (trials) analyzed for each formulation, so it was concluded that the procedure to prepare the sausages was adequate.

3.1. Composition, yield and emulsion stability

Chemical compositions of sausages with different additives are shown in Table 2, where some significant differences were found. Low salt addition to the batters (1.4 g of NaCl/100 g) produced low ash levels. Lipid content reflected the amount of fat or oil added plus the lipids contained in the meat, indicating that fat losses occurred during processing were low, thus the obtained emulsions were stable. Protein contents were higher than 12g/100 g product, and due to their origin (beef muscle, egg or dairy products), they were of high biological value.

Protein contents were higher in those formulations with added protein, as expected, with no significant differences among them ($P > 0.05$). However products containing HPMC presented similar high protein levels probably as a result of their lower moisture content and process yield ($P < 0.05$) (Table 2). Besides lipid, protein, and ash levels were higher in this formulation.

The incorporation of fish oil, instead of animal fat, offers a new healthy alternative to the consumer but has its challenges in terms of keeping this liquid oil within the meat product. Process yield depends on the ability of the protein matrix to immobilize both fat and water. However, in meat batters with extremely low-fat content, gelling ability and water retention capacity of non-meat ingredients may play a greater role rather than emulsion formation in determining thermal and storage stability of the products (Su, Bowers, & Zayas, 2002). In our study process yields ranged between 80.9 and 98.5 g/100 g (Table 2). Control formulation with fish oil (CO) had slightly lower process yield than control formulation with fat (CF) in spite of their similar water contents. This suggests that the difference in process yield might be attributed to

Table 2
Proximal composition, process yield, and weight loss by centrifugation (WLC) of different sausages formulations.

Formulation code*	Moisture g/100 g	Lipid g/100 g	Protein g/100 g	Ash g/100 g	Process yield g/100 g	WLC g/100 g
WPC	75.0^{b} (0.05)	6.0^{b} (0.02)	13.5^{a} (0.12)	2.6^{ab} (0.02)	98.5^{a} (0.02)	23.6^{bcde} (0.18)
WPCmod	74.2^{cd} (0.11)	6.2^{ab} (0.07)	13.4^{a} (0.07)	2.2^{b} (0.01)	98.1^{a} (0.08)	24.5^{bcd} (0.17)
MPr	74.4^{bcd} (0.08)	6.2^{ab} (0.09)	13.3^{ab} (0.08)	2.2^{b} (0.04)	98.1^{a} (0.21)	22.9^{def} (0.15)
EW	74.1^{d} (0.13)	6.0^{b} (0.04)	13.5^{a} (0.06)	2.4^{ab} (0.09)	98.2^{a} (0.15)	25.5^{bc} (0.23)
HPMC	72.0^{e} (0.14)	6.6^{a} (0.02)	13.4^{a} (0.09)	2.9^{a} (0.10)	80.9^{c} (0.08)	15.1^{g} (0.14)
MC	74.3^{cd} (0.17)	6.2^{ab} (0.10)	12.2^{c} (0.05)	2.3^{ab} (0.08)	96.7^{b} (0.10)	3.8^{i} (0.21)
XL	74.9^{bc} (0.12)	6.0^{b} (0.04)	12.4^{c} (0.10)	2.3^{ab} (0.07)	98.1^{a} (0.13)	7.5^{h} (0.19)
Carr	74.9^{b} (0.06)	6.1^{ab} (0.11)	12.2^{c} (0.05)	2.2^{b} (0.05)	97.9^{a} (0.08)	7.6^{h} (0.16)
CO	77.1^{a} (0.11)	6.0^{b} (0.04)	12.4^{c} (0.02)	2.2^{b} (0.01)	97.2^{b} (0.05)	28.0^{a} (0.19)
CF	76.7^{a} (0.16)	6.2^{ab} (0.12)	12.6^{bc} (0.15)	2.4^{ab} (0.10)	98.2^{a} (0.08)	26.0^{ab} (0.17)

*Formulation code according to Table 1. Means with same superscript within same column do not differ significantly ($P > 0.05$). SEM values were given between parentheses.

the formation of a less stable emulsion. Formulations with proteins (WPC, WPCmod, MPr, and EW), carrageenans (Carr), or XL gums produced yields similar to CF and higher than the control formulation with fish oil (97.2–98.5 g/100 g), indicating good thermal stability and water holding capacity of the meat emulsion matrix. These results were in agreement with Barbut and Choy (2007) who found that the use of dry whole milk, skimmed milk, caseinate, regular and modified whey, at 2 g/100 g level in a chicken breast meat system with 51 g/100 g water addition significantly reduced cooking loss compared with the control, with caseinate showing the best results. Atughonu, Zayas, Herald, and Harbers (1998) has also informed that whey protein concentrate and caseinate improved cook yield of beef and pork frankfurters.

The addition of cellulose derivatives (MC and HPMC) showed process yields similar or lower than CO, (96.7 and 80.9 g/100 g, respectively) in agreement with Foegeding and Ramsey (1986); of all the hydrocolloids assayed HPMC produced the lowest yield value (Table 2).

Although process yield averages showed small differences, liquid extracted from the systems varied considerably between formulations when force was exerted. The weight of liquid lost by centrifugation (WLC) ranged from 3.8 to 28 g liquid realeased/100 g, but it was not affected by the type of lipid used. The addition of hydrocolloids significantly affected weight losses by centrifugation as was expected from the known role of hydrocolloids in food systems. WLC values for products formulated with HPMC, MC, Carr, or XL were much lower than WLC of both controls, reflecting the improvement in good liquid retention when these additives are included in the matrix. MC produced the lowest WLC value, thus showing the highest capacity for liquid retention. A possible explanation, given in other study is that cellulose derivates enveloped the myofibrillar proteins thus reducing water holding capacity (Barbut & Mittal, 1996).

The HPMC formulation presented a liquid retention intermediate between proteins and hydrocolloids added sausages. HPMC sausages had a significantly lower process yield, thus after heat treatment the hydroxypropylmethylcellulose was more concentrated than the gums or non-meat proteins in the other formulations. Moisture for HPMC formulation was 72 g/100 g while the rest of the samples showed moisture values near 74.5 g/100 g. Therefore the low result of weight loss by centrifugation for HPMC was not necessarily due to a successfully integration of the hydrocolloids or an increase of the interactions between water and the network, but to a more concentrated matrix.

On the other hand, formulations with dairy proteins showed slightly lower values of WLC than CO sausages and similar to the CF products. From weight loss by centrifugation results it seems that the presence of caseins besides whey proteins in MPr formulations increases liquid retention by the matrix. There were no differences in WLC between WPC and WPCmod formulations although in a previous work (Marchetti, Andrés, & Califano, 2010) it was found that WPCmod proteins showed nearly half the solubility of WPC and lower denaturation enthalpy (by MDSC) than WPC (1.02 J/g and 2.24 J/g for WPCmod and WPC, respectively).

Egg proteins have no part in formation of protein structure and hence provide very little technological advantage; moreover it was reported that up to 3 g egg white/100 g had no influence on water- and fat-binding properties of pork meat batters (Hammer, 1992; Carballo, Barreto, & Jiménez-Colmenero, 1995). Our results agree with these authors, showing that the addition of egg white protein produced weight losses by centrifugation similar to the control formulation with fat.

The obtained results indicate that hydrocolloids have a higher capacity to bind water and restrict their molecular mobilities even under more drastic conditions, compared to the non-meat proteins selected (MPr, WPCmod, WPC, and EW).

3.2. Modulated differential scanning calorimetry (MDSC)

The thermograms of all the formulations assayed in this work, showed the characteristic water melting peak between −1 and −3 °C and the protein denaturation transitions in the range of 50–67 °C.

Although it is known that the effective latent heat of ice melting decreases with increasing concentration of the solution, the amount of the decrease depends on the solute. However this enthalpy variation in diluted solutions is small: for example in the case of NaCl solution as the concentration of solute increases from 0 to 5 g/100 g, the enthalpy decreases only from 333.9 J/kg to 330 J/kg (lower than 1 g/100 g, Kumano, Tatsunori, Akio, & Seiji, 2007). Therefore in Eq. (1) it was assumed for the calculations of the frozen water fraction the enthalpy of pure water.

Biopolymers addition produced an increase of non-frozen water content (Table 3). Some of the formulations (Carr, XL, MPr, and MC) doubled or tripled controls values. Water-polymer interaction affected water dynamics and decreased water mobility in the matrix (Hills, 2007). A similar effect produced by addition of different milk proteins to minced fish has been observed (Tsai, Unklesbay, Unklesbay, & Clarke, 1998). The highest values of non-frozen water corresponded to the sausages with hydrocolloids (except HPMC). The addition of non-meat proteins resulted in intermediate values of unfrozen water. The stronger water-hydrocolloid (Carr, MC, XL) interaction that results in higher non-frozen water content explains that process yields were higher than those obtained with non-meat proteins.

Endothermic events of meat proteins are typically grouped in three regions, with peak temperatures around 54–58 °C related to myosin (region I), 65–67 °C collagen and sarcoplasmic proteins (region II), and 80–83 °C related to actin (region III) (Martens & Vold, 1976; Wright, Leach, & Wilding, 1977; Tornberg, 2005). However

Table 3
Thermal parameters obtained from DSC analysis of sausages formulations.

Formulation code*	Non-frozen water (g/100 g total water)	T I (°C)	T II (°C)	ΔH denaturation ($J/g_{protein}$)	Glass transition temperature (°C)
WPC	19.34^{c} (0.23)	52.0^{b} (0.05)	66.1^{cd} (0.07)	8.92^{b} (0.02)	52.7^{cd} (0.02)
WPCmod	17.30^{de} (0.18)	51.6^{ab} (0.16)	65.4^{b} (0.26)	9.72^{a} (0.09)	52.3^{cd} (0.2)
MPr	22.92^{b} (0.14)	52.8^{c} (0.001)	66.6^{de} (0.12)	7.11^{c} (0.11)	53.4^{bc} (0.03)
EW	17.83^{d} (0.14)	52.3^{bc} (0.001)	66.2^{d} (0.05)	6.98^{c} (0.01)	53.2^{bc} (0.02)
HPMC	16.10^{e} (0.18)	51.0^{a} (0.02)	65.6^{bc} (0.17)	9.69^{a} (0.01)	51.7^{de} (0.3)
MC	23.93^{b} (0.21)	52.6^{c} (0.19)	66.6^{de} (0.16)	7.27^{c} (0.03)	53.3^{bc} (0.02)
XL	26.28^{a} (0.19)	53.3^{d} (0.13)	66.7^{e} (0.05)	6.90^{c} (0.03)	56.2° (0.7)
Carr	26.86^{a} (0.18)	54.5^{e} (0.03)	67.0^{e} (0.08)	7.57^{c} (0.07)	54.3^{b} (0.07)
CO	13.70^{f} (0.21)	51.6^{ab} (0.11)	64.5^{a} (0.05)	9.75^{a} (0.2)	51.1^{e} (0.08)
CF	8.74^{g} (0.17)	50.9^{a} (0.04)	64.7^{ab} (0.02)	9.87^{a} (0.05)	50.8^{e} (0.02)

*Formulation code according to Table 1. Means with same superscript within same column do not differ significantly ($P > 0.05$). SEM values were given between parentheses.

salts (NaCl, STPP) added to meat comminution can decrease the myofibrillar-proteins thermal stability, making the myosin and actin converge to the same denaturation temperature, evolving into practically a single endotherm (shoulder for the most thermolabile myosin fraction at ~52 °C) (Fernández-Martín, López-López, Cofrades, & Jiménez-Colmenero, 2009). Fig. 1 depicts the DSC traces obtained for the different sausages formulations. A complex endotherm appeared in the protein denaturation zone, which comprised several overlapping endothermic events typically observed in comminuted meat systems with added salts. Peaks II and III were contracted to one peak that according to the literature corresponds mainly to actin thermal transition (Kijowski & Mast, 1988; Graiver, Pinotti, Califano, & Zaritzky, 2006). These changes could be attributed to a partial breakdown of the myosin, indicating disassociation of the myosin heavy chain (Thorarinsdottir, Arason, Geirsdottir, Bogason, & Kristbergsson, 2002). As both peaks were very close, only the total enthalpy of transitions for the protein zone was calculated (Table 3). All the batters exhibited similar MDSC patterns. The different biopolymers incorporated to the formulations decreased water mobility of the systems producing changes in thermodynamic properties such as T_g, TI, and TII. The addition of some proteins (MPr, WPC, and EW) or hydrocolloids (Carr, XL, and MC) to the formulation with fish oil decreased the total enthalpy of protein denaturation and raised both peaks temperatures compared to the controls. Peak temperatures varied significantly ($P < 0.05$) for the formulations studied because of the different amounts of water available for the denaturation process (Table 3). When less water was available (increasing non-frozen water fraction) higher denaturation temperatures were observed (R^2 for TI = 0.89, $P < 0.05$ and R^2 for TII = 0.93, $P < 0.05$).

A subtle glass transitions in the reversing MDSC traces could also be observed. Most of the formulations presented a glass transition at a significantly higher temperature ($P < 0.05$) than both controls (Table 3). It has been previously reported higher T_g values for meat batters with olive oil and Sea Spaghetti seaweed added respect to the control (Fernández-Martín et al., 2009). When less water is available to act as plasticizer because it is "bound" by the added biopolymers, T_g increased. A positive correlation was found between T_g and the non-frozen water content (Fig. 2a, $R^2 = 0.81$, $P < 0.05$). The differences in the amount of non-frozen water explain the results obtained for process yield and weight of liquid loss by centrifugation. When the fraction of non-frozen water increased, there was a stronger biopolymer–water interaction, thus less water was exuded during thermal treatment (higher process yield or less liquid released by centrifugation), Fig. 2b.

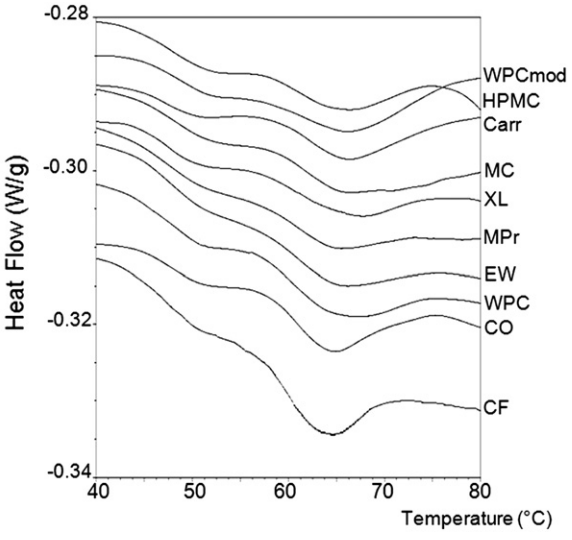

Fig. 1. DSC thermograms corresponding to heat denaturation of proteins for the different formulations studied (Exo up). Codes: whey protein concentrate (WPC), modified whey protein concentrate (WPCmod), milk proteins concentrate (MPr), dry egg white (EW), hydroxypropylmethylcellulose (HPMC), methylcellulose (MC), κ/ι carrageenans mixture (Carr), xanthan/locust bean gums (XL), control formulation with fat (CF) and control formulation with fish oil (CO).

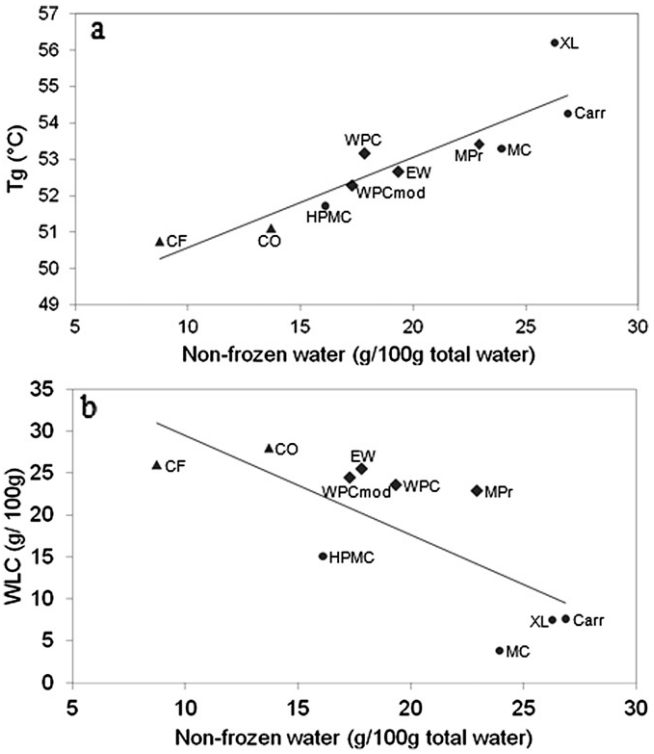

Fig. 2. Effect of non-frozen water content on: a) glass transition temperatures (T_g) of the meat batters, $R^2 = 0.81$, b) Weight loss by centrifugation (WLC). Codes: Whey protein concentrate (WPC), modified whey protein concentrate (WPCmod), milk proteins concentrate (MPr), dry egg white (EW), hydroxypropylmethylcellulose (HPMC), methylcellulose (MC), κ/ι carrageenans mixture (Carr), xanthan/locust bean gums (XL), control formulation with fat (CF) and control formulation with fish oil (CO).

3.3. Oscillatory dynamic experiments

Temperature sweeps were used to observe behavior upon heating since they are useful for determining the nature of protein matrix without damaging it (Tunick, 2011). Initially, G′ was always higher than G″ and loss tangent (tan δ = G″/G′) less than 0.25, showing a predominantly elastic behavior (Fig. 4), characteristic of weak gels even for the raw batters (Ross-Murphy, 1984). In this type of product, the macroscopic changes observed due to heat treatment are related to the effect of temperature on the constituents of the emulsion (Carp & Pilosof, 2007). As temperature increased bond-making led to structural changes that affected rheological properties increasing elastic moduli and decreasing tan δ. Gelation during heating (at 1 °C/min) characterized by the change in storage modulus (G′) is presented in Fig. 3. The curves showed a typical behavior of thermal gelation of meat systems where the main component corresponds to denaturation of myosin (Tornberg, 2005; Westphalen, Briggs, & Lonergan, 2005). Gel points were determined by locating the temperature at which tan δ was independent of frequency; denaturation temperatures observed by temperature sweeps appeared in the same range (53–58 °C) of those found by MDSC. Elasticity continued to develop during the heating period beyond gel point, corresponding to a progressive reinforcement of the gel network by addition of more molecules or

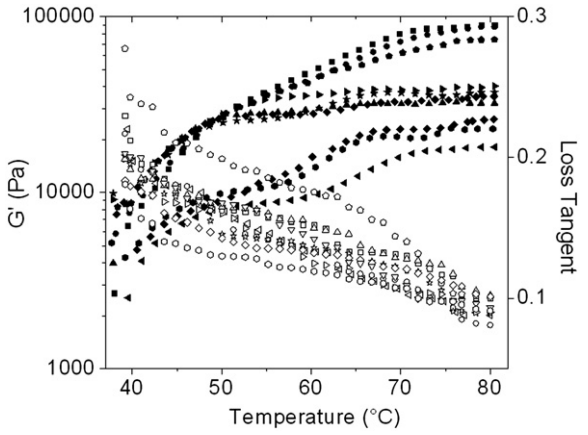

Fig. 3. Gelation curves representing the change in elastic modulus (G′, solid symbols) and loss tangent (open symbols) with temperature for: Whey protein concentrate (WPC, ►▷), modified whey protein concentrate (WPCmod, ★Ψ), milk proteins concentrate (MPr, ⬟□), dry egg white (EW, ▼▽), κ/ι carrageenans mixture (Carr, ■□), hydroxypropylmethylcellulose (HPMC, ⬢ν), methylcellulose (MC, ▲△), xanthan/locust bean gums (XL, ●○), control formulation with fat (CF,◆◇), and control formulation with fish oil (CO, ◄◁).

alterations within the network (Çakır, E. Allen Foegeding, 2011) and all the formulations reached the maximum G′ value above 75 °C. Table 4 shows the average maximum elastic moduli (G′) obtained.

Frequency sweeps experiments of the cooked batters at 25 °C showed that they behaved as viscoelastic solids (Fig. 4). Storage and loss moduli evolution with frequency was extremely dependent on macromolecule nature used as binder. Storage or elastic modulus (G′) had greater values than loss or viscous modulus (G″) in the tested frequency range, with a minimum of the latter at intermediate frequencies and a plateau region in G′. Loss tangent in the plateau zone presented values between 0.08 and 0.25, which corresponds to a gel-like behavior (Mours & Winter, 2000). Formulations containing carrageenans mixture or xanthan-locust bean gums showed the highest elastic moduli, reflecting the formation of an important three-dimensional network with even more solid-like characteristics than formulation containing beef tallow (CF); milk proteins concentrate addition resulted in products with similar rheological characteristics to CF; on the other hand, the

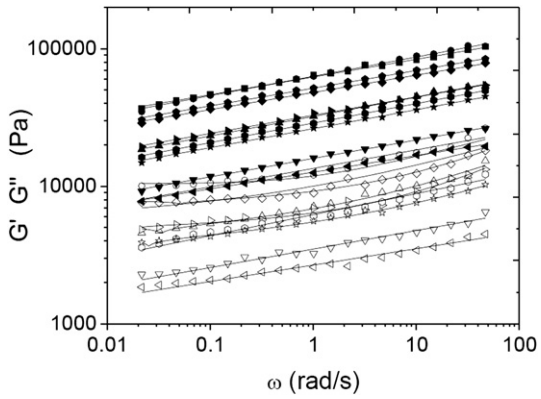

Fig. 4. Frequency dependence of storage (G′, solid symbols) and loss (G″, open symbols) moduli at 25 °C for the different thermally treated meat batters. (WPC, ►▷), modified whey protein concentrate (WPCmod, ★Ψ), milk proteins concentrate (MPr, ⬟□), dry egg white (EW, ▼▽), κ/ι carrageenans mixture (Carr, ■□), hydroxypropylmethylcellulose (HPMC, ⬢ν), methylcellulose (MC, ▲△), xanthan/locust bean gums (XL, ●○), control formulation with fat (CF, ◆◇), and control formulation with fish oil (CO, ◄◁). Solid lines (——) correspond to the predictions calculated from the broadened Baumgärtel, Schausberger, and Winter model.

Table 4
Mechanical and thermal parameters obtained from rheological and thermo-rheological analysis of sausages formulations.

Formulation code*	Plateau modulus (Pa)	Elastic modulus transition temperature (°C)	Maximum elastic moduli** (Pa)
WPC	20513^{de} (25)	56.1^{c} (0.3)	39794^{c} (9)
WPCmod	18260^{e} (10)	57.1^{b} (0.2)	36646^{d} (13)
MPr	26533^{cd} (16)	56.2^{c} (0.3)	73638^{b} (13)
EW	19663^{e} (30)	56.5^{bc} (0.1)	35074^{d} (10)
HPMC	12677^{f} (16)	55.9^{c} (0.08)	23028^{de} (7)
MC	21255^{de} (19)	53.8^{d} (0.1)	32367^{e} (9)
XL	37543^{a} (27)	58.3^{a} (0.2)	86084^{a} (22)
Carr	30100^{bc} (15)	57.8^{a} (0.3)	88800^{a} (21)
CO	7728^{g} (16)	56.4^{bc} (0.09)	17969^{g} (8)
CF	33443^{ab} (20)	55.9^{c} (0.2)	25677^{f} (10)

*Formulation code according to Table 1.
**Maximum elastic moduli (G′) was obtained from thermo-rheological temperature sweeps at 6.28 rad/s (1 Hz).
Means with same superscript within same column do not differ significantly ($P > 0.05$). SEM values were given between parentheses.

rest of the non-meat protein formulations resulted in less elastic systems, although always less viscous than CO.

By comparing Figs. 3 and 4, and Table 4, it can be seen the effect that cooling had on the cooked batters. Batters prepared with beef tallow (CF) showed a low value of G′ at the end of the heating zone, when fat was melted, while frequency sweeps of the cooked batter at 25 °C showed a significant increase of the elastic modulus (from 25570 Pa to 48640 Pa). G″ at 25 °C of CF sausages was as high as carrageenans formulations due to fat solidification. The rest of the formulations showed less marked changes due to cooling which, besides the expected relationship between rheological properties and temperature, may be attributed to the formation of additional non-covalent interactions among denatured proteins such as gelation of collagen.

3.4. Rheological data analysis

Although food products like sausages are complex matrices, they could be described with a certain approximation on the base of simplified models. The mechanical properties of the sausages might be described by certain response functions of the material also known as constitutive equations or rheological equations of state (Tschoegel, 1997; Andrés, Zaritzky, & Califano, 2008; Ranalli, Andrés, & Califano, 2012). These response functions can be evaluated by fitting a mathematical model to dynamic oscillatory experiments obtained within the linear state. G′ and G″ contains all the information needed for calculation of the relaxation time spectrum ($H(\lambda)$) which cannot be measured directly. In the range of small deformations, polymeric materials are expected to be characterized by a unique relaxation time spectrum, $H(\lambda)$. If the system can be thought as infinite Maxwell elements (spring and dashpot in series), it is possible to define a continuous relaxation spectrum of the material as a function of time. The expressions that relate the storage modulus (G′) and loss modulus (G″) with $H(\lambda)$ are as follows (Ferry, 1980, chap. 4)):

$$G'(\omega) = \int_0^{\lambda_{max}} H(\lambda) \frac{\omega^2\lambda^2}{1+\omega^2\lambda^2} \frac{d\lambda}{\lambda} \quad (3)$$

$$G''(\omega) = \int_0^{\lambda_{max}} H(\lambda) \frac{\omega\lambda}{1+\omega^2\lambda^2} \frac{d\lambda}{\lambda} \quad (4)$$

where ω corresponds to the applied angular frequency and λ are the relaxation times of the material.

Thus using an appropriate representation of the relaxation time spectrum, it is possible to model the dynamic moduli. The broadened Baumgärtel, Schausberger, and Winter (BSW) spectrum (Eq. (5)) describes experimental frequency sweep data (G′, G″) corresponding to linear, long, flexible molecules, with a broad distribution of molecular weights. The spectrum has the following form (Baumgärtel & Winter, 1989):

$$H(\lambda) = G_N^0\left[A\left(\frac{\lambda}{\lambda_0}\right)^{-n_0} + n_e\left(\frac{\lambda}{\lambda_e}\right)^{n_e}\right]\exp\left[-\left(\frac{\lambda}{\lambda_{\max}}\right)^{\beta}\right]\text{for} \quad \lambda \le \lambda_e H(\lambda) = 0 \quad \text{for} \quad \lambda > \lambda_e \tag{5}$$

where G_N^0 is the plateau modulus, n_e and n_0 are the slopes of the spectrum in the entanglement and high frequency glass transition regime, respectively, A is the glass transition front factor, and λ_e is the relaxation time corresponding to the beginning of the pseudo-terminal region. The exponent β controls the sharpness of the cut-off of the spectrum, $\lambda_{\max}$ is the longest relaxation time, and λ_0 is the crossover time to the glass transition.

In the present work, a non-linear method – the so-called IRIS method- was used to invert the integrals for G′ and G″ (Eqs. (3) and (4)) simultaneously, using the Rheo-Hub software IRIS (IRIS Development LLC, MA, USA) (Baumgärtel, De Rosa, Machado, Masse, & Winter, 1992; Baumgärtel, Schausberger, & Winter, 1990; Baumgärtel & Winter, 1989, 1992; Winter, 1997).

Fig. 4 shows the excellent fit of the predicted moduli to the experimental data.

One of the parameters of the regressed model is the plateau modulus (G_N^0) which reflects the molecular architecture of the polymers. It is proportional to the number of entanglements per unit volume and inversely proportional to the average molecular weight of the molecular segment between entanglements (Flory, 1953, chap. 9).

The plateau moduli predicted by the broadened BSW model for the different formulations assayed are shown in Table 4. There was a good agreement with those calculated directly from dynamic experiments, since G_N^0 was also estimated from the minimum in the loss tangent as follows: $G_N^0 = [G']_{\tan\,\delta\,\rightarrow\,\text{minimum}}$ (Bais, Trevisan, Lapasin, Partal, & Gallegos, 2005; Baumgärtel et al., 1992).

Taking into account that the linear viscoelastic functions always showed qualitatively similar frequency dependence, an empirical superposition method was applied, using the inverse of the plateau modulus G_N^0 as normalization factors "a" ($a = 1/G_N^0$). The normalized dynamic master curves of the storage and loss moduli can be seen in Fig. 5. All data fell into a single master-curve in reasonable approximation. The fact that these master curves could be achieved by applying an empirical vertical superposition method implies that the different additives did not modify the overall microstructural pattern of systems, but mainly influenced the level of interactions among macromolecular components.

3.5. Color and texture

Tables 5 and 6 show the effect of different binders added to the sausages formulation on average color and TPA parameters of the products. Yellowness (b^*) was higher in all formulations respect to CF due to the incorporation of fish oil. It has been previously reported that replacing 20 g pork backfat/100 g with olive oil increased b^* values of sausages (Muguerza, Fista, Ansorena, Astiasaran, & Bloukas, 2002). Replacing beef tallow with fish oil produced sausages with higher L^* values, except when HPMC or MC was added (Table 5). Other authors found that fermented sausages with 10–20 g pork backfat/100 g replaced by olive oil were lighter and more yellow than controls (Bloukas, Paneras, & Fournitzis, 1997). Sausages with HPMC showed the lowest lightness and the highest redness values, which could be related to the lower amount of water present which imply more concentration of solids.

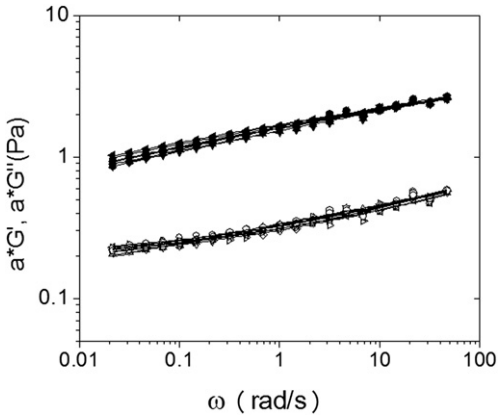

Fig. 5. Normalized dynamic master curves of the storage (G′, solid symbols) and loss (G″, open symbols) moduli, and their predictions by the broadened Baumgärtel, Schausberger, and Winter (BSW) model (solid lines), where "a" = $1/G_N^0$. Codes: (WPC, ►▷), modified whey protein concentrate (WPCmod, ★Ψ), milk proteins concentrate (MPr, ⬟□), dry egg white (EW, ▼▽), κ/ι carrageenans mixture (Carr, ■□), hydroxypropylmethylcellulose (HPMC, ⬟ν), methylcellulose (MC, ▲△), xanthan/locust bean gums (XL, ● ○), control formulation with fat (CF, ◆ ◇), and control formulation with fish oil (CO, ◄◁).

Fat replacement with fish oil had a strong impact on sausages texture. As it was expected, a reduction of 40.2% of hardness occurred when solid tallow fat was replaced by fish oil (Table 6). Nevertheless the incorporation of milk proteins concentrate, carrageenans, or xanthan-locust bean gums to the formulation with oil resulted in products with similar hardness to control formulation with fat ($P > 0.05$). The matrix was reinforced by their addition due to the gelling capacity of these biopolymers. The other additives produced lower hardness values than CF and slightly higher than CO ($P < 0.05$). HPMC and MC form thermo-reversible gels, thus, at room temperature they revert to the sol state. Although WPC also gels upon heating, the resulting gel strength is lower than for milk proteins because of the lack of caseins. Similar trends were obtained by other authors (Picone, Takeuchi, & Cunha, 2011).

A positive correlation was found between springiness and hardness ($R^2 = 0.86$, $P < 0.05$), as hardness increased the material became more elastic. Resilience which shows an instant elasticity of the material was not affected by proteins or hydrocolloids addition.

Chewiness, presented the same tendency as hardness.

Higher G′ values in the frequency sweep experiments indicated the formation of more covalent and non-covalent aggregates. Thus for Carr, MPr, and XL formulations higher amounts of aggregates produced a more cohesive matrix reflected in higher cohesiveness values obtained with TPA.

Table 5
Effects of non-meat protein or hydrocolloids addition on color parameters of sausages formulations.

Formulation code*	L^*	a^*	b^*
WPC	64.5^{bc} (0.08)	12.6^{c} (0.04)	11.4^{c} (0.02)
WPCmod	64.5^{bc} (0.09)	13.5^{b} (0.03)	11.7^{abc} (0.7)
MPr	66.0^{a} (0.05)	12.2^{c} (0.05)	11.0^{c} (0.05)
EW	65.1^{ab} (0.10)	12.7^{c} (0.04)	11.5^{bc} (0.6)
HPMC	58.7^{e} (0.05)	15.1^{a} (0.01)	11.3^{c} (0.08)
MC	61.9^{d} (0.07)	14.0^{b} (0.08)	12.4^{a} (0.04)
XL	64.8^{bc} (0.11)	13.7^{b} (0.06)	12.2^{ab} (0.04)
Carr	64.0^{c} (0.03)	13.6^{b} (0.06)	12.2^{ab} (0.04)
CO	63.4^{c} (0.07)	12.2^{c} (0.03)	11.7^{abc} (0.03)
CF	61.4^{d} (0.05)	14.0^{b} (0.02)	10.1^{d} (0.03)

*Formulation code according to Table 1. Means with same superscript within same column do not differ significantly ($P > 0.05$). SEM values were given between parentheses.

Table 6
Effect of non-meat protein or hydrocolloids addition on texture profile parameters of the assayed sausage formulations.

Formulation code*	Hardness (N)	Chewiness (N)	Cohesiveness (J/J)	Resilience (J/J)	Adhesiveness (Jx10^4)	Springiness (mm/mm)
WPC	6.44^{c} (0.10)	2.92^{bc} (0.04)	0.551^{cd} (0.010)	0.415^{b} (0.006)	0.59^{c} (0.2)	0.822^{cd} (0.005)
WPCmod	5.57^{fg} (0.08)	2.35^{bc} (0.05)	0.549^{cd} (0.010)	0.320^{c} 0.002)	0.47^{c} (0.5)	0.769^{ef} (0.010)
MPr	8.76^{b} (0.06)	4.19^{a} (0.02)	0.569^{a} (0.008)	0.413^{b} (0.010)	0.15^{c} (0.4)	0.841^{bc} (0.008)
EW	6.00^{de} (0.05)	2.66^{bc} (0.03)	0.548^{cd} (0.003)	0.403^{b} (0.009)	0.50^{c} (0.6)	0.791^{de} (0.015)
HPMC	5.82^{ef} (0.08)	2.38^{bc} (0.02)	0.543^{d} (0.002)	0.401^{b} (0.005)	3.70^{a} (0.8)	0.753^{fg} (0.012)
MC	6.45^{c} (0.04)	2.95^{b} (0.04)	0.551^{cd} (0.007)	0.446^{a} (0.004)	3.14^{a} (0.6)	0.831^{c} (0.007)
XL	9.00^{ab} (0.2)	4.34^{a} (0.06)	0.558^{bc} (0.005)	0.442^{a} (0.005)	2.26^{b} (0.4)	0.864^{ab} (0.020)
Carr	8.91^{ab} (0.09)	4.40^{a} (0.04)	0.566^{ab} (0.005)	0.433^{a} (0.007)	2.09^{b} (0.4)	0.872^{a} (0.011)
CO	5.15^{g} (0.04)	2.05^{c} (0.03)	0.548^{d} (0.007)	0.412^{b} (0.004)	3.45^{a} (0.7)	0.738^{g} (0.005)
CF	8.62^{b} (0.08)	3.93^{a} (0.04)	0.549^{d} (0.004)	0.413^{b} (0.003)	0.15^{c} (0.9)	0.815^{cd} (0.006)

*Formulation code according to Table 1. Means with same superscript within same column do not differ significantly ($P > 0.05$). SEM values were given between parentheses.

Cohesiveness mean values also depended on the additive used; formulations with milk proteins, or carrageenans presented the most cohesive matrix, following by CF and XL sausages. Adhesiveness was higher for control formulation with fish oil, MC, and HPMC formulations; while the addition of milk proteins gave products as adhesive as the control formulation with fat. Thus the loss of hardness, which was the most altered textural parameter when fish oil was used instead of solid fat, could be controlled with the addition of different macromolecules such as MPr, Carr, or XL.

It is interesting to mention that small strain results could be linked to the textural properties of the samples measured at large deformation, outside the viscoelastic linear ranges; a good correlation ($R^2 = 0.84$, $P < 0.05$) between the plateau modulus and hardness was found.

4. Conclusions

The addition of different binders to healthier meat sausages formulated with pre-emulsified fish oil produced stable products, with high process yields. The different macromolecules incorporated decreased water mobility of the systems reflected in changes in the amount of non-frozen water, which produced changes in the glass transition and denaturation peak temperatures. Viscoelastic behavior of cooked batters was satisfactory modeled using broadened BSW equation to predict the mechanical relaxation spectrum in the linear viscoelastic range. The different additives did not modify the overall microstructure pattern of systems, but mainly influenced the level of interactions among macromolecular components.

The addition of κ/ι-carrageenan, xanthan-locust bean gums, or milk proteins concentrate produced healthier meat sausages with adequate physicochemical and quality characteristics, similar to the traditional formulation containing beef tallow.

Acknowledgments

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