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Effect of prebiotic ingredients on the rheological properties and microstructure of reduced-sodium and low-fat meat emulsions

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ABSTRACT

The technological and rheological properties were evaluated for low-fat and reduced-sodium meat emulsions containing various levels of prebiotic fibers (inulin, FOS, polydextrose, and resistant starch) as fat and starch substitutes. Low emulsion stability was observed, mainly in the treatments containing inulin and polydextrose (3 and 6 g/100 g). Higher tenderness was observed in the low-fat bologna sausages containing prebiotic fibers. The prebiotic fibers influenced the color of the meat batters but not that of the bologna sausage, probably due to the curing reactions and fat melting and subsequent solidification reaction. The meat batters presented elastic behavior, demonstrated by a G' value that was higher than the G'' value during oscillatory tests. An increase in the gelation temperature may result from the addition of the fibers, which delayed the gelation reaction of the myosin. The microstructures showed a porous matrix in the treatments containing prebiotic fibers, and a compact and dense network was observed only in the control formulations and that one containing inulin, due to its chain length. Further studies are required to evaluate the suitable levels in low-fat and reduced-sodium meat emulsions of prebiotic fibers, including cassava starch, which it is not possible to remove completely from the formulations.

1. Introduction

Meat and meat products are important components of the current diet and an excellent source of proteins, iron, zinc, niacin and vitamins (Brewer, 2012). However, these products are also recognized for their high sodium and fat contents and a fatty acid profile rich in saturated fatty acids and cholesterol, which can increase the incidence of coronary heart disease (CVD), obesity, high blood cholesterol and certain types of cancer (Colmenero, 1996; Desmond, 2006; Hooper et al., 2001; Keeton, 1994; Zhang, Xiao, Samaraweera, Lee, & Ahn, 2010). Excessive sodium intake is considered a critical public health issue, and it has been correlated to an increase in hypertension (WHO, 2006).

Over recent decades, several public health organizations (American Heart Association, American Cancer Society) have proposed to limit the daily total fat intake to no more than 30 g/100 g of total calories, saturated fatty acids to less than 7 g/100 g of calories and a maximum of 300 mg cholesterol/day to further reduce the risk of CVD (Skulas-Ray, Flock, & Kris-Etherton, 2013). Regarding sodium intake, the World Health Organization (WHO) recommends a maximum of 5 g NaCl daily. These recommendations, consequently, have contributed to promoting extensive research on low-fat and low-sodium meat products, which is a big challenge to the meat industry due to the technological importance of these components (Ansorena & Astiasarán, 2004; Carrapiso, 2007; Jiménez-Colmenero, Reig, & Toldrá, 2006).

Sodium chloride plays an important role in the extraction of the myofibrillar proteins that are responsible for development of functional properties of emulsified meat products (bologna sausage and frankfurters), such as the water-holding capacity, gel formation

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and emulsification (Desmond, 2006). Sensorial acceptance and microbiological stability are also attributed to NaCl in emulsified meat products. Fat contributes to the flavor, texture, mouthfeel, overall sensation of lubricity and appearance of meat products (Brewer, 2012). Thus, the major problems in reducing fat and sodium chloride in finely comminuted meat products include the low sensorial acceptance and reduced global stability (Campagnol et al., 2013; dos Santos, Campagnol, Pacheco, & Pollonio, 2012; Vural, Javidipour, & Ozbas, 2004).

Many studies have reported the addition of functional ingredients as fat substitutes in emulsified meat products that significantly improve the nutritional value (Caceres, Garcia, Toro, & Selgas, 2004; Nowak, von Mueffling, Grotheer, Klein, & Watkinson, 2007; dos Santos et al., 2012; Santos et al., 2013). Among these compounds, prebiotic fibers enhance the growth of beneficial bacteria in the lower intestine, contributing to gastrointestinal health (Li, 2010). An immune function has been indicated by relevant scientific studies, in particular due to their resistance to hydrolysis by digestive enzymes (Homayouni et al., 2014; Jiménez-Colmenero, Carballo, & Cofrades, 2001).

FOS, inulin, resistant starch, and polydextrose are examples of potential prebiotic components which can be applied in meat emulsions to substitute for fat, based on their technological properties. FOS shows a neutral taste, stability over a wide pH and temperature range and has successfully been used in certain meat products (Caceres et al., 2004). Inulin has been added to many products, including sausages, meatballs and restructured products, and has shown good performance as a fat substitute due to its ability to form a gel when mixed with water (Álvarez & Barbut, 2013; García, Cáceres, & Selgas, 2006; Huang, Tsai, & Chen, 2011; Santos et al., 2013). Polydextrose is also odorless, showing high solubility in water (80 g/100 g at 20 °C), which allows the formation of high-viscosity solutions and the improvement of texture by replacing fat. Resistant starch can also be easily incorporated into food products due to its microparticulate structure that does not affect the appearance of the final product (Burdock & Flamm, 1999; Murphy, 2001; Niness, 1999; Sajilata, Singhal, & Kulkarni, 2006). However, there are few studies reporting the use of polydextrose and resistant starch in meat products, and the potential application of all of these fibers has not been sufficiently studied in emulsified meat products with simultaneous fat and sodium chloride reduction.

Therefore, the present study aimed to evaluate the effect of the addition of various prebiotic fibers on the rheological and technological properties and the microstructure of an emulsified meat product (bologna), with a simultaneous reduction of sodium chloride (NaCl) and pork-back-fat content.

2. Material and methods

2.1. Meat-batter formulation

Bovine raw material (knuckle – *M. quadriceps femoris*) and pork fat were obtained from a local market in Campinas (SP, Brazil) with guaranteed quality. The meat pieces were previously cleaned to remove the visible fat, cut into strips and then frozen. The pork fat was portioned, ground into 3-mm disks and stocked at −18 °C until use.

Bologna sausage formulations (FC1, FC2, FC3, F1–F10) were prepared based on a traditional emulsified meat product containing 60 g/100 g beef, 0.25 g/100 g sodium polyphosphate, 0.05 g/100 g sodium erythorbate, and 150 ppm sodium nitrite. Fructo-oligosaccharide (FOS) (NutraFlora® P95), resistant starch (Hi-Maize 260), inulin (Orafti® GR) and polydextrose (Sta-Lite® III) were added in two levels (3 and 6 g/100 g). Ice was added to attain 100 g/100 g for the formulation (Table 1).

All bologna sausage formulations were processed in a pilot plant on the same day according to industrial procedures. Condiments were not used because sensory evaluation was not the focus of the study. The portioned and frozen beef (−1 °C) was ground into 5-mm disks at the time of use and placed into a cutter (Mado, model MTK – 661, Germany) with NaCl and was comminuted for 3–4 min at low speed to extract myofibrillar proteins. The other additives were slowly added when the temperature reached 5–6 °C, and the temperature of the meat batters never exceeded 12 °C. A portion of the meat batter was packaged in plastic packages (30 × 50 cm) under vacuum and kept under refrigeration (4 °C) for further analysis. The other portion was embedded into impermeable cellulose wrappers (Clariant, ø 9 cm) with approximately 0.5 kg of product per package. The bologna sausage pieces were placed in a water bath for cooking, with a gradual increase in temperature until the pieces reached the final core temperature of 72–74 °C. After cooking (~2 h) the products were cooled in an ice bath, vacuum-packaged and stored at 4 °C before analysis.

2.2. Physical analysis of batters and bologna sausage

2.2.1. Emulsion stability

The emulsion stability was performed according to Parks and Carpenter (1987), with some modifications to the cooking process: 40 °C/30 min followed by 70 °C/30 min. The total amount of liquid released was expressed as a percentage of the sample weight.

2.2.2. Color measurements

The color was measured using a spectrophotometer CM-5 (Konica Minolta) with a 20-mm port size, illuminant D65, SCI and a 10° standard observer. CIELAB L^*, a^* and b^* values were determined as indicators of lightness, redness and yellowness, respectively. The refrigerated meat batters were placed in the sampler with at least 0.5 cm in height, and the bologna sausage samples were sliced (0.5-cm thick). Measurements were performed in triplicate for each treatment.

2.2.3. Instrumental texture

The instrumental texture was measured using a TA XT2i texture analyzer (Texture Technologies Corp., Scarsdale, NY) equipped with a 25-kg load cell, using Texture Expert V1.19 software (Stable Micro Systems). The meat batters were stored at 4 °C/24 h in a container (5 cm × 7 cm) for stabilization, and the force required to penetrate 2.5 cm material was measured at 2 mm/s using a 60° conical probe. The bologna sausage samples were cut into cylinders (20 mm × 20 mm), and the compression strength (50% of their original height) was measured with a cylindrical probe of 35 mm in diameter at 1 mm/s.

2.2.4. Rheological properties

The rheological behavior of the samples was evaluated by oscillatory shear measurements in a controlled-stress rheometer (AR 1500ex rheometer, TA Instruments, England) equipped with a circular parallel-plate (2-cm diameter, 1.5 mm gap). The samples of batters were gently placed onto the plate and allowed to equilibrate for 5 min at 4 °C. Frequency sweeps from 0.01 to 10 Hz were performed on the meat batters at 7 °C and a fixed strain of 1%. Temperature sweeps (1 °C/min) were performed in two steps to simulate the batter cooking: a heating step from 25 to 75 °C followed by a cooling step from 75 to 25 °C, both at 1 Hz and 60 Pa. The changes in the slope of the complex viscosity (η^*) *versus* temperature curve were maximized from the derivation of the data using the Savitzky and Golay filter (1964), and the gel point was determined when the slope of the log (η^*) was greater than 0.01 (Picone & Cunha, 2011).

Table 1
Formulations of low-fat and low-salt meat batters containing prebiotic fibers.

Ingredients	Composition (g/100 g)												
	FC1	FC2	FC3	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10
Pork fat	20.0	20.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Sodium chloride	2.0	1.0	2.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Cassava starch	5.0	5.0	5.0	5.0	–	–	–	–	–	–	–	–	–
FOS	–	–	–	–	3.0	6.0	–	–	–	–	–	–	1.5
Inulin	–	–	–	–	–	–	3.0	6.0	–	–	–	–	1.5
Resistant starch	–	–	–	–	–	–	–	–	3.0	6.0	–	–	1.5
Polydextrose	–	–	–	–	–	–	–	–	–	–	3.0	6.0	1.5
Ice	12.7	13.7	22.7	23.7	25.7	22.7	25.7	22.7	25.7	22.7	25.7	22.7	22.7

2.2.5. Scanning electron microscopy

The microstructure of samples was visualized by scanning electron microscopy (SEM), according to Jiménez-Colmenero, Herrero, Pintado, et al. (2010) and Julavittayanukul, Benjakul, and Visessanguan (2006), with some modifications. The bologna sausages were fixed with glutaraldehyde (3 g/100 g) in 0.1 mol/L phosphate buffer (pH 7.2–7.4), post-fixed with osmium tetroxide (1% with 0.2 mol/L phosphate buffer), washed, dehydrated in alcohol solutions with increasing concentrations and critical-point-dried (CPD030 Balzers Critical Point Dryer), sputter-coated with gold (SCD 050 Sputter Coater, Balzers) and scanned by SEM (JEOL JSM 5800 LV, Tokyo, Japan) at 10 kV. A large number of micrographs were acquired to select the most representative ones (500× magnification).

2.3. Statistical analysis

Analysis of variance was performed by using the ANOVA procedures of the SAS software (SAS statistical software, version 9.1) at a significance level of 5%. Tukey's test was used to evaluate differences between the mean values ($p < 0.05$).

3. Results and discussion

3.1. Emulsion stability

The stability of the different formulations was affected by the reduction in the NaCl and fat content as well as the starch substitution by the prebiotic fibers (Table 2). The highest significant values of fluid loss were observed in formulations containing inulin (F4 and F5) and polydextrose (F8 and F9), whereas the lowest values were observed in the control formulations (FC1, FC2, FC3, and F1) (0.20–1.97 g/100 g).

A large fluid loss was observed in the formulations containing prebiotic fibers (8.86–29.84 g/100 g). Among them, the formulations with FOS (F2 and F3) showed lower loss values. Additionally, the mix containing 1.5 g/100 g of each fiber did not increase the emulsion stability, resulting in low % fluid loss. This fact makes it possible to conclude that the sum of the fibers did not develop a synergistic effect regarding emulsion stability.

It should also be noted that the lower fat and NaCl contents resulted in larger amounts of water being added to the formulations, thus contributing to a greater liquid release after cooking (Crehan, Hughes, Troy, & Buckley, 2000). The fat reduction increased the moisture/protein ratio because the amount of meat was held constant (60 g/100 g), and the formulation (100 g/100 g) was completed with water. However, the large increase in the fluid loss of the prebiotic samples can be related to the removal of cassava starch (5 g/100 g) from the formulation because this ingredient (cassava starch) is recognized for its excellent property of gelatinization and water-holding capacity after heating and cooling.

3.2. Instrumental color

Because the color parameter significantly influences consumer acceptance, it is very important to study the color behavior in the meat batters to predict the changes occurring in the final product. Several authors have investigated the changes in the color parameters of meat products, generally in low-fat or low-sodium products (Caceres et al., 2004; Jiménez-Colmenero, Cofrades, Lopez-Lopez, et al., 2010; Mittal & Barbut, 1994). However, little is known about the effect of the addition of prebiotic fibers in a reformulated meat emulsion. Overall, the batter formulations containing prebiotic fibers exhibited darker colors than the control formulations.

Table 2
Percentage of liquid released after cooking (g/100 g), compressive strength (N) of bologna sausage and penetration force (N) of meat batters containing prebiotic fibers and low-fat and low-sodium content ($n = 39$).

Treatments	Fat (g/100 g)	Salt (g/100 g)	Fiber (g/100 g)	Fluid loss (g/100 g)	Penetration force (N)	Compression strength (N)
FC1	20.0	2.0	5% starch	0.50 ± 0.03 ^{e}	12.12 ± 0.76 ^{b}	53.14 ± 1.07 ^{ab}
FC2	20.0	1.0	5% starch	0.20 ± 0.18 ^{e}	14.40 ± 1.00 ^{a}	55.47 ± 2.40 ^{a}
FC3	10.0	2.0	5% starch	0.52 ± 0.49 ^{e}	9.05 ± 0.25 ^{c}	45.45 ± 4.79 ^{bc}
F1	10.0	1.0	5% starch	1.97 ± 0.42 ^{e}	7.27 ± 0.02 ^{c}	33.32 ± 1.14 ^{def}
F2	10.0	1.0	3% FOS	16.69 ± 0.40 ^{c}	6.98 ± 0.19 ^{c}	26.95 ± 1.20 ^{f}
F3	10.0	1.0	6% FOS	8.86 ± 1.61 ^{d}	7.16 ± 0.07 ^{c}	39.09 ± 1.35^{cd}
F4	10.0	1.0	3% IN	29.84 ± 1.91 ^{a}	7.65 ± 0.04 ^{c}	26.54 ± 0.31 ^{f}
F5	10.0	1.0	6% IN	24.64 ± 1.48 ^{ab}	8.81 ± 0.71 ^{c}	30.17 ± 1.70 ^{ef}
F6	10.0	1.0	3% RS	20.04 ± 2.33 ^{bc}	8.43 ± 0.01 ^{c}	30.74 ± 4.86 ^{ef}
F7	10.0	1.0	6% RS	19.99 ± 3.94 ^{bc}	7.97 ± 1.18 ^{c}	33.11 ± 1.98 ^{def}
F8	10.0	1.0	3% PD	27.26 ± 2.10 ^{a}	7.21 ± 0.21 ^{c}	26.98 ± 1.31 ^{f}
F9	10.0	1.0	6% PD	27.79 ± 2.35 ^{a}	7.47 ± 0.72 ^{c}	30.63 ± 1.57 ^{ef}
F10	10.0	1.0	1.5% each fiber	20.74 ± 0.91 ^{bc}	8.61 ± 0.08 ^{c}	38.19 ± 5.83 ^{cde}

$^{a, b, c, d, e, f}$ Means with the same letter in same column are not significantly different ($P < 0.05$). FOS = fructo-oligosaccharide, IN = inulin, RS = resistant starch and PD = polydextrose.

The color parameters of the meat batters and the bologna sausage are presented in Table 3. The low-fat content of FC3 and F1 contributed to differentiating the lightness (L^*), compared with those of the other control formulations FC1 and FC2. The formulations containing prebiotic fibers exhibited significantly lower L^* values than the control formulations FC1 and FC2. In contrast, no significant differences were observed when compared with FC3 and F1, except for the formulations containing polydextrose (F8 and F9), which presented the lowest lightness values.

Significant differences were observed with respect to the red color (a^*) of the meat batters when comparing the control formulations FC1, FC2 and FC3 and those with prebiotic fibers, except for the cases of the formulations F7 (6 g/100 g resistant starch), F8 (3 g/100 g polydextrose) and F10 (all fibers). The lowest a^* value (12.20) was observed in the formulation FC2, whereas the formulations containing 6 g/100 g FOS (F3) and 3 g/100 g inulin (F4) showed the highest values (17.32 and 17.28, respectively). Regarding the yellow color (b^*) of the batters, no significant difference was observed in all formulations.

Despite the significant difference observed for the L^* parameter of the meat batters with added prebiotic fibers, no significant difference was observed after cooking for bologna sausage containing low sodium and fat contents. In contrast, the lowest L^* values (59.1) were found in the formulations containing 3 and 6 g/100 g polydextrose (F8 and F9, respectively), with no significant difference when compared with the formulations of the other added prebiotic fibers. Regarding the a^* value, only the formulation with 6 g/100 g polydextrose (F9) differed from the others.

The lack of color change in the bologna sausages compared with the respective meat batters is probably related to the curing reactions that take place during the cooking process and to the fat melting and subsequent solidification. These last reactions lead to the reorganization of the components, including water, fat, and protein, which can remove the effect of the color parameters of bologna sausages, independent of the prebiotic fiber added.

3.3. Instrumental texture

The results for the penetration and compression tests performed on the meat batters and bologna sausages (Table 2) show significant differences with the reduction of the fat and salt levels. The control formulations FC1 and FC2 showed higher penetration and compression forces than the formulations containing prebiotic fibers, due to the excellent gel-forming ability of the myofibrillar proteins.

These results also highlight the role of fat in an emulsified meat network. Fat is critical for the development of flavor and texture properties, thereby contributing to the juiciness, flavor and overall palatability of the final product (Tobin, O'Sullivan, Hamill, & Kerry, 2012; Ventanas, Puolanne, & Tuorila, 2010). The network formed was very cohesive, probably because of the excellent quality of the raw meat (knuckle – *M. quadriceps femoris*) and the careful cleaning of the raw materials, including the complete removal of the subcutaneous connective tissue from the meat, before freezing to ensure a greater homogeneity of the batch in the milling stage.

When comparing the level of fiber addition, a trend toward higher values was observed for the compressive strength of the formulations containing 6 g/100 g fiber (F3, F5, F7, and F9) than for the formulations containing 3 g/100 g fiber (F2, F4, F6, and F8), which may be due to the high amount of water in these formulations, contributing to the lower compressive strength of the product. However, significant differences in the addition levels were observed only between the formulations containing FOS (F2 and F3). This could be because the presence of a higher content of soluble dietary fiber (F3) causes the gel structure to be more compact and therefore prevents proteins from retaining water, and this favors the formation of a more compact and stronger protein matrix, increasing the compressive strength (Caceres et al., 2004). It has been reported that the addition of FOS can increase the level of sweetness of meat products, but at the levels of 3 and 6 g/100 g, pepperoni formulations were well accepted by consumers (dos Santos et al., 2012). These results should be interpreted with future studies related to the microbiological stability and sensory acceptance of bologna sausage.

The effect of the fiber on the texture of the product is determined by the process conditions and, especially, by its molecular characteristics. The greater the chain length of the fiber, the higher the probability of it being unevenly distributed in the matrix with fewer interactions with the medium, reducing the binding properties of the meat protein structure and contributing adversely to the tenderness of the final product (Lopez-Lopez, Cofrades, Yakan, Solas, & Jimenez-Colmenero, 2010).

3.4. Rheological properties of the meat emulsion prior to thermal processing

The rheological results (Fig. 1) revealed that the formulations exhibited mechanical characteristics of solids because all samples showed $G' > G''$, which is related to elastic behavior. The

Table 3
Effect of low NaCl and low-fat contents in meat batters and bologna sausage, added of prebiotic fibers ($n = 39$).

Treatments	Meat batter	Meat batter	Meat batter	Bologna sausage	Bologna sausage	Bologna sausage
	L^*	a^*	b^*	L^*	a^*	b^*
FC1	61.33 ± 2.0 ^{a}	13.36 ± 1.01 ^{de}	18.99 ± 0.16 ^{a}	61.5 ± 1.1 ^{ab}	10.96 ± 0.4 ^{c}	10.64 ± 0.2 ^{bcde}
FC2	59.96 ± 0.4 ^{ab}	12.20 ± 0.21 ^{e}	18.85 ± 0.04 ^{a}	63.4 ± 0.7 ^{a}	11.12 ± 0.3 ^{c}	10.67 ± 0.1 ^{bcde}
FC3	55.17 ± 1.2 ^{bcde}	12.94 ± 1.02 ^{de}	18.94 ± 0.27 ^{a}	60.0 ± 1.5 ^{ab}	11.07 ± 0.3 ^{c}	10.07 ± 0.2 ^{f}
F1	55.67 ± 0.5 ^{abcd}	15.06 ± 0.15 ^{abcd}	19.34 ± 0.03 ^{a}	61.0 ± 0.2 ^{ab}	11.49 ± 0.2 ^{bc}	10.15 ± 0.2 ^{ef}
F2	52.95 ± 1.8 ^{cdef}	16.56 ± 0.15 ^{ab}	18.39 ± 0.05 ^{a}	62.0 ± 1.1 ^{ab}	11.03 ± 0.3 ^{c}	10.32 ± 0.2 ^{def}
F3	49.56 ± 1.8 ^{ef}	17.32 ± 0.18 ^{a}	18.40 ± 0.23 ^{a}	61.6 ± 1.4 ^{ab}	11.16 ± 0.2 ^{c}	10.39 ± 0.3 ^{def}
F4	52.52 ± 1.8 ^{cdef}	17.28 ± 0.43 ^{a}	17.65 ± 0.42 ^{a}	60.6 ± 1.4 ^{ab}	11.49 ± 0.1 ^{bc}	10.75 ± 0.2 ^{abcd}
F5	51.85 ± 1.9 ^{def}	16.14 ± 0.93 ^{abc}	18.28 ± 0.14 ^{a}	60.9 ± 1.6 ^{ab}	11.65 ± 0.4 ^{bc}	10.50 ± 0.2 ^{cdef}
F6	52.85 ± 2.2 ^{cdef}	16.49 ± 0.93 ^{ab}	18.38 ± 0.23 ^{a}	61.8 ± 2.2 ^{ab}	11.32 ± 0.6 ^{bc}	11.00 ± 0.2 ^{abc}
F7	57.84 ± 1.0 ^{abc}	12.88 ± 0.36 ^{de}	19.00 ± 0.24 ^{a}	62.6 ± 1.3 ^{ab}	11.10 ± 0.3 ^{c}	10.78 ± 0.2 ^{abcd}
F8	49.63 ± 2.0 ^{ef}	13.80 ± 0.94 ^{cde}	18.30 ± 0.88 ^{a}	59.1 ± 0.7 ^{b}	12.27 ± 0.3 ^{bc}	10.68 ± 0.1 ^{bcde}
F9	48.49 ± 0.1 ^{f}	16.11 ± 0.78 ^{abc}	17.59 ± 1.35 ^{a}	59.1 ± 1.5 ^{b}	12.73 ± 0.4 ^{a}	11.26 ± 0.2 ^{a}
F10	52.70 ± 0.6 ^{cdef}	13.96 ± 0.09 ^{bcde}	18.16 ± 0.46 ^{a}	60.5 ± 1.9 ^{ab}	11.90 ± 0.5 ^{abc}	11.06 ± 0.1 ^{ab}

$^{a, b, c, d, e, f}$ Means with the same letter in same column are not significantly different ($P < 0.05$). FC1 = 20% fat, 2% NaCl and 5% starch, FC2 = 20% fat, 1% NaCl and 5% starch, FC3 = 10% fat, 2% NaCl and 5% starch, F1 = 10% fat, 1% NaCl and 5% starch, F2= 10% fat, 1% NaCl and 3% FOS, F3 = 10% fat, 1% NaCl and 6% FOS, F4 = 10% fat, 1% NaCl and 3% inulin, F5 = 10% fat, 1% NaCl and 6% inulin, F6 = 10% fat, 1% NaCl and 3% resistant starch, F7 = 10% fat, 1% NaCl and 6% resistant starch, F8 = 10% fat, 1% NaCl and 3% polydextrose, F9 = 10% fat, 1% NaCl and 6% polydextrose and F10 = 10% fat, 1% NaCl, 1.5% FOS, inulin, resistant starch and polydextrose.

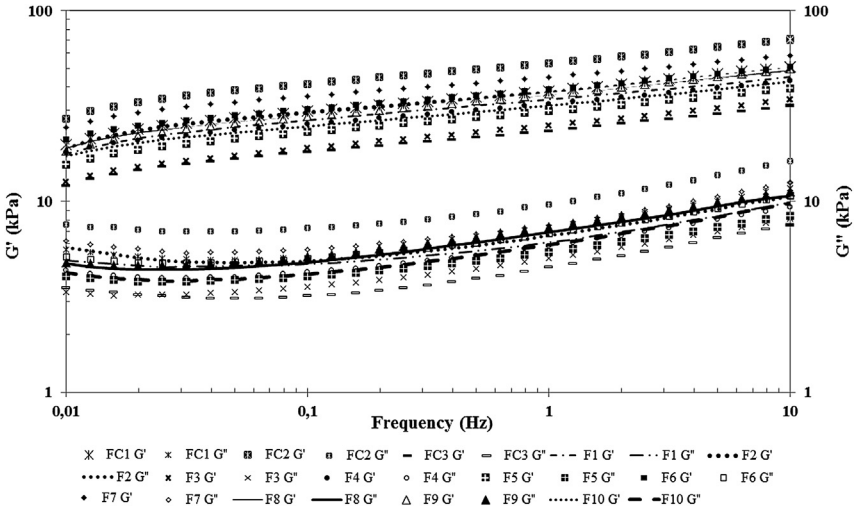

Fig. 1. Mechanical spectrum of the formulations at 7 °C and 1% deformation.

magnitudes of the elastic and loss moduli observed in the present study were similar to those obtained by Cardoso, Ribeiro, and Mendes (2012), who evaluated protein gels from various species of fish.

The slight increases in the G' and G'' moduli observed with the increase in the oscillation frequency is typical of the "plateau region" which is an intermediate zone of the mechanical spectrum between the terminal and the transition zones (Almdal, Dyre, Hvidt, & Kramer, 1993; Lorenzo, Checmarev, Zaritzky, & Califano, 2011; Savadkoohi, Shamsi, Hoogenkamp, Javadi, & Farahnaky, 2013). This type of behavior ($G' > G''$, $G'/G'' < 10$) is characteristic of a weak gel, can be correlated as a measure of density from the protein molecules at the oil/water interface and has been associated with the formation of a structural network in the emulsion (Franco, Raymundo, Sousa, & Gallegos, 1998).

3.5. Viscoelastic properties as a function of temperature

The storage (G') and loss (G'') moduli were used to measure the viscoelastic characteristics of the emulsified meat product during the cooking process. The control formulations showed similar behavior even with the reduction of sodium chloride (FC2), fat (FC3) content or both (F1) (Fig. 2).

It was observed during heating that there was a significant increase in the G' values for all formulations, unlike what was reported by other researchers, who observed first the reduction in G' due to breaking of the hydrogen bonds as the temperature increases (Ferris, Sandoval, Barreiro, Sánchez, & Müller, 2009; Savadkoohi et al., 2013). When comparing the control formulations with the formulations with added fiber, a more pronounced increase in G' was observed at the control formulations between 35 and 55 °C. For the formulations containing fibers, the increase in G' was slower and gradual, becoming more evident only after reaching 40–50 °C.

The gelation temperature of the batters obtained from the rheological measurements is shown in Table 4. In general, the gelation temperatures of the controls were significantly lower than those for the other samples, and the levels of fiber additives (3 and 6 g/100 g) did not affect significantly the gelation temperatures (46.97–49.32 °C). The significantly different values for the gelation temperatures between the control formulations (FC1, FC2, FC3 and F1) and those containing prebiotic fibers (F2–F10) is related to the formation of a more complex structure, requiring more energy to

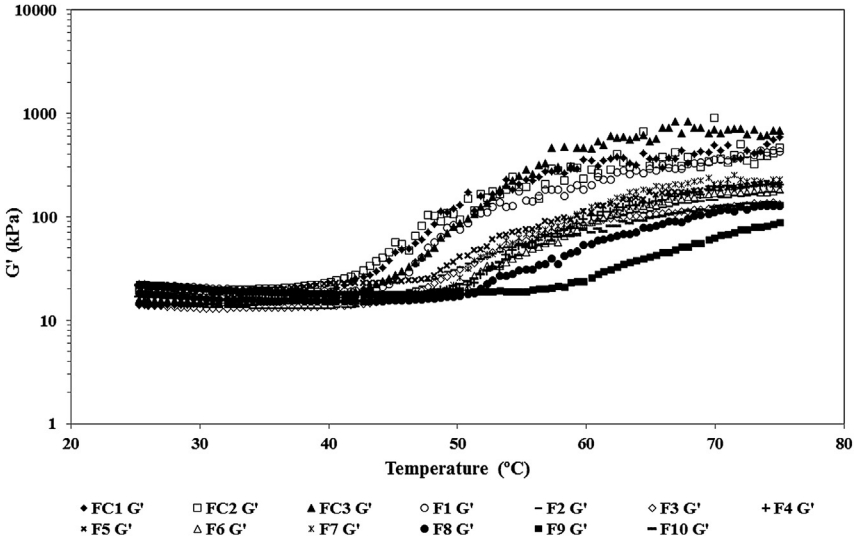

Fig. 2. Elastic modulus (G', kPa) *versus* temperature (°C) of the samples.

Table 4
Gelation temperatures of different formulations during heating ($n = 39$).

Treatments	Fat (g/100 g)	Salt (g/100 g)	Fiber (g/100 g)	Gelation temperature (°C)	End cooling (25 °C)	
					G' (kPa)	G'' (kPa)
FC1	20.0	2.0	5% starch	41.03 ± 0.47 ^{d}	534.50 ± 100.62 ^{a}	5.75 ± 2.08 ^{a}
FC2	20.0	1.0	5% starch	41.27 ± 0.80 ^{d}	262.65 ± 27.58 ^{bcd}	6.00 ± 0.06 ^{a}
FC3	10.0	2.0	5% starch	41.28 ± 0.35 ^{d}	520.45 ± 56.50 ^{a}	4.48 ± 1.96 ^{a}
F1	10.0	1.0	5% starch	44.85 ± 0.74 ^{c}	360.25 ± 95.46 ^{ab}	6.38 ± 3.92 ^{a}
F2	10.0	1.0	3% FOS	47.82 ± 0.12 ^{ab}	166.80 ± 14.71 ^{bcd}	3.57 ± 1.02 ^{a}
F3	10.0	1.0	6% FOS	46.97 ± 1.04 ^{bc}	128.78 ± 3.43 ^{cd}	3.22 ± 0.78 ^{a}
F4	10.0	1.0	3% IN	48.63 ± 0.53 ^{ab}	121.43 ± 20.97 ^{cd}	5.87 ± 0.66 ^{a}
F5	10.0	1.0	6% IN	48.85 ± 0.54 ^{ab}	193.98 ± 94.15 ^{bcd}	3.68 ± 0.18 ^{a}
F6	10.0	1.0	3% RS	48.92 ± 0.49 ^{ab}	200.88 ± 3.43 ^{bcd}	4.40 ± 0.24 ^{a}
F7	10.0	1.0	6% RS	46.97 ± 0.33 ^{bc}	326.90 ± 1.27 ^{abc}	7.81 ± 1.91 ^{a}
F8	10.0	1.0	3% PD	48.28 ± 0.12 ^{ab}	91.08 ± 54.13 ^{d}	3.31 ± 0.69 ^{a}
F9	10.0	1.0	6% PD	49.32 ± 0.54 ^{a}	76.87 ± 11.59 ^{d}	4.06 ± 0.71 ^{a}
F10	10.0	1.0	1.5% each fiber	48.68 ± 0.64 ^{ab}	121.90 ± 27.08 ^{cd}	4.27 ± 0.18 ^{a}

$^{a, b, c, d}$ Means with the same letter in same column are not significantly different ($P < 0.05$). FOS = fructo-oligosaccharide, IN = inulin, RS = resistant starch and PD = polydextrose.

break the bonds and component interactions leading to gel formation.

Comparing various formulations of added fiber (F2–F9) with the control formulation, significant differences can be observed for the gelation temperature, and the highest values are for the formulations with various added fibers (46.97–49.32 °C). Such behavior of the formulations containing fibers could be explained by the mechanism of myosin gelation. The addition of fibers may have retarded the aggregation of the globular head portion of myosin (first step of gelation) occurring at higher temperatures. Myosin gelation provides greater elasticity to the material mainly due to the hydrophobic and disulfide-sulfhydryl interactions between neighboring proteins with little contribution of hydrogen or other polar bonds (Ferris et al., 2009; Samejima, Ishioroshi, & Yasui, 1981; Savadkoohi et al., 2013).

Higher G' values were observed during heating for the control formulations than for those containing fibers (Fig. 2), and lower values were observed for the formulations containing polydextrose (F8 and F9) (Table 4). However, FC1 and FC3 showed higher G' at the end of the heating process, indicating that the reduction of NaCl was significant to decrease the G' moduli (Table 4). The behavior of the formulation containing all prebiotic fibers (F10) was similar to the formulations with fiber alone in relation to G' and was similar for all formulations with respect to G'' (data not shown).

A slight increase in G' was observed during cooling (data not shown), suggesting that the protein network continued to be developed, resulting in the final G' values presented in Table 4.

The magnitudes of G' were much higher than those obtained by Savadkoohi et al. (2013) due to the lower amount of protein present in the mechanically deboned meat (MDM). Additionally, the FC1 and FC3 (formulations without NaCl reduction) presented the highest G' values, contributing to the formation of a more cohesive and elastic structure. Thus, these values evidenced the higher elasticity of the meat emulsions based on the myofibrillar proteins of bovine origin, reinforcing that the higher the G' value, the firmer and more elastic a gel is formed, as previously described. These results could have an important impact on enhancing sensorial perceived toughness because a dense, elastic meat protein gel is sensorially preferred to a more brittle, grainy protein gel in an emulsion sausage (Tornberg, 2005).

3.6. Scanning electron microscopy (SEM)

Fig. 3 shows micrographs of the emulsified products. All control formulations exhibited a slightly rough and porous structure with spheres suspended in the protein network. These spheres may be fat globules that were not eliminated during preparation of the samples with glutaraldehyde (3 g/100 g) and alcohol solutions, or nongelatinized starch granules because the gelatinization temperature of cassava starch (65–70 °C) is similar to the thermal process applied to the samples (up to 74 °C) (Breuninger, Piyachomkwan, & Sriroth, 2009). In the control formulations, a greater number of pores was observed in the formulation with a lower fat content (FC3) due to the higher amount of water added, in agreement with Jiménez-Colmenero, Herrero, Pintado et al., (2010) and Alvarez, Xiong, Castillo, Payne, and Garrido (2012).

The formulations containing fibers showed less dense and less compact structures with similar porosity to the control formulations and with the presence of empty spaces. The high proportion of empty spaces can be interpreted as additional water due to the low fat content as well as the fiber addition, as previously discussed. In several micrographs, it is possible to visualize fat globules of various sizes and the protein network involving a portion of the fat globules (Morin, Temelli, & McMullen, 2004).

Although the rheological measurements (Figs. 1 and 2) and texture analysis (Table 2) showed no significant differences among the formulations containing fibers, the micrographs demonstrated that the formulations with inulin (F4 and F5) showed a more compact and denser topography than the formulations containing other fibers. This likely can be explained due to the long chain length of inulin and the degree of polymerization, which causes it to be less soluble and more viscous, and it can be used as a textural modifier to improve the texture (Niness, 1999; Wada, Sugatani, Terada, Ohguchi, & Miwa, 2005). However, formulations containing resistant starch (F6 and F7) presented very porous structure and a greater number of spheres on the surface, which are probably resistant starch granules because its gelatinization temperature is higher than the boiling point of water (Birt et al., 2013).

The micrographs of the bologna sausages containing polydextrose (F8 and F9) presented less dense and more porous structures but with fat globules of various sizes throughout the protein network. The formulation containing all of the prebiotic fibers (F10) revealed a structure with an irregular and quite porous surface, with some globules very similar to those observed in the formulations containing resistant starch (F6 and F7).

4. Conclusion

The different prebiotic fibers used in this study led to structural changes in the low-sodium and low-fat meat emulsions when

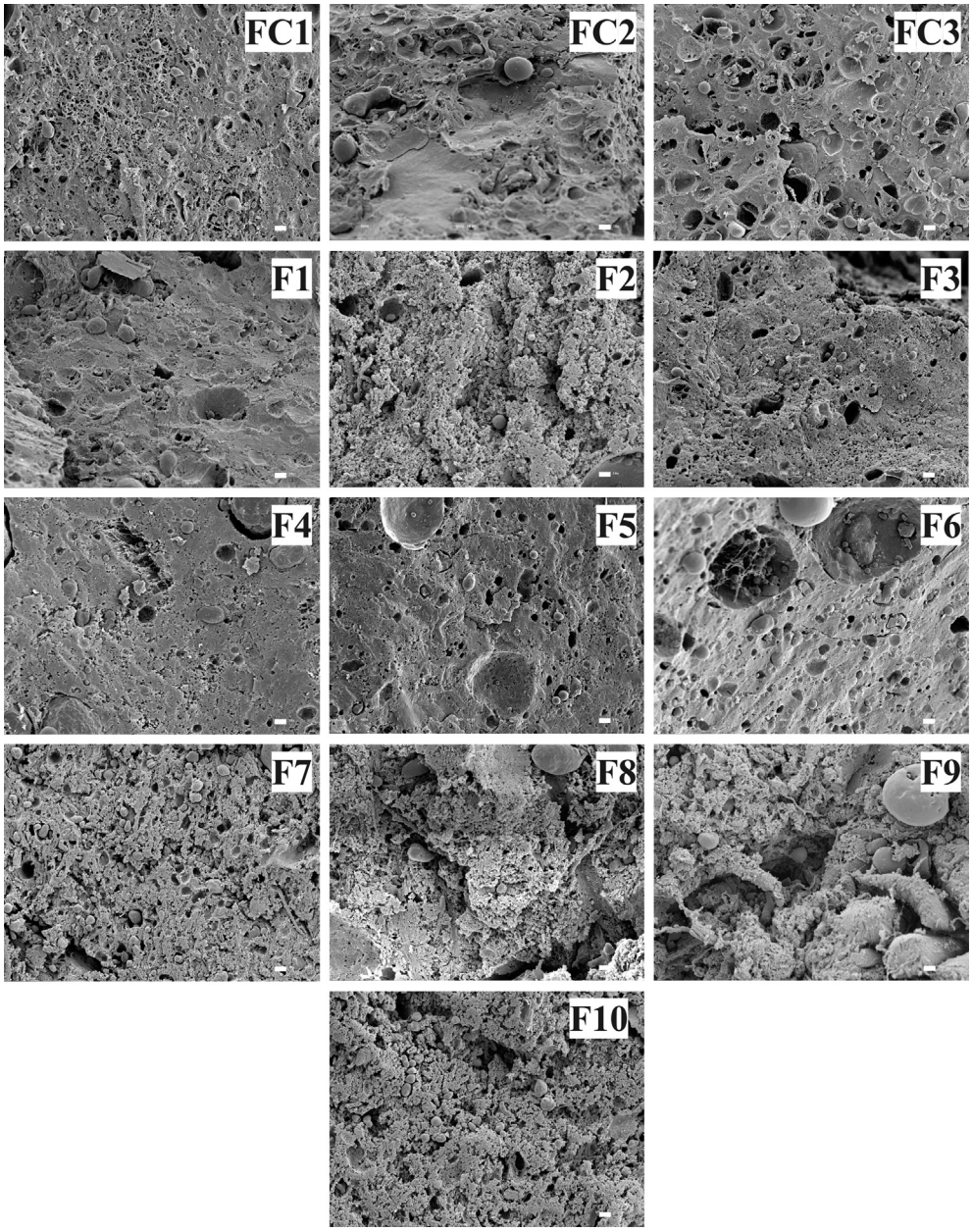

Fig. 3. Scanning electron microscopy (SEM) images of the bologna sausage.

compared with control formulations. The fiber addition delayed the aggregation of globular myosin heads; thus, the gelation process occurred at higher temperatures. The texture parameters were also affected with a significant difference observed in the compression strength. The microscopy assays showed porous structure in the formulations containing prebiotic fiber and more compact and denser structures in the control formulations. Thus, the results tell us that these changes could also affect the organoleptic properties of the products, impacting the consumer acceptability, due to the formation of a brittle protein gel in a bologna product. As a result, another extender ingredient able to reduce this effect is necessary, such as cassava starch, which was totally removed in this study. The simultaneous addition of a partial level of cassava starch and the prebiotic fibers would improve the stability of the meat emulsions, allowing the reliable production of a healthier bologna sausage. Finally, the impact of adding prebiotic fibers to emulsified meat products on the microbiological stability must be investigated along with the shelf life before it is advisable to recommend this beneficial reformulation.

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