Using inulin based of emulsion gels as fat substitute in salt reduced Bologna sausage

Running title: Emulsion gels as fat substitute in salt reduced Bologna sausage

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Abstract

BACKGROUND: A high-fiber emulsion gel (EG) containing inulin, soy protein isolate, and soybean oil was applied as animal fat replacer in reduced salt and fat Bologna sausage containing mechanically deboned chicken meat (MDCM), pork meat, and pork back fat. Technological and microbiological properties were evaluated for 60 days at 4 °C.

RESULTS: A reduction of 11-34% and 35-45% of fat and sodium were obtained in reformulated products, respectively. An increase in fiber content and polyunsaturated fatty acid was noticed in the formulations with EG. The addition of EG in Bologna increased L^* values and reduced a^* values comparing to control treatment. Microstructural properties of sausages exhibited a denser network with the presence of EG. A softer, more elastic, cohesive and resilient samples with a higher intensity of lipid oxidation ($p < 0.05$) were observed in EG added sausages. The NMR data shows that the presence of EG recovers the matrix that has been weakened due to reduction of fat

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and salt. Sensory evaluation showed that the incorporation of the emulsion gels resulted in acceptable scores.

CONCLUSION: These results suggest that inulin based emulsion gel is a potential fat substitute for developing healthier meat products, with better fatty acids composition and stable to chilled storage.

Keywords: dietary fiber; gelled emulsion; low-salt sausage; mechanically deboned chicken meat

1 INTRODUCTION

Processed meat is recognized for its nutrients content such essential amino acids, minerals (iron, zinc, selenium, etc.), vitamins (mainly B group), glutathione, ubiquinone which are important for growth and development. However, it also has the negative contribution to fat (mainly saturated fatty acid (SFA), cholesterol) and sodium daily intakes.¹ A recommendation to reduce the consumption of total fat (max. 30-35% of the total caloric value) and SFA (less than 10%) and also to increase the intakes of polyunsaturated fatty acids is suggested by health organization.² A sodium intake of less than < 5 g salt/day (2 g sodium/day) is also suggested.³

Emulsion gels (EGs) are structured oils and its may be suitable to improve the quality of fat in meat products. EG are generally formed by edible oils (from vegetable, marine, and others sources) in the dispersed phase, water in continuous phase, and structuring agents (proteins, fibers, etc) as emulsifiers, stabilizers and/or functional ingredients and have a solid-like lipid structure.⁴ The effect of adding EG in meat products such as

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burgers,^{5, 6} meat batters,⁷ and dry-fermented sausage⁸ have been reported. The main results derived from using structured healthier oil as animal fat replacer in meat products describe a better nutritional profile by reducing total and saturated fat contents and increasing polyunsaturated fatty acids. In some cases, the formulation of EG contains functional ingredients improving others nutritional properties such as adding fibers keeping the sensorial acceptance on these reformulated products.^{8, 9}

Vegetable proteins are recognized as a ingredients with great potential to increase functional properties of reformulated meat products. In particular, soy protein isolate (SPI) is an excellent emulsifier and gelling agent¹⁰ usually added in meat products formulations to improve technological properties.¹¹ Besides that, SPI presents good nutritional properties and is widely produced in the world¹² which allow this ingredient to have a low cost.

To perform the dispersed phase of the emulsion, a great alternative economically and nutritionally is soybean oil, due to its high production, availability and low value. Moreover, its fat content consists of 15-33% oleic acid (C18:1n-9), 43-56% linoleic acid (C18:2n-6), and 5-11% α -linolenic acid (C18:3n-3).¹³

Considering the possibility of adding a functional ingredient as part of the emulsion gel composition, inulin has been widely used in food and is commonly labeled as dietary fiber^{14, 15} Gels formed by inulin presents small crystals of approximately 100 nm in diameter that form clusters larger than 1-5 μ m, which retain a large amount of water in

the network, and its network properties may mimic fat crystals, which makes it a potential component to substitute fat in meat emulsion.^{16, 17}

Our previous studies have reported the optimization of an emulsion gel to be used as a fat replacer in meat emulsion¹⁸ and its application in frankfurter¹⁹ and Bologna sausage⁹. The results have shown us that it is a promising fat substitute by obtaining products with healthier nutritional profile and technological properties similar to a control product. However, the study of the stability in low cost and reduced-salt meat emulsions containing EG during shelf life is scarce in the literature suggesting that more thorough research needs to be conducted. Then, the goal of this study was to evaluate the effect of emulsion gel with soybean oil, SPI, and inulin in reduced fat and salt Bologna sausage containing MDCM regarding technological, microstructural, nutritional, microbial, and sensorial properties along 60 days of shelf life.

2 MATERIAL AND METHODS

2.1 Development of EG

EG was prepared as reported previously²⁰ with 4% soy protein (Dupont® Brazil), 50% soybean oil (Liza® Brazil), 16.5% inulin (ORAFIT GR, Clariant, USA), and 29.5% water.

2.2 Design and manufacture of Bologna sausages

Fresh pork *longissimus dorsi* muscle (72.2% moisture, 6.4% fat, 19.6% protein, and 1.9% ash), pork back fat (11.6% moisture, 84.2% fat, 3.6% protein, and 0.6 % ash), and

mechanically deboned chicken meat (68.4% moisture, 16.6% fat, 14.4% protein, and 1.2% ash), were obtained from a local marketplace in Campinas, SP. The pork meat was prepared by removing visible fat and connective tissue. The pork meat and pork back fat were grounded into 5 mm discs and then packed in vacuum and kept at -20 °C until their use.

Eight different batches of 6 kg were formulated according to Felisberto *et al.*¹⁴ Table 1. Bolognas were prepared in a cutter (MTK and 661 model, Mado, Germany) and embedded into impermeable cellulose casings (Viskase, 5.5 cm diameter) and package with approximately 200 g of batter per pieces. The cooking process was performed in a water bath (RSA 1708, RSA, Campinas, Brazil) gradually increasing the temperature until the sample core reached 72 – 74 °C (~1 h). The pieces were cooled in an ice bath (approximately 15 min) and kept at 4 °C. All samples were analyzed regarding to technological properties during shelf life (0, 15, 30, 45, and 60 days of storage at 4 °C). Microbiological and sensorial properties (day 10 of storage at 4 °C) were evaluated for PF20/NS (pork back fat 20%/normal salt), EG20/NS (emulsion gel 20%/normal salt – 2%), EG20/RS (emulsion gel 20%/reduced salt – 1%), PF10-EG10/NS (pork back fat 10% emulsion gel 10%/normal salt – 2%), and PF10-EG10/RS (pork back fat 10% emulsion gel 10%/reduced salt -1%) treatments.

2.2.1 Bologna sausages Emulsion stability

The stability of the emulsion was performed according to Colmenero *et al.*²¹ The total amount of fluid released containing water and fat from the raw sausage was expressed as a percentage in relation to initial weight of the batter. The analysis was carried out in five replicates.

2.3 Bologna sausages analysis

2.3.1 Proximate analysis, sodium content and energy value

Protein, ash, moisture contents and sodium content were performed according to AOAC²² at day 0. Bligh and Dyer²³ method was used to quantify fat content. From supplier data, the total dietary fiber (TDF) of inulin was estimated. Sodium content was measured with an inductively coupled plasma optical emission spectrometer (ICP OES) (Vista MPX, Varian, Mulgrave, Australia). The ICP OES operating conditions were: 1000 W of potency; 0.9 L/min of nebulizing rate; 1.5 and 15 L/min of flow rate of argon and the auxiliary gas, respectively; 10 and 3 s of integrating and reading times, respectively. λ (nm) used for sodium was 589.592. Values of 9 kcal/g for fat; 4 kcal/g for protein and carbohydrate and 2 kcal/g for dietary fiber were used to calculate energy content. All analysis were performed in triplicate.

2.3.2 Fatty acid profile - FAP

FAP was performed as reported by Paglarini *et al.*¹⁸ by using a GC with a capillary column (CGC 68650 Series GC System, Agilent, Santa Clara, California, USA) equipped with a flame ionization detector.²⁴ The methyl esters of fatty acids were separated on Agilent DB-23 capillary column (50% cyanopropyl-methylpolysiloxane), dimensions 60 m, Ø int: 0.25 mm, 0.25 µm film.

Atherogenic (AI) was calculated by using the equation $(C12:0 + 4 \times C14:0 + C16:0) / (C16:1, C17:1, C18:1n-9, C20:1, C18:2n-6, \text{ and } C18:3n-3)$ and thrombogenic index (TI) $(C14:0 + C16:0 + C18:0) / [(0.5 \times MUFA) + (0.5 \times \Sigma n-6) + (3 \times \Sigma n-3) + (\Sigma n-3 / \Sigma n-6)]$.²⁵

2.3.3 Instrumental pH, a_w and color

A Mettler Toledo pH meter (Columbus, OH, United States) was used to measure pH. The water activity (a_w) was analyzed with an Aqua lab CX-2 water-activity meter (Decagon Devices, Inc., Pullman, WA). CIELab L^* , a^* , and b^* were carried out using a CM-5 spectrophotometer (Konica Minolta, Tokyo, Japan), working with D65 illuminant, 10° observer angle and SCE mode. Euclidean distance ($\Delta E =$

$\sqrt{(L_{PF20/NS}^* - L_R^*)^2 + (a_{PF20/NS}^* - a_R^*)^2 + (b_{PF20/NS}^* - b_R^*)^2}$) was calculated to evaluate the difference between control (PF20/NS) and reduced salt and/or fat sausages -R (PF20/RS, PF10/NS, PF10/RS, EG20/RS, EG20/NS, PF10-EG10/NS, PF10-EG10/RS). Analyses were performed at days 0, 15, 30, 45 and 60 in triplicate.

2.3.4 Juiciness

Juiciness was performed according to the method proposed by Lucherik *et al.*²⁶ with some changes. Three cubes (dimensions 1x1cm) were taken from the middle of Bologna pieces. Cubes were individually weighed on 2 sheets of qualitative filter paper (Unifil, 9 cm) previously stored in a desiccator. Samples were compressed for 30s at 78.45 N with a texture analyzer (TA-xT2i Texture Technologies Corp., Scarsdale, NY), and filter paper was reweighed without sample. Juiciness was expressed as the % of fluid released from the sample during compression. The analysis was performed in triplicate at days 0, 15, 30, 45 and 60 of storage time.

2.3.5 Texture profile analysis (TPA)

TPA was determined at 4 °C using TA-xT2i texture analyzer (Texture Technologies Corp., Scarsdale, NY). Nine samples (2 cm thick and 2 cm Ø) of each treatment were submitted to two successive compression cycles (5 s between them) at 30% of their original thickness, speed of 1 mm/s, using a P-35 probe (stainless steel, 35 mm in diameter). The parameters of hardness (expressed in N), springiness, chewiness, and cohesiveness were measured at 0, 15, 30, 45 and 60 days of storage time.

Lipid oxidation Thiobarbituric acid reactive substances (TBARS) were determined as proposed by Bruna *et al.*²⁷ The measurements were taken at λ of 532 nm using a

spectrophotometer (Beckman, Model DU-70, Muskegon, USA). Results were presented as mg of malondialdehyde (MDA)/kg of sample and obtained from a standard curve of TEP (1,1,3,3-tetraethoxypropane). The analysis was carried out at 0, 15, 30, 45 and 60 days of storage time in quintuplicate.

2.3.6 Scanning Electron Microscopy (SEM)

The microstructure of the samples was evaluated according our previous study¹⁸ using a TM 3000 Tabletop Microscope (Hitachi High Technologies, Japan). 10 micrographs of two batches of each sample were taken at day 10.

2.3.7 Microbiological analysis

The total mesophilic aerobic bacteria (total viable count), total and thermotolerants coliforms, psychrotrophic bacteria, and lactic acid bacteria were quantified according to the methodology proposed by Compendium of Methods for the Microbiological Examination of Food²⁸ at days 0, 15, 30, 45 and 60 of storage time. Two pieces of Bologna were used per treatment, and the analyses were performed in duplicate. From 8 formulations of Bologna sausages 5 were selected to microbiological analysis (PF20/NS, EG20/NS, EG20/RS, PF10-EG10/NS, and PF10-EG10/RS). The criterion to select then was samples with added EG and a control to compare.

2.3.8 Low-field NMR spin–spin relaxation (T2) measurements

Water holding was investigated by measuring the NMR relaxation time T2 of the Bologna sausages after storage for 0, 30 and 60 days, according our previous study.²⁹ The measurements were performed utilizing a Bruker Minispec MQ20 NMR analyzer (Bruker Company, Tucson, USA) with 20 MHz of proton resonance frequency using approximately 1 g of the sample per replicate.

2.3.9 Sensory analyses

Sensory profile of Bologna was performed at the Laboratory of Sensory Analysis of the Institute of Food Engineering (FEA) - UNICAMP. Each sample (approximately 3 g, 5-10°C) was placed on plastic cups, labeled and presented in a balanced presentation order.³⁰ From 8 formulations of Bologna sausages 5 were selected to sensory analysis. The criterion to select then was samples with added EG and a control to compare. The Bolognas samples were evaluated by 120 untrained assessors (40 men, 80 women, aged 18-66) and the selection criterion was regular consumption of meat products.

2.3.9.1 Acceptance test and Purchase intention

Consumers were asked to evaluate the parameters aroma, color, flavor, texture, aroma, and overall liking on a hedonic scale from 1= disliked extremely to 9= liked extremely. We have included a purchase intention question using a 5-point scale from 1= would certainly not buy to 5= would certainly buy.

2.3.9.2 . *Check-all-that-apply questions*

19 descriptors linked to the sensory properties of Bolognas were evaluated by consumers in CATA questionnaire. The selection of the descriptors was based on previous studies performed in our laboratory (Meat and Meat products Laboratory, FEA/UNICAMP, Brazil). The sensory attributes appearance (pale color, and ideal color), flavor (spicy, oil, little spicy, juicy, mild, greasy, Bologna, salty, low salt, and salt in the right amount), texture (spongy, gritty, firm, rubbery, and soft), and aroma (Bologna, and mild), were used to describe the sensory profile of the Bolognas.

2.4 Statistical analyses

The experimental design was performed in three replicates by using a randomized block design. The differences between samples (except CATA data) were evaluated by analysis of variance using a General Linear Model (GLM) taking the treatments and storage time as fixed effect and the replicates as random effect by Tukey's test ($p < 0.05$). The trial version of Statistica 13 (Statsoft®, Dell software, Califórnia, EUA) was used.

CATA questionnaire was first analyzed by counting the frequency of mentioning each sensory descriptor for Bolognas,³¹ and a nonparametric Cochran's Q test was performed to detect significant differences between samples for each attribute.

Afterwards, correspondence analysis (CA) was executed considering the Chi-square distances³² and principal coordinate analysis was performed to correlate sensory attributes and overall liking using XLSTAT 2018 free trial (Addinsoft, New York, USA). Three samples per batch were analysed for the physicochemical and technological analysis. For sensorial evaluation, each panelist tasted five samples, one from each formulation, in a single session.

3 Results and discussion

3.1 Composition

As presented in Table 2, simple fat reduction strategy (PF10/NS and PF10/RS) caused an increase in the moisture content of the samples. Samples with EG (EG20/NS and EG20/RS) also presented higher moisture content than control normal fat ones ($p < 0.05$) probably due to the water addition in the EG formulation.

A fat reduction of approx. 21% was reached in Bolognas with EG (EG20/NS and EG20/RS) and approx 12% in Bologna with EG and animal fat (PF10-EG10/NS and PF10-EG10/RS). EG added in Bologna sausage slightly affected protein content. Reduced sodium products (PF20/RS, PF10/RS, EG20/RS, and PF10-EG10/RS) presented lower ash content than normal salt ones ($p < 0.05$), as expected. Generally, a sodium reduction of 39% was reached falling in the “reduced sodium” category.³³

A calculated dietary fiber content of 2.97% for EG20/NS and EG20/RS allows these samples to be labeled as "good source of fiber" by American rules.³⁴ A reduction of

approx. 16 and 9% in energy value of EG and EG+PF sausages, respectively, was obtained compared to controls samples (PF20/NS and PF20/RS). Samples with 10% of EG presented an estimated dietary fiber of 1.49%.

3.2 Juiciness and Emulsion stability

Related to juiciness values (Fig 1), reduced salt and fat sample (PF10/RS) presented higher values during all shelf life. Normal fat (PF20/NS and PF20/RS) and samples with added EG+PF (PF10-EG10/NS and PF10-EG10/RS) were less juicy during shelf life comparing to other ones ($p < 0.05$).

Reduced fat samples presented higher value of juiciness in the day 15 of shelf life and normal fat samples in the days 30 and 45 of chilled storage ($p < 0.05$). Samples from treatments with salt reduction generally presented higher value of juiciness than others. An unexpected result because one of the main functions of salt is to solubilize the functional myofibrillar proteins of meat and consequently increase juiciness in meat product.³⁵ It is important to highlight that control treatment (PF20/NS) showed values of juiciness close to samples with EG (Fig 1).

For the emulsion stability, a similar trend as for juiciness was observed. Bologna sausage with fat and salt reduction simultaneously (PF10/RS) released more liquid along the storage between all treatments while Bologna with EG (EG20/NS) was the most stable sample ($p < 0.05$) (Figure 1). MDCM is a meat ingredient commonly used to

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produce low cost meat products with protein level (approx. 14%) lower than lean muscle meat (20-24%). During production of MDCM a part of the myofibrillar proteins is damaged resulting in loss of functionality.³⁶ For these reasons, salt and fat reduction affects the quality of the final products resulting in a larger amount of liquid released during the cooking process. However, when emulsion gel was added to meat emulsion (EG20/NS, PF10-EG10/NS) the liquid released was close to zero probably due to the water and fat holding capacity of the hydrocolloids (especially inulin) added to the emulsion gels.^{8, 37}

3.3 Fatty acid profile

The fatty acid profile of Bologna sausages was improved in all treatments containing soybean oil as the lipid phase in EGs as can be observed in Table 3. A reduction of 47 and 25% of SFA and an increase of 87 and 42% of PUFA was reached for 100% and 50% substitution of pork back fat by EG, respectively (EG20/NS, EG20/RS, PF10-EG10/NS, and PF10-EG10/NS). In these samples, more than 70% of total fatty acids are unsaturated and it provides more than 20% of total energy of the product; then sausages could be considered as "high content unsaturated lipids".³⁸ These results are in according to Wolfer *et al.*³⁹ studying the replacement of pork back fat in frankfurter-type sausages by soybean oil oleogels structured with rice bran wax.

Although soybean oil is not a good source of n-3 if compared to the other vegetal oils such as canola and linseed,⁴⁰ values n-3 were increased from 2.36 (PF20/NS and RS)

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to 7.38 and 4.84 g/kg⁻¹ product (EG20 and PF10-EG10, respectively). EG20 samples had more than 0.6 g n-3 /100g product, which allows these products to be labeled as "rich in omega 3" by European legislation.³⁸ This oil has been selected for the emulsion gel formulations to be added as fat substitute in meat products^{19, 41} mainly due its healthier fatty acid composition (compared to pork back fat) and its high availability which reduces the cost of the final product. It seemed more compatible with the one of the most principal objectives of our work that is reformulating a low-cost formulation of Bologna sausage characterized by adding of 50% MDCM, a high value-added raw material widely used along the word. The searching for feasible strategies to reformulate meat products to be accessible to different target groups could effectively contribute to improve the nutritional status of population, including that one with low income. Then, despite the addition of EG reducing values of n-6/n-3 ratio, they are still higher than the recommendations to be lower than 4.⁴²

Related to PUFA/SFA ratio, the addition of EG in sausages increased significantly these values, indicating a better nutritional profile (Table 3). Previous studies that added structured vegetable oil to replace animal fat in meat products also indicated an improvement in PUFA/SFA ratio.⁷ A regular consumption of foods with a PUFA/SFA ratio lower than 0.45 may increase the occurrence of cardiovascular diseases.⁴³ Thrombogenic and atherogenic index (potential promoters of platelet aggregation) were also improved by EG addition. To maintain human health through the prevention of

coronary heart disease values for the AI and TI indexes, at 1 and 0.5, respectively, are expected.⁴⁴ Table 3 shows that only samples with EG (EG20/NS and EG20/RS) reached desirable values of TI and AI simultaneously.

3.4 pH, a_w , TBARS, and color (L^* , a^* , b^*) parameters of reformulated Bologna sausages

The pH values (Table 4) of Bolognas were affected by formulation and by chilled storage ($p < 0.05$), although typical values of pH for meat emulsion were found (6.13 to 6.46). In general reduced salt products presented higher pH values than normal salt samples ($p < 0.05$). Control (PF20/NS) and EG added treatments presented similar values of pH ($p > 0.05$), except in the day 60 of storage when control sample (PF20/NS) presented higher values. Similar pH range was reported previously when EG was added as fat substitute in frankfurter.⁴⁵

A_w values ranged from 0.970 to 0.985 and the reduction of sodium and animal fat simultaneously (PF10/RS, EG20/RS and PF10-EG10/RS) increased a_w in the samples ($p < 0.05$). During shelf life, a_w reached the higher values at day 60 for samples PF10/NS, PF10/RS, EG20/NS, EG20/RS, PF10-EG10/NS, and PF10-EG10/RS. In general, EG addition (EG20/NS, PF10-EG10/NS) do not affected a_w values comparing to control treatment (PF20/NS), with a_w values typical of emulsified meat products.⁴⁶ An interesting finding due to the importance of a_w for food quality and safety.

TBARS values of reformulated Bologna sausages ranged from 0.04 to 0.89 mg MDA/kg sample (Table 4) during chilled storage. It was observed that EG addition affected lipid oxidation in Bologna sausages. A more intense oxidation was perceived in EG added samples (EG20/NS and EG20/RS), followed by EG+PF (PF10-EG10/NS and PF10-EG10/RS) ($p < 0.05$). This increase in lipid oxidation was expected due to the higher concentration of PUFAs in treatments containing soybean oil as lipid phase of EGs when compared to controls added of pork back fat (Table 3), in accordance with previous studies.⁴³ According to Choi *et al.*,⁴⁷ different oils increase oxidation levels of frankfurters. Soybean oil was more prone to oxidation than pork back fat, olive oil, grape seed oil, corn oil, and canola oil in frankfurters.

During chilled storage, lipid oxidation remained steady until 30 days, with very similar results, not superior to 0.12 mg MDA/ kg sample. However, at 45 and 60 day, the TBARS values decreased for all treatments ($p < 0.05$) as opposed to reported in the literature by some authors.^{8, 48, 49} One possibility to explain these results is the reducing levels of malonaldehyde (MDA) precursors, during the secondary phase of the autoxidation process due to evolution of lipid oxidation.^{50, 51} However, it should be followed of a sensory degradation of the products causing consumer rejection, which has not happened in our study. Zhang *et al.*,⁵² regarding this point, investigated different TBARS methods aiming to correlate the data obtained to consumer study. The author shown there were no significant effects of TBARS values on the sensory results,

suggesting that untrained consumers cannot detect abnormal flavour development due to high levels of lipid oxidation (TBARS) as indicated by the TBARS test. Regardless of the differences in meat matrix, besides the use of nitrite in our treatments that could contribute for this decreasing along the time, the lipid oxidation investigation appears as a great point to be understood.

Despite these considerations, TBARS values near 0.5 mg / kg sample suggested beginning of oxidation, and values above 1.0 mg /kg sample are indicative of advanced oxidative status.⁵³ Then, all reformulated sausage presented values of TBARS lower than sensory rancidity detection (< 1.0). Wang *et al.*⁴⁸ reported that in emulsion gel, unsaturated oil is “structured” and the mobility of the reactants is restricted indicating why using EG is more suitable than adding the oil in a liquid form to guarantee a good oxidative stability. The addition of emulsion in different types of meat products as fat replacers have shown similar results, with TBARS values lower than 1.0 mg MDA /kg sample.^{5, 7, 45}

Color parameters (Table 4) show that control treatment (PF20/NS) presented lower values of lightness (L*) and higher values of a* than others (p < 0.05). L* values were higher (p < 0.05) in all EG added samples (EG20/NS, EG20/RS, PF10-EG10/NS, PF10-EG10/RS). In general, storage time did not affect the color of samples (p > 0.05). Values of Euclidian distance (ΔE) between 2 and 3.5 are an indicative that an inexperienced observer is able to perceive the difference while values among 3.5 and 5

every observer can easily see the difference.⁵⁴ Therefore, EG added samples were the most different samples comparing to PF20/NS treatment and can be easily differentiated from control by observer. These could be attributed to the ingredients used to form the emulsion gel (inulin, soy protein and soybean oil), given that the use of emulsion gel instead of animal fat does not perfectly mimic the color effect of pork back fat on the reformulated sausages. Color parameters of meat products are in general affected by emulsion gel addition. Barros *et al.*⁵ and Wang *et al.*⁴⁸ also found an increase in L* values when adding emulsion gel as a fat substitute in beef burgers and cooked style Harbin sausage, respectively.

3.5 Texture profile analysis (TPA) of reformulated Bologna sausages

Substituting pork back fat (PF) by EG in the Bologna sausages decreased the hardness ($p < 0.05$) while products with only fat and salt reduction (PF10/NS and PF10/RS) were softer and chewy ($p < 0.05$) than others (Table 5). This behavior indicates that replacing fat by EG seems to be a better strategy than only fat reduction without EG. Results reported in literature show that salt reduction decreases hardness in meat product,⁵⁵ however in this study fat reduction appeared to affect hardness more than salt reduction. During shelf life samples get harder for all treatments ($p < 0.05$), similar that was observed by other authors.⁵⁶

Sausages with EG (EG20/NS, EG20/RS) were more elastic and cohesive than control (PF20/NS) ($p < 0.05$) during the storage. In general, springiness was not affected by storage time and samples get more cohesive during shelf life (Table 5).

3.6 Microstructural and time domain NMR (TDNMR) results of reformulated Bologna sausage

Figure 2 shows that Bologna sausage with EG (EG20/NS and EG20/RS) exhibited a denser network, presentation a more continuous and compact matrix in accordance with previous studies.⁵⁷ When EG was added to replace partially PF the matrix presented structure similar to control treatment (Fig 2).

Sausages with salt reduction (PF20/NS, PF10/RS, EG20/RS, and PF10-EG10/RS) presented more empty spaces, probably due higher amount of free water in these samples, in accordance with emulsion stability data (Fig 1) when comparing samples with normal and reduced salt (for example, PF20/NS and PF20/RS, EG20/NS and EG20/RS) the reduced salt sample always released more liquid than normal salt one.

Figure 3-a shows that TDNMR T_2 relaxation curves for Bologna sausages presented a multiple-exponential distribution with clearly two distinct populations mainly related to water because about 80% of protons present in the meat samples comes from water. Entrapped or immobilized water (T_{2a}) presented a relaxation time between 10 and 100 ms and is linked to the highly organized protein or fiber bundles structures. While mobile water (T_{2b}) with relaxation time from 100 to 1000 ms refers to the water located between

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fiber bundles interacting weakly with charged group, also known as the extra-myofibrillar water containing the sarcoplasmic protein fraction.⁵⁸

The distribution of relaxation times in immobilized water (T_{2a}) (Fig 3b, Supplementary Material 1) showed a higher time when the fat and salt content was reduced (see samples PF10/NS and PF10/RS), $p < 0.05$. It can be associated with the increase in the mobility or the reduction of the interaction between mobility and the organized protein or fiber bundles structures. This result suggest that occurs an increasing in spaces that hold water as the fat and salt content was decreased in the Bologna sausages. According to Han *et al.*⁵⁹ a shorter T_{2a} may reflects a smaller size of the intrinsic structures in which the myofibrillar water is trapped and/or the local myofibrillar structures act as stronger relaxation sinks on the water molecules. However, with EG added treatments the values of time is recovered to near the controls probably by the fibers of the emulsion to make in parts the role of the fat, results similar to those obtained by juiciness and scanning electron microscopy evaluation. Comparing with texture data, especially for samples PF10/NS and PF10/RS, higher values of T_{2a} and lower values of hardness were obtained for these samples. The negative correlation between hardness and T_{2a} values was also observed by García *et al.*⁶⁰.

The behavior observed in mobile population (T_{2b}) (Fig 3b, Supplementary Material 1) shows a fluctuation in the average, might because change in the composition does not cause a sudden change in this population. These results suggested that mobile water is

not sensitive to changes occurring in the composition mainly because it is weakly bound to the matrix. In general control Bologna (PF20/NS) showed the lowest values of T_{2b} ($p < 0.05$).

Now probing the quantities of the two distinct populations, except for shelf life 15 days, it has a higher amount of mobile water population T_{2b} compared to immobilized water T_{2a} for the treatments control and with EG. On other hand, in the PF10/NS and PF10/RS samples this proportion was inverted ($p < 0.05$). This can probably be ascribed to the fat and salt reduction causing poor interaction (or increase of water holes) and for this reason is easier to remove mobile water. However the EG addition recovers the matrix that has been weakened due to reduction of fat and salt.

3.7 Microbiological properties of reformulated Bologna sausage

Total viable count (mesophilic colonies) remained low ($< 3.5 \log \text{ cfu/g}$) during chilled storage of Bologna sausages (Table 6). In general sample with salt reduction and EG addition presented the largest number of colonies (EG20/RS) ($p < 0.05$). Salt is an important ingredient in meat products ensuring microbiological stability mainly through water activity reduction.⁶¹ Lactic acid and psychrotrophic bacteria levels were lower than $1 \log \text{ cfu/g}$ and for coliforms was $< 3 \text{ NMP/g}$, (data not shown). These low levels of microorganism could be associated with the additives and seasonings added to the

Bolognas formulation and also good manufacturing practices ensuring that the product was safe for consumers.

3.8 Sensorial properties of reformulated Bologna sausages

As can be seen in Figure 4 all samples were judged acceptable (scores higher than 5). Excepting for color, no statistical difference ($p > 0.05$) were found between control (PF20/NS) and Bolognas with EG+PF with no salt reduction (PF10-EG10/NS) for texture, flavor, aroma, and overall liking. Samples with simultaneously salt and fat reduction and the addition of EG (EG20/RS and PF10-EG10/RS) scored lowest when compared to others samples in agreement with previous studies that have also found the negative impact that the reduction of fat and salt have on the sensory characteristics of emulsified meat products.⁶² Regarding purchase intention, samples presented 3.7, 3.0, 2.5, 3.6, and 2.90 scores for PF20/NS, EG20/NS, EG20/RS, PF10-EG10/NS, and PF10-EG10/RS, respectively.

The nonparametric Cochran's Q test was performed, and it shows that there were significant differences for 16 of 19 descriptors used to characterize the samples. Descriptors with less than 10% of frequency's mention were also removed.

By correspondence analysis (Figure 4b) three groups of samples with different sensory characteristics were perceived. The first group PF20/NS and PF10-EG10/NS were close to the descriptors firm, juicy, ideal color, right amount of salt, Bologna flavor,

Bologna aroma, and spicy flavor. According to Desmond³⁵ normal salt products are juiciest than reduced ones due to salt increase water holding capacity. The second group PF10-EG10/RS and EG20/RS were linked to mild aroma, low salt, little spicy, mild flavor, and pale color descriptors.

The third group was composed by EG20/NS sample, characterized as spongy, greasy, and with oil flavor. Overall liking attribute (Figure 4c) was associated to firm, Bologna flavor, juicy, ideal color, and right amount of salt descriptors, attributes characteristics of PF10-EG10/NS and PF20/NS samples.

When emulsion gel was applied as a fat replacer, sensorial results shown that the perception of flavor and aroma compounds was reduced. Previous studies shown that aroma compounds had lower released in systems more compact like emulsion gels.^{63, 64}

4 Conclusion

This study has demonstrated the potential use of inulin-based emulsion gel as a fat substitute in reduced fat and salt meat products. Replacing pork back fat by EG resulted in products with higher fiber content and a better fatty acid profile with higher amount of unsaturated fatty acids and lower content of SFA. The addition of EG makes sample more stable to heat treatment while microbiological and sensorial data were negatively impacted by simultaneously salt and fat reduction in Bologna sausage with EG. The shelf life study shown that samples with EG presented good results related to lipid

oxidation and NMR data, presenting alterations in textural and microstructural properties when compared to control. Bologna with EG seems to be a good option to obtain healthier meat product, and future studies are need to improve the properties when salt and fat are reduced simultaneously.

5 Conflict of interest

The authors declare no conflict of interest associated with this research.

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Figure 1. Emulsion stability (% exudated liquid) ■ at day 0 and juiciness evaluation (%) during chilled storage (■ day 0 □ day 15 ■ day 30 □ day 45 □ day 60) of Bologna sausages. Equal lower case letters for the same color are not statistically different ($p > 0.05$). NF-normal fat, RF-reduced fat, PF-pork back fat, EG- emulsion gel, NS-normal salt, RS-reduced salt. Bars show standard error

Figure 2. Effect of fat and salt reduction on microstructural properties in Bologna sausage with emulsion gel. NF-normal fat, RF-reduced fat, PF-pork back fat, EG- emulsion gel, NS-normal salt, RS-reduced salt

Figure 3. TDNMR - a) The TDNMR curve for the Bologna sausages samples exhibited a Log-Normal distribution deconvolution in two water populations. b) T_2 center relaxation time and c) relative area of the Bologna sausages samples in distinct shelf life (0, 15, 30, 45, and 60 days)

Figure 4. Sensory evaluation of Bologna sausages with emulsion gels. (a) Consumer test, (b) Representation of the samples and attributes in the first two dimensions of the correspondence analysis (CA) of the Bologna-sausages samples using CATA questions, (c) Correlation between sensory attributes and overall liking in the first two dimensions of the CA. NF-normal fat, RF-reduced fat, PF-pork back fat, EG- emulsion gel, NS-normal salt, RS-reduced salt

Figure 1.

Figure 2.

Figure 3.

Figure 4.

Table 1. Description of treatments (% w/w) of Bologna sausage elaborated with mechanically deboned chicken meat (MDCM) and inulin-based emulsion gel

Treatments	MDCM	Pork meat	PF	EG	NaCl	Water
PF20/NS	50	20	20	0	2	7.2
PF20/RS	50	20	20	0	1	8.2
PF10/NS	50	20	10	0	2	17.2
PF10/RS	50	20	10	0	1	18.2
EG20/NS	50	20	0	20	2	7.2
EG20/RS	50	20	0	20	1	8.2
PF10-EG10/NS	50	20	10	10	2	7.2
PF10-EG10/RS	50	20	10	10	1	8.2

Note: PF- pork back fat, EG- emulsion gel, NS- normal salt, RS- reduced salt, MDCM- Mechanically deboned chicken meat. Additives: 0.25 g/100 g sodium tripolyphosphate; 0.05g /100 g sodium ascorbate; 0.52 g/100 g seasoning and 150 ppm sodium nitrite.

Table 2. Chemical composition (g/kg⁻¹), sodium content (g/kg⁻¹), energy content (Kcal), and estimated total dietary fiber (g/kg⁻¹) of Bologna sausages

	Moisture	Fat	Protein	Ash	Carbohydrates*	Sodium	Energy content	TDF**
PF20/NS	593.4±2.8 ^g	248.5±6.6 ^a	126.9±2.7 ^{abc}	30.9±0.6 ^{ab}	0.00±0.00 ^d	8.06±0.10 ^b	275±4 ^a	-
PF20/RS	592.4±3.0 ^g	249.9±3.1 ^a	132.4±3.5 ^a	21.4±0.6 ^{cd}	3.7±0.32 ^{cd}	5.05±0.26 ^d	280±2 ^a	-
PF10/NS	675.6±3.7 ^b	163.3±5.6 ^e	120.5±3.3 ^{de}	30.5±0.3 ^b	11.5±0.81 ^{bcd}	8.63±0.18 ^a	200±3 ^d	-
PF10/RS	683.0±7.7 ^a	163.8±4.4 ^e	124.2±1.5 ^{cd}	20.5±0.2 ^d	10.5±0.57 ^{bcd}	4.72±0.06 ^e	201±4 ^d	-
EG20/NS	619.0±3.0 ^d	201.8±5.6 ^c	118.6±6.5 ^e	30.7±1.3 ^{ab}	35.1±9.1 ^a	7.58±0.26 ^c	235±3 ^c	29.7
EG20/RS	628.9±2.9 ^c	190.4±6.3 ^d	124.9±3.7 ^{cd}	21.6±0.3 ^c	38.0±6.8 ^a	4.91±0.07 ^{de}	229±4 ^c	29.7
PF10-EG10/NS	604.0±1.5 ^f	220.0±5.8 ^b	131.3±4.1 ^{ab}	31.5±0.2 ^a	17.7±6.4 ^{bc}	7.69±0.17 ^c	253±3 ^b	14.9
PF10-EG10/RS	612.0±6.4 ^e	218.5±5.2 ^b	126.0±1.1 ^{bc}	22.1±0.6 ^c	20.4±4.8 ^b	4.91±0.19 ^{de}	253±5 ^b	14.9

Note: * Carbohydrates (%) = 100%-moisture (%) - crude fat (%) - crude protein (%) - ash (%). **Values by calculation. **TDF- total dietary fiber. Estimated from Inulin fiber content (90%). Equal letters in the same column are not statistically different (p> 0.05). PF- pork back fat, EG-emulsion gel, NS-normal salt, RS-reduced salt.

Table 3. Fatty acid composition of Bologna sausage (g/kg⁻¹ product)

Fatty acid	PF20/NS and PF20/RS	PF10/NS and PF10/RS	EG20/NS and EG20/RS	PF10-EG10/NS and PF10-EG10/RS
C8:0 caprylic	0.12±0.00 ^{NS*}	0.12±0.00 ^{NS*}	0.12±0.00 ^{NS*}	0.12±0.00 ^{NS*}
C10:0 capric	0.16±0.00 ^a	0.10±0.00 ^c	0.06±0.00 ^d	0.11±0.00 ^b
C12:0 lauric	0.35±0.00 ^a	0.22±0.00 ^c	0.14±0.01 ^d	0.24±0.00 ^b
C14:0 myristic	3.00±0.01 ^a	1.82±0.00 ^c	0.99±0.01 ^d	1.95±0.01 ^b
C15:0 pentadecylic	0.21±0.00 ^a	0.16±0.00 ^c	0.16±0.00 ^c	0.18±0.00 ^b
C16:0 palmitic	59.81±0.20 ^a	39.64±0.07 ^c	34.80±0.70 ^d	46.52±0.19 ^b
C17:0 margaric	0.67±0.02 ^a	0.43±0.02 ^c	0.34±0.01 ^d	0.49±0.02 ^b
C18:0 stearic	28.08±0.15 ^a	16.88±0.08 ^c	11.48±0.22 ^d	19.35±0.13 ^b
C20:0 arachidic	0.57±0.01 ^a	0.35±0.01 ^b	0.57±0.03 ^a	0.56±0.01 ^a
C22:0 behenic	0.22±0.01 ^c	0.17±0.00 ^d	0.64±0.03 ^a	0.43±0.00 ^b
Σ SFA	93.20±0.34 ^a	59.91±0.11 ^c	49.30±0.98 ^d	69.96±0.32 ^b
C16:1 palmitolenic	7.57±0.03 ^a	5.50±0.03 ^c	3.99±0.03 ^d	5.70±0.03 ^b
C17:1 cis-10-heptadecenoic	0.43±0.01 ^a	0.26±0.00 ^c	0.18±0.00 ^d	0.30±0.00 ^b
C18:1n-9 oleic	101.5±0.36 ^a	65.24±0.13 ^c	59.54±1.43 ^d	79.12±0.35 ^b
C20:1 eicosenoic	1.81±0.08 ^a	0.99±0.04 ^c	0.56±0.02 ^d	1.15±0.05 ^b
Σ MUFA	111.3±0.4 ^a	72.00±0.15 ^c	64.27±1.46 ^d	86.27±0.39 ^b
C18:1 trans	0.62±0.22 ^a	0.38±0.10 ^b	0.20±0.00 ^b	0.40±0.11 ^b
C18:2 trans	0.10±0.00 ^c	0.04±0.00 ^d	0.28±0.02 ^a	0.19±0.00 ^b
C18:3 trans	0.01±0.00 ^c	0.01±0.00 ^c	1.03±0.06 ^a	0.52±0.00 ^b
C18:2n-6.linoleic	40.60±0.11 ^c	28.72±0.04 ^d	72.99±3.34 ^a	56.26±0.11 ^b
C18:3n-3.linolenic	2.36±0.01 ^c	1.78±0.00 ^d	7.38±0.38 ^a	4.84±0.01 ^b
Σ PUFA	42.97±0.12^c	30.50±0.04^d	80.37±3.72^a	61.10±0.12^b
PUFA/SFA	0.46±0.00 ^d	0.51±0.00 ^c	1.63±0.04 ^a	0.87±0.00 ^b
n-6/n-3	17.16±0.05 ^a	16.13±0.04 ^b	9.89±0.05 ^d	11.62±0.01 ^c
Thrombogenic index (TI)	1.09±0.00^a	1.04±0.00^b	0.51±0.01^d	0.78±0.00^c
Atherogenic index (AI)	0.47±0.00^a	0.46±0.00^b	0.27±0.00^d	0.37±0.00^c

Equal lower case letters in the same column are not statistically different ($p > 0.05$). Means±standard deviation. NS*, not significant. NS- normal salt; RS- reduced salt; EG- emulsion gel

Table 4. pH, a_w , TBARS values and color parameters of reformulated Bologna sausages during chilled storage

	Samples	Days					SEM
		0	15	30	45	60	
pH	PF20/NS	6.28 ^{CB}	6.26 ^{deBC}	6.25 ^{Cc}	6.19 ^{dB}	6.43 ^{abA}	0.01
	PF20/RS	6.35 ^{aB}	6.40 ^{aB}	6.35 ^{aB}	6.29 ^{aC}	6.46 ^{Aa}	0.01
	PF10/NS	6.29 ^{bcAB}	6.31 ^{cdAB}	6.31 ^{bAB}	6.23 ^{CB}	6.34 ^{bA}	0.01
	PF10/RS	6.35 ^{aB}	6.37 ^{abAB}	6.38 ^{aA}	6.28 ^{abC}	6.15 ^{cdD}	0.01
	EG20/NS	6.23 ^{dB}	6.26 ^{deA}	6.26 ^{CA}	6.18 ^{dC}	6.10 ^{dD}	0.01
	EG20/RS	6.30 ^{bC}	6.32 ^{bcB}	6.35 ^{aA}	6.26 ^{bD}	6.20 ^{cdE}	0.01
	PF10-EG10/NS	6.25 ^{dB}	6.25 ^{eB}	6.28 ^{CA}	6.20 ^{dC}	6.13 ^{cdD}	0.01
	PF10-EG10/RS	6.31 ^{bB}	6.34 ^{bcA}	6.36 ^{aA}	6.29 ^{aB}	6.23 ^{cC}	0.01
a_w	SEM	0.00	0.01	0.00	0.00	0.02	
	PF20/NS	0.973 ^{cNS*}	0.973 ^{dNS*}	0.973 ^{cNS*}	0.972 ^{dNS*}	0.974 ^{dNS*}	<0.0001
	PF20/RS	0.977 ^{bC}	0.981 ^{bB}	0.979 ^{bB}	0.984 ^{abA}	0.980 ^{bB}	<0.0001
	PF10/NS	0.973 ^{cC}	0.976 ^{cBC}	0.975 ^{cBC}	0.978 ^{cAB}	0.980 ^{bcA}	<0.0001
	PF10/RS	0.982 ^{aB}	0.983 ^{aAB}	0.982 ^{aAB}	0.984 ^{aAB}	0.984 ^{aA}	<0.0001
	EG20/NS	0.970 ^{dD}	0.972 ^{dC}	0.974 ^{cBC}	0.974 ^{dB}	0.976 ^{cdA}	<0.0001
	EG20/RS	0.979 ^{abC}	0.981 ^{bB}	0.981 ^{abB}	0.981 ^{bB}	0.985 ^{aA}	<0.0001
	PF10-EG10/NS	0.971 ^{cdC}	0.973 ^{dB}	0.973 ^{cBC}	0.973 ^{dB}	0.976 ^{cdA}	<0.0001
TBARS (mg MDA/ kg sample)	PF10-EG10/RS	0.981 ^{aB}	0.982 ^{abB}	0.980 ^{abB}	0.982 ^{abB}	0.985 ^{aA}	<0.0001
	SEM	0.000	0.000	0.000	0.001	0.001	
	PF20/NS	0.10 ^{dB}	0.12 ^{CA}	0.11 ^{cAB}	0.08 ^{cC}	0.05 ^{dD}	0.00
	PF20/RS	0.10 ^{dB}	0.12 ^{CA}	0.09 ^{cB}	0.04 ^{cD}	0.06 ^{dC}	0.00
	PF10/NS	0.09 ^{dA}	0.08 ^{cAB}	0.09 ^{CA}	0.07 ^{cB}	0.04 ^{dC}	0.00
	PF10/RS	0.09 ^{dB}	0.10 ^{cB}	0.12 ^{CA}	0.07 ^{cC}	0.07 ^{dC}	0.00
	EG20/NS	0.78 ^{bB}	0.76 ^{aB}	0.89 ^{aA}	0.46 ^{aC}	0.36 ^{aD}	0.03
	EG20/RS	0.84 ^{aAB}	0.77 ^{aB}	0.85 ^{aA}	0.50 ^{aC}	0.34 ^{aD}	0.03
L*	PF10-EG10/NS	0.45 ^{cB}	0.43 ^{bB}	0.52 ^{bA}	0.27 ^{bC}	0.20 ^{CD}	0.02
	PF10-EG10/RS	0.47 ^{CA}	0.48 ^{bA}	0.52 ^{bA}	0.28 ^{bB}	0.25 ^{bB}	0.02
	SEM	0.04	0.03	0.04	0.02	0.02	
	PF20/NS	63.29 ^{dNS*}	63.37 ^{eNS*}	63.16 ^{dNS*}	63.75 ^{dNS*}	63.58 ^{dNS*}	0.11
	PF20/RS	65.25 ^{cC}	65.74 ^{cABC}	65.91 ^{cAB}	66.33 ^{bA}	65.62 ^{bBC}	0.08
	PF10/NS	63.77 ^{dNS*}	63.94 ^{deNS*}	63.41 ^{dNS*}	63.49 ^{dNS*}	63.57 ^{dNS*}	0.09
	PF10/RS	64.37 ^{cdAB}	64.65 ^{dAB}	64.09 ^{dB}	65.06 ^{CA}	64.70 ^{cAB}	0.13
	EG20/NS	66.80 ^{abAB}	67.52 ^{abA}	67.16 ^{abAB}	67.54 ^{aA}	66.68 ^{aB}	0.10

	EG20/RS	67.80 ^{aA}	67.96 ^{aA}	67.65 ^{aAB}	67.50 ^{abAB}	66.64 ^{aB}	0.18
	PF10-EG10/NS	66.95 ^{abNS*}	66.73 ^{bcNS*}	66.23 ^{bcNS*}	66.55 ^{abNS*}	66.38 ^{abNS*}	0.09
	PF10-EG10/RS	66.57 ^{bNS*}	67.15 ^{abNS*}	66.60 ^{abcNS*}	67.09 ^{abNS*}	66.36 ^{abNS*}	0.11
	SEM	0.20	0.21	0.21	0.20	0.16	
a*	PF20/NS	11.27 ^a	11.21 ^a	11.39 ^a	11.14 ^a	11.01 ^a	0.06
	PF20/RS	10.49 ^{bcNS*}	10.36 ^{bcdNS*}	10.46 ^{bcdNS*}	10.23 ^{bNS*}	10.39 ^{bcNS*}	0.03
	PF10/NS	10.71 ^{bNS*}	10.69 ^{abNS*}	10.84 ^{bNS*}	10.81 ^{aNS*}	10.68 ^{abNS*}	0.06
	PF10/RS	10.65 ^{bcA}	10.63 ^{bcA}	10.78 ^{bcA}	10.32 ^{bAB}	10.10 ^{cB}	0.07
	EG20/NS	9.94 ^{dNS*}	9.96 ^{deNS*}	10.13 ^{deNS*}	10.07 ^{bNS*}	10.09 ^{cNS*}	0.03
	EG20/RS	9.88 ^{dNS*}	9.75 ^{eNS*}	9.84 ^{eNS*}	10.13 ^{bNS*}	10.03 ^{cNS*}	0.08
	PF10-EG10/NS	9.95 ^{dB}	10.10 ^{cdeAB}	10.31 ^{dA}	9.99 ^{bAB}	9.98 ^{cAB}	0.04
	PF10-EG10/RS	10.19 ^{cdNS*}	9.99 ^{deNS*}	10.34 ^{cdNS*}	10.09 ^{bNS*}	10.29 ^{bcNS*}	0.05
	SEM	0.06	0.07	0.06	0.06	0.06	
b*	PF20/NS	13.32 ^{abNS*}	13.27 ^{abNS*}	13.67 ^{abcNS*}	13.46 ^{bcNS*}	13.44 ^{abNS*}	0.05
	PF20/RS	13.20 ^{abcBC}	13.31 ^{abABC}	13.48 ^{bcdA}	13.07 ^{dC}	13.40 ^{abcAB}	0.04
	PF10/NS	12.78 ^{dB}	12.93 ^{abAB}	13.23 ^{dA}	13.21 ^{cdA}	12.95 ^{bcAB}	0.06
	PF10/RS	12.83 ^{cdB}	12.81 ^{bB}	13.35 ^{cdA}	13.13 ^{dAB}	12.86 ^{cB}	0.06
	EG20/NS	13.53 ^{aBC}	13.35 ^{aC}	13.92 ^{aA}	13.70 ^{abAB}	13.81 ^{aA}	0.04
	EG20/RS	13.48 ^{aNS*}	13.41 ^{aNS*}	13.78 ^{abNS*}	13.77 ^{aNS*}	13.75 ^{aNS*}	0.06
	PF10-EG10/NS	12.99 ^{bcdC}	13.26 ^{abBC}	13.88 ^{aA}	13.61 ^{abA}	13.54 ^{aAB}	0.07
	PF10-EG10/RS	13.43 ^{aAB}	13.24 ^{abB}	13.75 ^{abA}	13.66 ^{abAB}	13.84 ^{Aa}	0.06
	SEM	0.05	0.05	0.04	0.04	0.06	
ΔE	PF20/NS	-	-	-	-	-	-
	PF20/RS	2.11	2.61	2.75	3.22	2.49	-
	PF10/NS	0.91	0.95	0.46	0.51	0.75	-
	PF10/RS	1.34	1.59	0.94	2.02	1.89	-
	EG20/NS	3.76	4.43	4.08	4.43	3.62	-
	EG20/RS	4.72	4.91	4.61	4.38	3.60	-
	PF10-EG10/NS	3.90	3.63	3.14	3.51	3.36	-
	PF10-EG10/RS	3.45	4.07	3.46	3.99	3.26	-
	SEM						

Equal lower case letters in the same column are not statistically different ($p > 0.05$). Equal capital letters in the same row for the same parameter are not statistically different ($p > 0.05$). Note: NS*, not significant; PF- pork back fat; NS- normal salt; RS- reduced salt; EG- emulsion gel

Table 5. Texture Profile Analysis (TPA) of reformulated Bologna sausages during chilled storage

	Samples	Days					SEM
		0	15	30	45	60	
Hardness (N)	PF20/NS	9.95 ^{aC}	10.47 ^{aBC}	10.83 ^{aAB}	10.69 ^{aB}	11.46 ^{aA}	0.09
	PF20/RS	9.37 ^{abcB}	9.21 ^{bB}	9.61 ^{bB}	10.35 ^{aA}	10.77 ^{abA}	0.10
	PF10/NS	7.63 ^{dB}	7.86 ^{cAB}	7.58 ^{cB}	8.08 ^{bAB}	8.50 ^{cA}	0.09
	PF10/RS	7.41 ^{dB}	7.56 ^{cB}	7.75 ^{cB}	8.51 ^{bA}	8.57 ^{cA}	0.09
	EG20/NS	9.22 ^{bcC}	9.33 ^{bBC}	9.87 ^{bB}	10.76 ^{aA}	10.65 ^{bA}	0.09
	EG20/RS	8.90 ^{cC}	9.11 ^{bC}	9.85 ^{bB}	10.60 ^{aA}	10.09 ^{bAB}	0.09
	PF10-EG10/NS	9.64 ^{abC}	9.37 ^{bC}	9.60 ^{bC}	10.53 ^{aA}	10.02 ^{bAB}	0.10
	PF10-EG10/RS	9.38 ^{abcD}	9.55 ^{bCD}	10.18 ^{bBC}	11.22 ^{aA}	10.65 ^{bAB}	0.11
Springiness (mm)	SEM	0.08	0.09	0.10	0.11	0.10	
	PF20/NS	0.90 ^{bNS*}	0.89 ^{dNS*}	0.90 ^{bcNS*}	0.91 ^{abNS*}	0.89 ^{cNS*}	<0.001
	PF20/RS	0.89 ^{bNS*}	0.89 ^{cdNS*}	0.89 ^{cNS*}	0.90 ^{bNS*}	0.91 ^{bcNS*}	<0.001
	PF10/NS	0.91 ^{abNS*}	0.91 ^{bcNS*}	0.91 ^{abcNS*}	0.92 ^{aNS*}	0.92 ^{abNS*}	<0.001
	PF10/RS	0.90 ^{abB}	0.91 ^{bAB}	0.91 ^{abAB}	0.90 ^{abB}	0.92 ^{abA}	<0.001
	EG20/NS	0.91 ^{abC}	0.93 ^{aAB}	0.93 ^{aAB}	0.92 ^{abBC}	0.94 ^{aA}	<0.001
	EG20/RS	0.92 ^{aAB}	0.92 ^{abAB}	0.92 ^{aB}	0.92 ^{abAB}	0.93 ^{aA}	<0.001
	PF10-EG10/NS	0.90 ^{abNS*}	0.91 ^{bcNS*}	0.91 ^{aNS*}	0.92 ^{abNS*}	0.92 ^{abNS*}	<0.001
Cohesiveness	PF10-EG10/RS	0.90 ^{abNS*}	0.91 ^{bNS*}	0.91 ^{abNS*}	0.91 ^{abNS*}	0.91 ^{bcNS*}	<0.001
	SEM	0.00	0.00	0.00	0.00	0.00	
	PF20/NS	0.79 ^{cdeB}	0.79 ^{cB}	0.79 ^{dB}	0.81 ^{bA}	0.79 ^{dB}	<0.001
	PF20/RS	0.78 ^{eB}	0.80 ^{bcA}	0.80 ^{cdA}	0.80 ^{bcA}	0.80 ^{cdA}	<0.001
	PF10/NS	0.79 ^{bcC}	0.80 ^{abBC}	0.81 ^{abAB}	0.82 ^{aA}	0.81 ^{abAB}	<0.001
	PF10/RS	0.78 ^{deB}	0.80 ^{bcA}	0.80 ^{bcdA}	0.80 ^{cA}	0.80 ^{bcdA}	<0.001
	EG20/NS	0.80 ^{abB}	0.81 ^{aA}	0.81 ^{aA}	0.81 ^{bcAB}	0.81 ^{abA}	<0.001
	EG20/RS	0.80 ^{aB}	0.80 ^{abB}	0.80 ^{abcB}	0.80 ^{bcB}	0.82 ^{aA}	<0.001
Chewiness (N x mm)	PF10-EG10/NS	0.79 ^{bcdC}	0.80 ^{bcBC}	0.80 ^{bcAB}	0.80 ^{bcBC}	0.81 ^{abcA}	<0.001
	PF10-EG10/RS	0.78 ^{deB}	0.80 ^{abcA}	0.80 ^{cdA}	0.80 ^{cA}	0.80 ^{cdA}	<0.001
	SEM	0.00	0.00	0.00	0.00	0.00	
	PF20/NS	7.07 ^{aC}	7.33 ^{aBC}	7.68 ^{aAB}	7.82 ^{abA}	8.12 ^{aA}	0.07
	PF20/RS	6.55 ^{bB}	6.57 ^{bB}	6.87 ^{bB}	7.50 ^{bA}	7.81 ^{abA}	0.07
	PF10/NS	5.50 ^{cBC}	5.73 ^{cABC}	5.28 ^{cC}	6.11 ^{cAB}	6.35 ^{cA}	0.08
	PF10/RS	5.22 ^{cC}	5.47 ^{cC}	5.66 ^{cBC}	6.12 ^{cAB}	6.35 ^{cA}	0.07
	EG20/NS	6.69 ^{abC}	7.05 ^{abBC}	7.41 ^{abB}	7.95 ^{abA}	8.13 ^{aA}	0.08

EG20/RS	6.58 ^{bC}	6.76 ^{bC}	7.26 ^{abB}	7.84 ^{abA}	7.69 ^{abAB}	0.07
PF10-EG10/NS	6.90 ^{abB}	6.77 ^{bB}	6.77 ^{bB}	7.69 ^{abA}	7.46 ^{bAB}	0.10
PF10-EG10/RS	6.64 ^{abD}	6.96 ^{abCD}	7.41 ^{abBC}	8.15 ^{aA}	7.78 ^{abAB}	0.08
SEM	0.06	0.06	0.09	0.08	0.07	

Equal lower case letters in the same column are not statistically different ($p > 0.05$). Equal capital letters in the same row for the same parameter are not statistically different ($p > 0.05$). Note: NS*, not significant. PF- pork back fat; NS- normal salt; RS- reduced salt; EG- emulsion gel.

Table 6. Microorganisms count (log cfu/g) of reformulated Bologna sausage during chilled storage

Microorganism	Samples	Days				
		0	15	30	45	60
Total viable count (TVC)	PF20/NS	3.1±0.1 ^{abNS*}	2.9±0.1 ^{bNS*}	2.8±0.0 ^{bcNS*}	2.9±0.0 ^{aNS*}	3.0±0.0 ^{abNS*}
	EG20/NS	3.1±0.1 ^{abA}	2.9±0.0 ^{bAB}	2.7±0.1 ^{bcAB}	2.6±0.1 ^{bB}	2.9±0.3 ^{abA}
	EG20/RS	3.4±0.4 ^{Aab}	3.5±0.2 ^{aA}	2.9±0.0 ^{Bbc}	2.8±0.0 ^{abC}	3.3±0.4 ^{aABC}
	PF10-EG10/NS	2.9±0.1 ^{bB}	2.9±0.1 ^{bBC}	3.6±0.1 ^{aA}	2.7±0.1 ^{abCD}	2.6±0.0 ^{bD}
	PF10-EG10/RS	2.9±0.0 ^{bNS*}	2.7±0.3 ^{bNS*}	2.6±0.1 ^{cNS*}	2.8±0.3 ^{abNS*}	2.6±0.2 ^{bNS*}

Note: NS*, not significant; PF-pork back fat, EG- emulsion gel, NS-normal salt, RS-reduced salt. Equal capital letters in the same row for the same parameter are not statistically different ($p > 0.05$).