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Bacterial nanocellulose as novel additive in low-lipid low-sodium meat sausages. Effect on quality and stability

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ABSTRACT

Replacing animal fat with different oils has been proposed to improve fatty acid profile of meat emulsions. In this work a novel application of bacterial nanocellulose (BNC, 0–0.534 g of dry BNC/100 g batter) to low-lipid low-sodium meat emulsions formulated with pre-emulsified high-oleic sunflower oil is discussed. Process yield, water content, water activity, water holding capacity, color, texture, rheological characteristics, microstructure, and shelf-life were analyzed. Thermo-rheological curves showed a typical meat system gelation behavior where BNC addition produced a more solid-like three-dimensional network. Environmental Scanning Electron Micrographs of the systems revealed microstructure modifications in accordance with these results. Water-binding properties, hardness, cohesiveness, and chewiness increased when up to 0.267 g of dry BNC/100 g batter was added, while further additions had a negative impact on these attributes. Thus using oil pre-emulsified with BNC as fat mimetic had no negative effect on the quality properties of low-fat low-sodium meat sausages. A 45-days shelf-life under vacuum refrigerated storage was assured for this product.

1. Introduction

One of the key challenges facing the food industry is creating healthful food formulations. Meat products are an excellent source of the nutrients required for a good human health (Jiménez-Colmenero et al., 2012). However, meat and meat products are often associated with negative health claims. The main reason is the amount of fat, a high proportion of saturated fatty acids, and the presence of cholesterol. The high intake of these components is associated with some chronic diseases, such as cardiovascular diseases or some types of cancer and obesity (Valsta, Tapanainen, & Männistö, 2005; WCRC/AICR, 2007).

Nowadays, consumers are more aware of these food-related illnesses and choose health-oriented foods, leading the meat industry to develop a new category of foods that can meet this demand. Nevertheless, fat reduction in emulsified meat products such as sausages (frankfurter style) has many drawbacks in terms of achieving good appearance, flavor, and texture.

The simple fat replacement with water in meat emulsions results in high cooking and purge losses (Su, Bowers, & Zayas, 2000); also, higher water contents, directly alters the texture profile and juiciness of the product (Cierach, Modzelewska-Kapituła, & Szaciło, 2009). Several authors had employed different hydrocolloids (gums and non-meat proteins) with high water-binding capacity and gelling properties due to their ability to act as fat replacers or fats mimetics (Ayadi, Kechaou, Makni, & Attia, 2009; Brewer, 2012; do Amaral, Cardelle-Cobas, do Nascimento, Madruga, & Pintado, 2016; Hsu & Sun, 2006; Jiménez-Colmenero et al., 2012; Marchetti, Andrés, & Califano, 2013; Youssef & Barbut, 2011).

To modify fatty acid composition in meat emulsions vegetable or marine oils are commonly employed; thus a product with less cholesterol and a higher ratio of unsaturated to saturated fatty acids can be obtained (Olmedilla-Alonso, Jiménez-Colmenero, & Sánchez-Muniz, 2013). Oil inclusion also alters meat emulsions texture with a general trend to softer products (Álvarez, Xiong, Castillo, Payne, & Garrido, 2012), but some researchers had employed gums, non-meat proteins, or combinations of both that result in meat or chicken products with good functional and sensorial properties (Andrés, Zaritzky, & Califano, 2009; Jiménez-Colmenero, 2013).

Reformulation process and chilling storage affect product characteristics, but they do not produce safety issues or shelf-life constraints in frankfurters, suggesting that this can be a suitable strategy for functional frankfurters manufacture (Delgado-Pando, Cofrades, Ruiz-Capillas, & Jiménez-Colmenero, 2010).

Many industries including the food industry have recognized and

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embraced nanotechnology and nanomaterials, and commercial products are already being manufactured: One of those products is bacterial nanocellulose (BNC), produced by several bacterial, being *Gluconacetobacter xylinus* one of the most efficient producers (El-Saied, Basta, & Gobran, 2004; Klemm et al., 2006). BNC results similar to plant cellulose since both are composed of β-1,4-glucan chains (DP between 4000 and 10000 anhydroglucose units) with crystalline and non-crystalline zones.

BNC is a ribbon-shaped fibril with high crystallinity (up to 84–89%) that interacts with water, producing a hydrated nanofibrillated network with extraordinary water-binding properties. The structure has been described by Fink, Purz, Bohn, and Kunze (1997) as anhydrous nanofibrils in the range of 7 nm x 13 nm that appear hydrated as whole and are aggregated to flat microfibrils with a width of 70–150 nm, where water is outside of the crystalline cellulose nano-units and between these elements.

Potential uses of BNC in food technology include dressings, gravies, cultured dairy products and frozen dairy desserts. It is also a low-caloric additive, thickener, stabilizer, and texture modifier. As filler BNC has the highest water-holding capacity among commercial cellulose products (Dourado et al., 2016). Lin and Lin (2004), for example, had developed a very typical emulsified meat product (Chinese-style meatballs) containing BNC. BNC has been also employed by (Lin, Chen, & Chen, 2011) as fat replacer to produce surimi, resulting in products with superior water-holding capacity, retaining the original structure, and providing good mechanical properties. More recently, Corral, Cerrutti, Vazquez, and Califano (2015) used BNC to improve humidity and specific volume of French bread with a softer but more cohesive crumb than a control formulation without BNC.

This study was conducted to evaluate: i) the effect of adding different amounts of BNC to low-lipid low-sodium meat sausages formulated with pre-emulsified high oleic sunflower oil as fat source; physicochemical, rheological, and texture characteristics were analyzed; and ii) to evaluate storage stability of the products.

Meat industry could use this information to include BNC as an efficient additive to obtain low fat meat emulsions not only in meat sausages (as it is here proposed) but in other products as bologna, restructured meat, luncheon meat, etc.

2. Materials and methods

2.1. Materials

Two different meat lots were employed to produce independent replicates of low-lipid low-sodium (LLLS) sausages with different levels of BNC. For each lot, lean beef meat (*Adductor femoris* and *Semimembranosus* muscles) was obtained from local processors. Approximately 6 kg of meat (muscles from three different carcasses) without visible fat and connective tissue was passed through a grinder with a 0.95 cm plate (Meifa 32, Buenos Aires, Argentina). Ground meat was mixed to assure homogeneity avoiding intrinsic biochemical variability of different animals, divided in several batches of 500 g, 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). The employed fat source was high-oleic (HO) sunflower oil (C 18:1, 82.6%, Granix S.A. Vicente Lopez, Argentina). All the employed components were food-grade.

2.2. Bacterial nanocellulose production

BNC pellicles were produced by *Gluconacetobacter xylinus* NRRL B-42 kindly provided by Dr. Luis Ielpi (Fundación Instituto Leloir, Buenos Aires, Argentina) following the protocol of Foresti, Cerrutti, and Vazquez (2015). Previously, inocula were cultured for 48 h in Erlenmeyer flasks containing Hestrin and Schramm (HS) medium (in g/100 g): glucose, 2.0; peptone, 0.5; yeast extract, 0.5; anhydrous disodium phosphate, 0.27; citric acid, 0.115 (Hestrin, & Schramm, 1954). The pH was adjusted to 6.0 with dil. HCl or NaOH. Agitation was provided by an orbital shaker.

BNC pellicles were rinsed with water to remove culture medium, and then boiled in 2 g/100 g NaOH solution for 1 h in order to eliminate bacterial cells from the cellulose matrix. Then, pellicles were washed with distilled water till neutralization. All reagents used were analytical grade.

2.3. Sausage formulation and processing

The following components, expressed as g/100 g of raw meat batter, were included in all formulations: meat (67.57), HO-sunflower oil (5.00); NaCl (0.608), KCl (0.492), sodium tripolyphosphate (TPP, 0.50), sodium erythorbate (0.045), $NaNO_2$ (0.015), phytosterols (0.50), monosodium glutamate (0.02), ground pepper (0.2), nutmeg (0.05), and carminic acid (0.0032). NaCl, KCl, and TPP concentrations corresponded to the optimized formulation proposed by Marchetti, Argel, Andrés, and Califano (2015) to produce low sodium lean sausages with 5.0 g fish oil/100 g raw batter.

Adequate amounts of BNC pellicles several mm thick (2.137 g dry BNC/100 g hydrated gel) were homogenized in a blender for 4 min with additional water in order to reach a constant amount of water + BNC pellicles (25 g/100 g raw batter). BNC pellicle water content was previously determined by drying at 105 °C until constant weight was reached.

Six formulations were prepared with different amounts of BNC (g dry BNC/100 g batter): 0; 0.134, 0.200, 0.267, 0.401, and 0.534, in duplicate, one from each independent lot of meat, with the following codes: BNC0, BNC1, BNC2, BNC3, BNC4, and BNC5, respectively. BNC0 corresponds to a negative control formulation.

Sausages production was performed according to the methodology described by Andrés et al. (2009). Briefly, after meat packages were thawed (approximately 18 h at 4 °C), each batch was homogenized and grounded in a commercial food processor (Universo, Rowenta, Germany) with NaCl, KCl, and TPP in order to allow meat protein solubilization. Sodium nitrite and erythorbate were dissolved in cold water and then blended with HO-sunflower oil and the homogenized BNC, during 2 min to form a coarse emulsion. The obtained emulsion was added to ground meat with salts, processing all ingredients during 5 min afterward. Final batters temperatures ranged between 12 and 15 °C. Batters were immediately stuffed (vertical piston stuffer, Santini s.n.c., Marostica, Italy) into cellulose casing (22 mm diameter, Farmesa, Buenos Aires, Argentina), hand-linked, and placed in "cook-in" bags (Cryovac CN510, Sealed Air Co., Buenos Aires, Argentina) that were placed in a temperature-controlled water bath at 80 °C until a final internal temperature of 74 °C was reached (thermal treatment), assuring product safety. Thermal treatment time was determined in a preliminary test where the temperature was monitored by a type T (copper-constantan) thermocouple inserted in the center of a sausage, and connected to an acquisition system (TESTO175, Testo AG, Lenzkirch, Germany). It was set in 11.5 min including a safety margin of 30 s. After thermal treatment sausages in the bags were cooled immediately in an ice-water bath and stored at 4 °C until further analysis.

2.4. Rheological assays

2.4.1. Thermo-rheological assays

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). The static plate was equipped with a Peltier heating and cooling system with a temperature variation of ± 0.2 °C from the set point. A portion of raw meat batter was separated before stuffing and employed to perform the different rheological assays.

Sample was placed between serrated parallel plates (35 mm