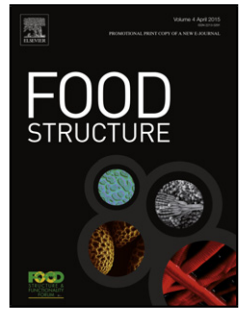

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Bacterial nanocellulose as novel additive in low-lipid low-sodium meat sausages. Effect on quality and stability.

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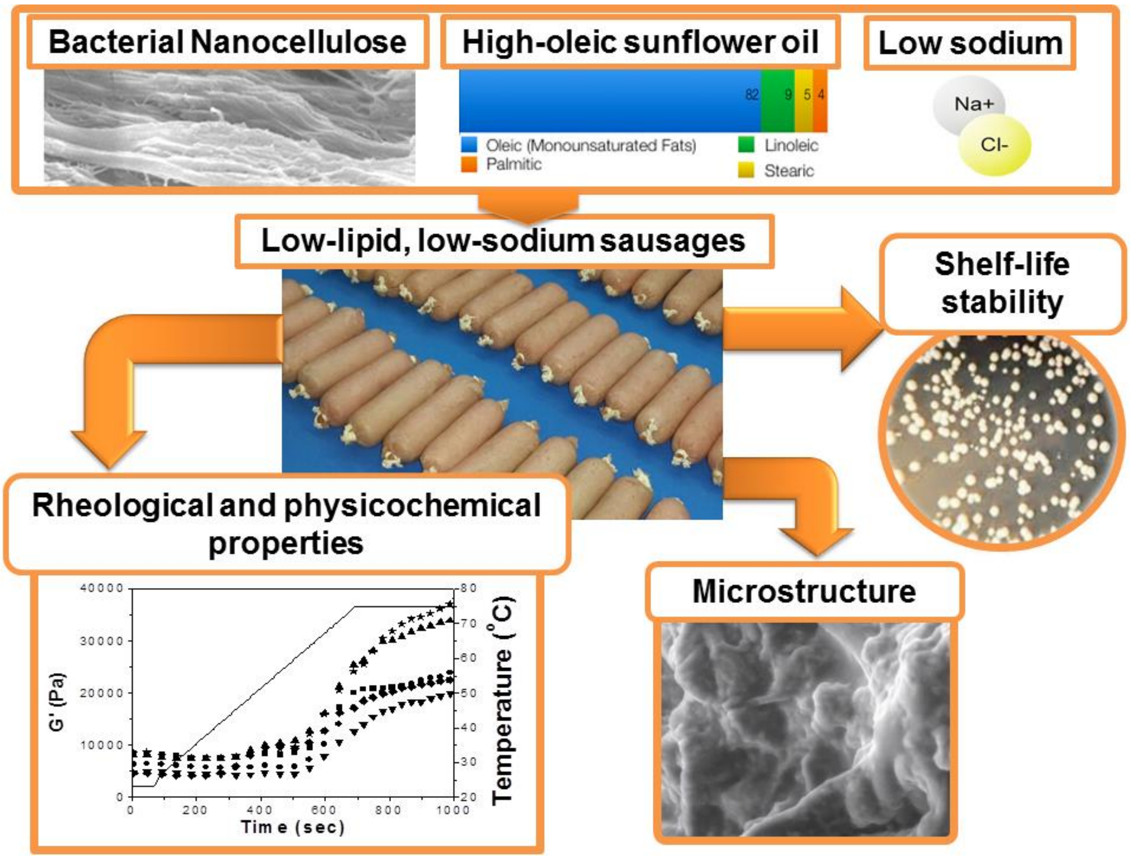

Highlights

- Bacterial nanocellulose (BNC) a novel biopolymer has great potential in meat industry.
- A more solid-like matrix was developed when BNC was added to meat emulsions.
- BNC increased water-binding properties, hardness, cohesiveness, and chewiness.
- Good stability of the products with BNC during 45-days of storage was achieved.
- BNC addition resulted into a suitable fat mimetic in low-fat emulsified meat systems.

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.

Keywords: bacterial nanocellulose; low-lipid meat emulsions; water-binding properties; texture; rheology.

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 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 (A*dductor femoris* and S*emimembranosus* 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 ºC, Sealed Air Co., Buenos Aires, Argentina), frozen, and stored at -20 ºC 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 minutes 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 ºC 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 ºC), 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 12–15 ºC. 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 ºC until a final internal temperature of 74 ºC 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 sec. After thermal treatment sausages in the bags were cooled immediately in an ice-water bath and stored at 4 ºC 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 ºC 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 diameter, 1 mm gap) and allowed to rest 3 min at 25 ºC for equilibration before starting the corresponding measurement Sample perimeters were covered with a thin film of silicone oil and the measuring system was covered with a special device to prevent evaporation during temperature sweeps.

Firstly, linear viscoelasticity range (LVR) was established by oscillatory stress sweep experiments at three temperatures: 25 ºC, 50 ºC, and 75 ºC. Afterwards temperature sweeps, and frequency sweeps were obtained within LVR stresses since they are useful for determining protein matrix characteristics without damaging it. Samples were sheared at a fixed frequency of 6.28 rad/s with a stress of 5.0 Pa during thermal ramps and isothermal processes. Thermal sweeps started by heating the sample to 75 ºC (heating rate 4.8 ºC/min) and isothermally holding it at 75 ºC for 5 min to complete the sol/gel transition. Changes in dynamic storage modulus (G'), loss modulus, (G''), and loss tangent (tan δ) were monitored continuously throughout the simulated gelling process. Thermo-rheograms presented correspond to average values of at least two replicates per formulation.

2.4.2. Frequency sweeps

To compared rheological characteristics of meat batters with those of the gelled product, raw batters were placed on the Peltier unit of the rheometer to obtain samples that perfectly fit the serrated parallels plates' gap (35 mm diameter, 1 mm height) heated to 75 ºC according to 2.4.1., and cooled from 75 ºC to 25 ºC at 10 ºC/min. Lastly, an isothermal step at 25 ºC was performed during 5 min. Frequency sweeps at 75 ºC and 25 ºC of thermally treated samples were performed in the LVR using a stress of 5.0 Pa. Storage modulus (G'), loss modulus (G''), and tan δ were recorded in the frequency range of 0.428 to 628 rad/s.

2.5. Physiochemical characterization of the products

2.5.1. Process yield

Process yield was determined by weighing the product before and after thermal treatment, and corresponds to the weight kept after heating (Andrés et al., 2009). Results were expressed as g/100 g in weight after thermal treatment (8 replicates per formulation).

2.5.2. Water activity and water content

Water activity (a_w) was determined in triplicate using an Aqualab 4TEV (Decagon Devices INC. MA, USA). Water content of the samples was determined in triplicate, heating at 105 ºC until constant weight was reached.

2.5.3. Water holding capacity

A modified method of Eide, Børresen, and Strøm (1982) was performed to evaluate stability of the matrices. Cylindrical samples from the center of the link were weighted (approximately 4 g), placed in Falcon tubes over glass beads, and centrifuged at 1200 *g* for 1min at 4 ºC. Liquids removed during centrifugation drained through the glass beads and were collected in the bottom of the centrifuge tube. Water holding capacity (WHC) was calculated as grams of remaining solid per 100 g of sausage (8 replicates per formulation).

2.5.4. Color

Color was measured at room temperature on the surface of recently cut transversal slices, using a ChromaMeter CR-400 colorimeter (Minolta Co., Ramsey, New Jersey, USA) and CIE-LAB parameters (lightness, L*, redness, a*, and yellowness, b*) were determined. Five measures were taken for each date point (8 replicates per formulation). The colorimeter was calibrated using a white tile (L* = 98.45, a* = − 0.10, b* = − 0.13; Minolta calibration plate), using an 8 mm aperture, illuminant D65 (6500 K color temperature) at a standard observation of 2°.

2.5.5. Texture Profile Analysis

Texture Profile Analysis (TPA) (Bourne, 1974; Brennan & Bourne, 1994) was performed on sausages in a controlled temperature room (20 ºC). Ten repeated measurements were taken and mean values were reported. Samples (1.5 cm thick and 1.7 cm diameter) were cut from the center of the links and compressed twice to 30% of their original height between flat plates using a TAXT2i Texture Analyzer (Stable Micro Systems, UK) with a 75 mm diameter probe at 0.5 mm/sec (SMSP/75), interfaced with a computer, using the software supplied by Texture Technologies Corp. Hardness (peak force of first compression cycle, N), cohesiveness (ratio of positive areas of second cycle to area of first cycle, J/J, dimensionless), adhesiveness (negative force area of the first byte represented the work necessary to pull the compressing plunger away from the sample, J), chewiness (hardness x cohesiveness x springiness, N), springiness (distance of the detected height of the product on the second compression divided by the original compression distance, mm/mm, dimensionless), and resilience (area during the withdrawal of the first compression divided by the area of the first compression, J/J, dimensionless) were determined (10 replicates per formulation).

2.5.8. Quality characteristics during the storage

After all the physicochemical determinations were analyzed one BNC level was selected according to the obtained results and sausages were manufactured following the methodology aforementioned (2.3), vacuum packed in Cryovac BB4L bags (PO_2: 0.35 $cm^3\ m^{-2}\ day^{-1}\ kPa^{-1}$ at 23 ºC, Sealed Air Co., Buenos Aires, Argentina) and stored at 4 ºC during 45-days (typical expected shelf-life for commercial products).
Water holding capacity and texture were periodically determined as described above (2.5.3.and 2.5.5).

2.5.8.1. Purge loss

Purge losses were performed by removing two packages from refrigerated storage at different times. Sausages were placed in funnels to drain (1 min), carefully bloated with filter paper to eliminate any liquid on the surface of the links, and weighed. Initial weight of the links was recorded at the beginning of refrigerated storage. Purge loss was reported as percentage of the initial weight (Andrés et al., 2009).

2.5.8.2. Lipid oxidation

In order to evaluate lipid oxidation in the sausages TBARS values were determined by quadruplicate according to Pennisi Forell, Ranalli, Zaritzky, Andrés, and Califano (2010). Results were expressed as mg malonaldehyde (MDA)/kg product.

2.5.8.3. Microbial analysis

Bacterial counts of the product were determined by duplicate at the beginning and the end of the refrigerated vacuum storage using the pour plate method according to Andrés, García, Zaritzky, and Califano (2006). Initial dilution was made by aseptically blending in a Stomacher blender (West Sussex, UK) 20 g of sample with 180 mL of 1 g/L of peptone solution for 1 min. Appropriate serial dilutions were plated with Plate Count Agar (PCA, Oxoid, Hampshire, UK) for total mesophilic aerobic count (incubated at 30 ºC for 2 d) and total psychrotrophic aerobic count (incubated at 4 ºC for 7 d), with Violet Red Bile Glucose Agar (Merck KGaA, Darmstadt, Germany) for *Enterobacteriaceae* (incubated at 37 ºC for 1 d), and with de Man, Rogosa, Sharpe agar (MRS agar, Oxoid) for lactic acid bacteria (incubated at 30 ºC for 2 d). Yeast Extract Glucose Chloramphenicol Agar (YGC agar, Merck KGaA) was used for mold and yeast counts (incubated for 5 d at 30 ºC). At the end of the storage, products were also tested for total coliform counts using the most probable number method (MPN) according to AOAC method 46016 (AOAC 1984), and sulfite-reducing *Clostridium* were enumerated in Tryptone Sulfite Neomycin Agar (TNS agar, Oxoid) (incubated at 30 ºC for 2 d). Data were expressed as log colony-forming units per gram of sample.

2.5.9. Microstructure

Small pieces (0.5 cm diam., 0.2–0.3 cm thick) were sliced from sausages, hand-broken and placed on aluminum stubs using double-sided tape expositing the side without a blade cutting effect. Immediately after, samples were examined using an electron scanning microscope FEI-Quanta 200 (Hillsboro, Oregon, USA) working on environmental mode.

2.6. Statistical Analysis

The entire trial was performed in duplicate. For each formulation (BNC0-5), results were expressed as mean ± standard error of the mean (SEM).

Analyses of variance were conducted separately on dependent variables considering each formulation as a level in a one-way factorial design. BNC level (treatment) was considered as a fixed effect and replicates were considered as random effect. Differences in means and F-tests were considered significant when $P < 0.05$. For simultaneous pairwise comparisons, least significance differences (LSD) test was chosen. All statistical procedures were computed using the SYSTAT software (SYSTAT, Inc., Evanston, IL).

3. Results and discussion

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

3.1. Rheological properties

Changes in elastic moduli of batters during thermal treatment as a function of processing time are shown in Figure 1. Figure 2A and 2B show, as examples, the evolution of elastic (G') and loss (G'') moduli for LLLS meat emulsions with BNC at both 75 ºC and 25 ºC, respectively. Table 1 shows storage (G') and plateau (G^0_N) moduli at 25 ºC and 75 ºC for the different formulations studied after heat treatment. Rheological measurements showed that G' changed much more dramatically than G'' during heating for all BNC level tested.

During temperature sweep runs (Figure 1) elastic modulus (G') of LLLS meat emulsions was always greater than loss modulus (G'') characteristic of weak gels (Ross-Murphy, 1995) with a predominantly elastic behavior ($\tan \delta = G''/G'$ less than 0.25), even for raw batters. As temperature increased during thermal treatment, different processes occurred leading to bond-making and structural changes that affected the rheological properties, increasing elastic moduli and decreasing tan δ (Marchetti et al., 2013; Marchetti et al., 2015). Thermal treatment of meat batters (from 25 ºC to 75 ºC) favored hydrophobic interactions and its main consequence was gel development.

Transition temperatures ranged from 51 to 53.5 ºC for the first slope (mainly myosin) and from 64 ºC to 72 ºC for the second slope (actin). BNC addition did not affect ($P >$ 0.05) temperature ranges for gelation process of this emulsified meat systems.

Incorporation of BNC impacted on meat batters properties increasing their elastic characteristics ($P < 0.05$), obtaining for BNC5 G' values 70% higher than the formulation without nanocellulose (Table 1). Rheological data of meat emulsions heated at 75 ºC showed that BNC addition had a significant impact on matrix structure.

Elastic properties (G') showed an increase with BNC up to 0.267 g/100 g (BNC3) while further BNC increments did not have a significant effect as can be observed (Table 1 and Figure 1).

Frequency sweeps could give information about meat system structure. Rheological data at 75 ºC indicated that LLLS meat emulsion with BNC behaved as viscoelastic solid (Figure 2A). Also, G' and G'' evolution with frequency was significantly affected by BNC content.

Comparing Figure 2A and B it is easy to notice that the cooling process increased the solid-like behavior of sausages mainly because of the H-bonding process (process that occurs at lower temperatures). All formulations showed higher G' values at 25 ºC respect to 75 ºC, in this case, tan δ ranged between 0.14 and 0.35, indicating that more solid gels were formed when samples were cooled. However the upper enhance was found when BNC was added ($P < 0.05$), reaching the highest G' for 0.267 g/100 g BNC addition.

Plateau modulus (G_N^0) is a parameter that is related to molecular architecture of the polymers in the studied system. It is proportional to the number of entanglements per volume unit and inversely proportional to the average molecular weight of the molecular segment between entanglements (Flory, 1953). It can be estimated from the minimum in the loss tangent as follows: $G_N^0 = [G']_{\tan\delta \rightarrow min}$ (Marchetti et al., 2013).

Plateau modulus of the samples (Table 1) followed a similar trend where BNC3 presented the most elastic properties. Emulsified meat gels with up to 0.267 g/100 g of dry BNC would have a larger number of entanglements and shorter segments between entanglements, i.e. a denser matrix. Storage moduli and G_N^0 at 25 ºC and 75 ºC of LLLS sausages reached its maximum when 0.267 g/100 g of BNC was added (BNC3), suggesting that a stronger three-dimensional network was developed, with a significant elastic behavior; apparently, further BNC addition affected protein gel formation impairing matrix densification.

3.2. Physicochemical aspects of the products

Process yields reflect system ability to retain water and oil during heating and cooling process and therefore the emulsified stability of meat batters.

Process yields of all sausages formulations were excellent (98.05 ± 0.3 g/100 g), with (BNC 1–5) or without (BNC0) BNC addition ($P > 0.05$). Thus, the addition of BNC did not adversely affect this parameter. It has been informed that emulsified meat systems with low fat content had very low fat losses and the amount of water released was up to ten times higher during the cooking process (Choi et al., 2010). Nevertheless a significant increase in water contents and a decrease in water activities of the products were obtained in the present study when BNC was included, as can be seen in Table 2. This information suggests that BNC incorporation increases water binding properties of the system. Beriain, Gómez, Petri, Insausti, and Sarriés (2011) informed similar water activities of Pamplona style sausages with olive oil as fat replacer and a partial NaCl substitution by KCl.

It has been reported by Su et al. (2000) that water retention is a very critical property, especially in high water added and NaCl reduced frankfurters. Also gelling properties and water binding properties of non-meat components play a great role in stabilizing meat emulsion. One way to estimated it is measuring WHC which reflects the strength or amount of water-matrix interactions.

The incorporation of BNC enhanced water binding properties since WHC of BNC1-BNC5 formulations were higher than BNC0 (Table 2). Higher WHC values are important to achieve in a formulation with a fat mimetic, since solid fat replacement with oil strongly diminishes binding capacity of a cooked meat emulsion (Marchetti et al., 2013). The highest WHC was founded in LLLS sausages with 0.267 g/100 g of BNC, while further additions did not improved it and seemed to have a small negative impact. In addition, a wide variety of hydrocolloids as fat mimetic and binders has been studied, some that enhanced water retention of meat systems (carrageenans 1 g/100 g, xanthan-locust bean gums mixture, 1 g/100 g, rice bran, 1 g/100 g, inulin powder, 1–6 g/100 g, fiber rich pea flour, 3.5 g/100 g, and chitosan, 0.25–1 g/100 g) (Álvarez et al., 2013; Choi et al., 2010; Marchetti et al., 2013; Pietrasik & Janz, 2010) and others which had showed a negative effect on water binding properties of similar meat systems, as hydroxypropyl-methylcellulose or methylcellulose, 1 g/100 g (Marchetti et al., 2013).

Table 2 also shows average color parameters. We found slight significant differences in lightness (L*) and redness (a*), but yellowness (b*) remained unchanged ($P > 0.05$). Lightness values were similar to low-fat meat systems with pre-emulsified fish oil and different hydrocolloids previously studied (Marchetti et al., 2013). It seems that BNC incorporation reduces L* at medium concentrations (0.134–0.267 g/100 g). In addition, LLLS sausages with BNC presented slightly lower redness values than the formulation without BNC ($P < 0.05$). However, Pietrasik and Gaudette (2015) stated that, although instrumental color parameter values showed some significant differences their practical importance is likely minimal because they did not have an impact on products consumer acceptance.

Table 3 shows textural profile analysis results. Hardness showed a linear increase with BNC addition up to 0.267 g/100 g (BNC3), implying that meat system structure was reinforced by nanocellulose due its high gelling capacity and possible protein-

hydrocolloid interactions that may had a significant role in structure and stability of many processed foods (Panyathitipong & Puechkamut, 2010).
However higher BNC contents showed a negative effect decreasing hardness to values similar to BNC0, debilitating or interfering with protein gel formation. Since water availability decreases as BNC is added (water added was constant), eventually meat protein-water interactions will be affected.
Springiness (Table 3) of LLLS sausages with BNC was slightly lower than BCN0 ($P < 0.05$). Similar results were reported by Zhou, Meng, Li, Ma, and Dai (2010) who found less springy systems when carrageenan was added to emulsified meat products but flaxseed gum exhibited a dual behavior, similar to BNC effect described in this paper. These authors stated that this second order behavior could be attributed to an optimum flaxseed gum content that might form a more stable network structure, which increased springiness, but an excess in flaxseed gum would increase meat batter viscosity before cooking that several air bubbles could be trapped during mixing and processing, so springiness would decrease. A dual trend was also noticed for product cohesiveness, being BNC3 the most cohesive formulation.
In both hardness and cohesiveness it was observed that BNC reinforced system structure, but from a critical concentration on BNC content interfered with the matrix and resulted in sausages with a softer and less cohesive texture.
Chewiness (hardness x cohesiveness x springiness) reflects hardness and cohesiveness patterns since BNC effect on these two parameters was similar; springiness has a less marked effect. Regarding resilience, the BNC had not a significant effect. Average value for all the formulations was 0.31 ± 0.04 J/J.
Several of the textural parameters measured in LLLS sausages with HO-sunflower oil and BCN presented values similar to a commercial product with 20 g/100 g pork fat content (Marchetti et al., 2013): hardness: 9.9 ± 0.1 N, springiness: 0.922 ± 0.002 J/J, cohesiveness: 0.440 ± 0.001 J/J, chewiness 4.05 ± 0.29 N, resilience: 0.484 ± 0.001 J/J, adhesiveness: 0.49 ± 0.08 $J \times 10^{-4}$. While BNC0 (control without BNC) exhibited a much softer texture profile.
These results obtained in large deformation assays such as TPA and water-binding properties (WHC), agreed with the rheological characteristics of thermally treated products. It is noticeable that small strain results, that did not alter sample microstructure, were affected by BNC incorporation in a similar way than textural properties measured outside the viscoelastic linear range such as hardness; an analogous correlation has been previously reported by Marchetti et al. (2013).

3.3. Matrix microstructure

Gelled meat emulsions without BNC (BNC0) and with 0.267 g/100 g of BNC (BNC3) that provided the best product characteristics can be observed, as an example, in Figure 3A and B, respectively. A three-dimensional network with more pores or sponge-like structure was observed for BNC0. BNC3 exhibited fewer cavities. The formation of these cavities has been described by Cavestany, Colmenero, Solas, and Carballo (1994) and it was related to the expansion of a number of components during product heat treatment, mainly water, oil, or air.

Morphological observations for these products indicated that characteristic of the continuous protein matrix was affected by BNC addition. LLLS sausages without BNC matrix showed a granular aggregated structure, while the addition of 0.267 g/100 g of BNC resulted in a continuous microstructure where protein aggregates were much more embedded into a homogeneous matrix. Microstructural changes agreed with related physicochemical characteristics of the studied meat emulsions. BNC inclusion produced a smoother and less sponge-like structure that can be related with higher cohesiveness, hardness, and chewiness and less springiness product. Other authors have reported that inclusion of fat replacers in emulsified meat systems had similar effects. Panyathitipong et al. (2010) reported that carrageenan addition to meat systems resulted in a smoother gel matrix and increased the compactness of protein gel network. Also sodium caseinate in meat emulsion resulted into a less spongy, showing a more continuous and compact structure (Delgado-Pando et al., 2011).

3.4. Storage stability of low-fat sausages with BNC

After analyzing all the data presented in sections 3.1 and 3.2, BNC3 formulation (0.267 g dry BNC /100 g batter) exhibited the most interesting physicochemical parameters, since above this BNC level the system matrix is negatively altered and quality attributes diminished (less WHC and hardness). Also, it exhibited a texture profile similar to a full fat commercial product (Marchetti, Andrés, & Califano, 2014). Thus, BCN3 formulation was chosen to study its behavior when stored in vacuum packaging for 45 days at 4 ºC because at this concentration it seems that BNC results into a good fat mimetic. Table 4 presents the parameters that exhibited significant differences ($P < 0.05$) during storage: WHC, purge loss, TBARS, hardness, cohesiveness, chewiness, and resilience.

Observed purge losses were low and similar to other low-fat meat emulsions (Candogan & Kolsarici, 2003; Marchetti, Andrés, & Califano, 2016) indicating that BNC addition to the meat system resulted into a stable emulsion. They increased until day 23, remaining constant thereafter. Water holding capacity also exhibited an increase from day 23. On the first weeks water released as purge loss corresponds to the weaker interactions with the solid matrix; water remaining in the systems is more strongly bonded, thus water released in the days following decreased and WHC increased. A similar trend was described by do Amaral et al. (2016) during storage of goat meat sausages with added chitosan.

Lipid peroxidation of meat emulsion was controlled since only an increase was observed at the end of storage, reaching very low TBARS numbers, below 0.5 mg MDA/kg which had been stated as the minimum detectable limit in pork meat (Lanari, Schaefer, Cassens, & Scheller, 1995). Vacuum packaging limits O_2 availability and also antioxidants present in the commercial oil and the additives in the product that have potential antioxidant activity were effective to limit lipid peroxidation.

Some textural attributes were altered during refrigerated storage, probably because of water released. Less water in the meat emulsion resulted into harder and more cohesive products; also more energy was needed during mastication process (higher chewiness), and slightly lower resilience. do Amaral et al. (2015) informed similar increases in hardness in low fat pork sausages with chitosan. Our results indicate that minor structural changes occurred probably not detectable by consumers (Marchetti et al., 2016). Springiness and adhesiveness were not altered during vacuum cold storage; average values were: 0.78 ± 0.01 mm/mm and 0.49 ± 0.06 $Jx10^{-4}$, respectively.

Color parameters of BNC3 sausages did not change during the 45-days storage. Average values were 63.1 ± 0.3, 15.7 ± 0.1, and 9.4 ± 0.1 for lightness (L*), redness (a*), and yellowness (b*), respectively.

Initially and after 45 days microbial counts were always below safety limits (Horita et al., 2016). Mesophilic microorganisms varied from 2.69 to 5.13 log CFU/g; psicrotrophic microorganisms from 2.64 to 5.24 log CFU/g; and lactic acid bacteria (LAB) from 2.22 to 4.29 log CFU/g. In addition, total coliforms count at the end of storage was less than 2 MPN/g and no sulfite-reducing Clostridium counts were detected.

From the storage assays it can be concluded that low-fat low-sodium meat emulsions with BNC formulated in this work had good stability and proper shelf-life.

4. Conclusion

Improvement of fatty acids profile achieved by replacing animal fat with vegetable oils leads to changes in several properties of thermally treated meat emulsions that could be controlled with the inclusion of suitable hydrocolloids. In this work bacterial nanocellulose was evaluated with this purpose. The addition of a very low concentration of BNC improved water-binding properties, increasing process yield, water content, and water holding capacity as a results of BNC great water affinity and specific contact area. Textural parameters were also improved, resulting in values closer to commercial products. The addition of BNC was not detrimental to shelf-life since quality parameters remained stable during the 45-days cold vacuum storage. BNC showed great potential to stabilize meat systems, and as a fat mimetic to replace beef tallow. Further studies, such as sensorial tests and escalation trials, should be performed in order to deepen and expand the applications horizon of this new food additive.

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Figure Captions

Figure 1. Gelation curves representing changes in storage modulus (G') vs. time during the heating and cooling processes. Codes: ■ BNC0; ● BNC1; ▲ BNC2; ◆ BNC3; ★ BNC4; ▼ BNC5. BNC0, BCN1, BCN2, BNC3, BCN4, and BNC5 formulations containing 0, 0.134, 0.200, 0.267, 0.401, and 0.534 g dry BNC/100 g batter, respectively.

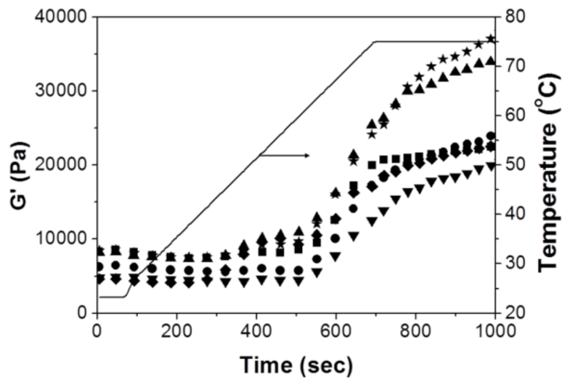
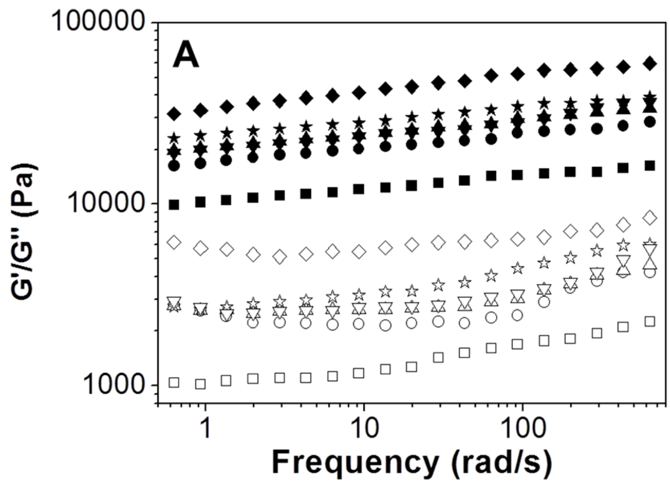


Figure 2. Frequency dependence of storage (G', solid symbols) and loss (G'', open symbols) moduli: **A)** after the thermal treatment at 75 ºC and **B)** after the cooling stage at 25 ºC. Codes: ■ BNC0; ● BNC1; ▲ BNC2; ◆ BNC3; ★ BNC4; ▼ BNC5. BNC0, BCN1, BCN2, BNC3, BCN4, and BNC5 formulations containing 0, 0.134, 0.200, 0.267, 0.401, and 0.534 g dry BNC/100 g batter, respectively.



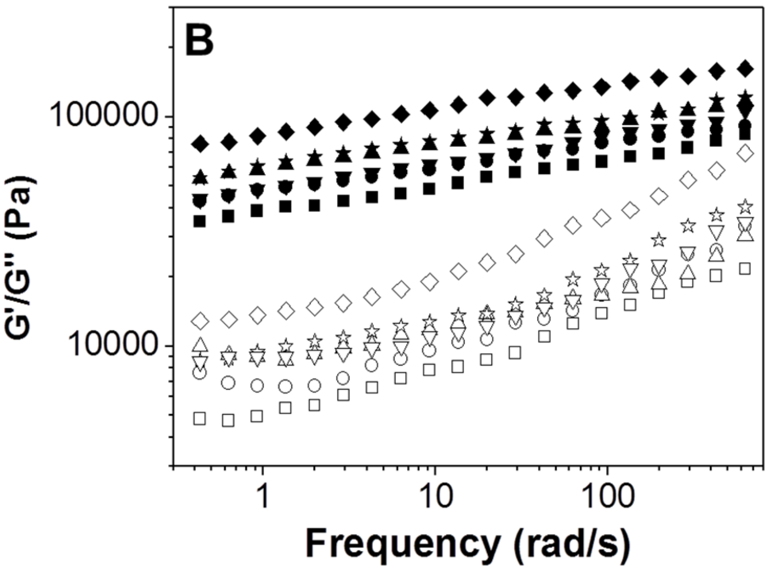
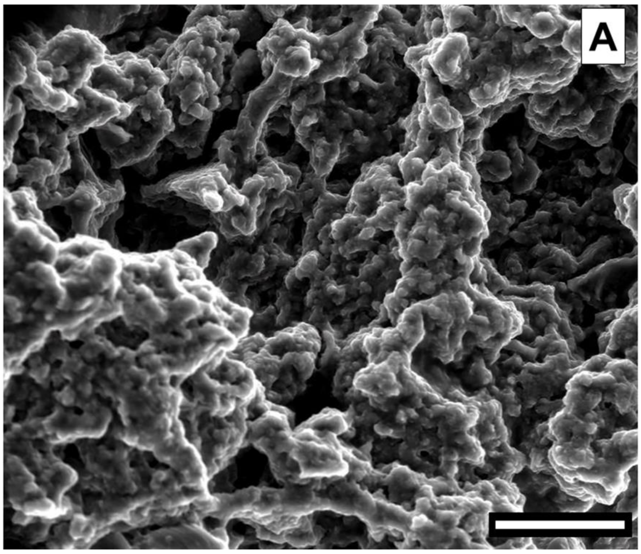


Figure 3. Environmental scanning microscopy of cooked low-fat meat emulsions **A)** without bacterial nanocellulose (BNC0) and **B)** with the addition of 0.267 g dry BNC/100 g raw batter (BNC3). White bars indicate 100 μm.



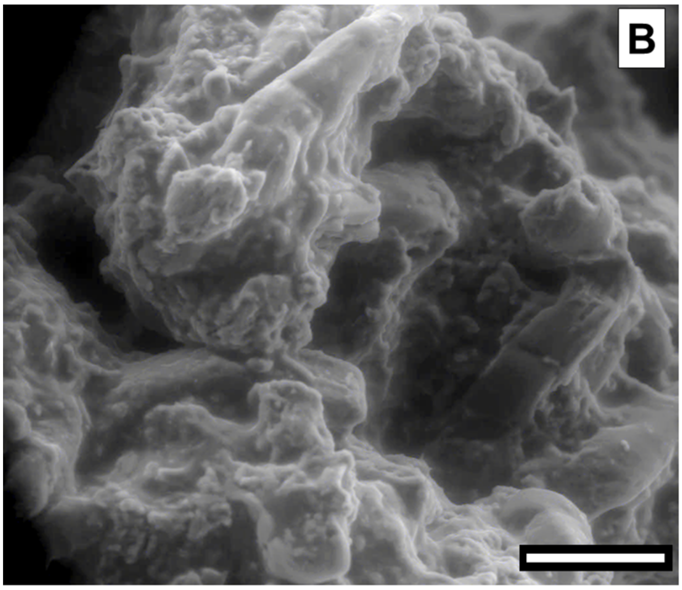

B

Table 1. Rheological properties (storage modulus G' and plateau modulus G^0_N) of studied meat products at 25 ºC and 75 ºC.

Formulation	**Raw batter (25º)**	**Cooked product (75 ºC)**		**Cooked product (25 ºC)**	
	G' (Pa)	**G' (Pa)**	**G^0_N (Pa)**	**G' (Pa)**	**G^0_N (Pa)**
BNC0	4921 ± 50[d]	15425 ± 800[d]	16400 ± 120[c]	40995 ± 300[d]	35875 ± 280[d]
BNC1	4668 ± 300[d]	21525 ± 900[c]	21625 ± 180[bc]	58335 ± 120[c]	52110 ± 240[c]
BNC2	6233 ± 120[c]	26508 ± 300[b]	26450 ± 300[ab]	76725 ± 400[b]	65040 ± 300[b]
BNC3	7798 ± 60[b]	31490 ± 420[a]	33790 ± 180[a]	95115 ± 700[a]	99930 ± 830[a]
BNC4	8059 ± 220[ab]	30958 ± 200[a]	27825 ± 100[ab]	78126 ± 500[b]	73410 ± 200[b]
BNC5	8368 ± 80[a]	31200 ± 850[a]	30530 ± 280[a]	60580 ± 800[c]	59605 ± 900[bc]

* Codes: BNC0, BCN1, BCN2, BNC3, BCN4, and BNC5 formulations containing 0, 0.134, 0.200, 0.267, 0.401, and 0.534 g dry BNC/100 g batter, respectively.

** Average values ± standard error of the mean. Different superscripts within the same column indicate significant differences according to a LSD pairwise comparison test.

Table 2. Water content, water activity (a_w), water holding capacity (WHC), and color parameters (L*, a*, b*) of low-lipid low-sodium sausages with different bacterial nanocellulose (BNC) contents.

Formulation	BNC (g/100 g)	Water content (g/100 g)	a_w	WHC (gH_2O/100 g)	Color parameter L*	a*	b*
BNC0	0	74.3 ± 0.2^c	0.993 ± 0.06^b	64.9 ± 0.7^c	64.7 ± 0.2^a	16.1 ± 0.1^a	9.45 ± 0.08
BNC1	0.134	74.9 ± 0.4^b	0.981 ± 0.08^{ab}	74.4 ± 0.8^b	64.7 ± 0.1^a	14.9 ± 0.1^b	9.67 ± 0.06
BNC2	0.200	75.1 ± 0.1^{ab}	0.981 ± 0.09^{ab}	75.3 ± 1.0^b	63.5 ± 0.2^c	15.3 ± 0.2^b	9.55 ± 0.06
BNC3	0.267	75.3 ± 0.2^{ab}	0.981 ± 0.10^{ab}	83.8 ± 0.7^a	63.7 ± 0.2^c	15.3 ± 0.1^b	9.64 ± 0.07
BNC4	0.401	75.4 ± 0.3^a	0.977 ± 0.05^a	78.7 ± 1.0^{ab}	64.5 ± 0.2^{ab}	15.2 ± 0.1^b	9.48 ± 0.05
BNC5	0.534	75.3 ± 0.2^{ab}	0.978 ± 0.08^a	78.9 ± 1.3^b	64.1 ± 0.1^{abc}	14.9 ± 0.1^b	9.47 ± 0.05

* Codes: BNC0, BCN1, BCN2, BNC3, BCN4, and BNC5 formulations containing 0, 0.134, 0.200, 0.267, 0.401, and 0.534 g dry BNC/100 g batter, respectively.

** Average values ± standard error of the mean. Different superscripts within the same column indicate significant differences according to a LSD pairwise comparison test.

Table 3. Texture profile analysis parameters of low-lipid low-sodium sausages with bacterial nanocellulose (BNC) added.

Formulation	Hardness (N)	Springiness (mm/mm)	Cohesiveness (J/J)	Chewiness (N)	Resilience (J/J)	Adhesiveness ($J\times10^{-4}$)
BNC0	7.75 ± 0.21[c]	0.864 ± 0.009[a]	0.447 ± 0.007[bc]	3.00 ± 0.17[b]	0.327 ± 0.021[a]	0.37 ± 0.11[a]
BNC1	8.92 ± 0.26[b]	0.785 ± 0.013[b]	0.479 ± 0.005[b]	3.35 ± 0.22[b]	0.253 ± 0.003[c]	0.28 ± 0.10[a]
BNC2	10.37 ± 0.32[b]	0.746 ± 0.010[b]	0.503 ± 0.006[b]	4.00 ± 0.24[b]	0.262 ± 0.004[b]	0.10 ± 0.03[b]
BNC3	13.08 ± 0.32[a]	0.788 ± 0.009[b]	0.539 ± 0.008[a]	5.55 ± 0.28[a]	0.270 ± 0.009[b]	0. 34 ± 0.07[a]
BNC4	7.80 ± 0.58[c]	0.824 ± 0.025[ab]	0.410 ± 0.007[c]	2.64 ± 0.31[b]	0.253 ± 0.004[c]	0.18 ± 0.06[b]
BNC5	8.81 ± 0.31[c]	0.759 ± 0.017[b]	0.420 ± 0.011[c]	2.81 ± 0.22[b]	0.256 ± 0.005[c]	0.15 ± 0.04[b]

* Codes: BNC0, BCN1, BCN2, BNC3, BCN4, and BNC5 formulations containing 0, 0.134, 0.200, 0.267, 0.401, and 0.534 g dry BNC/100 g batter, respectively.

** Average values ± standard error of the mean. Different superscripts within the same column indicate significant differences according to a LSD pairwise comparison test.

Table 4. Changes in water-binding capacity (WHC), purge loss, oxidative stability (TBARS), and textural parameters of low-lipid low-sodium sausages containing 0.267 g BNC/100 g batter during storage at 4 ºC under vacuum.

Storage Time (days)	**WHC (gH_2O/100 g)**	**Purge Loss (gH_2O/100 g)**	**TBARS (mg MDA/kg)**	**Hardness (N)**	**Cohesiveness (J/J)**	**Chewiness (N)**	**Resilience (J/J)**
0	80.5 ± 0.1^{b}	2.7 ± 0.4^{b}	0.125 ± 0.02^{b}	11.2 ± 0.5^{b}	0.476 ± 0.003^{c}	3.3 ± 0.1^{b}	0.806 ± 0.003^{a}
9	89.1 ± 0.9^{ab}	3.8 ± 0.4^{b}	0.110 ± 0.04^{b}	12.1 ± 0.8^{ab}	0.484 ± 0.004^{bc}	3.5 ± 0.3^{ab}	0.800 ± 0.002^{ab}
14	85.3 ± 1.4^{ab}	4.0 ± 0.3^{b}	0.149 ± 0.01^{b}	12.7 ± 1.0^{ab}	0.492 ± 0.003^{ab}	4.0 ± 0.3^{ab}	0.795 ± 0.002^{bc}
23	85.2 ± 1.0^{a}	5.1 ± 0.4^{a}	0.117 ± 0.01^{b}	11.8 ± 0.7^{ab}	0.499 ± 0.004^{a}	3.8 ± 0.3^{ab}	0.796 ± 0.002^{abc}
30	87.9 ± 1.6^{a}	5.9 ± 0.3^{a}	0.119 ± 0.01^{b}	13.8 ± 1.0^{a}	0.496 ± 0.003^{ab}	4.3 ± 0.4^{a}	0.796 ± 0.002^{abc}
37	90.5 ± 0.9^{a}	5.6 ± 0.3^{a}	N.D.	13.7 ± 0.7^{a}	0.498 ± 0.003^{ab}	4.4 ± 0.2^{a}	0.794 ± 0.002^{bc}
45	87.3 ± 1.3^{a}	5.1 ± 0.2^{a}	0.269 ± 0.09^{a}	13.9 ± 0.7^{a}	0.501 ± 0.005^{a}	4.4 ± 0.4^{a}	0.793 ± 0.003^{c}

*N.D. Not determined

** Average values ± standard error of the mean. Different superscripts within the same column indicate significant differences according to a LSD pairwise comparison test.