Nanofibrillated cellulose as functional ingredient in emulsion-type meat products.

Dolors Parés[a], M. Àngels Pèlach[b], Mònica Toldrà[a], Elena Saguer[a], Quim Tarrés[b], Carmen Carretero[a]

[a] Agrifood Technology Institute (INTEA)

[b] LEPAMAP Research Group

Universitat de Girona, Escola Politècnica Superior, C/Maria Aurèlia Capmany 61, 17003 Girona, Spain

Corresponding author: Dolors Parés

tel. +34 972418347

e-mail: dolors.pares@udg.edu

ORCID: 0000-0001-8438-5371

Abstract

The objective of this work was to introduce nanofibrillated cellulose (NFC) in the formulation of cooked emulsion-type sausages with the aim of assessing its feasibility to assume the role or compensate the lack of some conventional functional ingredients, such are polyphosphates, maize starch and sodium caseinate. For this aim, sausages based on standard commercial formula (Control) including all three ingredients, and sausages containing 0.5% NFC instead of phosphates and starch (NFC-1) or instead of phosphates, starch and sodium caseinate (NFC-2) were produced and characterized. In NFC-1 samples, 0.5% nanofibrillated cellulose succeed in replacing 0.5% polyphosphates and 1% starch without significantly altering the composition, nor negatively affecting the fat and water retention properties, neither of the raw batter or the cooked sausages. However, less stable meat batters and sausages with significantly reduced water holding capacity were obtained when 1.5% sodium caseinate, in addition to phosphates and starch, was also removed (NFC-2). Nevertheless,

results were hopeful enough to encourage further optimization studies, using several NFC concentrations and/or cellulose with different nanofibrillation degrees, in order to clarify whether it is possible to successfully replace also non-meat proteins in cooked emulsion-type sausages.

Keywords

Nanofibrillated cellulose; meat products; functional ingredients; polyphosphates; emulsion stability; Water Holding Capacity.

1. Introduction

Nanofibrillated cellulose (NFC) also referred to as nanocellulose, is a plant-derived biopolymer that exhibits attractive matrix properties and is potentially useful for a large number of industrial applications (Eichhorn et al. 2010; Klemm et al. 2011). Its high surface area and aspect ratio, rheological behavior, water absorption and absence of cytotoxic and genotoxic properties facilitate its use in food applications (Gómez et al. 2016). In these sense, in the literature review by Gómez et al. (2016), three different food applications of NFC are identified: (i) as a stabilizing agent, (ii) as a functional ingredient, and (iii) in food packaging. Although its use as a food additive was one of the applications already stated at early 80's (Turbak et al. 1982, 1983, 1984a, 1984b), nowadays, the most common application of nanocellulose in the food industry is as food packaging material. Nevertheless, recent advances in the development of cost-efficient NFC production have revived the interest in exploiting it as a potential food ingredient. It is worth noting that although a very recent EFSA re-evaluation concluded that there would be no safety concerns for the cellulose derivatives (EFSA ANS panel, 2018) the use of NFC as food ingredient would require approval under the Regulation (EU) 2015/2283 on novel foods to demonstrate that specific safety issues could not arise from its particular nanostructure.

Among the current food applications, its use as low-calorie thickener and suspension stabilizer in a wide variety of food products is being considered (Klemm et al. 2011; Lavoine et al. 2012; Endes et al. 2016). Its high viscosity at low concentrations promotes applications as thickener, flavor carrier and suspending agent in food (Bogati 2011).

NFCs can form web-like structures presenting high water holding capacity and several research works have also been focused on using NFC as stabilizer of oil-in-water (o/w) emulsions specially salad dressings or low-fat and fat free mayonnaise (Turbak et al. 1983; Xhanari et al. 2011; Winuprasith and Suphantharika 2015). These properties justify the

interest of assessing NFC as a potential alternative to the commonly used functional agents in emulsion-type meat products.

Emulsion-type meat batters, which consist of a homogenous fine mass with no visible particles of meat or fat, are used to produce cooked sausages. Basically, the meat batter or meat emulsion is a biphasic system composed of solid fat globules dispersed in a continuous aqueous matrix containing solubilized proteins, meat particles, connective tissue, insoluble proteins, and other material (Feiner 2006). Solubilized myofibrillar proteins, which are released from the muscle by disrupting the sarcolemma during the cutting process, are essential to achieve stabilized emulsions. Myofibrillar proteins stabilize both added water and fat in a three-dimensional matrix where fat particles remain dispersed in the viscous aqueous bulk due to the formation of a thin protein film around the fat globules.

The reasons that justify the use of several additives and non-meat ingredients in the formulation of meat emulsions are the need to successfully achieve the required stabilization of the raw batters as well as to improve the sensorial characteristics of the final products, often connected to their water holding capacity. Phosphates, non-meat proteins and polysaccharides are often used with these purposes. Phosphates, acting synergistically with salt, are considered as the most efficient additive for solubilising muscle proteins. Alkaline phosphates raise the pH and consequently increase the net negative charge of muscle proteins. These negative charges may cause better distribution of fat particles and prevent the clumping that occurs during over-chopping, and subsequent “fatting out” of the final product (Knipe 2004). Moreover, the enhanced water holding capacity (WHC) provided not only by phosphates but also by some nonmeat proteins, such as caseinate, egg, whey, gluten and wheat or soy protein, reduces cooking losses, and leads to increased yields, better texture and juicier products. In addition, polysaccharides are also very common in cooked sausages because of their high ability to bind water and their capacity to swell, thicken, or gellify in

aqueous solutions. Many of them also have the ability to emulsify fat, encapsulate fat particles, and stabilize emulsions when applied to comminuted meats.

Attending to the surface properties of nanofibrillated cellulose (NFC) and the growing interest in NFC food applications, the objective of this work was to assess its potential as stabilizing and water-binding agent in emulsion-type meat products, which in turn could result useful to attend the increasing pressure for the reduction of phosphate (Sherman and Mehta 2009; Tani et al. 2007) and/or to overcome the health concerns due to the allergenicity of several non-meat proteins. The work approach was in the form of a proof of concept as a preliminary step before carrying out optimization studies in the case of obtaining encouraging results.

For this aim, sausages based on standard commercial formula and NFC containing sausages were produced and characterized. The effects of using NFC to replace (1) phosphates and maize starch, and (2) phosphates, maize starch and sodium caseinate, were evaluated through determining the composition and emulsion stability of the raw batters, as well as the proximate composition, water-holding capacity (WHC), texture, microstructure, and internal colour of the cooked sausages.

2. Material and Methods

2.1 Preparation of nanofibrillated cellulose (NFC)

Nanofibrillated cellulose used in this work was produced by means of an enzymatic hydrolysis pretreatment followed by a mechanical homogenization. The cellulosic raw material used was a commercial bleached hardwood kraft pulp (BHKP) supplied by Torraspapel S.A. The hydrolysis pretreatment was performed using the commercial enzyme Novozym 476 (Novozymes A/S, Denmark), which contains 2% endo-β-1,4-glucanases with an activity factor of 4500 CNF-CA/g cellulose (tested over a carboxymethyl cellulose

substrate). The process was carried out according to the methodology reported by Tarrés et al. (2016a, 2016b). BKHP was dispersed at 1.5 % (w/w) in water in a laboratory pulper for 30 min at 3000 rpm. Then, the fibers were filtered until 10 % (w/w) dry matter and refined in a PFI mill for 4000 revolutions. This process was carried out to swell the fibers and thus to promote the activity of enzymes. Water and 0.1 N HCl were added to reach a pulp dry matter of 5% and pH of 4.8, respectively. Then, the suspension was heated until 50 ºC under constant stirring to avoid temperature gradients. At this step, the enzyme cocktail was dropped into the suspension and stirred for 4 h. The enzymatic process was stopped by heating the suspension to 80 ºC for 30 min. Enzyme dosage was fixed at 0.24 g of enzyme per kg of dried fibers. The enzymatically hydrolysed pulp was then washed with distilled water and kept at 4 ºC. Once cellulose fibers were enzymatically pretreated, they were subjected to high-pressure homogenization in a homogenizer Panda Plus 2000 (Gea Niro Soavi, Italy) 3 times at 300 bar, 3 times at 600 bar and 3 times at 900 bar. The characterization of NFC was performed according to cationic demand (225.43 μeq/g), carboxyl content (43.8 μmol/g) and yield of nanofibrilation (21.09%). Cationic demand was determined by colloidal titration using a Mütek PCD-04 charger analyser (BTG, S.L. UK). The carboxyl content was determined by ionic exchange between two defined pHs. The yield of nanofibrillation was determined by centrifuging an aqueous suspension of NFC in a vessel equipped with a nitrocellulose membrane. The retained solids were weighted and referred to the total amount of NFC added to the vessel, obtaining the non-fibrillated percentage.

The NFC obtained through the described process was in form of a gel with a dry matter content of 2.32%

2.2 Experimental design

Three trials of lab scale productions of cooked sausages were carried out. Each trial consisted on the production of three groups of sausages including control (C) and test sausages (NFC-1 and NFC-2) with different formulations, which are reported in Table 1. After separating part of the raw batter to be kept for further analysis, every trial allowed the production of at least 10 cooked sausages per treatment. The same raw material, pork meat and pork back fat, as well as the same bulk of NFC, were used in all the trials in order to minimise the variability factors between batches. As it is shown in the formula of the NFC-containing batches (Table 1), nanofibrillated cellulose was used instead of pentasodium tripolyphosphate and maize starch (NFC-1), or to replace polyphosphate, maize starch, and caseinate (NFC-2). The use of the NFC gel highly contributed to the water added to the NFC batches. Accordingly, the frozen water added in the test batches formulation was proportionally reduced as compared to the control.

2.3 Cooked sausages production

Fresh lean meat and pork fat were purchased from a local retail market and transported under refrigerated conditions. In the laboratory, the raw materials were cut into small pieces, which were kept at -18 ºC until use.

Sausages' productions were performed using a Thermomix TM-31 (Vorwerk, Wuppertal, Germany) food processor and the following procedure: partially thawed lean meat was chopped and homogenised with salts, polyphosphate (only in control batches), and around 70% of ice water (or frozen NFC gel) at a medium-high knife speed until the mass reached a tacky consistency (control temperature <4 ºC). The rest of frozen water (or frozen NFC gel) was added and the knife speed was increased until 10,000 rpm before introducing the caseinate (only in control and NFC-1 batches) along with the chilled fat (control temperature <12 ºC). Starch (only in control batches) and colour and flavour enhancers, sodium ascorbate

and sugar, were added before completing the emulsifying process (control temperature <15 ºC). Air was removed from the batter under vacuum (Tecnotrip EVT-7-G-TD-SD. Terrassa, Spain). Part of the raw batter (approx. 100 g) was stored under refrigeration and the rest was hand-linked in 150 mm-length sausages after being stuffed into 24 mm calibre multilayer polymer casings (®Nalobar, Kalle GmbH/Casings. Wiesbaden, Germany). Finally, sausages were cooked in a water bath at 80 ºC until reaching a minimum core temperature of 70 ºC and achieving a pasteurization value equivalent to $13D_{70}$ for *Streptococcus faecalis* (PV10/70=approx. 40 min). After cooling by showering with cold water, sausages were stored at 4±2 ºC until further analyses.

2.4 Analytical measurements

2.4.1 Emulsion stability

Emulsion stability was measured as described in (O'Flynn et al. 2014). Four portions of approximately 10 g (exact weight recorded) of raw batter were placed in a 50 mL polycarbonate centrifuge tubes and centrifuged for 1 min at 2000 x *g* (Sorvall RC-SC plus, Dupont Co, Newton, Connecticut, EUA). The samples were then heated in a water bath for 30 min at 70 °C and centrifuged again for 3 min at 2500 x *g*. The pelleted samples were removed and weighed; the percentages of total expressible fluid (TEF) were calculated from the weight loss provoked by the combined treatment of heating and centrifuging. The supernatants were poured into crucibles and weighed, dried overnight at 100±2 °C and reweighed. The residues remaining in the crucibles after drying were used to calculate the percentage of fat in the TEF.

2.4.2 Proximate analyses

Standard methods were used to analyse the proximate composition of raw batters and cooked sausages. Each product was analysed in representative triplicate samples. Moisture and ash contents were determined according to the Association of Official Analytical Chemists methods (AOAC 2000). Protein was estimated by multiplying by 6.25 the total Kjeldahl nitrogen (ISO 937, 1978); a semi-automatic digestion system (Gerhart KB20, Germany) and a distillation system (Buchi K314, Germany) were used. Total fat content was determined in duplicate by Sohxlet extraction using diethyl ether as solvent according to ISO 1443 (1973).

2.4.3 Water Holding Capacity

Water Holding Capacity (WHC) of cooked sausages was determined as described by (Hayes et al. 2005). Cores (10±1 g) of the products representative of each treatment were cut and placed in glass jars, closed and heated for 10 min in a water bath at 90 ºC. After heating, samples were cooled to room temperature, carefully removed from the jar using forceps, wrapped in cotton cheesecloth and placed into 50-mL polycarbonate centrifuge tubes (containing absorbent cotton wool at the bottom). Samples were centrifuged for 10 min at 9,000 x *g* (Sorvall RC-SC plus, Dupont Co, Newton, Connecticut, EUA) at 4 ºC. After centrifuging, the cheeseclothes were removed and sample weights were recorded. The results were reported as percentage (w/w) of water retained after centrifugation relative to the total water content of the sample. Three replicates were measured for each sample.

2.4.4 Colour

The internal colour was measured in triplicate on two different sausages of every batch with a MINOLTA CR-300 colorimeter (Minolta Co, Ltd., Japan) using diffuse illumination, a D_{65} light source and 2º-standard observer. The colorimeter had been previously calibrated using a standard white ceramic plate (Y=93.0, x=0.3158, y=0.3321). Lightness (L*), redness (a*) and

yellowness (b*) values were recorded (CIE La*b* colour system). The cylindrical coordinates, h° (hue angle in the CIE Lab colour wheel) and C* (chroma, relative saturation) were calculated as follows: $h^{\circ} = \arctan\left(\frac{b^*}{a^*}\right)$; $C^* = \sqrt{a^{*2} + b^{*2}}$.

2.4.5 Texture Profile Analysis

TPA was performed using a Texture Expert TA.XT2 (Texture Technologies Corp., Scarsdale, NY, USA). Three cores (20 mm diameter; 15 mm height) were cut from different sausages of every batch and were axially compressed by a two-cycle 50% compression test, using an aluminium 50 mm cylindrical probe. Determinations were performed at room temperature (22–23 °C). The established time for sample recuperation between both cycles was 3 s. Force-time deformation curves were recorded with a 25 kg load cell at a crosshead speed of 1 $mm \cdot s^{-1}$. The following parameters were considered: hardness (N), springiness (dimensionless), adhesiveness (N·mm), cohesiveness (dimensionless), and chewiness (N·mm), as defined by Bourne (2002).

2.4.6 Microstructure

Small pieces of sausages were fixed with a mixture (w/v) of 2.5% glutaraldehyde and 2% paraformaldehyde in 0.1 M cacodylate buffer pH 7.4, at 4 ºC during 2-4 h. After washing, samples were successively dehydrated in a graded series of ethanol, dried at the critical point and evaporated carbon in an Emitech K850 CPD (EMIntegrated Technology, Kent, UK) instrument, and coated with a 40 nm gold layer in an Emitech K550 diode sputtering coater. Examinations were carried out with a Hitachi S-4100 FE-SEM microscope (Hitachi High-Technologies Corporation, Tokyo, Japan). At least two representative pictures at each magnification (×100, ×200, ×500, and ×2000) were taken for every sample. Quartz PCI

software (Quartz Imaging Corporation, Vancouver, Canada) was used to acquire and process digital images.

2.5 Statistical analysis

The results in tables 2-5 were expressed as mean ± standard error of the mean (SE) of the three process replications (n=3) corresponding to the same formulation. The SPSS software package version 23.0 for Windows (IBM SPSS Statistical software Inc., Chicago, IL, USA) was used for statistical analysis. Batches (process replications) were considered as blocks in a randomized complete block design. Every trial (batch 1, 2 and 3) was considered as a block; and treatments (Control, NFC1 and NFC2) were assigned at random within each block. That is, all treatments were observed within each block. Analysis of variance (ANOVA) was performed to evaluate statistical significance of the formulation, as fixed effect; and batch as random effect. Tukey´s test was used for the *post hoc* analyses to compare means. The significance level for all tests was established at $P<0.05$.

3. Results and Discussion

In the approach of this study we assumed that the removal of phosphates in the NFC-containing batters would necessarily reduce the efficiency of muscular protein solubilization and in consequence would made more difficult to achieve stable emulsions and sausages showing good water holding capacity. Ruusunen et al. (2003) reported that non-meat ingredients are needed to produce frankfurters without phosphate in order to maintain texture and juiciness; and the same authors (Ruusunen et al. 2005) found significant increases in cooking losses in meat patties without phosphate. Our starting hypothesis was that NFC could act as water retention agent and also prevent coalescence of fat particles, thus offsetting the lack of phosphates and confidently making not necessary the use of other

polysaccharides, like starch, or even the use of nonmeat proteins, like casein, to obtain a final product with acceptable quality characteristics.

3.1 Composition and emulsion stability of raw batters

Table 2 shows the results on composition and stability of the raw batters corresponding to the three formulations. The moisture in the three samples was likely to reflect the extra amount of water added to compensate the weight of the removed ingredients in the NFC-containing formulations (Table 1). The control batter had a higher ash content ($P<0.05$) as compared to the two NFC-containing batters, probably attributable to the polyphosphates. Nevertheless, the mineral content was the only difference in composition between Control and NFC-1 samples. Conversely, NFC-2 also showed a moisture content higher ($P<0.05$) than the Control, and subsequently lower percentages in protein ($P<0.05$).

Several studies explain that nanocellulose is able to stabilize true emulsions by a mechanism which lies on the absorption of the dispersed nanocellulose at the oil-water interface to form steric barriers around the emulsion droplets, which prevent from coalescence (Cunha et al. 2014; Winuprasith and Suphantharika 2013, 2015). The report from Ström et al. (2013) also concluded that nanocellulose has interesting potential as stabilizing agent for food emulsions. In the present work the conventional formulation (Control sample) was the one that showed lower TEF percentage. However, statistical analysis only considers the Control TEF value significantly lower than that obtained in the NFC-2 formulation ($P<0.05$), but not different from the NFC-1, thus indicating some stabilizing capacity of NFC. Moreover, no differences between the fat content of the liquid released in the three formulations were obtained which might indicate that NFC resulted somewhat effective as fat binder. Contrasting our results, O'Flynn et al. (2014) observed the effect of phosphates (0, 0.25% and 0.5%) only on the percentage of fat in the TEF of raw batters of breakfast sausages, but not on the percentage of

released liquid. While other authors (Knipe et al. 1990; Keeton et al. 1984) reported that emulsions without phosphates showed a higher TEF and more fat losses as compared to emulsions containing 0.3 or 0.5% phosphate, attributed to the increase in solubilized proteins. Non-meat proteins, such are caseinate can be added to compensate unsatisfactory amounts of extracted salt-soluble proteins, and improve binding and emulsifying properties of low lean/high fat content sausages (Heinz and Hautzinger 2007) or, such is in this case, phosphate-free formulations. In the present work, 0.5% nanocellulose succeeded in the stabilization of meat emulsions without added phosphates and starch, but was not effective enough to also replace sodium caseinate.

3.2 Composition and WHC of cooked sausages

Relative to the composition of the final products (**Table 3**), although lower moisture could be expected in NFC-2 sausages according to the TEF values of the raw batters; the three samples (Control, NFC-1 and NFC-2) showed similar water contents. Probably the NFC-2 fluid losses during cooking were compensated by the slightly higher amount of water added to this formulation. Differences in ash content ($P<0.05$) were in agreement with the proximate analysis of the corresponding raw batters. Finally, no differences in fat content were obtained, so no fat separation occurred during the heat treatment of the three batters; whereas the actually minor variations in protein content, slightly higher in NFC-1 ($P<0.05$) as compared to the NFC-2 sausages, could likely be attributed to the casein removing.

The sausages corresponding to the conventional formulation (Control) were the ones showing the highest water holding capacity (**Table 3**); even so, the statistical analysis considers this WHC quite similar to those from NFC-1 samples. So, in our study 0.5% NFC acted as a water-holding agent at least as effective as 1% maize starch in cooked sausages without added phosphates. Leaving aside the effect of phosphates, this result is in agreement with

Ström et al. (2013), who reported that high amount of potato starch (1.4%) was required to lower the cooking losses to the same level as for cellulose microfibers (0.63%) in hamburgers. Moreover, the addition of a very low concentration of bacterial nanocellulose (BNC), showing great water affinity and specific contact area, was also reported to improve water-binding properties, increasing process yield, water content, and water holding capacity in sausages (Marchetti et al. 2017). On the contrary, the sausages obtained with the NFC-2 formulation showed much lower WHC (P<0.05). Thus might indicate that, despite having the same water content at the end of the production process, 0.5% NFC did not compensate the water binding capacity of 1.5% caseinate. NFC-2 sausages will likely lead to an excess of exudate inside the sausage containers during the storage and commercialization periods as well as to a drier and less juicy product after the culinary treatment before consumption.

It is widely known that the water retention value (WRV) of nanofibrillated cellulose is related to nanofiber size and consequently to the specific surface, which in turn is directly interconnected with the cationic demand (González et al. 2014). Accordingly, the use of NFC with cationic demand higher than that of NFC used in the present work (225.43 μeq/g) could be considered as a valid approach to obtain sausages showing better WHC. In this sense, the optimization of the enzymatic hydrolysis prior to the high-pressure homogenization process or its substitution by chemical pretreatments (e.g. TEMPO-mediated oxidation or carboxymethylation), which allow achieving cationic demands up to 1100 μeq/g (González et al. 2014), would lead to NFC with increased water retention properties.

3.3 Colour and textural properties

It can be seen in **Table 4** that Control and NFC samples showed basically the same chromatic properties since no significant differences between them were found in any of the CIE Lab coordinates. A slightly but not significantly darker colour in NFC samples was obtained,

while the Chroma and Hue, which were calculated from a* and b* values, were practically identical in the sausages from the three formulations. Anyway other authors have already stated that, some significant differences in instrumental colour parameter values have likely minimal practical effect on products consumer acceptance (Pietrasik and Gaudette 2015).

According to the results in **Table 5,** that shows the textural profile analysis parameters of the sausages, no significant differences between Control and NFC-2 products were found. Thus meaning that using 0.5% NFC instead of phosphates, starch and casein did not provoke any significant modification in texture parameters. Moreover, no significant differences in the springiness, cohesiveness and adhesiveness of the three samples could be observed, thus probably meaning that NFC was able to compensate the elasticity imparted by starch in the conventional formulation. Nevertheless hardness and chewiness were significantly higher (P<0.05) in NFC-1 than those in Control or NFC-2 samples. Milk proteins contribute not only to the emulsifying and binding properties but also to the structural and gelling qualities in sausages (Heinz and Hautzinger 2007). From our results it seems that the simultaneous presence of caseinate and NFC allowed obtaining a final product firmer than those obtained with a formulation that contains only casein or only NFC, suggesting an interesting collaborative interaction between both ingredients which reinforces the meat protein network. Marchetti et al. (2017) also reported that BNC addition up to 0.267% provoked a linear increase in hardness of low-fat low-sodium meat sausages, implying that meat system structure was reinforced by nanocellulose due to its high gelling capacity and possible protein-hydrocolloid interactions.

3.4 Microstructure

Figure 1 shows the SEM micrographs of sausages from Control, NFC-1 and NFC-2 formulations. A homogeneous network can be observed in the micrographs at any

magnification, nevertheless some differences in microstructure depending on formulation were obtained. Control and NFC-1, seemed to present a more compact structure and smaller cavities than NFC-2, which could be related to the degree of syneresis, because they showed also higher water retention capacity. Since either Control or NFC-1 formulations contained sodium caseinate, this result could be related with the well-known functionality of caseinate in both water holding and protein network structuring. Although the microstructure images are difficult to be correlated with the texture parameters, it is worthy to say that in some magnification (e.g. x500) a denser network can be observed in the NFC-1 images as compared to the control ones, which could be in agreement with the texture differences determined by the TPA analysis.

4. Conclusion

In conclusion, the results of the present work indicate that 0.5% NFC succeeded in replacing polyphosphates and starch, added at 0.5% and 1% respectively, in the conventional formulation. NFC-containing sausages without phosphates and starch showed similar composition and quality characteristics to those of control sausages. However, when caseinate was also removed from the list of ingredients, the nanofibers used in the present work were not suitable to obtain sausages of acceptable quality.

Considering the improvement range that can be provided by using higher NFC concentrations and/or cellulose with higher nanofibrillation degrees, further studies would be interesting in order to clarify whether it is possible to successfully replace also non-meat proteins in cooked emulsion-type sausages.

Acknowledgments

This work was financially supported by the University of Girona Project ref. MPCUdG2016 We gratefully acknowledge I. Lucena (MsC) for her assistance in the sausages production and the accurate lab work, and A.M. Aymerich for the helpful technical assistance.

References

Bogati, D.R. (2011). Cellulose based biochemicals and their applications. BSc thesis, Saimaa University of Applied Sciences, Finland.

Bourne, M. C. (2002). *Food texture and viscosity: Concept and measurement* (2nd ed.) New York: Academic Press Inc, 427.

Cunha, A. G., Mougel, J. B., Cathala, B., Berglund, L. A., & Capron, I. (2014). Preparation of double pickering emulsions stabilized by chemically tailored nanocelluloses. *Langmuir*, 30(31), 9327–9335.

EFSA ANS Panel (2018). Scientific Opinion on the re-evaluation of celluloses E 460(i),E 460(ii), E 461, E 462, E 463, E 464, E 465, E 466, E 468 and E 469 as food additives. EFSA Journal 2018; 16(1): 5047, 104 pp.

Eichhorn, S. J., Dufresne, A., Aranguren, M., Marcovich, N. E., Capadona, J. R., Rowan, S. J., Weder, C., Thielemans, W., Roman, M., Renneckar, S., Gindl, W., Veigel, S., Keckes, J., Yano, H., Abe, K., Nogi, M., Nakagaito, A. N., Mangalam, A., Simonsen, J., Benight, A. S., Bismarck, A., Berglund, L. A., & Peijs, T. (2010). Review: Current international research into cellulose nanofibres and nanocomposites. *Journal of Materials Science*, 45, 1-33.

Endes, C., Camarero-Espinosa, S., Mueller, S., Foster, E. J., Petri-Fink, A., Rothen-Rutishauser, B., Weder, C., & Clift, M. J. D. (2016). A critical review of the current knowledge regarding the biological impact of nanocellulose. *Journal of Nanobiotechnology*, 14(1), 78.

Feiner, G. (2006). *Meat products handbook. Practical science and technology*. Cambridge: CRC Press and Woodhead Publishing Limited.

Gómez H., C., Serpa, A., Velásquez-Cock, J., Gañán, P., Castro, C., Vélez, L., & Zuluaga, R. (2016). Vegetable nanocellulose in food science: A review. *Food Hydrocolloids*, 57, 178–186.

González, I., Alcalà, M., Chinga-Carrasco, G., Vilaseca, F., Boufi, S., & Mutjé, P. (2014). From paper to nanopaper: Evolution of mechanical and physical properties. *Cellulose*, 21(4), 2599–2609.

Hayes, J. E., Desmond, E. M., Troy, D. J., Buckley, D. J., & Mehra, R. (2005). The effect of whey protein-enriched fractions on the physical and sensory properties of frankfurters. *Meat Science*, 71(2), 238–243.

Heinz, G., & Hautzinger, P. (2007) *Meat Processing Technology for small-to-medium-scale producers*. RAP Publication 2007/20. FAO Regional Office for Asia and The Pacific. Bangkok.

Keeton, J. T., Foegeding, E. A., & Patana-Anake, C. (1984). A comparison of nonmeat proteins, sodium tri-polyphosphate and processing temperature effects on physical and sensory properties of frankfurters. *Journal of Food Science*, 49, 1462–1465.

Klemm, D., Kramer, F., Moritz, S., Lindström, T., Ankerfors, M., Gray, D., & Dorris, A. (2011). Nanocelluloses: A new family of nature-based materials. *Angewandte Chemie - International Edition*, 50(24), 5438–5466.

Knipe, C. L., Rust, R. E., & Olson, D.G. (1990). Some physical parameters involved in the addition of inorganic phosphates to reduced sodium meat products. *Journal of Food Science*, 55, 23–25.

Knipe, L. (2004). Use of phosphates in meat products. *Meat industry research conference, October 2: Ohio State University*. Presentation retrieved from the OSU Meat Science

Website on June 2011 at https://meatsci.osu.edu/sites/meatsci/files/imce/MIRCPhosphatesinMeatProducts10.04.ppt

Lavoine, N., Desloges, I., Dufresne, A., & Bras, J. (2012). Microfibrillated cellulose - Its barrier properties and applications in cellulosic materials: A review. *Carbohydrate Polymers*, 90(2), 735–764.

Marchetti, L., Muzzio, B., Cerrutti, P., Andrés, S. C., & Califano, A. N. (2017). Bacterial nanocellulose as novel additive in low-lipid low-sodium meat sausages. Effect on quality and stability. *Food Structure*, 14, 52-59.

O'Flynn, C. C., Cruz-Romero, M. C., Troy, D., Mullen, A. M., & Kerry, J. P. (2014). The application of high-pressure treatment in the reduction of salt levels in reduced-phosphate breakfast sausages. *Meat Science*, 96(3), 1266–1274.

Pietrasik, Z., & Gaudette, N. J. (2015). The effect of salt replacers and flavor enhancer on the processing characteristics and consumer acceptance of turkey sausages. *Journal of the Science of Food and Agriculture*, 95(9), 1845–1851.

Ruusunen, M., Vainionpää, J., Lyly, M., Lähteenmäki, L., Niemistö, M., Ahvenainen, R., & Puolanne, E. (2005). Reducing the sodium content in meat products: The effect of the formulation in low-sodium ground meat patties. *Meat Science*, 69(1), 53–60.

Ruusunen, M., Vainionpää, J., Puolanne, E., Lyly, M., Lähteenmäki, L., Niemistö, M., & Ahvenainen, R. (2003). Physical and sensory properties of low-salt phosphate-free frankfurters composed with various ingredients. *Meat Science*, 63(1), 9–16.

Sherman, R. A., & Mehta, O. (2009). Dietary phosphorus restriction in dialysis patients: Potential impact of processed meat, poultry, and fish products as protein sources. *American Journal of Kidney Diseases*, 54(1), 18–23.

Ström, G., Öhgren, C., & Ankerfors, M. (2013). Nanocellulose as an additive in foodstuff.

Innventia Report No 403, 1–28.

Tani, Y., Sato, T., Yamanaka-Okumura, H., Yamamoto, H., Arai, H., Sawada, N., Genjida, K., Taketani, Y., & Takeda, E. (2007). Effects of prolonged high phosphorus diet on phosphorus and calcium balance in rats. *Journal of Clinical Biochemistry and Nutrition*, 40(3), 221–228.

Tarrés, Q., Delgado-Aguilar, M., Pèlach, M. A., González, I., Boufi, S., & Mutjé, P. (2016a). Remarkable increase of paper strength by combining enzymatic cellulose nanofibers in bulk and TEMPO-oxidized nanofibers as coating. *Cellulose*, 23(6), 3939–3950.

Tarrés, Q., Saguer, E., Pèlach, M. A., Alcalà, M., Delgado-Aguilar, M., & Mutjé, P. (2016b). The feasibility of incorporating cellulose micro/nanofibers in papermaking processes: the relevance of enzymatic hydrolysis. *Cellulose*, 23(2), 1433–1445.

Turbak, A. F., Snyder, F. W., & Sandberg, K. R. (1982). Food products containing microfibrillated cellulose. US 4.341.807. *Washington, DC:US: Patent and Trademark Office.*

Turbak, A. F., Snyder, F. W., & Sandberg, K. R. (1983). Suspensions containing microfibrillated cellulose. US 4.378.381. *Washington DC:US: Patent and Trademark Office.*

Turbak, A. F., Snyder, F. W., & Sandberg, K. R. (1984a). Microfibrillated cellulose. US 4.483.743. *Whashington DC: US: Patent and Trademark Office*.

Turbak, A. F., Snyder, F. W., & Sandberg, K. R. (1984b). Suspensions containing microfibrillated cellulose. US 4.487.634. *Whashington DC:US: Patent and Trademark Office.*

Winuprasith, T., & Suphantharika, M. (2013). Microfibrillated cellulose from mangosteen (Garcinia mangostana L.) rind: Preparation, characterization, and evaluation as an emulsion stabilizer. *Food Hydrocolloids*, 32(2), 383–394.

Winuprasith, T., & Suphantharika, M. (2015). Properties and stability of oil-in-water emulsions stabilized by microfibrillated cellulose from mangosteen rind. *Food Hydrocolloids*, 43, 690–699.

Xhanari, K., Syverud, K., Chinga-Carrasco, G., Paso, K., & Stenius, P. (2011). Structure of nanofibrillated cellulose layers at the o/w interface. *Journal of Colloid and Interface Science*, 356(1), 58–62.

Fig. 1

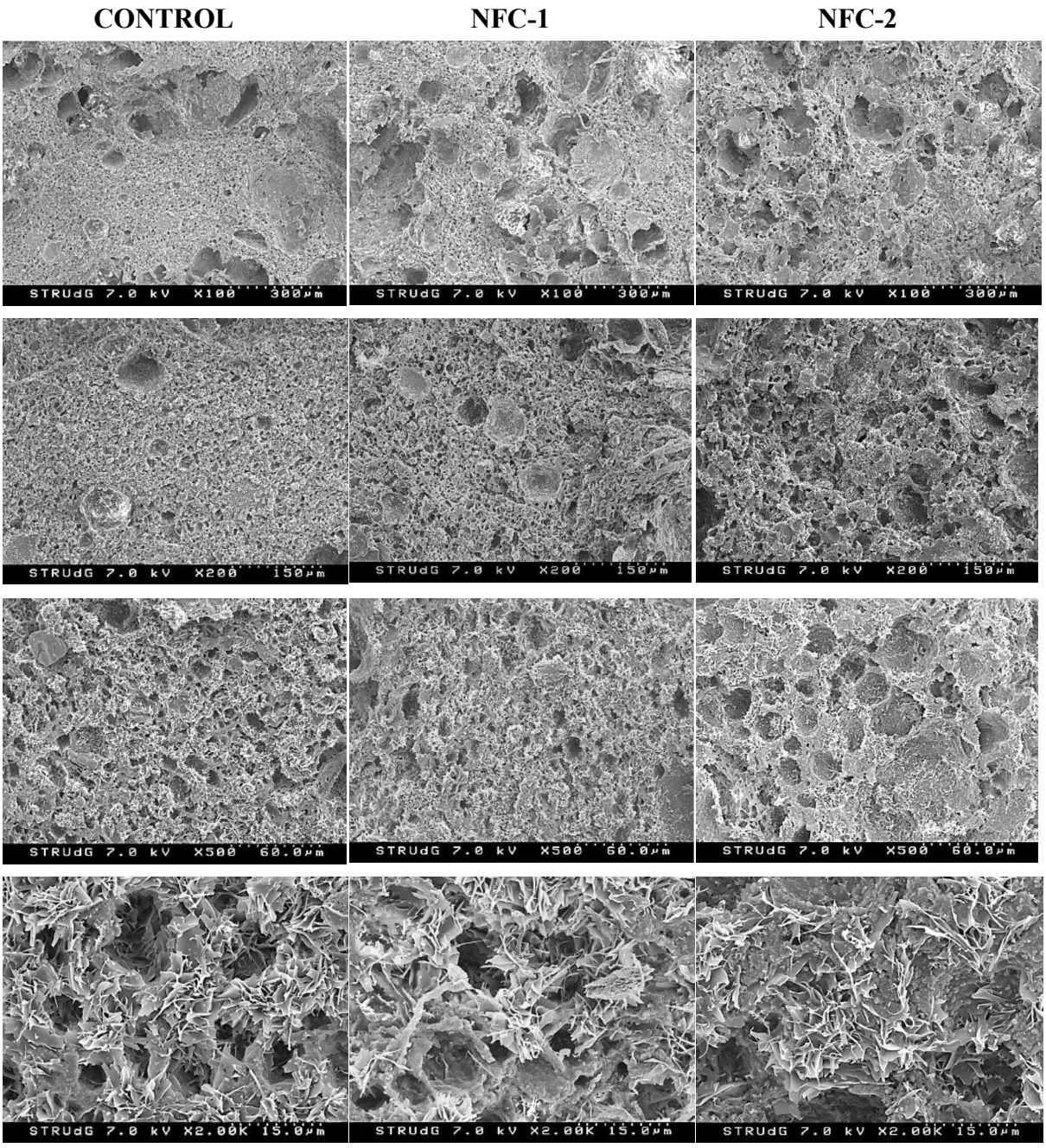

Figure caption

Fig. 1. Scanning electron micrographs of representative control and NFC-containing sausages. Magnification (from top to bottom) x100, x200, x500, x2000

Table 1. Formulation (g/kg) of control and NFC-containing sausages

Ingredient	**CONTROL**	**NFC-1**	**NFC-2**
Lean pork	400	400	400
Fat	220	220	220
Frozen water	327	126	141
Sodium chloride[a]	17	17	17
Sodium nitrite[a]	0.3	0.3	0.3
Sugar	5	5	5
Maize starch	10	0	0
Sodium caseinate[b]	15	15	0
Pentasodium tripolyphosphate[a]	5	0	0
Sodium ascorbate[c]	0.5	0.5	0.5
NFC	0	5	5
Water added with NFC gel	0	210.5	210.5

[a] Panreac Quimica, SA. (Barcelona, Spain).
[b] BDF Natural Ingredients SL (Barcelona, Spain).
[c] Induxtra de Suministros (Girona, Spain).

Table 2. Proximate analysis and Emulsion Stability (Total Expressible Fluid and fat content in TEF) of control and NFC-containing raw batters (mean±SE[1]; n=3)

	Raw batter composition (%)				Emulsion stability (%)	
Samples	Moisture	Protein	Fat	Ashes	TEF	Fat in TEF
CONTROL	62.97±0.50^{a}	9.70±0.04^{b}	23.88±0.37	2.60±0.01^{b}	2.91±0.38^{a}	5.65±0.24
NFC-1	65.74±0.44^{ab}	10.06±0.21^{b}	22.35±0.58	2.25±0.03^{a}	5.19±0.84^{a}	5.39±0.43
NFC-2	67.07±0.63^{b}	8.96±0.07^{a}	21.87±0.60	2.17±0.02^{a}	17.61±1.15^{b}	5.90±0.13

Different superscript letters in the same column indicate significant differences (P<0.05) between samples

[1]SE: Standard Error of the mean

Table 3. Proximate analysis and Water Holding Capacity of control and NFC-containing cooked sausages (mean±SE[1]; n=3)

	Cooked sausages composition (%)				WHC (%)
Samples	Moisture	Protein	Fat	Ashes	
CONTROL	64.33±0.36	10.02±0.10^{a}	24.67±0.47	2.49±0.05^{b}	79.85±1.28^{b}
NFC-1	66.01±0.48	10.62±0.18^{b}	23.13±0.61	1.97±0.18^{a}	73.54±0.71^{b}
NFC-2	66.44±0.51	9.97±0.20^{a}	23.48±0.06	1.93±0.08^{a}	56.78±2.89^{a}

Different superscript letters in the same column indicate significant differences (P<0.05) between samples.

[1]SE: Standard Error of the mean

Table 4. Internal colour parameters (CIE L*a*b*) of cooked sausages (mean±SE[1]; n=3)

Samples	Lightness (L)	Redness (a*)	Yellowness (b*)	Hue (H°)	Chroma (C*)
CONTROL	80.30±0.97	9.88±0.29	7.59±0.11	37.53±0.42	12.46±0.30
NFC-1	79.60±1.11	10.03±0.15	7.49±0.02	36.76±0.33	12.52±0.14
NFC-2	77.89±1.54	10.11±0.15	7.35±0.21	36.02±0.98	12.50±0.14

No significant differences $P>0.05$
[1]SE: Standard Error of the mean

Table 5. Texture profile attributes of cooked sausages (mean±SE[1]; n=3)

Samples	Hardness (N)	Springiness	Cohesiveness	Adhesiveness (N.mm)	Chewiness (N.mm)
CONTROL	14.23±0.29[a]	0.81±0.01	0.65±0.00	-0.32±0.04	107.86± 2.38[a]
NFC-1	17.93±1.25[b]	0.83±0.01	0.66±0.01	-0.31±0.01	142.40±9.87[b]
NFC-2	11.30±1.07[a]	0.82±0.01	0.60±0.02	-0.26±0.03	81.16±8.48[a]

Different superscript letters in the same column indicate significant differences (P<0.05) between samples

[1]SE: Standard Error of the mean