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Nanocellulose as a fat substitute to improve the quality of emulsified sausages: Effects of morphology and content

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Abstract: Two different morphologies of nanocellulose – cellulose nanofibres (CNFs) and cellulose nanocrystals (CNCs) with different contents were added to emulsified sausages to replace pork back fat in different proportions (10, 30, and 50%), and the colour, emulsion stability, texture characteristics and dynamic rheological behaviour of emulsified sausages were analysed. The results indicate substituting fat with nanocellulose led the L^* (lightness) and a^* (redness) values increased and the b^* (yellowness) values decreased of the emulsified sausages. Increasing nanocellulose concentration improved the emulsion stability of the sausages. In terms of emulsion stability, the use of CNFs outperformed CNCs, especially for samples substituted with 50% fat. In terms of texture, emulsified sausages where CNCs replaced fat showed lower hardness, viscosity, and chewiness compared to those with CNFs. The dynamic rheological results indicated that when the fat replacement levels were 10% and 30%, samples using CNFs as fat substitute had slightly higher storage modulus (G') values than those using CNCs. However, for 50% fat replacement, samples using CNFs as fat substitute had significantly higher G' values than those using CNCs, which demonstrates that higher CNFs concentrations are more conducive to the formation of a three-dimensional network structure.

Keywords: nanocellulose; fat substitute; emulsified sausages; morphology; texture; rheological properties

Traditional emulsified meat products contain up to 30% of animal fat; fat addition can effectively improve the quality of meat products, particularly, emulsion stability, texture, juiciness, taste, and flavour (Yoo et al. 2007; Zhuang et al. 2016). However, numerous studies have indicated that excessive intake of pork back fat may contribute to various chronic diseases, including hypertension, cardiovascular diseases, diabetes, and obesity (Larsson and Wolk 2006). Therefore, the production of emulsified meat products with low animal fat content is required. Nevertheless, reducing the fat content in meat products can decrease product quality and consumer acceptance, produce a rough texture, and reduce water and oil retention, leading to increased cooking loss. Additionally, it can have adverse effects on the flavour characteristics of the final product.

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Recently, an increasing number of fat substitutes, including lipid-based, protein-based, and carbohydrate-based substitutes, have been incorporated into meat products (Paglarini et al. 2020; López-Pedrouso et al. 2021). When added to meat products, cellulose or cellulose derivatives not only improve emulsion stability and enhance meat product quality but also serve as functional ingredients that increase dietary fibre levels in daily consumption. However, these powdered cellulose fibres are generally insoluble in water and have poor water-holding capacity, thus being used in relatively large amounts, which can result in uneven distribution and potentially lead to a rough texture in meat products, affecting the overall product quality. A nanoscale size of dietary fibres can improve their rheological properties and emulsion stability (Tavernier et al. 2016). In fact, incorporating nanoscale dietary fibres into meat products can decrease fat usage without compromising sensory quality (Gibis et al. 2015).

There were many studies on adding nanocellulose as a fat substitute to meat products. Wang et al. (2018) used a palm oil-based pickering emulsion (CPOE) containing 1% cellulose nanofibres (CNFs) as a fat substitute to replace 30% and 50% of the original fat in emulsified sausages, which reduced cooking loss, increased moisture content and brightness, and resulted in higher hardness, springiness, and chewiness. Zhao et al. (2018) found that incorporating 0.8% cellulose-based fibres in emulsified sausages effectively reduced fat content without compromising quality and sensory characteristics. Qi et al. (2020) added different CNFs morphology to emulsified sausages finding no significant differences in texture and microstructural properties among sausages with different CNFs morphology, except for variations in water and fat binding capabilities.

Nanocellulose generally has two morphologies: one is rigid rod-shaped particles with a diameter of 10–30 nm and a length of hundreds of nanometers (Salas et al. 2014; Du et al. 2016), commonly referred to as cellulose nanocrystals, sometimes referred to as nanocrystalline cellulose or cellulose nanowhiskers; the other type is flexible fibrous fibres with a diameter < 100 nm and a length ≥ 500 nm (Liu et al. 2016), commonly referred to as cellulose nanofibres, sometimes referred to as nanofibrillar cellulose. There have been no reports on the application of different morphology of nanocellulose instead of fat in emulsified sausages. Qi et al. (2020) conducted a study on different morphology of nanocellulose as additives for emulsified sausages, but this study only added nanocellulose to emulsified sausages and did not use nanocellulose as fat substitutes. Our study investigated adding different morphology of nanocellulose with different contents to emulsified sausages to replace pork back fat in different proportions (10%, 30%, and 50%). By analysing the colour, emulsion stability, texture characteristics and dynamic rheological behaviour of emulsified sausages, providing a theoretical foundation for the application of different morphology of nanocellulose in low-fat emulsified meat products.

MATERIAL AND METHODS

Materials. Fresh pork hind leg meat and pork back fat were purchased from a local supermarket, 48 h postmortem. Salt, starch, sugar, five-spice powder, chicken essence, and white pepper powder were also purchased from a local supermarket. Composite phosphate (sodium tripolyphosphate : sodium hexametaphosphate : sodium pyrophosphate ratio is 3 : 2 : 1 in weight), sodium erythorbate, soy protein isolate, red yeast rice pigment, and sodium nitrite were obtained from a food additive company. CNFs were purchased from Zhongshan Nanofiber New Materials, China (several micrometers long; diameter, 35 nm; solid content, 2.5%; viscosity, 6.65 Pa·s; crystallinity, 70%). CNCs were purchased from Shansi New Materials Technology, China (200 nm long; diameter, 10 nm; solid powder; crystallinity, 80%).

Preparation of emulsified sausages. Seven groups of emulsified sausages were prepared. The high-fat control group (C) consisted of 50% lean pork meat and 30% pork back fat, without cellulose-added. The other six groups were the cellulose-added groups, with 50% of lean pork meat, 10%, 30%, and 50% of fat were replaced by CNFs or CNCs, respectively. Among them, in two groups 2.5% CNFs and 2.5% CNCs were used to replace 10% pork back fat (final emulsified sausages contained 0.25% cellulose), named 10% F and 10% C, respectively. In another two groups 2.5% CNFs and 2.5% CNCs were used to replace 30% pork back fat (final emulsified sausages contained 0.5% cellulose), named 30% F and 30% C, respectively, and in the last two groups 2.5% CNFs and 2.5% CNCs was used to replace 50% pork back fat (final emulsified sausages contained 0.75% cellulose), named 50% F and 50% C, respectively.

The emulsified sausages were prepared as follows: All visible fat and connective tissue was removed from the thawed pork hind leg meat. The lean pork meat and pork back fat were separately ground into particle-free meat batter using a meat grinder (MR9401A; Morphyrichards, China). The ground fat was then weighed and

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emulsified with soy protein isolate, and mixed evenly with the lean pork meat. Then, 1/2 of iced water (containing cellulose nanofibres, corn starch, salt, composite phosphate, and sodium erythorbate) was added and mixed evenly. The remaining ice water, along with five-spice powder, white pepper powder, sugar, chicken essence, red yeast rice pigment, and sodium nitrite, were added to the meat batters and mixed until well combined. The Formulation of emulsified sausages is shown in Table 1. Finally, the meat batters were stuffed into natural sheep casings. The stuffed sausages (the initial temperature is 20 °C) were steamed in boiling water at 100 °C for 12 min, cooled, and stored at 4 °C.

Colour measurement. The colour of emulsified sausages was determined according to the method of Shang et al. (2022). Lightness (L^*), redness (a^*), and yellowness (b^*) were measured using a spectro colourimeter (Miniscan EZ; HunterLab, USA). Six measurements of these variables were performed for each of the three replicates.

Emulsion stability. Emulsion stability was determined according to Zhao et al. (2018) with some modifications. A total of 30 g of raw meat batters was centrifuged at 800 g for 5 min at 4 °C to remove air bubbles. Then, the samples were placed into an 80 °C water bath for 20 min and immediately inverted for 60 min at room temperature to release fat and water. Total fluid release (TR) was expressed as the percentage of total liquid released and the initial weight of sample. The water release (WR) was measured by drying the TR at 105 °C for 16 h and expressed as the percentage of dry weight and initial sample weight. The percentage of residual material after drying and the initial sample was considered as the fat release (FR).

Dynamic rheological measurement. The viscoelastic properties of meat batters during thermal gelation were continuously monitored using a rotary rheometer (MCR302; Anton Paar, Austria). According to Kang et al. (2014), parallel stainless-steel plates with a diameter of 50 mm and a gap of 1.0 mm were used. The raw meat batters were placed between the plates and heated from 20 to 80 °C at a heating rate of 2 °C per min. During heating, the samples were continuously sheared in oscillatory mode at a fixed frequency of 0.1 Hz. The change in storage modulus (G') was observed throughout the gelation process; each sample was measured three times for repeatability.

Table 1. Formulation of 7 groups of emulsified sausages [g·(100 g)$^{-1}$]

Ingredients	Treatments						
	control	10% F	30% F	50% F	10% C	30% C	50% C
Pork meat	50	50	50	50	50	50	50
Pork back fat	30	27	21	15	27	21	15
Ice water	20	13	9	5	13	9	5
2.5% CNFs	0	10	20	30	0	0	0
2.5% CNCs	0	0	0	0	10	20	30
Total	100	100	100	100	100	100	100
Corn starch	5	5	5	5	5	5	5
Salt	1.6	1.6	1.6	1.6	1.6	1.6	1.6
Sugar	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Composite phosphate	0.315	0.315	0.315	0.315	0.315	0.315	0.315
Sodium erythorbate	0.1	0.1	0.1	0.1	0.1	0.1	0.1
Soy protein isolate	0.85	0.85	0.85	0.85	0.85	0.85	0.85
Five-spice powder	0.4	0.4	0.4	0.4	0.4	0.4	0.4
White pepper powder	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Chicken essence	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Red yeast rice pigment	0.017	0.017	0.017	0.017	0.017	0.017	0.017
Sodium nitrite	0.012	0.012	0.012	0.012	0.012	0.012	0.012

CNFs – cellulose nanofibres; CNCs – cellulose nanocrystals; 10% F, 10% C – 2.5% CNFs and 2.5% CNCs were used to replace 10% pork back fat; 30% F, 30% C – 2.5% CNFs and 2.5% CNCs were used to replace 30% pork back fat; 50% F, 50% C – 2.5% CNFs and 2.5% CNCs were used to replace 50% pork back fat

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Texture profile analysis. The emulsified sausage samples were cut into cylindrical shapes at 20 mm height, and the hardness (N), springiness (ratio), cohesiveness (ratio), gumminess (N), and chewiness (N·mm) of the samples measured using a texture analyser (TA-XT plus; Stable Micro System, Great Britain). The testing conditions were the following: we used a P/75 probe; the pre-test and test speeds were both set to 2.0 mm·s^{-1}, the post-test speed was set to 5.0 mm·s^{-1}, the strain was set to 30%, and the trigger force was set to 5 g.

Data analysis. All experiments were repeated three times and the values were reported as means ± standard deviations. The one-way analysis of variance (ANOVA) procedure (SPSS, version 16.0) was used to analyse the results. The significant differences between the treatment samples were determined by Tukey's *b*-test (P < 0.05). Rheological properties were plotted using the software of Origin (version 2018).

RESULTS AND DISCUSSION

Colour. The effect of different nanocellulose morphology on the colour of emulsified sausage is shown in Table 2. Compared with the control group, samples using CNFs or CNCs to replace 10–50% fat showed a significant increase in L^* value (P < 0.05) with increasing pork back fat replacement, which may be due to the bright colour of CNFs and CNCs themselves (Zhao et al. 2018). Then a^* also showed a significant increasing trend (P < 0.05), which may be related to the antioxidant effect of nanocellulose. The addition of nanocellulose inhibited myoglobin conversion to metmyoglobin in meat, reducing the occurrence of browning (Jia et al. 2012). At the same time, b^* showed a significant decreasing trend (P < 0.05), consistent with Schuh et al. (2013), who considered that the b^* value of emulsified sausages corresponds to the fat concentration in the product, and decreased fat concentration correspondingly reduces the b^* value. At the same cellulose concentration to replace fat, samples using CNFs to replace partial fat have lower L^* and higher b^* than those using CNCs, which may be attributed to the colour of CNFs itself (Qi et al. 2020). The sample using CNFs as fat substitutes had a higher a^*, which may be attributed to the higher antioxidant activity of CNFs compared to CNCs, and further experiments are needed to verify the reasons.

Emulsion stability. Emulsion stability is used to evaluate the quality of sausages (Pereira et al. 2016). Nanocellulose has excellent emulsifying properties (Cunha et al. 2014; Winuprasith et al. 2015). The effect of different nanocellulose morphology on the emul-

Table 2. Colour parameters, emulsion stability, and textural profile of emulsified sausages

Index	Treatments						
	Control	10% F	30% F	50% F	10% C	30% C	50% C
Colour parameters							
L^*-value	60.79 ± 0.16^{a}	60.98 ± 0.03^{a}	61.32 ± 0.38^{ab}	61.69 ± 0.20^{bc}	61.03 ± 0.06^{ab}	62.05 ± 0.06^{cd}	62.63 ± 0.01^{d}
a^*-value	18.59 ± 0.09^{ab}	18.72 ± 0.15^{abc}	19.36 ± 0.07^{bc}	19.62 ± 0.26^{c}	18.35 ± 0.35^{a}	18.71 ± 0.23^{abc}	19.49 ± 0.40^{bc}
b^*-value	14.67 ± 0.13^{c}	14.39 ± 0.38^{bc}	14.31 ± 0.32^{bc}	13.39 ± 0.16^{ab}	14.37 ± 0.48^{bc}	13.46 ± 0.01^{ab}	13.09 ± 0.02^{a}
Emulsion stability							
Total released (%)	8.43 ± 0.43^{b}	10.05 ± 0.28^{a}	0.70 ± 0.02^{d}	0.37 ± 0.01^{d}	9.77 ± 0.06^{a}	2.85 ± 0.13^{c}	0.42 ± 0.02^{d}
Water released (%)	3.50 ± 0.36^{b}	2.51 ± 0.04^{c}	0.57 ± 0.02^{e}	0.37 ± 0.00^{e}	4.06 ± 0.07^{a}	1.32 ± 0.01^{d}	0.41 ± 0.01^{e}
Fat released (%)	4.74 ± 0.34^{c}	7.64 ± 0.19^{a}	0.13 ± 0.00^{e}	0.00 ± 0.00^{e}	5.67 ± 0.04^{b}	1.60 ± 0.02^{d}	0.01 ± 0.00^{e}
Textural profile							
Hardness (N)	13.34 ± 0.16^{b}	15.24 ± 0.34^{ab}	17.90 ± 1.10^{a}	13.46 ± 0.68^{b}	15.54 ± 0.59^{ab}	15.22 ± 0.49^{ab}	12.84 ± 1.02^{b}
Springiness (ratio)	0.91 ± 0.01^{a}	0.90 ± 0.01^{a}	0.91 ± 0.01^{a}	0.89 ± 0.01^{a}	0.90 ± 0.01^{a}	0.91 ± 0.01^{a}	0.89 ± 0.01^{a}
Cohesiveness (ratio)	0.85 ± 0.01^{a}	0.83 ± 0.01^{ab}	0.84 ± 0.01^{ab}	0.81 ± 0.01^{b}	0.81 ± 0.01^{ab}	0.84 ± 0.01^{ab}	0.83 ± 0.01^{ab}
Gumminess (N)	11.27 ± 0.13^{c}	12.54 ± 0.23^{b}	15.07 ± 0.23^{a}	11.00 ± 0.18^{c}	12.47 ± 0.08^{b}	13.15 ± 0.40^{b}	10.57 ± 0.21^{c}
Chewiness (N·mm)	10.22 ± 0.30^{cd}	11.34 ± 0.31^{bc}	13.72 ± 0.35^{a}	9.82 ± 0.28^{d}	11.25 ± 0.18^{bc}	11.82 ± 0.30^{b}	9.43 ± 0.28^{d}

$^{a–e}$ Mean values superscribed with different letters in the same row are significantly different (P < 0.05); CNFs – cellulose nanofibres; CNCs – cellulose nanocrystals; 10% F, 10% C – 2.5% CNFs and 2.5% CNCs were used to replace 10% pork back fat; 30% F, 30% C – 2.5% CNFs and 2.5% CNCs were used to replace 30% pork back fat; 50% F, 50% C – 2.5% CNFs and 2.5% CNCs were used to replace 50% pork back fat; L^* – lightness; a^* – redness; b^* – yeallowness

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sion stability of emulsified sausage is shown in Table 2. Compared with the control group, the FR and TR of the 10% F and 10% C samples (containing 0.25% CNFs and 0.25% CNCs, respectively) showed a significant increase ($P < 0.05$), while the WR of the 10% F sample was lower and that of the 10% C sample was higher than that of the control group, respectively. This indicates that 0.25% CNFs has stronger water retention than 0.25% CNCs. When the CNFs/CNCs content was 0.5% and 0.75%, the TR, WR, and FR showed a significant decrease ($P < 0.05$) compared with the control group, indicating that nanocellulose levels > 0.5% can better emulsify fat, and that the higher the cellulose concentration, the better the emulsification effect.

For samples with 30% and 50% fat replacement, CNFs had a better emulsion stability than CNCs, especially for samples with 50% fat replacement. This is consistent with the research of Gestranius et al. (2017) and Qi et al. (2020), the difference in emulsion stability is attributed to the different morphology of CNFs and CNCs. The slender morphology of CNFs (Liu et al. 2016) is more conducive to water and oil retention, while the short and hard morphology of CNC is insufficient in maintaining water and oil (Du et al. 2016).

Texture analysis. Adding non-meat ingredients can improve the gelation and structural properties of sausages, by enhancing protein cross-linking in the meat matrix (Heinz et al. 2007). Zhao et al. (2018) found that adding regenerated cellulose can enhance the hardness of emulsified sausages. The effect of different nanocellulose morphology on the texture of emulsified sausages is shown in Table 2. With increasing fat replacement, the hardness of emulsified sausages significantly increased ($P < 0.05$). When the fat replacement amount reaches 50%, the hardness began to decrease to the control level, which may be due to a sharp decrease in pork back fat content (Barbut et al. 2016). Emulsified sausages that use CNFs to replace pork back fat had slightly higher hardness than those using CNCs, which may be due to the formation of a good three-dimensional network structure by CNFs in emulsified sausages, resulting in a higher hardness (Liu et al. 2016). Springiness refers to the height or volume ratio of a deformed sample after compression, after removing the deformation force, to the conditions before deformation. Compared with the control group, there was no significant difference in the springiness of emulsified sausages using CNFs/CNCs to replace pork back fat, neither were differences between the two types of nanocellulose morphology ($P > 0.05$). Cohesiveness can be described as the retention structure of the compressed sample. Compared with the control group, only the 50% F group showed a significant decrease in cohesiveness ($P < 0.05$). Compared with the 50% F group, the 50% C group had higher cohesiveness, which may be due to the rigid structure of CNCs (Feng et al. 2022). Compared with the control group, gumminess of fat replaced samples showed first increasing and then decreasing trend. The sample replacing 30% fat had the highest value; gumminess in the 30% F group was higher than that of the 30% C group, which may be attributed to the high gumminess of CNFs itself (Feng et al. 2022). However, there was no significant difference in gumminess between the 10% F group and the 10% C group, and there was also no significant difference between the 50% F and 50% C groups. Chewiness refers to the energy required to chew a solid sample into a stable state during swallowing, being numerically expressed as the product of hardness, cohesiveness, and springiness. The chewiness trends of samples with different treatments are consistent with gumminess.

Rheological characteristics analysis. The rheological properties of raw meat batter during heating are represented by the storage modulus G' and the loss modulus G'', as shown in Figure 1. The G' of all measured samples was greater than G'', indicating that all samples show strong gel-like characteristics (Nacak et al. 2021). The transformation of G' reflects the conformational changes of myofibrillar proteins and myosin (Zhuang et al. 2016). G' undergoes three stages: *i*) with increasing temperature increases from 42 to 48 °C, G' shows a moderate increase caused by the dimerisation of myosin heads (Tornberg 2005); *ii*) from 49 to 54 °C, G' shows a trend of slowly decrease, this change is related to the degeneration of myosin tail (Li et al. 2022); *iii*) over 55 °C, G' increases sharply with increasing temperature so that raw meat batter changed from viscous sol to an elastic gel network (Kang et al. 2023).

Compared with the control group, the G' of the emulsified sausage using CNFs instead of fat showed an increasing trend, indicating that CNFs positively influenced the springiness of the emulsified sausage. Comparing all samples using CNFs as a fat substitute, no significant difference in G' between the 10% F group and the 30% F group, while that of the 50% F group showed a significant increase. Compared with the control group, at < 62 °C, the G' of emulsified sausages using CNCs to replace pork back fat showed an increasing trend, while > 62 °C, the G' of sausages in the 10% C group showed an increasing trend, while the G' of the emulsified sausage in the 30% C and 50% C groups showed

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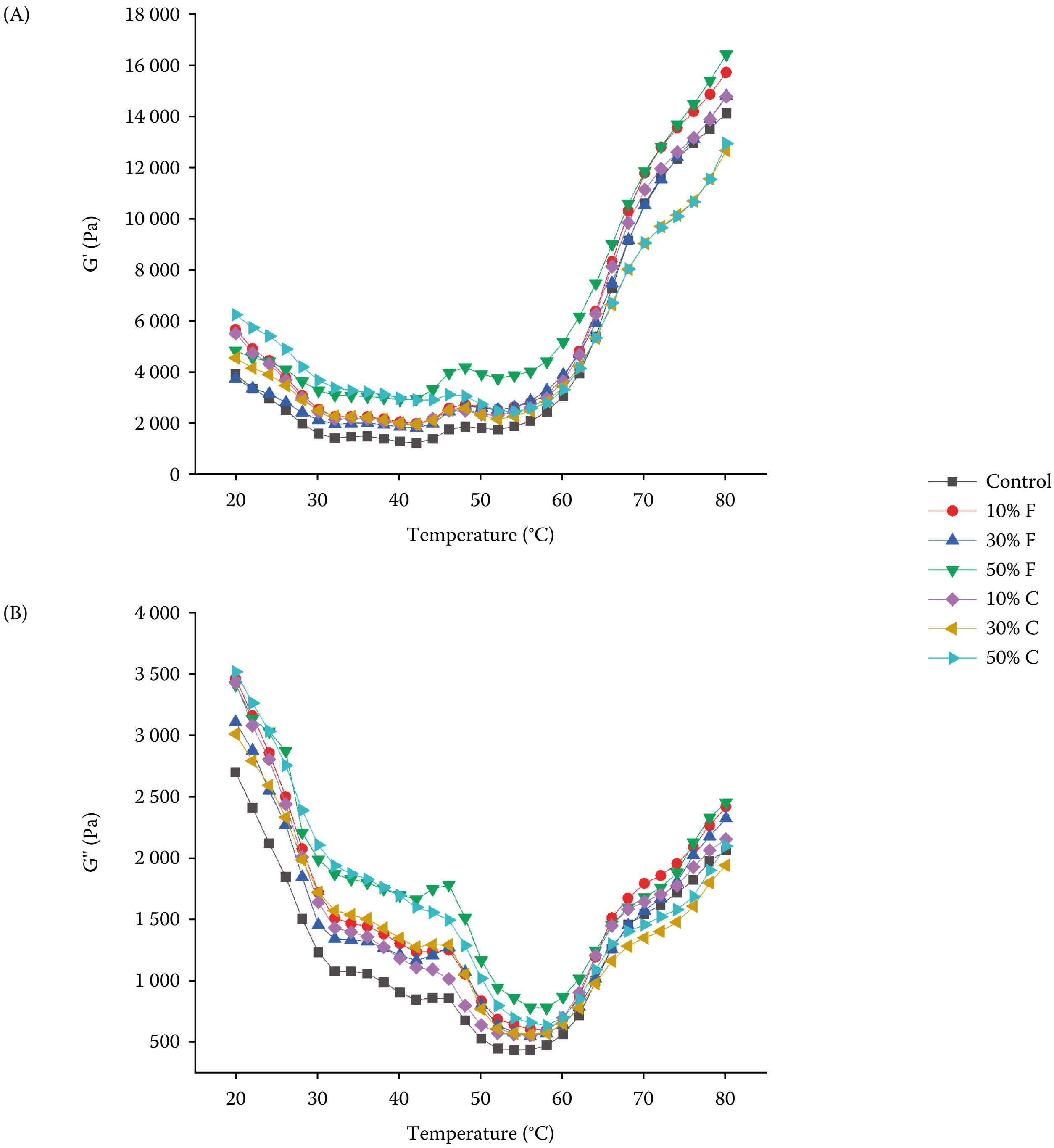

Figure 1. (A) Dynamic storage moduli (G') and (B) loss moduli (G'') for emulsified sausages

CNFs – cellulose nanofibres; CNCs – cellulose nanocrystals; 10% F, 10% C – 2.5% CNFs and 2.5% CNCs were used to replace 10% pork back fat; 30% F, 30% C – 2.5% CNFs and 2.5% CNCs were used to replace 30% pork back fat; 50% F, 50% C – 2.5% CNFs and 2.5% CNCs were used to replace 50% pork back fat

a decreasing trend. These results indicate that CNCs has a positive effect on the springiness of emulsified sausages at low temperatures, but a negative effect at high temperatures, especially for high-fat replacement. For the same level of fat replacement, at 10% and 30%, the G' of sausages using CNFs to replace fat was slightly higher than that of those with CNCs, and at 50% replacement, the G' of CNFs sausages was significantly higher than that of those with CNCs. Further evidence suggests that high CNFs concentrations are more conducive to the formation of three-dimensional network structures (Shang et al. 2024).

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Among all samples using CNFs as a fat substitute, at low temperature, the G'' of the 50% F group was the highest, and that of 10% F and 30% F groups was similar; being both higher than the control group. With increasing temperature, G'' increases more in sausages with CNFs than in controls. Among all samples using CNCs as a fat substitute, at low temperatures, with increasing fat replacement, the G'' of the emulsified sausage shows an increasing trend but at a higher temperature, only the G'' of emulsified sausages in the 10% C group was higher than in the control group, while that of those with 30% and 50% groups was lower than in the control. At the same replacement level, the G'' of emulsified sausages using CNFs was higher than that of those using CNCs, further indicating that CNFs has higher viscosity than CNCs (Shang et al. 2024).

CONCLUSION

Nanocellulose as a fat substitute can improve the quality of emulsified sausages. Emulsified sausages using CNFs instead of pork back fat have better emulsion stability, better viscoelasticity, and can more easily acquire a three-dimensional network structure than those using CNCs. The present research provides some basic dates for the application of nanocellulose in low-fat emulsified sausages. However, the safety of nanocellulose is currently controversial, and further research is needed to determine whether it can be used in production.

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