



The effect of homogenization and addition of polysaccharides on the viscoelastic properties of processed cheese sauce

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ABSTRACT

This study was conducted to determine the effect of 1-stage homogenization (OSH) and 2-stage homogenization (TSH) and the addition of polysaccharides [κ -carrageenan (CR) or furcellaran (FR) at levels ranging from 0.000 to 1.000% (wt/wt)] on the physicochemical, viscoelastic, and mechanical vibration damping properties of processed cheese sauces (PCS) after 30 d of storage ($6 \pm 2^\circ\text{C}$). The basic chemical properties (pH, dry matter content) were similar for all tested samples. Viscoelastic measurements indicated that PCS rigidity was directly proportional to increasing CR or FR concentration and to the application of homogenization. The interactions between the application of homogenization and the concentration of polysaccharides used were also significant. Compared with OSH, TSH did not lead to any further increase in the rigidity. The preceding results were also supported by data obtained from a nondestructive method of mechanical vibration damping. No changes in water activity were observed in any PCS sample. Overall, the addition of FR or CR appeared to be highly suitable for increasing the emulsion stability of PCS. If PCS products with softer consistency are desired, then a concentration of CR/FR $\leq 0.250\%$ (wt/wt) could be recommended together with OSH/TSH. For products for which a firmer PCS consistency is required, the addition of CR in concentrations of $\geq 0.500\%$ (wt/wt) or FR in concentrations of $\geq 1.000\%$ (wt/wt) together with OSH is recommended. Finally, as the concentration of polysaccharides increased, a darker PCS color was observed.

Key words: processed cheese sauce, homogenization, rheological properties, κ -carrageenan, furcellaran

INTRODUCTION

Processed cheese sauce (PCS) is a novel cheese-based product with a relatively high moisture content that is described as an oil-in-water emulsion. Given the lack of established standards or legal definitions regarding the usage of the term “cheese sauce” or PCS, the latter can be manufactured using various raw materials of dairy origin (natural cheese, rework, cheese powder, and whey protein powders). To obtain a high-quality final product with desirable organoleptic and functional properties, other nondairy ingredients of great importance, including emulsifying salts, stabilizers, preservatives, and flavor enhancers, are usually added during PCS manufacture (Saad et al., 2016; Shalaby et al., 2017; Salek et al., 2019; Szafránska et al., 2020). Hydrated and dispersed proteins (in particular casein) originating from the basic raw material (natural cheese or other dairy ingredients) are considered to be the main emulsifier in PCS and processed cheese (PC). The addition of emulsifying salts leads to the disruption of the calcium–phosphate-linked protein network (present in natural cheese), resulting in the release of casein fractions during the manufacture of PCS. Another function of emulsifying salts is to adjust the pH of the product (Kapoor and Metzger, 2008).

The consistency and emulsion stability of PCS can be extensively improved by the addition of hydrocolloids. In general, hydrocolloids constitute a large heterogeneous group of biopolymers that form viscous dispersions, gels, or both when dispersed in water. Hydrocolloids used in the dairy industry can be divided into polysaccharides or proteins according to their nature (Saha and Bhattacharya, 2010; Gao et al., 2017). Red seaweed (*Rhodophyceae*) polysaccharides (galactans), generally named carrageenans, are high-molecular-weight linear polysaccharides composed of a repeating carrabiose unit made up of 3-linked- β -D-galactopyranoses and 4-linked- α -D-galactopyranoses that may occur (especially in gelling carrageenans) in

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the form of the 3,6-anhydro derivative (**DA units**). Various hydroxyl groups can be substituted by ester sulfate, methyl groups, pyruvic acid acetal, and occasionally additional monosaccharide residues. Among the most important fractions of carrageenans are κ -, ι -, and λ -carrageenan, which vary according to the presence of the DA units and the number and position of the sulfate residues (Tuvikene et al., 2010; Robal et al., 2017; Saluri et al., 2019). κ -Carrageenan (**CR**) and ι -carrageenan are gel-forming carrageenans, while λ -carrageenan is only used as a thickening agent (Eha et al., 2021). Furcellaran (**FR**), another polysaccharide that naturally occurs in red seaweeds, is a sulfated galactan structurally similar to **CR**, however, the content of sulfate residues on the polysaccharide backbone is substantially different (Laos et al., 2007). Specifically, **FR** has one sulfate group per tetramer at position 4 of the galactose unit, while **CR** has one sulfate group per galactose dimer (Wurm et al., 2019). Therefore, **FR** has, on average, half the ester sulfate content of **CR** (Marangoni Júnior et al., 2021). Some authors have also referred to **FR** as a hybrid carrageenan composed of κ - and β -carrabiose units (Saluri et al., 2019). Essentially, **FR** can be used in much the same way as **CR**, but it has fewer commercial applications. Furcellaran and **CR** can form rigid and brittle gels, and therefore **FR** could be a notable alternative to **CR** (Eha et al., 2021). In particular, **FR** can be used in the manufacture of dairy desserts, confectionery, or meat products (as a gelling agent) and in beer manufacture (as a wort fining agent; Imeson, 2009; Saluri et al., 2019; Polášek et al., 2021; Saluri et al., 2021).

Generally, the PCS (and PC) manufacturing process can be characterized as the mixing and heating of preselected ingredients (85–110°C) in continuous shear until a homogeneous structure of a melted product is achieved (Kapoor and Metzger, 2008; Salek et al., 2019). In addition, high-pressure homogenization (**HPH**) of the hot melt can be included in the manufacturing process. High-pressure homogenization is a physical process in which a dispersed system (suspension or emulsion) is forced to flow at a high velocity through a narrow gap valve (a disruption valve), producing a smaller and narrow particle size distribution. Particles suspended in the fluid are thereby subjected to high mechanical stress, becoming twisted and deformed. Conventional homogenization, generally in the range of 5 to 50 MPa, is widely used in the food industry to stabilize emulsions (preventing creaming and coalescence), reduce particle size (dispersion), and mix ingredients (Augusto et al., 2013; Levy et al., 2021). While one-stage homogenization (**OSH**) is suitable for low-fat or high-viscosity products, 2-stage homogenization (**TSH**) is more effective for high-fat, high-solid, or low-viscosity

products. In **TSH**, higher pressure is usually used in the first stage, during which the diameter of the fat globules is reduced. The second stage is designed to break fat globule clusters formed in the first stage (Massoud et al., 2016).

Information on the behavior of **FR** in **PC** or **PCS** is scarce in the literature, although the related carrageenan groups have been studied in detail. Černíková et al. (2008, 2010), Hladká et al. (2014), Ahmad et al. (2016), and Chatziantoniou et al. (2019) have examined the effect of various additions of carrageenan on the rheological and sensory properties of **PC** as well as the possible replacement of emulsifying salts (partial/total) by carrageenan in **PC**. To the best of our knowledge, no study has described the application of **HPH** with the opposite pressure ratio. Further, the available literature does not include the impact of **OSH** or **TSH** on **PCS** properties. Therefore, the main objective of the current study was to obtain information on the impact of **HPH** (**OSH**, **TSH**, or both) and the addition of polysaccharides [**CR** or **FR** at concentrations of 0.125, 0.250, 0.500, 0.750, and 1.000% (wt/wt)] on **PCS** properties (physicochemical, viscoelastic, and mechanical vibration damping).

MATERIALS AND METHODS

Materials

To prepare the **PCS** samples, the following ingredients were used: Edam cheese blocks [7-wk maturity; 50% (wt/wt) **DM**; 30% (wt/wt) fat in **DM** (**FDM**; Agricol], butter [84% (wt/wt) **DM**, 82% (wt/wt) **FDM**; Fude + Serrahn Milchprodukte GmbH & Co. KG], emulsifying salts [monosodium dihydrogenphosphate (NaH_2PO_4), disodium hydrogenphosphate (Na_2HPO_4), tetrasodium diphosphate ($\text{Na}_4\text{P}_2\text{O}_7$), and sodium salt of polyphosphate with chain length $n \approx 20$; Fosfa a.s.], **FR** [Est-agar a.s.; $M_W = 2.55 \times 10^5$ Da, water gel strength according to Bloom = 480 g, 9.5% (wt/wt) moisture content], **CR** [Sigma-Aldrich s.r.o.; $M_W = 4.31 \times 10^5$ Da, water gel strength according to Bloom = 520 g, 1.2% (wt/wt) moisture content], mixture of monoacylglycerols and diacylglycerols (**MAG/DAG**; Brenntag **CR** s.r.o.), and ultrapure water (aquaMax Ultra 370 Series, YoungLin).

No animals were used in this study, and ethical approval for the use of animals was thus deemed unnecessary.

Experimental Design and PCS Sample Preparation

Furcellaran and **CR** were added separately to **PCS** at concentrations of 0.125, 0.250, 0.500, 0.750, and

1.000% (wt/wt). A control sample (**CS**) without polysaccharide addition was manufactured. All PCS samples were manufactured under the same conditions and designed to have a DM content of 33% (wt/wt) and an FDM of 66% (wt/wt). The target processing temperature was 90°C with a holding time of 1 min (total melting time 11–12 min); the manufacture protocol is described in detail in Buňka et al. (2013). The manufactured PCS samples were subjected to OSH ($p = 10$ MPa) or TSH [$p_1 = 10$ MPa (first stage) and $p_2 = 20$ MPa (second stage)] at 90°C using PandaPLUS equipment (GEA Niro Soavi). The PCS samples were divided into the following groups: nonhomogenized (**NH**), OSH, and TSH. The hot molten mass was then poured into polypropylene containers (cuboid shape; length, 95 mm; width, 75 mm; height, 30 mm) with sealable aluminum lids. The weight of each sample in a container was approximately 90 ± 5 g. The samples were left to cool and stored at $6 \pm 2^\circ\text{C}$ for 30 d. The samples were manufactured separately according to the formulations depicted in Table 1. A total of 99 batches of PCS were manufactured: 11 variants of polysaccharide types and concentrations (including CS) \times 3 homogenization regimens \times 3 repetitions. All analyses were performed 3 times ($n = 3 \times 3 = 9$) after 30 d of PCS storage. Figure 1 illustrates the experimental design.

Basic Chemical Analysis

The DM content was determined gravimetrically according to ISO 5534 (ISO, 2004). The pH was determined (at $22 \pm 1^\circ\text{C}$) by inserting a glass tip electrode of a calibrated pH meter (Edge; Hanna Instruments

Czech s.r.o.) directly into the samples. The recorded values were the mean of at least 9 replicates ($n = 9$).

Dynamic Oscillatory Rheology

An oscillatory shear rheometer (HAAKE RheoStress 1, Thermo Scientific; diameter of the parallel plate geometry = 35 mm, gap = 1 mm) was used to determine the viscoelastic properties of PCS ($20.0 \pm 0.1^\circ\text{C}$). Measurements were carried out in an oscillating mode within the region of linear viscoelasticity with a shear stress amplitude value of 2.0 Pa and a frequency ranging from 0.1 to 10.0 Hz. Stress sweeps were performed between 1 and 100 Pa, at a frequency of 10 Hz and a temperature of 20°C to determine the linear viscoelasticity region. The elastic (G' , Pa) and viscous (G'' , Pa) moduli were recorded as a function of the frequency f (Hz) using Rheowin Job software (v. 2.93, Haake). Furthermore, the phase shift (δ , degree) and the complex modulus (G^* , Pa) were determined according to Equations (1) and (2), respectively. For data presentation, a reference frequency of 1 Hz was selected.

$$\delta = \tan^{-1} G''/G', \quad [1]$$

$$G^* = \sqrt{(G')^2 + (G'')^2}. \quad [2]$$

Determination of Displacement Transmissibility

The mechanical vibration properties (and thus stiffness) of PCS were evaluated based on displacement

Table 1. Raw materials formulation and processing parameters of processed cheese sauce samples

Raw material and processing parameter	Ingredient composition, ¹ % (wt/wt)					
	CS	T ₁	T ₂	T ₃	T ₄	T ₅
Raw material						
Water	53.500	53.500	53.500	53.500	53.500	53.500
Natural cheese	22.600	22.475	22.350	22.100	21.850	21.600
Butter	20.600	20.600	20.600	20.600	20.600	20.600
Emulsifying salts ²	2.300	2.300	2.300	2.300	2.300	2.300
Polysaccharide	None	0.125	0.250	0.500	0.750	1.000
MAG/DAG ³	1.000	1.000	1.000	1.000	1.000	1.000
Processing parameter						
Agitation speed, rpm	3,000	3,000	3,000	3,000	3,000	3,000
Holding time, min	1	1	1	1	1	1
Target temperature, °C	90	90	90	90	90	90
Total time, min	10	10	10	10	10	10

¹CS = control sample (without polysaccharide addition); T₁, T₂, T₃, T₄, T₅ = samples with polysaccharide addition.

²Emulsifying salts: Na₂HPO₄, NaH₂PO₄, Na₄P₂O₇, sodium salt of polyphosphate with the mean length $n \approx 20$; (ratio 1.6:1.9:1.4:1.4).

³MAG/DAG = monoacylglycerols and diacylglycerols.

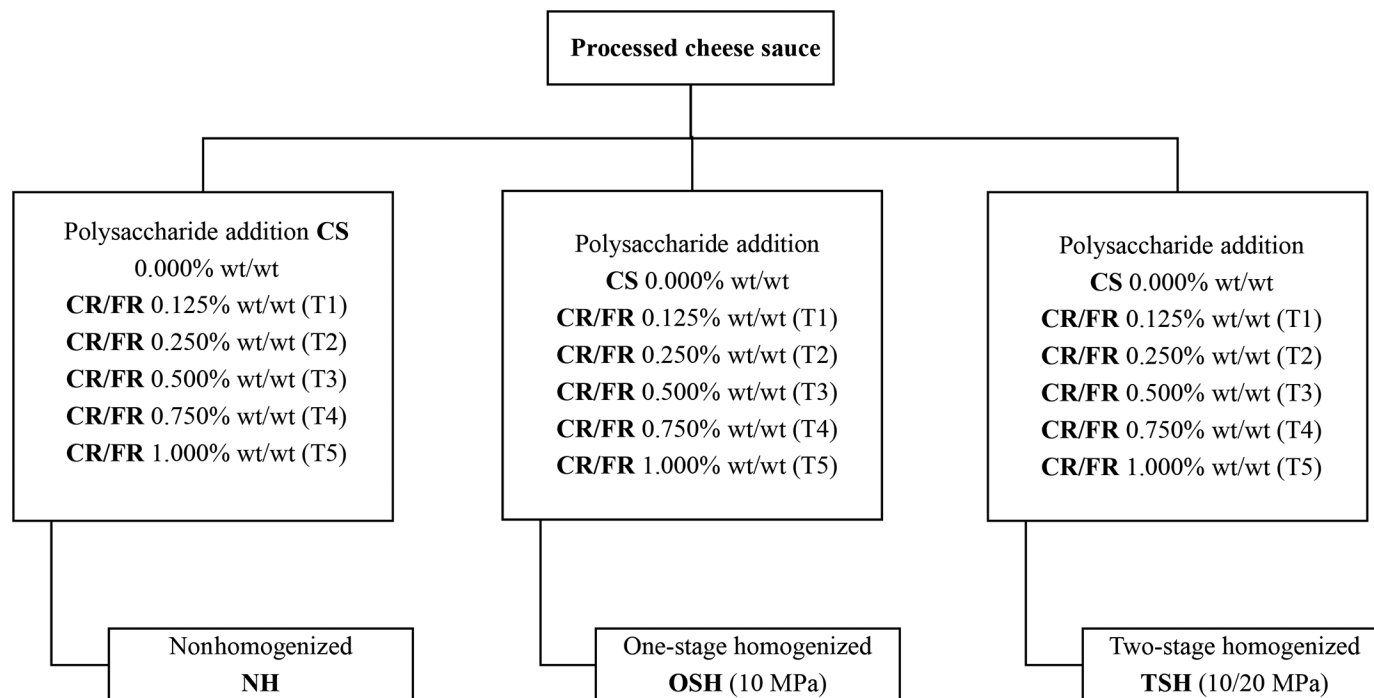


Figure 1. Schematic description of the experimental design with model processed cheese sauce samples manufactured with homogenization in 1 stage (OSH), 2 stages (TSH), and without homogenization (NH) in relation to the added type of polysaccharide: CR = κ -carrageenan; FR = furcellaran.

transmissibility (T_d ; unitless) as follows (Rao, 2005; Latif and Rus, 2014):

$$T_d = \frac{y_2}{y_1} = \frac{a_2}{a_1} = \frac{1 + (2\zeta \cdot r)^2}{\sqrt{(1 - r^2)^2 + (2\zeta \cdot r)^2}}, \quad [3]$$

where y_1 (m)/ a_1 ($\text{m}\cdot\text{s}^{-2}$) is the displacement/acceleration amplitude on the excitation (input) side of the investigated sample, y_2 (m)/ a_2 ($\text{m}\cdot\text{s}^{-2}$) is the displacement/acceleration amplitude on the free (output) side of the investigated sample, ζ (unitless) is the damping ratio, and r (unitless) is the frequency ratio of the circular frequency to the undamped natural frequency (Rao, 2005).

Under the condition $dT_d/dr = 0$, it is possible to obtain the frequency ratio r_0 from Equation 3. The displacement transmissibility T_d has its maximum value at r_0 (Rao, 2005):

$$r_0 = \frac{\sqrt{\sqrt{1 + 8\zeta^2} - 1}}{8\zeta^2}. \quad [4]$$

Equation 4 shows that the maximum value of displacement transmissibility is generally achieved at lower

values of the frequency ratio r with increasing damping ratio ζ .

Displacement transmissibility measurements of the investigated PCS were performed according to Salek et al. (2019) with minor modifications. Measurements were made for the mass load $m = 85$ g on the upper side of the dynamically loaded tested samples, which were placed in a metal bowl ($23 \pm 1^\circ\text{C}$). The samples were cuboid shaped with dimensions of 60 mm (length), 60 mm (width), and 10 mm (height).

Instrumental Color Measurement

The HunterLab UltraScan Pro Color Measurement Spectrophotometer (Hunter Associates Laboratory, Inc.) was used for instrumental color evaluation. The CIELAB color scale ($L^*a^*b^*$) was used, with D65 illuminant (standard daylight) and 10° angle. The parameters L^* (luminosity; 0 indicates black and 100 indicates white) and a^* and b^* [greenness (negative values) to redness (positive values) and blueness (negative values) to yellowness (positive values), respectively] were determined according to the International Commission on Illumination (CIE). The apparatus was calibrated in reflectance mode, with specular reflection excluded, using white (C6299 HunterLab Color Standard) and gray (C6299G HunterLab Color

Standard) reference plates. A 10-mm quartz cuvette was used for the readings.

Emulsion Stability Test

The emulsion stability measurements were performed according to Nikzade et al. (2012) with minor modifications. Samples of PCS of weight m_1 (5 ± 1 g) were transferred to 50-mL polypropylene centrifuge tubes (29.1-mm internal diameter; 114.4-mm height; conical bottom) sealed with plastic caps and then centrifuged at 6,000 rpm for a period of 20 min. After thoroughly removing the supernatant, the sediment was weighed (m_2). The emulsion stability (S) was calculated according to Equation 5 and the results are expressed as a relative percentage:

$$S = \frac{m_2}{m_1} \times 100. \quad [5]$$

Water Activity

The water activity (a_w) was measured ($25.0 \pm 0.1^\circ\text{C}$) with a 4TE dew point water activity meter (AquaLab, Decagon). To verify the precision of the result, a standard ($a_w = 0.92$ NaCl 2.33 molal in H_2O ; AquaLab, Decagon) was included before and during the measurements.

Statistical Analysis

The results obtained were evaluated for normal distribution (Shapiro-Wilk test; the significance level was 0.05; Unistat 6.5, Unistat Ltd.). The Shapiro-Wilk test is recommended on the basis of ISO 5479 (ISO, 1997) for the normal distribution testing of a small amount of data. However, the use of typical parametrical tests was rejected because the normal distribution was not acceptable for all data ($P < 0.05$). Therefore, the data were analyzed using a nonparametrical analysis of the variance of Kruskal-Wallis and Wilcoxon tests (Minitab 16 software; Minitab Ltd.), with the significance level set at 0.05. The above-mentioned statistical approach is in accordance with Granato et al. (2014), which describes statistical methods in food science and technology. The effects of homogenization and polysaccharide addition were evaluated separately. Additionally, the 2-way ANOVA was also applied for evaluation of the interaction effect of the independent variables (homogenization and polysaccharide addition) on the observed quality parameters of PCS.

RESULTS AND DISCUSSION

Basic Chemical Analysis

The results of the basic chemical analysis of PCS are presented in Table 2. The DM content of all model samples was not affected by the addition of polysaccharide or applied HPH ($P \geq 0.05$). The pH values of the samples ranged from 5.76 to 5.85 ($P \geq 0.05$), which could be considered suitable for the interaction of the present caseins with their uniform distribution in the PCS matrix leading to the formation of a smooth PCS consistency (Lee and Klostermeyer, 2001).

Dynamic Oscillatory Rheology

According to the results of the stress sweeps, a stress of 2.0 Pa was within the linear viscoelastic region in the tested PCS samples (Figure 2). Thus, the frequency sweeps were realized at 2.0 Pa. The development of elastic (G') and viscous (G'') moduli of the PCS samples (NH, OSH, TSH) in relation to the frequency (0.10–10.0 Hz) is shown in Figures 3 to 5 for the CS, CR, and FR samples, respectively. All tested samples showed a more elastic behavior ($G' > G''$; $P < 0.05$) across the entire frequency range. The G' and G'' values of the homogenized samples (both OSH and TSH with the same polysaccharide level) were significantly ($P < 0.05$) higher compared with the NH samples. High-pressure homogenization caused a reduction in the size of fat globules and protein particles present in PCS, leading to an increase in surface area. Furthermore, the proteins (forming the basic “skeleton” of PCS) were integrated into the membranes of newly formed fat globules (Lopez et al., 2015). Taken together, these actions are likely to increase the number of binding interactions in the system, thus supporting the creation of a more rigid PCS matrix and ensuring its improved physical stability as well (Mohammadi and Fadaei, 2018). Pluta-Kubica et al. (2021) reported that smaller fat globules allow the formation of a more compact protein matrix. In contrast to the typically applied ratios of HPH pressures, a higher pressure was used in the second stage of HPH than in the first stage. However, a closer look reveals that the application of TSH (and at the same time a higher homogenization pressure) did not lead to a further increase in G' and G'' compared with the OSH samples ($P < 0.05$). The curves of the CS and FR/0.125 TSH PCS samples were lower than those of the OSH samples. The excessive mechanical stress, which was applied to the melt during TSH, could lead to a partial collapse of the 3-dimensional matrix of the product. A

Table 2. Results of DM content (% wt/wt; $n = 3 \times 3 = 9$) and pH value ($n = 3 \times 3 = 9$) of the model processed cheese sauce with κ -carrageenan or furcellaran in concentrations of 0.125, 0.250, 0.500, 0.750, and 1.000% (wt/wt) compared with the control sample

Polysaccharide	Concentration, % (wt/wt)	Homogenization ¹	DM content, % (wt/wt)	pH value
Control sample		NH	32.70 ± 0.21 ^{a,A}	5.81 ± 0.02 ^{a,A}
		OSH	32.96 ± 0.24 ^{a,A}	5.82 ± 0.01 ^{a,A}
		TSH	33.01 ± 0.29 ^{a,A}	5.79 ± 0.02 ^{a,A}
Carrageenan	0.125	NH	32.49 ± 0.20 ^{a,A}	5.77 ± 0.02 ^{a,A}
		OSH	32.49 ± 0.24 ^{a,A}	5.80 ± 0.02 ^{a,A}
		TSH	32.48 ± 0.37 ^{a,A}	5.81 ± 0.01 ^{a,A}
	0.250	NH	32.65 ± 0.34 ^{a,A}	5.80 ± 0.02 ^{a,A}
		OSH	32.77 ± 0.43 ^{a,A}	5.76 ± 0.01 ^{a,A}
		TSH	33.11 ± 0.35 ^{a,A}	5.80 ± 0.03 ^{a,A}
	0.500	NH	32.22 ± 0.30 ^{a,A}	5.79 ± 0.02 ^{a,A}
		OSH	32.93 ± 0.36 ^{a,A}	5.80 ± 0.02 ^{a,A}
		TSH	32.33 ± 0.21 ^{a,A}	5.81 ± 0.03 ^{a,A}
	0.750	NH	33.00 ± 0.31 ^{a,A}	5.78 ± 0.02 ^{a,A}
		OSH	32.68 ± 0.30 ^{a,A}	5.78 ± 0.02 ^{a,A}
		TSH	32.41 ± 0.22 ^{a,A}	5.81 ± 0.01 ^{a,A}
1.000	NH	32.86 ± 0.26 ^{a,A}	5.80 ± 0.02 ^{a,A}	
	OSH	32.88 ± 0.25 ^{a,A}	5.81 ± 0.03 ^{a,A}	
	TSH	32.95 ± 0.39 ^{a,A}	5.81 ± 0.02 ^{a,A}	
Furcellaran	0.125	NH	32.89 ± 0.32 ^{a,A}	5.80 ± 0.02 ^{a,A}
		OSH	32.43 ± 0.38 ^{a,A}	5.81 ± 0.02 ^{a,A}
		TSH	31.84 ± 0.27 ^{a,A}	5.78 ± 0.02 ^{a,A}
	0.250	NH	32.61 ± 0.35 ^{a,A}	5.80 ± 0.02 ^{a,A}
		OSH	32.89 ± 0.32 ^{a,A}	5.79 ± 0.01 ^{a,A}
		TSH	32.05 ± 0.34 ^{a,A}	5.82 ± 0.02 ^{a,A}
	0.500	NH	32.48 ± 0.22 ^{a,A}	5.81 ± 0.02 ^{a,A}
		OSH	32.37 ± 0.33 ^{a,A}	5.80 ± 0.02 ^{a,A}
		TSH	31.94 ± 0.41 ^{a,A}	5.79 ± 0.02 ^{a,A}
	0.750	NH	32.21 ± 0.36 ^{a,A}	5.81 ± 0.03 ^{a,A}
		OSH	32.08 ± 0.41 ^{a,A}	5.81 ± 0.02 ^{a,A}
		TSH	32.67 ± 0.41 ^{a,A}	5.80 ± 0.01 ^{a,A}
1.000	NH	32.28 ± 0.32 ^{a,A}	5.81 ± 0.02 ^{a,A}	
	OSH	33.46 ± 0.40 ^{a,A}	5.80 ± 0.02 ^{a,A}	
	TSH	32.62 ± 0.27 ^{a,A}	5.83 ± 0.03 ^{a,A}	

^aMeans within a column (the difference between homogenization type; comparing the same polysaccharide and its concentration; the control sample was also included) followed by different superscript letters differ ($P < 0.05$). κ -Carrageenan and furcellaran were evaluated individually.

^AMeans within a column (the difference between the concentration of polysaccharide; comparing the same polysaccharide homogenization type; the control sample was also included) followed by different uppercase letters differ ($P < 0.05$). κ -Carrageenan and furcellaran were evaluated individually.

¹Samples were not homogenized (NH) or were homogenized in a 1-stage process (10 MPa; OSH) or a 2-stage process (10/20 MPa; TSH). Values are presented as mean ± SD.

similar phenomenon, albeit under different conditions, was previously described by Lee et al. (2003). This led us to conclude that TSH was not effective enough to influence the viscoelastic properties of this product type, compared with OSH.

Furthermore, increasing the polysaccharide concentration resulted in a significant increase in the G' and G'' curves (irrespective of HPH; $P < 0.05$) compared with CS, with the exception of the 0.125% FR sample ($P < 0.05$). The increase in sample rigidity was likely due to the coil-to-helix transition followed by aggregation of double helices to form a space-spanning network (Du et al., 2016). Previously, CR gel networks were reported to develop through a multitude of polymer chain associations, thus enhancing the formation of a

3-dimensional helix framework (Polášek et al., 2021). Chains are present as a random coil at temperatures above 50°C (soil state). However, at temperatures lower than 50°C, the chains are transformed into a helix, leading to the development of a gel when enough of the helix is formed to provide cross-links. In particular, during cooling, CR aligns 2 helical coils in a manner that orients its 4 sulfate groups toward each other, and charges are neutralized by divalent cations. Thereafter, a double helix is formed by hydrogen bonding (Trius et al., 1996).

According to Černíková et al. (2008), a denser network of polysaccharide chains can form with increasing CR concentrations, which results in the development of a more solid gel. However, a concentration of 0.125%

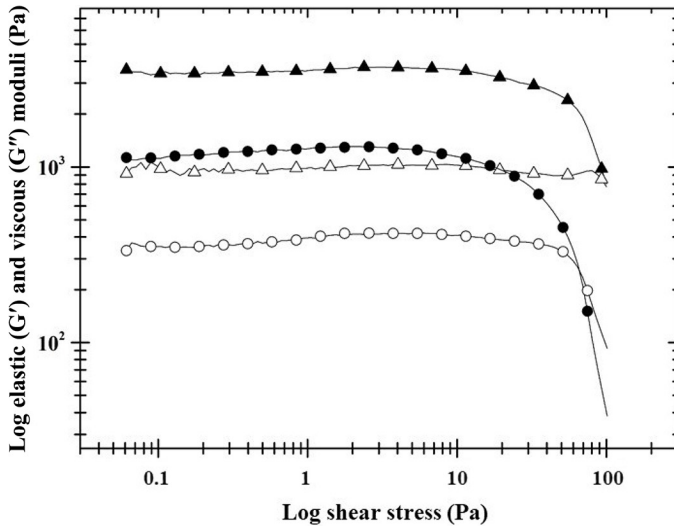


Figure 2. Stress sweeps of nonhomogenized processed cheese sauce samples made with κ -carrageenan (triangles) or furcellaran (circles) in a concentration of 0.500% (wt/wt). Elastic modulus (G' , closed symbols, Pa) and viscous modulus (G'' , open symbols, Pa).

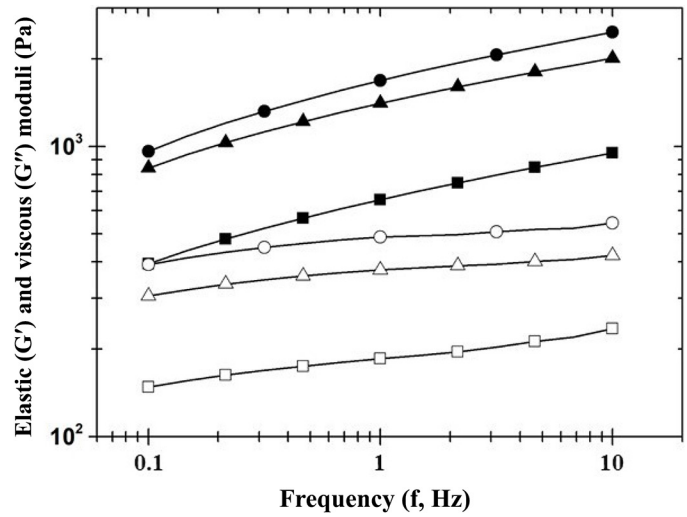


Figure 3. Effect of high-pressure homogenization on the viscoelastic properties (elastic moduli G' , closed symbols, Pa; viscous moduli G'' , open symbols, Pa) of control processed cheese sauce made without polysaccharide after 30 d of storage at $6 \pm 2^\circ\text{C}$. Samples were not homogenized (squares) or were homogenized in a 1-stage process (10 MPa, circles) or a 2-stage process (10/20 MPa, triangles; $n = 3 \times 3 = 9$).

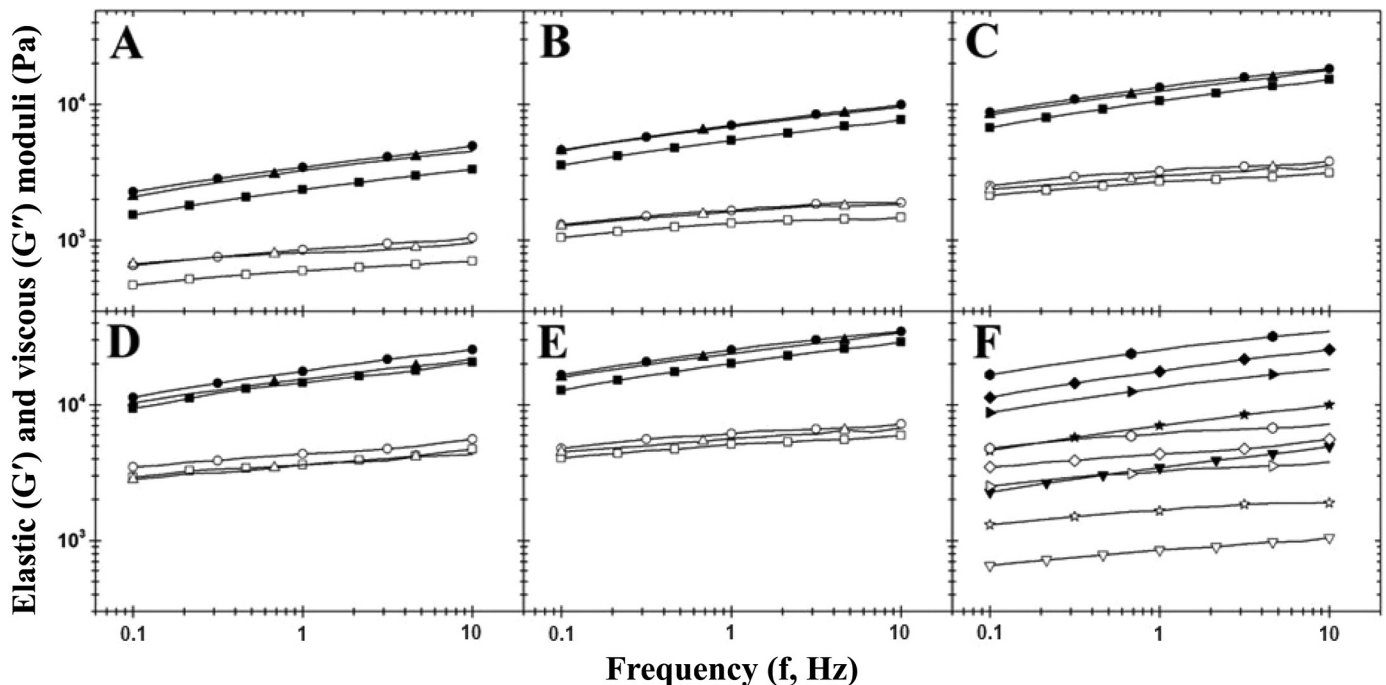


Figure 4. Effect of high-pressure homogenization on the viscoelastic properties (elastic moduli G' , closed symbols, Pa; viscous moduli G'' , open symbols, Pa) of the model processed cheese sauce made with κ -carrageenan in concentrations 0.125 (A), 0.250 (B), 0.500 (C), 0.750 (D), and 1.000% wt/wt (E) on frequency (f ; in the range of 0.1–10.0 Hz) after 30 d of storage at $6 \pm 2^\circ\text{C}$. Samples were not homogenized (squares) or were homogenized in a 1-stage process (10 MPa, circles) or a 2-stage process (10/20 MPa, triangles pointing up). For easy comparison, graph F shows the effect of polysaccharide concentration on the development of elastic (G' , closed symbols, Pa) and viscous (G'' , open symbols, Pa) moduli of model processed cheese sauce with κ -carrageenan in concentrations 0.125 (triangles pointing down), 0.250 (stars), 0.500 (triangles pointing right), 0.750 (diamonds), and 1.000% wt/wt (hexagons), with a 1-stage process (10 MPa) being used ($n = 3 \times 3 = 9$).

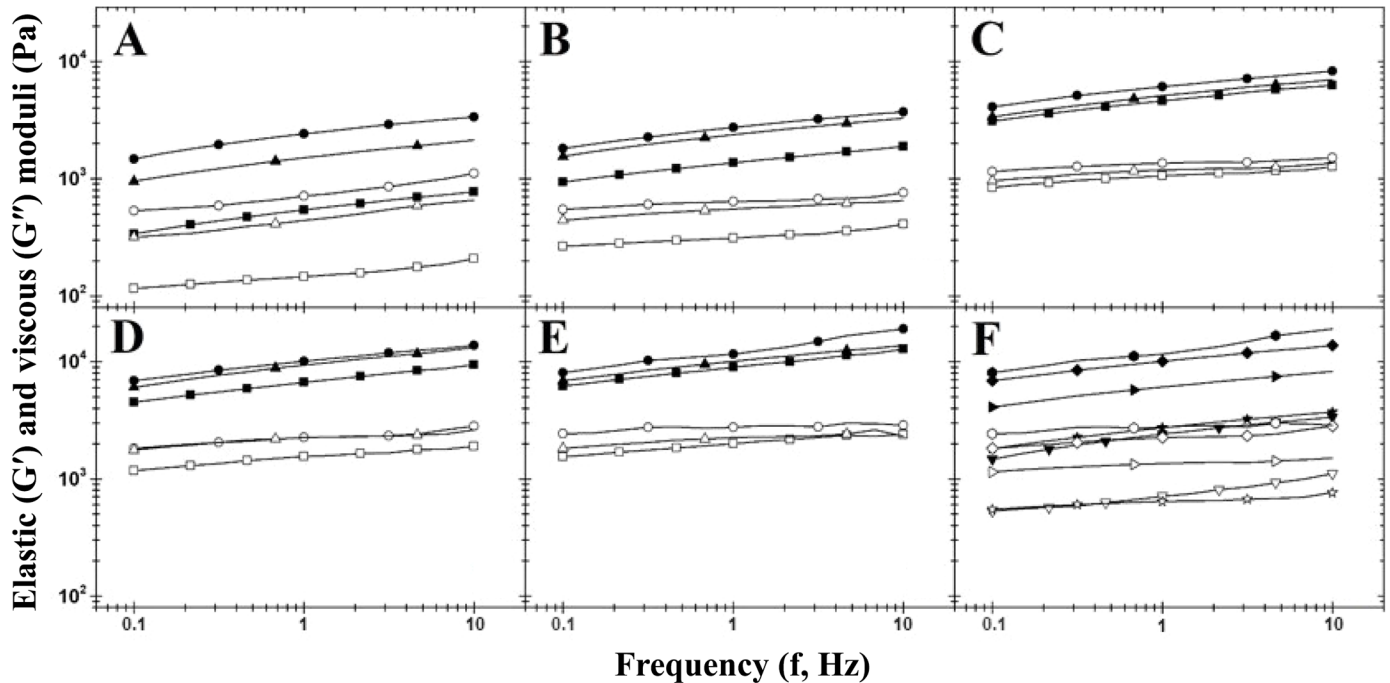


Figure 5. Effect of high-pressure homogenization on viscoelastic properties (elastic moduli G' , closed symbols, Pa; viscous moduli G'' , open symbols, Pa) of the model processed cheese sauce made with furcellaran in concentrations 0.125 (A), 0.250 (B), 0.500 (C), 0.750 (D), and 1.000% wt/wt (E) on frequency (f ; in the range of 0.1–10.0 Hz) after 30 d of storage at $6 \pm 2^\circ\text{C}$. Samples were not homogenized (squares) or were homogenized in a 1-stage process (10 MPa; circles) or a 2-stage process (10/20 MPa, triangles pointing up). For easy comparison, graph F shows the effect of polysaccharide concentration on the development of elastic (G' , closed symbols, Pa) and viscous (G'' , open symbols, Pa) moduli of model processed cheese sauce with furcellaran in concentrations 0.125 (triangles pointing down), 0.250 (stars), 0.500 (triangles pointing right), 0.750 (diamonds), and 1.000% wt/wt (hexagons), with a 1-stage process (10 MPa) being used ($n = 3 \times 3 = 9$).

(wt/wt) of FR appears to be insufficient for network formation (see Figure 4F, the comparison reported for OSH samples). In conclusion, CR must be present at a certain concentration to successfully induce the formation of a network and strengthen the gel. Notably, this threshold is influenced by many factors, such as pH, ionic strength, and other properties of the polysaccharide or protein matrix (Bourriot et al., 1999; Černíková et al., 2008). Compared with FR, the application of CR led to a statistically significant increase in sample rigidity, especially at higher concentrations, up to 0.500% (wt/wt; $P < 0.05$). The CR molecules were probably able to interact with the developed protein matrix, resulting in higher values of the monitored viscoelastic moduli. This interaction can occur between CR molecules and negatively charged carbonyl groups on the protein through cation bridging, or it may be a direct interaction between the CR molecules and the positively charged amino groups of the present protein. Other interactions such as hydrogen bonds, hydrophobic bonds, or covalent bonds may be involved in stabilizing the protein-polysaccharide matrix (Trius et al., 1996). Conformational alignments differ owing to the number of sulfate groups present in the polysaccharide

molecule that influence rigidity (Ruusunen et al., 2003; Zhang et al., 1994). Furcellaran has one ester sulfate group per tetramer, at position 4 of the galactose unit. Structurally, FR is related to CR, but it is less sulfated than CR (Jamróz et al., 2019). The observed viscoelastic differences between CR and FR samples, regardless of polysaccharide concentration and HPH application, could be explained by the above-mentioned statements. Furthermore, the interactions between homogenization use and added polysaccharide concentration were also significant ($P < 0.05$; the 2-way ANOVA was used).

The values of G^* and δ , as presented in Figure 6, also support the conclusion that an increase in the concentration of polysaccharides, with the exception of 0.125% (wt/wt) FR, coupled with the application of HPH, led to an increase in PCS rigidity ($P < 0.05$). Higher values of the phase angle indicate a tendency toward more viscous behavior, while lower values indicate a more elastic behavior (Buckley et al., 2013). The polysaccharide addition clearly resulted in higher G^* values than CS regardless of the type and concentration of polysaccharide (Figure 4). In the OSH sample with CR at a concentration of 1.000% (wt/wt), G^* was comparable (≈ 27 kPa, Figure 6A) to those of the “conven-

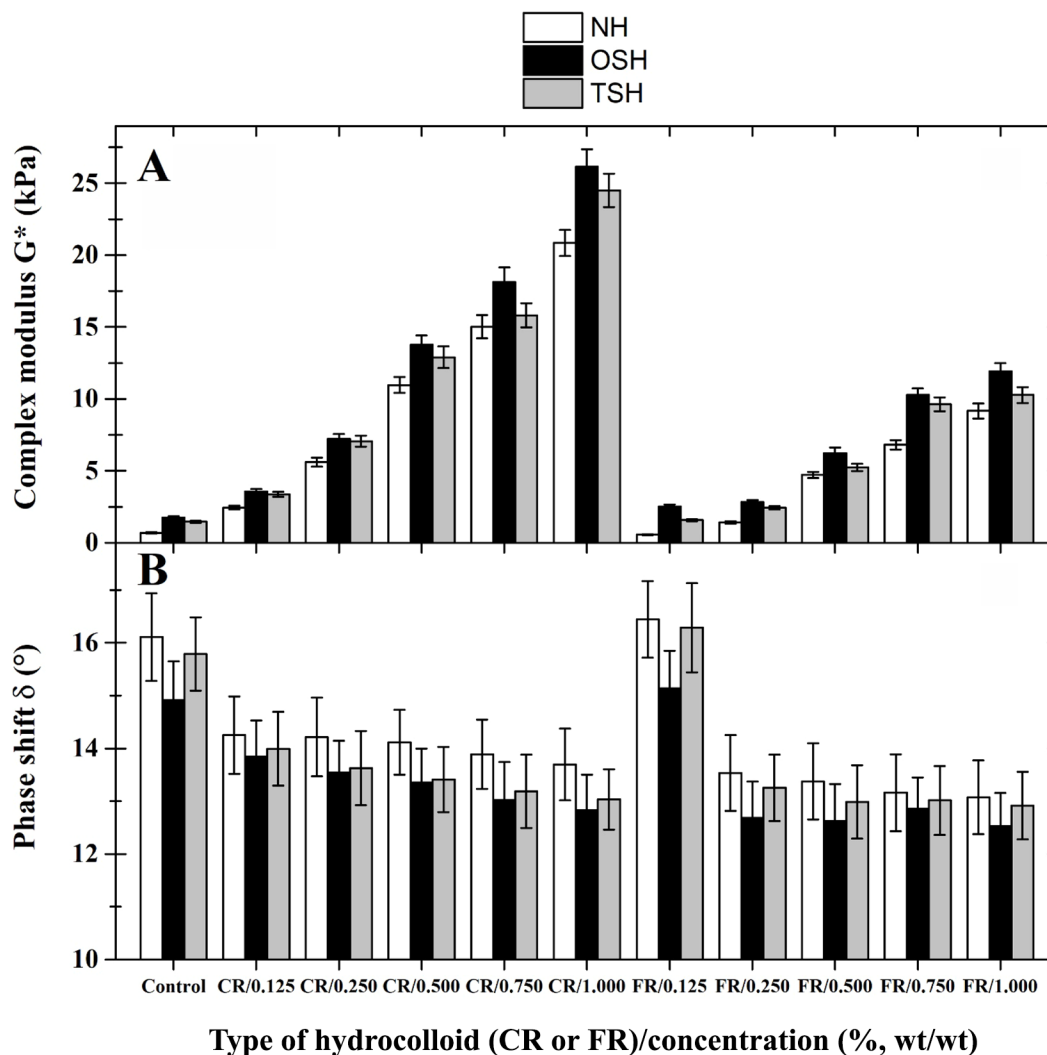


Figure 6. The effect of high-pressure homogenization on the development of complex moduli (G^* , kPa, A) and phase shift (δ , degrees, B) values at the reference frequency of 1 Hz of the model processed cheese sauce with κ -carrageenan (CR) and furcellaran (FR) at concentrations 0.125, 0.250, 0.500, 0.750, and 1.000% (wt/wt) and compared with the control sample. Samples were not homogenized (white columns, NH) or homogenized in a 1-stage process (10 MPa, black columns, OSH) or a 2-stage process (10/20 MPa, gray columns, TSH; $n = 3 \times 3 = 9$).

tional" spreadable PC with a DM content of 45% (wt/wt) [FDM of 50% (wt/wt)] previously presented in the work of Černíková et al. (2017). This result indicates a similar consistency of the product with PCS stabilized by CR addition and HPH (from this study) with 30% DM content.

Displacement Transmissibility

Examples of measured frequency dependencies of the PCS displacement transmissibility are shown in Figure 7. Resonant mechanical vibration (i.e., for $T_d > 1$) was generally found at low excitation frequencies. In contrast, vibration damping (i.e., for $T_d < 1$) was observed at higher excitation frequencies depending on

the homogenization process (NH, OSH, and TSH) and the type and concentration of polysaccharide (FR or CR).

The effect of HPH on displacement transmissibility is shown in Figure 7 (A and B). The lowest vibration damping properties were found for the NH samples ($P < 0.05$). This result is reflected in the higher values of the first resonance frequencies ($f_{R1} \approx T_{dmax}$) compared with the homogenized samples. For this reason, the NH samples are characterized by low viscous friction. In addition, vibration damping properties increased with HPH, which was accompanied by a decrease in the first resonance frequency of the OSH samples. However, TSH led to a slight decrease in viscous damping ($P < 0.05$) compared with the OSH samples. With an increasing

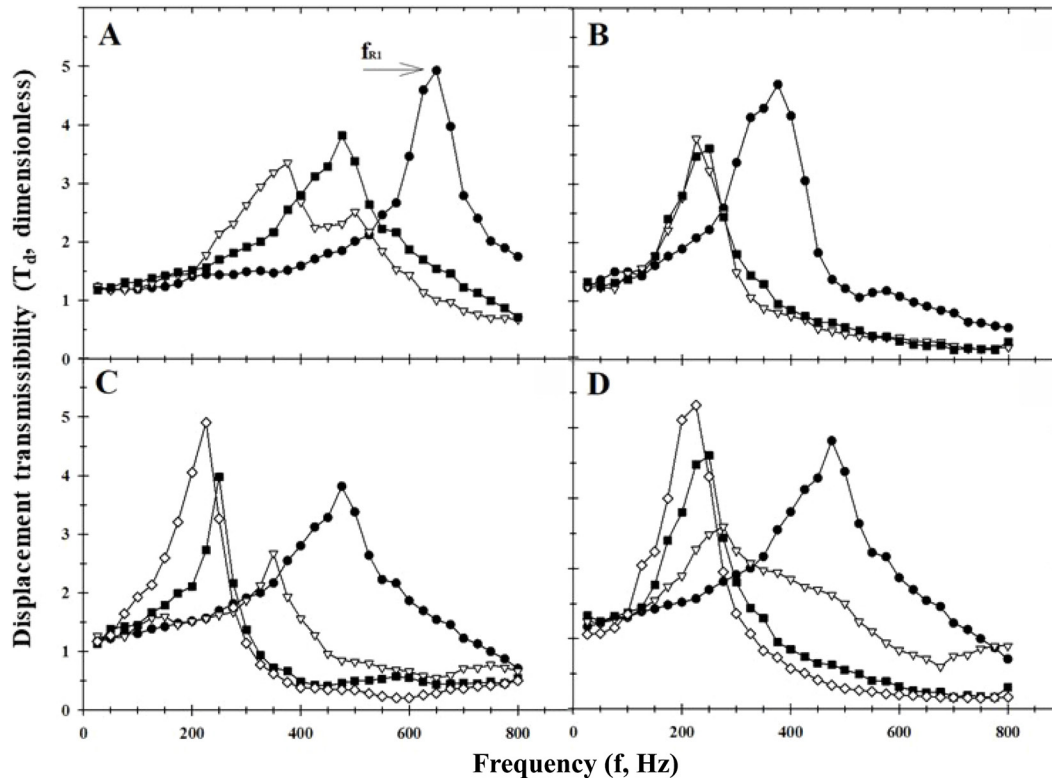


Figure 7. Effect of high-pressure homogenization on displacement transmissibility (T_d ; unitless) in relation to the frequency (f ; in the range of 2–800 Hz) after 30 d of storage at $6 \pm 2^\circ\text{C}$. (A) Control sample that was not homogenized (closed circle) or homogenized in a 1-stage process (10 MPa, open triangle) or a 2-stage process (10/20 MPa, closed square). (B) Model processed cheese sauce (PCS) with κ -carrageenan in concentration 0.500% wt/wt that was not homogenized (closed circle) or homogenized in a 1-stage process (10 MPa, open triangle) or a 2-stage process (10/20 MPa, closed square). (C) Model PCS with furcellaran homogenized in a 2-stage process in concentrations 0.125 (open triangle), 0.500 (closed square), and 1.000% (open diamond), wt/wt, compared with the control sample (closed circle). (D) Model PCS with κ -carrageenan homogenized in a 2-stage process in concentrations 0.125 (open triangle), 0.500 (closed square), and 1.000% (open diamond), wt/wt, compared with the control sample (closed circle; $n = 3 \times 3 = 9$). f_{R1} is the first resonance frequency.

degree of CS homogenization (Figure 7A), the peak position of the first resonance frequency was gradually changed from 657 Hz (NH) to 378 Hz (OSH) and 469 Hz (TSH). Similar results were also found in terms of the influence of HPH type on the dynamic mechanical behavior of cheese sauces containing 0.500% CR (wt/wt; Figure 7B) and for other samples examined, as shown in Table 3. This confirmed the validity of Equation 4, in which the peak position of f_{R1} was shifted to lower excitation frequencies with increasing damping ratio ζ .

The effect of polysaccharide concentration on the displacement transmissibility of the investigated TSH samples is demonstrated in Figure 7 (C and D). Higher concentrations of FR (Figure 7C) and CR (Figure 7D) in PCS were found to reflect a decrease in the peak position of the first resonance frequency and, therefore, an increase in the damping ratio ζ . Similar effects of the polysaccharide concentration in PCS on the first resonance frequency were observed for the tested NH and

OSH samples (Table 3). The lowest vibration damping properties were obtained for FR samples independently of HPH type. In contrast, the highest vibration damping properties and viscous friction were observed in PCS samples containing CR irrespective of the HPH process and the polysaccharide concentration.

The above-mentioned findings are in excellent accordance with the rheological analysis, in which the NH samples (independently of the polysaccharide concentration in PCS) were generally characterized by the lowest measured values of G'' and G^* . These findings were most likely due to the lower viscosity of the NH samples compared with that of the OSH and TSH samples. Therefore, the NH samples were characterized by a lower damping ratio ζ . This phenomenon is consistent with the validity of Equation 4, in which the frequency ratio r_0 generally increases with the decreasing damping ratio ζ , which is accompanied by an increase in the undamped natural frequency and the first resonance frequency (Rajoria and Jalili, 2005). Similar results

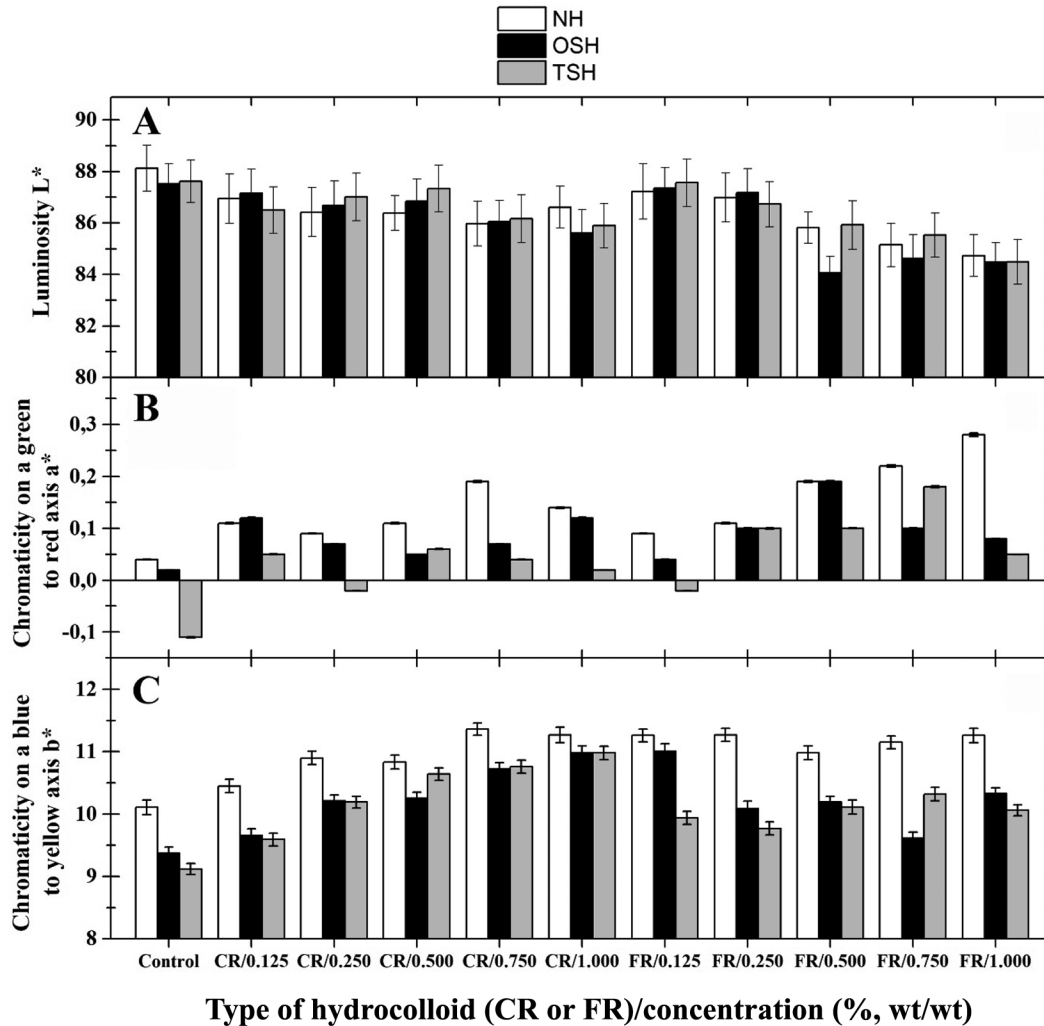


Figure 8. Effect of high-pressure homogenization on luminosity (L^* ; part A), chromaticity on a green to red axis (a^* ; part B), and chromaticity on a blue to yellow axis (b^* ; part C) of the model processed cheese sauce with κ -carrageenan (CR) and furcellaran (FR) at concentrations 0.125, 0.250, 0.500, 0.750, and 1.000% (wt/wt) compared with the control sample. Samples were not homogenized (white columns, NH) or were homogenized in a 1-stage process (10 MPa, black columns, OSH) or a 2-stage process (10/20 MPa, gray columns, TSH; $n = 3 \times 3 = 9$).

were found in terms of the effect of polysaccharide concentration in PCS independently of the HPH process, when a higher concentration of polysaccharide (CR or FR) generally led to higher values of G' and G^* and, subsequently, to lower values of f_{R1} . Based on the above statement, we conclude that the higher viscous friction of PCS, which is reflected in a higher conversion of mechanical energy into heat during forced oscillations (Martínez-Palou et al., 2011), generally led to a shift of the first resonance frequency peak position to lower frequencies.

Color Analysis, Stability, and Water Activity

The results of the color analysis of the PCS model samples are shown in Figure 8. All samples could be

characterized as pale yellow (Figure 8C) with a very slight red tint (aside from TSH CS, CR/0.250, and FR/0.125, for which negative a^* values indicated a green tint; Figure 8B). The addition of higher polysaccharide concentrations resulted in a darker PCS color ($P \geq 0.05$); however, lower L^* values were observed for the FR samples (Figure 8A; $P < 0.05$). A slight increase in yellowness was observed for higher concentrations of CR ($P < 0.05$). The OSH and TSH samples showed lower yellowness values compared with the NH samples. The effect of HPH on the other PCS color parameters was not proven. In general, HPH application and the addition of CR or FR could lead to the development of PCS samples with modified color properties following consumer preferences. If darker PCS is preferable, TSH could be applied with or without the

Table 3. First resonance frequency f_{R1} (Hz) of the model processed cheese sauce with κ -carrageenan or furcellaran addition in concentrations of 0.125, 0.250, 0.500, 0.750, and 1.000% (wt/wt) compared with the control sample

Polysaccharide type	Concentration, % (wt/wt)	Homogenization ¹		
		NH	OSH	TSH
Control	—	657 ± 28 ^{a,A}	378 ± 18 ^{b,A}	469 ± 19 ^{c,A}
Carrageenan	0.125	562 ± 23 ^{a,B,y}	249 ± 12 ^{b,B,y}	270 ± 11 ^{c,B,y}
	0.250	509 ± 22 ^{a,C,y}	238 ± 9 ^{b,BC,y}	246 ± 10 ^{b,C,y}
	0.500	379 ± 16 ^{a,D,y}	223 ± 10 ^{b,C,y}	241 ± 10 ^{b,CD,y}
	0.750	257 ± 11 ^{a,E,y}	194 ± 8 ^{b,D,y}	227 ± 11 ^{c,D,y}
	1.000	245 ± 11 ^{a,E,y}	185 ± 6 ^{b,D,y}	211 ± 8 ^{c,E,y}
Furcellaran	0.125	636 ± 29 ^{a,A,z}	250 ± 11 ^{b,B,y}	357 ± 17 ^{c,B,z}
	0.250	546 ± 21 ^{a,B,z}	251 ± 12 ^{b,B,z}	288 ± 13 ^{c,C,z}
	0.500	478 ± 21 ^{a,C,z}	243 ± 9 ^{b,BC,z}	249 ± 14 ^{b,D,y}
	0.750	386 ± 16 ^{a,D,z}	237 ± 10 ^{b,C,z}	245 ± 10 ^{b,DE,z}
	1.000	310 ± 14 ^{a,E,z}	193 ± 8 ^{b,D,y}	235 ± 11 ^{c,E,z}

^{a-c}Means within a line (the difference between homogenization type; comparing the same polysaccharide and its concentration; the control sample was also included) followed by different superscript lowercase letters differ ($P < 0.05$).

^{A-E}Means within a column (the difference between the concentration of polysaccharide; comparing the same the polysaccharide and homogenization type; the control sample was also included) followed by different superscript uppercase letters differ ($P < 0.05$).

^{y,z}Means within a column (the difference between the polysaccharide; comparing the same polysaccharide concentration and homogenization type) followed by different superscript lowercase letters differ ($P < 0.05$).

¹Samples were not homogenized (NH) or were homogenized in a 1-stage (OSH) or a 2-stage (TSH) process ($n = 3 \times 3 = 9$). Values are presented as mean ± SD.

addition of polysaccharide at a concentration greater than 0.125% wt/wt for CR and 0.250% wt/wt for FR. The color of food is a sensory attribute that can affect consumers' perception. Food producers need to have the expected color in their products because consumers associate certain colors with certain flavors. The color and appearance of food create expectations that affect consumers' preferences and food identification (Wadhvani and McMahan, 2012).

The stability of the PCS emulsion (Figure 9) exhibited a strong dependence on the concentration of polysaccharides ($P < 0.05$). The results obtained show that when CR was added at a concentration of 0.125% and 0.250% (wt/wt), higher stability values were obtained compared with samples with the same concentration of FR. This difference could be explained by FR having 1 sulfate ester group per tetramer at position 4 of the galactose unit, whereas CR has 1 sulfate ester group per dimer. Owing to the number of sulfate groups on polysaccharides, different conformational and constitutional alignments result, which influence application properties (Wurm et al., 2019). Samples with CR levels of 0.250 to 1.000% (wt/wt) and FR at levels of 0.500 to 1.000% (wt/wt) were stable (almost 100%), and no water release was observed ($P \geq 0.05$). The increasing stability of PCS could be explained by the high affinity of these polysaccharides for the water molecules to which they bind owing to a large number of hydroxyl groups (Saha and Bhattacharya, 2010). The stability

of the emulsion can also be promoted by the formation of protein-polysaccharide complexes (interaction between negatively charged polysaccharide and positively charged milk protein segments). However, these reactions were previously described primarily for micellar casein (Kováčová et al., 2010) and also for β -CN and α_S -CN (Lynch and Mulvihill, 1996). The effect of HPH became more significant when polysaccharide was absent (CS) or only present at relatively low levels (CR/0.125, FR/0.125, and FR/0.250). The stability of the samples increased in proportion to the increasing degree of HPH (NH < OSH < TSH; Figure 9). The application of HPH led to increased interactions, especially between fat and proteins, thus forming a stable oil-in-water emulsion.

As already mentioned, CR and FR bind relatively large amounts of water, so it can be assumed that, with their increasing concentration, free water will be removed and the PCS a_w will thereby be reduced. The a_w ranged from 0.9836 to 0.9882 (data not shown; $P \geq 0.05$) for all samples. Therefore, the effect of HPH was not demonstrated either. According to Buňková and Buňka (2017), the value of a_w in PC usually ranges from 0.91 to 0.96, which could inhibit the growth of some microorganism species. However, PCS has previously been mentioned to have a limited shelf life compared with conventional PC products (Saad et al., 2016). The results obtained in this study are comparable with those previously reported by Solowiej et al.

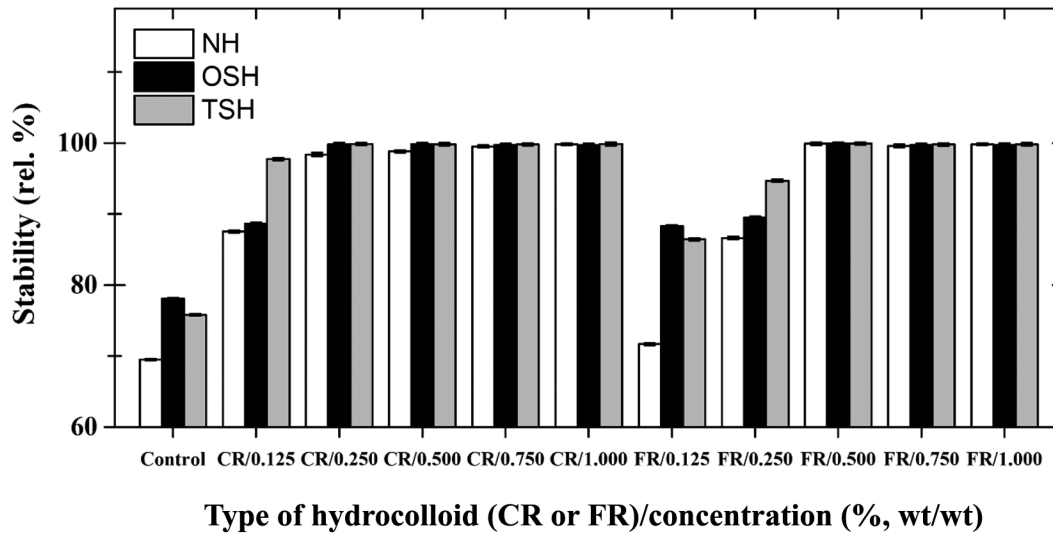


Figure 9. Effect of high-pressure homogenization on the emulsion stability (percentage, rel. %) of the model processed cheese sauce with κ -carrageenan or furcellaran in concentrations 0.125, 0.250, 0.500, 0.750, and 1.000% (wt/wt) compared with the control sample. The samples were not homogenized (white columns, NH) or were homogenized in a 1-stage process (10 MPa, black columns, OSH) or a 2-stage process (10/20 MPa, gray columns, TSH; $n = 3 \times 3 = 9$).

(2015, 2020). According to these authors, PCS should be stored at the refrigeration temperature to extend the shelf life of the product.

CONCLUSIONS

Increasing the concentration of polysaccharide and applying HPH increased the rigidity of PCS. Changes in mechanical stiffness were confirmed by a relatively fast and simple nondestructive method of mechanical vibration damping based on the peak position of the first resonant frequency. The use of HPH and polysaccharide also had a positive effect on the stability of the product emulsion. These findings indicate that FR is a promising alternative to CR for PCS manufacture with desirable functional and sensory properties. However, the dosage of FR should be higher than the dosage of CR to manufacture PCS with a similar consistency. A concentration of CR/FR $\leq 0.250\%$ (wt/wt) could be recommended together with OSH/TSH, when PCS products with softer consistency are desired. In contrast, for products in which a firmer PCS consistency is required, applying CR at concentrations of $\geq 0.500\%$ (wt/wt) or FR at concentrations of $\geq 1.000\%$ (wt/wt) together with OSH is possible.

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