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# Low-concentration salting of cod loins: The effect on biochemical properties and predicted water retention during heating

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## Abstract

Low levels of salt are frequently used to increase flavor and water retention in cod. This alters the biochemical properties of cod during heating. In this paper, properties needed to mathematically model moisture transfer during cooking – water holding capacity and storage modulus – were determined for cod containing 0.06, 1 and 3 g/100 g NaCl. Protein denaturation and microstructure was also investigated to increase the understanding of quality effects of salt during cooking. A model was established to investigate the effect of the measured storage modulus and water holding capacity on the predicted moisture retention during heating. Salting lead to a higher water holding capacity, less hardening of the muscle tissue during heating, diffused protein denaturation peaks and caused swelling of the muscle fibers. By interchanging the acquired variables in the model of coupled heat and moisture transfer, we found a higher predicted water retention during cooking of brined cod compared to unsalted cod. This knowledge may be utilized in creating modeling tools for optimization of cooking processes, which may support chefs and ready-to-eat meal producers in reducing weight loss and improving the texture and juiciness of their products.

## 1. Introduction

Mathematical modeling is a powerful tool which may be used in the food industry, catering and restaurants for creating cooking protocols tailored to the specific fish product at hand. Using physics-based mathematical modeling, it is possible to try out endless combinations of convection oven input parameters – such as temperature, relative humidity and heating time – and observe effects on quality parameters which may be included in the model as output. Such quality parameters may include texture – especially hardness or toughness; water holding capacity – which is related to juiciness; and color development (Kong, Tang, Rasco & Crapo, 2007; Ovissipour, Rasco, Tang & Sablani, 2017; Rabeler & Feyissa, 2018a). Moreover, such models can be combined with bacterial inactivation kinetics and kinetics of vitamin breakdown, and this offers opportunities for creating products which are both safe, healthy and tasty.

For modeling the moisture transfer during heating, it is commonly assumed that the muscle structure functions like a porous media, for which Darcy's law can be applied (Datta, 2007). Using this approach, properties describing both the muscle structure, the liquid being transported, and their interactions are needed. The driving force in Darcy's law has been hypothesized to be the swelling pressure that originates when proteins denature and muscle foods swell or shrink, which can be determined from the water holding capacity and storage modulus of a product (Barrière & Leibler, 2003; van der Sman, 2007). These properties have been determined for meat, chicken and cod, and the porous media approach has been used for modeling moisture transfer during heating of these foods (eg. Feyissa, Gernaey & Adler-Nissen, 2013; Rabeler & Feyissa, 2018b; Blikra, Skipnes & Feyissa, 2019). However, the properties of the muscle, especially the water holding capacity – which describes the ability of a food to hold on to water when a pressure is applied – may change drastically when salt is added. Salt is commonly applied to cod before heating, and thus it is important to know the extent of which water holding capacity and storage modulus are affected by salt concentration during heating.

Addition of low concentrations of salt (< 5.8 g/100 g NaCl) increase the ability of muscle foods to hold on to water, which decrease the amount of liquid exudated during storage of raw fish (Larsen & Elvevoll, 2008; Larsen, Olsen, Kristoffersen & Elvevoll, 2008) and during heating (Kong, Oliveira, Tang, Rasco & Crapo, 2008; Ofstad, Kidman, Myklebust, Olsen & Hermansson, 1996). The increased retention of moisture is caused by a displacement in the isoelectric point, changing the pH at which the muscle proteins bind water more effectively. In addition to being dependent on the salt concentration (Johnsen, Jørgensen, Birkeland, Skipnes & Skåra, 2009), the water holding capacity of fish show changes corresponding to the denaturation of major protein groups (Skipnes, Johnsen, Skåra, Sivertsvik & Lekang, 2011).

The other property needed to determine the swelling pressure during cooking, namely the storage modulus, is a rheological property which is correlated with the textural property of hardness. The storage modulus of extracted fish proteins and surimi pastes have been well documented. However, since the effect of salt on rheological properties depend on processing conditions and addition of other additives (eg. Cheow, Yu, & Howell, 1999; Kobayashi & Park, 2017), these results cannot be adopted directly to processing of lightly salted fish muscle. On the other hand, the hardness of brined fish muscle has been assessed using more traditional methods. Sensory analysis was used to assess the hardness of cod brined for 10 min in 5 g/100 g NaCl followed by heating at 95 °C for 10 min in a water bath (Esaiassen *et al*., 2004). The result indicated an increased softness of brined cod compared to unsalted samples. This correlates with measurements of the force required to pull bones from cod muscle, which decreased with increasing salt concentration in the brine from 1.5-6 g/100 g NaCl (Larsen *et al.*, 2008). As measured using a compression test, salmon brined in 1.5 g/100 g NaCl also showed a lower shear force after heating at 121.1 °C for 10-60 min compared to unsalted salmon (Kong *et al.*, 2008).

It is appropriate to mention that even though salting of fish, especially cod, is an ancient tradition, it is important to consider all foods in a more general perspective of food health. There is strong evidence that high levels of salt in the diet may cause unfavorable medical conditions, especially

raised blood pressure and in turn cardiovascular disease (He & MacGregor, 2009), and claims to reduce the amount of salt in processed foods have thus been raised (Asaria, Chisholm, Mathers, Ezzati & Beaglehole, 2007). In addition to demanding less salt, consumers also demand "natural foods" without artificial preservatives including phosphates which are commonly used to increase water retention in muscle foods (Bearth, Cousin & Siegrist, 2014; Evans, de Challemaison & Cox, 2010; Zink, 1997). It is important to offer consumers a choice to eat preservative-free food, however for the consumers who prefer fish products which are higher in flavor and juiciness, an option where a sufficiently low level of salt is used so that it does not cause damage to public health may be provided. It is our belief that complex modeling could be used to find this level. For restaurants, catering, and the ready-meal industry, adjusting the sodium content in the rest of the meal, for instance by serving cod with vegetables and low-sodium sauce, is also a suitable option.

The objective of this paper was 1) to investigate the changes in important moisture transfer properties for cod brined at low concentrations of salt, 2) to increase the understanding of the quality effects of salt on cod during cooking, and finally 3) to enable optimization of convection oven cooking of lightly salted cod through mathematical modeling.

## 2. Materials and methods

### 2.1. Raw material

Farmed Atlantic cod (*Gadus morhua*) weighing 2-4 kg were starved for 9 d, killed by a blow to the head, bled in seawater for 25 min, filleted (pre-rigor), and transported on ice to our lab (December 2017). The fillets were stored at 0-2 °C (5 d) to undergo *rigor mortis*, after which they were cut into pieces of 100-150 g. Untreated samples were quick-frozen in a blast freezer (<15 min) at -60 °C, vacuum packed at 7.66 kPa to avoid thawing, and stored at -80 °C until analysis to maintain freshness and avoid major changes in water state (Burgaard & Jørgensen, 2010). The remaining samples were brined in individual bags with 2 L brine (0, 1.5 and 4.5 g/100 g NaCl) per fish piece, for 48 h at 0-2 °C, lightly patted dry with a paper towel, followed by processing and storage as described for untreated samples. A flow chart summarizing the processing steps is given in Figure 1.

### 2.2. Characterization of raw material

Ten specimens were removed from the slaughter-line one at a time before bleeding. Their initial muscle pH and blood lactate was measured. Fish length ($L$; m), as well as mass of whole fish and liver ($W$, $LW$; kg) were used to calculate the hepatosomatic index ($HSI = \frac{LW}{W} * 100$) and condition factor ($K = \frac{W}{L^3} * 100$).

The weight gain during brining was analyzed by weighing 10 loins prior to and after brining, after lightly patting dry with a paper towel. Analysis of total aerobic count was performed according to NMKL 184 (2006) on raw material after processing and freezing (n=3). Final pH after processing and freezing was measured in a 1:1 solution of sample and 0.1 mol/L KCl (10-40 g each), using a Mettler Toledo Five Easy Plus pH meter (FEP20, Zürich, Switzerland) with an LE438 electrode. The measurement was performed in duplicate on four samples from different specimen.

### 2.3. Sample preparation

The temperature of frozen loins was gradually raised by following the steps shown in Figure 1, to allow cutting the cod pieces into samples of appropriate size. For analysis of water holding capacity

(WHC), salt and pH, frozen pieces of cod were cut horizontally into 3 mm thick slices using a meat slicer. For rheology, disks of 30 mm were cut from the muscle slices using a heavy duty round hollow punch – a hand-held device with a sharp-edged pipe attached to a handle. Any brown muscle and uneven areas were avoided during cutting. The white fish muscle surrounding the disks was finely chopped for DSC analysis. To avoid thawing of the samples, the preparation was performed in a chill room with circulating air at 0-2 °C, followed by vacuum-packing in appropriately sized polyethylene bags at a pressure which had previously shown not to thaw the samples (7.66 kPa). The samples were then put back for storage at -80 °C until analysis (<6 mo).

### 2.4. Salt concentration

The salt concentration after processing and freezing was measured as total chloride content by titration with silver chloride (Mettler Toledo T7), according to ISO 5943 IDF 88 with some deviations. 1.5 g homogenized sample was mixed with 50 mL distilled water at 55 °C for 1 h without subsequent blending. Two samples from the surface and core positions of four loins were analyzed for each parameter (n=2x4), except untreated cod which was analyzed without regard to position (n=8). Going forward, the salted samples (brined at 1.5 and 4.5 g/100g NaCl) are referred to using the average measured concentration of salt, 1 and 3 g/100 g NaCl, respectively (Table 1). The samples brined in pure water are referred to as “water-brined” samples, and the group “unsalted samples” include both water-brined and untreated samples.

### 2.5. Differential scanning calorimetry

Heat denaturation was analyzed using a Mettler Toledo DSC1 and a modified version of the methodology described by Skipnes, Van der Plancken, Van Loey & Hendrickx (2008). Samples of 51.4$\pm$6.5 mg were weighed into stainless steel crucibles (Mettler Toledo medium pressure Ø 7 mm with pin), sealed with a Viton O-ring and analyzed at a rate of 2.5 K/min from 2-100 °C. Deionized water corresponding to the amount of water in the sample (40 mg) was used in the reference pan to remove the noise of water from the resulting thermograms. Raw samples (n=6-8) and samples

isothermally heated in a GR150 water bath (10 min; n=2) at either 25, 30, 35, 40, 45, 55, 60, or 65 °C were used, except water-brined samples which were not heated at 30 °C. The heated samples were cooled in ice water (> 30 s) prior to DSC analysis. The thermograms were analyzed using the software StarE version 14.00 (Mettler Toledo). A spline baseline was used for analysis of all peaks. Data was reported as peak denaturation temperature (PDT) and normalized (residual) denaturation enthalpy ($h_{den}$) of each detected peak.

## 2.6. Water holding capacity

Water holding capacity (WHC) was analyzed as described by Skipnes, Østby & Hendrickx (2007), with some alterations. Briefly, 5.12±0.02 g of fish pieces were gently ripped to appropriate size and weighed into cooled, pre-weighed steel sample cups of height 37 and Ø36 mm (Skipnes *et al.*, 2007). The cups had an adjustable, central filter, allowing the fish sample to be rotated up until it touched the lid of the cup, and the expelled liquid was allowed to exit through the filter to the removable bottom. For analysis of cooked samples, the filled sample cup was isothermally heated in a water bath (GR150, Grant Instruments, Cambridge, UK). Each cup was heated at either 25, 30, 35, 40, 45, 55, 60, 65, 70 or 100 °C, before cooling in ice water for 5 min. The cups were kept cold until removal of the cook loss using paper towels, followed by centrifugation (Rotina 420R, Hettich, Tuttlingen, Germany) at 4 °C for 15 min at 528 g (Skipnes *et al.*, 2007; Skipnes *et al.*, 2011). WHC was determined as the remaining mass after centrifugation as a fraction of the original, raw mass and as g hold water /g dry weight (n=8). The gravimetrically determined (18 h, 105 °C) water content of representative samples (n=8) of each salt concentration was used in the calculation (Table 1).

## 2.7. Rheology

Rheological measurements were performed using a DHR-2 (TA Instruments, New Castle, DE, USA) with a 20 mm cross-hatched parallel plate and temperature control connected to a heat exchanger (P/N 953260.901 TGA), as described by Blikra *et al.* (2019). Samples were collected one-at-a-time from storage at -80 °C (n=10), thawed in ice water and put on the 0 °C Peltier plate. Temperature

ramps were performed at 1.0 Hz, from 0-100 °C, with a heating rate of 2.5 K/min, and with an initial axial force of 0.25±0.1 N. The strain % to be used for the tests were determined from amplitude sweeps performed at a minimum of three temperatures, to ensure testing in a linear viscoelastic region. The strain % determined were 0.05, 0.025, and 0.25 % for water-brined samples and samples containing 1 and 3 g/100 g NaCl, respectively. A solvent trap was placed around the sample and geometry to prevent heat loss and drying, and aluminum foil was placed around the solvent trap for additional prevention of heat loss.

### 2.8. Microstructure

Cross-sectional microstructure was analyzed using myotomes separated from muscle samples. In order to dissolve the collagen holding the sheets together and enable separation of the sheets, cubes of approximately 30 mm were vacuum packed (7.66 kPa) in polyethylene bags and heated for 20 min at 30 °C in a GR150 water bath. The samples were subsequently frozen in liquid nitrogen and stored at -80 °C until analysis (unsalted <13 mo; brined < 3 mo; Figure 1). Upon analysis, frozen samples were embedded in tissue freezing medium (Leica, 14020108926, Wetzlar, Germany), and cut into 10 µm slices at -20 °C using a Leica CM1860 UV cryostat. The slices were transferred to microscopy slides and stained with Orange G and Methyl blue (Sigma-Aldrich, St. Louis, MO, USA), followed by inspection using a Leica MZ8 stereo microscope (San Jose, CA, USA). Images were prepared with an integrated camera above the lens (VisiCam 10.0, VWR®, Leuven, Belgium). ImageJ (Version 1.52b, Fiji) was used for calibration of pixel size. For each sample group, the analysis was conducted in triplicate.

### 2.9. Model prediction

A mathematical model was established to investigate the effect of the measured difference in WHC and storage modulus on the predicted mass loss. A similar model was previously published (Blikra *et al*., 2019), but for convenience, a summary of the model is given below.

### 2.9.1. Governing equations

#### *2.9.1.1. Heat transfer*

The heat transfer is described by Eq. 1:

$$\rho c_p \left(\frac{\partial T}{\partial t}\right) + \nabla \cdot (-k\nabla T) + \rho_w c_{p,w} \boldsymbol{u_w} \cdot \nabla T = 0 \quad (1)$$

where $\rho$, $c_p$, and $k$ – the density (1060 kg/m$^3$), specific heat (3650 J/(kg K)) and thermal conductivity (0.515 W/(m K)) – are the material properties of the fish which are obtained from Skipnes *et al.* (2007). The thermophysical properties (density, $\rho_w$ (986 kg/m$^3$) and specific heat, $c_{p,w}$ (4190 J/(kg K)) of the liquid transported were approximated from the properties of water at 55 °C (Singh & Heldman, 2014). $\nabla$ is the three-dimensional del operator, i.e. partial derivative in x, y, and z direction ($\nabla = \partial/\partial x + \partial/\partial y + \partial/\partial z$). $u_w$ is the flow velocity of the liquid (m/s) given by Eq. 3, and $T$ is the temperature (K).

#### *2.9.1.2. Mass transfer*

Moisture transfer within the fish sample is based on the conservation of mass (Bird, Stewart, & Lightfoot, 2002), and given by Eq. 2:

$$\frac{\partial c}{\partial t} + \boldsymbol{\nabla} \cdot (-D_w \nabla c + \boldsymbol{u_w} c) = 0 \quad (2)$$

where $c$ is the moisture concentration (mol/m$^3$) and $D_w$ is the moisture diffusion coefficient ($4 \times 10^{-10}$ m$^2$/s; Valle & Nickerson, 1968) in the sample. Using a porous media approach (see Section 1), the velocity of the water inside the fish sample, $u_w$, was described by Darcy's law (Eq. 3):

$$\boldsymbol{u_w} = -\frac{\boldsymbol{\kappa}\, G'}{\mu_w} \nabla \left(C - C_{eq}\right) \quad (3)$$

where $\boldsymbol{\kappa}$ is the permeability of cod ($10^{-17}$ m$^2$; Datta, 2006), G′ is the storage modulus (Eq. 9), and $\mu_w$ is the dynamic viscosity of water (Pa s; Singh and Heldman, 2014), given by Eq. 4. $C$ is the mass fraction of water (kg/kg sample), and $C_{eq}$ is the water holding capacity (Eq. 8).

$$\mu_w = 2.414 \times 10^{-5} \times 10^{\frac{247.8}{T+133.15}} \quad (4)$$

### 2.9.2. Boundary conditions

#### *2.9.2.1. Heat transfer*

Convective boundary conditions were applied to all external surfaces of the fish sample (eg. Feyissa *et al.*, 2013):

$$\boldsymbol{n} \cdot (-k\nabla T) = (1 - f_h)\big(h(T_{oven} - T_s)\big) \quad (5)$$

where *h* is the convective heat transfer coefficient (55 W/(m$^2$ K)) and $T_{oven}$ is the temperature of the oven (146.8 °C). $f_h$ is a step function turning the heat transfer off when the surface temperature approaches 100 °C (see Feyissa *et al.*, 2013).

#### *2.9.2.2. Mass transfer*

The mass transfer boundary condition at the fish sample was applied to all external surfaces, except the bottom surface where a no flux condition was assumed. The evaporative flux was modeled using Eq. 6 (Feyissa *et al.*, 2013; Blikra *et al.*, 2019):

$$\boldsymbol{n} \cdot (-D_w\nabla c) = -f_{evap}\frac{h(T_{oven}-T_s)}{H_{evap}}\frac{C_s-C_{air}}{M_w} \quad (6)$$

where $f_{evap}$ is the measured fraction of the internal energy used for evaporation, given by Eq. 7 (Blikra *et al.*, 2019), $H_{evap}$ is the latent heat of evaporation ($2.3 \times 10^6$ J/kg), and $M_w$ is the molecular weight of water (18.02 g/mol). $C_s$ is the mass fraction of water at the surface of the sample, and $C_{air}$ is the relative humidity ($0.1[-]$).

$$f_{evap}(T) = 1 + \frac{-f_{max}}{1+exp\left(\frac{T-226.2}{15}\right)} \quad (7)$$

### 2.9.3. Model solution

The mathematical model was solved in COMSOL Multiphysics® version 5.4 using the Finite Element Method (FEM). The fish piece was modeled as a rectangle of 20x30x15mm. The geometry was meshed using a free tetrahedral distribution to increase the resolution along the edges of the fish, while the predefined “finer” setting was used for the remaining geometry (Blikra *et al.*, 2019).

In the computation, we took advantage of the geometrical symmetry to reduce the computational burden to $1/4^{th}$ of the required calculations. Thus, along the internal boundaries of the sample, symmetry boundary conditions were assigned to obtain a solution for each element in the full geometry (see Blikra *et al.*, 2019).

### 2.10. Statistical analysis

Statistical analysis was performed using Minitab® 18.1. One-way ANOVA with 95 % confidence interval and Tukey post-hoc test was performed for analysis of significant difference. For rheological analysis, measurements every 0.4 °C were collected. To simplify the statistical analysis, measurements every 5 °C were selected for analysis of variance.

## 3. Results and discussion

### 3.1. Characterization of raw material

The stress level experienced by the fish during the slaughter process was assessed using initial muscle pH and blood lactate, measured to $7.3 \pm 0.1$ and $2.5 \pm 0.7$ mmol/L, respectively, indicating low stress levels. Hepatosomatic index and condition (K) factor were used to assess the nutritional status of the fish. As found by Lambert & Dutil (1997), K-factor and HSI are indicators of muscle and liver energy in cod, respectively. The K-factor was found to be $1.35 \pm 0.1$ kg/m$^3$, which is comparable to other values reported for farmed cod (Hultmann, Tobiassen, Aas-Hansen, Phu & Rustad, 2016; Kristoffersen *et al.*, 2006a; Kristoffersen, Vang, Larsen & Olsen, 2007). The HSI was found to be $14.7 \pm 1.3$ [−], which indicates good nutritional status, and is slightly higher than other values reported for farmed cod (Kristoffersen *et al*., 2006a; Kristoffersen, Tobiassen, Steinsund & Olsen, 2006b; Kristoffersen *et al*., 2007).

During the brining process all samples absorbed weight, including samples brined in pure water. The amount of uptake increased with increasing salt concentration in the brine from 0 to 4.5 g/100 g (Table 1). No significant difference was found between the microbial load of samples undergoing the

256 different treatments. The total aerobic count averaged 2.3±0.3 cfu/g, indicating good microbial
257 quality.

258 ## 3.2. Differential scanning calorimetry

259 Differential scanning calorimetry (DSC) of untreated samples generally revealed three peaks (Figure
260 2). The peak maximum temperature found for the peaks of untreated cod in this work were in the
261 same range of what was found for farmed, unsalted cod (Skipnes *et al.*, 2008) and wild fresh cod
262 (Hastings, Rodger, Park, Matthews & Anderson, 1985). However, due to differences of the
263 instrument and setup, three peaks were identified instead of five and eight which were found in the
264 other studies. Based on the aforementioned work, peak 1 was attributed to myosin and residual
265 collagen, and peak 2 to sarcoplasmic proteins. Within peak 3 it was often possible to identify two
266 overlapping peaks: the smaller and of lower temperature was attributed to sarcoplasmic proteins,
267 and the larger to actin. In this study, these were analyzed as one peak, with peak denaturation
268 temperature corresponding to actin. It is a logical assumption that the majority of denaturation
269 enthalpy ($h_{den}$) of this peak comes from denaturation of actin, since the proportion of actin in cod
270 muscle greatly surpasses that of sarcoplasmic proteins. Furthermore, the peak denaturation
271 temperature (PDT) and $h_{den}$ for actin is known from literature (eg. Skipnes *et al*., 2008) to be in the
272 same range as peak 3 in the present study and does not correspond with observations of
273 sarcoplasmic proteins.

274 For samples that had not been heat-treated before the DSC analysis, the average PDT of myosin
275 (peak 1) was found at 35.8-46.2 °C, with higher values for unsalted specimen (Figure 3). Water-
276 brining shifted the myosin PDT significantly by 3.0 °C on average, from 43.2 for untreated samples to
277 46.2 °C. Salted samples showed a significantly lower myosin PDT and $h_{den}$ compared to unsalted
278 samples. The peak attributed to sarcoplasmic proteins was detected at average temperatures from
279 56.6-58.2 °C, with no significant differences between the sample groups. The exception was samples
280 containing 3 g/100 g NaCl, for which no peak was observed. The average PDT of actin (peak 3)
281 decreased with increasing salt concentration, from 76.1 °C for untreated samples to 66.2 °C for

282 samples containing 3 g/100 g NaCl. The denaturation enthalpy followed the same trend of decreasing
283 absolute value with increasing salt concentration, as was also reported for cod containing higher
284 concentrations of salt (7-20 g/100 g; Thorarinsdottir, Arason, Geirsdottir, Bogason & Kristbergsson,
285 2002).

286 When the samples were heated from 25-65 °C prior to DSC analysis, the residual denaturation
287 enthalpy ($h_{den}$) and PDT shifted (Figure 3). For all sample groups, the $h_{den}$ of myosin did not change
288 compared to raw samples after heating at 25 °C. For the salted samples, $h_{den}$ of myosin was
289 significantly smaller than for the unsalted samples, and the peak attributed to myosin disappeared
290 after heating at 30-35 °C. Sarcoplasmic proteins (peak 2) were completely denatured after heating at
291 55 °C or above for unsalted samples, and 50 °C or above for samples containing 1 g/100 g NaCl. After
292 heating the samples at 45 °C, the $h_{den}$ of untreated samples and samples containing 1 g/100 g NaCl
293 increased significantly in magnitude, indicating that peak 2 was partly covered by peak 1 during the
294 initial temperature range, and then grew as myosin was denatured. The same might be the case for
295 the sudden appearance of peak 2 after heating at 30 °C for samples containing 3 g/100 g NaCl. This
296 peak was not visible after heating at lower and higher temperatures. Actin seemed to be less heat-
297 stable for salted samples than for unsalted samples, as the $h_{den}$ of peak 3 decreased in magnitude
298 after heating at 45 °C or higher for samples containing 1 g/100 g NaCl, but generally remained at a
299 larger magnitude for unsalted samples until after heating at 70 °C. After heating at 40 °C or higher, all
300 peaks had disappeared completely from the thermographs of samples containing 3 g/100 g NaCl. This
301 indicates that for this salt concentration, any changes in 3D-conformational structure of the muscle
302 proteins after 40 °C were either too gradual or did not involve sufficient energy dissipation to be
303 visualized in the DSC thermographs.

304 ### 3.3. Water holding capacity

305 In Figure 4a, data for water holding capacity (WHC) of brined cod are shown with data for untreated
306 cod (Blikra *et al.*, 2019) as a function of heating temperature. As can be seen from the figure, salting
307 increased the WHC of the fish after heating at all tested temperatures. Samples containing 3 g/100 g

NaCl generally showed a higher WHC than the samples containing 1 g/100 g NaCl, although not always significant. This is in agreement with a previous study, where WHC of cod increased with increasing salt concentration in the brine added to ground cod, ranging from 0.3-1 g/100 g NaCl (Johnsen *et al.*, 2009).

In Figure 4a, the water-brined samples showed a lower WHC compared to the untreated samples. When considering the result in g hold water /g dry matter (Figure 4b), the results for untreated and water-brined samples were almost identical, showing that the absorbed water during brining could explain the difference in water holding capacity expressed as mass fraction of final to initial weight. The result for water-brined cod (Figure 4a) was similar to data for post-rigor fileted cod (Skipnes *et al.*, 2011), which is known to contain more water than pre-rigor fileted cod (Kristoffersen *et al.*, 2006a).

The experimental data was fitted to a function for the change in WHC with temperature (van der Sman (2007); Eq. 8):

$$C_{eq}(T) = C_{eq,0} - \frac{a_1}{1+a_2\, exp(-a_3(T-T_\sigma))} \quad (8)$$

where $C_{eq,0}$ is the initial WHC of raw sample, $T$ is the temperature in °C, $T_\sigma$ is the center of a logistic curve, and $a_1$, $a_2$, and $a_3$ are fitting parameters determined by trial-and-error (Table 1). The functions are shown in Figure 4a with the measured data. There was a general trend in all sample groups of higher WHC for samples not previously heated and samples heated at 30 °C, followed by a significant drop with a local minimum after isothermal heating treatments from 45-50 °C. For unsalted samples and samples containing 1 g/100 g NaCl, this drop corresponded to denaturation of peak 1, attributed to myosin.

It should be noted that while water-brined cod fit the sigmoidal curve type well, salted cod changed in a different manner. In contrast to water-brined samples and samples containing 3 g/100 g NaCl, the WHC of cod containing 1 g/100 g NaCl increased slightly after heating at 50-55 °C. The same increase was also observed after heating at 40-50 °C for untreated cod (Blikra et al., 2019). Since the

result was not significantly different from the results for samples treated at temperatures immediately lower or higher than 50-55 °C (namely 40-65 °C), this rise was not included in the parameters of Eq. 8. Cod containing 3 g/100 g NaCl showed a more gradual decrease in WHC with increasing temperature than all other sample groups, and as a consequence, the sigmoidal type equation could not capture all the measurement points. Rather, during the equation fitting we aimed to obtain a good prediction for a maximum of temperatures. The equation should be validated experimentally using the measured mass loss during heating. If substantial deviations are found between measured and predicted results, fitting the data to a more complex equation type can be considered.

### 3.4. Rheology

The storage modulus of cod samples with all salt concentrations tested followed the same overall trend of lower initial values (0-50 °C), then a rapid rise (50-70 °C), and finally a plateau (80-100 °C; Figure 5). The slope found between 50-75 °C was very similar for all sample groups. Significant differences between the storage modulus of some of the sample groups were found in the temperature range 0-40 °C, but none from 45-80 °C. However, there were some clear trends in the average values. The initial storage modulus for unsalted cod was higher than for the salted samples, which corresponds well with the decreased hardness of salted fish muscle compared to unsalted in the literature (Esaiassen et al., 2004; Kong et al., 2008; Larsen et al., 2008). Cod containing 3 g/100 g NaCl showed a different behavior than the other concentrations in that there was a local maximum in storage modulus observed between 40-44 °C for all parallels (n=10). This peak resulted in a significant difference in storage modulus between the samples containing 1 and 3 g/100 g NaCl at 40 °C. During DSC analysis of raw samples containing 3 g/100 g NaCl, a very low residual denaturation enthalpy of peak 3 (actin) was visible at a PDT of around 67 °C (Figure 3). However, after isothermal heat treatment at 40 °C for 10 min, this peak had disappeared, which indicates that actin easily denatures even at 40 °C in cod samples of this salt concentration. Thus, the local maximum in G' at 40-44 °C observed for samples of 3 g/100 g NaCl could be attributed to hardening as a consequence

of denaturation of actin. For the same salt concentration, a significant reduction in WHC occurred between isothermal heating temperatures of 35-40 °C (P=0.000; Figure 4). Since myosin was denatured after isothermally heating at 35 °C, the reduction in WHC of samples of this salt concentration could perhaps also be attributed to denaturation of actin. After reaching 50 °C, the G' of samples containing 3 g/100 g NaCl increased steadily until reaching around 75 °C, like the other samples, despite actin most probably already being denatured. The continued increase in G' could result from protein aggregation, which has been reported for isolated myosin from cod (Yongsawatdigul & Park, 1999), as well as for isolated myosin and myofibrils from white muscle of salmon (Lefevre, Fauconneau, Thompson, & Gill, 2007).

The change in storage modulus (G') as a function of temperature was fitted to sigmoidal curves (Figure 5; Eq. 9), as previously published for meat (Feyissa *et al.*, 2013), chicken breast (Rabeler & Feyissa, 2018a) and unsalted cod (Blikra *et al.*, 2019). In Equation 9, $G'_{max}$ and $G'_{min}$ are the average maximum and minimum storage moduli, respectively, *T* is the temperature (°C), and $g_1$ and $g_2$ are fitting parameters determined by trial-and-error, supplied in Table 1.

$$G'(T) = G'_{max} + \frac{G'_{min} - G'_{max}}{1 + \exp\left(\frac{T - g_1}{g_2}\right)} \quad (9)$$

## 3.5. Microstructure

Micrographs showing an average cross-sectional muscle structure for each sample parameter are shown in Figure 6. Muscle bundles can be seen as orange structures surrounded by white channels. The white channels can be regarded as areas where water can be transported during cooking without disrupting the structure. There were no visible spaces present between the muscle bundles of samples containing 3 g/100 g NaCl (Figure 6d), probably as a result of muscle structure swelling during brining, which has also been reported by other authors (Ofstad *et al.*, 1996). The water-brined samples (Figure 6b), on the other hand, showed a greater extent of widened channels than the other parameters (Figure 6a, c-d). It can be speculated that the water absorbed by these samples during brining was kept "free" in channels between the muscle structures. Since the water added did not

384 contain any solutes, less suspended particles per g extracellular water would be present in the water-
385 brined fish muscle than in the untreated, and thus a lower number of nucleation sites during freezing
386 (Burgaard, 2010). This could result in formation of fever and larger ice crystals compared to the other
387 sample groups and cause partial freeze denaturation of water-brined samples, which may have
388 caused the partially disrupted structure observed in Figure 6b. The elevated PDT of myosin observed
389 for water-brined samples compared to untreated samples (Section 3.1) could perhaps also be related
390 to this phenomenon. Moreover, the addition of brine (Figure 6c, d) could have caused better and
391 quicker freezing conditions due to the addition of solutes and thus nucleation sites in addition to
392 swelling. Therefore, it may be possible that the differences in microstructure not only reflect the
393 differences between salt concentrations directly, but also reflect the effect that these salt
394 concentrations have on protection of the muscle structure during this particular freezing regime
395 (Figure 1; Wolfe & Bryant, 2001).

396 ## 3.6. Model prediction

397 The model prediction showed that salting reduced the predicted mass loss in a concentration
398 dependent manner (Figure 7). This was in accordance with the expected result, since it is known that
399 brining in low salt concentrations decrease the amount of liquid loss during cooking (eg. Kong *et al.*,
400 2008; Ofstad *et al.*, 1996).

401 For water-brined samples, less water loss was predicted than for untreated fish (Figure 10). Based on
402 first-hand experience, however, we know that the water-brined samples lose more water upon
403 cooking than untreated samples. Storage modulus and WHC were the major variables measured in
404 this study; however, the permeability of the muscle also affects the modeled result (Blikra, *et al.*,
405 2019). In the simulations performed in this study, the permeability was assumed to be constant, and
406 therefore a fixed value was used for all sample parameters. However, since larger gaps were seen
407 between the muscle cells of water-brined samples than untreated samples (Figure 9) it is expected
408 that the permeability, in which pore size is a contributing factor (Datta, 2006), will be higher for the
409 former group, which would increase the predicted mass loss.

## 4. Conclusion

In this study, quality parameters of brined cod were investigated and used in a physics-based model to estimate water retention during heating. Equations describing the temperature dependency of the storage modulus and water holding capacity (WHC) for water-brined samples (0.06 g/100 g NaCl) and salted samples containing 1 and 3 g/100 g NaCl were developed. Samples containing 3 g/100 g NaCl showed significantly higher WHC than unsalted cod. Salting also showed a profound effect on the denaturation enthalpy and peak denaturation temperature of the three visible protein denaturation peaks. Salting lowered the denaturation temperature and reduced the magnitude of the observed residual enthalpy of the peaks attributed to myosin and actin. Considering the peak attributed to sarcoplasmic proteins, no difference was observed between untreated samples and samples containing 1 g/100 g NaCl. This peak was, however, camouflaged or denatured after heating at all tested temperatures except 30 °C for samples containing 3 g/100 g NaCl.

Mathematical modeling was used to investigate how the functions for storage modulus and WHC affected the predicted change in water content of cod during heating. The model prediction showed that cod containing 1-3 g/100 g NaCl had a higher water retention compared to unsalted samples, which was in agreement with experimental data obtained in other studies (Kong *et al.*, 2008; Ofstad *et al.*, 1996).

The model predictions remain to be quantitatively validated in later studies. This validation may be combined with an optimization study of a commercial product to prove the industrial impact of this innovative model. In addition, accurate values for the permeability of cod muscle is needed to acquire more accurate model solutions.

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**Table**

*Table 1. Fish characterization and model parameters.*

| | | | g/100 g NaCl in brine | | |
|---|---|---|---|---|---|
| | Property | Untreated | 0 | 1.5 | 4.5 |
| Characterization | Weight gain during brining (g 100/g initial weight, n=10) | – | 8±3[a] | 11±1[a] | 24±5[b] |
| | Final muscle pH (n=10) | 6.22±0.08[ab] | 6.27±0.06[a] | 6.23±0.02[ab] | 6.10±0.06[b] |
| | NaCl content (g/100 g, n=8) | 0.10±0.02[a] | 0.06±0.03[a] | 1.00±0.17[b] | 3.06±0.58[c] |
| | Moisture content (g/100 g, n=8) | 77.3±0.4[a] | 80.1±1.1[b] | 80.6±1.9[b] | 80.2±2.2[b] |
| Eq. 8. WHC | $C_{eq,0}$ | 0.82* | 0.70 | 0.85 | 0.98 |
| | $a_1$ | 0.12* | 0.13 | 0.17 | 0.32 |
| | $a_2$ | 23* | 23 | 25 | 20 |
| | $a_3$ | 0.42* | 0.35 | 0.42 | 0.08 |
| | $T_\sigma$ | 25* | 30 | 32 | 16 |
| Eq 9. G' | $G'_{max}$ (kPa) | 48±9* | 38±9 | 42±7 | 38±8 |
| | $G'_{min}$ (kPa) | 14±3* | 13±5 | 10±2 | 15±1 |
| | $g_1$ | 64* | 64 | 60 | 63 |
| | $g_2$ | 5* | 4 | 7 | 5 |

Values in a row not sharing a common letter are significantly different ($P < 0.05$).

*Data published by Blikra *et al.* (2019).

**Figure**

Figure 1. **Flow chart of processing and sample preparation steps.**

Figure 2. **Normalized examples of DSC thermographs for raw samples of all salt concentrations heated at 2.5 K/min from 2-100 °C.**

Figure 3. **Residual denaturation enthalpy ($h_{den}$) and peak denaturation temperature (PDT) of heated cod muscle of various salt concentrations.** Peaks 1 (A-B), 2 (C-D) and 3 (E-F) are shown for raw samples and samples isothermally heated for 10 minutes at 25-65 °C, ±SD (n=2). Cross: Untreated; Triangle: Water-brined; Diamond: 1 g/100 g NaCl; and Line: 3 g/100 g NaCl.

Figure 4. **Water holding capacity of raw and heat-treated cod muscle of various salt concentrations (±SD).** A) measurements (symbols) and equations (lines) in w/w percentage of raw sample weight; and B) measurements in g hold water /g dry water. Cross: Untreated; Yellow triangle: Water-brined; Orange diamonds: 1 g/100 g NaCl; and red circles: 3 g/100 g NaCl.

Figure 5. **Storage modulus of cod muscle of various salt concentrations.** A) Untreated; B) Water-brined; C) 1 g/100 g NaCl; D) 3 g/100 g NaCl. The dashed lines show measurements (n=10), and the solid lines show the corresponding equation. For clarity, SD is shown every 10 °C.

Figure 6. **Cross-sectional representative images of the microstructure of cod muscle of various salt concentrations (n=3).** A) Untreated; B) Water-brined; C) 1 g/100 g and d) 3 g/100 g NaCl.

Figure 7. **Model prediction of water retention in samples of cod muscle containing various salt concentrations**. Black: Untreated; Yellow: Water-brined; Orange: 1 g/100 g NaCl; and Red: 3 g/100 g NaCl.

Figure
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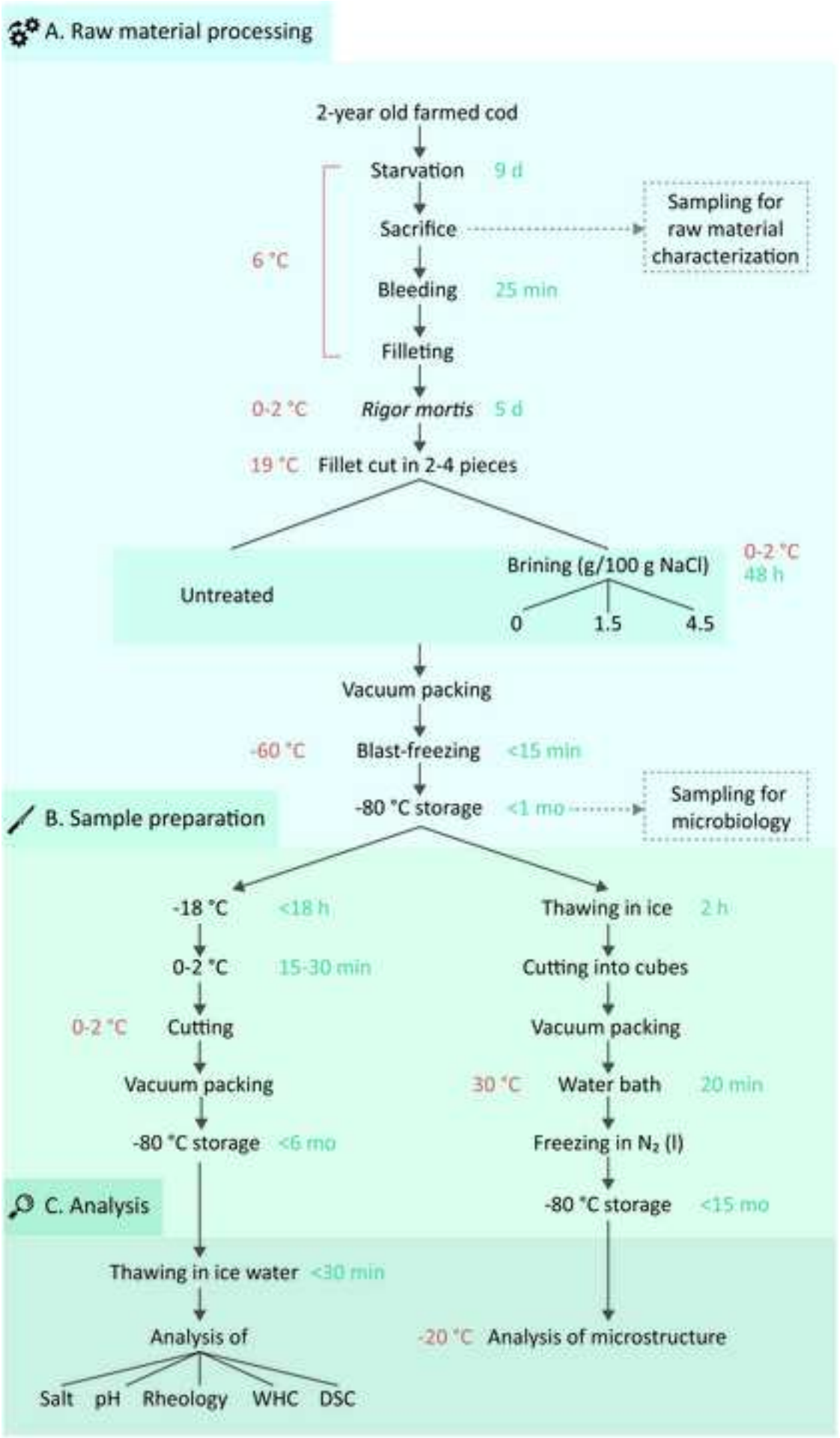

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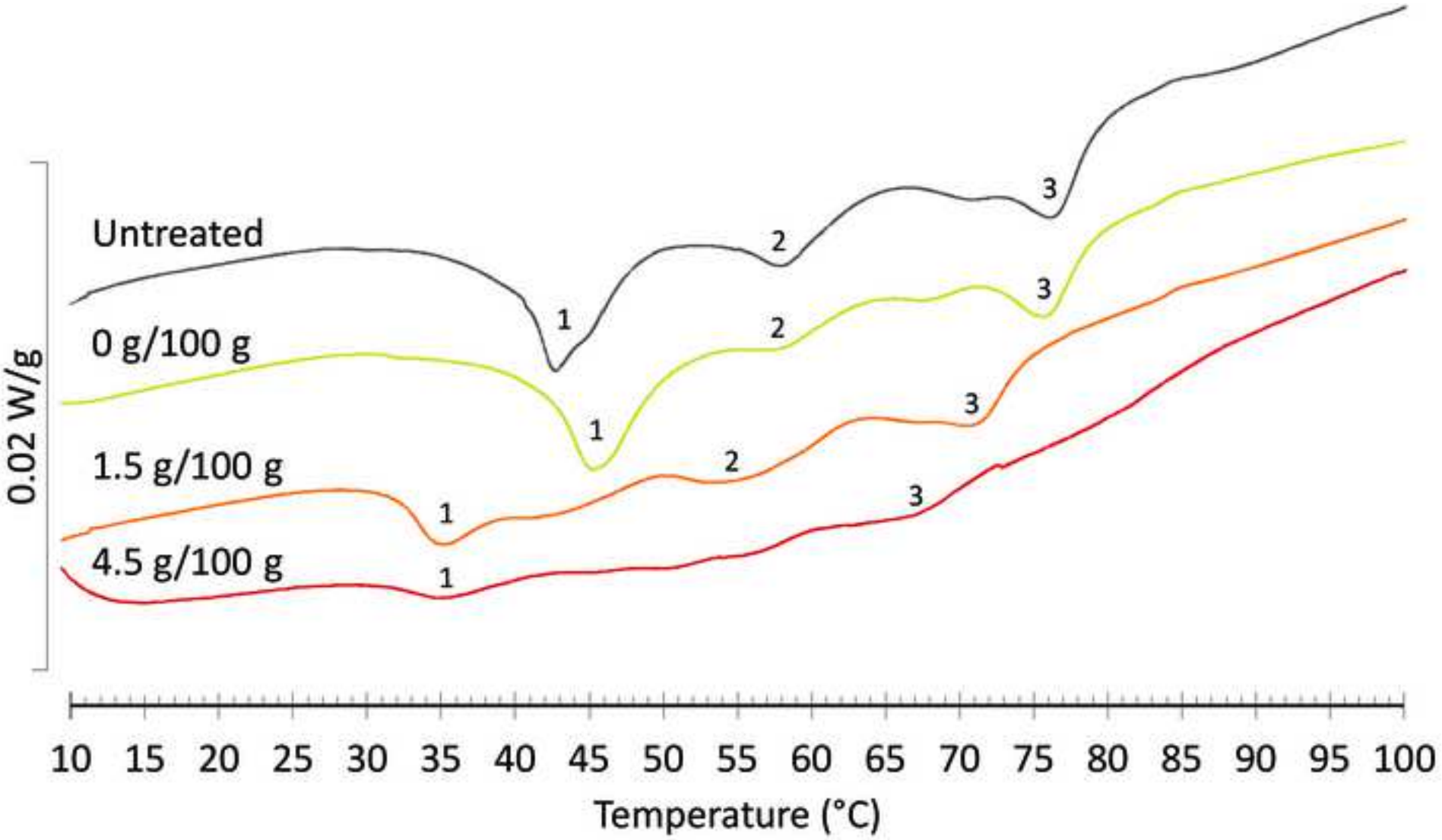

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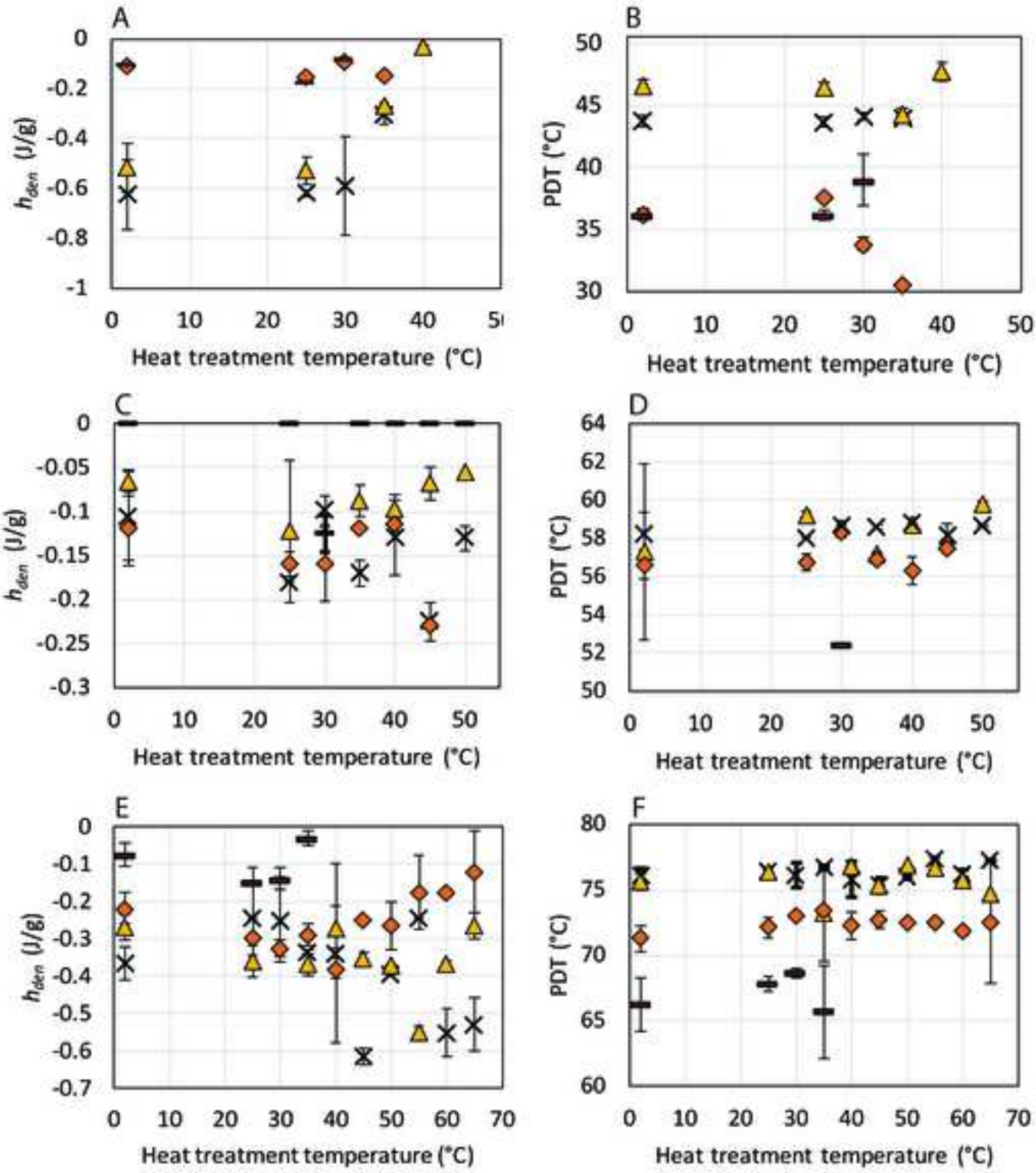

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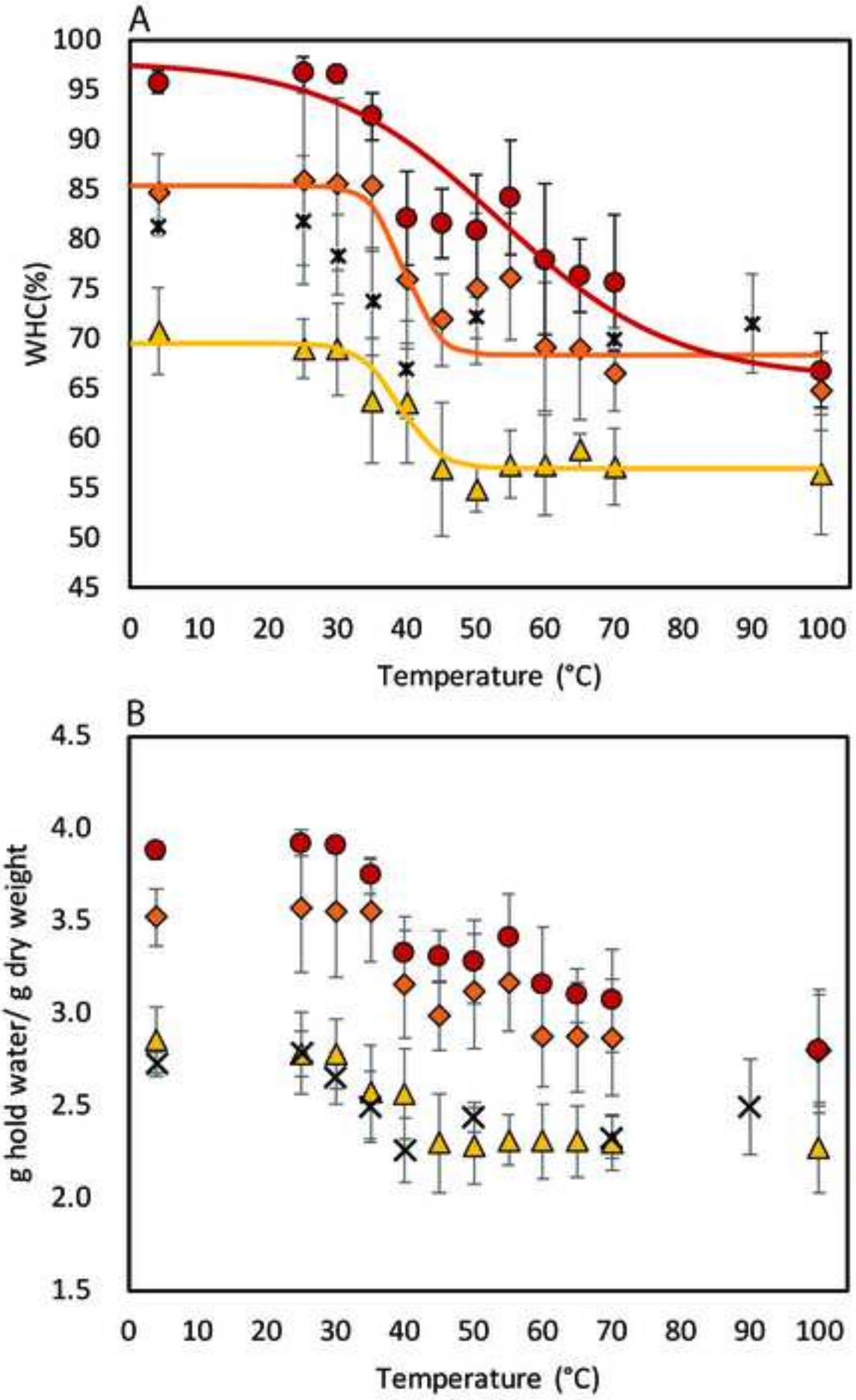
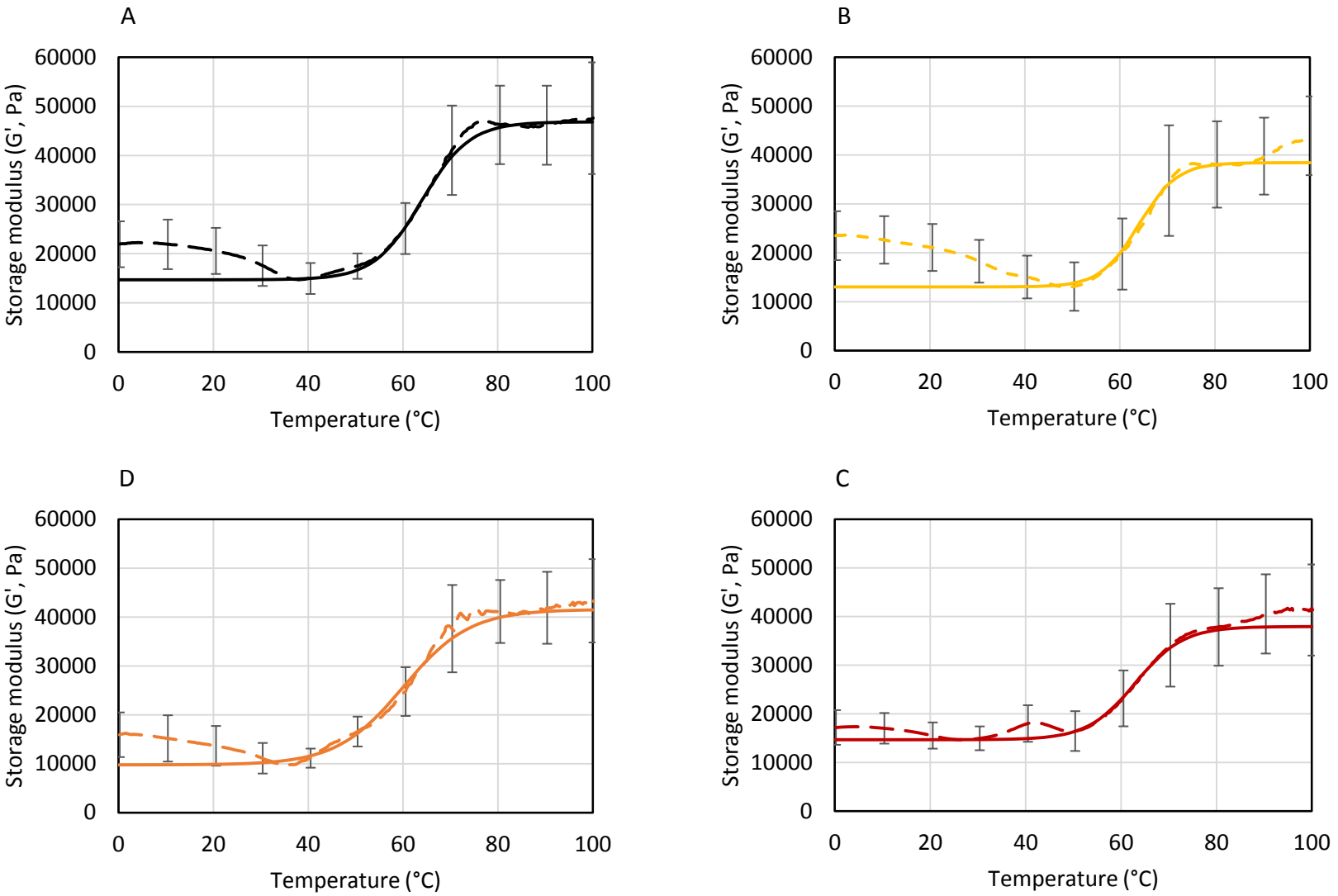
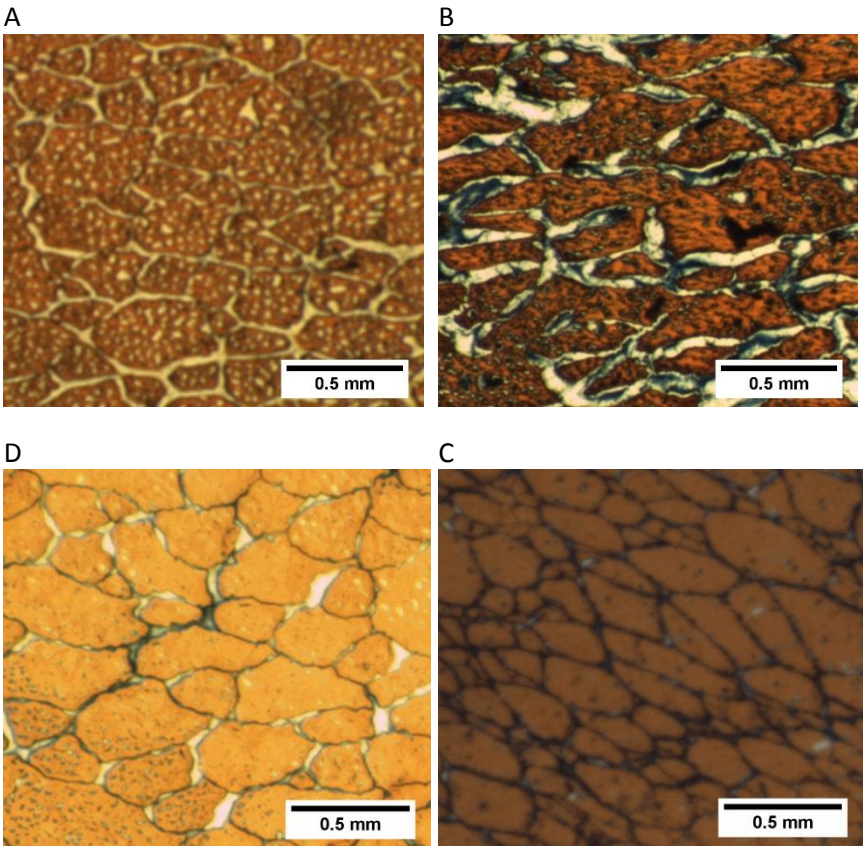


**Figure**


A
B
D
C
Storage modulus (G', Pa)
Temperature (°C)
0
10000
20000
30000
40000
50000
60000
0
20
40
60
80
100

**Figure**



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