

2 **Relationship between smoothing temperature, storage time, syneresis and rheological**
3 **properties of stirred yogurt**

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11 **Highlights**

- 12
- 13 • Smoothing at 10°C minimizes syneresis compared to higher temperature (15-30°C)
 - 14 • Rheological properties were improved for yogurt smoothed at 25 and 30°C.
 - 15 • Flow time through a Posthumus funnel could predict firmness of stirred yogurt.
- 16

17 **Abstract**

18 Six different smoothing temperatures were compared for nonfat yogurt and the changes in
19 syneresis and rheological properties observed for up to 22 days. Multiple linear regressions were
20 used to describe the syneresis, firmness, flow time, viscosity, and flow resistance and the
21 relationship between these properties, the smoothing temperature and the storage time. During
22 storage, viscosity, firmness, and flow time increased; syneresis and flow resistance remained
23 stable. Syneresis increased significantly ($P \geq 0.05$) with smoothing temperature (10 - 35 °C).
24 Other properties increased slightly ($P > 0.05$), and properties started to decrease above 30 °C.
25 Syneresis, viscosity, and flow resistance were more sensitive to smoothing temperature; firmness
26 and flow time were more sensitive to storage time. Lower smoothing temperature (10 °C) should

27 be used to minimize syneresis while smoothing temperature ranging from 25 to 30 °C is better to
28 improve rheological properties. Storage time must be considered to optimize these properties.

29 **1 Introduction**

30 Between 2005 and 2016, Canadian consumption of yogurts (set and stirred) increased
31 significantly (by 42.6%) (Canadian Dairy Information Centre, 2018). Unlike in set yogurt, the
32 additional operations of stirring, smoothing, and cooling to produce stirred yogurt break the acid
33 gel into a dispersion of brittle gel particles in the whey (Rasmussen, Janhoj, & Ipsen, 2007;
34 Zoon, 2003). This breakdown of the gel can affect the sensory quality of stirred yogurt in various
35 ways, such as expulsion of whey, decreased firmness and viscosity, and the appearance of lumps
36 that can be perceived in the mouth (Lucey, 2004).

37 Recently, results obtained by Guénard-Lampron, St-Gelais, Villeneuve, & Turgeon,
38 (2019) using a technical scale unit (30 L), have demonstrated that the smoothing and cooling
39 operations, comparatively to stirring and pumping, contribute most to the modulation of stirred
40 yogurt properties after 1 day of storage. . Several authors have also observed that smoothing is
41 crucial to obtaining a smooth yogurt, but this operation causes a significant breakdown in the
42 protein structure, which leads to lower values of viscosity of the yogurt (Cayot, Schenker,
43 Houzé, Sulmont-Rossé, & Colas, 2008; Mookoolall, Nöbel, & Hinrichs, 2016; Rasmussen et al.,
44 2007). The temperature of the yogurt during shearing is also critical in order to avoid viscosity
45 loss (Mookoolall et al., 2016). For example, Abu-Jdayil, Nasser, & Ghannam (2013) showed that
46 the higher the viscosity of the yogurt, the larger the viscosity loss observed during a shear
47 treatment. De Lorenzi, Pricl, & Torriano (1995) observed that temperature variations (between 4
48 and 20 °C) during the frequency sweep test of a full fat yogurt did not modified the G* values,

49 but a decrease of G^* was observed at 28 °C. Also, Afonso & Maia (1999) demonstrated that
50 viscosity of yogurt decreases when the temperature was increased (between 5 and 45 °C) and
51 that this effect was more pronounced for temperature above 25 °C. So far, the literature has
52 agreed that the smoothing operation must be carried out at about 20 °C in order to obtain a
53 yogurt with acceptable properties (Robinson, Lucey, & Tamime, 2007; A. Y. Tamime &
54 Robinson, 2007). Lucey (2004) observed that yogurt should not be smoothed when the gel is too
55 warm because the structure of the protein network would be too fragile. The smoothing of cooled
56 yogurt (10 °C) would also not be appropriate, comparatively to a yogurt cooled at 20°C which is
57 less viscous and therefore undergoes less damage by the mechanical stress. (Tamime &
58 Robinson, 2007). Guénard-Lampron, St-Gelais, Villeneuve, & Turgeon (2020) studied the
59 impact of the stirring operations, such as the smoothing temperature, on syneresis and
60 rheological properties of yogurts up to 22 days of storage. This study compared two smoothing
61 temperature (38 and 20 °C), but they were also linked to the operational sequence: smoothing
62 performed before the cooling (38 °C) or after the cooling (20 °C). Yogurt smoothed at 38 °C,
63 comparatively to those smoothed at 20 °C, showed higher flow time. However, this study did not
64 allow to dissociate the impact of the smoothing temperature from the impact of the operational
65 sequence. The literature does not include more specific data on the impact of smoothing at
66 different temperatures between a warm yogurt (ex: at the incubation temperature, 40°C) and a
67 cooled yogurt (ex: at the storage temperature, 4 °C). Moreover, the information reported in the
68 literature is based mainly on laboratory-scale stirring and smoothing operations, for example
69 using a syringe, which may not be representative of production conditions on an industrial scale.
70 A better understanding of the effect of different smoothing temperatures on the syneresis and
71 rheological and properties of yogurt in a context closer to the industrial reality is needed to

72 improve stirred yogurt quality. Consequently, the aim of the present study was to describe the
73 syneresis and rheological properties of yogurts smoothed at six different temperatures using a
74 technical scale unit and stored for up to 22 days.

75 **2 Materials and methods**

76 *2.1 Milk ingredients and starter*

77 Nonfat yogurts were produced with pasteurized skim milk (Laiterie Chalifoux Inc., Sorel-Tracy,
78 QC, Canada), low-heat skim milk powder (Agropur, Saint-Hyacinthe, QC, Canada), whey
79 protein concentrate (Agropur), and lactose (Saputo Inc., Montreal, QC, CA). A non-ropy
80 lyophilized culture of *Streptococcus thermophilus* and *Lactobacillus delbrueckii* ssp. *bulgaricus*
81 was used as described by Guénard-Lampron et al. (2019) to prepare the starter (Yo-Dolce; Biena,
82 Saint-Hyacinthe, QC, Canada). A non-ropy culture and a nonfat yogurt were chosen in order to
83 focus on the impact of the stirring process and more precisely on the smoothing temperature. For
84 all the yogurts produced, the average incubation time for the starter was 297 ± 11 min. Table 1
85 presents the composition of each ingredient.

86 *2.2 Yogurt production*

87 Skim milk was standardized to obtain a milk mixture at 0% fat, 4% total protein (casein-to-
88 whey-protein ratio of 2.8), and 14% total solids, rehydrated, homogenized in 2 stages (13.80 and
89 3.45 MPa), and heat-treated (94.5 °C for 5 min) as explained by Guénard-Lampron et al. (2019).
90 Each batch was made of 130 kg of skim milk and amount of each ingredient used is present in
91 Table 1. The treated milk mixture was incubated at 40 °C (Magelis unit; Schneider Electric,
92 Brossard, QC, Canada) in three 30 L cone-shaped spout yogurt vats. Yogurt vat was inoculated
93 with the starter (1.5% v/v), and the pH was measured (portable pH meter, model HI 99161;

94 Hanna Instruments, Laval, QC, Canada) until 4.7 was reached. The average incubation time for
95 the yogurts was 253 ± 20 min.

96 *2.3 Technical scale unit and stirring operations*

97 The technical scale unit used by Guénard-Lampron et al. (2019) and Guénard-Lampron, St-
98 Gelais, Villeneuve, & Turgeon (2020), which represents each sequential steps of the stirring
99 process (stirring in the yogurt vat, pumping, smoothing and cooling), was adapted to study the
100 effects of smoothing temperature (Fig. 1). A helical blade mixer (Fig.2) was used to perform the
101 stirring operation (10 min at 30 rpm) in the yogurt vat, and removable baffles were used during
102 the first 30 s of mixing, as explained by Guénard-Lampron et al. (2019). After 10 min of stirring,
103 the mixing speed was reduced to 15 rpm, and the yogurt was pumped using a positive gear pump
104 with a flow rate of 1.7 L/min (Seco DANA, model 210; Bronco Industries, BC, Canada) into
105 cylindrical stainless steel pipes (3.4 cm inner diameter, length of 4.4 m). Pressure was measured
106 after the pump by a digital pressure gauge (Distribution Qualtech, Saint-Hyacinthe, QC, Canada)
107 to allow detection of clogging of the smoothing nozzle. The yogurt was then presmoothed
108 (1.4 mm filter nozzle), cooled to one of the six smoothing temperatures under study (10, 15, 20,
109 25, 30, or 35 °C) with a plate heat exchanger (type A3-HBM; Alfa Laval, Lund, Sweden),
110 smoothed (425 µm filter nozzle), and cooled to 10 °C with a tubular heat exchanger (PG7757/84;
111 Sepak Industries Pty Ltd, Sydney, Australia). The heat exchangers were connected to a cold
112 water system in counterflow, and the temperature was controlled as described by Guénard-
113 Lampron et al. (2019).

114 The stirred yogurt was collected at the outlet of the technical scale unit in 175 mL
115 containers for all analyses except for the flow time, for which 500 mL containers were required

116 (Plastipak; GenPak, Boucherville, QC, Canada). The stirred yogurts were stored in a cold room
117 at 4 °C, and containers were chosen randomly for the syneresis and rheological analyses after 1,
118 3, 13, and 22 days.

119 *2.4 Yogurt analyses*

120 Analytical methods such as the determination of pH, total solids content (desiccation), fat
121 content (Mojonnier method), and total nitrogen, non-protein nitrogen, and non-casein contents
122 (Kjeldahl method) in the ingredients and yogurt milks taken before the heat treatment of the milk
123 mixture were performed as described by Guénard-Lampron et al. (2019). On day 1, 3, 13 and 22
124 of storage, microbial counts (M17 agar + 0.5% lactose for streptococci and MRS acidified for
125 lactobacilli), syneresis (centrifugation), firmness (TA-XT2 texture analyzer; Texture
126 Technologies Corporation, Scarsdale, NY, USA), apparent viscosity (Physica MCR301
127 rheometer; Anton Paar GmbH, Ostfildern, Germany), and consistency (Bostwick consistometer)
128 were performed using the methods provided by Guénard-Lampron et al. (2019). However, in the
129 present study, consistency (distance traveled in Bostwick) has been replaced by flow resistance
130 (maximal distance of the device minus distance travelled). The flow time of stirred yogurt
131 through a standard Posthumus funnel (Kutter, Singh, Rauh, & Delgado, 2011; Posthumus, 1954)
132 was added to the yogurt analyses. The flow time for 280 g of yogurt was recorded as a function
133 of mass using a balance (model P-2002, Pinnacle series; Denver Instrument, Mississauga, ON,
134 Canada) that was connected to a data acquisition system. Duplicates were performed for each
135 yogurt.

136 2.5 Data processing and statistical analyses

137 An empirical approach was used to describe each variable under study (syneresis, viscosity,
138 firmness, flow resistance, and flow time) depending on the smoothing temperature and the
139 storage time. The following polynomial equation was used:

140
$$y = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_{11} X_1^2 + \beta_{22} X_2^2 + \beta_{12} X_1 X_2 \quad (1)$$

141 where $\beta_0, \beta_1, \dots, \beta_{22}$ represent the regression coefficients, with β_0 as the constant, β_1 and β_2 as the
142 linear effect, β_{11} and β_{22} as the quadratic effect, and β_{12} as the effect of interactions; and X_1 and X_2
143 are, respectively, the independent variables of smoothing temperature and storage time. In order
144 to determine the regression coefficients and to minimize the error between the values measured in
145 the laboratory and those predicted by the model, the least squares estimation method of β (Eq. 2)
146 was used:

147
$$\hat{\beta} = (X^1 X)^{-1} X^1 y \quad (2)$$

148 where X^1 corresponds to the transpose of the matrix X , and $(X^1 X)^{-1}$ corresponds to the inverse of
149 the matrix $X^1 X$. The input variables were therefore smoothing temperature ($^{\circ}\text{C}$) and storage time
150 (days), and the output variables were syneresis, viscosity, firmness, flow resistance, and flow time.

151 The six smoothing temperatures were randomized and repeated three times. Statistical
152 analysis (split-plot statistical design) was carried out to compare the properties of the stirred yogurt
153 after 1, 3, 13, and 22 days of storage. The six temperatures were the main factor, and the number
154 of days of storage was the subplot factor. The GLM (General Linear Model) procedure of the SAS
155 software package (SAS Server Interface, version 2.5.14; SAS Institute Inc., Cary, NC) was used
156 to perform the statistical analyses. Two Pearson correlations were calculated with the CORR

157 procedure of the SAS software. The first one compared processing parameters (smoothing
158 temperature and storage time) and the properties of the stirred yogurt, and the second one compared
159 syneresis, viscosity, and firmness to flow resistance and flow time. Significant differences were
160 tested at $P \leq 0.05$. Correlations were considered for Pearson correlation coefficients over 0.50.

161 *2.6 Sensitivity analysis*

162 In order to analyze the effects of variation of the input variables on the outputs of the model, a
163 sensitivity analysis was carried out using the finite-difference method of Chokmani, Viau, &
164 Bourgeois (2001) and successfully used by (Bergeron Quirion, Villeneuve, Leblanc, & Delaquis,
165 2012; Mercier, Moresoli, Villeneuve, Mondor, & Marcos, 2013; Villeneuve & G elinas, 2007).
166 By using a reference scenario, each input parameter was varied within a specific range while
167 keeping the other parameters constant. In this study, smoothing temperature and storage time
168 were the input parameters. The reference scenario was smoothing at 20  C and storage of 7 days
169 with increments of 1  C (between 10 and 35  C) and 1 day (between 1 and 22 days). The outputs
170 of the model were syneresis, viscosity, firmness, flow resistance, and flow time. Critical input
171 parameters were expressed as % change in the model output per unit of change of the input
172 parameter. Relative sensitivity is not influenced by input parameters units or scales.

173 **3 Results**

174 *3.1 Composition of yogurt milk*

175 All milk mixtures had the same composition (fat: 0.18 ± 0.01 g/100g; total solids: 14.5 ± 0.1
176 g/100g; true proteins: 4.25 ± 0.09 g/100g; caseins: 3.13 ± 0.07 g/100g; whey proteins:
177 1.12 ± 0.01 g/100g; and casein-to-whey-protein ratio: 2.81 ± 0.04).

178 *3.2 Changes in microbial counts and pH*

179 During storage, a significant effect of storage time was observed on changes in bacterial
180 populations and pH. No significant interaction between smoothing temperature and storage time
181 was observed. Between day 1 and 13 of storage at 4 °C, the streptococci (8.40 ± 0.04 Log
182 CFU mL⁻¹) and lactobacilli (7.57 ± 0.09 Log CFU mL⁻¹) populations remained stable. After
183 22 days, the streptococci decreased slightly to 8.31 ± 0.04 and the lactobacilli decreased to
184 6.78 ± 0.09 Log CFU mL⁻¹. The pH of the yogurts decreased significantly, from 4.5 to $4.3 \pm$
185 0.01 , until day 13 and then remained stable.

186 *3.3 Changes in syneresis and rheological properties*

187 The effects of smoothing temperature and storage time on the stirred yogurt properties expressed
188 by the response surface are presented in Fig. 3. The polynomial equations used to describe each
189 property are presented in Table 2. The effect of storage time on syneresis was lower in
190 comparison with the effect of smoothing temperature (Fig. 3A). Syneresis of yogurt increased
191 with smoothing temperature (10 to 35 °C), but between 10 and 15 days of storage, syneresis was
192 similar for yogurts smoothed between 20 and 30 °C. The response surface had two parabolas, the
193 first limited from 10 to 20 °C and the second limited from 20 to 35 °C (Fig. 3A). For both
194 equations, the regression coefficients were much higher for smoothing temperature, and no
195 interaction coefficient was observed between smoothing temperature and storage time, as also
196 confirmed with the statistical analysis (Table 2).

197 Viscosity (Fig. 3B), flow resistance (Fig. 3C), firmness (Fig. 3D), and flow time (Fig. 3E)
198 increased as the smoothing temperature increased from 10 to 30 °C and then started to decrease
199 at 35 °C. The polynomial equations were therefore limited to smoothing temperatures from 10 to

200 30 °C. The regression coefficients for smoothing temperature and storage time were similar for
201 the equations for viscosity, flow resistance, and flow time, but for the firmness equation, the
202 regression coefficient for storage time was higher than the regression coefficient for smoothing
203 temperature (Table 2). For these properties, a weak interaction coefficient between smoothing
204 temperature and storage time was observed (Table 2), as also observed in the statistical analysis.
205 Fig. 3B also shows that, between 1 and 15 days of storage at 4 °C, viscosity was higher when the
206 smoothing temperature was between 25 and 30 °C. However, between 15 and 22 days, the
207 highest viscosity values were obtained at temperatures between 15 and 20 °C. Values for flow
208 resistance seemed stable over the 22 days of storage between 20 and 30 °C, whereas for the other
209 smoothing temperatures (10, 15, and 35 °C), a drop in flow resistance was observed after 22 days
210 of storage (Fig. 3C). Firmness (Fig. 3D) and flow time (Fig. 3E) also increased with storage time
211 (1 to 22 days).

212 *3.4 Sensitivity and Pearson correlation coefficients*

213 Fig. 4 presents the relative sensitivities to a variation of 1°C during smoothing (sensitivity to
214 smoothing temperature) and to a variation of 1 day during storage (sensitivity to storage time) of
215 the properties under study. Syneresis, viscosity, and flow resistance were more sensitive to
216 smoothing temperature than to storage time, whereas an opposite trend was observed for
217 firmness and flow time (Fig. 4). These results are in agreement with the Pearson correlation
218 coefficients, presented in Table 3, which indicated that storage time was positively correlated to
219 firmness or flow time (Table 3). In addition, flow time was about seven times more sensitive to
220 storage time than the other properties were. Viscosity and flow resistance were both more
221 sensitive to variations in smoothing temperature (Fig. 4), but no significant correlation was
222 observed between these properties and smoothing temperature (Table 3). Syneresis was very

223 sensitive (Fig. 4) and positively correlated (Table 3) to smoothing temperature. Between 10 and
224 20 °C, syneresis was 13 times more sensitive to a variation of 1 °C than to a variation of 1 day
225 (Fig. 4). The sensitivity of syneresis to storage time was similar for both smoothing temperature
226 intervals (10–20 °C and 20–35 °C) (Fig. 3).

227 Fig. 5 presents, more precisely, the relative sensitivity of syneresis to variations in
228 smoothing temperature, as provided by the two quadratic equations. The relative sensitivity of
229 syneresis is therefore presented for each temperature interval between the six temperatures
230 studied. The sensitivity of syneresis in the interval from 16 to 20 °C was 1.5 times higher than in
231 the intervals from 10 to 15 °C and 21 to 25 °C; 3 times higher than in the interval from 26 to
232 30 °C; and 10 times higher than in the interval from 31 to 35 °C. Syneresis was therefore much
233 less sensitive to variations in smoothing temperature above 30 °C.

234 Table 5 presents the Pearson correlation coefficients for the comparison of syneresis,
235 viscosity, and firmness to flow time and flow resistance. The coefficients of correlation indicate
236 that flow resistance and flow time were positively correlated to viscosity and that flow time was
237 even more positively correlated to firmness.

238 **4 Discussion**

239 During storage, syneresis and rheological properties of stirred yogurt depend on the entire
240 shear history that occurred during stirring operations (Fangary, Barigou, & Seville, 1999; Guénard-
241 Lampron et al., 2019; Mookoolall et al., 2016; Sodini, Remeuf, Haddad, & Corrieu, 2004). In the
242 present study, surface response, sensitivity analysis, and Pearson correlation indicate clearly that
243 syneresis was affected most by smoothing temperature. Even though post-acidification for all the
244 yogurts was similar, it would seem that the smoothing temperature modified the protein network,

245 which subsequently affected the restructuring of yogurt during storage. Mizrahi (2010) explained
246 that temperature, both during gel preparation and after production, changes the strength of
247 hydrogen bonds and hydrophobic interactions, which has the consequence of affecting the
248 association, dissociation, and configuration of the gel particles. In addition, temperature affects
249 osmotic pressure and gel contraction. Hinrichs & Keim (2007) demonstrated that hydrophobic
250 interactions represent 70% of the protein–protein interactions in skim milk yogurt after 7 days of
251 storage. Consequently, the modifications to hydrophobic interactions during smoothing at different
252 temperatures could have a major impact on the restructuring of the protein network and on the
253 expulsion of whey. Gilbert, Rioux, St-Gelais, & Turgeon (2020) also demonstrated that yogurt
254 smoothed in a rheometer at 42 °C had higher syneresis value and more heterogeneous
255 microstructure than yogurt smoothed at 20 °C. In addition, several authors agree that smoothing at
256 20 °C is ideal for obtaining high-quality yogurt, which is supposed to include lower syneresis
257 values (Robinson et al., 2007; Tamime & Robinson, 1999). In the present study, the lowest
258 syneresis values were obtained for yogurt smoothed at 10 °C, and the sensitivity results indicate a
259 major variation of syneresis for a smoothing temperature near 20 °C.

260 The smoothing temperature (10 to 30 °C) also increased the viscosity, flow resistance,
261 firmness, and flow time values. A similar observation was reported in the review by Mokoona
262 et al. (2016), who described a smaller decrease in viscosity when smoothing was performed at
263 20 °C in comparison with 6 °C. They also reported that greater loss of structure occurs when the
264 initial viscosity of a microgel suspension is higher. Abu-Jdayil, Nasser, & Ghannam (2013) have
265 also shown that increasing the casein content results in a more structured gel with higher viscosity
266 which result in a yogurt more sensitive to shear conditions. In the present study, the viscosity could
267 increase during cooling at lower temperature before the smoothing and this can lead to a greater

268 breakdown of the protein network during the smoothing at lower temperature and could explain
269 the lower values for rheological properties.

270 Viscosity, flow resistance, firmness, and flow time values also started to drop at 35 °C, and
271 their values were similar to those obtained at 10 °C. Smoothing yogurt at a temperature close to
272 the incubation temperature would damage the structure of the protein network because the network
273 would still be brittle (Lucey, 2004). In the present study, the yogurts incubated at 40 °C and
274 smoothed at 35 °C were subjected to mechanical stress caused by the plate heat exchanger as well
275 as by the smoothing filter nozzle at 35 °C, which could have greatly affected the brittle protein
276 network by, for example, breaking electrostatic interactions. These phenomena could explain the
277 lower values obtained for rheological properties at 35 °C. For both smoothing temperatures (10
278 and 35 °C), a difference in temperature of 25 °C (ΔT 25 °C) occurred during the cooling at 10 °C,
279 but at a different step in the stirring operation (after smoothing at 35 °C or before the smoothing
280 at 10 °C). Olsen (2003) observed a similar impact for the comparison of different filling
281 temperatures (10 to 25 °C) before a final cooling step at 5 °C. That author explained that yogurt
282 potted at 25 °C had a denser protein network (higher restructuration) than yogurt potted at 10 °C
283 did, possibly because shearing at a lower temperature implied a higher loss of protein structure.
284 However, Olsen (2003) did not test processing conditions involving cooling above 25 °C. In the
285 present study, it is possible that the protein structure would have difficulty rebuilding when yogurt
286 was smoothed at 10 °C and above 30 °C because of the high temperature difference (high ΔT).

287 In the present study, as post-acidification increased up to 13 days of storage (decrease of
288 pH from 4.5 to 4.3), viscosity, firmness, and flow time also increased. In addition, firmness and
289 flow time continued to increase up to 22 days. Increase in rheological properties during storage
290 was also observed by Serra, Trujillo, Guamis, & Ferragut (2009) and was related to post-

291 acidification (pH value not specified). During storage, flow resistance and syneresis stayed
292 relatively stable and were not very sensitive to storage time in comparison with the other
293 properties, which increased. The stability of flow resistance over time is difficult to explain but
294 could be due to the fact that the Bostwick consistometer analysis was less sensitive to the structural
295 changes in stirred yogurt. A decrease in syneresis as the pH decreased during storage was also
296 expected owing to the reabsorption of the whey through the gel particles, as reported by several
297 authors. For example, a decrease in syneresis was reported by Lorenzen, Neve, Mautner, &
298 Schlimme (2002) and was related to an increase of the titrable acidity from approximately 40 to
299 50°SH after 3 weeks of storage and by Prasad, Sherkat, & Shah (2013) for a decrease in pH from
300 4.5 to 4.2 after 4 weeks of storage.. However, Lucey (2001) reported that the expulsion of whey is
301 a consequence of an excessive rearrangement of gel particles. In the present study, the mechanical
302 stress caused by the stirring operation in the technical scale unit could have contributed to a more
303 stable and dense protein network that was able to maintain its capacity to retain the whey.

304

305 **5 Conclusions**

306 The present study demonstrated that smoothing temperature is a critical parameter for controlling
307 the syneresis and rheological properties of stirred yogurt during storage. The smoothing
308 temperature had the greatest effect on the syneresis of yogurt. A low smoothing temperature
309 (10 °C) would be better to minimize syneresis. However, this temperature was not optimal for
310 improving all yogurt properties. No matter the storage time (between 1 and 22 days), the
311 viscosity, flow resistance, firmness, and flow time tended to be lower for yogurts smoothed at
312 10 °C or above 30 °C. In order to improve these properties, a smoothing temperature between 25

313 and 30 °C could be recommended. The sensitivity analysis also demonstrated that each property
314 exhibited a different level of sensitivity to smoothing temperature and to storage time. A
315 correlation was established between firmness and flow time and could be further investigated to
316 predict the firmness from the Posthumus funnel flow. The next step will be to investigate the
317 relationship between the smoothing temperature and the change in the microstructure of the
318 protein network, which leads to the modification of the syneresis and rheological and properties
319 of stirred yogurt.

320

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331

332 **References**

- 333 Abu-Jdayil, B., Nasser, M. S., & Ghannam, M. (2013). Structure breakdown of stirred yoghurt in
334 a circular pipe as affected by casein and fat content. *Food Science and Technology*
335 *Research*, 19(2), 277–286. <https://doi.org/10.3136/fstr.19.277>
- 336 Afonso, I. M., & Maia, J. M. (1999). Rheological monitoring of structure evolution and
337 development in stirred yoghurt. *Journal of Food Engineering*, 42(4), 183–190.
338 [https://doi.org/10.1016/S0260-8774\(99\)00118-1](https://doi.org/10.1016/S0260-8774(99)00118-1)
- 339 Bergeron Quirion, S., Villeneuve, S., Leblanc, D. I., & Delaquis, P. (2012). *Thermophysical*
340 *properties and thermal behavior of leafy vegetables packaged in clamshells*. Retrieved from
341 <http://www.elsevier.com/copyright>
- 342 Canadian Dairy Information Centre. (2018). Consumption of dairy products (annual). Retrieved
343 from [http://www.dairyinfo.gc.ca/index_e.php?s1=dff-](http://www.dairyinfo.gc.ca/index_e.php?s1=dff-fcil&s2=cons&s3=conscdn&s4=dpcons&page=consdppl)
344 [fcil&s2=cons&s3=conscdn&s4=dpcons&page=consdppl](http://www.dairyinfo.gc.ca/index_e.php?s1=dff-fcil&s2=cons&s3=conscdn&s4=dpcons&page=consdppl)
- 345 Cayot, P., Schenker, F., Houzé, G., Sulmont-Rossé, C., & Colas, B. (2008). Creaminess in
346 relation to consistency and particle size in stirred fat-free yogurt. *International Dairy*
347 *Journal*, 18(3), 303–311. <https://doi.org/10.1016/j.idairyj.2007.06.009>
- 348 Chokmani, K., Viau, A., & Bourgeois, G. (2001). Analyse de l’incertitude de quatre modèles de
349 phytoprotection relative à l’erreur des mesures des variables agrométéorologiques d’entrée.
350 *Agronomie*, 21(2), 147–167. <https://doi.org/10.1051/agro:2001100>>
- 351 De Lorenzi, L., Pricl, S., & Torriano, G. (1995). Rheological behaviour of low-fat and full-fat
352 stirred yoghurt. *International Dairy Journal*, 5(7), 661–671. [https://doi.org/10.1016/0958-](https://doi.org/10.1016/0958-6946(95)00047-7)
353 [6946\(95\)00047-7](https://doi.org/10.1016/0958-6946(95)00047-7)
- 354 Fangary, Y. S., Barigou, M., & Seville, J. P. K. (1999). Simulation of Yoghurt Flow and
355 Prediction of Its End-of-Process Properties Using Rheological Measurements.
356 *TranslChemE*, 77(C), 33–39. <https://doi.org/10.1205/096030899532231>
- 357 Gilbert, A., Rioux, L.-E., St-Gelais, D., & Turgeon, S. L. (2020). Studying stirred yogurt
358 microstructure using optical microscopy: How smoothing temperature and storage time
359 affect microgel size related to syneresis. *Journal of Dairy Science*.
360 <https://doi.org/10.3168/JDS.2019-16787>
- 361 Guénard-Lampron, V., St-Gelais, D., Villeneuve, S., & Turgeon, S. L. (2019). Individual and

362 sequential effects of stirring, smoothing, and cooling on the rheological properties of nonfat
363 yogurts stirred with a technical scale unit. *Journal of Dairy Science*, 102(1).
364 <https://doi.org/10.3168/jds.2018-14565>

365 Guénard-Lampron, V., St-Gelais, D., Villeneuve, S., & Turgeon, S. L. (2020). Short
366 communication: Effect of stirring operations on changes in physical and rheological
367 properties of nonfat yogurts during storage. *Journal of Dairy Science*, 210–214.
368 <https://doi.org/10.3168/jds.2019-16434>

369 Hinrichs, J., & Keim, S. (2007). Process-induced stabilizing bonds in fermented milk products.
370 *Milchwissenschaft*, 62(4), 422–425.

371 Kutter, A., Singh, J. P., Rauh, C., & Delgado, A. (2011). Improvement of the prediction of
372 mouthfeel attributes of liquid foods by a posthumus funnel. *Journal of Texture Studies*.
373 <https://doi.org/10.1111/j.1745-4603.2011.00291.x>

374 Lorenzen, P. C., Neve, H., Mautner, A., & Schlimme, E. (2002). Effect of enzymatic cross-
375 linking of milk proteins on functional properties of set-style yoghurt. *International Journal*
376 *of Dairy Technology*, 55(3), 152–157. <https://doi.org/10.1046/j.1471-0307.2002.00065.x>

377 Lucey, J. A. (2001). The relationship between rheological parameters and whey separation in
378 milk gels. *Food Hydrocolloids*, 15(4–6), 603–608. [https://doi.org/10.1016/S0268-](https://doi.org/10.1016/S0268-005X(01)00043-1)
379 [005X\(01\)00043-1](https://doi.org/10.1016/S0268-005X(01)00043-1)

380 Lucey, J. A. (2004). Cultured dairy products: An overview of their gelation and texture
381 properties. *International Journal of Dairy Technology*, 57(2–3), 77–84.
382 <https://doi.org/10.1111/j.1471-0307.2004.00142.x>

383 Lucey, J. A., & Singh, H. (1998). Formation and physical properties of acid milk gels: A review.
384 *Food Research International*, 30(7), 529–542. [https://doi.org/10.1016/S0963-](https://doi.org/10.1016/S0963-9969(98)00015-5)
385 [9969\(98\)00015-5](https://doi.org/10.1016/S0963-9969(98)00015-5)

386 Mercier, S., Moresoli, C., Villeneuve, S., Mondor, M., & Marcos, B. (2013). Sensitivity analysis
387 of parameters affecting the drying behaviour of durum wheat pasta. *Journal of Food*
388 *Engineering*, 118(1), 108–116. <https://doi.org/10.1016/j.jfoodeng.2013.03.024>

389 Mizrahi, S. (2010). Syneresis in food gels and its implications for food quality. *Chemical*
390 *Deterioration and Physical Instability of Food and Beverages*, 324–348.
391 <https://doi.org/10.1533/9781845699260.2.324>

392 Mookonlall, A., Nöbel, S., & Hinrichs, J. (2016). Post-processing of fermented milk to stirred

393 products: Reviewing the effects on gel structure. *Trends in Food Science and Technology*,
394 54, 26–36. <https://doi.org/10.1016/j.tifs.2016.05.012>

395 Olsen, S. (2003). Microstructure and rheological properties of yogurt. In *Fermented Milk*
396 (Special Is). Brussels, Belgium: International Dairy Federation.

397 Olson, D. W., & Aryana, K. J. (2008). An excessively high *Lactobacillus acidophilus* inoculation
398 level in yogurt lowers product quality during storage. *LWT - Food Science and Technology*.
399 <https://doi.org/10.1016/j.lwt.2007.05.017>

400 Posthumus, G. (1954). Meten van de viscositeit. Een toestelletje voor het bepalen van de
401 viscositeit van enkele consumptiemelk. *Officieel Orgaan van de Koninklijke Nederlandse*
402 *Zuivelbond*, 4, 55–56.

403 Prasad, L. N., Sherkat, F., & Shah, N. P. (2013). Influence of Galactooligosaccharides and
404 Modified Waxy Maize Starch on Some Attributes of Yogurt. *Journal of Food Science*,
405 78(1), M77–M83. <https://doi.org/10.1111/j.1750-3841.2012.03004.x>

406 Rasmussen, M. A., Janhoj, T., & Ipsen, R. (2007). Effect of fat, protein and shear on graininess,
407 viscosity and syneresis in low-fat stirred yoghurt. *Milchwissenschaft*, 62(1), 54–58.

408 Robinson, R. K., Lucey, J. A., & Tamime, A. Y. (2007). Manufacture of Yoghurt. In A. Tamime
409 (Ed.), *Fermented Milks* (pp. 53–75). <https://doi.org/10.1002/9780470995501.ch3>

410 Sodini, I., Remeuf, F., Haddad, C., & Corrieu, G. (2004). The Relative Effect of Milk Base,
411 Starter, and Process on Yogurt Texture: A Review. *Critical Reviews in Food Science and*
412 *Nutrition*, 44(2), 113–137. <https://doi.org/10.1080/10408690490424793>

413 Tamime, A., & Robinson, R. (2007). Background to manufacturing practice. In *Tamime and*
414 *Robinson's Yoghurt: Science and technology* (3rd ed., pp. 13–161).
415 <https://doi.org/10.1533/9781845692612.13>

416 Tamime, A. Y., & Robinson, R. K. (1999). *Yoghurt science and technology* (2nd ed.). FL, USA
417 and Cambridge, UK: CRC Press and Woodhead Publishing.

418 Tamime, A. Y., & Robinson, R. K. (2007). *Yoghurt: Science and technology* (3rd ed.).
419 <https://doi.org/10.1533/9781845692612.162>

420 Villeneuve, S., & Gélinas, P. (2007). Drying kinetics of whole durum wheat pasta according to
421 temperature and relative humidity. *LWT - Food Science and Technology*.
422 <https://doi.org/10.1016/j.lwt.2006.01.004>

423 Wang, J., Guo, Z., Zhang, Q., Yan, L., Chen, Y., Chen, X., Liu, X.', Chen, W. & Zhang, H. P.

424 (2010). Effect of probiotic *Lactobacillus casei* Zhang on fermentation characteristics of set
425 yogurt. *International Journal of Dairy Technology*. [https://doi.org/10.1111/j.1471-](https://doi.org/10.1111/j.1471-0307.2009.00556.x)
426 [0307.2009.00556.x](https://doi.org/10.1111/j.1471-0307.2009.00556.x)

427 Zoon, P. (2003). *Viscosity, smoothness and stability of yogurt as affected by structure and EPS*
428 *functionality*. Brussels: International Dairy Federation.

429

430 **Table 1** Composition of milk ingredients and amount used in each batch.

| Ingredients ² | Components (%) ¹ | | | | | | Amount used in each batch (kg) |
|--------------------------|-----------------------------|-----------|-----------|-------------|-----------|-------------|--------------------------------------|
| | Total N | NPN | Casein | WP | TS | Fat | |
| Skim milk ³ | 3.1 ± 0.2 | 0.4 ± 0.1 | 2.0 ± 0.2 | 0.61 ± 0.03 | 8.3 ± 0.4 | 0.15 ± 0.01 | 130.0 |
| SMP | 34.2 | 0.7 | 26.7 | 6.8 | 97.7 | 0.007 | 6.64 ± 0.36 |
| WPC34 | 34.1 | 3.4 | 0.0 | 30.7 | 97.4 | 0.01 | 1.04 ± 0.02 |
| Lactose | 0.0 | 0.0 | 0.0 | 0.0 | 99.8 | 0.0 | 2.27 ± 0.12 |
| Starter | 4.1 | 0.08 | 3.2 | 0.8 | 11.7 | 0.001 | 0.45 |

431 ¹Total N, total nitrogen; NPN, non-protein nitrogen; WP, whey protein; TS, total solids.

432 ²SMP, low-heat skim milk powder; WPC34, 34% whey protein concentrate.

433 ³The values for skim milk are averages of the values measured with an FT 120 infrared analyzer
 434 (Foss North America, Eden Prairie, MN) in the milk used for all batches.

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438 **Table 2** Polynomial equations describing each property depending on the smoothing temperature (x_1 ; °C) and the storage time (x_2 ; days)

| Properties | Limitations | Polynomial equations | R^2 |
|------------------------|-----------------------|---|-------------------------|
| Syneresis (%) | $10 \leq x_1 \leq 20$ | $19.9573 - 2.0184x_1 + 0.0777x_1^2 + 0.1357x_2 - 0.0071x_2^2$ | 0.81 |
| | $20 \leq x_1 \leq 35$ | $54.6834 - 3.5650x_1 + 0.0682x_1^2 + 0.1525x_2 - 0.0075x_2^2$ | 0.92 |
| Viscosity (s^{-1}) | $10 \leq x_1 \leq 30$ | $1.3702 + 0.0171x_1 + 0.0200x_2 - 0.0007x_1x_2$ | 0.66 |
| Flow resistance (cm) | $10 \leq x_1 \leq 30$ | $13.2731 + 0.0564x_1 - 0.1213x_2 + 0.0055x_1x_2$ | 0.67 |
| Firmness (N/m^2) | $10 \leq x_1 \leq 30$ | $260.8744 + 1.9443x_1 + 4.8300x_2 - 0.0739x_1x_2$ | 0.94 |
| Flow time (min) | $10 \leq x_1 \leq 30$ | $-0.4634 + 0.3422x_1 + 0.1952x_2 + 0.0458x_1x_2$ | 0.91 |

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441 **Table 3** Pearson correlation coefficients (r) between processing parameters (smoothing
442 temperature and storage time) and properties of stirred yogurt ($n = 72$)

443 *** Significant correlation at $P < 0.001$.

| | Syneresis | Viscosity | Flow resistance | Firmness | Flow time |
|-----------------------|----------------|-----------|-----------------|----------------|----------------|
| Smoothing temperature | 0.55*** | -0.04 | 0.19 | 0.10 | 0.16 |
| Storage time | -0.02 | 0.20 | -0.10 | 0.81*** | 0.57*** |

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446 **Table 4** Pearson correlation coefficients (r) between syneresis, viscosity, and firmness and flow
447 resistance and flow time ($n = 72$)

| | Flow resistance | Flow time |
|-----------|-----------------|----------------|
| Syneresis | -0.30** | -0.11 |
| Viscosity | 0.50*** | 0.50*** |
| Firmness | -0.03 | 0.69*** |

448 **Significant correlation at $P < 0.005$.

449 ***Significant correlation at $P < 0.001$.

450

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452 Figure captions

453

454 **Fig. 1.** Technical scale unit consisting of stirring in the yogurt vat, presmoothing, cooling with a
455 plate heat exchanger (PHX), smoothing, and cooling with a tubular heat exchanger (THX).

456

457 **Fig.2.** Helical blade mixer. The dimensions are: A = 2.5 cm, B = 17.8 cm, C = 40.6 cm,
458 D = 7.6 cm and E = 15.2 cm.

459

460 **Fig. 3.** Response surfaces for the (A) syneresis (%), (B) viscosity (Pa*s), (C) flow resistance
461 (cm), (D) firmness (N/m²), and (E) flow time (min) of stirred yogurts depending on the
462 smoothing temperature (10, 15, 20, 25, 30 and 35 °C) and the storage time (1 to 22 days).

463

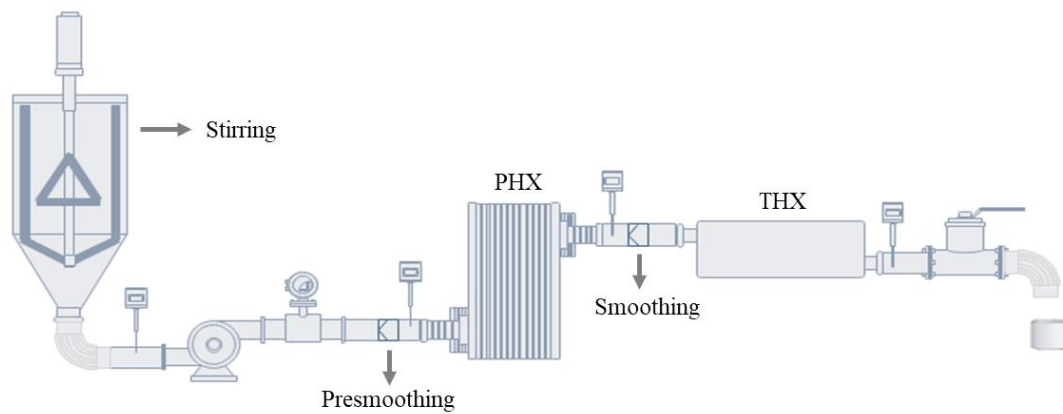
464 **Fig. 4.** Relative sensitivities of syneresis, viscosity, firmness, flow time, and flow resistance as a
465 function of storage time (black) or smoothing temperature (grey).

466

467 **Fig. 5.** Relative sensitivity of syneresis as a function of five smoothing temperature intervals.

468

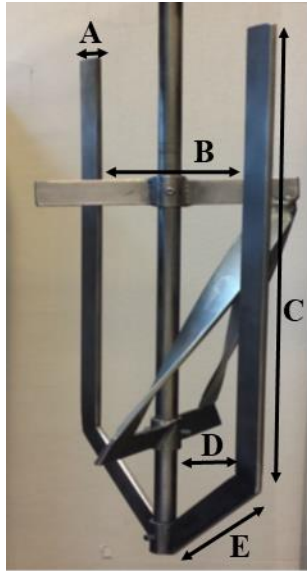
469 **Fig. 1**



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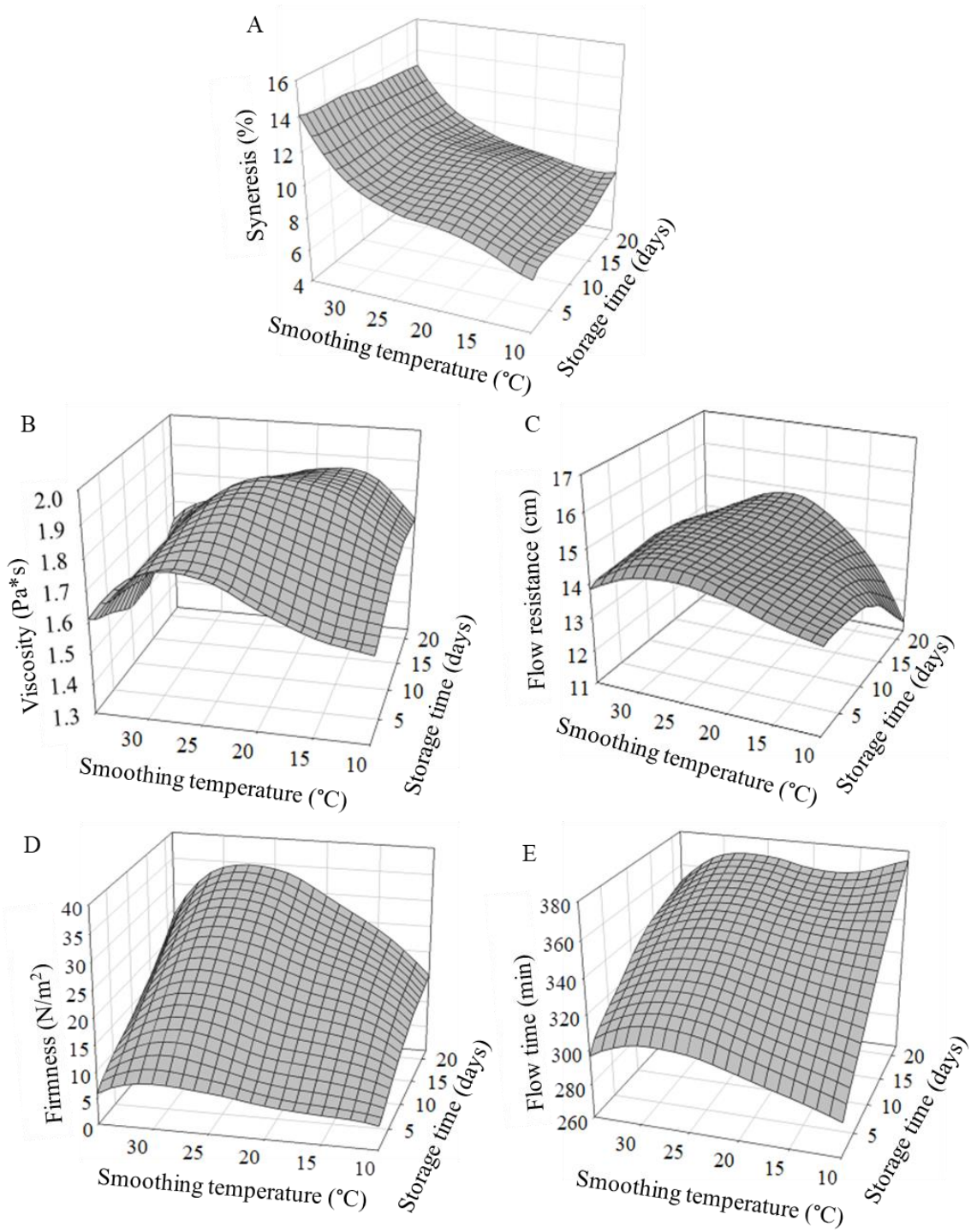
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472 **Fig. 2**

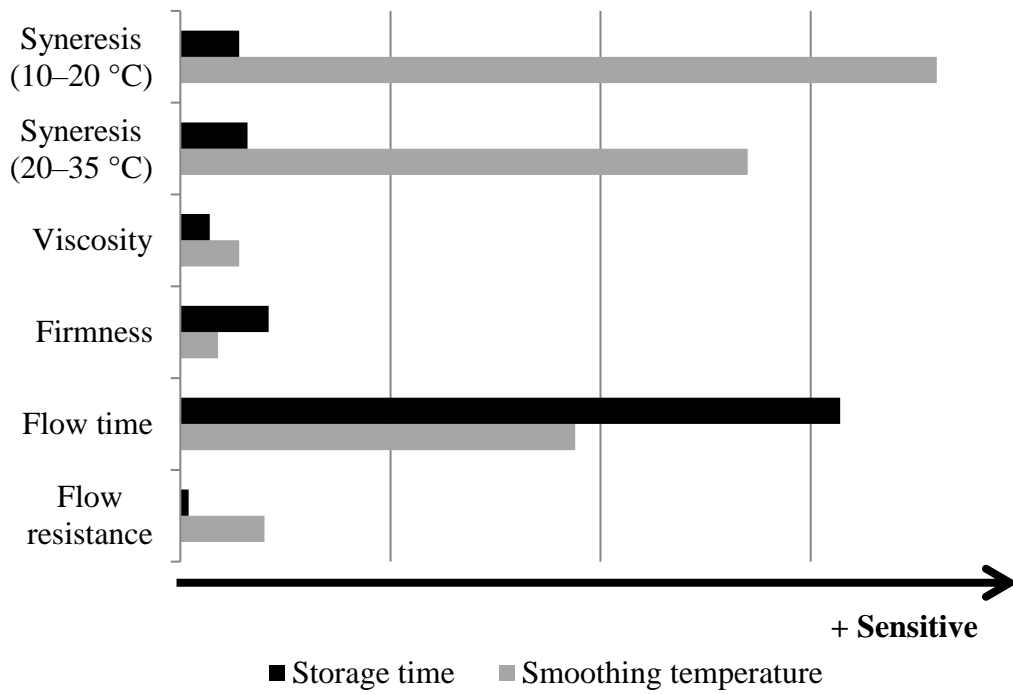


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478 **Fig. 4**

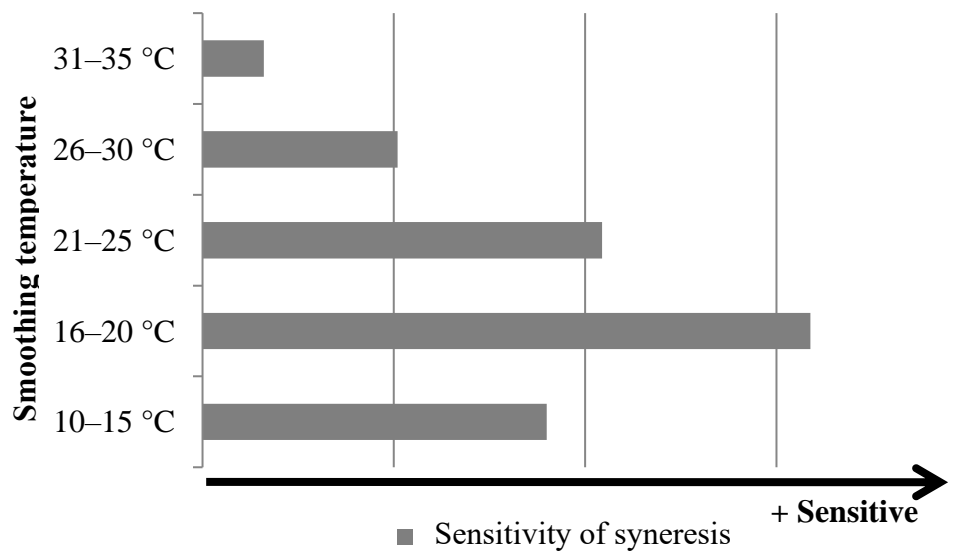


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481 **Fig. 5**

482



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