1   Characterization of syneresis phenomena in stirred acid milk gel using low frequency nuclear

2   magnetic resonance on hydrogen and image analyses.

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13 Abstract:

14 Water retention is an important quality attribute for yogurt. Classically, stirred yogurt water

15 retention is investigated using induced syneresis measurement (centrifugation), which does not

16 characterize spontaneous syneresis. Low-frequency nuclear magnetic resonance ([1]H-LF-

17 NMR) is a non-destructive technique to detect spontaneous syneresis. Experimental yogurt

18 from pasteurized skim milk, and commercial stirred yogurts were analyzed with ^{1}H-LF-NMR.

19 After Laplace's transformation of the signal, hydrogen atoms pools were differentiated

20 according to their mobility. Each hydrogen pool stood for a type of water mobility in the

21 matrices characterized by a relaxation time ($T_2(i)$), and a signal intensity ($I_2(i)$). Yogurt water

22 retention was assessed by induced syneresis and their structure was characterized using

23 microscopy. Low frequency ^{1}H-NMR detected four different water mobility groups in the

24 matrices. Among these, there was a signal from bulk water, and another attributed to the

25 separated serum (spontaneous syneresis). In experimental yogurts, spontaneous syneresis was

26 visible, resulting in induced syneresis higher than 50 %. Moreover, induced syneresis and

27 spontaneous syneresis detected by ^{1}H-LF-NMR were similar. In commercial yogurts, bulk

28 water mobility reduced with increasing protein content and protein network density. Induced

29 syneresis and bulk-water mobility correlated only in yogurts without gelatin. In the presence

30 of gelatin, the network was more open, probably favoring bulk water mobility. This study

31 shows that ^{1}H-LF-NMR associated with microscopy image analysis efficiently assesses and

32 describes yogurts water retention and spontaneous syneresis.

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34 Keywords: spontaneous syneresis, water-holding capacity, stirred yogurt, microstructure,

35 image analysis, time domain NMR.

36

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1. Introduction:

Milk gelation is responsible of the rheological transformation of milk from a Newtonian fluid to a semi-solid or solid product (Foegeding, Vardhanabhuti, & Yang, 2011). It is achieved through an enzymatic action, acidification or a combination of both. Yogurt is obtained using slow acidic gelation where casein micelles undergo changes that lead to the formation of a casein network entrapping serum (Dalgleish & Corredig, 2012). To simplify, gels can be considered as protein network in which serum is entrapped in pores. The serum can be released out of the network and form a serum layer that is unappealing to consumers. This phenomenon is called syneresis.

Syneresis depends on gel permeability (pore size and their interconnections) (R. Hinrichs, Götz, & Weisser, 2003) and the heterogeneity of the gel network (Lee & Lucey, 2006; Lee & Lucey, 2010; van Marle, 1998; Zoon, 2003). In stirred yogurts, the continuous protein network is disrupted by a smoothing step to produce microgels, which will re-associate during storage. A correlation between microgel size and syneresis is often mentioned in stirred yogurts, but studies report contradictory findings (Gilbert, Rioux, St-Gelais, & Turgeon, 2020; Körzendörfer, Temme, Schlücker, Hinrichs, & Nöbel, 2018; Küçükçetin, 2008; van Marle, 1998; Zhang, Folkenberg, Amigo, & Ipsen, 2016). Most of the differences can probably be explained by the shearing intensity of the fermented gel (Mokoonlall, Nöbel, & Hinrichs, 2016) and by the milk composition and the starter used (Hassan, 2008; Sodini, Remeuf, Haddad, & Corrieu, 2004). A reorganization of the protein network during storage also favors spontaneous syneresis (Lee, et al., 2006; Lee, et al., 2010; van Marle, 1998; Zoon, 2003). This leads to a decrease in the number of pores and an increase of their size to finally ease serum

60   expulsion from the gel (Lee, et al., 2010; J. A. Lucey, 2001; van Marle, 1998; van Vliet,

61   Lakemond, & Visschers, 2004) inducing syneresis and lowering water holding capacity

62   (WHC).

63   Multiple methods are available to investigate syneresis and most of them affect the yogurt

64   network integrity. In set-style yogurt, spontaneous syneresis can be measured by collecting the

65   exuded serum (John A. Lucey, Munro, & Singh, 1998). For stirred yogurt, syneresis induced

66   by centrifugation is often used, as reviewed by Sodini, et al. (2004). The result is largely

67   dependent on gel rigidity rather than gel WHC (J. A. Lucey, 2001). In literature, no

68   standardized protocol has been used, and centrifugation conditions differ in force, temperature

69   or duration (Sodini, et al., 2004), leading to diverging conclusions (Hassan, 2008). Another

70   limitation of this method is that it is not possible to distinguish spontaneous syneresis from

71   induced syneresis.

72   Low frequency nuclear magnetic resonance on protons (^{1}H-LF-NMR) is a non-destructive

73   technique to study water and fat inside complex matrices. ^{1}H-LF-NMR signals are analyzed

74   using discrete exponential fitting or mathematical transformation (continuous transformation)

75   to differentiate and characterize proton populations based on their mobility in the matrix. The

76   signal is decomposed in several pools described by their relaxation time (mobility) and their

77   relative signal intensity (proportion in the sample). Protons in water molecule will thus act as a

78   probe to discriminate different water mobility in the matrix. Discrete exponential fitting is

79   used to give a simple representation of water repartition without fitting the noise (Mariette,

80   Guillement, Tellier, & Marchal, 1996), but experimenters need to fix a minimum number of

81   pools with the risk to loose information (Mariette & Lucas, 2005; Mitchell, Gladden,

82   Chandrasekera, & Fordham, 2014; Peters, et al., 2016). Mathematical transformations such as

83   maximum entropy method (Mariette, et al., 1996) or Laplace transformation algorithms

84   (Mitchell, et al., 2014) transform ^{1}H-LF-NMR signals into spectra without any prior

85   assumption on the number of pools or their relaxation times (Tellier, Guillou‑Charpin, Le

86   Botlan, & Pelissolo, 1991). However, it should be mentioned that these transformation

87   methods can be sensitive to ghost peak from noise inside the signal decay and experimental

88   set-up (number of points recorded, recording duration, base-line quality…) (Mitchell, et al.,

89   2014; Tellier, et al., 1991). Yet, results are more consistent and better describe matrices

90   complexity (Tellier, Mariette, Guillement, & Marchal, 1993).

91   From studies on dairy protein solutions it has been demonstrated that at least one proton pool

92   interpreted as the proton mobility of bulk water localized between 100 and 1000 ms is found

93   in dairy matrices (Hills, Takacs, & Belton, 1990; Le Dean, Mariette, & Marin, 2004). A

94   second peak appearing at shorter relaxation times (1 to 100 ms depending on studies) has often

95   been mentioned, and it is sometimes referred as the proton exchanging between water and

96   protein (Hills, Takacs, & Belton, 1989; Hills, et al., 1990; Mok, Qi, Chen, & Ruan, 2008). In

97   dairy protein solutions, the bulk water mobility and the signal intensity due to proteins are

98   mainly controlled by the casein content (Le Dean, et al., 2004). In dairy gel, a strong

99   correlation between gel microstructures and ^{1}H-LF-NMR results is mentioned (Colsenet,

100   Mariette, & Cambert, 2005; Gianferri, Maioli, Delfini, & Brosio, 2007). Dairy gels tend to

101   display two to four pools depending on the signal decomposition used, their microstructure,

102   and their propension to syneresis (Gianferri, et al., 2007; Mok, et al., 2008; Møller, et al.,

103   2011). For example, one pool was attributed to serum separation from curd gel during cheese

104   ripening (Métais, Cambert, Riaublanc, & Mariette, 2006) or during yogurt drink

105   destabilization (Salomonsen, Sejersen, Viereck, Ipsen, & Engelsen, 2007). Water mobility

106   measured by ^{1}H-LF-NMR has already been used to characterize dairy gels syneresis (Ruth

107   Hinrichs, et al., 2004; R. Hinrichs, et al., 2003). In these studies, deuterium water was used to

108   perform a washout test to examine water entrapment in enclosed pore or in open capillaries.

109   However, no proton pool was attributed to specific water interactions inside the matrix. The

110 present study aimed to determine how ^{1}H-LF-NMR can be used to characterize and quantify

111 yogurt syneresis and how it relates with yogurt microstructure, specifically microgel sizes and

112 gel heterogeneity.

113

114 **2. Material and methods:**

115 *2.1. Dairy products*

116 Commercial pasteurized skim milk (around 3.6 % protein content and 8.4 % total solid) was

117 purchased in a local store. Skim milk permeate was obtained by ultrafiltration 10 kDa

118 ultrafiltration membrane (DESAL[TM] membrane PW2540F1074, GE Water & Process,

119 Oakville, ON, Canada) of commercial pasteurized skim milk. Native phosphocaseinate

120 powder was obtained from Ingredia (83.0 % casein content and 5.0 % whey protein content on

121 dry basis; Wapakoneta, OH, USA). Skim milk powder (**SMP**, 27.9 % casein content and 7.1

122 % whey protein content on dry basis), whey protein concentrate (**WPC**, 34.0 % whey protein

123 content on dry basis), whey protein isolate (**WPI**, 97.6 % whey protein content on dry basis),

124 and lactose were kindly donated by Agropur (Longueuil, QC, Canada). Dairy powder

125 compositions are described in Supplemented material (Table A.1).

126 Different commercial non-fat stirred yogurts were purchased between 8 and 40 days before

127 expiry date at a local store based on their protein content (10, 6, 5, 4 % named: Y10, Y6, Y5,

128 and Y4), the presence of polysaccharides (pectin, carob gum, carrageenan, starch; named as

129 Y4+P for example), or gelatin (identified with G in the name). The yogurts abbreviations and

130 compositions are described in Table 1.

131 *2.2. Protein solutions*

Native phosphocaseinate powder containing micellar caseins (**MC**) was solubilized in deionized water (**MC-W**) or in skim milk permeate (**MC-P**) to reach protein concentrations of 2, 4, 6 % (w/w). WPI was dissolved in permeate to reach concentrations of 0.5, 1, or 2 % (w/w). All solutions were stirred for at least 2 h at room temperature and stored at 4°C overnight. Heat treated WPI solutions (**WPI-HT**) were obtained by placing enclosed WPI aliquots into a boiling water bath for 15 min.

2.3. Milk formulated with dairy powders

Skim milk powder, whey protein concentrate, WPI, and lactose were mixed with water to prepare three formulations containing 14 % total solid and 3 % casein. Casein to whey protein ratios were adjusted to 1.5:1, 2.8:1, or 3.9:1. To obtained the different reconstituted milks, dairy ingredients were rehydrated overnight, homogenized (two-stage homogenizer, 137.8 and 34.5 bar) and heat treated at 95 °C, 5 min and cooled down quickly at 20 °C and stored at 4 °C overnight. Each condition was repeated three times.

2.4. Yogurt production

Experimental yogurts were designed from to be highly sensitive to syneresis. The commercial pasteurized skim milk was inoculated at 0.2 % with a commercial freeze-dried starter Yo-Dolce 1 (Biena, St-Hyacinthe, QC, Canada). The starter culture was fermented at 42 °C, and stored at 4 °C when the pH reached 4.6. The following day, 5 mL of starter were transferred into 200 mL of pre-warmed (43 °C) commercial pasteurized skim milk. Fermentation took place at 42 °C by aliquots of 20 mL in 50 mL falcon tubes, and 0.5 mL in ^{1}H-LF-NMR glass vials (see section 2.7). When pH reached 4.6 ± 0.05 (approximately 150 min), yogurt tubes and vials maintained at 42°C were separated in three groups: i) stirred 5 s at maximum power

156   with a vortex and placed at 4 °C (**YS42**), ii) immediately placed at 4 °C for two hours and then

157   vortexed for 5 s at maximum power (**YS4**), iii) immediately placed at 4 °C without stirring

158   (**YF**). Yogurt production was repeated twice.

159

160   *2.5. Gel structure and image analysis of yogurts and gels*

161   A homemade spacer of 0.175 ± 0.015 mm deep by 2.5×2.5 mm surface was built by sticking

162   coverslips on the sides of microscope slides using nail polish (Gilbert, et al., 2020).

163   Approximately 0.5 mL of stirred yogurt sample was spread gently on the homemade spacer.

164   Five random digital pictures (AM4515ZT4 Dino-Lite Edge Microscope, Dino, London, ON,

165   Canada) were taken at zoom 150 on the device. Each sample was analyzed in duplicate. It

166   allowed to visualize differences in gel density on the microscopic slides at the micrometer

167   scale (100 µm). With the digital camera, because the light does not go through the sample,

168   when a zone is whiter, the gel is denser. The whiter shapes on the images were assimilated to

169   aggregated clusters (gel fragments = microgels). Image analyses was used to count yogurt

170   microgels, and measure their surface areas on images (Gilbert, et al., 2020) using ImageJ2

171   software (https://imagej.net/ImageJ) (Rueden, et al., 2017). To differentiate particles from one

172   another, images were bipolarized according to the percentile threshold adjustment, and the

173   watershed process was applied. Particles on the border of images and under 200 μm^2

174   (detection limit measured using glass microspheres between 15 and 150 µm of diameter;

175   Quality Audit Standards, Malvern Ltd.) were not considered in the image analysis. Also,

176   protein network heterogeneity was determined using a method adapted from Küçükçetin,

177   Weidendorfer, and Hinrichs (2009) who studied stirred yogurt visual roughness. Briefly, they

178   studied gray level histogram distribution on randomly selected segments of images, while we

179   studied gray level histogram distribution on the total picture area. Moreover, they used images

180   at real scale, while we used a smaller scale (microscopic). In the present study, each pixel had

181 a value of gray representing the density of the protein network at that particular location on the

182 picture. When closer to 255 (white) this value represents a dense protein network at this

183 location. Conversely, dark pixel (close to 0) is associated with a loose protein network at that

184 location. A network heterogeneity index (**NHI**) which is equal to gray level variation on

185 pictures was defined (Gilbert, et al., 2020). Higher NHI represents a heterogeneous gel

186 network. Digital microscopic pictures were taken one day after production for the

187 experimental yogurt produced in the laboratory and between 8 and 40 days before the expiry

188 day for commercial yogurts.

189

190 *2.6. Induced syneresis*

191 Induced syneresis was measured at day one after production for the experimental yogurt

192 produced in the laboratory and between 8 and 40 days before the expiry day for commercial

193 yogurts. As described previously by Gilbert, et al. (2020), approximately 20 g of yogurt

194 samples were centrifuged at 238 g for 10 min at 10 °C (Eppendorf centrifuge 5804R V3.3,

195 Mississauga, ON, Canada; swinging bucket rotor Eppendorf Rotor A-4-44, Mississauga, ON,

196 Canada). The expulsed serum was carefully weighed and syneresis was calculated as the

197 percentage of serum expelled on the total yogurt weight.

198

199 *2.7. Low Frequency Nuclear Magnetic Resonance on Hydrogen (LF-^{1}H-NMR)*

200 For protein solutions (MC-P, PC-W, WPI, WPI-HT), reconstituted milks (caseins to whey

201 proteins ratio: 3.9, 2.8, 1.5), commercial pasteurized skim milk and commercial yogurts, five

202 to six drops of liquid samples were gently introduced into the ^{1}H-LF-NMR glass vials (height

203 ×OD = 40 × 8.2 mm; vial S1-W, Klaus Ziemer GmbH, Langerwehe, Germany) using a 3 ml

204 transfer pipette cut in diagonal and were closed using Teflon caps to prevent water loss.

205 Experimental yogurts were gelled and stirred (as described in section 2.4) *in situ* the [1]H-LF-

206 NMR glass vial preventing gel manipulation. LF-^{1}H-NMR was performed at day one after

207 production for protein solutions, reconstituted milk, and the experimental yogurt. Commercial

208 pasteurized skim milk and yogurts analyses was performed between 8 and 40 days before the

209 expiration date on the packaging.

210 NMR measurements were realized at 20 MHz in a Minispeq Mq20, using a 10 mm probe

211 (Bruker Optik GMbH, Rheinstretten, Germany) equipped with a variable temperature unit

212 BVT 3000 (Bruker Optik GMbH, Rheinstretten, Germany) to keep all at 4 °C. Proton

213 transversal relaxation decays were measured using the Carr-Purcell-Meiboom-Gill scan

214 sequence (CPMG) (32 consecutive scan, pulse separation (τ) = 0.5 ms, relaxation delay = 10

215 s). Each measurement was made in triplicate.

216 Laplace's transformation (CONTIN, Brucker, Milton, ON, Canada) was used to decompose

217 transversal signal decays. Data were fitted between 0.1 and 3000 ms generating a distribution

218 of relaxation time of the hydrogen in the matrix which refers to water mobility. In this

219 spectrum, each peak represented a pool of protons with its own mobility distribution.

220 Individual proton pools were described by a transversal relaxation time constant ($T_2(i)$), and a

221 relative intensity ($I_2(i)$). $T_2(i)$ was obtained using the peak position on the relaxation times

222 axis, while $I_2(i)$ was obtained using the area under the peak divided by the total area under the

223 curve (Han, Zhang, Fei, Xu, & Zhou, 2009).

224

225 *2.8. Experimental design and statistics*

226 Each experimental unit was repeated twice, except for reconstituted milks from dairy powders

227 that were repeated three times. Different data sets were generated depending on the structures

228 of the experimental units. 1) The effect of yogurt fermentation and stirring temperature on lab-

229 made yogurt induced syneresis and ^{1}H-LF-NMR measurements was assessed using a

230 randomized experimental design (2 repetitions); 2) The effect of micellar casein

231 concentrations and the dispersant (water or permeate) was assessed using a factorial design (2

232 repetitions); 3) The effect of whey protein concentrations and the heat treatment in WPI

233 solutions was assessed using a randomized split-plot design using WPI concentration as the

234 main plot and the heat treatment as the second plot with two repetitions (2 repetitions); 4) The

235 effect of casein to whey protein ratio in reconstituted milk was assed using a randomized

236 experimental design (3 repetitions); 5) The differences between commercial yogurts was assed

237 using randomized experimental design (2 repetitions).

238 Statistical analyses were carried out using the mixed procedure in the SAS software (SAS

239 Institute. release 9.4, Cary, NC, USA). Significant difference level was fixed at $p < 0.05$.

240 Normality assumption was verified using Shapiro Wilks' statistic, the residual plot was used to

241 look at variances homogeneity. Results are reported as the means $\pm$ standard error (SE).

242 Correlations between variables into the commercial stirred yogurt's data were also estimated

243 using Pearson's correlation coefficients.

244

245 **3. Results and discussion**

246 *3.1. Relationship between yogurt syneresis and ^{1}H-LF-NMR relaxation time distribution*

247 Yogurt manufacturing (use of commercial pasteurized skim milk and fast acidification) was

248 designed to ensure poor water holding capacities of the products. It translated as visible water

249 separation in the yogurts and induced syneresis values over 50 %. The induced syneresis

250 increased from 54.0 ± 0.7 (YF) to 62.5 ± 0.7 % (YS4) when the yogurt was stirred, this effect

251 being more pronounced when stirred at higher temperature (YS42, 69.0 ± 0.7 %, $p < 0.05$).

252 All yogurts presented four peaks in their transversal relaxation time distribution (Figure 1).

253 The two first populations ($T_2(1)$, $T_2(2)$ (< 130 ms) represented between 6 to 10 % of the total

254 signal and were not statistically different among the three yogurts (YF, YS4, YS42). $T_2(3)$

255 (260 ms), and $T_2(4)$ (1093 ms) explained together 90 to 94 % of the total signal. Although

256 $T_2(3)$ was similar for the three yogurts, the relative signal intensity, $I_2(3)$ of this peak changed

257 significantly ($p < 0.05$; Figure 1 insert A). Compared to YF, $I_2(3)$ decreased by 9 % and by 28

258 % when yogurt was respectively stirred at 4 °C and 42 °C (Figure 1 insert A). Simultaneously,

259 the fourth signal peak increased and included a larger range of relaxation times inducing an

260 increase of $T_2(4)$. $I_2(4)$ values increased from 4.5 % of the signal intensity for YF to 11 % for

261 YS4, and 33 % for YS42 (Figure 1 insert B) which corresponded to visual observations of

262 serum separation in yogurts tubes.

263 Microscopic observations of stirred yogurt (Figure 1 insert C) show the structures of YS4 and

264 YS42. YS4 is more homogeneous (light gray to white area on images) with fewer microgels

265 protein aggregates (denser white areas on images) and smaller pores (black areas on images)

266 than YS42. This gel was highly heterogeneous with large areas empty of network and areas of

267 dense compact network, which made the protein network mostly segregated from the serum.

268 YS42 higher induced syneresis, its heterogeneous open structure, and its higher $I_2(4)$ all

269 seemed correlated. Classically, in all dairy systems, one pool of water corresponding to the

270 mobility of bulk water is found between 100 and 1000 ms representing at least 50 % of the

271 signal (Gianferri, et al., 2007; Le Dean, et al., 2004; Møller, et al., 2011). The presence of a

272 population similar to $T_2(4)$ ($T_2(i)$ around or higher than 1000 ms) has often been reported in

273 gelled dairy systems. Métais, et al. (2006), Salomonsen, et al. (2007) or Peters, et al. (2016)

274 have shown the apparition of such a proton pool from expelled serum and water phase

275 separation (syneresis) with rennet gel, acidified milk drinks, and centrifuged solutions of

276 microparticulated whey proteins. But, to our knowledge, this peak has never been used to

277 quantify spontaneous syneresis yet. In the present work, induced syneresis was important (>

278 50 %), and is probably mainly explained by spontaneous syneresis since the gels were

279 formulated to be unstable and have a low water retention capacity. Moreover, microstructure is

280 known to influence syneresis (induced or spontaneous). Gilbert, et al. (2020) already linked

281 higher induced syneresis with heterogeneous structure of stirred yogurts. Thus, the

282 correspondence between induced syneresis results, network heterogeneity and the $I_2(4)$

283 suggests that $I_2(4)$ can be used to quantify spontaneous syneresis in yogurts.

284 As mentioned, between the three yogurt types no differences were seen in the two first proton

285 populations or the relaxation times of the bulk $T_2(3)$. As for $I_2(4)$, those peaks have never been

286 used to understand syneresis in the yogurt, and the signification of $T_2(1)$ and $T_2(2)$ remains

287 unclear in the literature. Both $I_2(1)$ and $I_2(2)$ values were below 10 % of the total signal.

288 According to Mitchell, et al. (2014) when analyzing results from mathematical transformation,

289 caution has to be taken with peaks representing less than 10 % of the signal intensity. They

290 might be artifacts from noise, mathematical transformation or insufficient signal time

291 recording, unless there is a physical reason justifying their existence. Also, when such peaks

292 appear, repeating the transformation with a higher fitting range can be used to test their real

293 existence. If these peaks are artifacts they will be displaced at the new fitting extremities, in

294 which case they should not be analyzed (Mitchell, et al., 2014). In the present work, no peak

295 moved when different fitting ranges were applied (results not shown). Artifacts from noise can

296 be avoided by sufficient number of scan, and insuring that the recordings include a baseline

297 three times longer than the decay (Bruker, 2006), which was the case in the present study.

298 Moreover, numerous studies on different food matrices in literature observed peaks at similar

299 relaxation times with intensities varying from 1 to 20 % using continuous transformation

300 (Han, Wang, Xu, & Zhou, 2014; Métais, et al., 2006; Møller, et al., 2011; Peters, et al., 2016;

301 Salomonsen, et al., 2007; Tananuwong & Reid, 2004). In the next sections other dairy systems

13

302   were studies using Laplace transformation in order to get more insights on the meaning of the

303   first peaks and see if they can also be used to predict syneresis in stirred yogurts.

304

305   *3.2. Comparison between ^{1}H-LF-NMR results of milk and yogurt*

306   *3.2.1. Effect of milk acidification on transversal relaxation time distribution*

307   The relaxation time distribution of commercial pasteurized skim milk presented two peaks

308   compared to four peaks for the firm yogurt. For both $T_2(1)$ or $I_2(1)$, no differences were

309   detectable between milk and yogurt (Figure 2-A). In yogurt, a peak appeared around 67 ms

310   and represented 1,0 % of the total signal. The third peak $T_2(3)$ in yogurt observed at a similar

311   relaxation time to the second peak of the milk. As its relaxation time is over 100 ms and its

312   signal relative intensity is over 60 %, it can be attributed to the bulk water mobility mentioned

313   earlier. Their relative intensities were both around 90 % of the total signal and yogurts' bulk

314   water mobility relaxation time was longer than the one of milk of milk ($p < 0.5$ - Figure 2-B).

315   Finally, the fourth peak detected over 1000 ms in yogurts was associated as the spontaneous

316   syneresis signal as described in section 3.1.

317   The evolution of the bulk water mobility was expected since in literature whatever NMR

318   probe (^{1}H or [17]O) or signal decomposition used, the bulk water mobility relaxation time

319   increases with acidification (Mariette, Tellier, Brule, & Marchal, 1993; Mok, et al., 2008;

320   Møller, et al., 2011; Torres, Mutaf, Larsen, & Ipsen, 2016) due to phenomena such as κ-casein

321   collapsing or casein micelle decalcification. It means that ^{1}H-LF-NMR is suitable to observe

322   protein hydration change during acidification (Mariette, 2003). However, under pH 5.5, other

323   phenomena linked to water mobility inside the gel start to influence ^{1}H-LF-NMR results

324   (Møller, et al., 2011). Møller, et al. (2011) used a continuous signal transformation and found

325   similar results. The only differences with the present study was that the $T_2(2)$ was detected in

both milk and yogurt, while it was found only in yogurt here and this may be related to higher solid content in the milk studied (15 and 25%) compared to 8% in the commercial milk studied here.

The $T_2(1)$, $T_2(2)$ and $T_2(3)$ proton populations have been described in many studies on dairy protein solutions (Table 2). The peak with higher relative signal intensity ($I_2(3)$) was interpreted as the mobility of protons in water molecules from bulk water; *i.e.* the water moving around macromolecules. However, there is no consensus on $T_2(1)$ or $T_2(2)$ meanings. Depending on studies, $T_2(1)$ is interpreted either as the protons from the water hydrating protein (bound water), the exchangeable protons of proteins, or the non exchangeable protons of proteins detected because of a possible signal maladjustment (Table 2). $T_2(2)$, which was detected when using continuous signal transformation, had no interpretation suggested in literature. Only few studies used the Laplace transformation (CONTIN) on dairy products mentioning the evolution of $T_2(2)$. Therefore, in the next section model dairy protein solutions were investigated, to get a better insight on the $T_2(2)$ using the Laplace's transformation.

3.2.2. Effect of protein type and concentration of dairy protein on $T_2(2)$

Casein and whey protein solutions presented two or three proton populations. In this section, focus is made on the second proton population and results related to other populations can be found in the Supplemented material (Figure A.1; Figure A.2). Water mobility of the micellar casein (MC) displayed three relaxation time peaks. $T_2(2)$ and its relative signal intensity $I_2(2)$ depended on MC concentration only ($p < 0.05$). The dispersant used to dilute MC solutions (water or permeate) had no influence on the second hydrogen population. $T_2(2)$, was only found in solution with 2 and 4% of MC and increased with decreasing MC concentration (Figure 3 A). Similarly, $I_2(2)$ peak intensity significantly decreased with increasing MC

350   concentration and above 4 % MC no peak was detected (Figure 3 B). In WPI solutions,

351   concentration affected $T_2(2)$ and $I_2(2)$ only with the heat-treated samples (WPI-HT $p < 0.05$)

352   and both $T_2(2)$ and $I_2(2)$ values were reduced with the highest WPI concentration (Figure 3 C,

353   D). In standardized milk, the CN:WP ratio (1.5, 2.8, 3.9) did not change ^{1}H-LF-NMR results

354   (data not shown). Only two proton pools were found, $T_2(1)$ and $T_2(3)$.

355   To summarize, the second peak is observed in MC solutions at concentrations below 6 %, in

356   heat treated whey proteins solutions but not in complex mixes such as milk, and with different

357   amounts of added whey protein. Based on the peak observed in MC solutions in the range of

358   concentrations found in milk a $T_2(2)$ peak would be expected. However milk has been heat

359   treated under conditions allowing whey protein interactions with the casein micelle to improve

360   water retention ability in acidified gels (Cayot, Fairise, Colas, Lorient, & Brulé, 2003; Sodini,

361   et al., 2004). The reduction in intensity observed after heat treatment of WPI confirms a role

362   for protein aggregation on water mobility in dairy systems. The fact that milk protein

363   composition (ratios) did not induce additional peaks including $T_2(2)$ suggests that in a complex

364   mixture as milk, water behavior is mainly explained by the $T_2(3)$ peak corresponding to bulk

365   water mobility. Previous attempts to interpret the $T_2(2)$ peak were inconclusive. In the present

366   study, this peak has been observed only in low protein content dairy solutions and

367   experimental yogurt gels. Even though the signal intensity $I_2(2)$ did not rise above 10 %, both

368    $T_2(2)$ and $I_2(2)$ evolved with protein concentration, aggregation and gelation. It strongly

369   suggests that $T_2(2)$ is not an artifact from the mathematical transformation. Moreover, the

370   experimental yogurt gels in which this pool appeared were highly heterogeneous and sensitive

371   to syneresis. In previous work, Gilbert, et al. (2020) observed that higher microstructural

372   heterogeneity of stirred yogurt gel was related to higher induced syneresis results. Possibly, as

373   for syneresis, $T_2(2)$ may be related to some structural features of yogurt gels such as microgel

374   sizes or gel heterogeneity (NHI).

375

3.3. General pattern and number of peaks

The general pattern of the ^{1}H transversal relaxation time distribution obtained by Laplace

transformation with yogurt is summarized in Figure 4. The first pool meaning is not clearly

identified; it could be the non-exchangeable protons of protein or water protons interacting

with proteins. The second pool $T_2(2)$ not present in all ^{1}H-LF-NMR study depends on the

model used to fit the signal decays. The use of a discrete exponential fitting method of milk

and yogurts leads most of the time to mono- or bi-exponential decays. Laplace's

transformation allows detection of $T_2(2)$ peak in yogurt, but no explanation was found in

literature and our results show that it is dependent on protein content. This pool disappeared

with high micellar casein concentration. $T_2(3)$ is commonly interpreted as the signal from bulk

water in gel or solutions (Colsenet, et al., 2005; Le Dean, et al., 2004; Mariette, et al., 1993;

Mok, et al., 2008; Møller, et al., 2011; Venu, Denisov, & Halle, 1997). Finally, $T_2(4)$

represents water protons in water exuded from gel (Métais, et al., 2006; Peters, et al., 2016;

Salomonsen, et al., 2007) and the results obtained from experimental stirred yogurts suggests

that its relative intensity can be used to quantify spontaneous syneresis.

In order to be able to estimate the application potential of this method in more complex

systems the next section presents ^{1}H-LF-NMR, induced syneresis and image analysis results

for commercial yogurts.

394

3.4. Potential use of 1H-LF-NMR to predict commercial yogurt syneresis

Commercial stirred yogurts induced syneresis varies from 1.4 to 14.4 % depending on the type

of yogurt ($p < 0.5$; Figure 5-A). Yogurt with high protein content (Y10) was among the

samples with the lowest syneresis values. Syneresis increased with lower protein content (Y6)

399   even when polysaccharides were used as stabilizers (Y5P and Y4P). The combination of

400   gelatin and polysaccharides resulted in very low values of induced syneresis. This is in

401   agreement with previous studies reporting that higher protein content and gelatin addition

402   significantly reduce yogurt syneresis (induced or spontaneous) (Fiszman, Lluch, & Salvador,

403   1999; Keogh & O'Kennedy, 1998; Pang, Deeth, Prakash, & Bansal, 2016; Sodini, et al., 2004).

404   Structural organizations of yogurt gels were probed using microscopy (Figure 5-E). As Y10

405   was too thick to be spread in the spacer used for sample preparation, it was not analyzed.

406   Microgels (dense white shapes) and serum (black areas) can be distinguished. This type of

407   microstructure has already been characterized by Gilbert, et al. (2020) and yogurt was

408   previously described as microgels suspension by Zoon (2003), Mokoonlall, et al. (2016).

409   Image analysis allowed quantifying microgel sizes and heterogeneity in the gel. The type of

410   yogurt impacted microgel surface and gel heterogeneity index (p <0.05; Figures 5-C and D).

411   Y4P had the largest microgels (3400 µm^2), and yogurts with gelatin had the smallest microgels

412   (2200 µm^2). Pang, Deeth, Sharma, and Bansal (2015) reported an increase dairy gel porosity

413   probed by confocal microscopy when gelatin was added into a yogurt formulation at 4.5 %

414   protein content. Previously, Fiszman, et al. (1999) described the microstructure of yogurts (10

415   or 15% SMP) with gelatin as a suspension of aggregated caseins into a gelatin gel. Yogurts at

416   4 % protein content (Y4P) presented more open and heterogeneous structure (more dark

417   regions, high NHI values). Y4P had a highly heterogeneous structure with large dense

418   microgels (highest NHI value). Higher protein content resulted in intermediate microgel sizes

419   and a homogeneous structure (low NHI). In table 3, Pearson's correlations between the

420   different measured characteristics of yogurts are presented. A correlation of 0.78 (Table 3) was

421   found between induced syneresis and microgel surface (Figure 5 A and C) in accordance with

422   similar behaviors reported by both Körzendörfer, et al. (2018) and Gilbert, et al. (2020).This

423   set of commercial products showcases different gel structures and water holding capacities and

424 has therefore been used to test the ability of the ^{1}H-LF-NMR method to probe water mobility

425 in yogurt.

426 Commercial yogurts presented three to four proton pools. The relaxation time of the first peak

427 $T_2(1)$ was similar for all yogurt types (data not shown). The second peak was present only in

428 yogurt at 4 % protein content (Y4P; Y4PG-1, Y4PG-2) as observed with MC solutions. $T_2(2)$

429 was similar among those yogurts ($T_2(2)$ = 49 ms, data not shown). The presence of stabilizers

430 influenced the relative signal intensity $I_2(2)$ (p <0.5; data not shown) which was about 1.6 % in

431 Y4PG-1 and Y4PG-2, while it was about 4.8 % in Y4P. The type of yogurt also significantly

432 impacted the bulk water mobility, $T_2(3)$ (p <0.5; Figure 5-B) and its relative signal intensity

433 $I_2(3)$ (p <0.5; data not shown). $I_2(3)$ was higher than 85 % in all samples. Bulk water mobility,

434 $T_2(3)$, decreased with increasing protein content of yogurts (Y10 < Y6 ≈ Y5 <Y4) and yogurts

435 containing 4% protein and stabilizers showed higher bulk water mobility. Interestingly, a

436 correlation was observed between $T_2(3)$ and induced syneresis in yogurt without gelatin (0.92,

437 Table 3). Commercial yogurt formulations included a mixture of stabilizers as carrageenan,

438 carob, pectin and starch (Table 1) used in unknown concentrations. However, a different

439 behavior is observed in yogurt formulations containing gelatin as high $T_2(3)$ values were not

440 associated with high syneresis values. Gelatin is known to have a high impact on water

441 retention in yogurt (Fiszman, et al., 1999; Pang, et al., 2016; Sodini, et al., 2004). Furthermore,

442 the difference in water retention in absence of gelatin is confirmed with the presence of a

443 proton pool with a relaxation time over 1000 ms ($T_2(4)$) and 0.1 % signal intensity ($I_2(4)$) for

444 Y10, Y6, Y5P and Y4P. $T_2(4)$ and $I_2(4)$ were not influenced by the yogurt type studied ($T_2(4)$=

445 1218 ms; $I_2(4)$= 0.6 %, data not shown). Conversely to experimental yogurt results (section

446 3.1) suggesting that $I_2(4)$ may help to quantify spontaneous syneresis it did not correlate with

447 induced syneresis in commercial yogurts (Table 3). Induced syneresis values of commercial

448 yogurts were below 15 % compared to values larger than 50% for experimental yogurts. $I_2(4)$

449 values (data not shown) were variable and not significantly different between samples. It is

450 possible that commercial stirred yogurts had very few or no expelled serum. A correlation of

451 0.79 ($p < 0.0001$) was also found between bulk water mobility relaxation time $T_2(3)$ and NHI

452 (Table 3) meaning that yogurt with less protein, more open structures and heterogeneous

453 density (high NHI) had the highest bulk water mobility. Therefore, $T_2(3)$ would be a good

454 index of bulk water mobility inside the gel depending on microstructure.

455 In commercial yogurt samples, only yogurts at 4 % of protein content induced a $T_2(2)$ peak.

456 As mentioned earlier, the value of $I_2(2)$ from the Y4P yogurt was more than twice higher than

457 the two others. When looking at pictures and their corresponding NHI there might be a link

458 between $I_2(2)$ and NIH which is confirmed by a correlation of 0.98 (Table 3). Heterogeneous

459 structure is observed for all three yogurts where higher NIH values are indicating more open

460 structures with visible separations between microgels and serum. Higher $I_2(2)$ found in Y4P

461 could be due to the presence of larger areas with low protein concentration in the stirred gel

462 (darker zones).

463 To summarize, considering the correlations between microstructural descriptors, the

464 characteristics of the pools of water mobility $T_2(2)$ and $T_2(3)$, and induced syneresis, there is

465 great potential for the microscopic image analysis and the ^{1}H-LF-NMR methods to be used as

466 predictors of yogurt syneresis. Image analysis permitted to characterize the gel heterogeneity

467 at a macroscopic level, which was correlated to the $T_2(3)$ values and the presence of a $T_2(2)$.

468 Syneresis was also correlated to microgel surface and $T_2(3)$ values. Therefore, microgel

469 surfaces and $T_2(3)$ could be used to characterize water holding capacity. $T_2(2)$ and $I_2(2)$ are

470 related to yogurt protein content and gel heterogeneity.

471 In the future, larger experiment should be planned to build a predictive model (e.g.: partial

472 least square regression) of syneresis based on $T_2(3)$, microgel surface area, and NHI.

473   Also, results showed that gelatin plays a different role on water mobility compared to

474   polysaccharides. Even though $T_2(3)$ of yogurt with gelatin were among the highest, the

475   induced syneresis results were the lowest. Moreover, the fourth peak ($T_2(4)$), the expelled

476   water pool disappeared from the spectrum. Image analysis and ^{1}H-LF-NMR techniques were

477   able to discriminate the yogurt with gelatin from all other yogurts. However, in the future

478   more work is needed to understand how gelatin modifies the water mobility inside the yogurt

479   and how it relates with yogurt microstructure.

480   For the first time microstructural descriptors of yogurt gel and water mobility measurements

481   were correlated, which provided an improved understanding of 1H-LF-NMR data obtained by

482   Laplace's transformation. Moreover $T_2(2)$ was related to structural features of stirred yogurt

483   gels.

484   **4. Conclusion**

485   LF-^{1}HNMR has already been identified as a method of interest to understand water behavior

486   in dairy systems from milk to gel. Depending on the mathematical model used to fit magnetic

487   signal decays, findings differed slightly. The present study highlights the use of Laplace's

488   transformation to provide structural information of dairy matrices as gel heterogeneity. It also

489   allows distinguishing between spontaneous syneresis and bulk water mobility inside the gel.

490   Signals were influenced by composition, presence of protein aggregates, and gel network

491   structures. Four proton pools were found in yogurts, and the last two pools (peaks) were

492   related to induced syneresis and spontaneous syneresis. $T_2(3)$ indicates bulk water mobility in

493   network, which depended on serum entrapment (water holding capacity of the gel). It mainly

494   explained induced syneresis of commercial yogurts. The fourth proton, $T_2(4)$, resulted from

495   spontaneous syneresis, and its relative signal intensity, $I_2(4)$ could be used to quantify

496   spontaneous syneresis.

497   Both the sample composition and the gel microstructure influenced the transversal relaxation

498   time distribution. However, ^{1}H-LF-NMR in combination with digital microscopy image

499   analysis, allows to characterize gels microstructure since data between image analysis and

500   low-frequency NMR correlate quite well. ^{1}H-LF-NMR and image analysis could be method to

501   predict syneresis behaviors of yogurt by differentiating serum mobility inside the gel and

502   spontaneous syneresis, which is a promising asset. The method is less destructive than the

503   classical centrifugation method, the sample stays intact during measurements. Consequently,

504   measurements could be realized repeatedly during storage on the same sample. Moreover, [1]H-

505   LF-NMR has potential for development in an in-line installation for industries with the

506   arrivals of new sensor (NMR-MOUSE) and image analysis is quick and affordable.

507

508   Acknowledgments

514

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516   References

517   Bruker. (2006). The minspec users guide: using inverse Laplace transformation for relaxation
518   studies with the minispec. In (1.0 ed.). Rheinstetten Germany.

519   Cayot, P., Fairise, J.-F., Colas, B., Lorient, D., & Brulé, G. (2003). Improvement of
520   rheological properties of firm acid gels by skim milk heating is conserved after stirring.
521   *J. Dairy Res., 70*(4), 423–431.

522   Colsenet, R., Mariette, F., & Cambert, M. (2005). NMR relaxation and water self-diffusion
523   Studies in Whey protein solutions and gels *J. Agric. Food Chem., 53*(17), 6784-6790.

524   Dalgleish, D. G., & Corredig, M. (2012). The structure of the casein micelle of milk and Its
525   changes during processing. *Annu. Rev. Food Sci. Technol., 3*, 449-467.

526   Fiszman, S. M., Lluch, M. A., & Salvador, A. (1999). Effect of addition of gelatin on
527   microstructure of acidic milk gels and yoghurt and on their rheological properties. *Int.
528   Dairy J., 9*(12), 895-901.

529   Foegeding, A., Vardhanabhuti, B., & Yang, X. (2011). Dairy systems. In *Practical food
530   rheology: An interpretative approach* (pp. 133-172): Wiley Online Library.

531   Gianferri, R., Maioli, M., Delfini, M., & Brosio, E. (2007). A low-resolution and high-
532   resolution nuclear magnetic resonance integrated approach to investigate the physical
533   structure and metabolic profile of Mozzarella di Bufala Campana cheese. *Int. Dairy J.,
534   17*(2), 167-176.

535   Gilbert, A., Rioux, L. E., St-Gelais, D., & Turgeon, S. L. (2020). Studying stirred yogurt
536   microstructure using optical microscopy: How smoothing temperature and storage time
537   impact microgel sizes related to syneresis. *J. Dairy Sci., 103*, 2139–2152.

538   Han, M., Wang, P., Xu, X., & Zhou, G. (2014). Low-field NMR study of heat-induced
539   gelation of pork myofibrillar proteins and its relationship with microstructural
540   characteristics. *Food Res. Int., 62*, 1175-1182.

541   Han, M., Zhang, Y., Fei, Y., Xu, X., & Zhou, G. (2009). Effect of microbial transglutaminase
542   on NMR relaxometry and microstructure of pork myofibrillar protein gel. *Eur. Food
543   Res. Technol., 228*(4), 665-670.

544   Hassan, A. N. (2008). ADSA Foundation Scholar Award: Possibilities and challenges of
545   exopolysaccharide-producing lactic cultures in dairy foods. *J. Dairy Sci., 91*(4), 1282-
546   1298.

547   Hills, B. P., Takacs, S. F., & Belton, P. S. (1989). The effects of proteins on the proton N.M.R.
548   transverse relaxation times of water. *Mol. Phys., 67*(4), 903-918.

549 Hills, B. P., Takacs, S. F., & Belton, P. S. (1990). A new interpretation of proton NMR
550   relaxation time measurements of water in food. *Food Chem., 37*(2), 95-111.

551 Hinrichs, R., Götz, J., Noll, M., Wolfschoon, A., Eibel, H., & Weisser, H. (2004).
552   Characterisation of the water-holding capacity of fresh cheese samples by means of
553   low resolution nuclear magnetic resonance. *Food Res. Int., 37*(7), 667-676.

554 Hinrichs, R., Götz, J., & Weisser, H. (2003). Water-holding capacity and structure of
555   hydrocolloid-gels, WPC-gels and yogurts characterised by means of NMR. *Food
556   Chem., 82*(1), 155-160.

557 Keogh, M. K., & O'Kennedy, B. T. (1998). Rheology of stirred yogurt as affected by added
558   milk fat, protein and hydrocolloids. *J. Food Sci., 63*(1), 108-112.

559 Körzendörfer, A., Temme, P., Schlücker, E., Hinrichs, J., & Nöbel, S. (2018). Vibration-
560   induced particle formation during yogurt fermentation–Effect of frequency and
561   amplitude. *J. Dairy Sci., 101*(5), 3866–3877.

562 Küçükçetin, A. (2008). Effect of heat treatment and casein to whey protein ratio of skim milk
563   on graininess and roughness of stirred yoghurt. *Food Res. Int., 41*(2), 165–171.

564 Küçükçetin, A., Weidendorfer, K., & Hinrichs, J. (2009). Graininess and roughness of stirred
565   yoghurt as influenced by processing. *Int. Dairy J., 19*(1), 50–55.

566 Le Dean, A., Mariette, F., & Marin, M. (2004). 1H nuclear magnetic resonance relaxometry
567   study of water state in milk protein mixtures. *J. Agric. Food Chem., 52*(17), 5449-
568   5455.

569 Lee, W.-J., & Lucey, J. A. (2006). Impact of gelation conditions and structural breakdown on
570   the physical and sensory properties of stirred yogurts. *J. Dairy Sci., 89*(7), 2374–2385.

571 Lee, W.-J., & Lucey, J. A. (2010). Formation and physical properties of yogurt. *Asian-Aust. J.
572   Anim. Sci., 23*(9), 1127–1136.

573 Lucey, J. A. (2001). The relationship between rheological parameters and whey separation in
574   milk gels. *Food Hydrocoll., 15*(4-6), 603-608.

575 Lucey, J. A., Munro, P. A., & Singh, H. (1998). Whey separation in acid skim milk gels made
576   with glucono‐δ‐lactone: effects of heat treatment and gelation temperature. *J.
577   Texture Stud., 29*(4), 413-426.

578 Mariette, F. (2003). NMR Relaxometry and MRI for food quality control: Application to dairy
579   products and processes. In P. S. Belton, A. M. Gil, G. A. Webb & D. Rutlege (Eds.),
580   (RSCpublishing ed., pp. 209-221). Cambridge, UK: The Royal Society of Chemistry.

581 Mariette, F., Guillement, J. P., Tellier, C., & Marchal, P. (1996). Chapter 10 Continuous
582   relaxation time distribution decomposition by mem. In D. N. Rutledge (Ed.), *Data
583   Handling in Science and Technology* (Vol. 18, pp. 218-234): Elsevier.

Mariette, F., & Lucas, T. (2005). NMR signal Aanalysis to attribute the components to the solid/liquid phases present in mixes and ice creams. *J. Agric. Food Chem., 53*(5), 1317-1327.

Mariette, F., Tellier, C., Brule, G., & Marchal, P. (1993). Multinuclear NMR study of the pH dependent water state in skim milk and caseinate solutions. *J. Dairy Res., 60*(02), 175-188.

Métais, A., Cambert, M., Riaublanc, A., & Mariette, F. (2006). Influence of fat globule membrane composition on water holding capacity and water mobility in casein rennet gel: A nuclear magnetic resonance self-diffusion and relaxation study. *Int. Dairy J., 16*(4), 344-353.

Mitchell, J., Gladden, L. F., Chandrasekera, T. C., & Fordham, E. J. (2014). Low-field permanent magnets for industrial process and quality control. *Prog. Nucl. Magn. Reson. Spectrosc., 76*, 1-60.

Mok, C., Qi, J., Chen, P., & Ruan, R. (2008). NMR relaxometry of water in set yogurt during fermentation. *Food Sci. Biotechnol., 17*(5), 895-898.

Mokoonlall, A., Nöbel, S., & Hinrichs, J. (2016). Post-processing of fermented milk to stirred products: Reviewing the effects on gel structure. *Trends Food Sci. Technol., 54*, 26–36.

Møller, S. M., Whittaker, A. K., Stokes, J. R., Gidley, M. J., Andersen, U., & Bertram, H. C. (2011). Molecular Water Motions of Skim Milk Powder Solutions during Acidification Studied by17O and1H Nuclear Magnetic Resonance and Rheology. *J. Agric. Food Chem., 59*(18), 10097-10103.

Pang, Z., Deeth, H., Prakash, S., & Bansal, N. (2016). Development of rheological and sensory properties of combinations of milk proteins and gelling polysaccharides as potential gelatin replacements in the manufacture of stirred acid milk gels and yogurt. *J. Food Eng., 169*, 27-37.

Pang, Z., Deeth, H., Sharma, R., & Bansal, N. (2015). Effect of addition of gelatin on the rheological and microstructural properties of acid milk protein gels. *Food Hydrocoll., 43*, 340-351.

Peters, J. P. C. M., Vergeldt, F. J., Van As, H., Luyten, H., Boom, R. M., & van der Goot, A. J. (2016). Time domain nuclear magnetic resonance as a method to determine and characterize the water-binding capacity of whey protein microparticles. *Food Hydrocolloids, 54, Part A*, 170-178.

Rueden, C. T., Schindelin, J., Hiner, M. C., DeZonia, B. E., Walter, A. E., Arena, E. T., & Eliceiri, K. W. (2017). ImageJ2: ImageJ for the next generation of scientific image data. *BMC Bioinformatics, 18*(1), 529.

619 Salomonsen, T., Sejersen, M. T., Viereck, N., Ipsen, R., & Engelsen, S. B. (2007). Water
620   mobility in acidified milk drinks studied by low-field 1H NMR. *Int. Dairy J., 17*(4),
621   294-301.

622 Sodini, I., Remeuf, F., Haddad, S., & Corrieu, G. (2004). The relatives effect of Milk Base,
623   starter, and process on Yogurt texture: A revew. *Crit. Rev. Food Sci. Nutr., 44*, 113–
624   137.

625 Tananuwong, K., & Reid, D. (2004). DSC and NMR relaxation studies of starch-water
626   interactions during gelatinization. *Carbohydr. Polym., 58*(3), 345-358.

627 Tellier, C., Guillou‑Charpin, M., Le Botlan, D., & Pelissolo, F. (1991). Analysis of
628   low‑resolution, low‑field NMR relaxation data with the Padé‑Laplace method.
629   *Magn. Reson. Chem., 29*(2), 164-167.

630 Tellier, C., Mariette, F., Guillement, J. P., & Marchal, P. (1993). Evolution of water proton
631   nuclear magnetic relaxation during milk coagulation and syneresis: Structural
632   implications. *J. Agric. Food Chem., 41*(12), 2259-2266.

633 Torres, I. C., Mutaf, G., Larsen, F. H., & Ipsen, R. (2016). Effect of hydration of
634   microparticulated whey protein ingredients on their gelling behaviour in a non-fat milk
635   system. *J. Food Eng., 184*, 31-37.

636 van Marle, M. E. (1998). *Structure and rheological properties of yoghurt gels and stirred
637   yoghurts.* Universiteit Twente, Enschede, Netherlands.

638 van Vliet, T., Lakemond, C. M. M., & Visschers, R. W. (2004). Rheology and structure of
639   milk protein gels. *Curr. Opin. Colloid Interface Sci., 9*(5), 298-304.

640 Venu, K., Denisov, V. P., & Halle, B. (1997). Water 1H Magnetic Relaxation Dispersion in
641   Protein Solutions. A Quantitative Assessment of Internal Hydration, Proton Exchange,
642   and Cross-Relaxation. *J. Am. Chem. Soc., 119*(13), 3122-3134.

643 Zhang, L., Folkenberg, D. M., Amigo, J. M., & Ipsen, R. (2016). Effect of exopolysaccharide-
644   producing starter cultures and post-fermentation mechanical treatment on textural
645   properties and microstructure of low fat yoghurt. *Int. Dairy J., 53*, 10–19.

646 Zoon, P. (2003). Viscosity, smoothness and stability of yogurt as affected by structure and
647   EPS functionality. In *Fermented Milk.* (pp. 280–289). Brussels, Belgium: International
648   Dairy Federation.

649

650

651

Figure Legend

653

654 Figure 1: Transversal relaxation time distribution of yogurt gels made from commercial
655 pasteurized skim milk: set gel (YF: ——— ;■), stirred gel at 4 °C (YS4: ——— ;■), stirred gel
656 at 42 °C (YS42: ——— ;■) and relative signal intensity (A) $I_2(3)$; B) $I_2(4)$. Each bar is the
657 mean ± SE (n = 2). Letters (a, b, c) indicate significant statistical differences (p <0.05).
658 Images on the top right are microscopic images of yogurt stirred at 4 °C (YS4) and 42 °C
659 (YS42), the white scale bars represent 250 µm.

660

661 Figure 2: Transversal relaxation time dispersion of commercial pasteurized skim milk (- - - -)
662 and its corresponding set yogurt gel made in laboratory (———). Characteristic transversal
663 relaxation times $T_2(1)$ (A), $T_2(3)$ (B) and their relative signal intensities $I_2(1)$ (A), $I_2(3)$ (B) for
664 commercial pasteurized skim milk (■) and its corresponding set gel (■). Each bar is the mean
665 ± SE (n = 2). Letters (a, b or A) indicate significant statistical differences (p <0.05).

666

667 Figure 3: Transversal relaxation time $T_2(2)$ (A, C) and its relative signal intensity $I_2(2)$ (B, D)
668 of native micellar casein solutions (MC) (A, B) and whey protein isolate solutions (WPI) or
669 heat treated whey protein isolate solutions (WPI-HT) dispersed in milk permeate at 0.5 % ( ),
670 1 % (■) and 2 % (■) (w/w) (B, D). Each bar is the least square mean ± standard error of the
671 mean (A; B; D: n = 6 ; C: n = 2). Letters (a, b, c) indicate significant statistical differences (p
672 <0.05).

673

674 Figure 4: Schematic representation of ^{1}H relaxation time distribution pattern for yogurt

675

676   Figure 5: Characterization of commercial stirred yogurt at 4 % protein, 5 % protein, 6 %

677   protein, 10 % protein: syneresis values (A), transversal relaxation time $T_2(3)$ (B); particle

678   surfaces (C); network heterogeneity index (NHI) (D); and microscopic images (E) (scale bars

679   represent 250 µm). NA= Not analyzed. Each bar represents the least square mean ± SE (n =

680   2). Letters (a, b, c) indicate significant statistical differences (p <0.05).

681

682

683   Table 1: Commercial stirred yogurts
684

Yogurt[1]	Protein content (%)	Stabilizer
Y10	10	-
Y6	6	-
Y5P	5	Pectin + carob
Y4P	4	Pectin + carrageenan + starch
Y4PG-1	4	Starch + gelatin
Y4PG-2	4	Pectin + starch + gelatin

685

686   [1]Commercial yogurts acronyms: Y=yogurt, the number indicates the protein content; P=

687   presence of polysaccharides; G= presence of gelatin.

688

689   Table 2: Comparison of the proton pools and their interpretation determined by ^{1}H-LF-NMR using transversal relaxation time analyses on milk or

690   dairy solutions at pH 6.6.

691

Samples	SD[4]	NPP[5]	$T_2(1)$ ms	$T_2(2)$ ms	$T_2(3)$ ms	$I_2(1)$ %	$I_2(2)$ %	$I_2(3)$ %	Interpretation of		References
									$T_2(1)$	$T_2(2)$	
RM[1] from SMP[2]	DF	1 to 2	30-100	-	60-400	NP[10]	-	NP	Protein exchangeable protons - Diffusion	-	Hills, et al. (1990)
RM from LH-SMP[2] (8-10 % w/w)	CT	2	0.3	-	177	4.5	-	96	Protein non-exchangeable protons	-	Mariette, et al. (1993)
RM from LH-SMP (9 % w/w)	CT	1	-	-	80	-	-	100	-	-	Tellier, et al. (1993)
RM from LH-SMP (9 % w/w)	DF	1	-	-	90	-	-	100	-	-	Tellier, et al. (1993)
Dairy protein solution and milk (CNC[3] 3-15 %)	CT + DF	1-2	0.8-133	-	25-203	0-10	-	90-100	Protein non-exchangeable protons	-	Le Dean, et al. (2004)
Whey protein powder solutions (1.8-30.1 % w/w)	DF	1-2	0-1.3	-	80-1774	10	-	90	Protein non-exchangeable protons	-	Colsenet, et al. (2005)
Whole milk + SMP (4 % w/w)	DT	2	≈ 50	-	≈ 130	≈ 2	-	≈ 98	Water protons hydrating protein	-	Mok, et al. (2008)
RM from SMP (15 or 25 % w/w)	CT	3	1.2-1.6	4-13	80-133	1-3	0.5-2	90-98	ND	ND	Møller, et al. (2011)
RM from SMP + WPC (TS[3]=17 or 20 %, PC[3]=7.8 %)	CT + DF	3	1-10	≈ 50	200-250	NP	NP	> 90	ND	ND	Salomonsen, et al. (2007)

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RM from SMP + WPC or MWP[2] (PC = 6.5 % w/w)	DF	2	50-100	-	150-250	0-90	-	10-100	Water protons at protein surface	-	Torres, et al. (2016)

692

693   [1]RM= Reconstituted milk

694   [2]SMP= Skim milk powder, LH-SMP =Low heat skim milk powder, WPC= Whey protein concentrate, MWP= Microparticulated Whey protein

695   [3]CNC= Casein content, TS= Total solids, PC=Protein content

696   [4]Signal decomposition (SD) corresponds to a

697   discret fitting (DF) and/or continuous transformation (CT).

698   [5]NPP = Number of proton pool

699   NP = Not presented; ND = Not discussed

Table 3: Pearson's correlation[1] between ^{1}H-LF-NMR results and induced syneresis and image analysis of commercial stirred yogurts.

	All yogurt types			Yogurt without gelatin		
	Induced syneresis	NHI[3]	Microgel surface[3]	Induced syneresis	NHI[3]	Microgel surface[3]
$T_2(1)$	0.11	-0.33	0.06	-0.59	-0.54	-0.53
$T_2(2)^2$	0.45	0.74	0.3	NA[4]	NA	NA
$T_2(3)$	0.1	*0.79*	0.05	*0.92*	*0.99*	0.78
$T_2(4)$	-0.11	-0.22	-0.4	0.22	-0.35	-0.29
$I_2(1)$	0.11	0.54	-0.13	0.81	0.55	0.25
$I_2(2)^2$	0.35	*0.98*	0.54	NA	NA	NA
$I_2(3)$	-0.46	*-0.91*	-0.42	*-0.89*	*-0.91*	-0.59
$I_2(4)$	0.52	-0.15	0.09	0.4	-0.18	-0.39
Induced syneresis[3]	1	0.26	*0.78*	1	0.61	*0.79*
NHI[3]	0.26	1	0.52	0.61	1	0.83
Microgel surface[3]	*0.78*	0.52	1	*0.79*	0.83	1

[1] Coefficient of correlation in bold and italic are significant at $p < 0.01$.

[2] The correlation was established only on yogurts at 4 % protein content because other concentrations did not have this peak.

[3] The correlations was established without the yogurt at 10 % protein content

[4] NA= Not analyzed, the number of data point was insufficient to calculate a correlation