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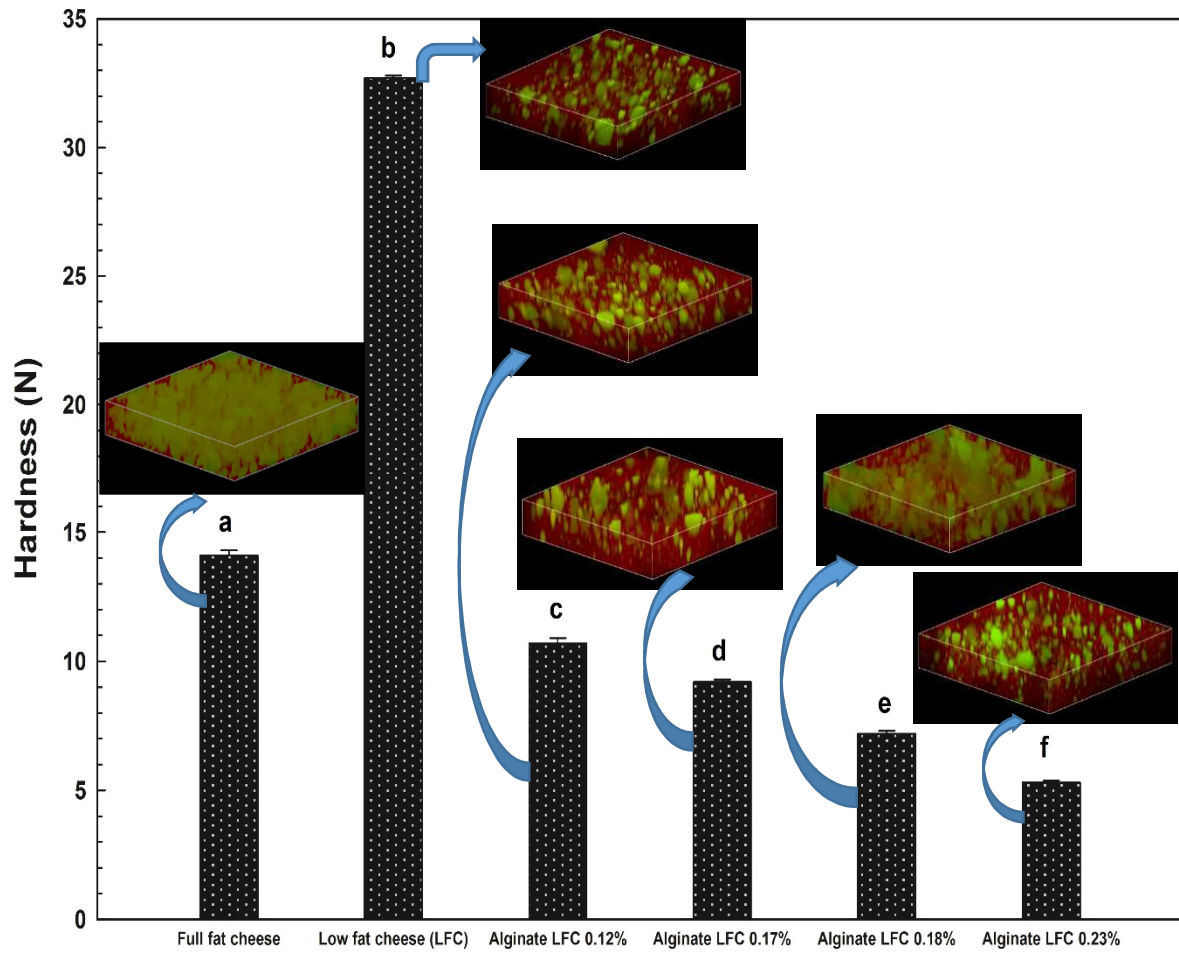
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ACCEPTED

1 **Modifying textural and microstructural properties of low fat** 2 **Cheddar cheese using sodium alginate**

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9 **Abstract**

10 Low fat Cheddar cheese (LFC) with up to 91% fat reduction were prepared using four levels
11 of sodium alginate (alginate): 0.12 (LFCA1), 0.17 (LFCA2), 0.18 (LFCA3) and 0.23% (w/w)
12 (LFCA4). Control full fat cheese (CFFC) and control low fat cheese (CLFC) were used for
13 comparison. Physical characteristics, namely texture profile, microstructure, transverse
14 relaxation time (T_2) distribution (measured by low-field NMR) and color were analysed
15 periodically during ripening until 180 days. Texture profile analysis illustrated a significant
16 improvement in texture of alginate added LFC ($P < 0.05$) as compared to CLFC. The textural
17 attributes of LFCA1 ripened for 30 days were comparable to CFFC ripened for 60 days and
18 beyond. A close resemblance in textural attributes between alginate added LFC and CFFC,
19 not previously reported when using other fat replacers, was observed. Scanning electron
20 micrograph (SEM) images revealed that alginate added LFCs had smoother surfaces as
21 compared to CFFC and CLFC, and the dense and compact protein matrix characteristic of
22 CLFC was not observed. Confocal laser scanning microscopy (CLSM) suggested that the fat
23 particle size, area and volume were affected in all LFCs due to their lower fat level and these
24 parameters increased during ripening in CFFC. NMR results revealed increase in higher
25 mobility water fraction in alginate added cheese compared to CFFC and CLFC. Hunter L , a
26 and b values for alginate added LFCs indicated that they were whiter than CLFC and less
27 yellowish than CFFC at the beginning of ripening; the color of some of the alginate added
28 LFCs was comparable to CFFC after 120 days of ripening. Overall, addition of alginate
29 significantly improved the textural, microstructural properties and color of LFCs, affirming
30 its potential as a promising texture modifier.

31 **Key words:** Low fat cheese; Cheese, Alginate; Cheddar; Rennet and Milk

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34 **1 Introduction**

35 Cheese contains complex matrix of milk protein, fat, lactose, water and minerals (Mistry &
36 Anderson, 1993). Fat provides smoothness and it acts as a filler between protein network in
37 cheese. Decreasing the fat content increases the density of protein network and decreases the
38 moisture to protein ratio in cheese, which consequently increases the hardness in LFC
39 (Johnson, 2016; Rogers, McMahon, Daubert, Berry, & Foegeding, 2010). Cheese develops
40 undesirable hard and rubbery texture when fat is reduced (Mistry, 2001; Rogers et al., 2010;
41 Zisu, 2005). Texture of a food material is an attribute resulting from a combination of
42 physical and chemical properties, and is perceived mainly by the sense of touch, sight and
43 hearing (Buffa, Trujillo, Pavia, & Guamis, 2001). Body and texture of cheese are important
44 parameters for its consumer acceptance and are reflection of its microstructure (Buffa et al.,
45 2001; Mistry & Anderson, 1993).

46 A clear understanding of the role of fat and its replacers in the development of cheese
47 microstructure is imperative to produce LFC with smoother texture (Mistry & Anderson,
48 1993). There are several reports on the size and shape of milk fat particles in cheese
49 visualized by scanning electron microscopy (SEM) (Cunha, Dias, & Viotto, 2010; Ong,
50 Dagastine, Kentish, & Gras, 2011, 2012; Wang et al., 2014). Microstructure of reduced fat
51 cheese and LFC revealed fewer fat particles in a large stretch of protein network, whereas full
52 fat cheese exhibited the protein network interspersed with numerous fat particles (Drake,
53 Boylston, & Swanson, 1996a). Furthermore, low fat hard cheese such as Cheddar may give a
54 dull appearance due to reduction in light scattering properties of milk fat particles (Mistry &
55 Anderson, 1993). Hence, color is also a very important parameter for the quality evaluation
56 of cheese as it is regarded as a primary factor by the consumers when making a buying
57 decision (Pinho, Mendes, Alves, & Ferreira, 2004).

58 Various modification techniques and strategies have been applied to produce LFC with
59 characteristics comparable to its full fat counterpart (Banks, 2004; Chatli, Gandhi, & Singh,
60 2017; Drake & Swanson, 1995). Approaches towards improving LFC include increasing
61 moisture to protein ratio (using various fat replacers), hydrolysing some proteins, altering
62 protein-protein interactions and creating large filler phase (Banks, 2004; Mistry, 2001).
63 Carbohydrate based fat replacers (starch, pectin, beta glucan, modified starch etc.) when
64 added in cheese, strongly bind water (increasing the moisture to protein ratio) and work in a
65 manner that mimics the mouth feel of fat (Aryana & Haque, 2001; Diamantino, Beraldo,
66 Sunakozawa, & Penna, 2014). In addition, protein based (micro-particulated proteins, whey

67 protein isolate, gelatin, egg protein etc.) and fat based replacers have been used to
68 manufacture LFC. Several researchers have reported improvement in textural properties of
69 low-fat Cheddar cheese using fat replacers such as Dairy Lo™, Simplesse™, Novagel™ and
70 Stellar™ and Avicel Plus® CM 2159 (Küçüköner, 1996), β -glucan (Konuklar, Inglett,
71 Warner, & Carriere, 2004; Sahan, Yasar, Hayaloglu, Karaca, & Kaya, 2008) and lecithin
72 (Drake, Truong, & Daubert, 1999). Among hydrocolloids, alginate can be used as a fat
73 replacer. Few patents (Hine, 1994; Liot & Stenbaek, 2014; Merrill & Singh, 2014) provide
74 reference to potential use of alginate (as a powder, micro gel or as a slurry) as an
75 ingredient in low fat cheese, but details about its effect on textural and microstructural
76 properties of cheese are lacking. No scientific published research study has utilized alginate
77 alone as a fat replacer in a low-fat Cheddar cheese milk. A recent study has included alginate
78 at a higher concentration (0.3%) to improve properties of low-fat Mozzarella cheese made
79 from buffalo milk (Chatli et al., 2017). Effect of adding alginate on cheese microstructure
80 was also not included in that study.

81 In this study, sodium alginate (alginate) was chosen as a fat replacer to prepare low-fat
82 Cheddar cheese. It was hypothesized that the textural properties of LFCs would improve due
83 to the higher water binding capacity of alginate. Furthermore, alginate gel particles
84 (generated *in situ* due to cross-linking of alginate molecules by Ca^{2+} present in milk and any
85 added calcium chloride) would act like hydrated filler particles in protein network of the
86 LFC. Formation of *in situ* alginate particles in milk in the presence of Ca^{2+} has been
87 confirmed by our recent study (Khanal, Bhandari, Prakash, & Bansal, 2017).

88 The objective of this study was to determine the effect of addition of alginate in the cheese
89 milk on physical characteristics such as texture, microstructure and color of low fat Cheddar
90 cheese and to compare those with the control full fat cheese (CFFC) and control low fat
91 cheese (CLFC).

92 **2 Materials and Methods**

93 **2.1 Materials**

94 Commercially available skim milk (0.11g/100 g fat), cream (39.5 g/100 g fat) and skim milk
95 powder (SMP) (moisture: 3.9 g/100 g, protein: 32.5 g/100 g, fat: 0.8 g/100 g, lactose: 55
96 g/100 g, minerals: 7.8 g/100 g) were used. Starter culture FD-DVS R-707 (*Lactococcus lactis*
97 *subsp. lactis* and *Lactococcus lactis subsp. cremoris*) was obtained from Chr. Hansen Pty.
98 Ltd., VIC, Australia. Rennet (Chymax plus, FPC, 200 IMCU /mL) was purchased from

99 Cheeselinks, VIC, Australia. Sodium alginate (Grindsted[®] alginate FD 155) was obtained
100 from Danisco, NSW, Australia.

101 **2.2 Cheese Making**

102 Alginate added LFCs were manufactured using four levels of alginate. Table 1 shows six
103 different formulations of milk used to prepare cheese including CFFC and CLFC. All
104 samples were prepared in triplicate.

105 Cheddar cheese was prepared according to the method described by Bansal et al. (2009) with
106 some modifications. Briefly, milk was standardised mixing skim milk and cream using
107 Pearson's square method (Tamime & Robinson, 2007). Appropriate levels of stock solution
108 (5 g/ 100 g) of alginate were added to milk to achieve the desired alginate concentration.
109 Dilution of solids due to addition of alginate solution was compensated by adding skim milk
110 powder. The final volume of cheese milk was 20 L for all formulations. The cheese milk was
111 cooled and equilibrated to 32°C in cheese vats. FD-DVS R-707 culture was propagated in
112 skim milk at 32°C (50 U / 500 mL) according to manufacturer's instructions. The propagated
113 culture (0.1 g/100 g of cheese milk) was mixed with cheese milk homogenously followed by
114 addition of CaCl₂ (1.5 mM) and then incubated at 32°C for 30 min. Rennet was added at a
115 rate of 200 µL/1000 mL, then the milk was left for 45 min without any disturbance for
116 coagulation. Curd was cut into cubes (1.5 cm³ in size) after 45 min and heated for 10 min
117 without stirring. Then the curd was cooked at 39°C until the pH reached 6.2, at which point
118 the whey was drained. After whey drainage, the curd was cheddared until the pH reached 5.2.
119 The curd was then milled, salted at 2.5% (w/w of the curd), moulded and pressed (550 kPa)
120 for 18 h. The pressed cheese was vacuum-packed in air-tight plastic bags and ripened at 8°C.
121 The cheese samples were analysed at day 7 (D7), day 30 (D30), day 60 (D50), day 120
122 (D120) and day 180 (D180) from the date of manufacture.

123 **2.3 Compositional analysis**

124 Moisture (Vacuum oven, 925.10), fat (Gerber method, 989.05.), protein (Kjeldhal method,
125 2001.14) and total ash (muffle furnace, 923.03) content in cheese were determined according
126 to method described in AOAC (2005). All compositional parameters of cheese were
127 determined at D7 of ripening period.

128 **2.4 Texture profile analysis (TPA)**

129 TPA was conducted according to Lashkari, Khosrowshahi Asl, Madadlou, & Alizadeh (2014)
130 with some modifications using TA-XT2 Texture Analyser (Stable Micro Systems, UK). A

131 flat probe of 35 mm diameter was attached to the moving cross head. Cylindrical cheese
132 samples (12 × 10 mm), taken from a depth of 5 mm in the cheese block at 8°C with a cork
133 borer, were placed in air-tight plastic bags, kept refrigerated at 4°C for 4 h to equilibrate and
134 then set aside at 21 ± 1°C for 45 min. Samples were compressed in two cycle tests at a speed
135 of 1.2 mm/sec with 33% deformation from the initial height of the sample. Textural
136 parameters such as hardness, cohesiveness, gumminess, chewiness and springiness were
137 determined. Cheese samples were analysed for textural attributes at D7, D30, D60, D120,
138 D180 of the ripening period. Hardness (N) was recorded as the maximum force during the
139 first compression cycle. Springiness was the height regained after the first compression.
140 Cohesiveness was considered as the ratio of positive force area under the second and first
141 compression cycle. Gumminess was calculated as hardness × cohesiveness and chewiness
142 was calculated as gumminess × springiness (Frau, Simal, Femenia, Sanjuán, & Rosselló,
143 1999). Each sample was analysed in duplicate.

144 **2.5 Color measurement**

145 Color measurements on cheese were made using Minolta Konica Chroma Meter CR-400
146 (Konica Minolta, INC, Japan). Hunter *L*, *a* and *b* values for color measurements were
147 determined. The instrument was calibrated with a white tile ($Y = 94.93$, $x = 0.3131$, $y =$
148 0.3197) (Pinho et al., 2004). Duplicate analysis was carried out for each sample.

149 **2.6 Microstructure analysis**

150 **2.6.1 Confocal laser scanning microscopy (CLSM) and image analysis**

151 Microstructure of cheese was analysed using Olympus Fluoview FV1000 BX2 upright
152 confocal laser scanning microscope (CLSM, Zeiss, Berlin, Germany). Cheese samples were
153 prepared according to Auty, Twomey, Guinee, & Mulvihill (2001) with some modifications.
154 Briefly, cheese samples were cut into 10 mm × 10 mm × 1 mm thick strips with a razor blade.
155 Nile Red (1 mg/10 g in ethanol) was used to label the fat and Rhodamine B (10 mg/100 g in
156 ethanol) was used to label the protein in cheese. To see the dual images of both fat and
157 protein, mixtures of Rhodamine B and Nile red (1:1) were used. Samples were examined
158 using 63× magnification objective and confocal illumination was obtained by an air-cooled
159 Ar/Kr laser. Rhodamine B was excited at 555 nm and Nile red was excited at 488 nm. The
160 pinhole diameter was 1 Array Unit. RGB color images (8-bit, 1024 pixel in size) were
161 acquired using a zoom factor of 1 with averaging of 2. Zen software was used to acquire
162 digital images. Images obtained from two different wavelengths were combined in the

163 overlaid images in which Rhodamine B stained protein appeared red, Nile red stained fat
164 appeared green and air pockets along with voids appeared black.

165 CLSM micrographs were analysed using ImageJ Software (Research Services Branch,
166 Maryland, U.S.A.). Particle counts, area covered and average size of fat particles were
167 determined using “Analyse particles” menu of ImageJ software. During image analysis, Pixel
168 (1024) and area of the sample (101.6 μm) were used to set the scale of the images. The
169 images were then flattened using band pass filters and adjusted with the color threshold to
170 transform it to a binary image with all fat particles appearing as black pixels and all protein
171 appearing as white pixels. The average area, count and average size of fat particles calculated
172 were only representative of 2D images of the cheese and not the absolute of the whole cheese
173 samples. Bins for the range of different sized data of fat particles were created from all the
174 images to construct histograms to illustrate the distribution of fat particles in cheese. This
175 method has been previously used to compare mean diameter of fat particles obtained from
176 laser diffraction and CLSM (Ong, Dagastine, Kentish, & Gras, 2010). Image analysis of 3D
177 images was carried out by (Fiji Is Just) ImageJ (Laboratory for Optical and Computational
178 Instrumentation, Wisconsin, USA). Images were opened in a green (fat) channel by splitting
179 the channels and processed by median filter of 2-pixel radius. Images were subjected to
180 thresholding process prior to determining the volume occupied by fat particles in 3D images
181 by 3D object counter. Six replicates micrographs of each treatment of cheese were used for
182 image analysis.

183 **2.6.3 Scanning electron microscopy (SEM)**

184 SEM imaging was carried out according to Aryana & Haque (2001). Briefly, the cheese
185 samples were sliced in 1 mm \times 1 cm \times 1 cm strips. Samples were first fixed in 2.5%
186 glutaraldehyde (solution prepared in water at pH 5.5) overnight at room temperature and
187 washed three times with water for 10 minutes for each wash. Then, samples were dehydrated
188 with series of ethanol concentrations from 10 to 100%. Dehydration was performed for 10
189 minutes for each ethanol concentration. The samples were then frozen and fractured under the
190 liquid nitrogen with a cooled razor blade. Fractured samples were thawed in 100% ethanol
191 followed by washing in fresh ethanol. Finally, samples were critical point dried in a Tousimis
192 Autosamdri 815 (Tousimis Automatic, Rockville, USA). Samples were mounted on stubs
193 with double-sided carbon sticky tape and coated with a thin layer (15 nm thickness) of
194 iridium in a Baltek iridium coater (Baltek, USA). A high vacuum SEM (Philips XL30
195 scanning electron microscope) (Philips, Tokyo, Japan) at 10 kV was used to view each

196 sample at a magnification of 4000 \times . The SEM was used to visually compare the images of
197 different cheese samples.

198 **2.6.4 Low field-nuclear magnetic resonance (LF-NMR)**

199 The moisture and fat distribution in cheese samples (CFFC, CLFC and LFCA1 at 180 d
200 ripening time) measured as transverse relaxation time (T_2) was determined by LF-NMR. The
201 T_2 has been used to represent the water retention in cheese and indicates interactions of
202 protons within its vicinity (Lilbæk et al., 2006). LF-NMR measurement was performed using
203 a MesoMR23-060V-I NMR analysing system (Niumag Corporation, Shanghai, China)
204 equipped with 25 mm diameter probe. The magnetic field strength was 0.52 ± 0.05 T and the
205 corresponding resonance frequency for protons was 21.3 MHz. Approximately 0.5 g of
206 sample was placed in NMR tube and then inserted in to NMR probe. The T_2 was measured
207 using the Carr-Purcell-Meiboom-Gill (CPMG) sequence with 3000 echoes and 4 scan
208 repetitions. The SIRT algorithm was employed in the 100,000-iterative fitting. The intensity
209 of the resulting T_2 distribution spectrum was normalized by the weight of sample. All the
210 measurements were performed in duplicate.

211 **2.7 Statistical analysis**

212 Data analysis was performed using Minitab-16 statistical software (Minitab Inc., USA).
213 General linear model of analysis of variance (ANOVA) and Tukey's comparison was used to
214 study differences between means at 95% confidence limit ($P < 0.05$).

216 **3 Results and discussions**

217 **3.1 Compositional analysis of cheese**

218 Composition of different cheese samples is shown in Table 2. As expected, significant
219 difference ($P < 0.05$) in moisture, fat and protein content was observed in all LFCs compared
220 to that of CFFC. There was a reduction in fat content by 84, 90, 91, 82.5, and 87 % in CLFC,
221 LFCA1, LFCA2, LFCA3 and LFCA4, respectively, compared to that of CFFC. Higher level
222 of protein in all LFCs in this study was in accordance with the findings of Aryana & Haque
223 (2001); Kumar et al. (2011) and Kavas, Oysun, Kinik, & Uysal (2004). Higher amount of
224 protein and moisture in LFCs were also reported by other researchers when using different fat
225 replacers such as Simplese[®]D-100, starch and Dairy-Lo[™] (Katsiari & Voutsinas, 1994;
226 Koca & Metin, 2004; Lobato-Calleros, Ramírez-Santiago, Vernon-Carter, & Alvarez-
227 Ramirez, 2014). Moisture content was increased in alginate added LFCs due to higher water

228 holding capacity of the alginate. Owing to higher water retention capacity of fat replacers, the
229 driving force involved to expel the water from the cheese curd is lowered (McMahon,
230 Alleyne, Fife, & Oberg, 1996).

231 **3.2 Texture profile analysis (TPA) of cheese**

232 All cheese samples were analysed for the textural parameters during ripening from D7 to
233 D180 (Table 3). At D7, alginate added LFCs showed significantly ($P<0.05$) lower hardness,
234 chewiness and gumminess than CLFC and CFFC, whereas their cohesiveness and springiness
235 did not differ from that of CFFC, except LFCA3 (for springiness).

236 When hardness was compared over the ripening period from D30 to D180, it decreased
237 significantly ($P<0.05$) in all cheese samples as ripening progressed. At each ripening time,
238 the hardness, gumminess and chewiness of CLFC were significantly higher ($P<0.05$) than
239 CFFC, whereas all alginate added LFCs demonstrated significantly ($P<0.05$) softer, less
240 gummy and less chewy characteristics than both CFFC and CLFC. The textural attributes
241 demonstrated were improved with increasing alginate concentration, LFCA4 being least hard,
242 gummy and chewy at each time point. From D30 onwards, the textural attributes of LFCA1
243 were comparable to that of CFFC that was matured for longer than 60 days. For example, the
244 hardness of LFCA1 at D30 was comparable to that of CFFC at D60 and so on. Increased
245 hardness in CLFC compared to CFFC was associated with reduction in fat content and the
246 resulting high protein density which makes the cheese highly resistant to deformation (Cunha
247 et al., 2010). The decrease in hardness in alginate added LFCs could be attributed to
248 alginate's capacity to bind water, thus increasing the moisture content of cheese, and to form
249 discrete gel particles in situ in the presence of Ca^{2+} in cheese milk (Khanal et al., 2017) where
250 fat replacers are used, water plays a role of plasticizer in between protein molecules and thus
251 makes the cheese softer (Sahan et al., 2008). In addition, interactions between protein and
252 polysaccharide are crucial to develop the structure and stability of the product, and types of
253 polysaccharide and their charge are responsible to govern the nature of these interactions
254 (Hosseini et al., 2013). Furthermore, higher protein content is another factor for the harder
255 texture in CLFC. Sahan et al. (2008) illustrated decrease in gumminess in low fat Kashar
256 cheese added with Avicel Plus® CM 2159 or β -glucan; and Volikakis, Biliaderis, Vamvakas,
257 & Zerfiridis (2004) with commercial oat β -glucan. According to Sahan et al. (2008),
258 reduction in gumminess was caused by the removal of fat from cheese.

259 Springiness did not change in each sample over the ripening period from D30 to D120.
260 Similar observation was reported by Sahan et al. (2008) with other fat replacers in low fat

261 Kashar cheese. Springiness decreased ($P < 0.05$) in LFCA3 and LFCA4 as compared to CFFC
262 and CLFC at D120 and onwards, while no significant differences were observed between
263 LFCA1, LFCA2, CFFC and CLFC at all time points. Addition of alginate affected the
264 cohesiveness of cheese (Table 3), but the effect was dependent on the concentration of
265 alginate and the age of cheese. CLFC was more cohesive ($P < 0.05$) than CFFC at all time
266 points of ripening period. LFCA1 was more cohesive than CFFC until D120, whereas
267 LFCA2, 3 and 4 were not different with CFFC. At D180, no differences in cohesiveness was
268 detected between alginate added LFCs and CFFC. The denser protein matrix in CLFC is
269 associated with higher springiness and cohesiveness (Lobato-Calleros, Robles-Martinez,
270 Caballero-Perez, & Vernon-Carter, 2000). With increasing quantity of alginate in cheese,
271 cohesiveness decreased as compared to CLFC and became similar to CFFC. Other fat
272 replacers such as β -glucan concentrate (Volikakis et al., 2004), Simplese® D-100 and
273 Novagel™ NC-200 (Romeih, Michaelidou, Biliaderis, & Zerfiridis, 2002) have been also
274 associated with the decrease in cohesiveness in different types of LFCs..

275 Results of TPA suggested that there was a continuous improvement in all textural parameters
276 in all cheeses during ripening from D7 to D180 and this was due to on-going proteolysis
277 (Romeih et al., 2002). Textural attributes changed with increased alginate concentration and
278 similar trends were reported by adding other fat replacers such as lecithin (Drake et al.,
279 1999), Simplese® and Dairy-Lo™ (Kavas et al., 2004), β -glucan hydrocolloid suspension
280 (Konuklar, Inglett, Carriere, & Felker, 2004) and soy protein isolate. Increase in alginate
281 concentration formed softer rennet gel and resulted in lower G' in our previous study,
282 indicating alginate particles acted as fillers in protein matrix to soften the texture of gel
283 (Khanal et al., 2017). Texture of cheese is directly influenced by water holding capacity
284 (WHC) of the rennet gel. The WHC of protein gels is influenced by the interactions between
285 milk proteins and sodium alginate. Protein-polysaccharide interactions that affect WHC
286 capacity include electrostatic forces, hydrogen bonds, covalent bonds, excluded volume,
287 hydrophobic interactions, ionic bridging and Van der Waals interactions (Chen, Chen, &
288 Hsieh, 2016; Yao et al., 2018). In case of alginate, interaction is facilitated by hydrophobic or
289 hydrogen bonding between proteins and its hydroxyl groups of mannuronic or guluronic acid
290 residues (Chen et al., 2016).

291 The TPA parameters of LFCA1 closely resembled to those of CFFC; such a close
292 resemblance in textural parameters of a low fat cheese with full fat cheese has not been

293 previously reported when using other fat replacers (Drake, Herrett, Boylston, & Swanson,
294 1996b; Koca & Metin, 2004; Konuklar, Inglett, Carriere, et al., 2004; Oliveira et al., 2010).

295 **3.2 Color measurement**

296 Comparison of Hunter *L*, *a* and *b* values within each time point of ripening period revealed
297 significantly lower ($P < 0.05$) *L* and *b* values of all LFCs than those of CFFC (Table 4), CLFC
298 being the lowest, indicating they were darker and less yellow as compared to CFFC. Similar
299 decrease in *L* value was also reported by Deegan, Holopainen, McSweeney, Alatossava, &
300 Tuorila (2014) in reduced fat cheese. Significantly lower *b* value in CLFC was due to the
301 difference in yellowness attributed to low fat percentage as compared to CFFC (Cunha et al.,
302 2010). Deep yellow color in CFFC is due to effective light scattering by large amounts of fat
303 globules (Deegan et al., 2014). The *L* and *b* values increased by increasing alginate
304 concentration in LFCs. The *L* value of LFCA2, LFCA3 and LFCA4 at D120; and LFCA3 and
305 LFCA4 at D180 were not significantly different ($P > 0.05$) to CFFC. Increase in lightness (*L*
306 value) by adding alginate was attributed to increase in moisture to protein ratio, which
307 subsequently increases the surface area occupied by scattering centres (Rahimi,
308 Khosrowshahi, Madadlou, & Aziznia, 2007). Furthermore, similar increase in *L* value have
309 also been reported using gum tragacanth and Salatrim[®] as fat replacers in low and reduced fat
310 Mozzarella cheese by Rahimi et al. (2007) and by Rudan, Barbano, & Kindstedt (1998) in
311 low fat white brined cheese. All LFCs showed significantly ($P < 0.5$) higher *a* values
312 (negative) compared to that of CFFC at D7, D30 and D60 but not at D120 and D180. The
313 negative *a* value found in this study indicated tendency of the samples towards green color
314 (Pinho et al., 2004). During ripening, the difference between *L* values of CFFC and LFCA3
315 and LFCA4 were narrowing and the LFCA4 was not significantly ($P < 0.05$) different than the
316 CFFC at D120 and D180.

317 **3.4 Cheese Microstructure**

318 Representative 2D (Figures 1, 2 and 3) and 3D CLSM images (Figures 4 and 5) clearly
319 demonstrates that CFFC samples exhibited more fat particles (as expected) and the number of
320 fat particles decreased in all LFCs samples. Fat particles are more scattered in D7 samples (as
321 seen in both 2D and 3D images) and coalesced as ripening progressed, especially in CFFC
322 due to the presence of higher amount of fat as compared to LFC samples. Pronounced
323 clumping and coalescence of fat particles have been previously reported with increased fat
324 content in cheese (Guinee, Auty, & Fenelon, 2000). The 2D images were further analysed to

325 determine parameters such as area, size and numbers of fat particles, whereas 3D images
326 were used to determine their volume.

327 Image analysis of CLSM micrographs revealed abundant numbers of small particles (ranging
328 from up to $0.5 \mu\text{m}$) in all cheese samples throughout the ripening process (Figures 6, 7 and 8).
329 Fat particles of $>0.6 \mu\text{m}$ were present in larger number in CFFC as compared to LFCs. As
330 evident in their respective 2D and 3D images, the size of fat particles in CFFC profoundly
331 increased during ripening from D7 to D180 whereas their size in LFCs increased subtly.

332 Fat particles in all LFCs were significantly smaller ($P<0.05$) in size, area (in 2D images) and
333 volume (in 3D images) as compared to CFFC over the ripening period (Figures 9 A and B
334 and D). The size and area of the fat particles increased ($P<0.05$) from D7 to D180 in CFFC
335 but not in LFCs. The volumes, area and size of fat particles in alginate added LFCs were not
336 different from CLFC (except LFCA2 for volume), suggesting alginate did not affect these
337 parameters. The fat particles in this study were larger as the cheese milk was not
338 homogenized. Results by Ong et al. (2010) also reported larger fat particles (the mean
339 diameters of up to $9 \mu\text{m}$) for raw un-homogenised milk. Large numbers ($P<0.05$) of fat
340 particles were present in CFFC at D7 and D120 as compared to D180 (Figure 9 C). Wang, Li,
341 Wang, & Özkan (2010) also reported total numbers and area covered by fat particles in CFFC
342 were higher due to inclusion of more amount of fat in milk used for cheese preparation. At
343 D180, number of fat particles detected in CFFC and all LFCs samples were not different,
344 possibly due to coalescence of fat particles in CFFC during cheese ripening.

345 Though CLSM provided information regarding difference in effect on fat particle size, area
346 and volume in all LFCs compared to that of CFFC, we could not able to observe alginate
347 particles by CLSM despite the use of alginate specific staining. Hence, images were further
348 viewed through SEM in an attempt to visualise the alginate. The alginate was not observed in
349 SEM either (Figure 10). However, SEM images revealed (Figure 10) increased smoothness in
350 cheese with increasing alginate concentration and no noticeable phase separation between
351 protein and alginate was seen. Due to de-lipidation during sample preparation for SEM, voids
352 spaces were left intact where fat particles used to be in the samples (Aryana & Haque, 2001).
353 This fact is further evident by CFFC showing more and larger voids and more open structure
354 as compared to LFCs. On the other hand, there was a dense protein network with less
355 numbers of voids present in CLFC. Similar microstructural images were also observed by
356 Diamantino et al. (2014); and Lobato-Calleros et al. (2007) for CFFC and CLFC. The surface

357 of the LFCA1 displayed an increase in porous and spongy character, smoothness and
358 cohesive appearance, this porous microstructure could provide more space for water
359 entrapment and result in a softer texture. Addition of alginate could have interfered with the
360 aggregation of caseins resulting in the formation of inhomogeneous casein network with
361 porous and smooth microstructure. Also, alginate being negatively charged polysaccharide
362 interacts with positively charged proteins at low pH and forms highly structured open porous
363 protein network (Chen et al., 2016). The protein network seemed to be covered over in a
364 cheese having higher alginate concentration. Some small white aggregates were scattered
365 over the protein network (indicated by red arrow in Figure 10) in all alginate added LFCs.
366 Such aggregates were also noted by Drake et al. (1996b) when using lecithin as a fat replacer
367 in reduced fat cheese.

368 **3.5 LF-NMR results**

369 Fig. 11 shows the transverse relaxation time (T_2) spectra of CFFC, CLFC and LFCA1. The T_2
370 and the corresponding peak area can reflect the mobility and distribution of molecules (e.g.,
371 water and fat) containing hydrogen protons in a cheese matrix, respectively. A longer
372 transverse relaxation time indicates lower binding energy and higher mobility of molecules
373 containing hydrogen protons. The cheese matrix affects the relaxation of protons in water
374 owing to interactions between macromolecules and water. Hence, different states of water
375 molecules yield a spectrum of transverse relaxation time (T_2) (Altan, Oztop, McCarthy, &
376 McCarthy, 2011). The relaxation is not only affected by water translation and rotation of
377 molecules, chemical exchange between water molecules and biopolymers or other solutes
378 also have an impact on it (Gianferri, Maioli, Delfini, & Brosio, 2007).

379 Multiple relaxation times in cheese are due to its heterogeneous matrix. The protons in less
380 mobile and more mobile fractions of water in cheese corresponds to the components with
381 shorter and longer relaxation time, respectively (Altan et al., 2011). Generally, three peaks
382 were observed in the T_2 distribution spectrum of cheese samples. The first peak (T_{21}) between
383 the shortest relaxation time of 0.05-2 ms corresponded to protons of the tightly bound water
384 molecules accumulated in the large open channel of the protein network (Bordoni et al.,
385 2011). The second peak (T_{22}) between the medium relaxation time of 3-30 ms was designated
386 to protons of water molecules entrapped inside the protein gel- network within the cheese
387 matrix (Gianferri et al., 2007). Finally, the third peak (T_{23}) between the longest relaxation
388 time of 40-400 ms was ascribed to protons of fat molecules within the cheese matrix. As
389 shown in Table 5, no significant differences were observed in T_{21} , T_{22} and T_{23} relaxation

390 times between CLFC and CFFC, while the T_{21} , T_{22} and T_{23} relaxation times of LFCA1 were
391 longer than CLFC and CFFC, suggesting a higher mobility of water and lipid molecules in
392 LFCA1. This relaxation time data was in accordance with the moisture data in Table 2.
393 Moreover, no significant differences were observed in T_{21} peak area between LFCA1 and
394 CLFC, while the T_{21} (A_{21}) peak area of LFCA1 and CLFC was larger than that of CFFC,
395 which might be attributed to the lower number of hydrophilic compounds (e.g., protein and
396 water) in CFFC. For the major peak T_{22} (A_{22}), the peak area was not significantly different
397 between CLFC and CFFC, while the corresponding peak area of LFCA1 was significantly
398 ($P < 0.05$) larger compared to CLFC and CFFC. Therefore, these results suggested that the
399 increase in water content in alginate added low-fat cheese was mainly caused by an increase
400 in the amount of water in fraction T_{22} . This increase in higher mobility water fraction in
401 LFCA might be responsible for its softer texture (Table 3). In addition, the T_{23} peak area of
402 LFCA1, CLFC and CFFC were generally in agreement with the corresponding fat content
403 (Table 2). The relaxation time of cheese is sensitive to the level of water and ratio of protein
404 to water (Chaland, Mariette, Marchal, & De Certaines, 2000). Similar kind of easily
405 distinguishable relaxation time for fat and water proton molecules was reported by Chaland et
406 al. (2000) in cheese samples.

407

408 **4. Conclusions**

409 This study investigates the effect of adding alginate to the development of texture, colour and
410 microstructure of LFCs. Present results indicated that fat reduction in cheese led to increase
411 in hardness, denser microstructure and poor color development. Addition of alginate in LFCs
412 improved these attributes, making alginate added cheese (at as low as 0.12% addition)
413 comparable to CFFC. Furthermore, alginate added LFCs were softer, more cohesive, chewier
414 and smoother than CLFC and CFFC; and one of the combinations (LFCCA1) closely
415 resembled CFFC in terms of textural parameters. It was possible to see the relaxation time of
416 water protons and fat protons by LF-NMR and provided insights into the existence of fat and
417 water in cheese. NMR results verified presence of high amount of higher mobility water in
418 alginate added LFC which might contribute to its softer texture. Study on digestibility,
419 tribology and sensory properties of alginate added LFCs will be the focus of future research.

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 597 PhD Thesis), Victoria University, Werribee Campus, VIC, Australia.

598 Table 1. Composition of milk and levels of alginate used for Cheddar cheese making.

S N	Samples code	Fat (g / 100 g)	Protein (g / 100 g)	Added (g / 100 g)
1	Full fat control cheese (CFFC)	2.92 ± 0.02 ^a	3.54 ± 0.09 ^a	0
2	Low fat control cheese (CLFC)	0.44 ± 0.02 ^b	3.70 ± 0.13 ^a	0
6	Low fat cheese (LFCA1)	0.48 ± 0.01 ^b	3.60 ± 0.06 ^a	0.12
7	Low fat cheese (LFCA2)	0.47 ± 0.01 ^b	3.68 ± 0.12 ^a	0.17
3	Low fat cheese (LFCA3)	1.08 ± 0.01 ^c	3.74 ± 0.06 ^a	0.18
4	Low fat cheese (LFCA4)	1.04 ± 0.02 ^c	3.78 ± 0.08 ^a	0.23

599 Fat and protein content are expressed as the mean ± standard error (n = 6). Means in a single
 600 column with different superscripts are significantly different (P<0.05).

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618 Table 2. Composition of Cheddar cheese with or without added alginate at different levels.

Cheese	Total fat (g/100g)	Total protein (g/100g)	Moisture (g/100g)	Ash (g/100g)
CFFC	31.5 ± 0.7 ^a	29.5 ± 0.6 ^d	34.5 ± 0.5 ^d	4.8 ± 0.1 ^b
CLFC	5.0 ± 0.2 ^{bc}	42.2 ± 0.6 ^a	41.9 ± 0.5 ^c	5.9 ± 0.2 ^a
LFCA1	3.1 ± 0.2 ^d	35.8 ± 0.5 ^b	50.3 ± 0.3 ^b	5.6 ± 0.02 ^b
LFCA2	2.7 ± 0.1 ^d	35.6 ± 0.4 ^b	51.7 ± 0.7 ^b	5.5 ± 0.1 ^a
LFCA3	5.5 ± 0.4 ^b	33.0 ± 0.3 ^c	52.1 ± 0.3 ^b	4.9 ± 0.03 ^b
LFCA4	3.8 ± 0.1 ^{cd}	31.7 ± 0.4 ^c	54.6 ± 0.5 ^a	4.8 ± 0.1 ^b

625 All results are expressed as the mean ± standard error (n = 6). Means in a single column with
626 different superscripts are significantly different (P<0.05). DB is on dry basis.

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649 Table 3. Textural characteristics of Cheddar cheese with or without added alginate at
 650 different levels obtained by texture analyser.

Ripening time	Sample	Hardness (N)	Springiness	Gumminess (N)	Chewiness	Cohesiveness
D7	CFFC	39.79 ± 0.9 ^B	0.94 ± 0.0 ^A	28.38 ± 0.70 ^B	26.88 ± 0.5 ^B	0.71 ± 0.03 ^B
	CLFC	55.19 ± 0.70 ^A	0.92 ± 0.01 ^{AB}	45.82 ± 1.3 ^A	42.03 ± 1.6 ^A	0.82 ± 0.02 ^A
	LFCA1	32.85 ± 0.40 ^C	0.94 ± 0.01 ^{AB}	24.06 ± 0.8 ^C	22.60 ± 0.7 ^C	0.73 ± 0.02 ^B
	LFCA2	26.26 ± 0.31 ^D	0.91 ± 0.003 ^{AB}	20.24 ± 0.31 ^D	18.45 ± 0.4 ^D	0.77 ± 0.01 ^{AB}
	LFCA3	24.15 ± 0.72 ^D	0.90 ± 0.01 ^B	18.19 ± 1.0 ^{DE}	16.42 ± 1.1 ^{DE}	0.75 ± 0.02 ^{AB}
	LFCA4	20.04 ± 0.95 ^E	0.92 ± 0.01 ^{AB}	14.81 ± 0.42 ^E	13.67 ± 0.5 ^E	0.73 ± 0.01 ^B
D30	CFFC	21.70 ± 0.30 ^d	0.97 ± 0.007 ^a	17.81 ± 0.24 ^d	17.26 ± 0.21 ^d	0.82 ± 0.82 ^{defgh}
	CLFC	44.55 ± 0.45 ^a	0.95 ± 0.003 ^{abc}	39.52 ± 0.47 ^a	37.72 ± 0.40 ^a	0.89 ± 0.88 ^a
	LFCA1	18.17 ± 0.11 ^{ef}	0.95 ± 0.004 ^{abc}	15.70 ± 0.07 ^e	15.02 ± 0.06 ^e	0.86 ± 0.86 ^{abc}
	LFCA2	15.26 ± 0.33 ^h	0.91 ± 0.003 ^{bcdefgh}	12.78 ± 0.28 ^g	13.14 ± 0.56 ^g	0.84 ± 0.83 ^{cdef}
	LFCA3	8.22 ± 0.09 ^{lm}	0.92 ± 0.02 ^{bcdefgh}	6.76 ± 0.10 ^{kl}	6.20 ± 0.17 ^{kl}	0.82 ± 0.82 ^{defgh}
	LFCA4	6.27 ± 0.04 ^{no}	0.90 ± 0.01 ^{defgh}	5.19 ± 0.06 ^{mn}	4.71 ± 0.08 ^{mn}	0.82 ± 0.82 ^{defg}
D60	CFFC	19.01 ± 0.20 ^e	0.95 ± 0.01 ^{abcd}	15.34 ± 0.25 ^{ef}	14.56 ± 0.32 ^{ef}	0.80 ± 0.006 ^{fgh}
	CLFC	44.64 ± 0.23 ^a	0.95 ± 0.004 ^{abc}	39.60 ± 0.42 ^a	37.78 ± 0.48 ^a	0.89 ± 0.006 ^a
	LFCA1	17.00 ± 0.09 ^g	0.92 ± 0.0 ^{abcdefg}	14.67 ± 0.12 ^{ef}	13.62 ± 0.11 ^{fg}	0.86 ± 0.005 ^{abc}
	LFCA2	14.10 ± 0.23 ⁱ	0.92 ± 0.005 ^{abcdefg}	11.95 ± 0.23 ^{gh}	11.09 ± 0.22 ^h	0.85 ± 0.003 ^{bcd}
	LFCA3	8.10 ± 0.06 ^m	0.91 ± 0.007 ^{bcdefgh}	6.70 ± 0.03 ^{kl}	6.14 ± 0.06 ^{kl}	0.83 ± 0.005 ^{defg}
	LFCA4	5.70 ± 0.08 ^o	0.90 ± 0.01 ^{efgh}	4.66 ± 0.07 ^{mn}	4.18 ± 0.05 ^{mn}	0.82 ± 0.002 ^{defgh}
D120	CFFC	17.93 ± 0.21 ^{fg}	0.96 ± 0.01 ^{ab}	14.43 ± 0.30 ^e	13.86 ± 0.41 ^{efg}	0.81 ± 0.01 ^{gh}
	CLFC	37.41 ± 0.25 ^b	0.93 ± 0.01 ^{abcdefg}	32.70 ± 0.35 ^b	30.50 ± 0.37 ^b	0.87 ± 0.005 ^{ab}
	LFCA1	14.91 ± 0.22 ^{hi}	0.92 ± 0.005 ^{abcdefg}	12.47 ± 0.19 ^{gh}	11.50 ± 0.20 ^h	0.85 ± 0.003 ^{bcd}
	LFCA2	12.32 ± 0.1 ^j	0.92 ± 0.007 ^{bcdefgh}	10.11 ± 0.06 ⁱ	9.3 ± 0.10 ⁱ	0.82 ± 0.008 ^{defgh}
	LFCA3	7.2 ± 0.12 ^{mn}	0.89 ± 0.008 ^{gh}	5.7 ± 0.09 ^{lm}	5.10 ± 0.11 ^{lm}	0.80 ± 0.006 ^h
	LFCA4	5.41 ± 0.11 ^o	0.89 ± 0.009 ^{efgh}	4.30 ± 0.06 ⁿ	3.85 ± 0.07 ^{mn}	0.80 ± 0.008 ^h
D180	CFFC	14.1 ± 0.18 ⁱ	0.94 ± 0.07 ^{abcde}	11.50 ± 0.17 ^h	10.8 ± 0.17 ^h	0.82 ± 0.008 ^{efgh}
	CLFC	32.7 ± 0.10 ^c	0.94 ± 0.009 ^{abcdef}	28.3 ± 0.14 ^c	26.6 ± 0.26 ^c	0.90 ± 0.002 ^{abc}
	LFCA1	10.7 ± 0.20 ^k	0.91 ± 0.006 ^{cdefgh}	8.9 ± 0.17 ^j	8.1 ± 0.18 ^{ij}	0.83 ± 0.004 ^{defg}
	LFCA2	9.2 ± 0.09 ^l	0.92 ± 0.007 ^{bcdefgh}	7.5 ± 0.10 ^k	6.9 ± 0.08 ^{jk}	0.81 ± 0.00 ^{fgh}
	LFCA3	7.2 ± 0.13 ^{mn}	0.90 ± 0.008 ^{fgh}	5.7 ± 0.09 ^{lm}	5.10 ± 0.07 ^{lm}	0.80 ± 0.007 ^h
	LFCA4	5.3 ± 0.11 ^o	0.87 ± 0.01 ^h	4.23 ± 0.10 ⁿ	3.4 ± 0.09 ⁿ	0.80 ± 0.007 ^h

651 All results are expressed as the mean ± standard error (n = 6). Means in a single column with
 652 different superscripts are significantly different (P<0.05).

653 ^{abc} denotes comparison between cheeses over the time period from D30 to D180.

654 ^{ABC} denotes comparison between cheeses at D7.

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665 Table 4. Hunter L, a and b values of cheese obtained by colorimeter.
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Ripening time	Samples	<i>L</i>	<i>a</i>	<i>b</i>
Day 7	CFFC	65.5 ± 0.4 ^a	-5.5 ± 0.4 ^b	20.9 ± 0.9 ^a
	CLFC	44.4 ± 1.8 ^c	-4.4 ± 0.3 ^a	9.9 ± 0.6 ^d
	LFCA1	52.7 ± 0.5 ^d	-4.9 ± 0.3 ^a	11.3 ± 0.4 ^c
	LFCA2	58.2 ± 0.4 ^c	-4.8 ± 0.2 ^a	12.5 ± 0.9 ^b
	LFCA3	58.7 ± 0.4 ^c	-4.4 ± 0.2 ^a	12.7 ± 0.3 ^b
	LFCA4	60.7 ± 0.9 ^b	-4.8 ± 0.2 ^a	12.6 ± 0.3 ^b
Day 30	CFFC	64.2 ± 1.8 ^a	-5.2 ± 0.4 ^b	20.8 ± 0.3 ^a
	CLFC	45.6 ± 0.9 ^d	-4.5 ± 0.4 ^a	8.5 ± 0.2 ^d
	LFCA1	53.5 ± 1.4 ^c	-4.3 ± 0.3 ^a	11.0 ± 0.4 ^c
	LFCA2	54.0 ± 0.6 ^c	-4.4 ± 0.2 ^a	11.0 ± 0.7 ^c
	LFCA3	58.6 ± 1.2 ^b	-4.4 ± 0.1 ^a	12.2 ± 0.7 ^b
	LFCA4	58.3 ± 0.8 ^b	-4.4 ± 0.2 ^a	12.0 ± 0.7 ^b
Day 60	CFFC	63.1 ± 2.3 ^a	-5.5 ± 0.2 ^a	20.1 ± 1.5 ^a
	CLFC	46.2 ± 0.6 ^d	-4.6 ± 0.3 ^b	8.1 ± 0.4 ^c
	LFCA1	54.6 ± 1.2 ^c	-4.6 ± 0.2 ^a	11.0 ± 1.0 ^b
	LFCA2	54.8 ± 1.2 ^c	-4.5 ± 0.58 ^a	10.7 ± 0.3 ^b
	LFCA3	58.3 ± 2.2 ^b	-4.4 ± 0.2 ^a	11.9 ± 0.7 ^b
	LFCA4	58.8 ± 0.4 ^b	-4.6 ± 0.3 ^a	11.7 ± 1.1 ^b
Day 120	CFFC	64.5 ± 1.0 ^a	-4.9 ± 0.3 ^a	21.0 ± 1.5 ^a
	CLFC	48.7 ± 0.7 ^c	-4.4 ± 0.04 ^a	12.1 ± 1.5 ^d
	LFCA1	59.6 ± 1.0 ^b	-4.9 ± 0.2 ^a	15.4 ± 0.8 ^c
	LFCA2	65.9 ± 0.9 ^a	-5.2 ± 0.1 ^a	17.1 ± 0.7 ^{bc}
	LFCA3	65.9 ± 0.7 ^a	-4.8 ± 0.3 ^a	18.8 ± 0.8 ^b
	LFCA4	65.8 ± 0.4 ^a	-5.2 ± 0.2 ^a	17.5 ± 0.9 ^b
Day 180	CFFC	66.0 ± 1.1 ^a	-4.4 ± 0.1 ^a	21.6 ± 0.5 ^a
	CLFC	49.2 ± 0.2 ^c	-4.0 ± 0.1 ^a	10.6 ± 0.2 ^d
	LFCA1	56.9 ± 1.2 ^b	-4.2 ± 0.1 ^a	13.0 ± 0.3 ^c
	LFCA2	58.7 ± 0.3 ^b	-4.2 ± 0.2 ^a	13.5 ± 0.4 ^c
	LFCA3	65.9 ± 0.7 ^a	-4.5 ± 0.04 ^a	16.3 ± 0.2 ^b
	LFCA4	64.1 ± 0.1 ^a	-4.3 ± 0.3 ^a	14.8 ± 1.0 ^{bc}

667 All results are expressed as the mean ± standard error (n = 6). Means in a single column
 668 within a ripening time block with different superscripts are significantly different (P<0.05).
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677 Table 5. The transverse relaxation time (T_2) and corresponding peak area.

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Cheese	Relaxation time			Peak area		
	T_{21}	T_{22}	T_{23}	A_{21}	A_{22}	A_{23}
CFFC	0.5 ± 0.0^a	9.3 ± 0.0^a	81.3 ± 8.0^a	382 ± 25^a	2157 ± 30^a	627 ± 42^a
CLFC	0.4 ± 0.1^a	9.3 ± 0.0^a	93.5 ± 9.2^a	536 ± 56^b	2099 ± 176^a	79 ± 25^b
LFCA1	0.7 ± 0.3^b	14.2 ± 0.0^b	251.6 ± 140.8^b	476 ± 43^c	2521 ± 78^b	21 ± 5^c

679 Relaxation time are expressed as the mean \pm standard error (n = 6). Means in a single column
 680 with different superscripts are significantly different (P<0.05).

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720 Figure captions

721 Figure 1. 2D images of cheese samples at D7 obtained from CLSM. Images from A to F are
722 CFFC, CLFC, LFCA1, LFCA2, LFCA3, LFCA4 cheese samples, respectively. The fat
723 particles and protein network are stained green and red, respectively.

724 Figure 2. 2D Images of cheese samples at D120 obtained from CLSM. Images from A to F
725 are CFFC, CLFC, LFCA1, LFCA2, LFCA3, LFCA4 cheese samples, respectively. The fat
726 particles and protein network are stained green and red, respectively.

727 Figure 3. 2D images of cheese at D180 samples obtained from CLSM. Images from A to F
728 are CFFC, CLFC, LFCA1, LFCA2, LFCA3, LFCA4 cheese samples, respectively. The fat
729 particles and protein network are stained green and red, respectively.

730 Figure 4. 3D Images of cheese (D120) samples obtained from CLSM. Images from A to F are
731 CFFC, CLFC, LFCA1, LFCA2, LFCA3, LFCA4 cheese samples, respectively. The fat
732 particles and protein network are stained green and red, respectively.

733 Figure 5. 3D Images of cheese (D180) samples obtained from CLSM. Images from A to F are
734 CFFC, CLFC, LFCA1, LFCA2, LFCA3, LFCA4 cheese samples, respectively. The fat
735 particles and protein network are stained green and red, respectively.

736 Figure 6. Fat particle size distribution in D7 samples. A-F distributions are for CFFC, CLFC,
737 LFCA1, LFCA2, LFCA3, LFCA4 cheese samples, respectively. Six replicate images were
738 used for the distribution analysis of each cheese sample.

739 Figure 7. Fat particle size distribution in D120 samples. A-F distributions are for CFFC,
740 CLFC, LFCA1, LFCA2, LFCA3, LFCA4 cheese samples, respectively. Six replicate images
741 were used for the distribution analysis of each cheese sample.

742 Figure 8. Fat particle size distribution in D180 samples. A-F distributions are for CFFC,
743 CLFC, LFCA1, LFCA2, LFCA3, LFCA4 cheese samples, respectively. Six replicate images
744 were used for the distribution analysis of each cheese sample.

745 Figure 9. Average size, area and total number of fat particles in 2D images during ripening
746 (A-C) and volume covered by fat particles at 180D, obtained by 3D image analysis (D) in six
747 different optical fields. All results are expressed as the mean \pm standard error ($n = 6$). Means
748 in a single figure with different letters are significantly different ($P < 0.05$).

749 Figure 10. Images of cheeses (at D180 old) obtained from SEM. Images from A to F are
750 CFFC, CLFC, LFCA1, LFCA2, LFCA3, LFCA4, respectively. Small white aggregates are
751 scattered over the protein network (indicated by red arrow) in alginate added cheese.

752 Figure 11. Transverse relaxation spectra of 180 days aged cheese showing distribution of
753 transverse relaxation time (T_2) obtained by LF-NMR.

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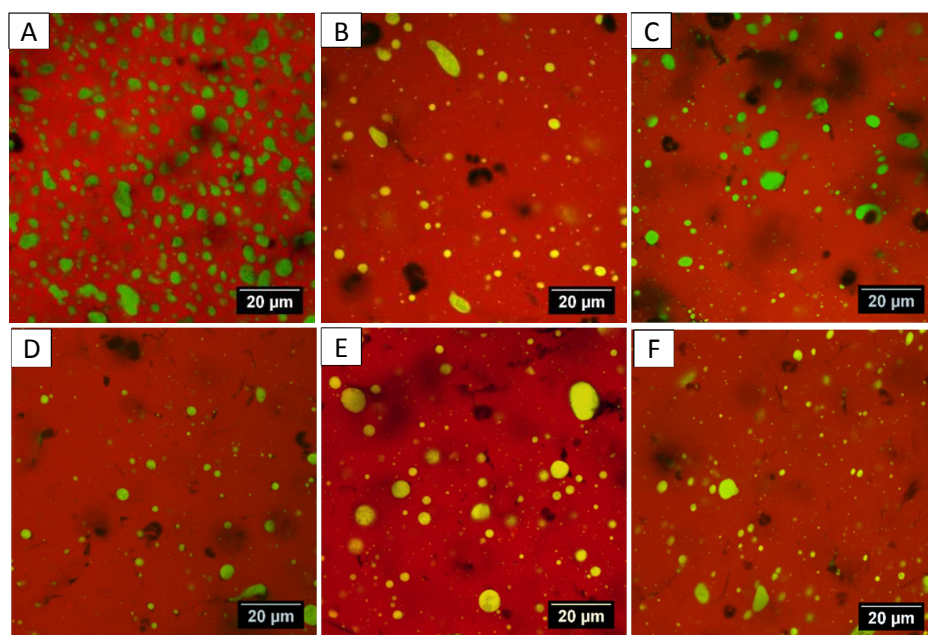


Figure 1.

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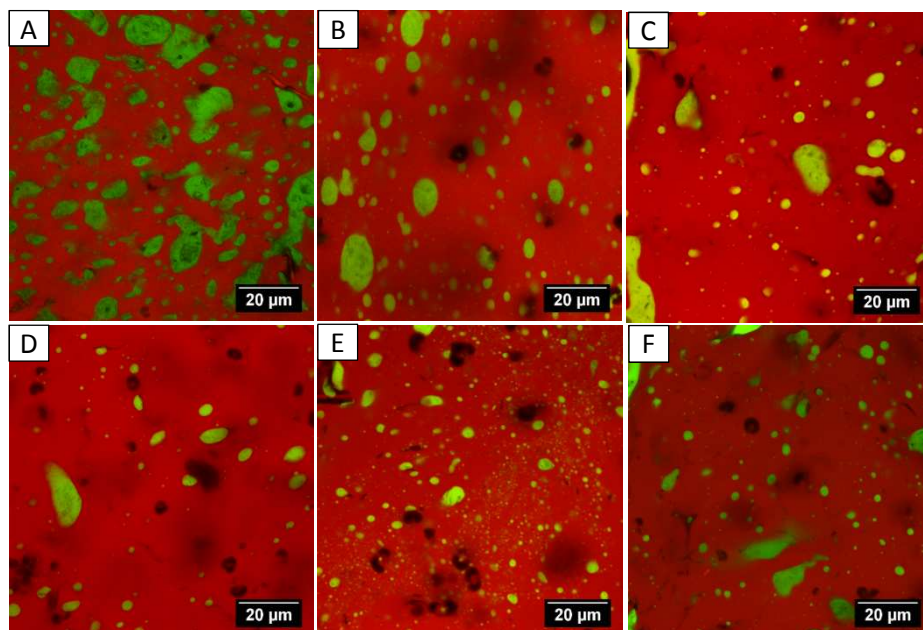
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**Figure 2.**

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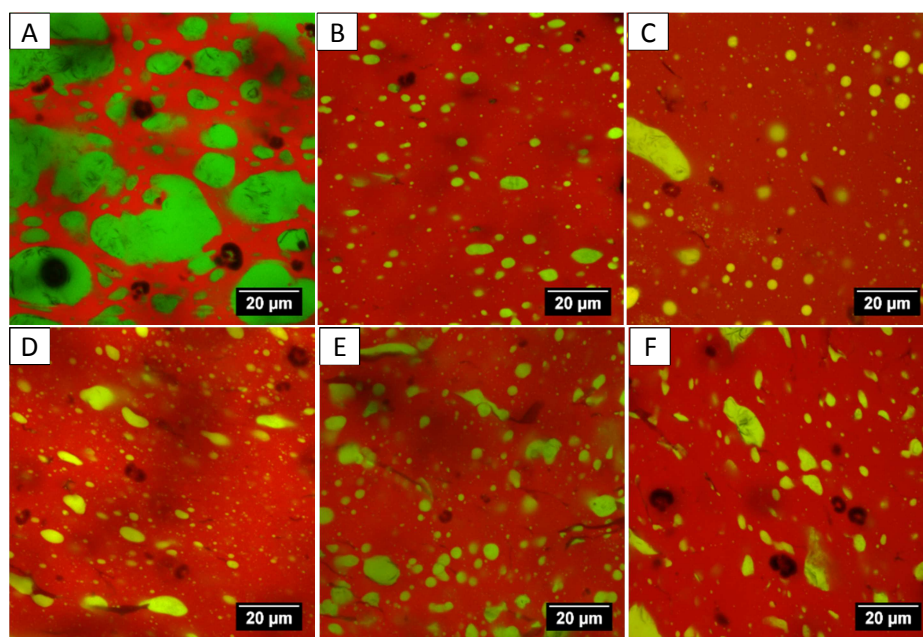


Figure 3.

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886 **Figure 4.**

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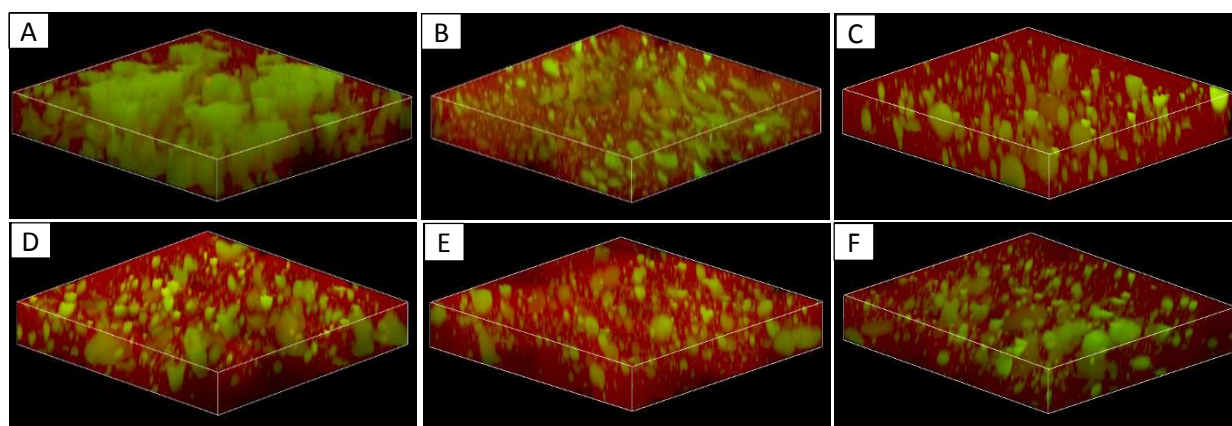
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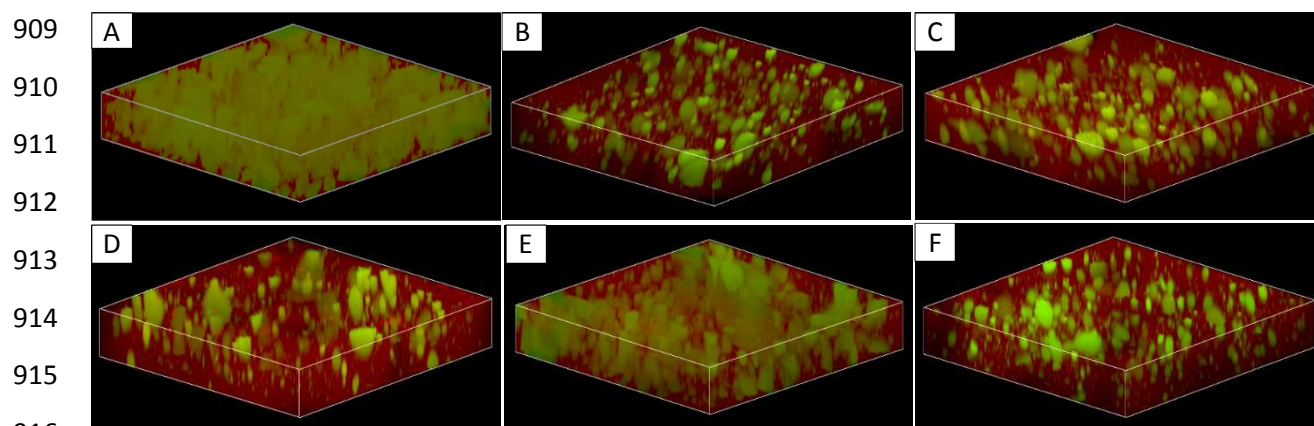
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917 **Figure 5.**

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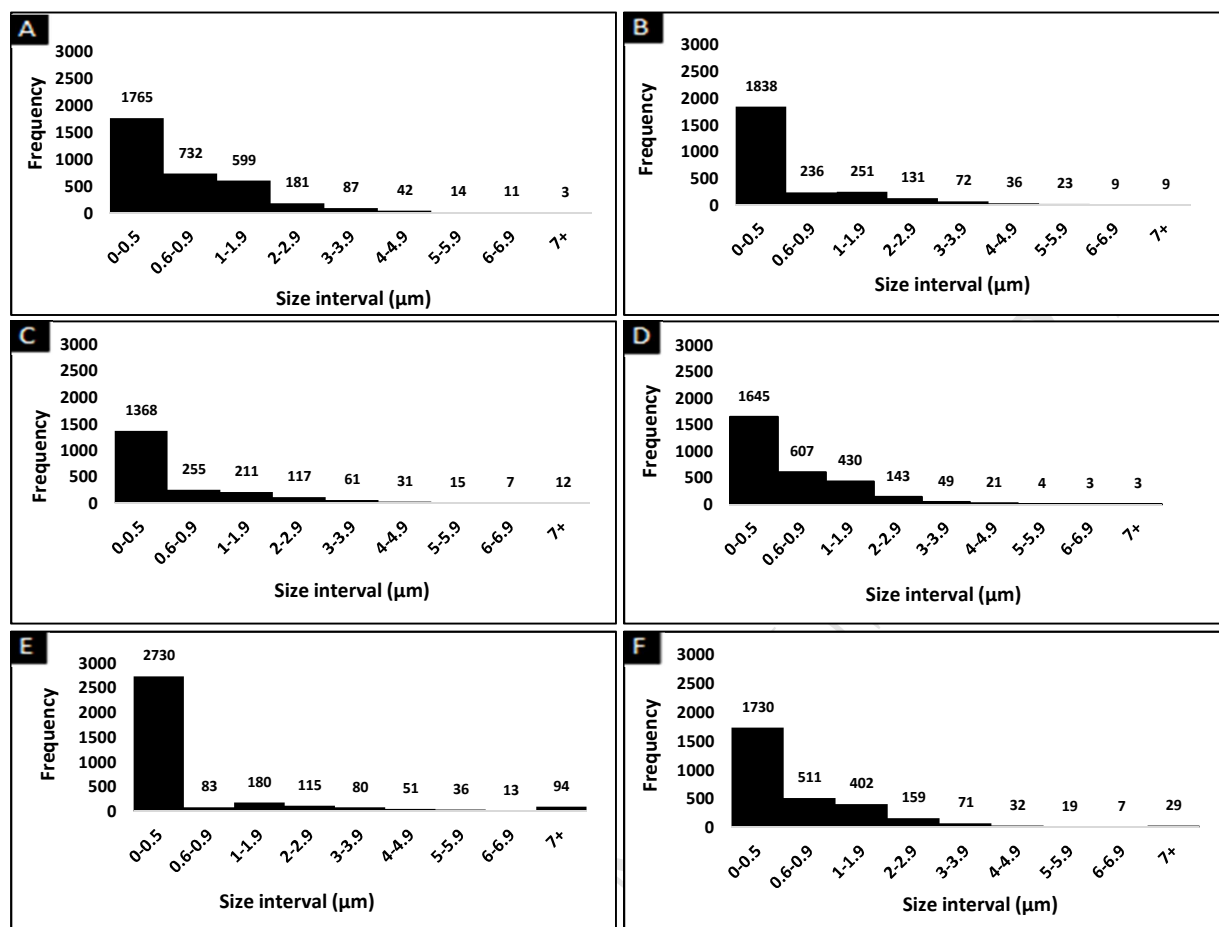
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938 **Figure 6.**

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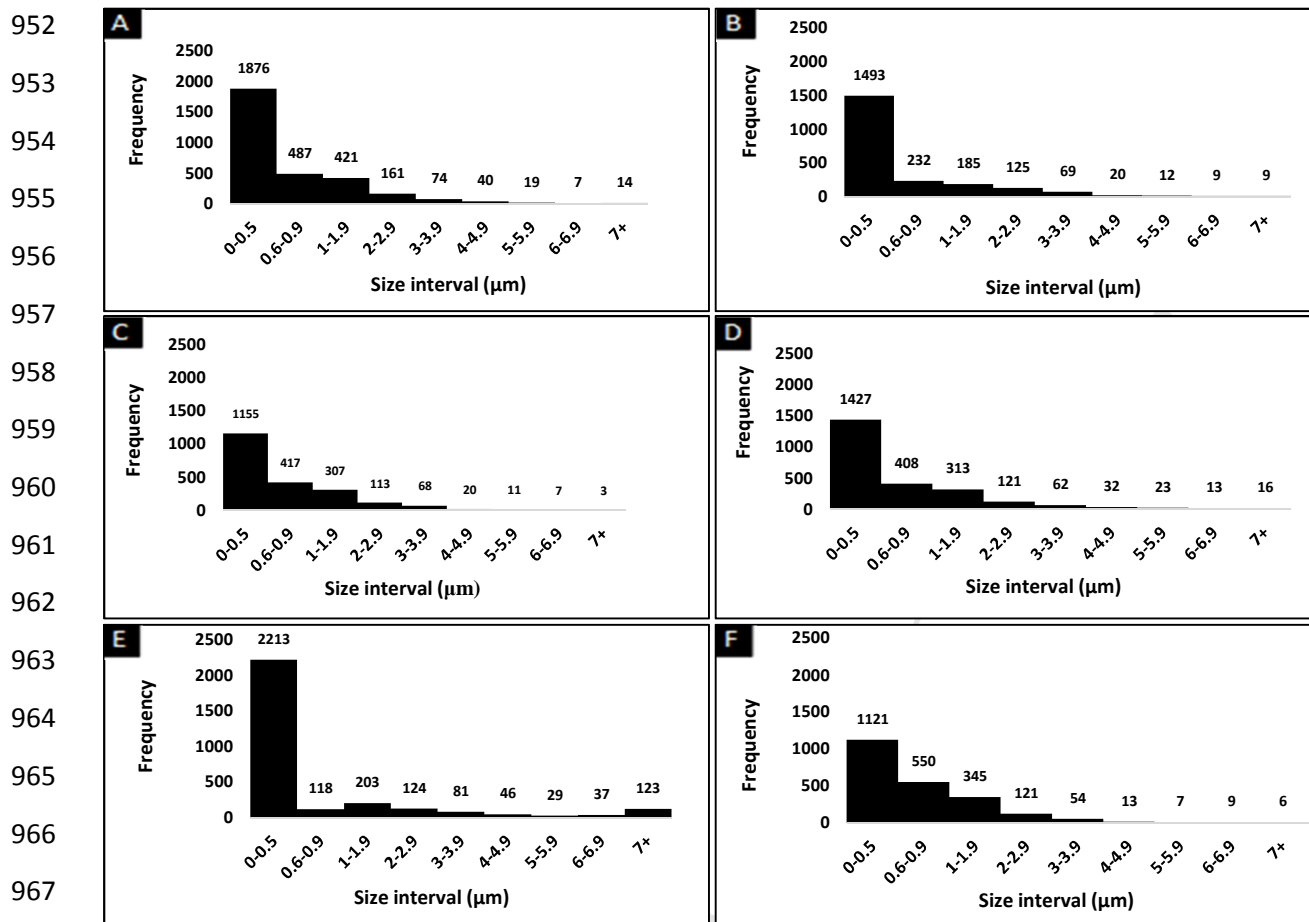
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968 **Figure 7.**

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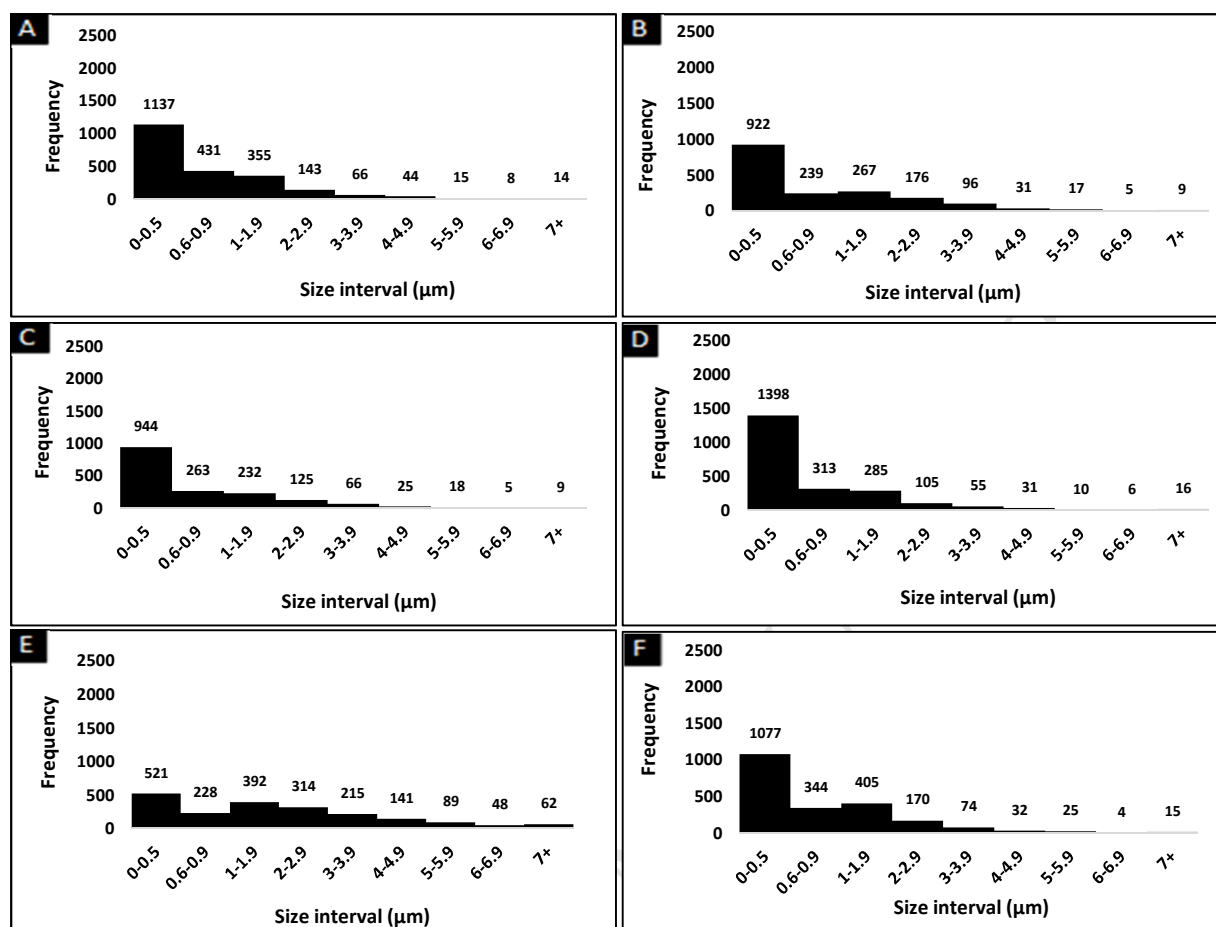
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984 **Figure 8.**

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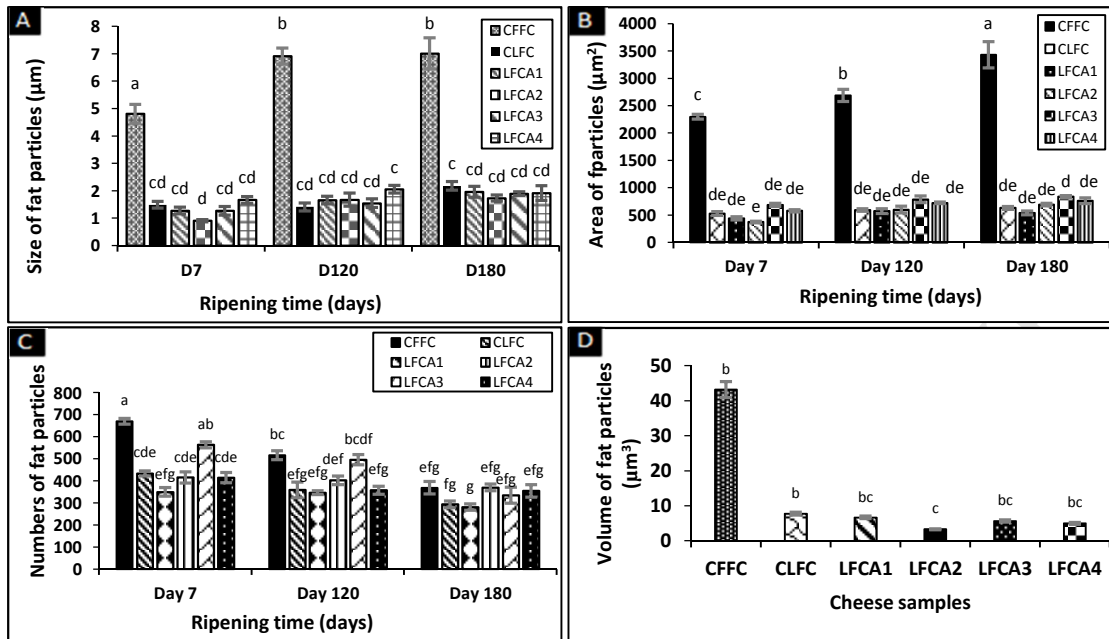
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1009 **Figure 9.**

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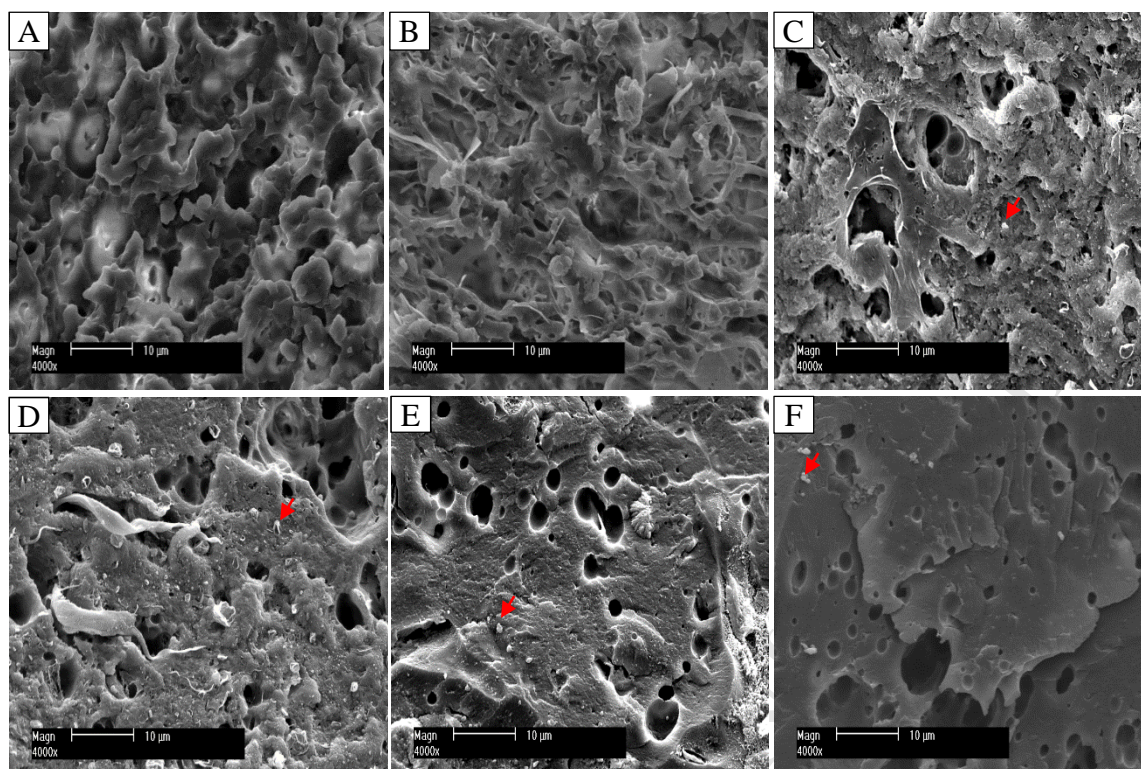
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1040 **Figure 10.**

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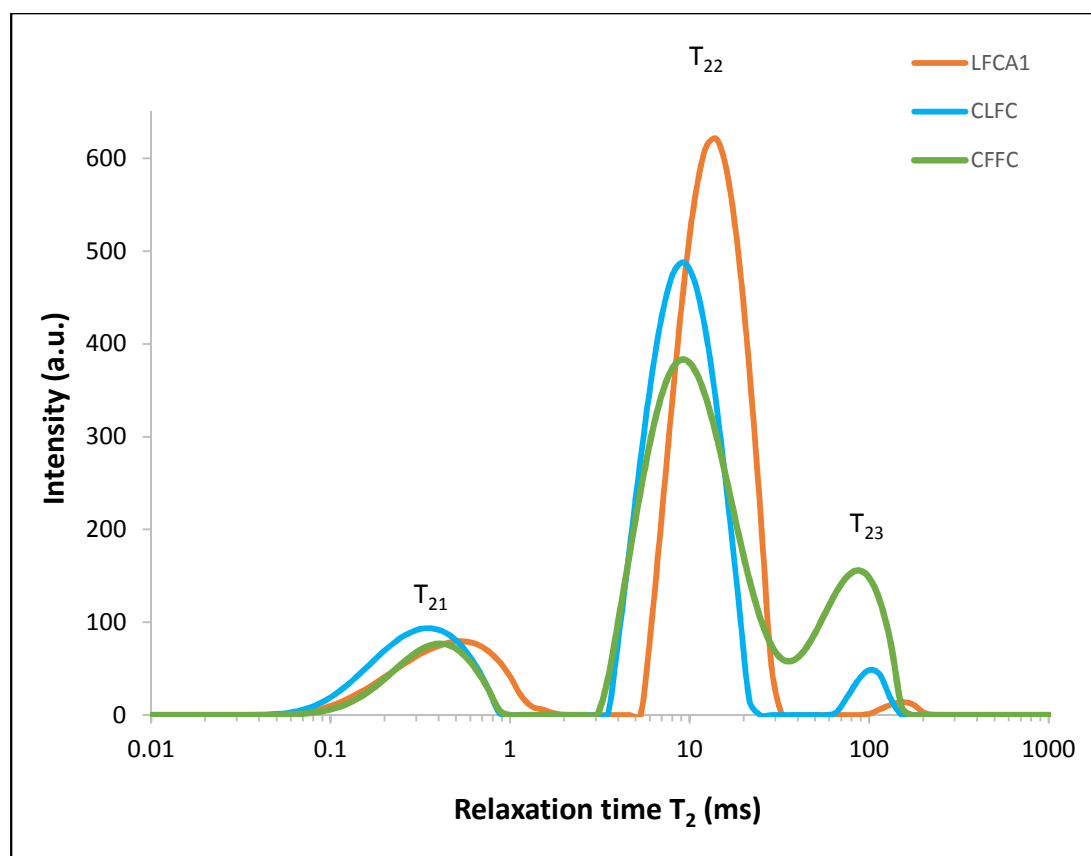
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1057 **Figure 11.**

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Highlights

1. Low fat Cheddar cheese (LFC) with four concentrations of alginate were prepared.
2. Fat level in alginate added LFC was reduced by up to 91% as compared to control full fat cheese (CFFC).
3. Textural properties of one of the alginate cheese containing only 3.1% fat were similar to those of CFFC.
4. Microstructure revealed smoother texture in alginate added LFC compared to control LFC.
5. Micrographs suggested there was effect on the fat particle size, area and volume in all LFCs.
6. Higher mobility water fraction was found in alginate added cheese compared to CFFC and CLFC by NMR.
7. Color of some of the alginate added LFCs was comparable to CFFC after 120 days of ripening.