

Effect of seasonal variation on the composition and properties of raw milk destined for processing in the UK

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1 **Effect of Seasonal variation on the composition and properties of raw milk**
2 **destined for processing in the UK**

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7 **Abstract (150 words max)**

8 The composition and physical properties of raw milk from a commercial herd were studied
9 over a one year period in order to understand how best to utilise milk for processing
10 throughout the year. Protein and fat levels demonstrated seasonal trends, while minerals and
11 many physical properties displayed considerable variations, which were apparently unrelated
12 to season. However, rennet clotting time, ethanol stability and foaming ability were subject to
13 seasonal variation. Many significant interrelationships in physico-chemical properties were
14 found. It is clear that the milk supply may be more suited to the manufacture of different
15 products at different times of the year or even on a day to day basis. Subsequent studies will
16 report on variation in production and quality of products manufactured from the same milk
17 samples described in the current study and will thus highlight potential advantages of
18 seasonal processing of raw milk.

19 **Highlights – 3-5 bullet points – max 85 characters including spaces**

- 20 - Physical and chemical properties of commercial raw milk studied throughout year
21 - Rennet clotting, ethanol stability and foaming ability displayed seasonal variation
22 - Significant interrelationships in physico-chemical properties were found
23 - Clearer guidelines on the seasonal processing of raw milk to be resolved.

24

25 **1. Introduction**

26 The composition of raw bulk milk is of prime importance for the manufacture of products in
27 the UK, and there is significant interest in variations in the composition and physico-chemical
28 properties of raw milk. In general, the composition of milk varies with season, stage of
29 lactation, feeding, health status of the cow, milking interval, genetic factors and other day to
30 day variation (Heck, van Valenberg, Dijkstra and van Hooijdonk, 2009).

31 The effects of seasonal variation on milk composition have been reported by many
32 researchers and it is clear that the concentrations of many constituents and the physico-
33 chemical properties vary throughout the year to different extents (DairyCo, 2013). Heck et al.,
34 (2009) reported lower fat and protein contents in summer than in winter milk. This could be
35 attributed to the different temperatures and feed composition, because cows consume more
36 dry feed in winter, whereas in summer they eat grass and stay outside for longer (Fox and
37 McSweeney, 2003). Some of this variation is well established and predictable. For example,
38 DairyCo (2013) reported that in the years 2009 to 2013, fat levels from the UK national herd
39 gradually decreased from January to July, followed by a sharp increase to more than 4.20 %
40 in August and September, and remained constant in October, November and December.
41 Protein content followed a similar trend but with less variation. From November to April,
42 protein content declined steadily from 3.35 % to 3.23 %, followed by a constant period from
43 April to July, and finally increased slightly from July to November. While these trends are
44 repeated annually, it is notable that there was significant year on year variation in the absolute
45 values. Also it should be noted that these trends are the means of the national herd, and
46 greater variation would be expected when considering individual milk supplies. Different
47 regions and feeding regimes result in different seasonal effects on the main components of
48 raw milk. Compared with the UK, the lowest protein content observed by O'Brien, Mehra,

49 Connolly and Harrington, (1999a) in Ireland was in March, which was probably due to lower
50 intake of feed energy with indoor feeding.

51 These seasonal changes cause problems, but also allow opportunities for dairy manufacturers.
52 For example, it is well established that butter spread ability is better when produced from
53 summer fat compared with winter fat, due to the higher proportion of unsaturated fatty acids
54 when cows are maintained on pasture in summer (Schmidt and Van Vleck, 1974). In the
55 cheese industry, extended rennet clotting times can result in either disruption of production
56 schedules or the failure to form a coagulum (Schmidt and Van Vleck, 1974). In addition to
57 cheese manufacturing, seasonal variation in milk composition probably causes a range of
58 problems in the manufacture of casein powder, whipping cream and liquid milk (Murphy and
59 O'Brien, 1997).

60 Variation in raw milk properties, such as pH, Ca^{2+} and mineral content, can also have a
61 pronounced effect on the manufacture of different dairy products (Faka, Lewis, Grandison
62 and Deeth, 2009). For example, according to Faka et al., (2009), higher Ca^{2+} and lower pH
63 was generally correlated with poor heat stability in skim milk powder (SMP) and vice versa,
64 and it is well known that calcium chloride addition can reduce the rennet coagulation time
65 and increase the curd firmness in cheese-making (Tsioulpas, 2005). On-Nom, Grandison and
66 Lewis (2010) found that Ca^{2+} concentration increased as pH decreased, and both parameters
67 decreased as temperature increased. Casein micelle size, buffering capacity, viscosity and
68 foaming ability are other important physico-chemical properties, which are related to the
69 natural and induced variations in the composition of milk (Salaun, Mietton, and Gaucheron,
70 2005; Fox and McSweeney, 2003).

71 The aim of this study was to determine the composition and physical properties of raw milk
72 from a commercial herd over the period from August 2011 to October 2012. Although
73 seasonal variation in milk composition and properties has been studied previously, the current

74 study is focussed on the practical relationship of these variations to dairy product
75 manufacture.

76

77 **2. Materials and methods**

78 *2.1 Milk samples*

79 Raw bulk milk was collected from the University of Reading Centre for Dairy Research. The
80 herd consists of an average of 550 lactating Holstein cows and the milk is sold commercially.
81 The animals are year-round calvers, and the majority are maintained indoors on total mixed
82 rations, while approximately 30% spend the summer months on grass. The composition and
83 physical properties were measured every two weeks. All analyses of raw milk were carried
84 out in triplicate. pH, ionic calcium, lactose, protein, fat and total solids content, rennet
85 clotting time (RCT), ethanol stability (ES), percentage of dry sediment, density, viscosity,
86 buffering capacity (BC), casein micelle size, freezing point depression (FPD), foaming ability
87 were measured at 20°C within 24 hours of milking. A total of 25 bulk milk samples were
88 studied during the period August 2011 to October 2012.

89 *2.2 Chemical analysis*

90 The protein, fat, lactose, urea, somatic cell count (SCC) and total casein concentrations were
91 measured in raw milk using a Lactoscope (Quadrachem Laboratories Ltd, London, UK)

92 pH was measured using a Sentron 3001 pH meter (Sentron Europe BV, ZH Roden, Dutch),
93 which was calibrated with standard buffer solutions of pH 4.0 and 7.0.

94 Ionic calcium (Ca^{2+}) was measured using a Ciba Corning 634 ISE Ca^{2+} /pH analyser (Ciba-
95 Corning Diagnostic Limited). The instrument was calibrated in the millivolt (mV) output
96 mode with solutions of 0.50, 1.00, 2.50 and 5.00 mM Ca^{2+} daily, prior to use. There was a

97 linear relationship between log (ionic calcium) and mV output, with correlation coefficients
98 greater than 0.99.

99 To determine total solids content (TS), raw milk samples (5 g) were accurately weighed and
100 poured into stainless aluminium dishes and placed in an oven (100°C) to constant weight. The
101 residual dry weight of raw milk was considered as the total solids.

102 To measure buffering capacity (BC), 4.0 mL 0.1 M HCL solution was added to 25 mL raw
103 milk sample and left for 1 h at room temperature. The pH difference before, and 1 hour after,
104 acid addition was considered to be the buffering capacity, and was expressed in pH units. The
105 fall in pH accompanied by this procedure is similar to the fall in pH when milk is heated from
106 20 °C to 120 °C.

107 Ash content was measured using the AOAC method (2005) employing a Precisa 125A
108 balance. Dry ashing was preferred to wet digestion. Results were expressed as % (w/v)
109 percentage of ash in milk. Each sample was measured in triplicate.

110 Total calcium and magnesium concentration were determined according to the AOAC
111 official method of analysis 991.25 (AOAC, 2005), employing a Pye Unicam SP9 Atomic
112 Absorption Spectrophotometer (Atomic absorption Spectrometer novAA350, Analytik Jena
113 AG, Germany) and using a calcium/magnesium lamp at a wavelength of 422.7 nm and 282.5
114 nm respectively. This method involves dry ingestion of milk samples followed by dissolution
115 of the resulting ash in concentrated nitric acid (69% HNO₃, Fisher Scientific, Loughborough,
116 UK). The calcium in the samples (100ml) reacts with the added lanthanum chloride solution,
117 1ml (10%; Fisher Scientific, Loughborough, UK).

118 Total citrate concentration was determined by HPLC (High Performance Liquid
119 Chromatography) according to Garnsworthy, Masson, Lock and Mottram (2006). The HPLC
120 apparatus consisted of an Agilent 1100 Isocratic Pump, an auto-sampler, a variable

121 wavelength detector, and a Prevail™ Organic Acid, 5µm Column (150 x 4.6mm) (Alltech,
122 Deerfield, US). Data were analysed by ChemStation software.

123 Total phosphorus concentration was determined according to IDF standard 42B (International
124 Dairy Federation, 1990).

125 ***2.3 Physical analysis***

126 Dry sediment and ethanol stability (ES) were measured according to Chen, Grandison and
127 Lewis, (2012).

128 A range of hydrometers (ranged from 1.000 to 1.050 g/ml) were used to determine the
129 density of raw milk.

130 Kinematic viscosity was determined by a capillary BS/U tube viscometer (PoultenSelfe and
131 Lee Ltd., Essex, UK). Raw milk was well shaken before the analysis. All measurements took
132 place at room temperature (20°C). The kinematic viscosity was calculated by multiplying the
133 flow time by the instrument constant. Types B, C, and D BS/U tube viscometers were used.
134 The nominal constants for each type are 0.01, 0.03 and 0.1. cSt s⁻¹ (1 cSt s⁻¹ = 10⁻⁶ m²s⁻¹)
135 respectively.

136 Freezing point depression (FPD) was measured for milk samples by using an Advanced Milk
137 Cryoscope 4L2 (Advance Instruments Inc., Metuchen, NJ, USA).

138 Rennet coagulation time (RCT) was measured according to Tsioulpas (2005).

139 The average casein micelle size of raw milk was measured with a Zeta Master (Malvern
140 Instruments, Malvern, UK) according to Chen et al., (2012).

141 Foaming ability was determined at 65⁰C by the air bubbling method developed by Huppertz
142 (2010).

143 ***2.4 Statistical analysis***

144 Statistical analysis of all data used Xlstat, 2012 and Statistical Package for the Social
145 Sciences (SPSS 18) software. All variables were centred and normalised using SPSS 18
146 normality test (explore). The Spearman correlation method in Xlstat was used to establish
147 whether the correlation coefficients between parameters were significant. Mean values,
148 number of determinations, regression, univariate analysis and seasonal variations were
149 calculated using SPSS 18 one-way ANOVA. The threshold levels of significance of $p < 0.05$,
150 0.01 and 0.001 were used in all analysis. Seasonal variations in raw milk were categorised
151 into four groups as shown in Table 1.

152 Measurements were made in triplicate and inserted into the database. Principal component
153 analysis (PCA) statistical method was performed with the Xlstat, 2012 software.

154 **3. Results and discussion**

155 The milk samples were analysed in detail in order to investigate how milk composition varies
156 throughout the year. The milk was also processed under standardised conditions to make a
157 variety of dairy products in order to investigate how selected quality attributes of these
158 products are influenced by milk composition. This paper presents the results for properties of
159 the raw milk.

160 ***3.1 Chemical properties of raw milk***

161 Table 2 presents the average values, range and seasonal variation for composition of raw milk
162 collected over the period August 2011 to October 2012. Higher total solids were found in
163 autumn than in summer but there was no significant seasonal variation over the spring and
164 winter. It was observed by O'Brien et al., (1999a) that there was a reduction in total solids
165 content in January to April and July to August which is in agreement with the current study.
166 Raw milk produced in the autumn period had a significantly higher fat content than in other
167 periods which is broadly in line with the UK national statistics (DairyCo, 2013).

168 Compared with fat, the protein and casein concentrations showed less variability, ranging
169 from 2.89 to 3.56 %. However, significantly higher protein content was observed in spring
170 compared to the summer and autumn periods. This would not be expected from UK national
171 statistics (DairyCo, 2013), which would predict higher levels of protein in the autumn and
172 winter months, and may reflect the particular feeding regime used for this herd.

173 The average concentration of urea was 3.95 mM, ranging from 2.65 mM in October to 5.44
174 mM in November, although there was no specific significant seasonal variation. There were
175 no significant correlations between urea and other components of raw milk. This contrasts
176 with Giaccone, Todaro and Scatassa (2007) who reported considerable seasonal variation in
177 urea level. This difference could be attributed to the farming methods used at the Reading
178 University farm where there was no strict time schedule in moving from indoor feeding to
179 pasture feeding.

180 There was a little variation in the level of lactose which ranged from 4.52 to 4.69 % with no
181 significant seasonal differences. This is not surprising as lactose is well known to be one of
182 the least variable milk components.

183 pH showed a similar seasonal trend to the protein, which was significantly higher in spring
184 than in summer and autumn. However, buffering capacity (BC) did not display any
185 significant seasonal trend.

186 Generally the levels of minerals and citrate were within the normal range found for cows'
187 milk, and while there were fluctuations, there were no significant differences between
188 seasons. The total calcium and magnesium concentrations followed a similar trend. This is in
189 general agreement with O'Brien, Mehra, Connolly and Harrington (1999d) who showed that
190 calcium, magnesium and chloride concentrations fluctuated considerably but showed no
191 definite trend over the year, and that these minerals were predominantly influenced by

192 lactation stage, but were also influenced by feed type (Keogh, Kelly, O'Keeffe and Phelan,
193 1982).

194 The ash content for raw milk fluctuated greatly over the period of study, but no significant
195 seasonal difference was observed. The maximum ash content (1.03%) was observed in
196 August and minimum content (0.53%) was found in October. Rao and Mishra (2010)
197 reported that ash content was not significantly influenced by season, breed and lactation stage,
198 but much narrower variations were observed compared to the current study.

199 The Ca^{2+} concentration ranged from 1.68 to 2.55 mM (which corresponds to 5.7 to 8.7% of
200 the total Ca), with no significant seasonal differences. Although bulking of milk will reduce
201 these variations, raw milk destined for processing will still be subject to considerable
202 variations in Ca^{2+} and pH, which may have significant effects on its processing behaviour.
203 Grimley, Grandison and Lewis (2009) monitored changes in minerals throughout the spring
204 flush period and reported that Ca^{2+} was reduced from 1.48 to 1.40 mM at this time, whereas
205 total divalent cations were reduced from 35.4 to 33.4 mM. There was no evidence to suggest
206 that any of the milk samples were unsuitable for processing, in terms of poor heat stability or
207 poor coagulation properties.

208 Buffering capacity (BC) of raw milk was constant throughout the year. The average value of
209 BC, expressed as pH differential value, was 0.84 ± 0.02 . Conversely, Harris, Tong, Vinkl,
210 Izeol and Jimenez-Flores (2002) observed that the highest BC values were found in
211 December and the lowest in September in California, using a method based on titration of
212 milk to pH 4.0. Again, farming methods and feeding regime probably contributed to
213 differences in the study.

214 The BC depends mainly on the composition and distribution of minerals and proteins
215 between aqueous and solid phases. Salaun et al., (2005) proposed that BC is determined by

216 soluble phosphate, colloidal calcium phosphate, citrate, bicarbonate, caseins and whey
217 proteins. However, with the exception of a positive correlation with somatic cell count, no
218 significant correlation between BC and compositional properties was observed in the current
219 study.

220 *3.2 Physical properties of raw milk*

221 Values, ranges and seasonal variations in physical properties and various markers of milk
222 stability are given in table 3. The viscosity and density displayed some fluctuations but no
223 significant seasonal variation.

224 The average casein micelle size was 163 nm, which was slightly smaller than reported by
225 Glantz, Devold, Vegarud, Lindmark Månsson, Stålhammar and Paulsson (2010). The range
226 was from 132 nm in August to 202 nm in October. However, no significant seasonal variation
227 was observed. Holt and Muir (1978) reported that the average size of casein micelles
228 followed a pronounced seasonal trend with smaller average sizes in summer compared to
229 winter. The present study showed a similar trend in that the average sizes in summer and
230 winter were 162 nm and 167 nm respectively, but the difference was not statistically
231 significant.

232 The FPD displayed a relatively narrow range, as would be expected because it is a
233 consequence of the osmotic balance between the milk and the blood (Shipe, 1959). However
234 the values were significantly greater in winter than in the spring and summer. Changes in
235 temperature and diet are considered to be primarily responsible for the seasonal effect on
236 milk FPD (Henno, Ots, Jōudu, Kaart and Kärt, 2008). However, the latter authors stated that
237 the increase in milk freezing point was not caused by the lack of energy or protein from the
238 feed ration, but was probably due to the increased water intake due to the increased
239 temperature and sunshine hours as suggested by Bjerg, Rasmussen and Nielse (2005).

240 The RCT, ES, foaming ability and sediment formation are all properties which relate directly
241 to the processing of milk, and it is notable that all displayed significant seasonal variation
242 (Table 3). The raw milk samples produced in spring had significantly longer RCT than in the
243 autumn. Average values were 21 min in spring compared to 17 min in autumn, which would
244 imply considerable coagulation time differences in rennet cheese manufacturing. This
245 variation could be attributed to the pH value in raw milk. It is well known that reduced pH
246 leads to a reduction in RCT. (e.g. Lucey, 2002).

247 The average ES was higher (93%) than that reported by Chavez, Negri, Taverna and Cuatrin
248 (2004), where about one third of samples had an ES less than 72%. All samples were above
249 74%, which is the suggested limiting value for for UHT processing (Shew, 1981). Ethanol
250 stability followed the same seasonal trend as RCT with significantly higher values in spring
251 than autumn.

252 Dry sediment formation followed a different trend with significantly higher values in the
253 summer and autumn compared to the winter. However, the importance of this finding is not
254 clear as sediment in raw milk may be unrelated to other compositional properties, being at
255 least partly related to extraneous debris in the milk (reference).

256 Foaming ability displayed the greatest range of all parameters measured (8.5 times difference)
257 and in common with the RCT and ES, values were highest in the spring, in this case higher
258 than the other three seasons. (discussed in section 3.3.7)

259

260 ***3.3 Interrelationships between physical and chemical parameters***

261 Significant correlations between the main milk components and properties are shown in
262 Tables 4 and 5. It should be noted that not all the significant correlations will be causal, some
263 may be coincidental or linked indirectly to other parameters.

264 Fat content was strongly correlated with total solids, pH and protein content, and weakly with
265 total calcium and phosphorus, while protein content was significantly correlated with pH and
266 total casein content.

267 The average value of total solids was 12.78 % which gave a strong positive correlation
268 coefficient ($p < 0.001$) to the fat content as shown in Table 4. Also, it was weakly correlated
269 with total ash and calcium content.

270 Lactose content was weakly correlated with total ash, citrate and SCC content.

271 As shown in Table 4, there were positive correlations between pH and protein and total
272 casein content, but a negative correlation with fat content. Pastorino, Hansen and McMahon
273 (2003) reported that the most significant effect of decreased pH was to promote mineral
274 solubilisation and casein dissociation from casein micelles, both of which altered milk
275 properties by affecting the extent and nature of protein interactions.

276 A weak but significant negative correlation between pH and Ca^{2+} was found, which agrees
277 with On-Nom et al., (2010). It is well established (Fox and McSweeney, 2003) that reducing
278 milk pH results in solubilisation of micellar calcium, which would result in increased Ca^{2+} .

279 Keogh et al., (1982) reported that there were significant positive correlations between citric
280 acid and fat content and between soluble calcium and fat content, but no correlation was
281 found between fat content and citrate in the present study.

282 A number of weak but significant correlations were found among the mineral, citrate, lactose
283 and SCC concentrations, but it is not clear which of these were of biological significance.

284 RCT and ES were strongly positively correlated, which is not surprising as both are
285 expressions of the stability of the casein micelle system (Tsioulpas, 2005). Both parameters
286 were significantly related to pH, protein, casein and fat levels. The correlations with fat are
287 likely to be coincidental or linked indirectly to other parameters. The correlations with pH are
288 to be expected as lowering pH is known to reduce casein micelle stability and the
289 relationships with protein and casein are probably at least partly secondary to the
290 relationships with pH (Fox and McSweeney, 2003). RCT correlated with Ca^{2+} , and it is
291 surprising that ES did not display a similar correlation, as other researchers (e.g. Davis and
292 White, 1958) have reported that ethanol stability was inversely related to Ca^{2+} concentration.
293 The explanation could be that ES was not only very high for this sample set but the range was
294 also much narrower than those found by other investigators. Also ES was only measured up
295 to 100%, and a number of samples displayed ES values of 100% (N.B. it is possible to have
296 values >100% if more concentrated ethanol solutions are used).

297

298 **3.3.1 Rennet Clotting Time (RCT)**

299 RCT was highly positively correlated with pH, protein, casein and ES, but negatively
300 correlated with Ca^{2+} , fat and total phosphorus content (Table 5). The strong positive
301 correlation with pH would be expected from earlier studies. Lucey (2002) showed that the
302 activity of rennet increased with decrease of pH, while other studies have demonstrated that
303 the aggregation of destabilised micelles increases at lower pH due to solubilisation of
304 micellar calcium phosphate, a decrease in net surface charge and dissociation of casein from
305 the micelles (Fox and McSweeney, 2003). The Ca^{2+} was negatively correlated with RCT, in
306 agreement with Tsioulpas (2005) who showed that increasing Ca^{2+} would accelerate milk
307 coagulation because Ca^{2+} in the serum phase reduces the negative charge on the surface of

308 casein micelles, weakening repulsion and accelerating their coagulation. Fox and McSweeney
309 (2003) suggested that initiating coagulation required a minimum concentration of Ca^{2+} of
310 about 1.5 mM.

311 Jõudu, Henno, Kaart, Püssa and Kärt (2008) showed that an increase in milk protein, casein,
312 casein fractions, and the casein number decreased the rennet coagulation time of milk which
313 was not in agreement with the current study. The weak negative correlation between RCT
314 and fat content observed in Table 5 is probably secondary to the correlations of fat with pH
315 and protein (Table 4).

316 The negative correlation of RCT with total phosphorus content corresponds with the findings
317 of McMahan, Brown, Richardson and Ernstrom (1984) who found that addition of phosphate
318 reduced coagulation time with a minimum at 0.01 M added phosphate. However higher levels
319 of addition (0.04 M added phosphate) increased coagulation time above control levels. They
320 suggested that the calcium phosphate system was extremely slow in equilibrating, and at low
321 added phosphate, it enhanced coagulation, whereas at high phosphate the equilibrium was
322 forced sufficiently in the direction of complex formation with Ca^{2+} that coagulation was
323 retarded.

324 There was a significant positive correlation between RCT and ES, which could be attributed
325 to Ca^{2+} and pH in raw milk because higher concentration of Ca^{2+} and lower pH value resulted
326 in the shorter RCT and poor ES in raw milk as described above.

327

328 **3.3.2 Casein micelle size**

329 As discussed previously, large casein micelles would be expected to cause a longer RCT,
330 poorer heat stability (Chen et al., 2012) and weaker curd compared with smaller ones. As

331 shown in Table 4, there was a positive significant correlation between casein micelle size and
332 total phosphorus content. Holt and Muir (1978) reported that the average size of casein
333 micelles correlated positively with the amount of colloidal phosphorus per unit weight of
334 casein, and negatively with casein-bound calcium. Micelle size has been shown to vary, not
335 only between feeding regimens and regions (Devold, Brovold, Langsrud and Vegarud, 2000),
336 but also between protein genotypes in different breeds. The casein micelle size was also
337 related to milk pH (table 5). Milk pH correlated negatively with casein micelle size, implying
338 that a higher milk pH would result in smaller native casein micelles, which was in contrast to
339 data of Glantz et al., (2010), although the reason for this disparity is not clear. With the
340 exception of total phosphorus content, none of the mineral components was significantly
341 related to casein micelle size, which is also in contrast to Glantz et al., (2010). Total protein
342 content was significantly correlated to the casein micelle size, which is in agreement with
343 results found previously in individual cows (Devold et al., 2000). The fact that there was no
344 correlation with the total calcium content was also in agreement with studies on bulk tank
345 milk and milk from individual cows (Devold et al., 2000).

346

347 ***3.3.3 Viscosity and density***

348 The average value of viscosity of was 1.93 cSt ($1 \text{ cSt} = 10^{-6} \text{ m}^2 \text{ s}^{-1}$), with a range from 1.52 to
349 2.36 cSt (Table 3). It has been shown by Fernandez-Martin (1972) that at room temperature,
350 milk viscosity increased either with increasing fat content, when solids-not-fat content was
351 kept constant, or with increasing solids-not-fat content when fat content was constant. There
352 is general agreement that milk viscosity is a non-linear function of total solids content.
353 However, according to Table 5, viscosity was highly significantly positively correlated with

354 pH and casein content, but not with total solids or fat content, although the correlation with
355 pH may be secondary to that with casein.

356 According to Table 2, the highest density was 1.031 g/ml and the lowest was 1.026 g/ml,
357 which fall within the normal range of 1.025-1.035 g/ml, reported by Scott, Robinson and
358 Wilbey (1998). The density of raw milk did not vary significantly with seasonal changes over
359 the year.

360 ***3.3.5 Somatic Cell Counts (SCC)***

361 Contrary to the reports of some authors (Klinkon, Zadnik and Nemec, 2000), the raw milk
362 produced in winter had significantly more SCC than in other seasons, presumably reflecting
363 differences in herd management. The SCC results of Rajčević, Potočnik and Levstek (2003)
364 were in partial agreement with the current study. SCC in raw milk ranged from 65000 to
365 357000 and correlated positively and significantly with lactose and phosphorus content, and
366 negatively with total calcium and citrate content in raw milk in the present study.

367 Rajčević et al., (2003) reported that a statistically significant negative correlation occurred
368 between SCC and lactose content in milk, which was in contrast to the present study.

369 Compared with former researchers, the significant positive correlation in our results could be
370 attributed to the different feed, regions and even milking methods.

371 ***3.3.6 Freezing Point Depression (FPD)***

372 Freezing point depression was significantly higher in winter than in spring and summer
373 (Table 3) with a range comparable to that reported by Shipe (1959) – i.e. 515 to 530 m°C.

374 Changes in temperature and diet were considered to be primarily responsible for the seasonal
375 effect on milk FPD (Henno et al., 2008). Pinkerton and Peters (1958) suggested that
376 differences in environmental temperature and animal feed were contributing factors to the

377 seasonal variations that they observed. However, Henno et al., (2008) pointed out that the
378 increase in milk FPD was not caused by the lack of energy or protein from the feed ration
379 used but was probably due to the increased water intake with increased temperature and
380 sunshine hours. No significant correlation between FPD and compositional parameters was
381 found, which implies that FPD was a strong independent parameter in raw milk. The freezing
382 point of cows' milk is relatively constant as a consequence of osmotic equilibrium in milk
383 and blood (Shipe, 1959).

384

385 ***3.3.7 Foaming ability***

386 The foaming ability (the time to produce a foam) of raw milk in the summer period was
387 significantly lower than in spring, autumn and winter (Table 3). It was significantly positively
388 correlated with protein and casein content in raw milk (Table 5) which suggests that higher
389 protein content in raw milk would cause the longer foaming times. This result is counter-
390 intuitive and contrasts with Marinova et al., (2009) who reported that the foaming ability of
391 milk increased with the protein concentration, until a constant value was reached.

392 Foaming ability did not correlate significantly with milk pH, although the pH range was
393 narrow. However, Augustin and Clarke (2008) indicated that when pH was decreased to 5.6,
394 an increase in foaming ability was observed, but this pH is well below the values measured in
395 the current study.

396 The mineral components in milk presumably play a significant part in determining the
397 foaming ability properties due to their large effect on the casein micelle (Augustin and Clarke,
398 2008). However, no correlation was observed in the present study. Augustin and Clarke
399 (2008) reported that addition of calcium chelators, such as citrate and EDTA, could improve

400 the foaming ability and stability in milk. Such enhanced foaming ability in milk could
401 possibly be attributed to the higher proportion of non-micellar casein, which could adsorb
402 onto the air interface.

403 Gambini, Castagnetti and Losi (1995) reported that foaming capacity of milk also tended to
404 decrease with increasing SCC due to the strongly correlated with increased plasmin
405 concentration and proteolysis in milk. However, no significant relation was observed in the
406 present study.

407 ***3.3.8 Principal Component Analysis***

408 PCA was carried out on the whole data set consisting of 25 samples and the 23 variables. The
409 similarity map defined by principal components PC 1 and PC 2 showed a discrimination of
410 samples according to the different seasons. Considering the PCA similarity map defined by
411 principal components 1 and 2, the milk samples were separated according to principal
412 component 1 (37.79 % of the total variance; Figure 1). This component clearly showed the
413 effect of the seasonal variations. Most samples in spring and summer were located on the
414 positive part of the similarity map, whereas autumn samples were on the negative part. Raw
415 milk in spring and summer were characterised by higher pH, ash content, foaming ability, ES,
416 RCT, total casein and protein content. In contrast, the properties of raw milk in autumn were
417 characterised by higher fat content and CM size than in spring and summer. Autumn milk
418 also exhibited higher Ca^{2+} , urea, TS, SCC and percentage of dry sediment than spring and
419 summer milk, but the contributions of these variables were less significant. Similarly, spring
420 milk contained higher citrate, total Mg, Total Ca and viscosity, but these attributes were less
421 significant due to being closer to the origin of the coordinate. BC, total phosphorus, density
422 and lactose were not subject to seasonal variations, since these variables were located close to
423 the origin of the coordinate (Figure 1) which is in agreement with Table 2 and 3. However,

424 FPD was not influenced by seasonal variations from the similarly map which was in contrast
425 to Table 3 which, again, implied that FPD was a strong independent parameter in raw milk.
426 In addition, two summer milk samples located in the bottom left position could probably be
427 attributed to the sampling time since they were the final samples in summer period. This short
428 interval between the last sample in summer and the first sample in autumn probably explains
429 the similar physico-chemical properties. Overall this analysis shows that the physico-
430 chemical properties of the spring and autumn milks were quite distinct, whereas the summer
431 and winter milks were much less differentiated.

432 **4. General Discussion**

433 The aim of this study was to determine the extent of variations in composition and physico-
434 chemical properties of bulk milk from a commercial herd in the UK throughout the year.

435 Seasonal variations were found for some properties and correlations between some physical
436 properties and milk composition have been highlighted. PCA was used to distinguish
437 between the samples and was able to differentiate between autumn and spring milk samples.

438 The novelty of this study is that these milk samples were then used to produce a range of
439 products under standardised conditions in order to determine how selected quality attributes
440 of the products were influenced by milk composition and other properties.

441

442 The observed variation in different parameters could be related to seasonal effects in some
443 cases but seemed to be more difficult to explain. Much of the data was consistent with earlier
444 studies on seasonal variation, while other parameters varied less widely or displayed less
445 seasonality than may have been expected.. This may be because previous studies were from

446 different geographical areas, or employed different, in some cases outdated, farming practices
447 such as diets or calving patterns.

448 Many interrelationships in physical and chemical parameters were observed and it is clear
449 that the milk supply would be more suited to the manufacture of different products at
450 different times of the year or even on a day to day basis. Subsequent studies will report on
451 variation in production and quality of products manufactured from the same milk samples
452 described in the current study. In this way it is hoped to provide some clearer guidelines on
453 the relationships in milk composition, properties and product manufacture, including seasonal
454 factors.

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Seasons		Definitions	Sample size
Spring	SP	March, April and May	6
Summer	SM	June, July and August	6
Autumn	A	September, October and November	9
Winter	W	December, January and February	4

565 **Table 1 The definition and sample sizes of different seasons**

Compositional Properties	Mean±SD	Range	Seasonal variation
pH	6.79±0.35	6.73 - 6.87	SP>SM and A;W > A
Ca ²⁺ (mM)	2.05±0.25	1.68 - 2.55	NS
TS (%)	12.78±0.29	12.31 - 13.31	A > SM
Protein (%)	3.29±0.16	2.89 - 3.56	SP>SM and A
Total casein (%)	2.36±0.09	2.08 - 2.52	SP>SM and A
Fat (%)	4.08±0.36	3.62 - 4.77	A > SP, SM and W
Lactose (%)	4.59±0.44	4.52 - 4.69	NS
BC	0.84±0.02	0.78 - 0.88	NS
Ash (%)	0.71±0.14	0.53 - 1.03	NS
Total Ca (mM)	29.3±1.78	24.5 - 31.5	NS
Total Mg (mM)	5.11±0.34	4.21 - 5.81	NS
Total P (mM)	9.04±0.53	8.22 - 10.1	NS
Total Citrate (mM)	27.5±2.12	22.6 - 33.6	NS
Urea (mM)	3.95±0.40	2.65–5.44	NS
SCC (cells ml ⁻¹ x 10 ³)	155±63	65 – 357	W > SP, SM and A

Table 2 Composition of raw milk collected over the period August 2011 to October 2012 (Results are mean ± Standard deviation, n= 25.)

SP=Spring; SM=Summer; A=Autumn; W=Winter;NS=Non-significant difference (p<0.05)

566 *Total solids=TS; Buffering capacity= BC; Somatic cell count=SCC*

Physical properties	Mean±SD	Range	Seasonal variation
Density (g/cm ³)	1.028±0.01	1.026 - 1.031	NS
Casein micelle size (d.nm)	163±16	132 - 202	NS
*Viscosity (cSt)	1.93±0.21	1.52 - 2.36	NS
FPD (m°C)	523±3	514 - 530	W > SP and SM
Foaming ability (s)	88±47	24 - 205	SP > SM, A and W
RCT (min)	18.6±3.1	12.3 - 24.0	SP > A
ES %	93±5	84 - 100	SP > A
Sediment (%)	0.09±0.02	0.03 - 0.13	SM and A > W

*1 cSt = 10⁻⁶ m²s⁻¹

Table 3 Physical and stability properties of raw milk over the period August 2011 to October 2012 (Results are mean ± Standard deviation, n= 25.)

SP=Spring; SM=Summer; A=Autumn; W=Winter; NS=Non-significant difference (p<0.05)

567 *Freezing point depression=FPD; Rennet coagulate time=RCT; Ethanol stability=ES*

Components	Correlation coefficient (p)
pH/Ca ²⁺	-0.471*
pH/Protein	0.631***
pH/Total casein	0.658***
pH/Fat	-0.525**
TS/Fat	0.630***
TS/Total Ca	-0.406*
TS/Total ash	0.482*
Protein/Fat	-0.614**
Protein/Total Casein	0.597**
Fat/Total Ca	-0.441*
Fat/Total P	0.414*
Lactose/Total citrate	-0.452*
Lactose/Total ash	-0.511*
Lactose/SCC	0.444*
Total Ca/Total Mg	0.446*
Total Ca/Total citrate	0.476*
Total Ca/SCC	-0.457*
Total Mg/Total citrate	0.464*
Total Mg/Total ash	-0.473*
Total P/SCC	0.492*
Total citrate/Total ash	0.438*
Total citrate/SCC	-0.498*

Table 4 Correlation coefficients between raw milk compositional parameters over the period from August 2011 to October 2012

*Significance levels: * P < 0.05; ** P < 0.01; *** P < 0.001*

568 *Total solids=TS; Somatic cell count=SCC*

569

Components	Correlation coefficient (<i>p</i>)
Density/Lactose	0.545**
Density/SCC	0.447*
Density/Foaming ability	0.469*
Casein micelle size/pH	-0.525**
Casein micelle size/TS	0.410*
Casein micelle size/Protein	-0.494*
Casein micelle size/Fat	0.666***
Casein micelle size/Total phosphorus	0.408*
Viscosity/pH	0.555**
Viscosity/Total casein	0.646***
Foaming ability/Protein	0.587**
Foaming ability/Total casein	0.526**
RCT/pH	0.629***
RCT/Ca ²⁺	-0.557**
RCT/Protein	0.678***
RCT/Fat	-0.470*
RCT/Total phosphorus	-0.653***
RCT/Total casein	0.559**
RCT/Viscosity	0.421*
RCT/Foaming ability	0.459*
RCT/ES	0.639***
ES/pH	0.757***
ES/Protein	0.663***
ES/Fat	-0.488*
ES/Total casein	0.629***
ES/Casein micelle size	-0.436*
ES/Viscosity	0.417*
BC/SCC	0.488*

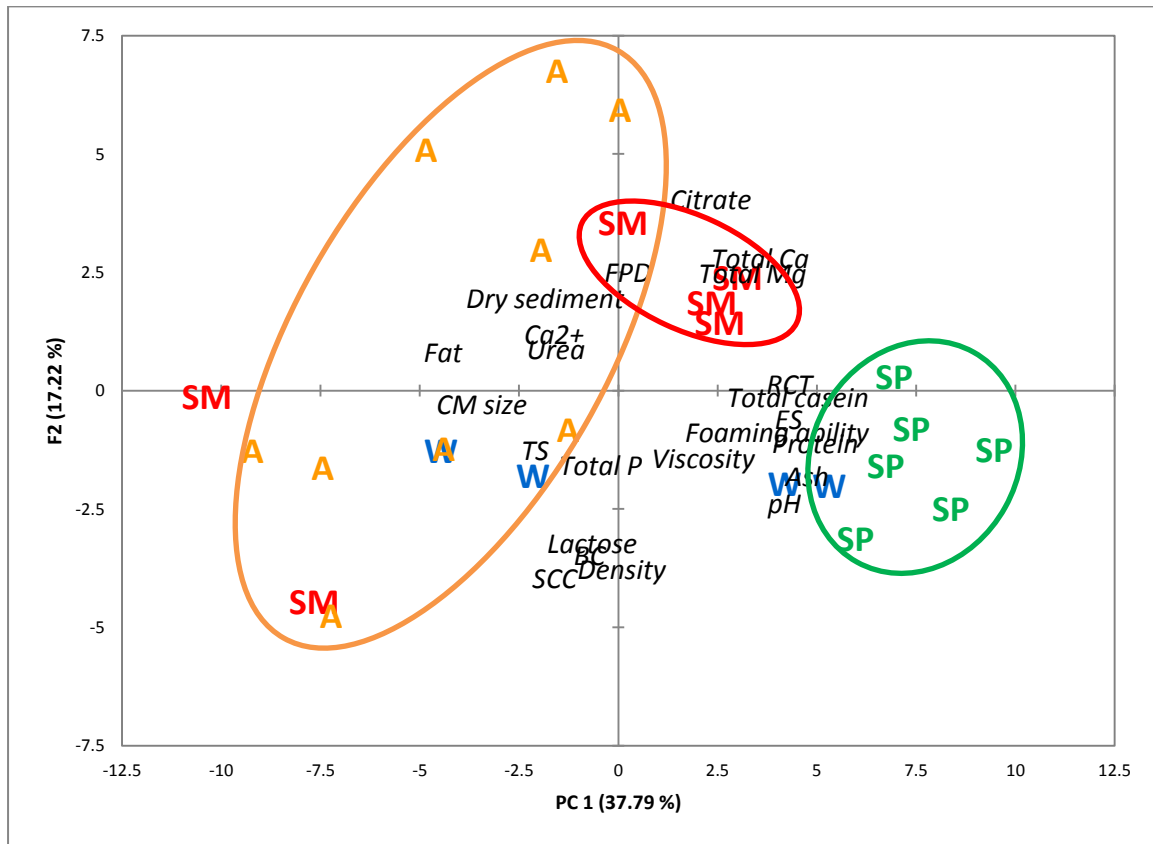
Table 5 Correlation coefficients between raw milk compositional parameters and physical properties over the period from August 2011 to October 2012

*Significance levels: * P < 0.05; ** P < 0.01; *** P < 0.001*

570 *Somatic cell count=SCC; Rennet coagulate time=RCT; Ethanol stability=ES; Buffering*

571 *capacity= BC*

572



574

575 **Figure 1**576 **Figure captions**

577 **Figure 1. Effect of the seasonal variation on the physico-chemical properties of raw milk**
 578 **according to principal component analysis (PCA) similarity map, determined by**
 579 **principal components PC 1 (37.79 %) and PC 2 (17.22 %).**

580 *SP=Spring; SM=Summer; A=Autumn; W=Winter;*

581 *Total solids=TS; Buffering capacity= BC; Somatic cell count=SCC; Freezing point depression=FPD;*

582 *Rennet coagulate time=RCT; Ethanol stability=ES; CM size= casein micelle size; Ca²⁺=Ionic*

583 *calcium*