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Title: Improvement of the quality of low-fat cheese using a two-step strategy

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**Abstract:** A two-step strategy was applied to overcome the quality changes observed in low-fat cheese. Cheese texture was first improved using microparticulated whey proteins and buttermilk as ingredients added to the cheese milk. Second, the flavour of the cheese was improved by selected *Lactobacillus* ssp. isolated from good-quality cheese.

A 10 % fat Dutch cheese, Norvegia, with the optimal mixture of microparticulated whey proteins and buttermilk had an improved texture compared to the regular cheese without any additional ingredients. The obtained optimal recipe for cheese texture was subsequently used, and *Lactobacillus* (Lb.) casei TINE36 or Lb. plantarum TINE18 isolated from good quality cheese were added as adjuncts for flavour production. It was found that the adjunct *Lactobacillus* ssp. also influenced the texture of the cheese, making it less firm.

1 **Improvement of the quality of low-fat cheese using a two-step strategy**

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12 **Abstract**

13 A two-step strategy was applied to overcome the quality changes observed in low-fat cheese.

14 Cheese texture was first improved using microparticulated whey proteins and buttermilk as

15 ingredients added to the cheese milk. Second, the flavour of the cheese was improved by

16 selected *Lactobacillus* ssp. isolated from good-quality cheese.

17

18 A 10 % fat Dutch cheese, Norvegia, with the optimal mixture of microparticulated whey

19 proteins and buttermilk had an improved texture compared to the regular cheese without any

20 additional ingredients. The obtained optimal recipe for cheese texture was subsequently used,

21 and *Lactobacillus (Lb.) casei* TINE36 or *Lb. plantarum* TINE18 isolated from good quality

22 cheese were added as adjuncts for flavour production. It was found that the adjunct

23 *Lactobacillus* ssp. also influenced the texture of the cheese, making it less firm.

24

25 **1. Introduction**

26 Consumers often regard cheese with a reduced fat content as having unsatisfactory quality;  
27 the cheese is often too elastic and has a cohesive texture and a deficient flavour. Measures to  
28 improve the quality of reduced-fat cheese have been the focus of researchers for the past 20  
29 years (Banks, 2004). However, reduced-fat cheese is still regarded as having inferior quality.  
30 The strategy is often to improve both texture and flavour at the same time. However, an  
31 alternative strategy to achieve improvements in the quality of low-fat cheese may be to work  
32 on improving texture and flavour separately.

33

34 Microparticulated whey protein (MWP) is made by the controlled denaturation and shear of  
35 whey protein concentrates which later form gel particles. **Microparticulated whey protein**  
36 may be tailored to specific applications by controlling the degree of denaturation of the whey  
37 proteins, the shear used in the processing and the composition of the whey preparation  
38 (**Sanchez** & Paquin, 1997). The functional properties of MWP are often better compared to  
39 native proteins, and MWP may be used as fat replacers (Kelly, Huppertz, & Sheehan, 2008;  
40 Renard, Lavenant, Sanchez, Hemar, & Horne, 2002). The particles of MWP in cheese must  
41 be small enough not to interrupt the casein network adversely during coagulation, but they  
42 must be large enough to be entrapped in the matrix and not lost with the whey.

43 **Microparticulated whey protein** can break the casein network in the same manner as fat  
44 globules, but it also has a higher water binding capacity than the casein network, therefore  
45 rendering the cheese softer and likely to be regarded by consumers as having a higher fat  
46 content than cheese without MWP (Steffl, Hafenmair, Hechler, & Hinrichs, 1999).

47

48 Buttermilk (BM) is a valuable by-product of butter making. Buttermilk is rich in milk fat  
49 globule membranes (MFGM) and membrane components with an increased amount of

50 phospholipids, sphingolipids, glycoproteins and other minor compounds compared to  
51 skimmed milk (Morin, Jiménez-Flores, & Pouliot, 2007). The MFGM components have been  
52 reported to be of particular nutritional interest and are also reported to have beneficial health  
53 effects (Dewettinck et al., 2008). In previous studies using buttermilk in pizza cheese  
54 manufacture (Govindasamy-Lucey, Lin, Jaeggi, Johnson, & Lucey, 2006; Govindasamy-  
55 Lucey et al., 2007), commercial buttermilk from creameries was used; however, the authors  
56 did not report the type of heat treatment that was used for the cream and buttermilk. The  
57 buttermilk had most likely been subjected to a severe heat treatment because the treatment of  
58 cream at 85-95 °C for at least 15 seconds or more is often recommended for butter-making.  
59 Such heat treatment of the cream for butter-making implies a certain degree of denaturation  
60 of the whey proteins in the cream, and the whey proteins may attach to the MFGM. This  
61 effect will also most likely influence the properties of the buttermilk used for cheese-making,  
62 such as an increased water binding capacity of the cheese. In the present study and in a  
63 previous experiment (Romeih, Moe, & Skeie, 2012), sweet buttermilk was used. The  
64 buttermilk was subjected to a controlled temperature protocol from raw milk until the  
65 addition of buttermilk to the cheese vat. Romeih et al. (2012) showed that the buttermilk  
66 added had an emulsifying effect on the fat globules in the cheese, and the cheese structure  
67 was observed to be smoother and softer by scanning electron microscopy. This softening  
68 effect of buttermilk on cheese structure has also been shown by other researchers (Mistry,  
69 Metzger, & Maubois, 1996; Poduval & Mistry, 1999).

70

71 *Lactobacillus* ssp., a part of the non-starter lactic acid bacteria (NSLAB) flora in cheese, is  
72 important for the flavour formation in cheese (Beresford, Fitzsimons, Brennan, & Cogan,  
73 2001). The starter culture suppliers offer a range of adjunct cultures of lactobacilli, in  
74 addition to the different lactococci starters, for increased flavour formation in cheese.

75 However, different lactobacilli give different flavour profiles, depending on the type and  
76 strain used, the technology used during production and the development and evolution of the  
77 microbial flora during ripening. Lactobacilli have also been connected to the development of  
78 off-flavours in cheese (Urbach, 1995). In an ongoing project, we are aiming to identify  
79 isolates of lactobacilli from Norwegian cheeses with excellent quality. A number of these  
80 isolates have been used in the study presented here.

81

82 Norvegia is a Gouda type cheese which is one of the most popular cheeses in Norway. This  
83 cheese is sold as a cheese with and without a rind and with fat contents ranging from 16 % to  
84 45 % fat in dry matter (FDM). The reduced-fat varieties do not have high acceptance among  
85 Norwegian consumers and constitute only 1.7 % of the total production of Norvegia, which is  
86 approximately 23 000 tonnes annually.

87

88 The objective of this study was to address the low-fat cheese problem with a two-step  
89 approach. The first step was with respect to texture; the objective was to determine the  
90 optimal combination of BM and MWP as ingredients to be added to the cheese milk to  
91 improve the texture of low-fat Norvegia cheese. In step 1, flavour was not the focus. Step 2  
92 involved an approach to flavour, where the best combination of BM and MWP, as decided in  
93 step 1, was used, and selected strains of *Lactobacillus (Lb.) casei/paracasei* or *plantarum*  
94 isolated from commercial cheeses with excellent flavour were added to the cheese milk to  
95 improve both the texture and flavour of the low fat Norvegia cheese.

96

## 97 **2. Materials and Methods**

### 98 **2.1. Buttermilk**

99 The buttermilk was made by the continuous churning of sweet cream. The cream prior to  
100 churning was pasteurised at 72-73 °C for 15 s. After churning, the fat was removed from the  
101 BM by separation, and the BM was pasteurised at 72-73 °C for 15 s. The BM was  
102 subsequently transported from the creamery at <4 °C to the pilot plant of the university and  
103 used within 1 to 3 days of churning. Before addition of BM to the cheese milk, the BM was  
104 pasteurised again at 72-73 °C for 15 s and cooled to 32 °C before addition to the cheese vat.  
105 The average composition of the BM was 8.8 % dry matter, 3 % protein and 0.5 % fat, and the  
106 pH was 6.6.

107

## 108 2.2. Microparticulated whey proteins

109 The liquid MWP, delivered from TINE SA, was produced from whey protein concentrate 60  
110 % (WPC60) by heating to 85-90 °C in the presence of a high shear rate. The whey protein  
111 denaturation in the liquid MWP was > 85%. The MWP contained particles between 1 and 10  
112 µm, to simulate milk fat globules. In step 1 of the investigation, the age of the fluid MWP  
113 used varied between 4-13 days, while in step 2, the age was 5 and 7 days. The average  
114 composition of the MWP was 13.5 % dry matter and 7.4 % protein, and the pH was, on  
115 average, 6.3.

116

## 117 2.3. Cheese Milk

118 The milk for the cheese was obtained from the university herd (Norwegian University of Life  
119 Sciences, Ås, Norway). The milk was skimmed and pasteurised (72 °C, 15 s) and  
120 standardised in the vat to a fat content of 1.0 % with pasteurised cream (74 °C, 15 s).  
121 Buttermilk and/or MWP were added to the vat as described in section 2.6. and the cheese  
122 milk was stirred for at least 50 minutes before rennet addition.

123

124 2.4. *Adjuncts*

125 The two adjunct cultures used were isolated from high quality Norvegia cheeses; *Lb.*  
126 *plantarum* TINE18 was isolated from a 16 % fat cheese, and *Lb. casei/paracasei* TINE36  
127 was isolated from a 28 % full-fat cheese. The strains were isolated and characterised as  
128 described by Porcellato et al. (2012). Because we could not distinguish *Lb. casei* from *Lb.*  
129 *paracasei* by the methods used, the name *Lb. casei* TINE36 will be used for the remainder of  
130 this paper. The adjunct lactobacilli were inoculated (1 %) in De Man, Rogosa, Sharpe broth  
131 (MRS, Difco, Sparks, MI, USA) and incubated at 30 °C for 20 h. The inoculation in the  
132 cheese vats was 0.11 % for both *Lactobacillus* ssp.

133

134 2.5. *Cheese making*

135 Washed-curd, brine salted cheese was made with 10 % fat in the cheese, yielding 20 % fat in  
136 dry matter (FDM<sup>-1</sup>). The cheese was made from 350 L milk as described by Skeie, Lindberg  
137 and Narvhus (2001) with several modifications. The starter culture used was Probat Visbyvac  
138 505 (Danisco, Copenhagen, Denmark), and the pre-ripening of milk and starter was  
139 performed for 45 min at 30.5 °C. The rennet used was Naturen Premium 225 (Chr. Hansen,  
140 Hørsholm, Denmark), and the coagulation time (from set to cut) was 36 (±2.5) min. Whey  
141 drainage was 45 % (vol/vol), and water addition was between 30 and 40 % (vol/vol). The  
142 scalding temperature was 35.5 (±0.5) °C, and the process was performed for 25-30 min.  
143 Plastic cheese moulds (Laude b.v., Ter Apel, The Netherlands) yielding 5-kg cheese wheels  
144 were used. The cheeses were salted in a saturated brine at 10 °C for 10 h. Before and after  
145 cheese-making, the dairy equipment were washed and disinfected with steam. The cheese  
146 was kept for 10 days at 10 °C and plastic coated twice with Ceska-coat (Producan, Kolding,  
147 Denmark). The cheese was later stored in the curing room for 14 days at 19 °C and was  
148 vacuum-wrapped in plastic bags and stored at 4 °C for the remaining ripening period.



149

## 150 2.6. *Experimental design*

151 Two different cheese making experiments were designed, hereby referred to as step 1 and  
152 step 2.

153

154 Step 1: Cheese was made using two experimental factors, i.e., the addition of either MWP or  
155 BM to the cheese milk, in 3 replicate blocks. The experimental factor MWP was added at  
156 three levels, 0, 3 and 6 % of the milk volume. The experimental factor BM was added at two  
157 levels, 0 and 15 % of the milk volume. Each replicate block consisted of 6 cheese vats made  
158 over 2 days; all the replicate blocks were made in a period of 5 days. As the cheese of each  
159 replicate block were made over 2 days, 2 extra control vats with no addition of MWP and BM  
160 were made to adjust for the possible variation in milk quality and composition. In total, 20  
161 vats of cheese were made. Chemical and microbial analyses of the cheese were performed  
162 after 24 h and 6 weeks, and sensory and texture analyses were made after 12 weeks of  
163 ripening.

164

165 Step 2: Cheese was made with the addition of 3 % MWP and 15 % BM to the cheese milk  
166 and with one experimental factor, namely, the inoculation of adjunct cultures, in three levels  
167 and in 2 replicate blocks. The three levels of adjunct were the following: no adjunct addition,  
168 the addition of *Lb. plantarum* TINE18 or the addition of *Lb. casei* TINE36. In total, 6 vats of  
169 cheese were made. Chemical and microbial analyses of the cheese were performed after 24 h,  
170 8, 16, 20 and 28 weeks. Sensory and texture analyses were made after 16, 20 and 28 weeks of  
171 ripening.

172

## 173 2.7. *Sampling and analysis of milk and cheese*

174 Sampling for the gross composition and microbial analyses were made according to the IDF-  
175 standard 50C (IDF/FIL, 1995). Microbial counts, pH and dry matter were measured  
176 immediately after sampling. Dry matter was determined according to IDF standard 4A  
177 (IDF/FIL, 1982), salt was determined according to IDF standard 88 (IDF/FIL, 1988), fat was  
178 determined according to IDF standard 222 (IDF/FIL, 2008) and protein was determined  
179 according to IDF standard 20B (IDF/FIL, 1993). The pH of the samples was measured as  
180 described by Skeie, Lindberg & Narvhus (2001). Presumptive lactococci were enumerated on  
181 M17 broth (Merck, Darmstadt, Germany) with 15 g L<sup>-1</sup> Bactoagar (Saveen Werner AB,  
182 Malmö, Sweden) after aerobic incubation for 2 days at 30 °C. Presumptive lactobacilli and  
183 *Leuconostoc* ssp. were enumerated on *Lactobacillus* selective agar (LBS agar, Difco) after  
184 anaerobic incubation in an anaerobic incubator (W.C. Hearaeus GmbH, Hanau, Germany)  
185 with 10 % v/v CO<sub>2</sub> for 4 days at 30 °C.

186

187 Cheese hardness was measured using the Texture Profile Analysis (TPA) technique on a TA-  
188 XT2i Texture Analyser (Stable Micro Systems (SMS) Ltd., Surrey, UK) with a measuring  
189 cell of 25 kg and an SMS P/45 flat aluminium plunger (Ø 75 mm). From each cheese, 12  
190 cylinders (15 mm in height, Ø 23 mm) were sampled, packed in aluminium foil and tempered  
191 at 15 °C before analysis. The samples were compressed axially in two consecutive cycles  
192 without yield with 75 % deformation from the initial sample's height at a 1 mm s<sup>-1</sup> rate of  
193 force application. The result of the 6 samples which was most consistent was further used in  
194 the statistical analyses. The force required to attain a given deformation or the maximum  
195 force during the first compression is the TPA hardness, as measured in Newtons.

196

197 Volatile compounds were determined in headspace vials containing 10 g of grated cheese,  
198 sealed with 20-CBT-3 Teflon coated septa and aluminium crimp caps using headspace gas

199 chromatography (HSGC) according to the method of Narvhus, Østeraas, Mutukumira and  
200 Abrahamsen (1998) and with modifications described by Skeie, Kieronczyk, Næss and Østlie  
201 (2008).

202

203 Organic acids and lactose were analysed using high performance liquid chromatography  
204 (HPLC) as described by Skeie, Narvhus, Ardö, Thorvaldsen and Abrahamsen (1997) and  
205 Skeie et al. (2001) with modifications described by Skeie et al. (2008).

206

207 Sensory quality gradings were made by at least 3 trained quality assessors at TINE SA using  
208 a scale extending from 1 to 5, where 5 is a very high-quality cheese. The cheeses were  
209 evaluated as a full fat Norvegia by the quality grading panel, and the cheese was fit for sale as  
210 a full-fat Norvegia if it had a grade higher than 3. Sensory profiling of 20 texture and flavour  
211 attributes were made by 5-7 trained assessors from TINE SA using a scale of 1-9. Before the  
212 profiling of the experimental cheese, the assessors agreed upon the attributes using a  
213 reference cheese as a standard. A hedonic sensory evaluation (liking) was performed by a  
214 trained panel of 5 assessors at the Norwegian University of Life Sciences using a scale from 1  
215 to 5, where 5 was liked very much.

216

## 217 *2.8. Statistical treatment of data*

218 Significant differences ( $P < 0.05$ ) between replicate blocks and treatment factors were found  
219 by ANOVA using SAS Enterprise guide 4.0 (SAS Institute Inc., Cary, NC, USA). In step 1,  
220 the treatment factors were the replicate block, MWP and BM, and their interaction MWP  $\times$   
221 BM. In step 2, the treatment factors were the replicate block and the adjunct culture of  
222 lactobacilli. An ANOVA was made at each ripening step. Principal component analysis was  
223 made by using the Unscrambler X 10.0.1 (CAMO Process AS, Oslo, Norway). The organic

224 acid data and the volatile component data were weighted by dividing each response variable  
225 by the standard deviation of that variable, while the sensory data sets were not weighted. A  
226 full cross-validation was used for the validation of the data set.

227

### 228 **3. Results**

#### 229 *3.1. Step 1. Texture approach*

230 As shown in Table 1, the addition of 15 % BM reduced the dry matter and protein content of  
231 the cheese milk, while the addition of MWP increased the dry matter and protein content of  
232 the milk. The pH of the cheese milk was not influenced by the addition of BM. The addition  
233 of 6 % MWP did reduce the pH weakly, but it was statistically significant. In cheese 24 h  
234 after the start of the cheese making process and after 6 weeks of ripening (Table 2), the  
235 addition of BM and MWP lowered the content of dry matter compared to cheese without  
236 these additions. The pH of the cheese 24 h after the start of cheese-making was not  
237 influenced by the additions, but after 6 weeks of ripening, the pH was lower in cheeses with 6  
238 % MWP (Table 2). The salt in moisture was 2.9 % on average and was only slightly  
239 influenced ( $P<0.02$ ) by the addition of 6 % MWP, which increased the salt in moisture to an  
240 average of 3 % (results not shown). Fat in dry matter was 21.4 % on average and was not  
241 influenced by the experimental factors. Only the addition of BM influenced the set to cut  
242 time, which increased significantly ( $P<0.05$ ) by 3 min with the addition of 15 % BM.

243

244 The addition of MWP and BM significantly ( $P<0.0001$ ) reduced the hardness of the cheese,  
245 as measured by texture analysis (Figure 1) and sensory profiling (Figure 2). The sensory  
246 profiling showed that addition of MWP and BM, separately and in combination, moved the  
247 cheeses from the firm, dry and grainy area to a more doughy and soluble character. The  
248 doughiest cheeses were those with a combination of BM and 6 % MWP. None of the cheeses

249 obtained a texture quality grade  $>3$ , which was needed to be comparable with full-fat  
250 Norvegia cheese (Table 3). The liking panel could differentiate among the treatments, and  
251 cheeses with added BM or 3 % MWP obtained significantly better texture liking scores; the  
252 cheese with the combination of BM and 3 % MWP had the highest liking score. As shown in  
253 Table 3, the addition of 6 % MWP was not beneficial for texture, as these cheeses obtained  
254 the lowest score both by the quality grading panel and by the liking panel. This cheese was  
255 also the doughiest cheese, regardless of BM addition. The sensory profiling (Figure 2)  
256 revealed that the cheeses with added MWP and BM had a more acidic and pungent flavour  
257 than did cheeses without these additions. Because the combination BM and 3 % MWP  
258 yielded cheese with the highest texture likings, this cheese was used in step 2, despite its  
259 somewhat acidic and pungent flavour.

260

### 261 3.2. Step 2. Flavour approach.

262 The cheeses produced in step 2 were made from cheese milk with 15 % BM, 3 % MWP and  
263 one of the two different strains of adjunct lactobacilli. These cheeses were more similar in  
264 composition than the cheeses made in step 1, and no significant differences in gross  
265 composition or pH were found among the cheeses. The dry matter of the cheese was, on  
266 average,  $42.51 \pm 1.44$  % 24 h after the start of cheese-making and  $49.07 \pm 0.81$  % after 16  
267 weeks of ripening. Fat in dry matter was, on average,  $22.01 \pm 0.94$  %, and salt in moisture was  
268  $2.88 \pm 0.11$  % after 16 weeks of ripening. The adjuncts did not significantly influence the pH  
269 of the cheeses; the pH increased from  $5.22 \pm 0.02$  in the young cheese to  $5.51 \pm 0.06$  after 16  
270 weeks of ripening. However, the control cheese generally had the highest pH throughout  
271 ripening. No significant difference between the cheeses was found with respect to hardness as  
272 measured by the texture analyser; the TPA hardnesses were 78, 60 and 55 N in the control  
273 and in the cheeses with added *Lb. plantarum* TINE18 and *Lb. casei* TINE36, respectively.

274

275 All the cheeses had high cell numbers on LBS agar at 24 h, between log 7.5 (control) and 8.5  
276 (cheeses with adjunct) cfu g<sup>-1</sup>. A mesophilic aromatic starter with *Leuconostoc* ssp. was used;  
277 therefore, high cfu numbers on the LBS agar were also expected for the control cheeses 24 h  
278 after the start of cheese-making. After 8 weeks of ripening, the cell numbers enumerated on  
279 LBS in the control cheese were reduced to log 6.7 cfu g<sup>-1</sup>, while the cheese with added *Lb.*  
280 *casei* TINE36 had an average cell number of log 7.7 cfu g<sup>-1</sup> and the cheese with added *Lb.*  
281 *plantarum* TINE18 had an average of log 8.1 cfu g<sup>-1</sup> at this stage. During further ripening, the  
282 cell numbers on LBS agar decreased for the control cheese and for the cheese with added *Lb.*  
283 *casei* TINE36 (log 6.05 and 7.14 cfu g<sup>-1</sup>, respectively, after 28 weeks of ripening), while the  
284 cell numbers remained stable in the cheese with added *Lb. plantarum* TINE18. The adjuncts  
285 clearly influenced the ripening of the cheese, as shown by the development of organic acids  
286 and volatile flavour compounds throughout ripening (Figure 3 and Figure 4, respectively). As  
287 shown in Figure 3, the control cheese and cheese with added *Lb. casei* TINE36 clustered  
288 closely together, while the cheese with added *Lb. plantarum* TINE18 (the ellipse in Figure 3)  
289 differed from these two because of its higher content of formic and acetic acids. The PCA  
290 analysis of the volatile flavour compounds clustered the treatments clearly into three groups,  
291 with the control cheese having the lowest development of volatile aroma compounds  
292 throughout ripening. Additionally, the cheeses with added adjuncts were grouped clearly into  
293 two groups, with the cheese with added *Lb. plantarum* TINE18 exhibiting the highest content  
294 of most of the identified volatiles.

295

296 The adjuncts significantly ( $P < 0.05$ ) influenced the flavour profile of the cheeses (Figure 5).  
297 However, more surprisingly, these adjuncts also had a significant ( $P < 0.05$ ) influence on the  
298 texture perceived by the sensory profiling analysis. The cheeses with the adjunct culture were

299 considered less firm and less dry than the control cheese. As shown in table 4, all of the  
300 cheeses made in step 2 obtained a quality grading higher than 3 for both texture and flavour,  
301 proving a quality comparable to full-fat Norvegia. However, the quality was highest after 20  
302 weeks and declined after 28 weeks ripening, indicating that the cheese did develop in an  
303 undesirable direction during the prolonged ripening period. The terms used to describe the  
304 quality deficiency, particularly for the 28-week cheeses, were grainy, cohesive, pungent and  
305 acid. However, the liking panel did appreciate these cheeses notably, giving several of them a  
306 score of 4 out of 5 for flavour. The quality grading panel gave the cheese with added *Lb.*  
307 *plantarum* TINE18 the lowest score for flavour (table 4) during the evaluation at 20 weeks,  
308 while the liking panel liked this cheese and the control cheese better than the cheese with  
309 added *Lb. casei* TINE36. Furthermore, at 28 weeks, this difference was significant ( $P<0.05$ ).  
310 No further significant differences between the treatments were found by the sensory gradings,  
311 neither by the quality nor by the liking panel.

312

#### 313 **4. Discussion**

314 The use of a two-step strategy for the improvement of the quality of low-fat Norvegia seemed  
315 to be successful. However, a premise in these experiments was to use sensory methods for  
316 profiling and determining the liking of the cheeses in addition to the traditional quality  
317 grading. It would have been optimal to do consumer tests, as well, but that approach would  
318 have required more cheese, and it would have been much more costly and out of the scope for  
319 this work. By the approach used, the cheeses could be made in a pilot plant, and the number  
320 of experimental cheese vats could be minimised. Using a one-step approach with the same  
321 experimental factors would have required at least 36 cheese-makings (using 2 replicate  
322 blocks), and cheese-makings of the same replicate blocks would have had to be spread over

323 3-4 days. In the two step approach, the number of cheese makings was reduced to 26, and it  
324 allowed us to use the 3 replicate blocks in step 1.

325

326 Only the addition of BM to cheese milk influenced the set to cut time, while MWP did not  
327 influence the observed coagulation time. An increased set to cut time and a reduced gel  
328 firmness caused by BM addition was also observed by Morin, Pouliot and Britten (2008),  
329 who linked the reduced coagulation properties of the cheese milk with added BM to the heat  
330 treatment of the cream before churning and to the MFGM components of the BM. By adding  
331 different commercial MWPs to milk, Lucey and Gorry (1994) observed little effect on rennet  
332 coagulation, while Fenelon and Guinee (1997), Guinee et al. (1997) and Schenkel, Samudrala  
333 and Hinrichs (2011) reported impaired rennet coagulation properties.

334

335 The texture measurements of the cheeses produced in step 1 showed that cheeses with BM  
336 and MWP added to the cheese milk had a reduced hardness, and the sensory profiling  
337 analysis indicated that these cheeses had a somewhat pungent and acidic flavour. As shown  
338 by Saint-Eve, Laverjat, Magnan, Déléris, and Souchon (2009), the texture perception of  
339 model cheeses with exactly the same texture was influenced by the flavour of the cheese, and  
340 the flavoured cheese had a much better texture grading than did the unflavoured cheese. The  
341 texture of the cheeses from step 1 was not acceptable according to the quality grading panel  
342 when comparing these low fat cheeses with the texture of a full-fat variety of the same cheese  
343 (Norvegia). This reduced acceptability is most likely due to a confounding with the inferior  
344 flavour of these cheeses, as all the cheeses produced in Step 2 were evaluated according to  
345 the same standard and had a better flavour quality. The texture analysis and the sensory  
346 profiling showed that BM and MWP had a beneficial effect on the texture of the low-fat  
347 cheese, making it less firm, less cohesive and less dry, which is in accordance with the



348 findings of others for both BM (Mistry et al., 1996; Poduval & Mistry, 1999; Romeih et al.,  
349 2012) and MWP (Fenelon & Guinee, 1997; Lobato-Calleros, Robles-Martinez, Caballero-  
350 Perez, Aguirre-Mandujano, & Vernon-Carter, 2001; Lucey & Gorry, 1994; Schenkel et al.,  
351 2011). However, using 6 % MWP, the cheese became too doughy and had a textural quality  
352 which was not appreciated neither by the quality graders nor by the liking panel. In this  
353 experiment, the optimal addition of MWP was therefore 3 %. The BM reduced the firmness  
354 of the cheese, and a combination with 3 % MWP seemed to have a positive effect on the  
355 texture properties of the cheese; however, a combination of BM and 6 % MWP resulted in a  
356 cheese that was overly doughy. Based on the observations from the experiments in step 1, it  
357 was decided to continue to step 2 with the 3 % MWP and 15 % BM additions to the cheese  
358 milk.

359

360 The control cheese in step 2 had a slightly softer texture than the corresponding cheese in step  
361 1, as measured on the texture analyser, but the differences as evaluated by the quality graders  
362 were considerable. The control cheese now obtained a notably good texture grading, which  
363 was within the standard for full fat Norvegia after 20 weeks of ripening. The texture quality  
364 was, however, reduced by further ripening. In step 1, the sensory evaluation was made after  
365 12 weeks, thus the cheese may have been too young, and this characteristic might explain the  
366 differences obtained in the quality gradings in the two different steps. It might be that the  
367 optimal texture of this cheese can be obtained between 12 and 28 weeks of ripening.

368 Therefore, further work has to be undertaken to determine the ripening time necessary for  
369 optimal texture quality and to find the ageing period wherein the quality of the cheese is  
370 satisfactory. The reduction of cheese firmness caused by the adjunct lactobacilli was  
371 somewhat surprising, as the species added are considered weakly proteolytic. However, a  
372 reduced firmness of the cheese with added adjunct *Lb. casei* and *Lb. plantarum* have also

373 been observed by other researchers (Hynes et al., 2003; Sallami, Kheadr, Fliss, &  
374 Vuilleumard, 2004).

375

376 The development of the organic acids and the volatile flavour compounds showed that the  
377 adjunct lactobacilli influenced the production of flavour compounds in the cheeses, which has  
378 also been shown in previous experiments with washed-curd cheese varieties (Antonsson,  
379 Ardo, Nilsson, & Molin, 2002; Skeie et al., 2001; Skeie et al., 2008). The biochemical  
380 changes caused by the adjuncts were also reflected in the sensory profiling analysis.

381 However, the results of the sensory grading and liking were not influenced by the adjunct  
382 lactobacilli, and the grading panel and the liking panel responded conversely to the effects of  
383 the adjunct cultures. The grading panel liked the cheese with added *Lb. plantarum* TINE18  
384 the least, which means that this lactobacilli most likely gives the cheese a flavour that is  
385 somewhat unusual for full fat Norvegia. However, this strain was isolated from a high-quality  
386 Norveiga cheese with 16 % fat, where it was dominant. Østlie, Eliassen, Florvaag and Skeie  
387 (2004) found that Norvegia cheese is usually dominated by *Lb. casei/paracasei*, and it is  
388 possible that the dominance of *Lb. plantarum* in low-fat Norvegia is producing a flavour in  
389 the low fat variety that diverges from the full fat Norvegia. However, the liking panel liked  
390 the cheese with added *Lb. plantarum* TINE18 and the control cheese the most, while they  
391 liked the cheese with *Lb. casei* TINE36 the least. Therefore, determining consumer opinions  
392 of low-fat Norvegia with different adjuncts of lactobacilli would be an interesting further  
393 study.

394

## 395 **5. Conclusions**

396 The texture of low-fat Norvegia was improved by adding 15 % BM and 3 % MWP to the  
397 cheese milk, and these additions reduced the firmness and the rubbery texture of the cheese.

398 The adjunct lactobacilli used influenced the texture positively, while the effects on the  
399 flavour were more conflicting. The sensory profiling attributes showed a clear effect of the  
400 adjuncts on the cheese flavour; however, the cheese with added adjuncts did not obtain a  
401 better quality grading or liking compared with the control cheese.

402

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408 Chemistry, Biotechnology and Food Science for their assistance during cheese-making and  
409 for their assistance with the microbial and chemical analyses of the cheese.

410

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512 characterization of the microflora in Norway cheese during ripening. *International Journal*  
513 *of Food Microbiology*, *94*, 287-299. doi:10.1016/j.ifofoodmicro.2004.01.012

514

515

516 Table 1. Dry matter, protein and pH of cheese milk with added buttermilk (BM) and or  
 517 microparticulated whey proteins (MWP) before starter addition (Step 1). Values are means  
 518 and SD. The p-statistics of each experimental factor and the significant ( $P<0.05$ ) differences  
 519 between the least square mean (LSM) of each level of MWP are shown in the last four rows  
 520 of the table.

BM	MWP	Dry matter %		Protein %		pH	
		Mean	SD	Mean	SD	Mean	SD
0	0	10.08	0.08	3.47	0.05	6.65	0.04
0	3	10.20	0.11	3.56	0.01	6.65	0.04
0	6	10.28	0.08	3.69	0.04	6.63	0.02
15	0	9.98	0.04	3.42	0.03	6.66	0.03
15	3	10.10	0.07	3.48	0.06	6.62	0.03
15	6	10.20	0.12	3.59	0.01	6.61	0.08

p-statistics of the experimental factors ( $P<0.05$ , ns= not significant )				
Day		0.0001	ns	0.002
BM		0.0001	0.003	ns
MWP		0.0001	0.0002	0.03
LSM: MWP		0<3<6	0<3<6	0,3>6

521

522

523 Table 2. Dry matter and pH of cheese with added buttermilk (BM) and or microparticulated  
 524 whey proteins (MWP) analysed 24 h after the start of cheese-making and after 6 weeks of  
 525 ripening (Step 1). Values are means  $\pm$  SD. The p-statistics of each experimental factor, the  
 526 interaction BM  $\times$  MWP and the significant ( $P < 0.05$ ) differences between the mean of each  
 527 level (LSM) of MWP are shown in the last four rows of the table.

BM	MWP	Dry matter %				pH			
		24 h		6 w		24 h		6 w	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
0	0	45.70	0.92	50.38	0.96	5.24	0.05	5.45	0.03
0	3	43.08	0.66	48.74	0.36	5.19	0.06	5.40	0.17
0	6	41.85	1.67	47.72	2.36	5.17	0.04	5.40	0.10
15	0	42.81	0.53	49.01	1.15	5.22	0.04	5.44	0.06
15	3	42.10	0.27	47.75	1.34	5.20	0.03	5.43	0.08
15	6	40.28	1.14	45.80	0.82	5.18	0.06	5.31	0.12
p-statistics of the experimental factors ( $P < 0.05$ , ns= not significant )									
Day		ns		ns		ns		0.0100	
BM		0.0040		0.0200		ns		ns	
MWP		0.0007		0.0030		ns		0.0300	
BM $\times$ MWP		ns		ns		ns		ns	
LSM: MWP		0>3>6		0>3>6				0,3>6	

528

529

530 Table 3. The texture grading of the quality and the liking panel for cheese with added  
 531 buttermilk (BM) and/or microparticulated whey proteins (MWP) analysed after 12 weeks of  
 532 ripening (Step 1). Values are means  $\pm$  SD. The p-statistics of each experimental factor, the  
 533 interaction BM  $\times$  MWP and the significant ( $P < 0.05$ ) differences between the mean of each  
 534 level (LSM) of MWP are shown in the last four rows of the table.

BM	MWP	Texture quality grading		Texture liking	
		Mean	SD	Mean	SD
0	0	2.63	0.48	2.67	0.47
0	3	2.83	0.44	3.44	0.51
0	6	2.46	0.42	3.11	0.19
15	0	2.94	0.38	3.72	0.25
15	3	2.72	0.42	3.94	0.10
15	6	2.22	0.10	2.83	0.17

p-statistics of the experimental factors ( $P < 0.05$ , ns= not significant )	
Day	ns
BM	0.0050
MWP	0.0080
BM $\times$ MWP	0.0400
LSM: MWP	6,0 < 3

535

536

537



538 Table 4. The texture and flavour gradings of the quality and the liking panel for the cheese  
 539 with 15 % buttermilk (BM), 3 % microparticulated whey proteins (MWP) and different  
 540 adjuncts analysed after 20 and 28 weeks of ripening (Step 2). Values are means  $\pm$  SD.  
 541 Significant ( $P < 0.05$ ) differences within the means for each ripening time are shown with  
 542 different superscript lower case letters.

Age	Adjunct	Quality Grading				Liking			
		Flavour		Texture		Flavour		Texture	
		Mean	SD	Mean	SD	Mean	SD	Mean	SD
20	Control	3.58	0.12	3.67	0.24	4.00	0.24	3.92	0.12
20	<i>Lb. plantarum</i> TINE18	3.33	0.24	3.92	0.12	4.08	0.12	3.92	0.12
20	<i>Lb. casei</i> TINE36	3.50	0.00	3.92	0.12	3.75	0.12	4.00	0.24
28	Control	3.08	0.12	3.25	0.12	4.00 <sup>a</sup>	0.24	3.67	0.24
28	<i>Lb. plantarum</i> TINE18	3.08	0.12	3.58	0.35	4.08 <sup>a</sup>	0.12	3.58	0.12
28	<i>Lb. casei</i> TINE36	3.08	0.12	3.50	0.24	3.50 <sup>b</sup>	0.24	3.50	0.00

543

544

545 **Figure captions.**

546

547 Figure 1. Hardness, in Newton (N), of cheese with added buttermilk (BM) and/or  
548 microparticulated whey protein (MWP), as measured by the Texture Profile Analysis (TPA)  
549 technique (Step 1). No BM addition: —■—, 15 % BM addition •••●•••.

550

551 Figure 2. Scores (a) and loadings (b) of the principal component analysis (PCA) of the  
552 sensory profiling attributes of cheese with no added buttermilk (BM) (black) or with 15 %  
553 BM (grey) and microparticulated whey protein (MWP); 0 % MWP (—), 3 % MWP (— —  
554 —) and 6 % MWP (••••••••) after 12 weeks of ripening (Step 1). Sample marking: Replicate  
555 block (A, B, C)- % BM- % MWP. The first and the second principal component (PC)  
556 explained 86 and 5 % of the variation, respectively.

557

558 Figure 3. Scores (a) and loadings (b) of the principal component analysis (PCA) of the  
559 development of organic acids during ripening of the control cheese without an adjunct (0) and  
560 cheese with added *Lb. plantarum* TINE18 (18)(ellipse) or *Lb. casei* TINE36 (36) from 0 to  
561 28 weeks (Step 2). Each point represents the average of two replicate blocks. All the cheeses  
562 had an addition of 15 % buttermilk (BM) and 3 % microparticulated whey protein (MWP).  
563 Sample marking: Adjunct-weeks of ripening. The first and the second principal component  
564 (PC) explained 68 and 18 % of the variation, respectively.

565

566 Figure 4. Scores (a) and loadings (b) of the principal component analysis (PCA) of the  
567 development of volatile aroma compounds during ripening of the control cheese without an

568 adjunct (0, —) and cheese with added *Lb. plantarum* TINE18 (18, — — —) or *Lb. casei*  
569 TINE36 (36, ······) from 0 to 28 weeks (Step 2). Each point represents the average of two  
570 replicate blocks. All the cheeses had an addition of 15 % buttermilk (BM) and 3 %  
571 microparticulated whey protein (MWP). Sample marking: Adjunct-weeks of ripening. The  
572 first and the second principal component (PC) explained 49 and 19 % of the variation,  
573 respectively.

574

575 Figure 5. Scores (a) and loadings (b) of the principal component analysis (PCA) of the  
576 sensory profiling attributes of the control cheese without an adjunct (0, —) and cheese with  
577 added *Lb. plantarum* TINE18 (18, — — —) or *Lb. casei* TINE36 (36, - - -) evaluated after 20  
578 and 26 weeks of ripening (Step 2). All the cheeses had an addition of 15 % buttermilk (BM)  
579 and 3 % microparticulated whey protein (MWP). Sample marking: Replicate block (A, B)-  
580 adjunct-weeks of ripening. The first and the second principal component (PC) explained 79  
581 and 14 % of the variation, respectively.

582

583 **Figure captions for colour figures to be used in the web version**

584 Figure 3. Scores (a) and loadings (b) of the principal component analysis (PCA) of the  
585 development of organic acids during ripening of the control cheese without an adjunct (0,  
586 black) and cheese with added *Lb. plantarum* TINE18 (18, blue) or *Lb. casei* TINE36 (36,  
587 red) from 0 to 28 weeks (Step 2). Each point represents the average of two replicate blocks.  
588 All the cheeses had an addition of 15 % buttermilk (BM) and 3 % microparticulated whey  
589 protein (MWP). Sample marking: Adjunct-weeks of ripening. The first and the second  
590 principal component (PC) explained 68 and 18 % of the variation, respectively.

591

592 Figure 4. Scores (a) and loadings (b) of the principal component analysis (PCA) of the  
593 development of the volatile aroma compounds during ripening of the control cheese without  
594 an adjunct (0, black —) and cheese with added *Lb. plantarum* TINE18 (18, blue — — —) or  
595 *Lb. casei* TINE36 (36, red ······) from 0 to 28 weeks (Step 2). Each point represents the  
596 average of two replicate blocks. All of the cheeses had an addition of 15 % buttermilk (BM)  
597 and 3 % microparticulated whey protein (MWP). Sample marking: Adjunct-weeks of  
598 ripening. The first and the second principal component (PC) explained 49 and 19 % of the  
599 variation, respectively.

600

601 Figure 5. Scores (a) and loadings (b) of the principal component analysis (PCA) of the  
602 sensory profiling attributes of the control cheese without an adjunct (0, black —) and cheese  
603 with added *Lb. plantarum* TINE18 (18, blue — — —) or *Lb. casei* TINE36 (36, red - - -)  
604 evaluated after 20 and 26 weeks of ripening (Step 2). All the cheeses had an addition of 15 %  
605 buttermilk (BM) and 3 % microparticulated whey protein (MWP). Sample marking:

606 Replicate block (A, B)-adjunct-weeks of ripening. The first and the second principal  
607 component (PC) explained 79 and 14 % of the variation, respectively.

608

609

Figure 1.

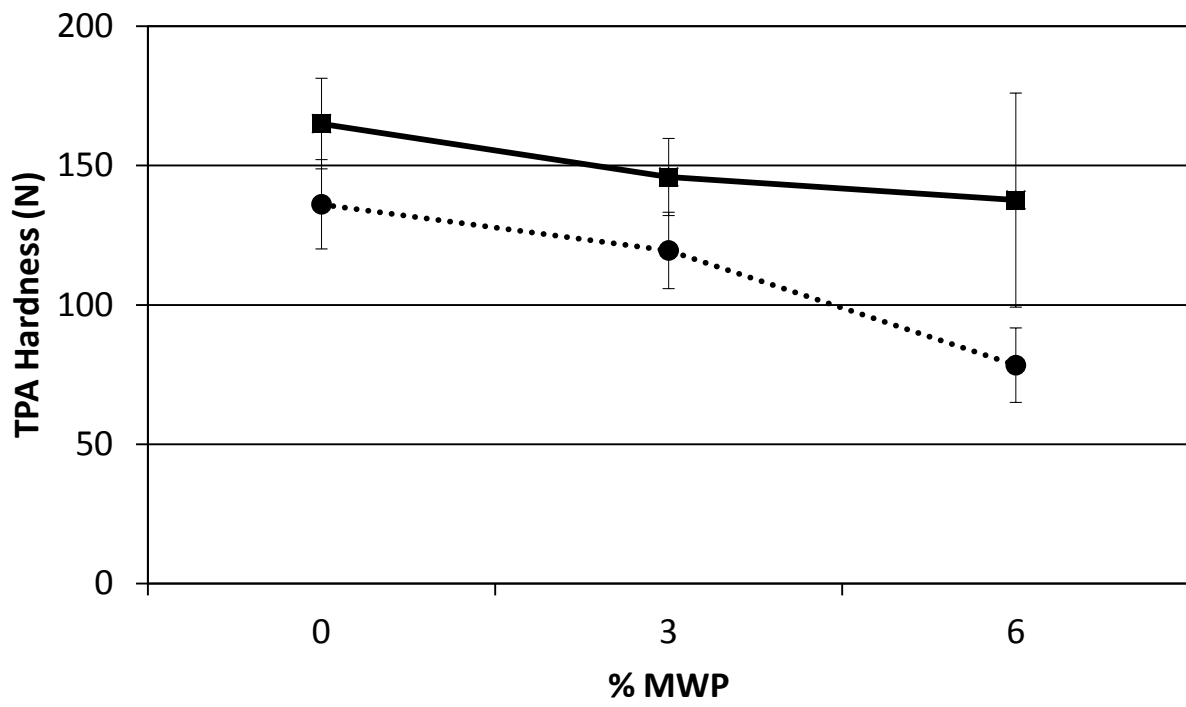
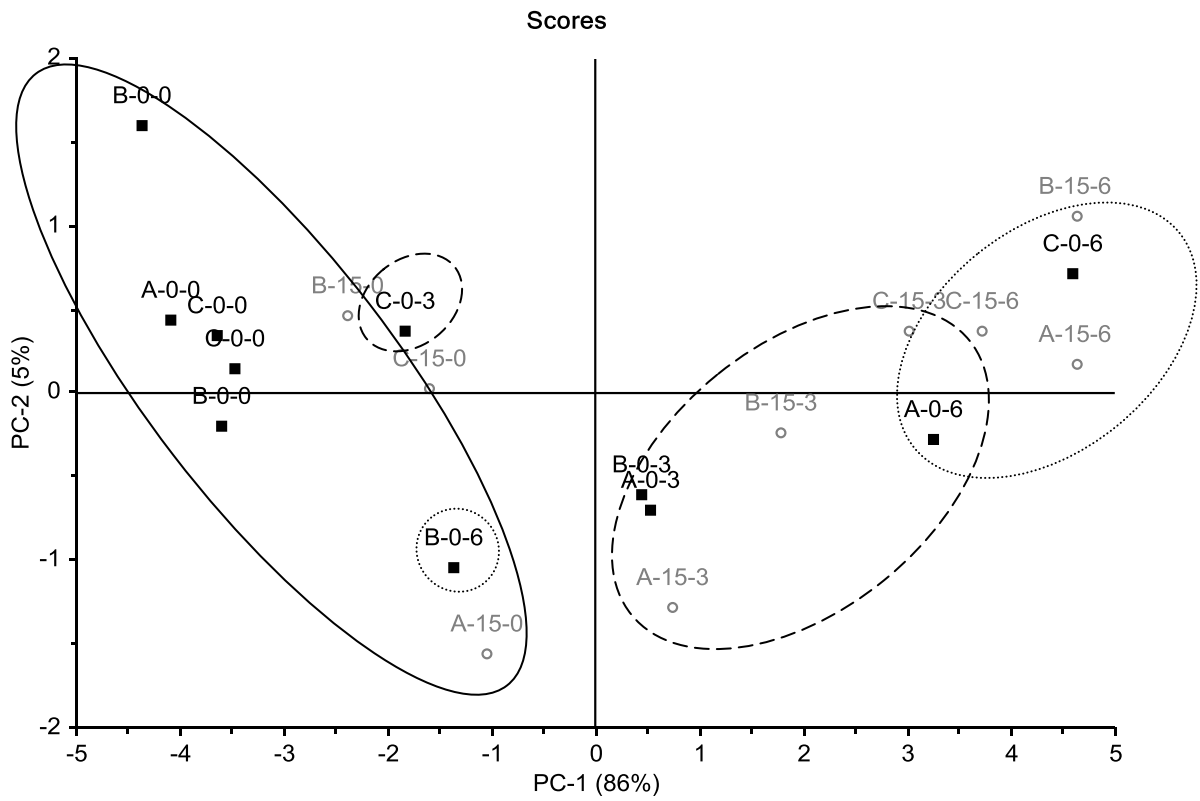


Figure 2.

a)



b)

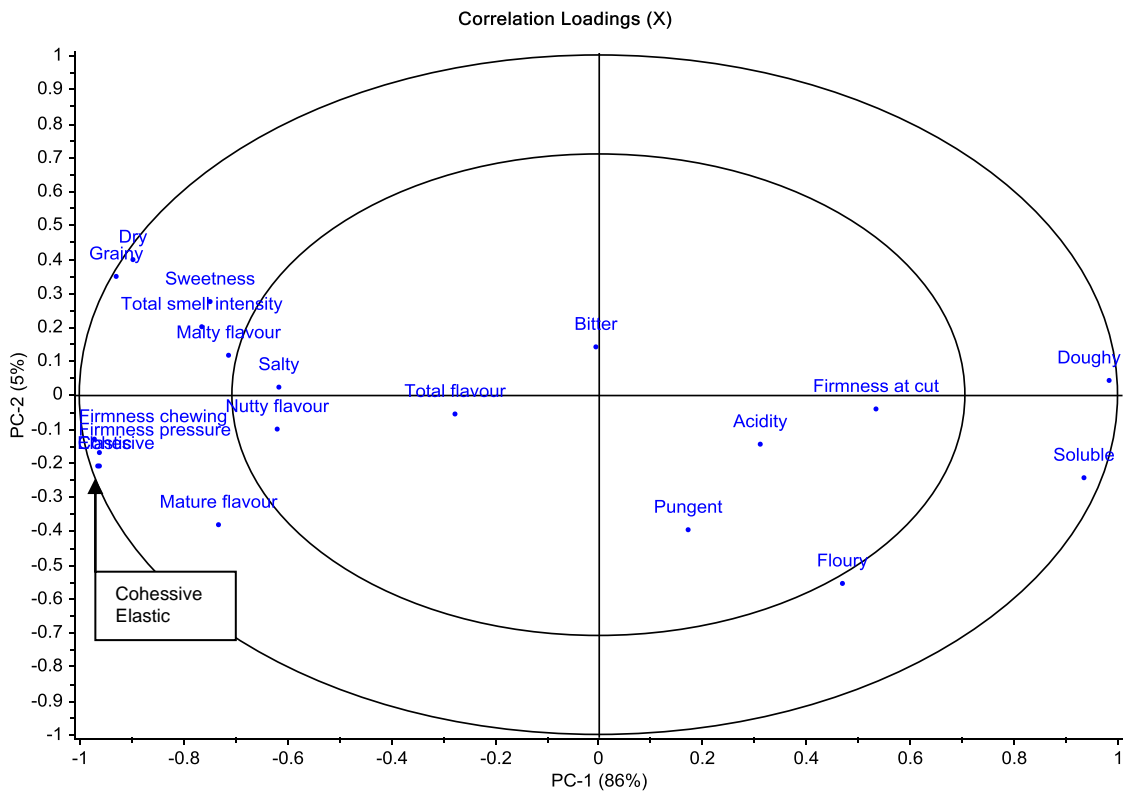
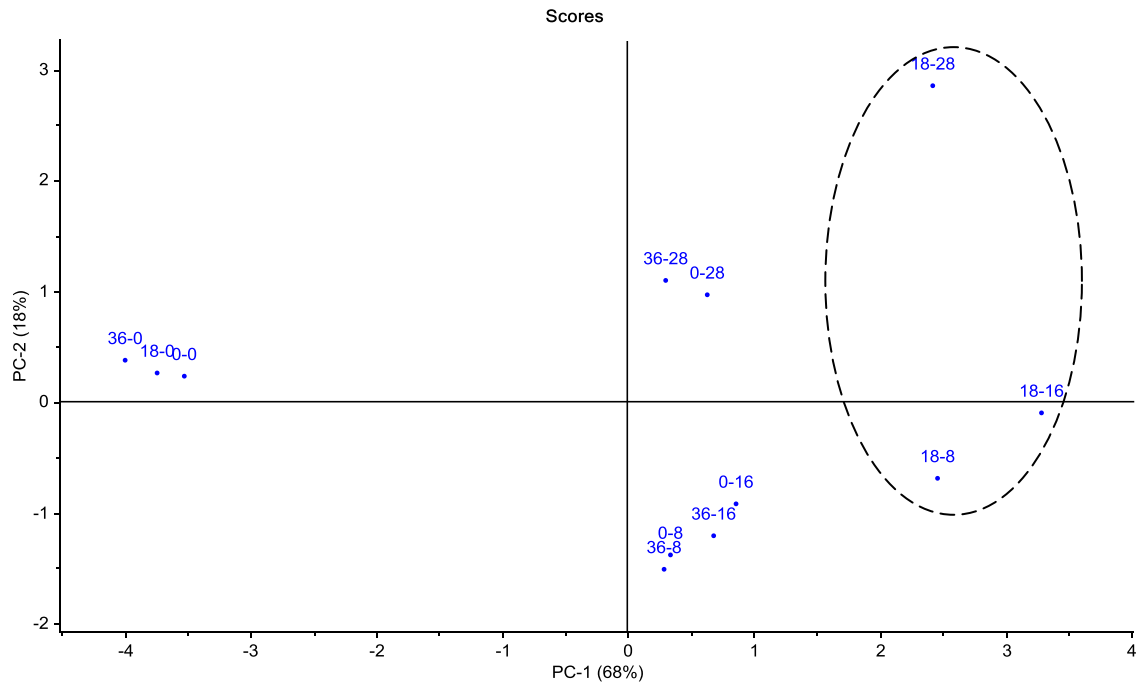


Figure 3.

a)



b)

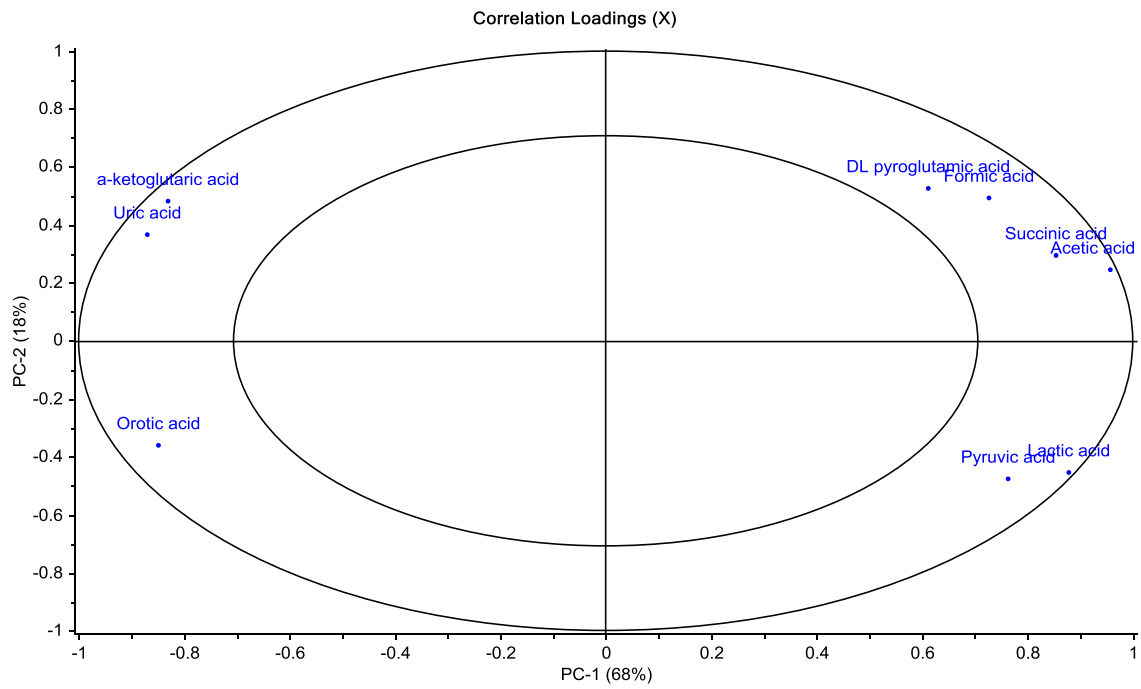
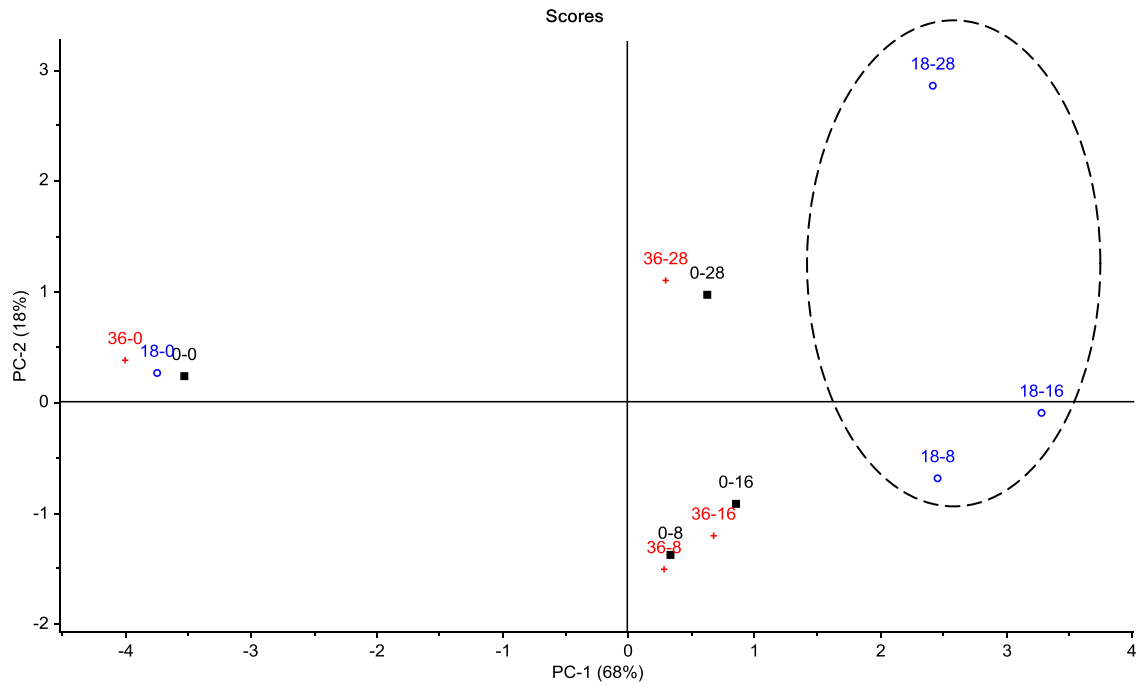




Figure 3. Colour version for the web

a)



b)

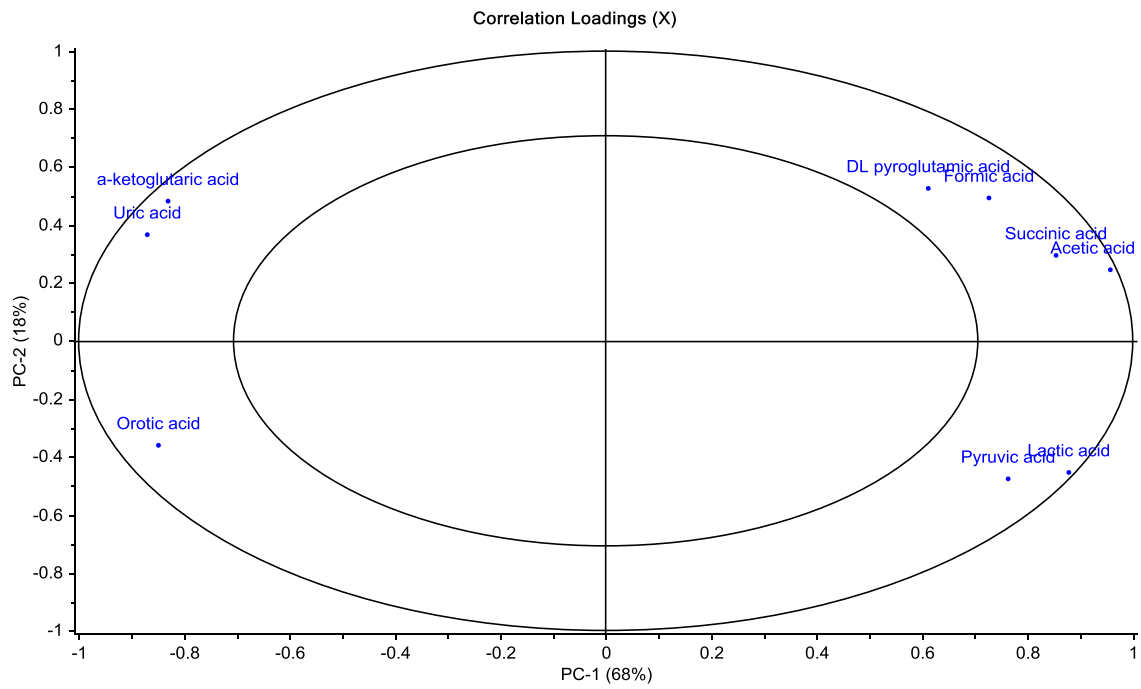
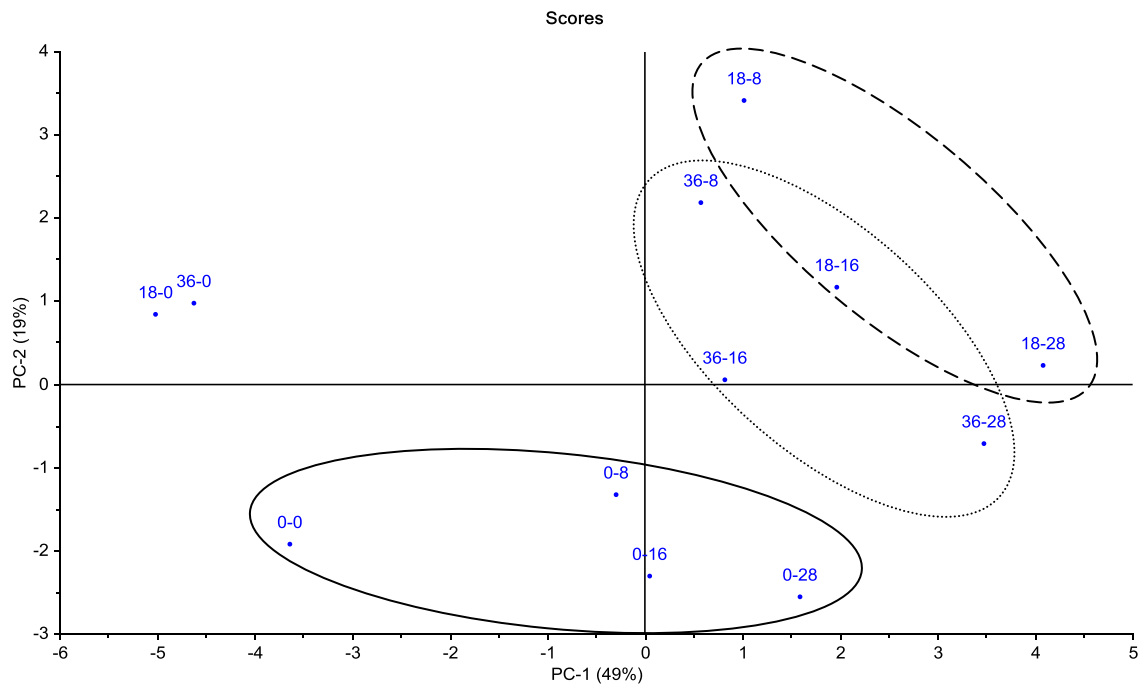


Figure 4

a)



b)

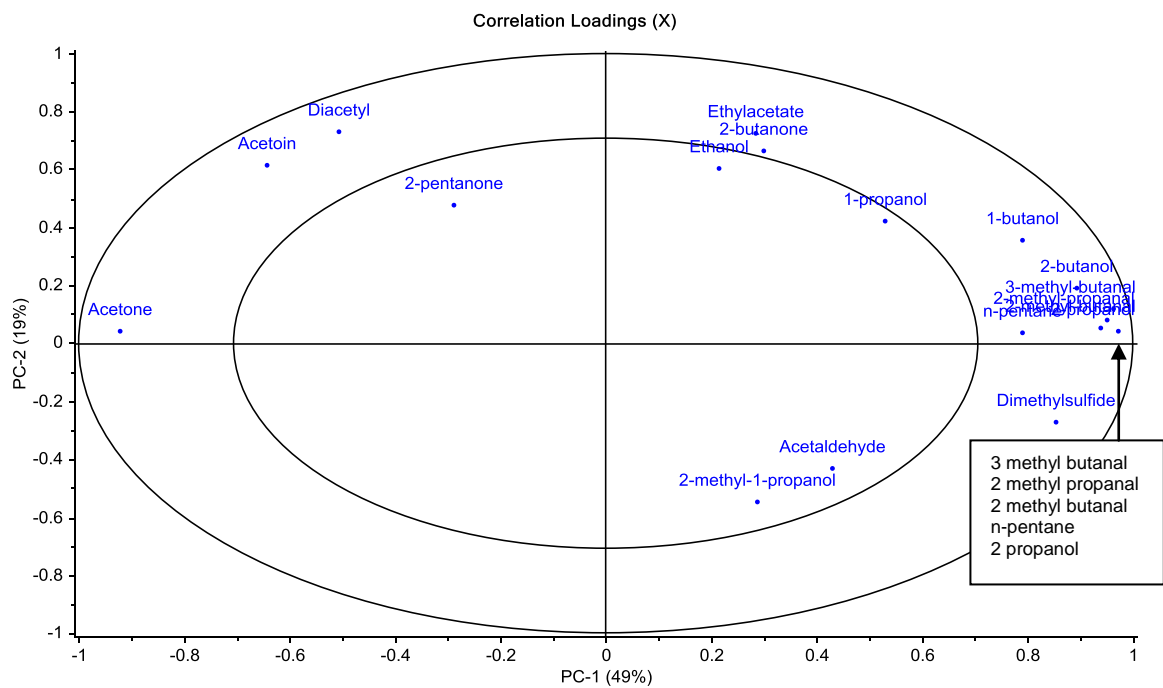
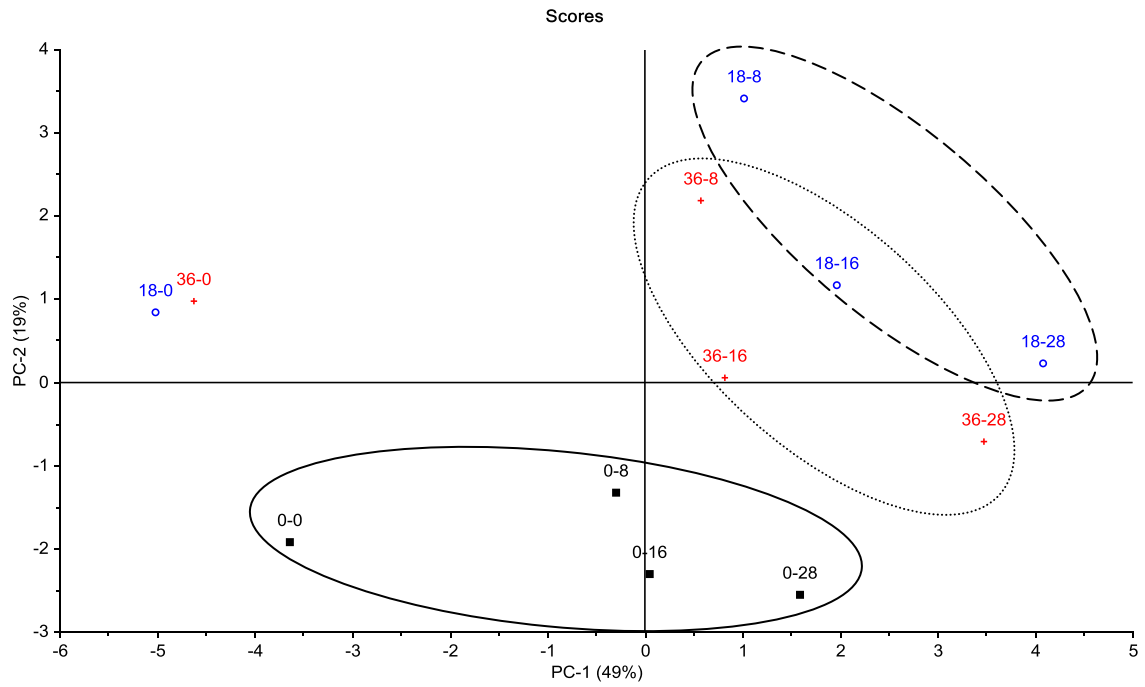


Figure 4 colour

a)



b)

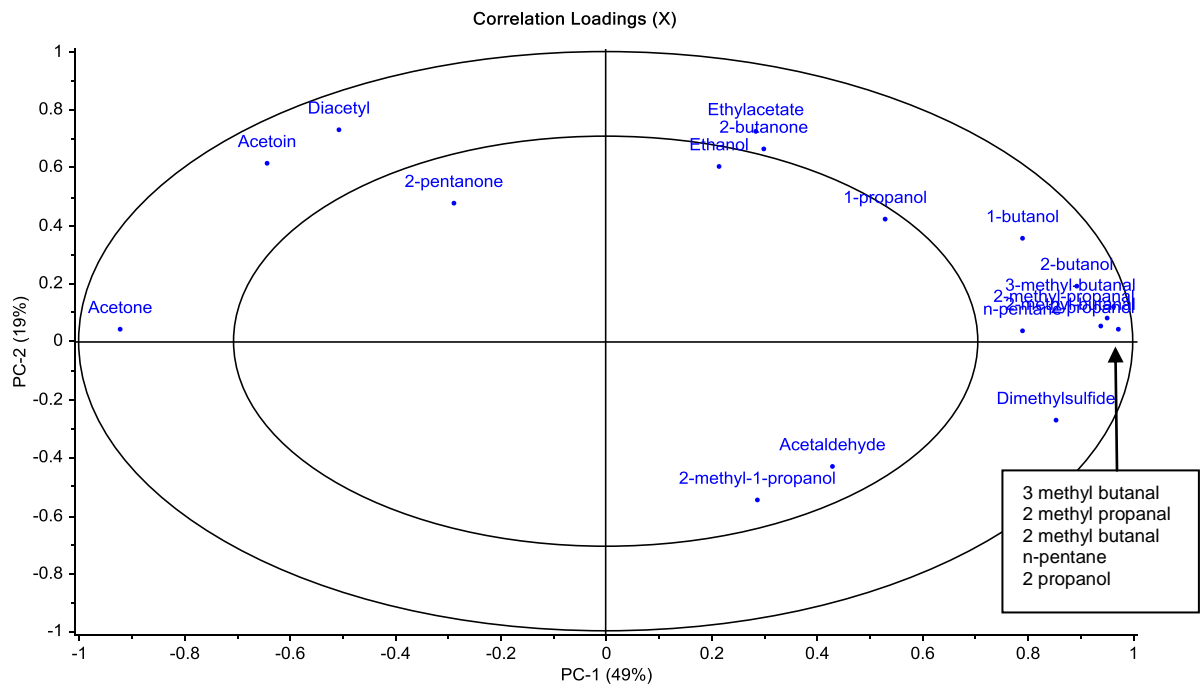
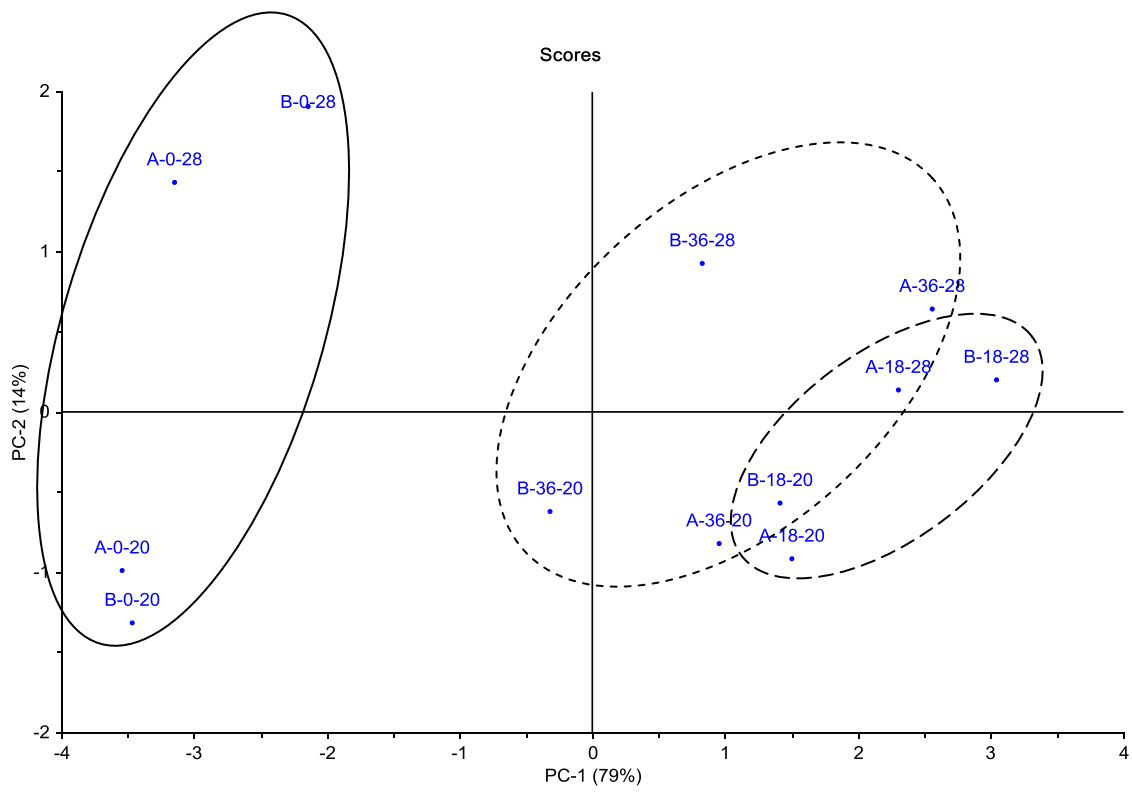


Figure 5

a)



b)

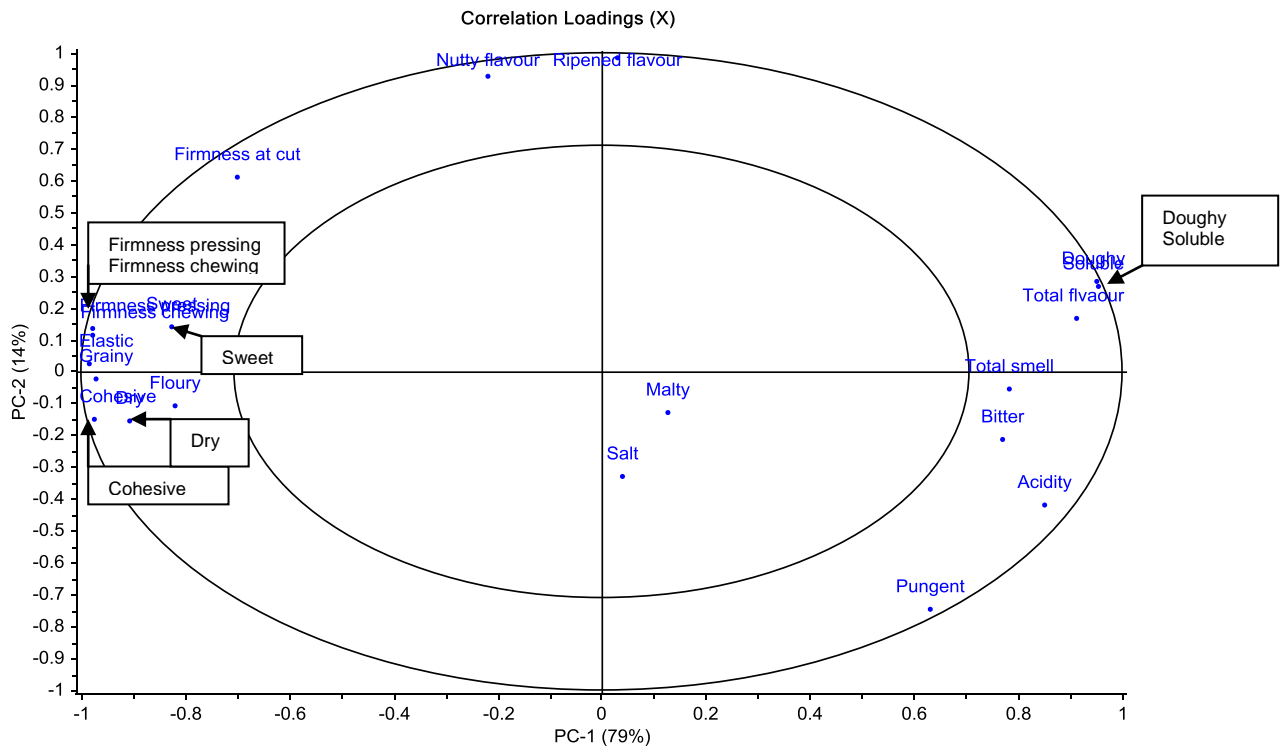
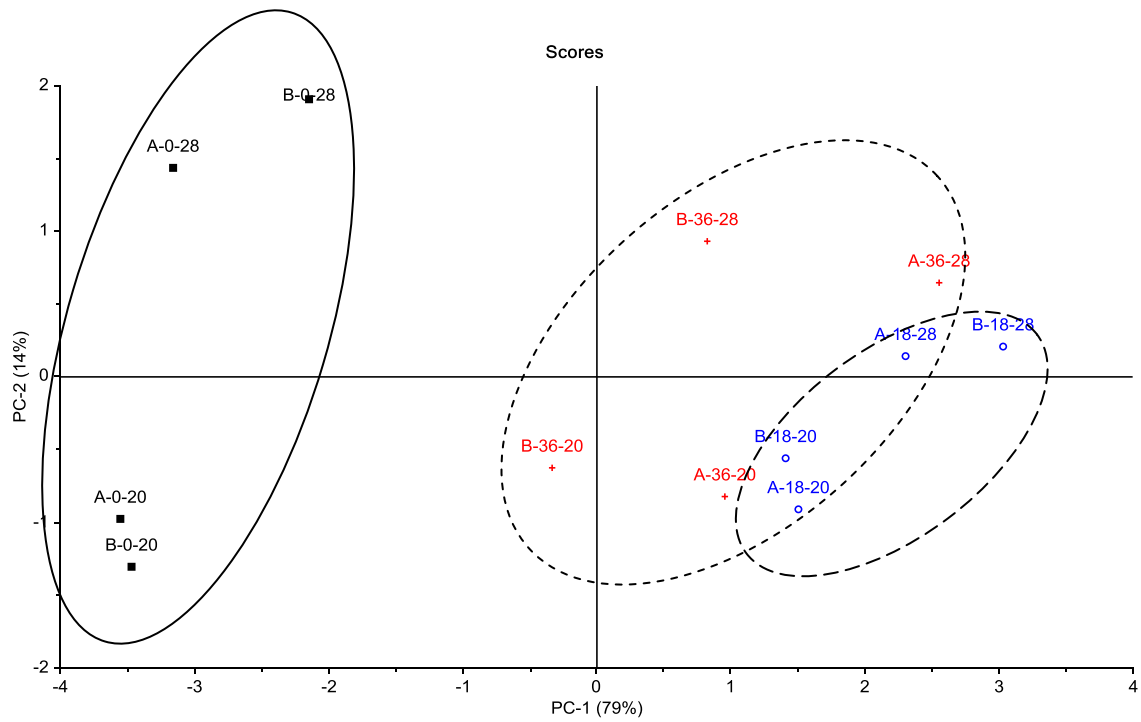


Figure 5 Colour

Figure 5

a)



b)

