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Additional Information

1 **Effect of the inclusion of citrus pulp in the diet of goats on**
2 **cheeses characteristics**

3

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15

16 **Abstract**

17 The differences between the physicochemical (water content, water activity,
18 pH, NaCl, fat, color, and texture) and sensory (descriptive analysis and
19 consumer test) characteristics of cheeses made from the milk of goats fed on
20 a typical control diet and goats fed on a diet incorporating orange pulp were
21 compared. The addition of orange pulp leads to obtain cheeses (i) with a

22 lower pH and water activity, but with a higher fat content, (ii) that are lighter
23 and with a more yellowish-green hue and (iii) higher in color purity, hardness
24 and adhesiveness, although they are less elastic and cohesive. Thus, the
25 incorporation of orange pulp into the goat's diet affected not only the
26 presence of holes in the cheese, but also its hardness, goat taste and salty
27 taste, which were associated with a higher score of consumer acceptance.

28

29 **Key words:** Goat, diet, cheese, orange pulp, physicochemical properties,
30 sensory analysis

31

32 **Highlight**

33 ▶▶ The addition of orange pulp to the diet affect physicochemical cheese
34 properties. ▶▶ The evolution of properties with the ripening time was similar to
35 the control cheese. ▶▶ Chesses obtained with the orange pulp diet present a
36 higher level of consumer acceptance and a greater intention of purchase.
37 ▶▶ The use of milk of goats fed with the orange diet improves the sensory
38 quality of the obtained cheeses

39

40 **1. Introduction**

41 The nutritional needs of animals are met from two major food groups: rich in
42 energy and / or protein concentrates and fibrous foods, mainly forages. It is
43 essential to know the chemical composition of the diet, its nutritional value
44 and the amount in which it can be ingested by goats. Nowadays, because of

45 to the low profitability of the small ruminants sectors, it is very important to
46 reduce the cost of their diet (Garg et al, 2013).

47 The use of agroindustrial by-products for ruminant feed is an economical and
48 environmentally sound way for food processors to reduce waste discharge
49 and to decrease waste management costs. Historically, the use of
50 agroindustrial by-products in areas where the natural forage is insufficient
51 has been recommended (Martinez and Medina, 1982 Martinez et al, 1998.).
52 Actually, its use seems widespread and covers areas where natural forages
53 are surplus. The choice of agroindustrial by-products supplementation should
54 be based on economical (low price), nutritional (dense in nutrients), and
55 toxicological (free of toxins or other substances) considerations. Large
56 amounts of waste from citrus processing for juice extraction is available in
57 Spain. Citrus pulp, the main citrus by-product, is a high energy content by-
58 product that can partly replace cereal grains in animal rations with no
59 adverse effect on milk yield or composition. The use of citrus pulp in
60 formulated feeds depends largely on the availability and its relative cost-
61 effectiveness when compared with other alternative raw materials. Citrus
62 pulp is primarily a feedstuff with low protein and high carbohydrate contents,
63 which differs substantially from the chemical composition of conventional raw
64 materials. The nutrient content of citrus by-product feedstuff is influenced by
65 different factors, including the source of the fruit and the type of processing.
66 In general, all citrus by-products are suitable for inclusion in ruminant diets
67 because of their ability to ferment high-fiber feeds in the rumen (Arbabi et al.,
68 2008). The effects of incorporating citrus pulp into cow, sheep and goat diets

69 on the physical quality of the milk has been studied (Bampidis and Robinson,
70 2006; Jaramillo et al., 2006). However, its effect on physico-chemical and
71 sensory characteristics of the obtained dairy products is important (Scholz,
72 1995; Lebecque et al., 2001) and, to date, it is not available.

73 The aim of this study was to compare between cheeses made from the
74 milk of goats fed on a conventional diet and on a diet supplemented with
75 orange pulp. The physico-chemical and sensory properties during cheeses
76 ripening were studied.

77

78 **2. Material and methods**

79 **2.1. Samples**

80 24 Murciano Granadina (a Spanish breed) goats, part of the small-ruminant
81 farm of the Universitat Politècnica de València (UPV), in the sixth month of
82 lactation were adapted to experimental conditions and for 14 days from, were
83 fed on a control diet. Later, the 24 goats were divided into two groups,
84 according to their productive characteristics and the composition of their milk.
85 These two groups followed a 46-day pilot phase, during which the goats were
86 fed on 2 different although nutritionally equivalent diets, the same control diet
87 (CD) used in the pre-experimental period and a diet incorporating orange
88 pulp (OPD). Randomly, one of the groups was fed in the first 23 days on the
89 CD and the other one on the OPD. The diets were changed in the second
90 period. For each animal, both diets contained 1500 g of alfalfa and 200 g of
91 straw. In addition, the CD contained 1300 g of feed supplement and the OPD
92 included 100 g of feed supplement, 50 g of soy and 2500 g of orange pulp

93 per animal. The CD contained: 17 g protein/100 g of dry matter and a net
94 energy of 0.82 fodder units (FU) (Belloin, 1988.). The OPD contained 16.33 g
95 protein/100 g dry matter and a net energy of 0.82 FU units fodder milk/kg dry
96 matter. The components of the diets were supplied by a local company
97 (Piensos y Cereales Noalles S.L., Valencia), and the rations were prepared
98 daily, just before the goats were fed.

99

100 **2.2. *Manufacture of the cheeses***

101 The cheeses were prepared in the cheese factory of UPV. The raw milk was
102 obtained at the same day as the cheeses were manufactured. Upon receipt,
103 the milk was filtered and kept in refrigeration until the start of the processing.
104 The process started by the addition of the commercial starter culture
105 (Laboratorios Arroyo, Santander, Spain) when the milk reached the
106 appropriate temperature setting for the bacteria (28 - 30 °C). After that,
107 calcium chloride and the rennet (Laboratorios Arroyo, Santander, Spain)
108 were added in order to initiate the formation of a firm curd. After coagulation
109 (about 35 min), the curd was cut with large knives to allow the elimination of
110 the serum. The curd was placed into moulds and pressed to facilitate the
111 blending of the curd grains and to accelerate the whey separation. After this
112 stage, the cheeses were salted in brine (22 °Baume concentration) to
113 eliminate more whey and to make possible the formation of the cortex.
114 Finally, the cheeses went through a ripening stage of 60 days inside
115 chambers with controlled temperature (11-12 °C) and relative humidity (80-
116 85). The minimum ripening time was in accordance with the current Spanish

117 regulations for making cheeses made from raw milk, which weight less than
118 1.5 kg (España, 2006). The final products had an average weight of 800 g.
119 Cheeses made from the milk of goats fed on CD are named CCh and those
120 made from the milk of goats fed on OPD are named OPCh.

121

122 **2.3. Physico-chemical characterization of cheeses**

123 Cheeses were characterized throughout ripening process in the chambers at
124 1, 40 and 60 days from the start. The crust was removed when preparing the
125 samples for analysis.

126

127 **2.3.1. Water content**

128 The water content of the cheeses was measured in triplicate following the
129 official AOAC 926.08 method (1997).

130

131 **2.3.2. Water activity**

132 The water activity (a_w) of the samples, previously homogenized, was
133 measured in triplicate by using a dew point hygrometer (Decagon Devices
134 Inc, Aqualab 4TE, USA).

135

136 **2.3.3. pH**

137 The pH was measured using a pH meter (Crison Instruments, S.A., Basic 20,
138 Spain) with a penetration electrode. The readings were taken in different
139 parts of the cheese, making a minimum of six readings per sample.

140

141 2.3.4. Sodium chloride

142 An automatic chloride analyzer (Sherwood Scientific, Chloride Analyzer 926,
143 U.K.) was used to determine the sodium chloride content in the cheeses,
144 following the equipment instructions.

145

146 2.3.5. Fat content

147 The Soxhlet method was used to separate the fat from cheese. It is a little
148 time-consuming and labor intensive method and its validity has been tested
149 by comparing the determinations of fat in cheese products with those
150 analyzed by conventional methods, such as the Roesse-Gottlieb, the Gerber,
151 the Schmidt-Bondzynski-Ratzlaff and the Babcock (García-Ayuso et al.,
152 1999; Purcarea, 2009).

153

154 2.3.6. Color

155 Color measurement was done by using a Minolta, CM 3600D (Tokyo, Japan)
156 spectrophotometer which provided the CIE-L*a*b* color coordinates (10°
157 observer and D65 illuminant) (Hutchings, 1999). Readings were obtained at
158 room temperature (22±1 °C) directly on the surface of cylindrical samples, 10
159 mm in height and 20 mm in diameter. At least 20 replicates were carried out
160 for each sample. In order to quantify the color difference caused by ripening,
161 the ΔE^* was calculated (Eq. 2) between cheeses kept inside the ripening
162 chambers for 1 and 60 days. This equation was also used to quantify the
163 color difference between CCh and OPCh samples ripened for 60 days.

164

165
$$\Delta E^* = \sqrt{\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2}} \quad (2)$$

166

167 The color difference is graded as follow (Bodart et al., 2008): $\Delta E^* < 1$ color
168 difference could not be detected by the human eye; $1 < \Delta E^* < 3$ minor color
169 differences could be detected by the human eye, depending on the hue, and
170 $\Delta E^* > 3$ color differences would be detected by the human eye.

171

172 ***2.3.7. Textural properties***

173 Texture measurements were performed using a TA-XT Plus Texture
174 Analyzer (Stable Micro Systems, U.K.). A Texture Profile Analysis (TPA) was
175 carried out at room temperature (22 ± 1 °C) using a 45 mm diameter plunger
176 (P/45). A sample (10 mm in height and 20 mm in diameter) was compressed
177 to 50% of its height at a constant deformation rate of 1 mm s^{-1} , leaving 5 s
178 between the first and the second compressions. At least 20 replicates were
179 carried out for each sample. The parameters obtained from the test were:
180 hardness (maximum force registered during the first compression cycle),
181 adhesiveness (negative area registered after the first compression cycle),
182 cohesiveness (ratio of the positive areas under the first and the second
183 compressions), springiness (height recovered by the sample during the time
184 elapsed between the end of the first compression and the start of the second
185 compression cycles), gumminess (energy required to disintegrate a semi-
186 solid food) (Rosenthal, 1999).

187

188 ***2.4. Sensory analysis***

189 The samples were assessed in a standardized tasting room, which was
190 equipped with individual booths (ISO, 2007). The data acquisition was
191 performed using Compusense five releases 5.0 software (Compusense Inc.,
192 Guelph, Ontario, Canada).

193

194 2.4.1. Descriptive sensory analysis

195 Descriptive sensory analysis was carried by a panel of 11 assessors (25-55
196 years old) with experience in assessing similar products, which were trained
197 for the descriptive sensory analysis (ISO, 2012) to select the descriptors by
198 using a consensual checklist method, as described by Lawless and Heymann
199 (1998). During the training sessions, the assessors proposed a list of sensory
200 attributes for the product, their definitions and how to evaluate each attribute
201 in an open session. This procedure was proposed by Stone and Sidel (2004)
202 in order to obtain a complete description of a product's sensory properties.
203 The panel reached a consensus and the following descriptors were chosen:
204 appearance (presence of holes), goat cheese odor, foreign odor, hardness in
205 mouth, pastiness, goat cheese taste, salty taste, acid taste, fatty taste and
206 foreign taste.

207 Panel members were trained in the use of scales with reference samples. A
208 10-cm unstructured scale was used throughout the training period. Panel
209 performance was checked by analysis of variance (ANOVA) for
210 discrimination ability and reproducibility of the panelists using the statistical
211 software Senpaq (V. 4.2). Twelve 30-min training sessions were required for
212 the panel members to reach homogeneous evaluation

213 The samples were rated in duplicate, in two separate sessions, on a 10-cm
214 unstructured scale. The data was analyzed by using experimental design of
215 balanced complete blocks. The two set of goat cheese samples, CCh and
216 OPCh, were presented to the assessors with randomly assigned three-digit
217 codes in a randomized order on plastic trays at 22 °C. Each panel member
218 assessed two samples per session. Water was provided to rinse the mouth
219 between each sample.

220

221 *2.4.2. Consumer test*

222 Eighty two untrained consumers from 18 to 50 years old (30% men and 70%
223 women), which frequently eat goat cheese, took part in the consumer test.
224 The consumers evaluated the two samples (CCh and OPCh), coded with
225 random three-digit numbers following a balanced complete block
226 experimental design. Consumer acceptance testing was carried out using a
227 9-point hedonic scale (9 = like very much; 1 = dislike very much). The
228 consumers scored their liking for the “appearance”, “color”, “texture”, “taste”
229 and “overall acceptance”.

230 In addition, the consumers answered to a questioner in which it was asked if
231 they perceived a strange odor or taste with “yes” or “no”. The consumers also
232 evaluated their intention to purchase the cheeses on five-point category
233 scales with the ends anchored with “I would definitely buy it” through to “I
234 would definitely not buy it” and a neutral central point: “maybe I would or
235 maybe I would not buy it.”

236

237 **2.5. Statistical analysis**

238 A one-way analysis of variance (ANOVA) was used to see if the effect of goat
239 feed on the physico-chemical parameters of cheeses was significant. The
240 least significant differences were calculated by Tukey's test and the
241 significance at $P < 0.05$ was determined using the software Statgraphics Plus
242 (V. 5.1). A two-way analysis of variance (ANOVA) was applied to each
243 descriptor in the descriptive sensory analysis, considering the assessors, the
244 samples and their interaction as factors. A one-way analysis of variance
245 (ANOVA) was applied to the consumer test in order to study the effect of the
246 sample on the consumer acceptability scores. In both analyses, the least
247 significant differences were calculated by Tukey's test and the significance at
248 $P < 0.05$ was determined. Sensory statistical analyses were performed using
249 XLSTAT 2009.4.03 statistical software (Microsoft, Mountain View, CA).

250

251 **3. Results and discussion**

252

253 **3.1. Physico-chemical characterization of cheese**

254

255 The two diets did not affect significantly the pH, a_w or fat content of fresh
256 cheeses (Table 1). However, the water and NaCl contents were higher in
257 cheeses newly made from goats fed on OPD. On the whole, as ripening
258 progressed the pH, the NaCl content and the fat content of the two cheeses
259 increased, even when data are compared on dry basis, while water content
260 and a_w decreased. These results coincide with those obtained by Freitas and

261 Malcata (2000). The increase of pH during ripening may reflect proteolytic
262 processes, which release certain basic amino acids, NH_3 and lactate
263 decomposition (Brito et al., 2003). The decrease in a_w during ripening occurs
264 as a result of increase in solid concentration and due to the production of low
265 molecular weight non-protein nitrogen compounds and soluble solutes
266 derived from glycolysis, proteolysis and lipolysis processes (Marcos et al.,
267 1979). Hence, the addition of orange pulp to the animal diet led to cheeses
268 with lower values of pH and a_w , but with a higher content of both fat and NaCl
269 at the end of the ripening period studied.

270

271 **3.2. Evaluation of color in cheeses**

272 The obtained color characteristics agree with those reported by Chacón-
273 Villalobos and Pineda-Castro (2009) when analyzing goat cheese under
274 similar conditions. After manufacture, OPCh samples had higher L^* and
275 negative a^* values and lower values of b^* in comparison to CCh samples
276 (Table 2). The higher water content of OPCh might favor the product's
277 lightness (Álvarez et al., 2007; Caro et al., 2014). As far as ripening time was
278 concerned, only a significant increase in b^* was found only at 60 days.
279 Similar trends were reported by Rohm and Jaros (1996) and Buffa et al.
280 (2001). According to Lucas et al. (2008), the increase in b^* values relates to
281 proteolysis and browning reactions. With respect to the color difference
282 brought about by the ripening stage, this was of 3.8 units (standard deviation
283 0.4) for CCh samples and of 2.0 units (standard deviation 0.2) for OPCh
284 samples. Only for CCh was this difference greater than 3 units, indicating

285 color changes that may be perceived by the human eye (Bodart et al, 2008).
286 On day 60, the color differences between the two cheeses were in the order
287 of 5.8 units (standard deviation 0.6), indicating that consumers will be able to
288 differentiate between them.

289

290 **3.3. Texture evaluation of cheese**

291 During ripening, the samples became significantly harder and more adhesive,
292 while the springiness, cohesiveness and gumminess decreased significantly
293 (Table 3). At the end of the ripening stage, OPCh samples exhibited greater
294 hardness and adhesiveness and less springiness, cohesiveness and
295 gumminess, as compared with CCh. In general, these changes are
296 consistent with previous results under similar settings (Lucey et al., 2004,
297 Theophilou and Wilbey, 2007). The increase in adhesiveness and the
298 decrease in springiness may be caused by the rupture of the fat globule
299 membrane. A lower degree of springiness also decreases the resistance to
300 deformation, leading to less cohesiveness (Muller, 1977).

301

302 **3.4. Sensory analysis of cheeses**

303 The results from the descriptive sensory analysis showed that goat feed had
304 some effects on several descriptors of the cheeses. As can be seen in Fig. 1,
305 panelists only detected significant differences in the descriptors “appearance
306 (presence of holes)”, “hardness in mouth”, “goat cheese taste” and “salty
307 taste”. OPCh had higher values of hardness in mouth, goat cheese taste and
308 salty taste, but lower values of appearance (presence of holes) in

309 comparison with CCh. The higher salty taste, hardness and goat cheese
310 taste that was found in OPCh may be related to the higher content of sodium
311 chloride, higher instrumental hardness and higher fat content obtained for
312 this sample in comparison to CCh. This higher fat content favored the
313 palatability of the product and, therefore, the greater cheese taste found by
314 the panelists. In fact, other authors found less flavor and taste in cheeses
315 with lower fat content (Childs and Drake, 2009; Deegan et al., 2014).

316 As a complement to the descriptive analysis, a consumer test was carried out
317 to evaluate the acceptance of the cheeses. The liking scores for the
318 “appearance,” “color,” “texture” “taste” and “overall acceptance are shown in
319 Fig. 2. Only taste and overall acceptance were significantly different between
320 the samples being the OPCh scores higher. These results suggest that the
321 most important attribute, which affect the overall sample acceptance, is the
322 taste. When the results were analyzed for the question concerning whether
323 consumers perceived a strange odor or taste in the samples, it was found
324 that 87% and 95% of consumers did not perceive any strange odor in CCh
325 and OPCh, respectively. Nevertheless, 40% of the consumers perceived a
326 certain, strange taste in CCh (bitter, spicy and acidic) and only 20% of the
327 consumers claimed that they detected a mild goat cheese taste. This result
328 may explain the significant differences in consumer acceptance of the taste
329 of the studied samples. Finally, from evaluation of the responses regarding
330 the intention to purchase the samples, it was found that only 39% of the
331 consumers would buy the CCh, while 53% would buy the OPCh.

332

333 **4. Conclusions**

334 Adding orange pulp to the diet of goats affected some physicochemical
335 properties of the cheeses, although the evolution of these properties with the
336 ripening time was similar in both types of cheese. Cheeses made with the
337 incorporation of orange pulp to the diet had higher level of consumer
338 acceptance and a greater intention of purchase, probably because the
339 consumers perceived greater hardness, more marked salty taste and goat
340 taste. Hence, the use of milk of goats fed with the orange pulp diet improves
341 the sensory quality of the obtained cheeses.

342

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347

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448

449 **FIGURE CAPTIONS**

450

451 Figure 1. Descriptive sensory analysis of cheeses ripened for 60 days (mean
452 values marked with * represent significant differences at $p < 0.05$).

453 Figure 2. Consumer scores (mean values marked with different letters for
454 each attribute indicate significant differences between samples at $p < 0.05$).

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