Composition, proteolysis and volatile profile of Strachitunt PDO cheese

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Key Words:	Strachitunt cheese, proteolysis, volatile organic compounds, dual-curd method of production

Interpretive Summary

Composition, Proteolysis and Volatile Profile of Strachitunt PDO Cheese. *By F. Masotti et al.* Strachitunt, an Italian blue-veined cheese, was given the Protected Designation of Origin (PDO) in 2014. The double curd method of production makes Strachitunt unique among PDO cheeses. In this research, 10 Strachitunt samples made weekly over a 4 mo period by the same cheese maker and ripened 75−77 d were analyzed to measure the main chemical properties. Differently from other blue varieties, Strachitunt showed an acid paste and a less important proteolysis. Also the profile in volatile organic compounds was particular, being characterized by the prevalence of esters and alcohols, while ketones were less abundant than other blue-cheeses. The large variability of values of the above mentioned parameters in the equally ripened samples was attributed to the specific artisanal processing technology.

Running head: CHEMICAL CHARACTERIZATION OF STRACHITUNT CHEESE

Composition, proteolysis and volatile profile of Strachitunt PDO cheese

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ABSTRACT

Strachitunt, a blue-veined Italian cheese, received the Protected Designation of Origin (PDO) label in 2014. Its unique technological feature is represented by the dual-curd method of production. Strachitunt is produced from raw bovine milk with or without the inoculation of

natural starter cultures of lactic acid bacteria, while the addition of secondary cultures of mould spores is not permitted by the product specification. Physico-chemical properties, proteolysis and volatile profile of Strachitunt were investigated in 10 cheese samples (ripened 75 d) made throughout springtime 2015 and provided by the main cheese maker. Overall, composition parameters showed a large variability among samples. Cheese was characterized by an acid paste (pH 5.46) and a lower extent of proteolysis in comparison to other blue-veined varieties. The main chemical groups of volatile organic compounds were alcohols and esters, whereas ketones represented only a minor component. The erratic adventitious contamination by mould spores of the cheese milk, the unique dual-curd method of cheese making and the large time variability between the piercing time and the end of ripening were responsible of both the distinctive analytical fingerprint and the scarce standardization of this blue-veined cheese.

Key words: Strachitunt cheese, proteolysis, volatile organic compounds, dual-curd method of production

INTRODUCTION

Strachitunt, an Italian blue-veined cheese made in Valtaleggio, a restricted area of Lombardy region, in 2000 was inserted among the most valuable traditional products made in this region, and in 2014 it was given the Protected Designation of Origin (**PDO**) (Commission Implementing Regulation, 2014). This artisanal PDO cheese, produced using a traditional method and complying with regulatory manufacturing standards, provides an annual production around 50 tons. Raw cow milk of Italian Brown breed is used according to the product

specification (Announcement EU, 2016). Bacteria promoting acid development in Strachitunt curd can be present as "wild" microflora or added as autochthonous natural starters. The use of natural starters is adopted only in the hot periods of the year, and it is intended (i) to achieve a uniform rate of milk acidification, (ii) to control unwanted bacteria, (iii) to balance the variability of microbial population, (iv) to affect positively curd syneresis and cheese rheology and (v) to favour rennet action. The technological feature of Strachitunt is the so called dual-curd method of production, based on the mix of a 12-h-old curd, called "cold curd", with a fresh curd, called "hot curd" (in a 1 : 10 ratio, respectively), to make the body of the final cheese. Apart from Beacon Fell traditional Lancashire, a crumbly hard cheese (Commission Regulation, 1996), Strachitunt is the only PDO cheese produced applying this method. No addition of mould spores is permitted during Strachitunt cheese making. The dual-curd mixing, promoting the formation of cavities entrapping air into the paste, coupled to the cheese piercing is known to favour the natural growth of moulds. The cylindric wheel, ripened at least 75 d, weighs 4–6 kg and the characteristic appearance of the paste is ivory-white with blue-green veins. An uneven vein distribution in the matrix is normally observed, and it is ascribed to the variability in piercing time from the 30th to the 50th d of ripening.

A previous research (Belotti et al., 2003) described the microbiological evolution in Strachitunt during ripening (from 1 to 3 mo). The lactic acid bacterial microflora of Strachitunt consisted of micrococci, enterococci, thermophilic and mesophilic lactobacilli. Heterofermentative lactobacilli produced micro holes in the paste promoting the growth of moulds. Generally in blue-veined cheeses, the production of lactic acid by thermophilic bacteria promotes the rapid growth of *Penicillium roqueforti*, which is the prevailing mould of these varieties. *Penicillium roqueforti* is the main donor of proteolytic enzymes, released when the

mould dies and lyses, leading to the formation of water soluble nitrogen and free amino acids acting as precursors of volatile compounds (Lawlor et al., 2003). In addition, *Penicillium* spp. are also the main lipolytic microbiota in these cheese varieties. The lipid fraction is the major contributor to the development of taste and aroma profiles in blue-veined cheeses (Preedy et al., 2013). Enzymatic reactions produce a large number of volatile organic compounds (**VOCs**) contributing to cheese aroma. In particular, in blue-veined cheeses the main contribution to flavour is ascribed to methyl ketones produced by the ß-oxidation of free fatty acids followed by a decarboxylation reaction (Voigt et al., 2010).

The scope of the present study was to provide a chemical portrait of PDO Strachitunt. For this purpose, we surveyed the physico-chemical characteristics, proteolysis and the volatile profile of the cheese ready for consumption supplied by the main cheese maker over a 4 mo period. We studied also the effect of milk inoculation with natural starters, a procedure allowed by the standard manufacturing protocol of this blue-veined cheese to better standardize cheese characteristics during summertime.

MATERIALS AND METHODS

Cheese Manufacturing and Sampling

Ten samples of Strachitunt cheeses were made consecutively on a weekly basis during the springtime in 2015 and ripened 75−77 d. These no commercial cheese samples were provided by the main cheese maker belonging to the "Consorzio per la tutela dello Strachitunt Valtaleggio" (protection body for PDO Strachitunt). Cheese samples from A to H (Table 1) were

obtained by milk renneted without starter inoculation. Differently, the milk-in-vat of samples I and L was added with a starter culture produced through backslopping (Figure 1).

Cheese sampling scheme (n = 8 + 2) was representative of the allowed technological variant consisting in the milk inoculation with a starter culture during the hot period of the year to guarantee a better standardisation of the final product. Each sample consisted of a wheel of cheese. After rind removal (10 mm) the cheese matrix was homogenised and sampled for analyses.

Composition and Physico-Chemical Analyses

The following analyses were carried out on Strachitunt cheese samples: total solids by drying at 102 °C (ISO, 2004a), water activity by dew-point measurement (ISO, 2012) with an Aqua Lab 3TE Water Activity System apparatus (Decagon devices Inc., Pullman, WA, USA), pH by pH-meter pH25 (Crison Instruments SA, Barcelona, Spain) with a puncture electrode (Crison Instruments SA), fat by gravimetric method (ISO, 2004b) and total nitrogen by Kjeldahl method (ISO, 2008).

Proteolysis was evaluated by measuring the nitrogenous fractions by Kjeldahl method according to ISO Standard (ISO 2011). The procedure consisted in the separation of the pH 4.4-soluble nitrogen (**pH 4.4-SN**) from a citrate solution of the cheese. This fraction was further extracted with 12% trichloroacetic acid (**TCA**) or phosphotungstic acid (**PTA**) to obtain TCA-soluble nitrogen (**TCA-SN**) or PTA-soluble nitrogen (**PTA-SN**), respectively.

Urea Polyacrylamide Gel Electrophoresis (Urea PAGE)

The pH-4.4 insoluble fractions of the cheeses were analysed by urea PAGE according to the method of Andrews (1983) with modifications of Veloso et al. (2004) on 10% resolving gel. Electrophoresis was performed on a Mini vertical electrophoresis unit (SE250, Hoefer, Holliston, MA, USA) at a constant voltage of 60 V, with a power supply EPS 500/400 (GE Healthcare, Upsalla, Sweden). Gels were stained with Coomassie Brilliant Blue G-250. Band identification was performed by comparison with those of a commercial sample of sodium caseinate (Fonterra, Auckland, New Zealand) and with the electropherograms of Bertolino et al. (2011).

Volatile Organic Compounds Analysis

VOCs were extracted from the headspace of cheese by means of solid phase micro extraction and analyzed by gas chromatography-mass spectrometry (**SPME GC/MS**) procedure according to Cornelli et al. (2015), slightly modified for better evaluation of the acidic fraction. Briefly, 1 mL of a 0.12 M H_2S0_4 solution was added to 5 g of cheese along with 2 g of NaCl immediately before volatile extraction, all other parameters being the same previously described. Semi-quantitative evaluation of VOCs was carried out by integrating the peak area of the characteristic ion (Q_{ion}) using the MS-Chemstation software (Agilent Technologies, Santa Clara, CA, USA). Data were shown as the mean value of two replicates.

Statistical Analysis

The physico-chemical parameters and the nitrogen fractions of the Strachitunt cheese samples were submitted to one-way analysis of variance (**ANOVA**) followed by Tukey post hoc test, taking 0.05 as the limit of significance. Data analyses were carried out with Minitab® software (Release 17, 2016, State College, PA, USA). Principal component analysis (**PCA**) was

146 performed on VOCs, classified as chemical classes. Results were graphically represented by the
147 projection of the first two principal components (**PC**).
148
149 **RESULTS AND DISCUSSION**
150
151 ***Composition and Physico-Chemical Parameters of Strachitunt***
152 The main physico-chemical characteristics of Strachitunt cheese samples weekly
153 produced in the same factory over a 4 mo period (March-June) and matured 75−77 d are reported
154 in Table 1. Mean dry matter (**DM**) content was 543.0 g/kg (ranging from 522.1 g/kg to 568.3
155 g/kg), a value similar to that reported for Gorgonzola (532 g/kg) and other internal mould-
156 ripened cheeses (Fernández-Salguero, 2004). Some differences are usually expected between
157 batches of the same type of blue-veined cheese (Preedy et al., 2013). The measured water
158 activity was 0.960, a value in line with that of Gorgonzola and Bleu Bresse (Fernández-Salguero,
159 2004). The mean protein content was 397.2 g/kg expressed on DM, and it varied from 383.8 to
160 409.4 g/kg being in the range of data reported in literature (Fernández-Salguero, 2004). Also the
161 fat level (501.9 g/kg on DM, on average) was similar to the contents of other blue-veined
162 varieties (Fernández-Salguero, 2004). The differences between batches with or without starter
163 (Table 1) were not significant ($P > 0.05$) for the above mentioned parameters. Differently, pH
164 value of samples inoculated with starter (5.11) was significantly ($P < 0.05$) lower than that
165 observed in the starter-free batches (5.53). Typically, the pH of blue-veined cheeses increases
166 throughout ripening, reaching the level 6.5–6.8 at 90 d ripening (Prieto et al., 2000). The
167 consumption of lactic acid by moulds and the deamination of free amino acids are responsible of
168 such high pH level (Fox et al., 2000). The significantly lower pH values observed in Strachitunt

169 in relation to other blue varieties were attributed to the less extensive proteolytic phenomena, as
170 a consequence of the lack of inoculation of mould spores.
171
172 ***Evaluation of Strachitunt Proteolysis***
173 The growth of *P. roqueforti* throughout the matrix of blue-veined varieties sensitively
174 enhances the proteolysis affecting both flavour and texture of the mature cheese (Fox et al.,
175 2000). The piercing of Strachitunt is realized after 30–50 d of ripening. On this basis, it is
176 expected that both main biochemical processes, proteolysis and lipolysis, are slowed down. A
177 distinctive phenomenon in the blue-veined cheeses is the extensive proteolysis caused by lactic
178 acid bacteria, the coagulant enzyme (chymosin) and mainly by *Penicillium* spp. (Prieto et al.,
179 2000; Cantor et al., 2004)
180 Proteolysis of Strachitunt samples was firstly evaluated by measuring the nitrogenous
181 fractions. The relative distribution of nitrogen in the pH 4.4-SN, TCA-SN and PTA-SN fractions
182 is shown in Table 1. The pH 4.4-SN is a heterogeneous fraction representing an accepted index
183 of the degree of proteolysis (Diezhandino et al., 2015). This fraction was on average 16.89% on
184 TN in the 10 cheese samples and no significant ($P > 0.05$) effects were observed among the
185 samples with or without the inoculation of starter cultures. This nitrogen level evidenced the
186 reduced occurrence of proteolytic phenomena in comparison to other mould ripened cheeses
187 (Prieto et al., 2000; Diezhandino et al., 2015). A survey on the characteristics on the main
188 European blue-veined cheeses reported values of pH 4.4-SN more than two fold higher, ranging
189 from 55.9% for Cabrales to 72.6% (on TN) for Roquefort (Fernández-Salguero, 2004).
190 TCA-SN consists of small peptides (2–20 residues) and free amino acids deriving from
191 the hydrolysis of intermediate peptides (Sousa et al. 2001). This nitrogen fraction reached 9.51%

192 expressed on TN (Table 1) and no significant ($P > 0.05$) differences were observed between the
193 batches without or with inoculation of starter (9.13% and 11.03%, respectively). The TCA-SN
194 content expressed as percentage of pH 4.4-SN (56.3%) was low in relation to that reported in
195 literature for other blue-veined varieties (Zarmpoutis et al., 1997; Diezhandino et al., 2015)
196 proving the lower proteolysis extent of Strachitunt cheese.

197 PTA-SN consisting of small peptides (< 600 Da) and free amino acids was 2.86%
198 expressed on TN and about ten folds lower than the levels recorded in other blue-veined cheeses
199 (Zarmpoutis et al., 1997; Diezhandino et al., 2015). Also the contents of this nitrogen fraction
200 were not significantly different between the batches without or with starter ($P > 0.05$). Overall,
201 data on the contents of the different nitrogen fractions indicate that Strachitunt cheese ready for
202 consumption (75 d) is subjected only to limited proteolytic phenomena.

203 Subsequently, proteolysis of Strachitunt was evaluated by urea PAGE. The
204 electropherograms of the pH 4.4-insoluble fractions of the cheese samples showed appreciable
205 differences in number and intensity of bands (Figure 2). Some profiles almost overlapped, in
206 particular A *vs* B and D *vs* I. In blue-veined cheeses, both α_s-casein (CN) and β-CN are expected
207 to be completely hydrolysed at the end of ripening (Fox et al., 2000). In our work, intact β-CN
208 was present in all lanes suggesting that its degradation by action of plasmin was only partial,
209 likely because the pH value of the cheeses (on average 5.46) was lower than the optimal for this
210 enzyme (7.5). Accordingly, in all samples (Figure 2), the occurrence of low mobility products
211 corresponding to γ-CN, formed by plasmin from β-CN, was less pronounced than that reported
212 in literature for other varieties of blue-veined cheeses (Diezhandino et al., 2015). Indeed, the
213 presence of γ-CN in blue-veined cheeses like Stilton, Danablu, Cashel, Chetwynd and

214 Gorgonzola was attributed to a high level of plasmin activity, in view of the high pH of these
215 cheeses (Zarmpoutis et al., 1997).

216 The α_{s1}-CN band was evident in samples C–L, but faded away in A and B. The first
217 hydrolysis product of this CN fraction by chymosin, represented by α_{s1}-CN f(24–199) (α_{s1}-I-
218 CN), was revealed in samples C–L. The electrophoretic patterns A and B lacked α_{s1}-I-CN band,
219 but showed other low molecular degradation products migrating faster and probably
220 corresponding to the further degradation of this peptide. In blue-veined cheeses, after
221 sporulation, enzymes from *P. roqueforti* hydrolyse among the others also α_{s1}-I-CN, changing the
222 peptide profile (Fox et al., 2000). The distinguishing patterns of samples A and B was paralleled
223 by the highest levels of the three nitrogen fractions in relation to that of samples C–L (Table 1).
224 Such results were consistent with those of Zarmpoutis et al. (1997) who coupled the low extent
225 of CN hydrolysis with the low values for SN, TCA-SN and PTA-SN in some Irish blue cheeses
226 in comparison to Danablu, Stilton and Gorgonzola.

227 A qualitative evaluation of the electrophoretic profiles (Figure 2) suggested that
228 Strachitunt samples were characterized by noticeable heterogeneity in the proteolytic phenomena
229 and no differences were observed between the batches with or without inoculation of starter
230 culture. Likely the adventitious secondary microflora developed differently as a result of variable
231 selective conditions (pH, water activity, temperature, time of piercing and development of
232 environmental moulds).

233

234 ***Analysis of VOCs***

The VOCs were identified in the head space of Strachitunt cheese samples by SPME-GC/MS (Table 2). Twenty-nine compounds were identified: 1 aldehyde, 5 alcohols, 10 esters, 6 ketons and 7 acids. Other components were present at trace levels.

Only the aldehyde 3-methyl buthanal was revealed with a mean area of 55 x 10^3 arbitrary units. This branched aldehyde, considered in different cheese varieties as an important odour-active substance, is formed by *P. roqueforti* from leucine *via* Strecker degradation (Fox et al., 2000). Generally, aldehydes are expected to be low in the ripened cheese due to their conversion to alcohols or acids. Their absence in Strachitunt could be attributed to the catabolic oxidation to the corresponding acid along ripening.

Alcohols represented 9.5% of all VOCs, the majority being primary alcohols. Ethanol was the only linear-chain alcohol detected, and it was the component with the largest fluctuations (CV 117%) among this chemical group. Ethanol is formed through lactose fermentation by starter bacteria and yeasts, and it is reported to have a role as precursor of ethyl esters (Corrëa Lelles Nogueira et al., 2005). Two methyl alcohols were identified, 2-methyl-1-propanol and 3-methyl-1-buthanol, this last being the most abundant, in agreement with results reported in literature for blue cheeses (Gallois and Langlois, 1990; Moio et al., 2000). These branched-chain alcohols are formed by reduction of the corresponding aldheydes, which are derived from the metabolism of specific amino acids (Corrëa Lelles Nogueira et al. 2005). A minor component was 2-heptanol, a secondary alcohol, formed through enzymatic reduction of methyl ketones by *P. roqueforti* metabolism (Cantor et al., 2004). Two-alkanols from 2-propanol to 2-nonanol, typical components of the flavour of blue cheeses (Engels et al., 1997), were not found. All samples of Strachitunt showed a distinctive abundance of 2-phenyl ethanol (2.2 x 10^6 arbitrary units). This aromatic alcohol formed by the metabolism of amino acids essentially by yeasts is

reported as an important odour compound in blue cheeses (Gallois and Langlois, 1990). Moio et al. (2000) reported that alcohols in Gorgonzola represented more than 30% of the neutral volatiles, a value similar to that measured for Strachitunt (25%). However, in our study a different relative distribution of alcohols was observed.

A prominent group of volatiles in Strachitunt consisted of esters. Among the 10 compounds identified, the major ones (82.3%) were both methyl and ethyl esters of free fatty acids. The occurrence and variety of ethyl esters in Strachitunt could be related to the abundant presence of the precursor molecule, ethanol. The ester profile of Strachitunt was characterised also by 2-phenyl-ethyl acetate and 2-phenyl-ethyl isobutanoate. In this case, their presence was related to that of the aromatic alcohol 2-phenyl ethanol. Esters, accounting for 53.8% of neutral volatiles in Strachitunt, are reported to provide fruity notes and likely could be responsible of the aromatic note of Strachitunt. An abundant amount of esters was revealed also in Roquefort, while in Gorgonzola and other blue-veined cheeses esters represented only a minor fraction (Gallois and Langlois, 1990; Moio et al., 2000; Wolf et al., 2011).

Ketones consisted of 6 components and represented 9.0% of Strachitunt VOCs (Table 2), but this percentage was exactly halved when cheese sample H was not taken into consideration, resulting as outlier for most ketones for Grubbs' test ($P < 0.05$). In this last case, the GC/MS total peak area of this chemical group ranged from 2.1% (sample A) to 8.8% (sample I) of all volatiles. Ketones are known as the most abundant volatiles in blue-veined cheeses (Fox et al., 2000), and their production is affected by the physiological state of mycelium, pH, salt content and concentration of fatty acids (Wolf et al., 2011). The ketones more abundant in Strachitunt were 2-alkanones (methyl ketones) with odd number of carbon atoms (from C_5 to C_9), in particular the area of 2-eptanone was 42.0% of all ketones in accordance with the predominance

of this compound reported in blue cheeses (Fox et al., 2000). Both 2-butanone and acetone were present in similar low amounts (219 and 273 x 10^3 arbitrary units, respectively) when excluding sample H. Acetoin was the less abundant compound (133 x 10^3 arbitrary units) among ketones. Methyl ketones in Strachitunt represented the minor contributors (22.1%) among neutral volatile constituents with the exception of aldehydes, probably because of their conversion to secondary alcohols. This percentage further decreased (12.2%) when cheese sample H was not considered. As a comparison, this chemical group represented 50–70% of neutral volatiles in French blue cheeses like Roquefort, Bleu des Causses, Bleu d'Auvergne and in Gorgonzola (Gallois and Langlois, 1990; Moio et al., 2000).

Generally, blue cheeses are characterized by extensive lipolytic phenomena. In Strachitunt acids accounted for 59.8% of VOCs. Large variations were observed among samples, with peak areas ranging from 27 x 10^6 to 132 x 10^6 arbitrary units (samples A and G, respectively). Among the 7 identified acids, butanoic and hexanoic acids were the most abundant, while lower amounts were measured for acetic, octanoic and decanoic acids. In addition to n-acids, also branched-chain acids (2-methyl propanoic acid and 3-methyl butanoic acid), deriving from amino acid metabolism (valine and leucine, respectively) were found in high amounts. In comparison to Gorgonzola (Moio et al., 2000), Strachitunt showed a high relative level of butanoic acid and 3-methyl-butanoic acid while octanoic acid and decanoic acid were present in low amounts.

On the whole, the differences in the VOCs composition of the 10 samples of Strachitunt were likely attributable to the uneven growth and development of the adventitious microflora expected in an artisanal environment (Williams and Withers, 2010). Moulds are known as the major source of volatiles in blue-veined cheeses (Fox et al., 2000). The VOCs balance differed

304 from that of other known Italian blue-veined cheeses like Gorgonzola. This last has a typical
305 piquant note promoted by the prevailing presence of methyl-ketones (Moio et al. 2000), while
306 esters and alcohols (responsible for a mild flavour) prevailed in Strachitunt. Among these last
307 components, branched-chain and aromatic compounds deriving from amino acid catabolism
308 were the most relevant. These data suggested that metabolic pathways, other than lipolysis,
309 contributed to VOCs formation. It was noticeable that cheese samples I and L obtained by
310 natural starter inoculation resulted less rich in volatiles than samples A–H, although the
311 differences were not significant ($P > 0.05$).

312 PCA was adopted as a means to visualize any relation among VOCs data. Ten cheese
313 samples and 5 chemical classes of VOCs, selected as input variables, were adopted in a
314 correlation matrix. Three PC cumulatively explained 86.1% of the total variability in the volatile
315 profile of the samples analysed. Based on scree plots (eigenvalue > 1), the first 2 PC, explaining
316 72.3% of the overall variance (Figure 3), were adopted. The loading vectors in the PC1 *vs* PC2
317 cartesian diagram ran in all the 4 quadrants of the plot (Figure 3a). The opposite curves
318 suggested that the corresponding variables were negatively correlated. An exception was
319 represented by alcohols and aldehydes with high positive loadings (> 0.5) lying close to each
320 other so that being positively correlated. On the opposite side acids prevailed for negative
321 loading. In relation to PC2, esters and ketones showed the major absolute loadings >|0.6|. The
322 distance among cheese samples in the score plot is proportional to their relationship. A scattered
323 distribution of cheese samples was observed in the score plot (Figure 3b). PC1 distinguished
324 samples C and D for their high levels of alcohols and aldehydes and sample A for large amounts
325 of esters. PC2 differentiated sample G for acids content and sample H for the high level of

ketones. No clear distinction was possible between cheese samples A–H *vs* I–L. Summing up, multivariate analysis evidenced a large variability of the VOCs profile in Strachitunt samples.

CONCLUSIONS

This study reported for the first time the physico-chemical properties, the proteolytic and volatile profiles of Strachitunt cheese. Main features of this Italian PDO cheese were an acid pH of the paste, the low hydrolysis of casein fractions and the abundance of esters and alcohols among VOCs. No significant variations of these parameters could be ascribed to the addition of natural starter. The resulting analytical portrait is characterized by both its distinctiveness in comparison to that of other blue-veined varieties and the large variability of the obtained data. The scarce standardization of this blue-veined cheese was attributed to the erratic adventitious contamination by mould spores of the cheese milk, the dual-curd method of cheese making and the large time variability between the piercing time and the end of ripening. Results obtained represent an added value and a contribution for an extended comprehension of this variety of cheeses.

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Table 1

Composition and physico-chemical parameters of Strachitunt PDO cheeses made during springtime and ripened 75−77 d. A−H, samples starter-free; I−L, samples made with starter inoculation of lactic acid bacteria.

Parameter	A	B	C	D	E	F	G	H	I	L	Mean	SD
DM (g/kg)	522.1^{f}	538.6^{d}	528.6^{e}	547.3^{c}	541.2^{d}	546.3^{c}	538.9^{d}	568.3^{a}	562.2^{b}	536.9^{d}	543.0	14.0
Fat (g/kg DM)	487.3^{d,e}	504.5^{b,c}	512.8^{b}	498.6^{c,d}	497.2^{c,d}	495.1^{c,d}	515.5^{b}	491.5^{d,e}	481.8^{e}	534.7^{a}	501.9	15.6
Protein* (g/kg DM)	401.6^{a,b}	392.2^{b,c}	400.4^{a,b}	399.5^{a,b}	401.9^{a,b}	409.4^{a}	383.8^{c}	384.8^{c}	399.0^{a,b}	399.7^{a,b}	397.2	8.0
Water activity (a_w)	0.960^{b}	0.960^{b}	0.975^{a}	0.957^{b,c}	0.964^{b}	0.947^{c}	0.957^{b,c}	0.963^{a,b}	0.958^{b}	0.956^{b,c}	0.960	0.007
pH	5.22^{e}	5.70^{a,b}	5.62^{b}	5.39^{d}	5.49^{e}	5.48^{c}	5.47^{c}	5.75^{a}	5.08^{f}	5.24^{e}	5.46	0.22
pH 4.4-SN (% TN)	20.26^{a}	19.02^{b}	16.26^{e}	13.38^{i}	14.84^{h}	16.71^{d}	15.24^{g}	17.16^{c}	15.78^{f}	20.26^{a}	16.89	2.31
TCA-SN (% TN)	10.93^{b}	10.57^{b,c}	8.86^{d}	7.87^{e}	8.11^{e}	8.70^{d}	9.00^{d}	8.97^{d}	10.46^{c}	11.59^{a}	9.51	1.28
PTA-SN (% TN)	4.04^{a}	3.50^{b}	2.61^{c}	2.02^{f,g}	1.88^{g}	2.31^{d,e}	2.51^{d}	2.12^{e,f}	4.20^{a}	3.47^{b}	2.87	0.86

* N x 6.38; DM, dry matter; SN: soluble nitrogen; TN: total nitrogen; TCA-SN: soluble nitrogen in trichloroacetic acid;
PTA-SN: soluble nitrogen in phosphotungstic acid.
Each value is the mean of two determinations.
Values with different letters in the same row are statistically different ($P < 0.05$).

Table 2

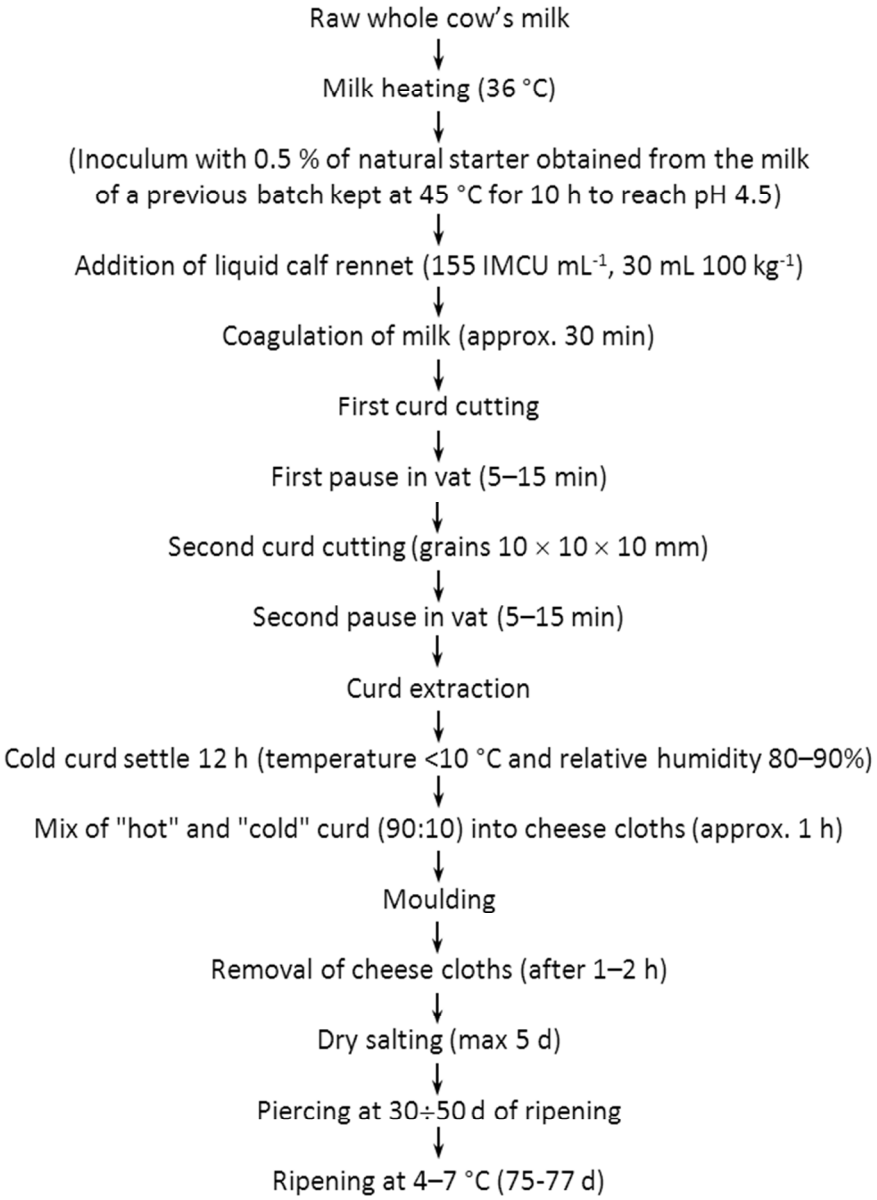
Volatile organic compounds of Strachitunt PDO cheeses made during springtime and ripened 75 d. A-H, samples starter-free; I-L, samples made with starter inoculation of lactic acid bacteria. Data expressed as arbitrary units (x 10^3) of the peak area of Q_{ion} (in brackets).

Chemical class	A	B	C	D	E	F	G	H	I	L	Mean	SD
Aldehydes												
3-methyl butanal (41)	28^{b,c}	42^{b}	210^{a}	195^{a}	27^{b,c}	28^{b,c}	12^{b,c}	11^{b,c}	6^{b,c}	4^{c}	55	76
Alcohols												
ethanol (45)	2529^{b}	804^{d,e}	2431^{b}	9713^{a}	1990^{b,c}	1302^{c,d}	1960^{b,c}	41^{e}	1087^{d}	1309^{c,d}	2317	2708
2-methyl 1-propanol (43)	701^{a}	274^{c}	462^{b}	414^{b}	164^{d}	126^{d,e}	53^{e,f}	102^{d,e,f}	72^{e,f}	45^{f}	241	219
3-methyl 1-butanol (55)	8746^{a}	4682^{b,c}	9120^{a}	7676^{a}	4683^{b,c}	3555^{c,d}	884^{e,f}	5517^{b}	2344^{d,e}	778^{f}	4798	3021
2-heptanol (45)	51^{d}	223^{b,c}	160^{b,c,d}	247^{b}	159^{b,c,d}	220^{b,c}	56^{d}	650^{a}	103^{c,d}	245^{b}	212	171
2-phenyl ethanol (91)	992^{d,e}	1982^{d}	3172^{c}	1283^{d,e}	5875^{a}	4798^{b}	753^{e}	1196^{d,e}	1004^{d,e}	782^{e}	2183	1829
Esters												
ethyl acetate (43)	35379^{a}	2243^{d}	11658^{b}	5168^{c}	1385^{d}	875^{d}	1395^{d}	2^{d}	656^{d}	180^{d}	5894	10939
ethyl butanoate (71)	50^{e}	9^{e}	56^{e}	777^{b}	262^{d,e}	204^{d,e}	1267^{a}	<1^{e}	347^{c,d}	554^{b,c}	347	523
ethyl hexanoate (88)	122^{b}	50^{b}	282^{b}	250^{b}	1220^{b}	1543^{b}	23610^{a}	3660^{b}	102^{b}	1779^{b}	2894	7309
isobutyl acetate (43)	3329^{a}	2515^{b}	2520^{b}	147^{d}	50^{d}	24^{d}	675^{c}	239^{c,d}	11^{d}	5^{d}	951	1301
isoamyl acetate (43)	13745^{b}	18945^{a}	20271^{a}	2793^{d}	888^{d,e}	572^{d,e}	7411^{c}	1010^{d,e}	188^{e}	151^{e}	6597	8086
isoamyl propionate (57)	310^{c}	942^{a}	192^{c,d}	106^{d,e,f}	49^{d,e,f}	40^{e,f}	138^{d,e}	619^{b}	3^{e,f}	<1^{f}	240	309
isoamyl butanoate (71)	112^{d,e}	79^{d,e}	33^{e}	88^{d,e}	1231^{c}	1599^{b}	2042^{a}	66^{d,e}	7^{e}	226^{d}	548	769
isoamyl hexanoate (70)	19^{c,d}	10^{d}	10^{d}	15^{c,d}	210^{b,c}	275^{b}	1028^{a}	13^{c,d}	1^{d}	38^{c,d}	162	319
2-phenylethyl acetate (104)	10053^{a}	5352^{b,c,d}	7198^{a,b}	3139^{c,d,e}	978^{e}	728^{e}	6648^{a,b,c}	2306^{d,e}	65^{e}	76^{e}	3654	3478
2-phenylethyl isobutanoate	20^{b}	86^{b}	122^{b}	291^{b}	4070^{a}	3716^{a}	261^{b}	262^{b}	11^{b}	10^{b}	885	1591
Ketones												
acetone (43)	413^{b}	354^{b,c}	437^{b}	184^{b,c}	321^{b,c}	341^{b,c}	195^{b,c}	759^{a}	123^{c}	92^{c}	322	195
2-butanone (43)	609^{b}	107^{b}	205^{b}	87^{b}	284^{b}	212^{b}	33^{b}	12836^{a}	417^{b}	20^{b}	1481	3994
3-hydroxy 2-butanone (45)	180^{b}	168^{b}	106^{b,c}	101^{b,c}	119^{b,c}	100^{b,c}	95^{b,c}	106^{b,c}	296^{a}	64^{c}	133	67
2-pentanone (43)	408^{b}	350^{b}	266^{b}	984^{b}	1119^{b}	1707^{b}	1634^{b}	9440^{a}	1823^{b}	249^{b}	1798	2755
2-heptanone (43)	476^{d}	1497^{c,d}	640^{d}	3202^{b,c}	3155^{b,c}	4994^{b}	1604^{c,d}	20767^{a}	1593^{c,d}	884^{c,d}	3872	6069
2-nonanone (43)	111^{d}	2110^{b}	184^{d}	1702^{b,c}	852^{c,d}	1320^{b,c,d}	703^{c,d}	8410^{a}	166^{d}	576^{c,d}	1613	2481

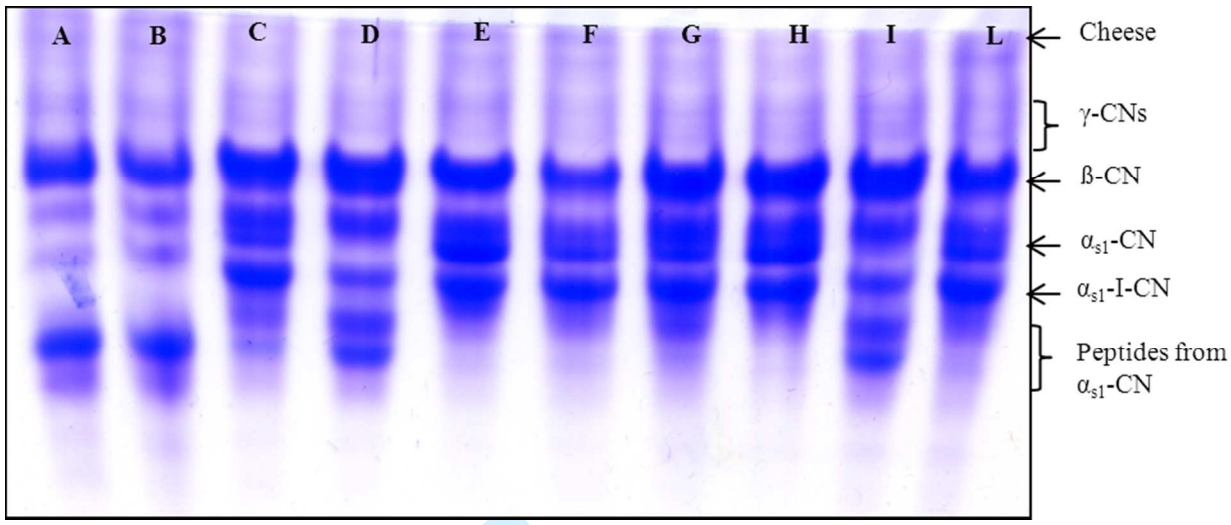
Acids												
acetic acid (60)	1634^{d}	1468^{d}	1045^{d}	833^{d}	2100^{d}	2406^{b,c,d}	3935^{a,b}	2243^{c,d}	4304^{a}	3658^{a,b,c}	2383	1246
2-methyl propanoic acid (43)	461^{c}	427^{c}	1022^{b,c}	772^{b,c}	2981^{a}	2801^{a}	748^{b,c}	2525^{a}	1563^{b}	559^{c}	1386	1014
butanoic acid (60)	10030^{f}	19748^{d,e}	3498^{g}	18320^{e}	25058^{c,d}	30558^{b,c}	55969^{a}	31392^{b}	20749^{d,e}	32465^{b}	24726	14356
3-methyl butanoic acid (60)	3122^{d}	4181^{d}	9538^{b,c}	8810^{b,c}	20025^{a}	17947^{a}	4048^{d}	10027^{b}	7955^{b,c}	7158^{c}	9290	5663
hexanoic acid (60)	11139^{d,e}	14582^{d}	2489^{f}	9184^{d,e,f}	23087^{b,c}	27656^{b}	50178^{a}	27690^{b}	4560^{e,f}	20950^{c}	19094	14141
octanoic acid (60)	1171^{b}	1573^{a,b}	234^{b}	1385^{b}	3171^{a,b}	4520^{a,b}	14215^{a}	7758^{a,b}	430^{b}	4998^{a,b}	3928	4317
decanoic acid (60)	240^{b}	181^{b}	36^{b}	89^{b}	421^{b}	629^{b}	2664^{a}	89^{b}	106^{b}	724^{b}	518	791

SD: Standard deviation. Each value is the mean of two determinations. Values with different letters in the same row are statistically different ($P < 0.05$).

Masotti – Figure 1



Masotti – Figure 2



Masotti – Figure 3

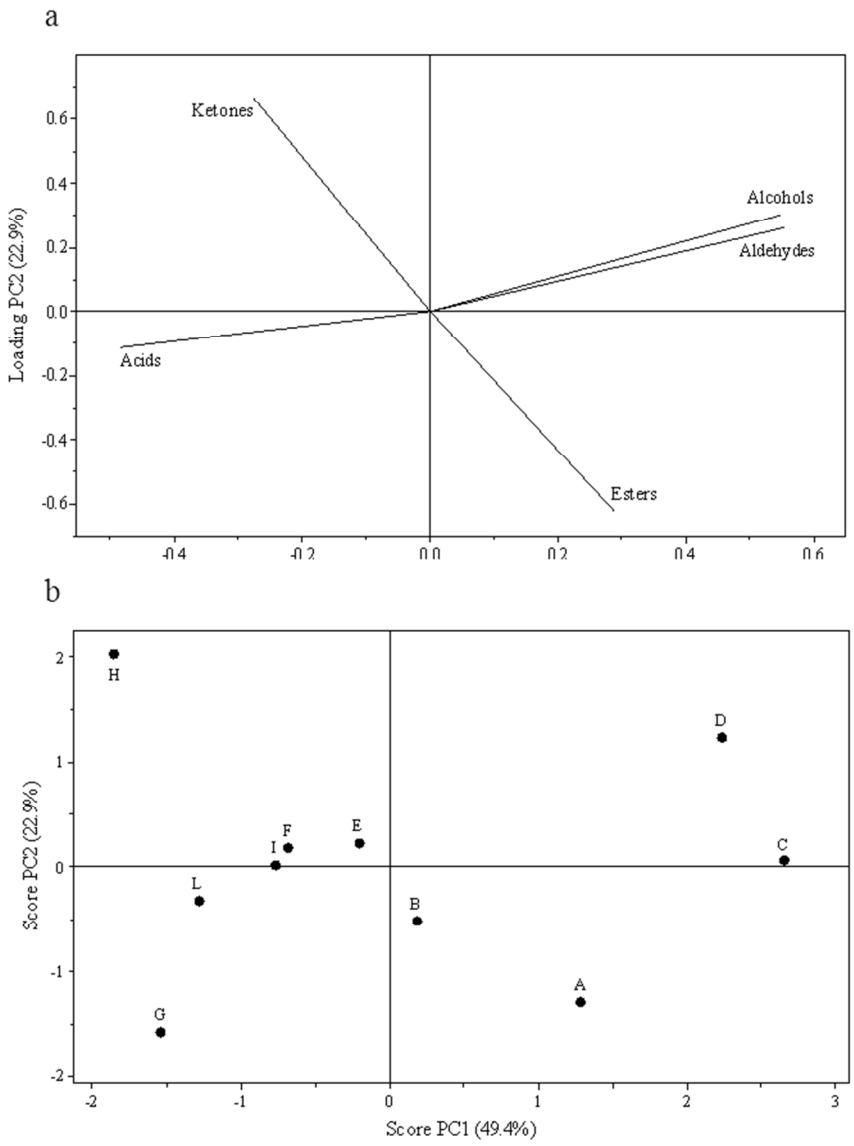


Figure captions

Figure 1

Flowchart for Strachitunt cheese making

Figure 2

Urea-polyacrylammide gel electrophoresis patterns of the pH 4.4-insoluble fraction of Strachitunt cheeses ripened 75 d. Lanes A–H, samples starter-free; lanes I–L, samples made with starter inoculation of lactic acid bacteria. CN, casein.

Figure 3

Loading (a) and score (b) plots of the principal components (PC) 1 and 2 describing the variations among chemical classes of VOCs and among Strachitunt cheese samples, respectively.