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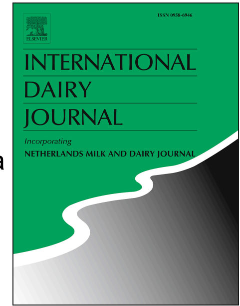

Biochemical changes to milk following treatment by a novel, cold atmospheric plasma system

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1 **Biochemical changes to milk following treatment by a novel, cold atmospheric plasma system**

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25 **Abstract**

26

27 It has previously been shown that non-thermal (i.e., cold) plasma can successfully
28 decontaminate milk from *E. coli*. This study investigated the possible biochemical changes to the
29 protein, free fatty acids and volatiles profiles of whole raw milk samples following application of
30 cold plasma. Raw milk was treated with a cold plasma system at intervals of 0, 3, 6, 9, 12, 15 and
31 20 min. Significant changes were observed for 1 octanol ($P<0.05$), 2 heptanone ($P<0.01$), 2
32 hexenal ($P<0.01$), 2 octenal ($P<0.05$), nonanal and benzaldehyde ($P<0.001$). Plasma treatment did
33 not result in significant changes to the lipid composition of raw milk. However, exposure to cold
34 plasma significantly increased the total aldehyde content following 20 min treatment. No significant
35 difference was observed in the total ketone or alcohol levels.

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38 **1. Introduction**

39

40 The accomplishment of plasma physicists to generate low temperature plasmas at
41 atmospheric pressure has provided a great chance for the application of this phenomenon in areas
42 where 'thermal' or 'hot plasmas' cannot be used. Such areas include the textile, medical and food
43 industries. Cold plasmas have been investigated for their potential in many applications (Fridovitch,
44 1995; Korachi, Turan, Senturk, Sahin, & Aslan, 2009; Laroussi, Alexeff, & Wang, 2000), including
45 sterilisation and decontamination (Gurol, Ekinci, Aslan, & Korachi, 2012; Korachi, Gurol, & Aslan,
46 2010; Korachi et al., 2009; Perni, Liu, Shama, & Kong, 2008; Ragni et al., 2010). The recent
47 advances in cold plasma have allowed scientists to successfully develop many different systems,
48 with parameters that can be adjusted to the material, such as voltage, gas type and temperature
49 (Eliasson & Kogelschatz, 1991).

50 Over the last decade, research in 'cold plasma' at atmospheric pressure has shown
51 decontaminating properties for various materials, including living cells, meat, poultry, milk, water,
52 fresh fruit and vegetables, due to its ability to kill a wide range of microorganisms, including
53 bacteria (*Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus*
54 *mutans, Bacillus subtilis*), yeasts (*Candida albicans*), fungi (*Aspergillus niger*) and green algae
55 (Berardinelli, Vannini, Ragni, & Guerzoni, 2012; Deng et al., 2007; Gurol et al., 2012; Kelly-
56 Wintenberg et al., 1998; Korachi et al., 2009, 2010).

57 The success of such investigations has led to the question of the feasibility of this
58 technology as an alternative processing system to current thermal techniques that can be detrimental
59 to the quality of the food product (Gould, 2000; Korachi et al., 2009). Since consumer demands for
60 more 'natural tasting products' have increased, so has the demand for such cold plasma processing
61 technologies.

62 A previous investigation on the biocidal efficacy of a cold plasma system for the
63 decontamination of liquids has successfully shown its ability to totally eradicate microorganisms

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64 from water (Korachi et al., 2009). Furthermore, the same system with some adjustments has also
65 been investigated for its decontaminating ability on milk contaminated with *E. coli* (Gurol et al.,
66 2012), where a three log cycles decrease was observed.

67 Despite many studies on the decontaminating ability of plasma, there are limited
68 investigations into the effect of cold plasma on the food product(s) itself. This study aims to
69 provide further insight into the possible biochemical changes that may occur following application
70 of plasma on milk.

71

72 **2. Materials and methods**

73

74 *2.1. Milk samples*

75

76 Raw milk samples of Grey Steppe breed (3.5 % fat) were obtained from a local farm in
77 Istanbul, Turkey. Samples were placed on ice until delivered to the laboratory where they were
78 processed immediately. Confirmation of viable microbial growth (colony forming units, cfu) was
79 obtained by inoculating 100 µL of the raw milk sample onto Tryptone Soya Agar (TSA) and violet
80 red bile agar (VRBA) and incubating at 37 °C for 48 h.

81

82 *2.2. Plasma corona discharge setup and application*

83

84 An atmospheric plasma discharge system previously described for testing the
85 decontamination potential of *E. coli* in milk (Gurol et al., 2012) was used in this study. Briefly, the
86 system consisted of a 9 kV AC power supply, two tungsten electrodes (0.8 mm radius) and a simple
87 ballast circuit. A high voltage was applied between the upper electrode tip and the liquid surface.
88 The tip of the electrode was kept at a distance of 8 mm from the milk surface. A current of 90 mA
89 was measured to flow into in the plasma corona and the temperature was kept below 35 °C. Fifteen

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90 mL of milk samples were pipetted into sterile petri dishes (100 mm × 25 mm) and treated with cold
91 plasma for exposure times of 3, 6, 9, 12, 15 or 20 min as previously described (Gurol et al., 2012).
92 Experiments were carried out three times with five replicates per experiment.
93 The light emission intensities of the plasma discharge were determined by way of a UV-
94 visible emission spectrometer and a TCD-1304 Toshiba CCD sensor (Baki, Istanbul, Turkey),
95 manufactured by the Laser Technologies Laboratory of Kocaeli University; Turkey as previously
96 described (Gurol et al., 2012). The optical resolution of the spectrometer was 1.6 nm and slit
97 resolution was 600 lines per mm. The integration time of the data collection was selected to be 10
98 ms for each spectroscopic data. The spectroscopic data was taken by a light falling on the surface of
99 an optical fibre, where the tip of this fibre was kept at 0.7 cm above the milk surface. At this
100 distance, the recorded emissions were not affected by milk surface effects. The emissions were
101 recorded between wavelengths of 350 and 800 nm.
102
103 *2.3. Assessment of lipolysis*
104
105 The presence of free fatty acids (FFAs) in milk samples was determined following plasma
106 application. Lipid extraction was carried out according to the method described by Lopez-Lopez,
107 Castellote-Bargallo, and Lopez-Sabater (2001) with some modifications and standards used as
108 previously described by (Lanciotti et al., 2006). Dichloromethane-methanol (2:1) (Sigma, Munich,
109 Germany) was added to milk and the mixture was mechanically agitated in a shaker (Hotech,
110 Taipei, Taiwan) and then centrifuged at 3000 × *g*. After washing with sterile distilled water, the
111 organic phase was filtered and the solvent was removed in a rotatory evaporator (Heidolph,
112 Schwabach, Germany).
113 Lipids were extracted by methylation using n-hexane (Merck, Darmstadt, Germany) and 2 M
114 potassium hydroxide in methanol (Merck, Darmstadt, Germany). After evaporation under N_2 flux,
115 diazomethane (Sigma, Munich, Germany) was added. Fatty acid methyl ester analysis was carried

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116 out using an Agilent Hewlett-Packard 7890GC gas chromatograph (GC) equipped with a mass
117 spectrometry (MS) detector (Hewlett-Packard 5970 MSD, CA, USA) and a 30 m × 0.32 i.d. fused
118 silica capillary column coated with a 0.2 µm film of Carbowax (Supelco, Bellefonte, CA, USA) as
119 the stationary phase. The identification of the individual FFAs of milk samples was based on the
120 comparison of the retention times of the unknown FFAs with those obtained from the known FFA
121 standards (Sigma). The identification of FFAs was also carried out by computer matching of their
122 mass spectral data with those of the compounds contained in the Agilent Hewlett-Packard NIST 98
123 and Wiley version 6 Mass spectral data base. The quantification of FFA level of milk samples was
124 performed using C11:0 as an internal standard at concentration of 100 µL per 2.5 mg of fatty acid
125 esters.
126
127 *2.4. Identification of volatile compounds*
128
129 Volatile compounds for each milk sample were evaluated by GC-MS/solid-phase micro-
130 extraction (SPME) analysis as previously described (Lanciotti et al., 2006). A divinylbenzene-
131 carboxen-polydimethylsiloxane-coated fibre (65 µm) and a manual SPME holder (Supelco) were
132 used for the SPME of volatile compounds in milk. For peak detection, an Agilent 7890 gas
133 chromatograph (Agilent Technologies, Santa Clara, FL, USA) coupled to an Agilent 5970 mass
134 selective detector was used. This system was operated in electron impact mode with an ionisation
135 voltage of 70 eV. The column used was a Chrompack CP-Wax 52 CB capillary column (50 m ×
136 0.32 mm i.d.; Chrompack, Middetburg, Netherlands). The temperature was adjusted to 50 °C for 2
137 min and then raised 1 °C every minute up to 65 °C and after that 5 °C per min to 220 °C. The
138 temperatures of the injector, interface, and ion source were 250, 250, and 230°C, respectively.
139 Injections were carried out in splitless mode, and the carrier gas was helium with a constant flow
140 rate of 1 mL min^{-1}. Volatile compounds were identified using mass spectra databases
141 (NIST/EPA/NIH version 2005). The quantification of the main volatile compounds was performed

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142 on the basis of calibration curves obtained by adding pure standards to 5 mL of milk samples and
143 prepared as previously described for volatile compound analysis.
144
145 *2.5. Statistical analysis*
146
147 All experiments were repeated three times with 5 replicates per experiment. Data were
148 statistically analysed using the Minitab version 16 statistical software. One-way analysis of
149 variance (ANOVA) was applied to the data to determine significant differences among the different
150 plasma treatment. Tukey test was used for comparison of sample (significant level $P<0.05$).
151
152 **3. Results and discussion**
153
154 Although several studies have described the use of cold plasma for food decontamination
155 purposes, the effect of such applications on the food product itself has not been studied. We have
156 previously reported that, following 3 min of cold plasma application on milk, a significant 54%
157 reduction in the population of *E. coli* was observed, with a minimal effect on the colour and pH of
158 the milk (Gurol et al., 2012). This follow-up study was carried out to determine whether the cold
159 plasma treatment of milk resulted in any changes to the chemical composition of cold plasma
160 treated milk.
161 Light emission intensity spectroscopy results (Fig. 1) showed identical results to those
162 shown in our previous study (Gurol et al., 2012). The peaks observed in the spectrum were
163 identified using the NIST Atomic Spectra Database (Ralchenko, Kramida, & Reader, 2008). The
164 peak locations (A-H in Fig. 1) produced by the discharge were as follows: A, C-II (585.22 nm); B,
165 N-I (670.48 nm); C, O-I (700.19 nm); D, O-II (712.89 nm); E, O-III (749.28 nm); F, N-I (760.88
166 nm); G, N-II (776.22 nm); H, O-III (780.75 nm) where the Latin numbers I, II and III next to the

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167 atomic symbols corresponded to singlet, doublet, and triplet, respectively. The peaks B, D, and G
168 corresponded to maximum intensities of nitrogen and oxygen.

169 The spectra obtained from the tip of the electrode in the air showed peaks identified as
170 oxygen (O), nitrogen (N) and carbon (C). This is to be expected since the plasma system operates in
171 air. It has been suggested that these ions accelerate towards the liquid surface, creating ozone and
172 other active species with very short lifetimes, and thus vastly reducing any toxic effects (Lukes,
173 Clupek, Babicky, Janda, & Sunka, 2005).

174 However, although these ions give rise to an antimicrobial effect, they could also be
175 detrimental to the chemical composition of treated food products, especially those that are high in
176 nutritious value. Milk is such a product, with a complex structure, which is known to be easily
177 affected by processing treatments.

178

179 *3.1. Assessment of lipolysis*

180

181 The effect of cold plasma treatment on the FFA composition of milk is summarised in Table
182 1. Total saturated chain fatty acids (SFAs) detected were between C8:0 – C20:0, monounsaturated
183 fatty acids (MUFAs) were C14:1, C16:1 *cis/trans*, C18:1, C19:1, C20:1 *cis/trans*, and
184 polyunsaturated fatty acids (PUFAs) were C18:2, C20:4 and C20:3.

185 Approximately 64% of FFAs were SFAs and the remainder MUFAs and PUFAs with
186 approximate percentages of 27% and 3%, respectively. The SFA concentration was seen to
187 decrease from 64.4% to 63.6% within the first 3-5 min of plasma application. However, following 5
188 min cold plasma treatment, the total SFAs gradually increased to 65.8% (20 min). Despite these
189 observations, no significant changes were observed in total FFAs concentrations compared with
190 control non-treated samples. Application of cold plasma displayed a larger effect on
191 polyunsaturated fatty acids (PUFAs) in milk, which were seen to decrease from 3.0% to 2.8% after
192 only 3 min treatment and further decreased to 2.5% following 20 min of treatment. Overall, the

193 predominant fatty acids observed pre-plasma application were hexadecanoic acid (C16:0), oleic
194 acid (C18:1) and stearic acid (C18:0), which made up approximately 32%, 24% and 15% of the
195 fatty acid content of the whole milk, respectively.

196 These results are in agreement with previous studies that found C16:0 to be the major fatty
197 acid in milk (Guler, Cakmak, Zengin, & Aktumsek, 2010; Prandini, Sigolo, Tansini, Brogna, &
198 Piva, 2007). The concentration of hexadecanoic acid was seen to decrease from 32% to 30% after 3
199 min application and then increase to 32.5% following further treatment (20 min). Oleic acid was the
200 most abundant MUFA, at approximately 24% in samples before and after plasma treatment. The
201 amount of oleic acid increased from 23.9% to 24.2% and then 24.7% following 3 and 6 min of
202 plasma application, respectively, and then decreased to 23.1% after 20 min. Oleic acid has
203 previously been documented to be predominant MUFA in dairy products from Turkey (Guler et al.,
204 2010; Seckin, Gursoy, Kinik, & Akbulut, 2005). Application of cold plasma for 20 min caused a
205 slight reduction in the amount of stearic acid, from 15.3% to 14.1%. The changes in fatty acids may
206 be attributed the dehydrogenation of stearic acid caused by the oxygen radicals produced during
207 plasma treatment, resulting in an increase in oleic acid. The decrease in oleic acid after 20 min
208 could be indicative of an opposing or reversible reaction produced by the H and OH plasma species.

209 Comparison of levels of C18:0, C12:0, and C10:0 showed C18:0 to decrease, while short-
210 chain fatty acids (C10:0 and C12:0) increased following plasma application. This may suggest that
211 cold plasma treatment results in a hydrolytic effect on long-chain SFAs. Conversely, the free
212 radicals such as hydroperoxyl radicals, superoxide radicals, and singlet oxygen are described as
213 attacking PUFAs (Doroszkiewicz, Sikorska, & Jankowski, 1994) which generate shorter fatty acids
214 (Farr & Kogoma, 1991). On the other hand, the active species formed during plasma discharge can
215 initiate lipid peroxidation and produce hydroperoxide, which may be further converted into
216 secondary oxidation products such as aldehydes or shorter chain fatty acyl compounds (Benedetti,
217 Competi, Fulceri, & Esterbauer, 1984; Kappus, 1985; Mead, 1976). However, further studies are
218 needed to elucidate the observed changes to confirm these assumptions.

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219 Differences between pre- and post-cold-plasma-treated milk samples were detected.
220 However, statistical analysis revealed that the changes to the fatty acid profiles at different exposure
221 times were not significant. This suggests that this cold plasma system does not significantly affect
222 the fatty acid composition of milk for treatment times up to 20 min.

223

224 *3.2. Volatile compounds*

225

226 More than 50 volatile organic compounds (VOCs) were identified in control and plasma
227 treated raw milk samples (Table 2). Ketones, aldehydes, alcohols and to a lesser extent
228 hydrocarbons were detected in all pre and post - treated milk samples.

229 Overall, a significant change was observed in total aldehyde content ($P<0.01$), while no
230 significant changes were observed in the total composition of ketones and alcohols ($P>0.05$). A
231 marked increase was observed in the level of total aldehydes (20.8±5.1 µg) following 20 min cold
232 plasma treatment. Both qualitative and quantitative changes were observed when pre- and post-cold
233 plasma samples were compared. In particular, a significant quantitative change was observed in the
234 presence of 2 hexenal ($P<0.01$) and 2 octenal ($P<0.05$) and a highly significant change was
235 revealed for the presence of nonanal and benzaldehyde ($P<0.001$). The increase in these aldehydes
236 could be attributed to the degradation of several unsaturated fatty acids found in milk, e.g., oleic
237 and linoleic acids (Benedetti et al., 1984; Kappus, 1985; Mead, 1976), by auto-oxidation and/or the
238 spontaneous decomposition of hydroperoxides, which have been found to result in the production of
239 aldehydes (Vazquez-Landaverde, Torres, & Qian, 2006). Such degradation could be a result of the
240 reactive species seen to be produced by plasma.

241 The aldehydes detected by GC/MS-SPME analysis in whole milk following cold plasma
242 were 2-butenal, 2-methyl-propenal, hexanal, heptanal, 2-hexenal, octanal, 2-heptanal, nonanal, 2-
243 octenal, 3-furaldehyde, 3-cyclohexene-1-carboxaldehyde, decanal, 2-nonenal, and benzaldeyde.
244 Aldehydes such as 2-butenal, heptanal, 2-heptanal, 3-cyclohexane-1-carboxaldehyde, and 2-

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245 nonenal, which were not detected in untreated control samples, were observed in post treated cold
246 plasma samples. Other previously present aldehydes increased with exposure time to cold plasma.
247 The observed increase in the content of aldehydes post cold plasma treatment could be due to the
248 detrimental effect of the produced reactive species by the plasma system (N and OH, and NO)
249 (Ragni et al. 2010).

250 The ketones detected in both pre and post plasma treatment were, acetone, 2-butanone, 2-
251 pentanone, methyl-isobutyl ketone, 5-methyl-3-hexanone, 4-methyl-2-hexanone, 4-methyl-3-
252 penten-2-one, 2,6-dimethyl-4-heptanone, 2-heptanone, 2-octanone, 2,5-octanedione, 2-nonanone,
253 3,5-octadien-2-one, and 2-undecanone. Of the ketones, only 2 heptanone was observed to be
254 significantly different (P<0.01) when compared with control, nontreated milk samples. Slight
255 increases were detected in the concentrations of methyl-isobutyl-ketone, 4-methyl-3-penten-2-one,
256 and 2,6-dimethyl-4-heptanone, while a decrease was observed in 4-methyl-2-hexanone, and 2-
257 nonanone compared with nontreated control samples. However, these changes were not found to be
258 statistically significant.

259 Overall, in terms of the total volatile composition, alcohols were found in lower amounts
260 compared with ketones and aldehydes. Ethanol, 2-hexanol, 5-methyl-3-hexanol, 1-octanol and 2-
261 hexanol were observed as the most predominant alcohols in control milk samples. Total alcohol
262 profiles of plasma treated samples displayed an increase in concentration from 0.8 µg before cold
263 plasma application to approximately 2.1 µg post cold plasma treatment (20 min), with a significant
264 change observed in 1 octanol (P<0.05), which increased with increasing exposure time to cold
265 plasma.

266 It is of interest that quantitative changes to 2-butanone, dimethyl sulphide, ethanol and 2-
267 propanone, which are well known to be related to the off-flavour and degeneration of milk (Gordon,
268 & Morgan, 1972; Keller & Kleyn, 1972; Reddy, Bassette, Ward, & Dunham, 1967; Shipe et al.,
269 1962), were found to be not significant in this study, even after 20 min of plasma application.
270

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271 **4. Conclusion**

272

273 A cold plasma corona discharge system previously tested for its decontamination potential
274 of *E. coli* in milk was assessed for its effect on the chemical composition of raw milk. No
275 significant changes were observed to the lipid composition of milk, although significant changes
276 were seen to affect several volatile compounds following cold plasma treatment. Further studies are
277 required to confirm the potential of cold plasma as an alternative technology in milk
278 decontamination.

279

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281

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284

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286

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Figure legend

Fig. 1. Emission spectrum of the plasma discharge on milk surface: A, C-II (585.22 nm); B, N-I (670.48 nm); C, O-I (700.19 nm); D, O-II (712.89 nm); E, O-III (749.28 nm); F, N-I (760.88 nm); G, N-II (776.22 nm); H, O-III (780.75 nm).

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1 **Table 1**

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2 Fatty acid composition of milk treated with the cold plasma system for different treatment times. [a]

Compound	Control	Treatment time (min)					
		3	6	9	12	15	20
C10:0	-	1.97±0.03	0.78±0.16	1.42±0.04	1.33±0.51	2.11±0.17	1.51±0.72
C11:0	0.71±0.56	0.23±0.09	0.48±0.16	0.30±0.18	0.64±0.41	0.35±0.04	0.39±0.25
C12:0	2.57±0.66	3.04±0.62	2.56±0.59	2.86±0.66	2.60±1.12	2.90±0.69	2.89±0.83
C13:0	0.09±0.03	0.12±0.02	0.11±0.02	0.12±0.02	0.11±0.03	0.11±0.02	0.08±0.08
C13 iso	0.20±0.03	0.25±0.01	0.22±0.04	0.24±0.06	0.21±0.03	0.23±0.04	0.22±0.04
C14:1	1.05±0.17	1.23±0.05	1.18±0.05	1.19±0.14	1.20±0.13	1.15±0.12	1.15±0.20
C14:0	10.78±0.80	11.41±0.41	11.28±0.45	11.45±0.55	11.57±0.55	11.16±0.42	11.93±0.87
i-C14:0	0.39±0.03	0.47±0.02	0.44±0.05	0.45±0.07	0.44±0.03	0.43±0.06	0.42±0.06
a-C14:0	0.85±0.05	1.00±0.06	0.95±0.10	0.96±0.16	0.96±0.06	0.93±0.15	0.92±0.11
C15:0	1.73±0.06	1.87±0.04	1.84±0.06	1.76±0.03	1.80±0.13	1.76±0.15	1.76±0.20
C15:0 iso	0.51±0.02	0.58±0.03	0.54±0.08	0.57±0.08	0.54±0.06	0.54±0.08	0.52±0.07
C16:1 (trans-Δ^9)	0.12±0.01	0.14±0.02	0.14±0.01	0.13±0.02	0.13±0.02	0.13±0.02	0.12±0.03
C16:1(cis-Δ^9)	1.91±0.04	1.94±0.04	1.95±0.02	1.96±0.10	1.93±0.06	1.88±0.06	1.85±0.22
C16:1 (Δ^{11})	0.03±0.01	0.03±0.01	0.03±0.01	0.03±0.00	0.03±0.01	0.03±0.01	0.03±0.00
C16:0	31.97±1.29	29.98±0.71	30.92±1.65	31.30±0.46	31.50±0.88	30.64±0.98	32.52±1.95
C16:0 iso	0.56±0.02	0.64±0.04	0.61±0.06	0.63±0.09	0.60±0.05	0.59±0.09	0.55±0.06
C16:0 ante	0.82±0.02	0.95±0.08	0.91±0.11	0.93±0.16	0.91±0.11	0.87±0.15	0.80±0.06
C17:0	1.04±0.03	1.13±0.04	1.11±0.07	1.11±0.12	1.06±0.08	1.06±0.11	0.99±0.12
C18:2	2.53±0.11	2.26±0.14	2.39±0.26	2.37±0.12	2.40±0.04	2.41±0.19	2.16±0.40
C18:1 (cis-Δ^9)	23.90±0.70	24.18±3.40	24.66±3.18	23.30±0.64	23.11±1.26	23.26±1.23	23.12±2.20
C18:1 (trans-Δ^9)	0.36±0.03	0.33±0.02	0.25±0.22	0.34±0.03	0.33±0.06	0.35±0.03	0.34±0.04
C18:1 (Δ^{11})	1.27±0.01	1.04±0.07	1.07±0.09	1.17±0.09	1.12±0.16	1.17±0.17	1.16±0.14
C18:0	15.31±0.39	13.99±0.89	14.58±1.06	14.77±0.68	14.38±0.63	14.62±0.96	14.06±1.44
C19:1	0.12±0.02	0.15±0.02	0.14±0.03	0.15±0.02	0.14±0.01	0.14±0.02	0.13±0.00
C19:0	0.08±0.01	0.13±0.04	0.11±0.01	0.11±0.02	0.09±0.02	0.10±0.03	0.10±0.00
C20:4	0.17±0.03	0.18±0.01	0.15±0.03	0.15±0.03	0.14±0.03	0.16±0.03	0.17±0.01
C20:3	0.13±0.01	0.13±0.01	0.12±0.01	0.11±0.02	0.10±0.01	0.12±0.01	0.12±0.01
C20:1 n9 (cis 11)	0.16±0.03	0.19±0.01	0.18±0.02	0.17±0.02	0.16±0.02	0.18±0.03	0.17±0.03
C20:0	0.26±0.03	0.27±0.01	0.28±0.03	0.26±0.04	0.24±0.01	0.29±0.03	0.26±0.03
Total SFA	64.37±0.56	63.58±3.36	63.79±2.60	64.51±0.85	64.88±1.76	64.80±0.94	65.75±1.57
Total MUFA	27.28±0.53	27.84±3.36	28.26±3.23	26.92±0.59	26.68±1.20	26.77±1.04	26.44±1.75
Total PUFA	3.00±0.11	2.75±0.15	2.88±0.22	2.84±0.11	2.82±0.05	2.89±0.16	2.47±0.67

3

4 [a] Data (μg g^{-1}) are means ± standard deviation; means in the same row were not significantly different (*P*

5 >0.05); -, not detected.

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9 **Table 2**

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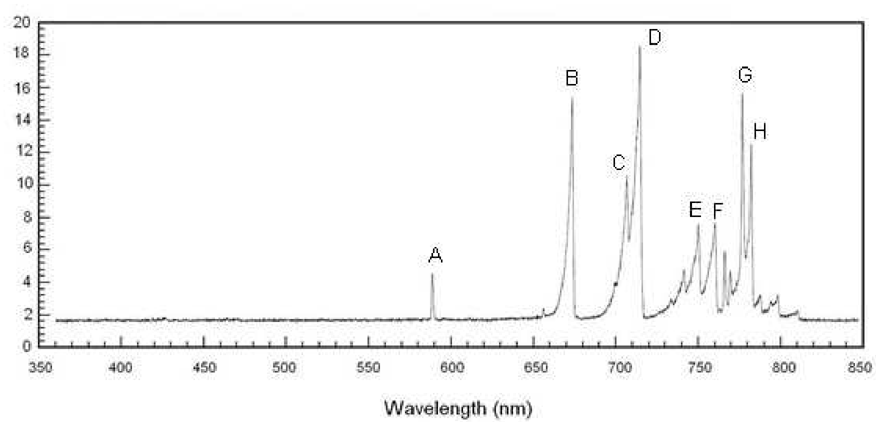
10 Volatile organic compounds (with retention times, RT) detected in whole milk before and after plasma treatment. [a]

Volatile compound	RT (min)	Control	Treatment time (min)					
			3	6	9	12	15	20 min
Octane	4.278	0.29±0.12	-	0.16±0.04	0.23±0.12	0.24±0.18	0.09±0.02	0.13±0.02
Acetone	4.761	0.43±0.08	0.30±0.16	0.31±0.16	0.13±0.04	0.19±0.09	0.24±0.10	0.27±0.16
1-Octene	5.036	0.12±0.09	-	-	-	0.19±0.06	0.29±0.09	0.20±0.04
2-Methylpropenal	6.062	-	-	-	-	0.14±0.01	0.28±0.08	0.20±0.03
2-Butanone	6.463	0.06±0.03	0.11±0.08	0.11±0.06	-	0.12±0.10	0.16±0.08	0.12±0.05
Ethanol	7.173	0.21±0.15	0.37±0.17	0.45±0.11	0.90±0.47	0.95±0.57	0.80±0.13	1.07±0.40
2 Pentanone	8.333	0.70±0.28	0.89±0.65	0.41±0.20	0.32±0.02	0.40±0.22	0.70±0.69	0.78±0.88
Methyl-isobutyl-ketone	8.973	0.27±0.09	0.26±0.12	0.45±0.17	0.40±0.04	0.44±0.04	0.40±0.10	0.47±0.14
1-Decene	9.515	-	-	-	-	-	0.31±0.12	0.50±0.28
2-Butenal	9.869	-	0.07±0.04	0.29±0.36	0.08±0.02	0.11±0.11	0.14±0.08	0.19±0.18
5-Methyl-3-hexanone	10.461	0.43±0.21	0.39±0.21	0.32±0.03	0.29±0.01	0.28±0.08	0.22±0.08	0.25±0.00
Hexanal	10.623	2.12±1.82	1.25±0.68	1.46±0.65	1.55±0.56	1.51±0.26	1.58±0.46	2.85±0.94
4-Methyl-2-hexanone	11.424	1.13±0.42	0.72±0.26	0.79±0.06	0.67±0.04	0.85±0.37	0.64±0.15	0.83±0.50
Ethyl-benzene	11.634	0.30±0.21	-	0.24±0.04	0.25±0.14	0.37±0.22	0.52±0.34	0.79±0.47
4-Methyl-3-penten-2-one	11.783	3.45±0.95	2.60±1.85	4.61±0.44	3.86±0.93	4.77±1.07	4.11±0.15	3.45±0.44
2,6-Dimethyl-4-heptanone	12.417	4.00±2.43	2.54±2.17	3.88±1.35	3.17±1.56	4.20±1.88	4.10±0.37	3.76±1.50
2-Heptanone**	12.664	1.96±0.82	0.84±0.52[a]	0.63±0.42[a]	0.42±0.07[a]	0.27±0.23[a]	0.74±0.00[a]	0.15±0.02[a]
Heptanal	12.724	-	-	0.27±0.22	0.25±0.13	0.56±0.40	0.59±0.48	0.93±0.26
2-Hexanol	13.098	1.17±1.31	0.70±0.63	0.44±0.11	0.42±0.12	0.52±0.10	0.46±0.11	0.36±0.21
5-Methyl-3-hexanol	13.350	0.13±0.08	0.15±0.07	0.18±0.03	0.15±0.03	0.19±0.03	0.21±0.01	0.10±0.14
2-Hexenal**	13.464	0.78±0.28	0.52±0.29	0.31±0.05	0.64±0.28	0.55±0.08	0.62±0.15	1.88±0.61[a]
Cloro 1 octane	13.808	0.48±0.57	0.21±0.09	0.15±0.07	0.20±0.06	0.27±0.31	0.29±0.19	0.55±0.25
Styrene	14.157	0.17±0.10	0.16±0.06	0.47±0.37	0.29±0.24	0.69±0.28	1.75±0.90	0.89±1.05
2,2,4,6,6-Pentamethyl-3-Heptene	14.231	0.60±0.78	-	-	-	-	-	-
2-Octanane	14.478	-	-	-	-	0.07±0.01	0.09±0.04	0.15±0.04
Octanal	14.558	0.50±0.40	0.64±0.78	0.51±0.46	0.43±0.30	0.54±0.37	0.77±0.44	1.29±0.75
2,5-Octanedione	14.974	-	0.15±0.15	-	-	-	-	-
2-Heptanal	15.270	-	0.33±0.45	0.07±0.01	0.08±0.00	0.06±0.03	0.11±0.07	0.33±0.41
Tetradecane	15.904	0.12±0.10	0.12±0.08	0.04±0.01	0.07±0.01	0.11±0.04	0.09±0.02	0.12±0.02
2-Nonanone	16.127	0.72±0.42	0.35±0.06	0.42±0.28	0.39±0.08	0.32±0.02	0.29±0.09	0.34±0.04
Nonanal***	16.223	0.68±0.14	3.49±2.05	1.14±0.13	1.68±0.40	3.26±1.42	2.17±0.33	8.80±0.61[a]
2-Octenal*	16.914	0.07±0.04	2.26±3.83[a]	-	0.24±0.12	0.26±0.07	0.25±0.10	0.56±0.69
3 Furaldehyde	17.377	0.38±0.23	0.74±0.69	0.24±0.17	0.29±0.20	0.34±0.30	0.56±0.22	0.76±0.53
3-Cyclohexene-1-carboxaldehyde	17.558	-	-	0.05±0.01	0.07±0.02	0.11±0.08	0.18±0.08	0.13±0.03
Decanal	17.748	0.20±0.13	0.20±0.17	0.15±0.05	0.20±0.02	0.17±0.08	0.27±0.16	0.29±0.14
1-Octanol*	18.297	0.23±0.21	0.17±0.17	0.13±0.11	0.18±0.07	0.15±0.08	0.20±0.07	0.62±0.18[a]
2-Nonenal	18.386	-	0.27±0.35	0.04±0.01	0.04±0.02	0.04±0.02	0.06±0.02	0.24±0.27
Benzaldehyde***	18.446	0.21±0.16	0.29±0.06	0.46±0.23	0.48±0.18	0.66±0.16	1.45±0.50[a]	1.04±0.08[b]
Nonadecane	18.736	1.25±0.77	0.21±0.03	0.38±0.23	0.30±0.14	0.26±0.23	0.43±0.46	0.28±0.36
2-Undecanone	19.082	0.18±0.12	0.12±0.03	0.10±0.08	0.09±0.04	0.08±0.02	0.11±0.09	0.09±0.00
Total ketones		12.02±2.26	11.17±0.87	11.66±1.49	9.65±2.62	12.02±2.70	10.60±0.77	10.49±1.33
Total aldehydes**		7.30±0.56	3.34±1.24	5.84±2.90	6.75±2.90	7.25±2.93	7.60±0.01	20.79±5.13[a]
Total alcohols		0.80±0.23	1.45±0.40	1.19±0.17	1.70±0.59	1.83±0.63	1.68±0.25	2.06±0.53

11 [a] Data (µg) are means ± standard deviation (-, not detected); means followed by the different superscript letter within the same

12 row are significantly different at a level indicated after the compound name: **P*<0.05; ***P*<0.01; ****P*<0.001.



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