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

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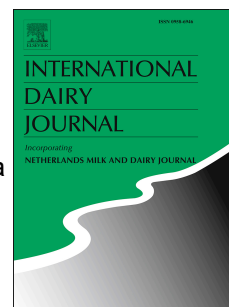
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**Biochemical changes to milk following treatment by a novel, cold atmospheric plasma system**

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**Abstract**

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

## 1. Introduction

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

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

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

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

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

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

## 2. Materials and methods

### 2.1. Milk samples

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

### 2.2. Plasma corona discharge setup and application

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

mL of milk samples were pipetted into sterile petri dishes (100 mm × 25 mm) and treated with cold plasma for exposure times of 3, 6, 9, 12, 15 or 20 min as previously described (Gurol et al., 2012). Experiments were carried out three times with five replicates per experiment.

The light emission intensities of the plasma discharge were determined by way of a UV-visible emission spectrometer and a TCD-1304 Toshiba CCD sensor (Baki, Istanbul, Turkey), manufactured by the Laser Technologies Laboratory of Kocaeli University; Turkey as previously described (Gurol et al., 2012). The optical resolution of the spectrometer was 1.6 nm and slit resolution was 600 lines per mm. The integration time of the data collection was selected to be 10 ms for each spectroscopic data. The spectroscopic data was taken by a light falling on the surface of an optical fibre, where the tip of this fibre was kept at 0.7 cm above the milk surface. At this distance, the recorded emissions were not affected by milk surface effects. The emissions were recorded between wavelengths of 350 and 800 nm.

### 2.3. *Assessment of lipolysis*

The presence of free fatty acids (FFAs) in milk samples was determined following plasma application. Lipid extraction was carried out according to the method described by Lopez-Lopez, Castellote-Bargallo, and Lopez-Sabater (2001) with some modifications and standards used as previously described by (Lanciotti et al., 2006). Dichloromethane-methanol (2:1) (Sigma, Munich, Germany) was added to milk and the mixture was mechanically agitated in a shaker (Hotech, Taipei, Taiwan) and then centrifuged at  $3000 \times g$ . After washing with sterile distilled water, the organic phase was filtered and the solvent was removed in a rotatory evaporator (Heidolph, Schwabach, Germany).

Lipids were extracted by methylation using n-hexane (Merck, Darmstadt, Germany) and 2 M potassium hydroxide in methanol (Merck, Darmstadt, Germany). After evaporation under  $N_2$  flux, diazomethane (Sigma, Munich, Germany) was added. Fatty acid methyl ester analysis was carried



out using an Agilent Hewlett-Packard 7890GC gas chromatograph (GC) equipped with a mass spectrometry (MS) detector (Hewlett-Packard 5970 MSD, CA, USA) and a 30 m  $\times$  0.32 i.d. fused silica capillary column coated with a 0.2  $\mu$ m film of Carbowax (Supelco, Bellefonte, CA, USA) as the stationary phase. The identification of the individual FFAs of milk samples was based on the comparison of the retention times of the unknown FFAs with those obtained from the known FFA standards (Sigma). The identification of FFAs was also carried out by computer matching of their mass spectral data with those of the compounds contained in the Agilent Hewlett-Packard NIST 98 and Wiley version 6 Mass spectral data base. The quantification of FFA level of milk samples was performed using C11:0 as an internal standard at concentration of 100  $\mu$ L per 2.5 mg of fatty acid esters.

#### 2.4. Identification of volatile compounds

Volatile compounds for each milk sample were evaluated by GC-MS/solid-phase micro-extraction (SPME) analysis as previously described (Lanciotti et al., 2006). A divinylbenzene-carboxen-polydimethylsiloxane-coated fibre (65  $\mu$ m) and a manual SPME holder (Supelco) were used for the SPME of volatile compounds in milk. For peak detection, an Agilent 7890 gas chromatograph (Agilent Technologies, Santa Clara, FL, USA) coupled to an Agilent 5970 mass selective detector was used. This system was operated in electron impact mode with an ionisation voltage of 70 eV. The column used was a Chrompack CP-Wax 52 CB capillary column (50 m  $\times$  0.32 mm i.d.; Chrompack, Middelburg, Netherlands). The temperature was adjusted to 50  $^{\circ}$ C for 2 min and then raised 1  $^{\circ}$ C every minute up to 65  $^{\circ}$ C and after that 5  $^{\circ}$ C per min to 220  $^{\circ}$ C. The temperatures of the injector, interface, and ion source were 250, 250, and 230 $^{\circ}$ C, respectively. Injections were carried out in splitless mode, and the carrier gas was helium with a constant flow rate of 1 mL min<sup>-1</sup>. Volatile compounds were identified using mass spectra databases (NIST/EPA/NIH version 2005). The quantification of the main volatile compounds was performed

on the basis of calibration curves obtained by adding pure standards to 5 mL of milk samples and prepared as previously described for volatile compound analysis.

### 2.5. Statistical analysis

All experiments were repeated three times with 5 replicates per experiment. Data were statistically analysed using the Minitab version 16 statistical software. One-way analysis of variance (ANOVA) was applied to the data to determine significant differences among the different plasma treatment. Tukey test was used for comparison of sample (significant level  $P < 0.05$ ).

## 3. Results and discussion

Although several studies have described the use of cold plasma for food decontamination purposes, the effect of such applications on the food product itself has not been studied. We have previously reported that, following 3 min of cold plasma application on milk, a significant 54% reduction in the population of *E. coli* was observed, with a minimal effect on the colour and pH of the milk (Gurol et al., 2012). This follow-up study was carried out to determine whether the cold plasma treatment of milk resulted in any changes to the chemical composition of cold plasma treated milk.

Light emission intensity spectroscopy results (Fig. 1) showed identical results to those shown in our previous study (Gurol et al., 2012). The peaks observed in the spectrum were identified using the NIST Atomic Spectra Database (Ralchenko, Kramida, & Reader, 2008). The peak locations (A-H in Fig. 1) produced by the discharge were as follows: 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) where the Latin numbers I, II and III next to the

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

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

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

### 3.1. Assessment of lipolysis

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

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

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

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

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

Differences between pre- and post-cold-plasma-treated milk samples were detected.

However, statistical analysis revealed that the changes to the fatty acid profiles at different exposure times were not significant. This suggests that this cold plasma system does not significantly affect the fatty acid composition of milk for treatment times up to 20 min.

### 3.2. Volatile compounds

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

Overall, a significant change was observed in total aldehyde content ( $P<0.01$ ), while no significant changes were observed in the total composition of ketones and alcohols ( $P>0.05$ ). A marked increase was observed in the level of total aldehydes ( $20.8\pm5.1\text{ }\mu\text{g}$ ) following 20 min cold plasma treatment. Both qualitative and quantitative changes were observed when pre- and post-cold plasma samples were compared. In particular, a significant quantitative change was observed in the presence of 2 hexenal ( $P<0.01$ ) and 2 octenal ( $P<0.05$ ) and a highly significant change was revealed for the presence of nonanal and benzaldehyde ( $P<0.001$ ). The increase in these aldehydes could be attributed to the degradation of several unsaturated fatty acids found in milk, e.g., oleic and linoleic acids (Benedetti et al., 1984; Kappus, 1985; Mead, 1976), by auto-oxidation and/or the spontaneous decomposition of hydroperoxides, which have been found to result in the production of aldehydes (Vazquez-Landaverde, Torres, & Qian, 2006). Such degradation could be a result of the reactive species seen to be produced by plasma.

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

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

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

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

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

#### 4. Conclusion

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

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#### References

- Benedetti, A., Competi, M., Fulceri, R., & Esterbauer, H. (1984). Cytotoxic aldehydes originating from the peroxidation of liver microsomal lipids, *Biochimica et Biophysica Acta*, 192, 172-181.
- Berardinelli, A., Vannini, L., Ragni, L., & Guerzoni, M. E. (2012). Impact of atmospheric plasma generated by a dbd device on quality-related attributes of "abate fetel" pear fruit. In Z. Machala et al. (Eds.), *Plasma for bio-decontamination, medicine and food security. NATO science for peace and security series A: Chemistry and biology* (pp. 457-467). New York, NY, USA: Springer Science and Business Media.
- Deng, S., Ruan, R., Mok, C., Huang, G., Lin X., & Chen, P. (2007). Inactivation of *Escherichia coli* on almonds using nonthermal plasmas. *Journal of Food Science*, 72, 62-66.



- 297 Doroszkiewicz, W., Sikorska, I., & Jankowski, S. (1994). Studies of the influence of ozone on  
 298 complement-mediated killing of bacteria. *FEMS Immunological and Medical Microbiology*,  
 299 9, 281–285.
- 300 Eliasson, B., & Kogelschatz, U. (1991). Nonequilibrium volume plasma chemical processing. *IEEE*  
 301 *Transactions on Plasma Science*, 19, 1063–1077.
- 302 Farr, S. B., & Kogoma, T. (1991). Oxidative stress responses in *Escherichia coli* and *Salmonella*  
 303 *typhimurium*. *Microbiology Reviews*, 55, 561–585.
- 304 Fridovitch, I. (1995). Superoxide radicals and superoxide dismutases. *Annual Reviews of*  
 305 *Biochemistry*, 64, 97-112.
- 306 Gordon, D. T., & Morgan, M. E. (1972). Principal volatile compounds in feed flavored milk.  
 307 *Journal of Dairy Science*, 55, 905–912.
- 308 Gould, G. W. (2000). New and emerging physical methods of preservation. In B. Lund, T. C.  
 309 Baird-Parker, & G. W. Gould (Eds.) *The microbiological safety and quality of food* (pp.  
 310 277-293). Gaithersburg, MD, USA: Aspen Publishers.
- 311 Guler, G. O., Cakmak, Y. S., Zengin, G., & Aktumsek, A. (2010). Fatty acid composition and  
 312 conjugated linoleic acid (CLA) content of some commercial milk in Turkey. *Kafkas*  
 313 *Universitesi Veterinerlik Fakültesi Dergisi*, 16, 37-40.
- 314 Gurol, C., Ekinci, F. Y., Aslan, N., & Korachi, M. (2012). Low temperature plasma for  
 315 decontamination of *E. coli* in milk. *International Journal of Food Microbiology*, 157, 1-5.
- 316 Kappus, H. (1985). Lipid peroxidation: mechanisms, analysis, enzymology and biological  
 317 relevance, In H. Sies (Ed.) *Oxidative stress* (pp. 273-310). New York, NY, USA: Academic  
 318 Press.
- 319 Keller, W. J., & Kleyn, D. H., Jr. (1972). Headspace gas chromatography for objectively  
 320 determining intensity of flavor in raw milk. *Journal of Dairy Science*, 55, 574–576.
- 321 Kelly-Wintenberg, K., Montie, T. C., Brickman, C., Roth, J. R., Carr, A. K., Sorge, K., et al.  
 322 (1998). Room temperature sterilization of surfaces and fabrics with a one atmosphere



uniform glow discharge plasma. *Journal of Industrial Microbiology and Biotechnology*, 20, 69-74.

Korachi, M., Gurol, C., & Aslan, N. (2010). Atmospheric plasma discharge sterilization effects on whole cell fatty acid profiles of *Escherichia coli* and *Staphylococcus aureus*. *Journal of Electrostatics*, 68, 508-512.

Korachi, M., Turan, Z., Senturk, K., Sahin, F., & Aslan, N. (2009). An investigation into the biocidal effect of high voltage AC/DC atmospheric corona discharges on bacteria, yeasts, fungi and algae. *Journal of Electrostatics*, 67, 678–685.

Lanciotti, R., Vannini, L., Patrignani, F., Iucci, L., Vallicelli, M., Ndagijimana, M., et al. (2006). Effect of high pressure homogenisation of milk on cheese yield and microbiology, lipolysis and proteolysis during ripening of Caciotta cheese. *Dairy Research*, 73, 216-26.

Laroussi, M., Alexeff, I., & Wang, W. L. (2000). Biological decontamination by nonthermal plasma. *IEEE Transactions on Plasma Science*, 28, 184–188.

Lopez-Lopez, A., Castellote-Bargallo, A. I., & Lopez-Sabater, M. C. (2001). Comparison of two direct methods for the determination of fatty acids in human milk. *Chromatographia*, 54, 743-747.

Lukes, P., Clupek, M., Babicky, V., Janda, V., & Sunka, P. (2005) Generation of ozone by pulsed corona discharge over water surface in hybrid gas–liquid electrical discharge reactor. *Journal of Physics D: Applied Physics*, 38, 409-416.

Mead, J. (1976). Free radical mechanisms of lipid damage and consequences for cellular membranes. In W. A. Pryor (Ed.) *Free radicals in biology* (pp. 51-68). New York, NY, USA: Academic Press.

Perni, S., Liu, D. W., Shama, G., & Kong, M. (2008). Cold atmospheric plasma decontamination of the pericarps of fruit. *Journal of Food Protection*, 71, 302–308.

- 347 Prandini, A., Sigolo, S., Tansini, G., Brogna, N., & Piva, G. (2007). Different level of conjugated  
348 linoleic acid (CLA) in dairy products from Italy. *Journal of Food Composition Analysis*, 20,  
349 472-479.
- 350 Ragni, L., Berardinelli, A., Vannini, L., Montanari, C., Sirri, F., Guerzoni, M. E., et al. (2010). Non-  
351 thermal atmospheric plasma device for surface decontamination of shell eggs. *Journal of*  
352 *Food Engineering*, 100, 125-132.
- 353 Ralchenko, Yu., Kramida, A. E., Reader, J. (2008). NIST atomic spectra database (Ver. 3.1.5).  
354 Gaithersburg, MD, USA: National Institute of Standards and Technology  
355 <http://physics.nist.gov/asd3>.
- 356 Reddy, M. C., Bassette, R., Ward, G., & Dunham, J. R. (1967). Relationship of methyl sulfide and  
357 flavor score of milk. *Journal of Dairy Science*, 50, 147-150.
- 358 Seckin, A. K., Gursoy, O., Kinik, O., & Akbulut, N. (2005). Conjugated linoleic acid (CLA)  
359 concentration, fatty acid composition and cholesterol content of some Turkish dairy  
360 products. *Food Science and Technology*, 38, 909-915.
- 361 Shipe, W. F., Ledford, R. A., Peterson, R. D., Scanlon, R. A., Geerken, H. F., Dougherty, R. W., et  
362 al. (1962). Physiological mechanism involved in transmitting flavors and odors to milk. II.  
363 Transmission of some flavor components of silage. *Journal of Dairy Science*, 45, 477-480.
- 364 Vazquez-Landaverde, P. A., Torres, J. A., & Qian, M. C. (2006). Effect of high-pressure-moderate  
365 temperature processing on the volatile profile of milk. *Journal of Agricultural and Food*  
366 *Chemistry*, 54, 9184-9192.

**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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**Table 1**

Fatty acid composition of milk treated with the cold plasma system for different treatment times.<sup>a</sup>

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 <sup>9</sup> )	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 <sup>9</sup> )	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 <sup>11</sup> )	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 <sup>9</sup> )	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 <sup>9</sup> )	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 <sup>11</sup> )	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

<sup>a</sup> Data (μg g<sup>-1</sup>) are means ± standard deviation; means in the same row were not significantly different (*P* >0.05); -, not detected.

**Table 2**

ACCEPTED MANUSCRIPT

Volatile organic compounds (with retention times, RT) detected in whole milk before and after plasma treatment. <sup>a</sup>

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 <sup>a</sup>	0.63±0.42 <sup>a</sup>	0.42±0.07 <sup>a</sup>	0.27±0.23 <sup>a</sup>	0.74±0.00 <sup>a</sup>	0.15±0.02 <sup>a</sup>
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 <sup>a</sup>
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 <sup>a</sup>
2-Octenal*	16.914	0.07±0.04	2.26±3.83 <sup>a</sup>	-	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 <sup>a</sup>
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 <sup>a</sup>	1.04±0.08 <sup>b</sup>
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 <sup>a</sup>
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

<sup>a</sup> Data (µg) are means ± standard deviation (-, not detected); means followed by the different superscript letter within the same

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

