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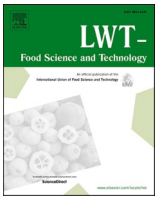

The effects of cold plasma technology on physical, nutritional, and sensory properties of milk and milk products

Nooshin Nikmaram *, Kevin M. Keener

University of Guelph, School of Engineering, Guelph, Ontario, Canada

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ABSTRACT

Thermal processes such as high-temperature-short-time (HTST) are utilized in the dairy industry to maintain milk safety and shelf life. However, thermal processes while ensuring milk safety can negatively impact milk quality including protein denaturation, non-enzymatic browning, loss of vitamins, and flavor changes. Cold plasma is a rapid and non-thermal technique that can pasteurize milk to ensure food safety while maintaining the milk quality. Recent papers have highlighted the advantages of cold plasma treatment on reducing bacteria and their enzymes in raw milk and dairy products; however, these reviews lack examination of cold plasma's impact on milk and dairy product quality. This research review focuses on the effects of cold plasma treatment on quality of milk and dairy products. Reported quality information includes viscosity, color, particle size, fat, protein, lactose, as well as sensory properties. Cold plasma processes can be optimized to maximize bacterial reduction without significantly impacting milk and dairy product quality; however, non-optimized cold plasma processes, while effective in achieving significant microbial reduction, can result in product quality losses, including lipid oxidation, protein aggregation, and off-flavor. Thus, future cold plasma research studies need to consider microbial inactivation in parallel with quality impact and consumer sensory.

1. Introduction

Cow's milk is readily consumed throughout the world. The composition of cow's milk is comprised of approximately, 870 g/L water, 490 g/L lactose, 340 g/L fat, 33 g/L proteins and 7 g/L vitamins and minerals. The food safety of milk is generally preserved by thermal processing (pasteurization and sterilization), fermentation, and culturing (cheese making) which also provides extended shelf-life (Kelly & Meena, 2020).

Thermal processing is used to ensure food safety and shelf-life of dairy products for the past 100 years. Pasteurization conditions include either not less than 62.8 °C or more than 65.6 °C for at least 30 min or not less than 71.7 °C for at least 15 s (High-temperature-short-time, HTST) (Juffs & Deeth, 2007). There are two sterilization processes to produce milk with a long shelf life at room temperature. 'Canned' (Sterilized) milk is processed by in-container sterilization at 115–120 °C for 10–20 min. Alternatively, ultra heat temperature (UHT) milk is processed at 135–150 °C for only a few seconds in a continuous flow process and aseptically filled into sterile containers.

Unfortunately, traditional pasteurization or sterilization methods significantly impact milk quality resulting in non-enzymatic browning, loss of vitamins, and flavour (Coutinho et al., 2018). Alternatively, consumers are seeking less processed and "fresher" foods including dairy products that retain a maximum nutritional content while maintaining a high level of safety and long shelf-life without "cooking". In this regard, novel food preservation technologies that can inactivate microorganisms and enzymes in food with minimal heating and are gaining interest. These novel methods include high hydrostatic pressures, pulsed electric fields, ultrasounds, and cold atmospheric plasma (Kim, Lee, Choi, & Kim, 2014).

Plasma is an ionized gas composed of ions, free electrons, atoms and molecules. For discharge to open-air atmospheres, the reactive oxygen species (ROS) and reactive nitrogen species (RNS) are the most important active plasma agents formed. Table 1 indicates an overview of created species during cold plasma treatment. These species are extensively evaluated for their anti-microbial effects against a range of microorganisms, including bacteria, molds, and yeasts (López et al., 2019). Half-life which is the time taken for the concentration of a reactant to

* Corresponding author. University of Guelph, School of Engineering, College of Engineering and Physical Sciences, Albert Thornbrough Building, Rm 3107, 50 Stone Road East, Guelph, ON, NIG 2W1, Canada.
E-mail address: nnikmara@uoguelph.ca (N. Nikmaram).

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drop to half its original value, is also presented in Table 1.

Cold plasma technology offers many advantages in food industry such as short processing times (between a few seconds to minutes), being effective at room temperature which is important for heat-sensitive products, and low-energy requirement, shown in Fig. 1. (Kim et al., 2014). Published reviews focus on microbial decontamination of dairy products using cold plasma; however, they lack substantial information on milk and dairy product quality changes resulting from cold plasma (Rathod, Kahar, Ranveer, & Annapure, 2021) (Coutinho et al., 2018). The focus of this review is to highlight the quality impacts from cold plasma on milk and dairy product quality.

2. Explanation of process and treatment conditions

Plasma is a quasi-neutral ionized gas state composed of ions, free electrons, atoms and molecules in their fundamental or excited states with a net neutral charge. Atmospheric [pressure] cold plasma (ACP) is a partially ionized gas state where the ionization is approximately 5% or less of the gas and the gas temperature is maintained at approximately room temperature (Ekezie, Sun, & Cheng, 2017). The non-equilibrium conditions cause generation of free electrons to be stripped from a very small number of gas molecules based on their ionization potential. These free elections then collide with unionized molecules leads to radicals. This process that is called a cascade reaction results in generation of a variety of reactive species such as radicals and reactive gas species (Misra & Jo, 2017).

There are many plasma sources used for food treatment including plasma jets, dielectric barrier discharges (DBD's), corona discharges, and microwave discharges (Misra & Jo, 2017). The two most common plasma sources reported in literature for treatment of milk include the DBD and corona discharge. A DBD device consists of two electrodes and at least one or usually two dielectric barriers (mainly to avoid any arc transition). Whereas, a corona discharge is a weakly luminous discharge that usually appears at atmospheric pressure near sharp electrode geometries (points, edges or thin wires) (Misra & Jo, 2017).

There are many factors that influence plasma reactive gas species (RGS) generation and milk treatment effectiveness including plasma device geometry, electrode design, implemented pressure, voltage, treatment time, direct or indirect mode of exposure, applied humidity, distance between electrodes, working gas, and sample volume. The researchers' challenge to identify the most effective combination of factors to achieve the targeted intended effect (*e.g.,* pasteurization) without undesirable consequences (*e.g.,* lipid peroxidation) (Misra & Jo, 2017). Table 2 summarizes published cold plasma studies on milk and dairy products which measured quality attributes that are the most important factors for the inactivation of microorganisms.

3. Physical properties

3.1. Viscosity

Milk is an emulsion with dispersed fat and protein compounds in an aqueous (watery) environment. Milk viscosity is dependent on its composition and processing conditions (Manoharan, Stephen, & Radhakrishnan, 2020). A study conducted by Alcă;ntara et al. (2012) indicated that viscosity decreased as temperature and moisture content increased; and increased as fat, lactose, protein and minerals contents increased. Viscosity is an important physical property that correlates to "mouthfeel" and is used as a quality control measurement (Wagoner, Çakır-Fuller, Shingleton, Drake, & Foegeding, 2019). As an example, an inline viscometer is used to track coagulation of milk to produce curd. The increasing viscosity of the developing curd can be used to help decide the cut time. Viscosity varies due to composition differences such as fat content and protein content, and processing conditions such as the pasteurization temperature, pasteurization time, and homogenization pressure. The viscosity of skim cows' milk is approximately 1.56 mPa s at room temperature and the viscosity of whole cows' milk is approximately 2.00 mPa s (Khalifa & Ghanimah, 2013).

Study #2 in Table 2 shows a reduction in viscosity of raw milk from 1.62 mPa s (control sample) to 1.60 and 1.57 mPa s for direct plasma exposure at a flow rate 3.0 and 6.0 ml/min, respectively (Manoharan et al., 2020). However, these differences in viscosity are negligible in milk processing. This viscosity reduction has been previously observed in a high voltage cold plasma treatment process and was documented to results from oxidation of proteins and lipids (Sarangapani, Ryan Keogh, Dunne, Bourke, & Cullen, 2017). There was no further investigation of the milk chemistry changes resulting in the viscosity reduction in either of these studies. Another study by Silveira et al. (2019) indicated that plasma treated samples (400 W and 50 kHz) exhibited lower flow consistency index ($p \leq .05$), with values varying from 5.148 to 9.385 mPa s. They concluded that mild plasma treatment generates a lower viscosity and lower flow consistency index in the milk. If higher viscosity and consistency are important parameters to the beverage quality, it is advised to use more drastic processing conditions.

Table 1
Typical lifetimes of selected reactive species that may be generated by cold plasma and potential effects on milk.

Species	Molecular formula	Potential effects on milk	Lifetime (T1/2, s)	Gas condition	Reference
Reactive Oxygen Species (ROS)					
Hydroxyl radical	OH·	Protein aggregation, Fat oxidation, Enzyme inactivation	$<10^{-9}$	Water	Buxton, Greenstock, Helman, and Ross (1988)
Ozone	O_3	Protein aggregation, Fat oxidation, Color change	150 s	Air	Klockow and Keener (2009)
Hydrogen peroxide	H_2O_2	Not reported	Up to 8 h	Water	Cooper and Zepp (1989)
Singlet oxygen	$^1O_2$	Protein aggregation, Fat oxidation	10^{-6} s	Water	Phaniendra, Jestadi, and Periyasamy (2015)
Superoxide anion	$O_2^{\cdot-}$	Enzyme inactivation	10^{-6} s	Water	Phaniendra et al. (2015)
Reactive Nitrogen Species (RNS)					
Peroxynitrite	$ONOO^-$	Not reported	10^{-2} s	Cells and tissues	Ferrer-Sueta and Radi (2009)
Nitric oxide	NO·	Not reported	Up to 10 s	Air-saturated solution	Kohen and Nyska (2002)
Dinitrogen tetroxide	N_2O_4	Not reported	Seconds	Air	Phaniendra et al. (2015)
Dinitrogen pentoxide	N_2O_5	Not reported	Seconds	Air	Phaniendra et al. (2015)
Nitrate	NO_3^-	pH reduction	up to 3.8 h	Plasma in blood samples	Zeballos et al. (1995)
Nitrite	NO_2^-	Not reported	Between 20 min and 45 min)	Plasma in blood samples	Zeballos et al. (1995)

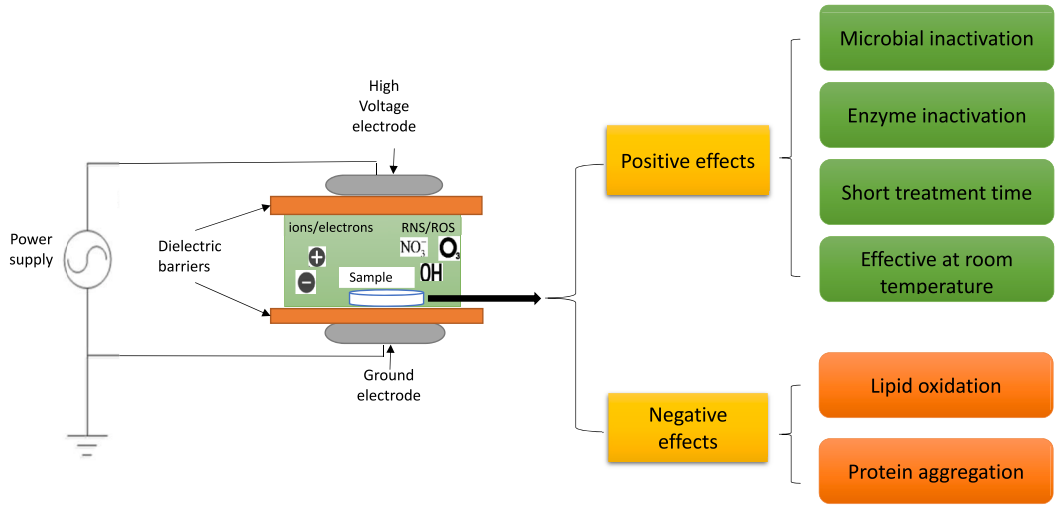

Fig. 1. The schematic representation of a dielectric barrier discharges (DBD) cold plasma along with positive and negative effects.

Recently, Wu, Luo, Zhao, M, & Mu. (2021) investigated the effect of a DBD system as summarized in #1, Table 2. The authors found that milk viscosity increased from 1.8 to 2.2 mPa s as voltage increased from 40 to 80 V, respectively. Interestingly, this value is between the viscosity of traditional UHT milk (2.2 mPa s) and pasteurized milk (1.75 mPa s). According to the findings, low-intensity treatment would lead to the shear thinning of the fluid due to the viscidity ingredient, such as carbohydrate, fat, and protein in milk with adhesion broken into small units by treatment (Wu, Luo, Zhao, M S. M., & Mu, 2021).

In summary, the effect of cold plasma treatment on viscosity of milk can vary significantly. Long treatment time (>5min), high voltage (app. 80 V), and high flow rate (*e.g.,* 30 mL/min at 15 min) lead to higher viscosity. Depending on the plasma condition such as voltage, treatment time, and flow rate, viscosity can be optimized.

3.2. Color

Milk color is an important sensory property that not only affects consumer choice of one product over another, but also it has a close relationship with the quality of dairy products. Milk color varies from a yellowish white to nearly white depending on various reasons including cattle breed, feed composition, stage of lactation, milking-time, and seasonal calving (Scarso et al., 2017). Color is defined by the International Commission on Illumination based on a three-dimensional color space with three primary coordinates of L*, a*, b*, where L* indicates lightness or darkness, a* redness or greenness, and b* yellowness or blueness. The total color difference (ΔE) is the standard calculation metric which correlates the human visual judgment of differences between two perceived colors (Browder, 2018). Based on the classification given by Cserhalmi, Sass-Kiss, Tóth-Markus, & Lechner. (2006), ΔE can be evaluated as non-noticeable (0–0.5), slightly noticeable (0.5–1.5), noticeable (1.5–3), visible change (3–6), and great change (6–12). According to (Bermúdez-Aguirre, Fernández, Esquivel, Dunne, & Barbosa-Cánovas, 2011), for raw skim milk these values are as follow: L*: 85.35, a*: −3.55, and b*: 1.81. Similarly, for raw whole milk colorimeter values have been reported as: L*: 88, a*: −0.88, and b*: 4.42 (Cheng, Barbano, & Drake, 2018).

Cold plasma generates ROS such as ozone when air and oxygen are used as the working gas. The ratio of different ROS species depend on many factors including the working gas, plasma treatment time, mode of exposure, and plasma source. For example, the presence of water vapor in working gas will shift the ROS from ozone into greater peroxides which show much less lipid oxidation (Feizollahi, Iqdiam, Vasanthan, Thilakarathna, & Roopesh, 2020). Study #2 in Table 2 found ΔE values in plasma-treated milk were 0.91 and 1.58 for milk treated for 6 and 3 ml/min flow rate (Manoharan et al., 2020). Whereas, Gurol, Ekinci, Aslan, & Korachi. (2012) reported no significant change in the color after plasma treatment (#3) of raw milk up to 15 min of treatment time. The total color difference (ΔE) for cow's milk after 9 min of plasma treatment with 9 kV was 0.25 while longer exposure to plasma (20 min) caused slightly higher color differences with a ΔE of 0.52.

Alternatively, Kim et al. (2015) achieved non noticeable ΔE after 5 min (ΔE: 0.27) and 10 min (ΔE: 0.50) of treatment. They hypothesised that higher L* might be related to higher number of fat globules that are able to diffract light more effectively. Moreover, according to Popov-Raljić, Lakić, Laličić-Petronijević, Barać, & Sikimić. (2008) an increase in b* is an indicator that determines nonenzymatic milk reactions, also known as Maillard's reactions. This reaction starts with binding of aldehyde group of lactose with ε-amino group of the lysyl – residues (amino-acid radical, or residue of amino-acid lysine) from different milk proteins (Popov-Raljić, Lakić, Laličić-Petronijević, Barać, & Sikimić, 2008).

In a study by Wu et al. (2021) the ΔE values of sample treatment with DBD at 80 V for 120 s were in the range between UHT (36.02) and pasteurization (9.13), therefore, color of treated samples was considered to be in the range of the accepted color in public.

In study #9 from Table 2, the ΔE was less than 1.5 for milk powder after 120 s of plasma treatment (Chen et al., 2019). They observed a slight decrease in the L* value (lightness), a slight increase in a* value, and a slight decrease in b* value represented slight brown pigment formation in milk powder. The largest ΔE for treated samples was 1.14 which is still within the range of slightly noticeable (0.5–1.5). It was proven that oxidation of milk proteins was affected by cold plasma which was known to induce a minor yellowing effect that could be picked by the colorimeter (Segat, Misra, Cullen, & Innocente, 2015). Another study applied cold plasma (#10) on queso fresco cheese reported that treatments in dry air and MA65[1] induced some color changes in the samples, they were not perceptible with the naked eye due to the low total color difference (ΔE < 1.5) (Wan, Misra, Li, & Keener, 2021). A significant decrease in L*-value and increase in a*-value for sliced cheddar cheese treated with a DBD-plasma system (at 15 kHz) was observed (Yong et al., 2015) which might be due to longer treatment time (10 min).

Therefore, to have insignificant change in color of dairy products, it is advised to keep the treatment time below 5 min. As higher b* (e.g., >6) indicates nonenzymatic milk reactions, low oxygen concentration in working gas is suggested to avoid fat and protein oxidation which can

[1] MA65: Modified Air that consists of 65% of O_2, 30% CO_2, and 5% of N_2.

Table 2
Effect of cold plasma technology on cow's milk.

#	Plasma source	Plasma source condition	Analysis performed	Results	Reference
1	DBD-type plasma	Voltage: 40, 50, 60, 70, and 80 V, Treatment time: 15, 30, 60, 90, and 120 s Current: 2 ± 0.2 A Sample volume: 10 ml	Microbial analysis (*S. aureus*, *E. coli*, and *L. monocytogenes*), Morphology (scanning electron microscope), Fourier-transform infrared spectroscopy (FTIR), Color pH Viscosity Lipid peroxidation	• Highest value of ΔE with 60 V (120 s) treatment samples and smallest ΔE for 50 V (120 s) condition • Higher viscosity after 120 s treatment time and higher voltage (>60 V) • Lipid peroxidation increased with both higher voltage and longer treatment time	Wu et al. (2021)
2	DBD-type plasma	Voltage: 2 kV, Current: 57 mA, electrode distance: 1.5 cm, reduced pressure (16 Pa), Sample volume: 300 ml Working gas: air Temperature: room temperature	Microbial analysis Coliform), pH conductivity Color Viscosity Protein, Fat, Lactose	• No detrimental effects during plasma interaction on physicochemical properties, and nutrient contents	Manoharan et al. (2020)
3	Atmospheric corona discharge	Voltage: 9 kV, Treatment time: 0, 3, 6, 9, 12, 15 and 20 min Working gas: air Temperature: below 35 °C	Microbial analysis (*E. coli*), pH, Color, Storage (test cold conditions of 4–7 °C)	• No significant change to the pH (6.7 ± 0.05) and color values (L*: 81.39, b*: 6.36, and a*: −1.46),	Gurol, Ekinci, Aslan, and Korachi (2012)
4	Encapsulated DBD plasma	Power: 250 W, Frequency: 15 kHz, Treatment time: 5 and 10 min Pressure: ambient pressure, Sample volume: 10 mL, Temperature: not reported	Microbial (*E. coli*, *L. monocytogenes*, and *S. Typhimurium*), pH, Color	• Higher L* (89.70) and b* (6.19) values, lower a* (−2.47) after 10 min of treatment, • No effect on fatty acid concentration	Kim et al. (2015)
5	DBD-type plasma	Treatment time: 5, 10 and 15 min, Working gas: nitrogen in low humidity, Plasma flow rates: 10, 20 and 30 mL/min, Exposure: indirect mode, Sample volume: 120 mL, Temperature: 21–25 °C	Particle size, Viscosity, Thermal behavior, Microstructure	• Higher particle size (18.29 ± 0.81) cold plasma at gas flow of 20 mL/min for 5 min compared to the pasteurized sample (11.51 ± 0.42), • Higher consistency (744.39 ± 24.19) compared to the pasteurized product (14.83 ± 0.08)	Coutinho et al. (2019)
6	Corona discharge	Voltage: 9 kV AC, Current: 90 mA, Distance from the milk surface: 8 mm, Treatment times: 3, 6, 9, 12, 15 and 20 min, Temperature: below 35 °C	Protein, Free fatty acids, Volatiles profiles	• Significant changes after 20 min for 1 octanol (from 0.23 μg ± 0.21–0.62 μg ± 0.18), 2 heptanone (from 1.96 μg ± 0.82 to 0.15 μg ± 0.02), 2 hexenal (from 0.78 μg ± 0.28–1.88 μg ± 0.61), 2 octenal (0.07 μg ± 0.04–0.56 μg ± 0.69), nonanal (from 0.68 μg ± 0.14–8.80 μg ± 0.61) and benzaldehyde (from 0.21 ± 0.16 to 1.04 ± 0.08), • No change to the lipid composition, • Higher aldehyde content after 20 min	Korachi et al. (2015)
7	DBD atmospheric cold plasma	Voltage: 70 kV, Treatment time: 1, 5, 10, 15, 30, and 60 min, Working gas: air distance between the two electrodes: 44 mm pressure: atmospheric pressure, Sample volume: 30 mL Temperature: not reported	Ozone concentration, Color, pH, Protein carbonyls, Free sulfhydryl content, Emulsifying capacity, Foaming properties	• Mild oxidation in the proteins after 15 min according to protein-bound carbonyl groups (3 AU Abs compared to control: app. 0.25 AU Abs), • Lower free SH groups after 30 min (1.8×10^{-5} mol/L SH/g) compared to control (3.2×10^{-5} mol/L SH/g), • Lower foaming (from 90% to 70%) and emulsifying capacity (from 0.3 to app. 0.17 a.u. Abs) after 60 min compared to control	Segat et al. (2015)
8	DBD atmospheric cold plasma	Voltage: 15 kV, a bipolar square-waveform, 2-W average power, Working gas: air	Microbial (*E. coli*, *S. typhimurium* & *L. monocytogenes*), pH, Color, TBARS values, Sensory evaluation	• With increasing the treatment time, pH and L*-values decreased, TBARS and b*-values increased. • No significant difference regarding (ΔE) • significant reductions in flavor and overall acceptance as well as an increase in off-odor	Yong et al. (2015)
9	DBD atmospheric cold plasma with fluidized bed plasma treatment chamber	Voltage: 4.4 kV, Treatment time: up to 120 s, Working gas: pure nitrogen gas (99.9% purity), Flow rate: 8–20 L/min	Microbial (*C. sakazakii*), OES, Color, X-ray diffraction (XRD) analysis, Amino acid composition, Phenolic content	• No significant changes were observed regarding color changes (ΔE < 1.5), crystallinity, amino acid composition, or phenolic content after 120s-CAP treatment.	Chen et al. (2019)
10	DBD-type plasma	Voltage: 60, 80, or 100 kV, Treatment time: up to 5 min,	Microbial (*L. innocua and E. coli*), pH, Color,	• Minimal quality changes in pH, moisture, color and lipid oxidation were observed • No significant changes was found regarding texture	Wan et al. (2021)

(*continued on next page*)

Table 2 (*continued*)

#	Plasma source	Plasma source condition	Analysis performed	Results	Reference
		Working gas: MA65 and dry air, distance between the two electrodes: 28 mm	Moisture Optical Emission Spectroscopy (OES), Optical Absorption Spectroscopy (OAS), TBARS value, Texture profile analysis		
11	DBD-type plasma	Power supply: 400 W and 50 kHz, Treatment time: 5, 10, and 15 min, Working gas: Nitrogen, Flow rate: 10, 20, and 30 mL/min, Sample volume: 120 mL	Microbial (coliforms and *Salmonella* sp.), pH, Viscosity, Particle size, Differential scanning calorimetry (DSC) analysis,	• Plasma treatment resulted in higher pH and higher flow behavior indexes. • Milder plasma processing conditions (lower flow rate with shorter treatment time) if low viscosity and consistency is required • More drastic processing conditions (higher flow rate for longer time) if higher viscosity and consistency is desired • Plasma treatment did not affect thermal properties (melting temperature and enthalpy)	Silveira et al. (2019)

lead to higher yellowness.

3.3. Particle size distribution

Particle size (comprised of lipoprotein molecules), especially particle size of fat globules, has a high impact on the flavor, mouthfeel, and emulsion stability of milk (Coutinho et al., 2019). Thus, particle size is considered as a factor to evaluate the quality control of milk. Particle size can vary upon different factors such as cow's breed, feeding composition, cow's age, time of the year, and production process such as homogenization, skimming, etc. A bimodal characteristic distribution was found in raw milk: a 0.20-μm peak, corresponding to casein micelles, and a second peak of 3.7 μm, corresponding to milk fat globules. Size of fat globules in milk varies from 0.10 μm to 15 μm (Thum, Roy, Everett, & McNabb, 2021). When milk is required to be stored for several weeks, the diameter of fat globules should be reduced to size less than about 0.7 × 10−6 m when they exhibit Brownian movement and prevent formation of cream layer (Sahu & Mallikarjunan, 2012).

Cold plasma treatment of whole chocolate milk, study #5 in Table 2, after 5 min results in milk particles with higher surface areas and smaller volume diameters in chocolate milk (Coutinho et al., 2019). This treatment resulted in increased particle size of the of small particles (10–100 μm) and increased volume of particles with large diameters (100–1000 μm). ROS presented in the cold plasma processing can result in mild oxidation of the proteins, increases in carbonyl groups and surface hydrophobicity, and reductions of free SH groups. The reduction of SH groups implies in the formation of disulphide cross-links, both intra or intermolecular, resulting in aggregation of the proteins. This aggregation results in products with larger particles (Coutinho et al., 2019). Similar results were achieved in the study #11 in Table 2 by (Silveira et al., 2019) for the whey beverages treated by cold plasma processing. They reported that 50 ml exposure with nitrogen gas during cold plasma processing resulted in particle distribution similar to that observed for the pasteurized product (1000 μm). An increase in the particle surface area and the number of small particles (10 μm) was observed after plasma processing with higher flow rate (20–30 ml/min) which is due to the increased shear rate, leading to the formation of smaller particles.

There is an inverse correlation between the creaminess of milk and particle size. Thus, milk with higher number of smaller particles is creamier than those with large particles (Janhøj, Bom Frøst, & Ipsen, 2008). Considering the consumers' preference about higher viscosity of chocolate milk, cold plasma processing of chocolate milk can increase the viscosity of the final product due to alteration in the particle size which is more attractive from a consumer's viewpoint. In this study, by increasing the treatment time to 15 min, the consistency increased to 744.39 ± 24.19 (mPa.s^n) in comparison with control sample 14.83 ± 0.08 (mPa.s^n) (Coutinho et al., 2019). Protein oxidation causes the peptide chain to break and can lead to protein aggregation, cross-linking, and conformational changes, and consequently changes in proteins' physicochemical properties such as viscosity, color, and particle size (Li et al., 2019). This subject will be discussed in detail in protein section (5.1.).

In conclusion, cold plasma treatment longer than 5 min results in protein oxidation due to generation of ROS and subsequently larger particle size. However, higher flow rate (30 ml/min) causing smaller particle size results from higher gas velocity.

4. Nutrition

4.1. Protein

Milk proteins (32 g/L–38 g/L in whole milk) are an important component in milk products as they influence the physical, chemical, and sensory characteristics. They are categorized in two primary groups which in whole cow's milk consist of caseins (80%) and whey proteins (20%) (Davoodi et al., 2016). There are four main types of caseins: αsl-, αs2, (β+γ) and κ-casein with the ratio of about 0.45:0.11:0.33:0.11. Whey proteins, on the other hand, consist of α-lactalbumin (α-LA), β-lactoglobulin (β-LG), serum albumin and immunoglobulin (Sharma & Singh, 2020).

A DBD atmospheric cold plasma of 70 kV for 15 min caused a mild oxidation (about 3 AU[2] Abs) of the proteins as measured by the amount of protein-bound carbonyl groups compared to the control sample (app. 0.25 AU Abs) using a spectrophotometer (reaction with 2,4-Dinitrophenylhydrazine method) (Segat et al., 2015). Higher amount of carbonyl groups was observed which could be attributed to the modifications of a number of amino acid side chain groups, especially with NH− or NH_2 or by peptide bond cleavages. There was a reduction in free SH groups from approximately 3.2 × 10^{-5} mol/L SH/g for the control sample to about 1.8 × 10^{-5} mol/L SH/g after 30 min of treatment. Disulphide cross-linked formation is a method to characterize protein aggregation. Disulphide cross-linked bonds commonly occur during heat treatment, and is related to denaturation of whey proteins and formation of aggregates between b-LG and k-CN (Guyomarc'h, Law, & Dalgleish, 2003).

Protein oxidation was measured by the amount of protein-bound carbonyl groups. It was observed that more aggregation was reported by increasing the treatment time from 15 min (2.5 AU Abs) to 30 and 60 min (app. 3–3.5 AU Abs) (Segat et al., 2015).

The generated ROS (e.g., hydroxyl radical) have been attributed to the increased cross-linking of free amino acids with sulphur-containing

[2] Absorbance is measured in absorbance units (AU).

amino acid side chains such as cysteine and consequently protein aggregation (Meinlschmidt et al., 2016). It was found by (Manoharan et al., 2020) that application of low-pressure cold plasma treatment did not alter the protein content of raw milk (35.3 g/L ± 0.06) compared to the control sample (34.7 g/L ± 0.17).

A study on non-fat dry milk (#9) showed no significant change on amino acid profile (Chen et al., 2019). They explained that the atomic oxygen and hydroxyl radicals, which can be detected at 777 nm and 309 nm of the emission spectrum, respectively, are known to have the highest oxidation potential (2.86 and 2.42 V, respectively) within all ROS generated by plasma and are capable of oxidizing amino acid. However, neither species were detected in the optical emission spectra. This may be due to low humidity (~35%) adapted in the CAP treatments reduced the amount of atomic oxygen generated by N_2 (Chen et al., 2019). Additionally, the duration of CAP treatment (120 s) in the current study was much shorter than the durations in previous studies.

Overall, depends on the applied condition, cold plasma effect may cause no significant change if low pressure (16 Pa) or nitrogen gas are applied as there is no ROS. It may result in mild oxidation or significant aggregation if air and high voltage (60 kV) and long treatment time over 30 min is used due to higher concentration of generated ROS. Therefore, to minimize protein change, treatment conditions need to be tailored.

4.2. Fat

In dairy cows, milk fat is organized in milk fat globules (MFG), which are droplets of triglycerides surrounded and stabilized in the aqueous phase by a 3-layer membrane of proteins and phospholipids that is derived from the outer leaflet of the endoplasmic reticulum and the apical plasma membrane of the cell (Lu et al., 2016). Fatty acid composition is different between different sized MFG (e.g., higher amount of polyunsaturated fatty acid presents in smaller MFG).

In 2015, Kim et al. found that an encapsulated DBD plasma source (#4) among all fatty acids, only butyric acid concentration (0.6 g/L of milk) changed after 10 min treatment compared to control (0.7 g/L of milk). Free radicals such as Hydroxyl radical, generated during cold plasma treatment has been stated to react with unsaturated fatty acids and resulting in a break down of the double and consequently cellular injury (Kim et al., 2015). However, the obtained data in this study showed that encapsulated DBD plasma source shows the minimum impact on fatty acid composition in milk.

Fat oxidation is another concern in treated milk sample with cold plasma technology. In this regard, the same study conducted by Kim et al. (2015) used Thiobarbituric acid reactive substances (TBARS) test to examine lipid oxidation and claimed that there is no significant difference in TBARS value after plasma treatment of milk sample. The double bonds of unsaturated fatty acids in milk such as Oleic acid and linoleic acid are especially vulnerable to ozone attack (Uknowledge & Yang, 2016). The previous cold plasma study did not find any fat oxidation in milk based on TBARS values (0.029 (mg malondialdehyde/kg)) for 10 min treatment compared to 0.027 (mg malondialdehyde/kg) for control sample (Kim et al., 2015).

An atmospheric plasma discharge system (#6) has also been tested on milk to evaluate fat oxidation (Korachi et al., 2015). They reported a one percent lower concentrations of short chain fatty acid[3] 63.6% during the first 5 min of treatment, there was an increase to up 65.8% after 10 min. These results are in agreement with Wu et al. (2021) who reported that higher voltage led to higher lipid peroxidation in milk. Lipid peroxidation occurs, when any free radical attacks and abstracts hydrogen from a methylene groups (CH2) in a fatty acid (LH) which results in the formation of a carbon centered lipid radical (L•). The lipid radical can react with molecular oxygen to form a lipid peroxyl radical (LOO•). These lipid peroxyl radicals can further propagate the peroxidation process by abstracting hydrogen atoms from the other lipid molecules. Likely, free radicals such as hydroperoxyl radicals and singlet oxygen are generated which have been shown to attack PUFA's generating shorter fatty acids (Ayala, Muñoz, & Argüelles, 2014).

(Korachi et al., 2015) reported that treated milk sample did not vary from the control sample in terms of total FFAs concentrations. Overall, the predominant fatty acids observed pre-plasma application were palmitic 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.

The same study also investigated the changes in aldehyde, ketones, and alcohols content. A remarkable difference was achieved in total aldehyde content after 20 min (20.79 μg ± 5.13) in comparison with control sample (7.30 μg ± 0.56), by contrast no significant changes were observed in the total composition of ketones (after 20 min: 10.49 μg ± 1.33, control: 12.02 μg ± 2.26) and alcohols (after 20 min:2.06 μg ± 0.53, control: 0.80 μg ± 0.23) (Korachi et al., 2015). 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 by auto-oxidation and/or the spontaneous decomposition of hydroperoxides, which have been found to result in the production of aldehydes. Such degradation could be a result of the reactive species seen to be produced by plasma.

Treated milk with a low-pressure (16 Pa) plasma system (#2) indicated a fat loss after plasma processing (Manoharan et al., 2020). Authors described the reason as of using non-homogenized fresh cows' milk; the sticking of the fat to the tubes was observed when passed under reduced flow rates. Without homogenization, fat molecules in milk will stick together, while homogenizing milk prevents this separation from occurring by breaking the molecules down to such a small size that they remain suspended evenly throughout the milk.

Lipid oxidation was reported higher based on TBARS values in DBD (15 kHz) treated sliced Cheddar cheese (0.183 ± 0.027 mg malondialdehyde/kg after 10 min of treatment) compared to the control sample (0.132 ± 0.021 mg malondialdehyde/kg) (Yong et al., 2015).

As higher ROS including OH radical and atomic oxygen will lead to higher level of fat oxidation, minimizing the concentration of ROS via lower voltage, shorter treatment time (below 10 min) would result in lower fat oxidation. Lower concentration of O_2 in the initial working gas such as pure nitrogen would also lead to lower fat oxidation.

4.3. Carbohydrates (Lactose)

The main carbohydrate in cow's milk is lactose. Lactose concentration varies slightly in milk (4.5–5.2 g/100 g) with several factors such as diet, time of year, and husbandry practices. It has a vital role in fermented milk products including yoghurt and cheese where lactose is used as substrate by lactic acid bacteria (Mourad, Bettache, & Samir, 2014).

Limited research has been conducted on the possible impact of cold plasma treatment on cow's milk lactose. Manoharan et al. (2020) applied a DBD-type plasma system (#2) on raw milk and measured lactose content. They reported a significant reduction in lactose content (44.8 g/L) at 3 ml/min flow compared to the control sample (46.4 g/L). This reduction in lactose content has been associated to the interaction of OH radical with lactose and consequently abstracting a proton from disaccharide lactose to form sugar-free radical.

5. Sensory

Sensory evaluation is critical for every application of milk. It is necessary to understand the sensory qualities of milk in part because of the widespread familiarity of fluid milk and its typical sensory profile. Unfortunately, there are no published studies examining the sensory properties of cow's milk treated with cold plasma. However, there are

[3] Short-chain fatty acids (SCFA) are fatty acids with aliphatic tails of five or fewer carbons (*e.g.* butyric acid).

some research on the effect of cold plasma on sensory properties of cheese. For example, Yong et al. (2015) reported significant reductions in flavor and overall acceptance as well as an increase in off-odor of sliced cheddar cheese. Similar results were found by Lee et al. (2012) where they also reported a significant reduction in flavor and odor of sliced cheese using DBD plasma system (3.5 kV, helium and He/O_2 mixture gas, for 1, 5, 10, and 15 min). Free radicals, which are precursors of lipid hydroperoxides, can cause lipid oxidation and result in the production of secondary oxidation products including alkanes, aldehydes, alcohols, ketones, and acids. These lipid oxidation by-products produce off-odors described as metallic, fishy, rancid, and oxidized (Yong et al., 2015).

6. Consumer acceptance

Consumers accept or reject new technologies for many different reasons such as ethical, safety, and environmental concerns. Due to lack of understanding of consumer perception, many novel processes and technologies struggle to gain widespread consumer acceptance. Therefore, evaluating consumers' view point is an important factor in the early stages of food product development (Coutinho et al., 2021).

One study has investigated consumers' perception of the processing of chocolate milk drinks by cold plasma (Coutinho et al., 2021). They evaluated the consumers' perception (n = 1085) about processing chocolate milk drinks by cold plasma. The consumers were asked about familiarity and willingness to buy the product, sensory attributes, and perceived quality compared to traditional technologies. Based on their collected data, consumers' concern was the possible negative impact of new technologies on health, quality of the product, as well as environment. However, the majority of volunteers (72.3%) were willing to purchase the chocolate milk drink processed by cold plasma, mainly if the price is similar to that of the conventional product. For the rest of the participants, the price was not the main concern.

The willingness to buy the new product (considering they are affordable to purchase) increases with the belief that the new technologies would not bring negative health effects, while it decreases with the unfamiliarity with new technologies, doubts about the information provided by the media, or no perceived quality compared to the traditional technology. Therefore, it is important that the food industry provide consumers with accurate, understandable, and clear information so that the fear could be overcome (Coutinho et al., 2021).

7. Conclusion

Cold plasma is a promising technology to process milk and dairy products with minimum quality change when the conditions are optimized. Tailoring the cold plasma treatment conditions including gas type, voltage, treatment time, and plasma source can achieve non thermal pasteurization or sterilization of milk and dairy products. Additional research is needed to further understand this complex process.

Declaration of competing interest

This manuscript has not been submitted to, nor is under review at, another journal or other publishing venue.

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