Indian J Dairy Sci 76(4): 356-363
https://doi.org/10.33785/IJDS.2023.v76i04.007

RESEARCH ARTICLE

Effect of cold plasma on the quality parameters of custard apple juice milk beverage

Shifa Sanofer Khair K M[1], G Sujatha[2*] and Rita Narayanan[3]

Received: 02 December 2022 / Accepted: 21 February 2023 / Published online: 18 August 2023

Abstract: This study is aimed to investigate and compare the physicochemical and sensory quality parameters which are associated with the freshness of the developed Custard apple juice milk beverage (CAJMB) subjected to cold plasma (CP) technology. The developed beverage was subjected to CP generated from two different configurations of Dielectric Barrier Discharge plasma (DBD) viz., High Voltage Atmospheric Cold Barrier Discharge plasma system (HV ACBD plasma system) and High Voltage Cold Barrier Discharge (HVCBD) Plasma torch/ Gun using Argon gas. The treatments were performed for different processing times (2 & 3 min) at an applied voltage gradient of 35 kV and 30 kV respectively. The treatments did not have any potential change in the sensorial quality attributes evaluated immediately after processing compared to untreated control sample. However, a noteworthy difference was observed in pH and titratable acidity after processing, directly proportional to increase in treatment conditions concerning voltage and processing time. The pH and acidity at defined intervals during storage showed a slow rate of reduction as compared to the control. In the control sample levels of pH and acidity reached to an unacceptable range in 3 to 5 days of storage, due to the formation of lactic acid by subsequent microbial growth. Whereas, CP treated sample using HV ACBD plasma system at 35kV for 3min exhibited promising results among other treatments and remained stable at refrigerated condition, with pH and acidity levels within the expected acceptable range for 9 to 10 days of storage.

[1]College of Food and Dairy Technology, Koduveli, Chennai -52

[2]Department of Food Process Engineering, College of Food and Dairy Technology, Koduveli, Chennai -52

[3]Department of Food Processing Technology, College of Food and Dairy Technology, Koduveli, Chennai -52

G. Sujatha (✉)
Department of Food Process Engineering, College of Food and Dairy Technology, Koduveli, Chennai -52
Email: sujathabhusham@gmail.com

Keywords: Cold plasma, Dielectric Barrier Discharge (DBD), Plasma Torch/ Jet, Custard Apple Milk Beverage, Physicochemical properties, Shelf life

Introduction

The demand for healthier minimally processed ready-to-drink (RTD) fresh fruit beverages with new choices and flavours is constantly increasing in the market. This is especially for combinations of fruit juices and milk with natural and added vitamins, minerals and fiber, which are consumed as functional foods in recent times. Milk contains lactose, fatty acids, proteins and various micronutrients such as minerals and trace elements. Therefore, the hazard of contamination by spoilage-causing and infective microbes in milk and fruit-based RTD beverages is of apprehension because these are consumed without any further processing after purchase from retailers. Custard apple (*Annona squamosa*) is a climacteric and very perishable fruit with 2 or 3 days of shelf life when ripened (Solanke et al. 2019). But it is a great source of iron, phosphorous, potassium and vitamin C. Also, it has numerous medicinal and nutritional factors containing a pool of sodium, magnesium, ascorbic acid, fiber and sugars, etc., with excellent antioxidant activity (Solanke et al. 2019). Thus, there is a necessity to utilize the nutritional as well as relish its flavour in the form of value-added food products to reduce post-harvest losses. Various value-added products of custard apple such as jams, ice creams, squash, crush, nectars, etc., which are highly processed are on market (Solanke et al. 2019). Milk beverages are generally thermally processed by pasteurization, retort processing and sterilization which improves the product safety and shelf life by destroying the microbial population. But these heat treatments lead to denaturation of essential proteins, non-enzymatic browning, nutritional loss, and higher alterations in the quality attributes including physicochemical and sensorial parameters (Pan et al. 2019). For such reasons, there is an essential need for non-thermal processing interventions. In current times, many non-thermal techniques such as Pulsed Electric Field (PEF), High Pressure Processing (HPP), Ultraviolet (UV) and Ultrasonication (US), and Ozone treatments were highly explored to increase the keeping quality of the food product while maintaining the quality characteristics of the treated food (Coutinho et al. 2018).

Cold Plasma (CP) is a newly emerging technology that is explored in recent periods among the other novel nonthermal technologies for the destruction of microbes and as a complementary to thermal techniques (Liao et al. 2017). Plasma is often described as the fourth state of matter with increasing energy from the gaseous state to energized plasma state containing elements like Reactive Oxygen Species (ROS), Reactive Nitrogen Species (RNS), positive ions, negatively charges atoms, neutrals, UV, free radicals, etc., (Liao et al. 2017). The element generated from plasma is reported to be the cause of the microbial inactivation but which element is particularly responsible and also the principle behind the influence of CP on Physico-chemical properties, interaction with biomolecules, Etc. are still under study (Coutinho et al. 2018). Two types of plasma have been classified as thermal and non-thermal plasma differentiated by their thermal equilibrium between ions and electrons. In non-thermal plasma the ions and electrons are in thermal equilibrium hence it is maintained at almost room temperature making it practically cold, which we can touch with bear hands thus causing no damage to the subjected food products but affecting the membranes of micro-organisms due to their relatively smaller cell size and structure (Surowsky et al. 2015). CP is generated by applying very high voltage to gas molecules. This high power can be generated by different means viz., alternating or direct current, microwaves, radio frequency, pulsed power source, etc., (Montejano et al. 2019). Various configurations and device geometries have been proposed for cold plasma generation among which Atmospheric pressure plasma (ACP) by Dielectric Barrier Discharge (DBD) system with symmetric electrode geometry and plasma jet/ plasma torch are widely explored for processing food products because of their simple design (Bourke et al. 2018).

Thus, this study mainly aims to evaluate the effect of plasma on the physico-chemical and sensorial quality parameters and keeping quality of the developed RTD custard apple juice milk beverage (CAJMB) using different methods of plasma generation of DBD viz., High Voltage Atmospheric Cold Barrier Discharge plasma system (HV ACBD) parallel plate plasma and High Voltage Cold Barrier Discharge (HV CBD) plasma torch system.

Materials and Methods

Experimental setup and experimental design

Cold plasma treatment of the CAJMB sample was carried out in two different methods of novel CBD non-thermal plasma systems. The systems used in this study were High Voltage Atmospheric pressure CBD plasma system and HV CBD plasma torch/ Gun (Zeonics Systech Defence and Aerospace Engineers (P) Ltd.,). The former system setup consists of two parallel plate electrodes measuring 400×400mm, made of stainless steel. The bottom electrode is grounded whereas the upper electrode is connected to a high voltage power supply (50kV, 50Hz) and can be adjusted according to the size of the sample. About 50 mL of the sample was poured into the sterile borosil Petri plates of 90mm diameter and 15.5 mm depth. The depth of the sample was 14.5 mm. the gap between the high voltage electrode and the surface of the sample was kept constant at 10mm and the total gap between the two electrodes was 25.5 mm. The treatments were conducted with an input voltage of 35kV at two different treatment times (2 min and 3 min). The later system setup consists of two electrodes made of stainless steel. The upper electrode or the power electrode is a rod-shaped electrode surrounded by a glass tube acting as the dielectric in this CBD system. The bottom electrode is a stainless-steel plate electrode that is connected to the ground. In this system, argon gas is used as the plasma medium. The samples were poured into the sterile borosil glass beaker of volume 150mL. The experiment was conducted at 30kV for 2 min and 3 min respectively. The treated samples from both systems were transferred to sterilized PET bottles using sterile syringes, labelled and stored at refrigerated conditions until further analysis and throughout the storage studies.

Preparation of Custard Apple Juice Milk Beverage (CAJMB)

Fresh custard apple was procured from the local vendors. Custard apple pulp was prepared according to the procedure of (Ramesh et al. 2017) with slight modifications. The mucilage and the seed were separated manually. The pulp was blended using a blender and was filtered using a muslin cloth and was stored in a separate sterile PET bottle. The custard apple milk beverage was prepared by mixing 65% pasteurized cow milk, 35 % custard apple pulp and 5% of powdered sugar, in a mixing jar and blended well using a blender (Bakane et al. 2016). The prepared beverage was stored in sterile PET bottles at 4°±2° C temperature for further non-thermal plasma processing within 24hrs.

pH

The pH of the treated and control CAJMB sample was analysed using a digital hand pH meter (Eco Testr pH 1, Eutech instrument, OAKTON) after calibrating with pH 4 and pH 7 buffer standards solutions.

Titratable acidity

The treated and control CAJMB samples were evaluated for titratable acidity, 10 ml of sample was taken in a conical flask or beaker and titrated with 0.1 N sodium hydroxide using phenolphthalein as an indicator. The endpoint was noted when a pink color was noticed and persisted for about 30 sec (AOAC International methods, 1999). the volume of NaOH consumed was noted and further, the acidity was calculated in terms of lactic acid and expressed as ml/ L. The titratable acidity was computed using the relationship in the equation.

$$\text{Acidity} = \frac{\text{Base titrant mL} \times \text{Normality of base} \times \text{Acid factor}}{\text{Sample volume mL}}$$

Sensory evaluation

Sensory evaluation of control and treated samples was done by a subjective test based on a 9-point hedonic scale (Gupta, 1976). Instructions were given to the panellists to rinse their mouths with drinking water after tasting every sample. The panellists were instructed to examine the sample for the attributes of colour, mouthfeel and flavour.

Shelf life and storage studies

The pH and acidity of the stored samples were assayed at steady intervals of 2 days throughout the storage period.

Statistical analysis

SPSS version 20 statistical software (SPSS, Inc., United States) was used for data analysis. All the experiments were done in six replicates. The statistical differences in the analysed parameters of the plasma-treated CAJMB samples considering the varying voltage and treatment time were calculated using Analysis of variance (ANOVA). Multivariate Duncan's test ($p < 0.05$) was used to evaluate statistical differences between treatments and a significant difference was defined at $p < 0.05$. The developed CAJMB samples were treated with maximum treatment conditions from both the systems viz., T_2 - 35kV, 3min and T_4- 30kV, 3min. These two treatments were statistically analysed using the results of the pH and TA obtained to compare the impact of CP generated from different configurations of the CBD system used in this study on CAJMB.

Results and Discussion

pH

The pH values of the control and treated samples processed using both the HV ACBD parallel plate system and the HV CBD plasma torch over the storage period are shown in Table 1 and Table 3. The table depicts a significant decrease in the pH of the treated samples with an increase in voltage and treatment time compared to the control untreated sample immediately after processing. The pH of the fresh control sample was 6.39 ± 0.018 and the pH of the HV ACBD plasma plate system treated samples immediately after processing was between the mean of 6.29 and 6.32 respectively. The pH of the HV CBD plasma torch treated samples ranged between mean values of 6.3 and 6.33. Despite the statistical difference, the values of pH ranged within the expected levels. However, there was no significant difference in the pH within the treatments after processing. The decline in pH in treated samples compared to the control was 0.07 and 0.1, 0.09 and 0.06 for the HV ACBD plate system and HV CBD plasma torch respectively. The pH value showed a highly significant descending trend in both control and treated samples throughout the storage period, even though there was no significant difference observed between the control and treated samples of CAJMB in both the systems during the second day of storage.

Titratable acidity

Titratable acidity (TA) of the cold plasma processed CAJMB was highly significantly higher to the control sample. There was a significant increase in the titratable acidity in the samples immediately after processing as shown in Table 2 and Table 4. The TA of the fresh control sample was 0.128 ± 0.002 whereas the treated samples T_1 and T_2 had TA of mean in the range of 0.148 and 0.162 at the immediate point of the assay. The titratable acidity of each of the treated CAJMB also showed a considerable significant difference between each other. The TA of the samples processed in the HV CBD plasma torch system ranged between 0.151 and 0.144 for treatments T_3 and T_4 correspondingly. However, there was no significant difference within the treatments as it was observed within the treated samples processed in HV ACBD parallel plate plasma system. The observed titratable acidity values at specified intervals showed a noteworthy and exceedingly significant increase in the control and treated samples across the storage period, although there was no substantial difference during the second day of storage.

Sensory

Sensory evaluation of the control and treated custard apple milk beverage (CAJMB) was carried out immediately after the processing (zeroth day). Though there was a significant increase in titratable acidity and decrease in pH, the sensory scores for colour, mouthfeel and flavour of the developed cold plasma processed CAJMB showed no significant difference ($P > 0.05$, from Table 7.) compared to the control sample. The results obtained for the processed samples exhibit much liking and acceptable scores of more than 9.00 (liked extremely).

Statistical comparison of the physicochemical quality parameters of the cold plasma treated samples in the HVACP CBD plasma system and HV CBD plasma torch

_From Table 5 and Table 6, it was noticed that the pH and TA of the treatments T_2 and T_4 were significantly different but in a very narrow range at the beginning of the storage period excepting the second day. Also, a trivial increase in temperature by 2° to 3° was observed in the HV CBD plasma-torch treated samples. However, there was a noteworthy difference in the decline of pH and increase in TA between the treated samples (T_2 and T_4) as the storage days increased (on the 10^{th} day). It was noted that the pH and acidity of the T_2 treated samples at the end of the 10^{th} day showed an acceptable level for the developed CAJMB compared to the T_4 treated sample and control sample. From Tables 5 and 6, a gradually decreasing drift and ascending level of pH and TA could be observed as it moved towards the tenth day of storage. pH and TA as one of the primary quality attributes of a food product since the increase in TA and decrease in pH can be associated with the growth of lactic acid bacteria, especially in milk products (data not presented). Hence, from Tables 5 and 6 it is observed that the HV ACBD plasma system has shown a slow decline in the pH and TA and prolonged the acceptable level of the sample till the 10th day of storage. Whereas, the control sample and T_4 sample reached unacceptable levels the same level on the 2nd day and 6th day of storage respectively.

Effect of the generated High Voltage Cold Barrier Discharge plasma on the parameters analysed (pH, TA and sensorial attributes) in the developed CAJMB

The results found in this study, from Tables 1, 2, 3, and 4 showed a significant decline in pH and an increase in TA after CP processing. These two parameters viz., pH and TA are the primary factors that contribute to the freshness of food samples. This decrease in PH and increase in TA immediately after treatment might be potentially due to the reaction of plasma-generated reactive species like reactive oxygen species (ROS) and Reactive nitrogen species (RNS) with the moisture (i.e., water molecules) present in the food sample. (Muhammad et al.2018). These reactions lead to the formation of acidic compounds such as nitric acid, H_2O_2, and nitrous acids formed by the hydrolysis of NO and NO_2 reactive species and other elements in plasma-treated liquid samples (Zheng et al. 2019) resulting in a lessening of pH (Liu et al. 2015). Increased reduction in pH and increase in TA has been largely noted in the cases where nitrogen is used as the gas medium (Misra et al. 2016). The surge in acidity may also be owing to the split-up of amino acids (AA) that exists in the sample by plasma reactive species and diffusion of the same in the sample solution (Buβler et al. 2015) attributed to the formation of carboxylic groups from AA disintegration. The increase in the intensity of the voltage applied, gas flow rate and the treatment

Table 1: pH of HV ACBD plasma processed CAJMB at refrigerated condition during storage period

Treatment	pH						F VALUE
	0th day	2nd day	4th day	6th day	8th day	10th day	
T_1 – 35 kV, 2min	$6.32±0.021^{Ae}$	$6.30±0.017^{Ade}$	$6.25±0.030^{Bd}$	$6.14±0.018^{Bc}$	$6.07±0.017^{Bb}$	$5.72±0.018^{Ba}$	118.054**
T_2 – 35kV, 3 min	$6.29±0.017^{Ae}$	$6.25±0.030^{Ade}$	$6.20±0.018^{Bcd}$	$6.17±0.017^{Bbc}$	$6.12±0.018^{Bb}$	$6.02±0.007^{Ca}$	26.040**
C	$6.39±0.018^{Bf}$	$6.24±0.017^{Ae}$	$6.10±0.018^{Ad}$	$5.81±0.017^{Ac}$	$5.20±0.021^{Ab}$	$5.00±0.017^{Aa}$	1134.616**
F VALUE	7.406 *	2.143^{NS}	11.467**	189.259**	753.142**	1203.152**	

A, B, C Values having varying alphabetical superscripts are significantly different corresponding to treatment voltage and treatment time (between treatments) (p < .05);
a,b,c,d,e Values having varying alphabetical superscripts are significantly different corresponding to storage period (p Â .05).
Superscripts in the F value indicates: ** - Highly significant (p< 0.01); * - significant (p< 0.05); NS – Non significant

Table 2: Acidity of HV ACBD plasma processed cajmb at refrigerated condition during storage period

Treatments	ACIDITY						F VALUE
	0th day	2nd day	4th day	6th day	8th day	10th day	
T_1–35 kV, 2min	$0.148±0.002^{Ba}$	$0.181±0.030^{Aab}$	$0.206±0.002^{Bbc}$	$0.211±0.018^{Abc}$	$0.224±0.017^{Abc}$	$0.258±0.018^{Ac}$	4.690**
T_2–35kV, 3 min	$0.162±0.001^{Ca}$	$0.175±0.021^{Aab}$	$0.173±0.014^{Aab}$	$0.207±0.017^{Aabc}$	$0.219±0.018^{Abc}$	$0.225±0.007^{Ac}$	3.260*
C	$0.128±0.002^{Aa}$	$0.213±0.002^{Ab}$	$0.237±0.002^{Bb}$	$0.287±0.018^{Bc}$	$0.312±0.007^{Bcd}$	$0.340±0.021^{Bc}$	41.860**
F VALUE	88.371**	0.923^{NS}	14.037**	6.583*	12.466**	12.545**	

A, B, C Values having varying alphabetical superscripts are significantly different corresponding to treatment voltage and treatment time (between treatments) (p < .05);
a,b,c,d,e Values having varying alphabetical superscripts are significantly different corresponding to storage period (p Â .05).
Superscripts in the F value indicates: ** - Highly significant (p< 0.01); * - significant (p< 0.05); NS – Non significant

Table 3: pH of HVCBD plasma processed CAJMB at refrigerated condition during storage period

Treatments	pH						F VALUE
	0th day	2nd day	4th day	6th day	8th day	10th day	
T_3-30 kV,2min	$6.33±0.014^{Ad}$	$6.27±0.017^{Ac}$	$6.23±0.030^{Bc}$	$6.15±0.017^{Bb}$	$6.10±0.018^{Bb}$	$5.70±0.007^{Ba}$	150.475**
T_4-30 kV,3min	$6.30±0.017^{Ad}$	$6.26±0.019^{Acd}$	$6.21±0.030^{Bc}$	$6.12±0.018^{Bb}$	$6.07±0.017^{Bb}$	$5.76±0.018^{Ca}$	92.893**
C	$6.39±0.018^{Bf}$	$6.24 ±0.017^{Ae}$	$6.10±0.018^{Ad}$	$5.81±0.007^{Ac}$	$5.20±0.021^{Ab}$	$5.00±0.017^{Aa}$	1134.616**
F VALUE	8.700**	0.728^{NS}	7.047*	168.072**	735.320**	781.670**	

A, B, C Values having varying alphabetical superscripts are significantly different corresponding to treatment voltage and treatment time (between treatments) (p < .05);
a,b,c,d,e Values having varying alphabetical superscripts are significantly different corresponding to storage period (p Â .05).
Superscripts in the F value indicates: ** - Highly significant (p< 0.01); * - significant (p< 0.05); NS – Non significant

time results in the amplified concentration of different plasma-produced elements in the liquid products (Yong et al. 2015), such as ROS, RNS, free radicals, ions, energized and non-energized atoms (Pankaj et al. 2017a). This might lead to the production of higher acidogenic molecules, influencing the pH of the sample (Yong et al. 2015; Helmke et al. 2011). The increase in acidity in the sample (CAJMB) distinguished after CP processing might be caused by consecutive reactions such as oxidation of aldehydes ignited by the plasma species such as O_3, OH and NO with water molecules present in the aqueous-air interphase (Liu et al. 2010). It could also be due to the breakdown of hydroxyl radical produced in the plasma discharge process as reported by Muhammad et al. (2019) for the CP-treated Tiger Nut Milk (TNM) and other researchers in various studies (Coutinho et al. 2019). The impact of plasma on the physico-chemical quality parameters such as the difference in pH of the food sample is also attributable to its buffering capacity, protein and phosphate content as in milk (Ponraj et al. 2017) and acid content (Kim et al. 2015) which has been observed in cases cold plasma treated Guava Fruit juice Whey Beverage (GFWB) and orange juice and milk (M. R. Silveira et al. 2019; Xu et al. 2017; Manoharan et al. 2021). Though there was a decrease in pH and acidity, it was only in a narrow range, causing no effect on the major biomolecule dissolving power in the treated food samples of CAJMB. pH and acidity are considered important quality traits in processed food samples whose sweeping alteration can impact the sensorial parameters and shelf life (Pankaj et al. 2018). Noteworthy slight changes in those parameters did not affect the quality of the developed beverage in this study (CAJMB), which was practically shown by the non-significant change in the sensorial parameters examined after CP treatment which has been reported by various workers (Li and Xiong, 2021; Eazhumalai et al. 2021).

Table 4: Acidity of HVCBD plasma processed CAJMB at refrigerated condition during storage period

Treatments	ACIDITY						F VALUE
	0^{th} day	2^{nd} day	4^{th} day	6^{th} day	8^{th} day	10^{th} day	
T_3-30 kV,2min	0.151 ± 0.003^{Ba}	0.170 ± 0.017^{Aab}	0.209 ± 0.003^{Abc}	0.224 ± 0.017^{ABbc}	0.231 ± 0.018^{Ac}	0.242 ± 0.007^{Ac}	8.085**
T_4-30 kV,3min	0.144 ± 0.002^{Ba}	0.196 ± 0.017^{Aab}	0.212 ± 0.006^{Ab}	0.222 ± 0.018^{Ab}	0.237 ± 0.017^{Ab}	0.245 ± 0.018^{Ab}	6.310**
C	0.128 ± 0.002^{Aa}	0.212 ± 0.005^{Ab}	0.237 ± 0.002^{Bbc}	0.287 ± 0.018^{Bcd}	0.312 ± 0.007^{Bde}	0.340 ± 0.021^{Be}	40.971**
F VALUE	23.436**	2.174^{NS}	14.932**	4.426*	9.280**	11.113**	

A, B, C Values having varying alphabetical superscripts are significantly different corresponding to treatment voltage and treatment time (between treatments) ($p < .05$);
a,b,c,d,e Values having varying alphabetical superscripts are significantly different corresponding to storage period ($p < .05$).
Superscripts in the F value indicates: ** - Highly significant ($p< 0.01$); * - significant ($p< 0.05$); NS – Non significant

Table 5: Comparative analysis of pH of effective treatments of each configuration of the plasma systems

Treatments	pH						F VALUE
	0^{th} day	2^{nd} day	4^{th} day	6^{th} day	8^{th} day	10^{th} day	
T_2 – 35kV, 3min	6.29 ± 0.017^{Ae}	6.25 ± 0.030^{Ade}	6.20 ± 0.018^{Bcd}	6.17 ± 0.017^{Cbc}	6.12 ± 0.018^{Bb}	6.02 ± 0.007^{Ca}	26.040**
T_4 – 30kV, 3min	6.30 ± 0.017^{Ad}	6.26 ± 0.019^{Acd}	6.21 ± 0.030^{Bc}	6.12 ± 0.018^{Bb}	6.07 ± 0.017^{Bb}	5.76 ± 0.018^{Ba}	92.893**
C	6.39 ± 0.018^{Bf}	6.24 ± 0.017^{Ae}	6.10 ± 0.018^{Ad}	5.81 ± 0.007^{Ac}	5.20 ± 0.021^{Ab}	5.00 ± 0.017^{Aa}	1134.616**
F VALUE	10.603**	0.167^{NS}	7.274*	180.405**	753.142**	1230.006**	

A, B, C Values having varying alphabetical superscripts are significantly different corresponding to treatment voltage and treatment time (between treatments) ($p < .05$);
a,b,c,d,e Values having varying alphabetical superscripts are significantly different corresponding to storage period ($p < .05$).
Superscripts in the F value indicates: ** - Highly significant ($p< 0.01$); * - significant ($p< 0.05$); NS – Non significant

Table 6: Comparative analysis of Titratable Acidity of effective treatments of each configuration of plasma systems

Treatments	ACIDITY						F VALUE
	0^{th} day	2^{nd} day	4^{th} day	6^{th} day	8^{th} day	10^{th} day	
T_2–35kV, 3min	0.162 ± 0.001^{Ca}	0.175 ± 0.021^{Aab}	0.173 ± 0.014^{Aab}	0.207 ± 0.017^{Aabc}	0.219 ± 0.018^{Abc}	0.225 ± 0.007^{Ac}	3.260*
T_4–30kV,3min	0.144 ± 0.002^{Ba}	0.196 ± 0.017^{Ab}	0.212 ± 0.006^{Bbc}	0.222 ± 0.018^{Abc}	0.237 ± 0.017^{Abc}	0.245 ± 0.018^{Ac}	6.310**
C	0.128 ± 0.002^{Aa}	0.212 ± 0.005^{Ab}	0.237 ± 0.002^{Bb}	0.287 ± 0.018^{Bc}	0.312 ± 0.007^{Bcd}	0.340 ± 0.021^{Bd}	40.971**
F VALUE	78.234**	1.410^{NS}	12.380**	5.858*	11.084**	13.506**	

A, B, C Values having varying alphabetical superscripts are significantly different corresponding to treatment voltage and treatment time (between treatments) ($p < .05$);
a,b,c,d,e Values having varying alphabetical superscripts are significantly different corresponding to storage period ($p < .05$).
Superscripts in the F value indicates: ** - Highly significant ($p< 0.01$); * - significant ($p< 0.05$); NS – Non significant

Table 7: Sensory analysis of custard apple juice milk beverage before and after CP processing

CBD - Configuratio	Treatments	SENSORY – 0th d		
		COLOUR	MOUTHFEEL	FLAVOUR
HV CBD Parallel	T_1 – 35 kV, 2min	9.92 ± 0.083	9.83 ± 0.105	9.42 ± 0.083
Plate plasma	T_2 – 35kV, 3 min	9.92 ± 0.083	9.75 ± 0.112	9.50 ± 0.129
HV CBD Plasma	T_3- 30 kV, 2min	9.92 ± 0.083	9.83 ± 0.105	9.42 ± 0.154
Torch using Argon gas	T_4 - 30 kV, 3min	9.92 ± 0.083	9.67 ± 0.167	9.50 ± 0.183
Control	C	9.92 ± 0.083	9.83 ± 0.105	9.67 ± 0.167
	F VALUE	0^{NS}	0.377^{NS}	0.481^{NS}

A, B, C Values having varying alphabetical superscripts are significantly different corresponding to treatment voltage and treatment time (between treatments) ($p < .05$);
a,b,c,d,e Values having varying alphabetical superscripts are significantly different corresponding to storage period ($p < .05$).
NS – Non significant

The significant decline in pH and a significantly slight increase in titratable acidity (TA) post CP processing observed in this study with CAJMB was similar to the results of Eazhumalai et al. (2021) in the treatment of oat milk. In the research, where cashew apple juice was treated with a spark DBD plasma, similar results of a slight descending level in pH were observed which was maintained during storage (Illera et al. 2019) supporting the discoveries of Dasan & Boyaci (2018) in the CP processed sour cherry nectar. In their study, Eazhumalai et al. (2021) reported that, decrease in pH and an increase in TA were directly proportional to significant increase in treatment time and no substantial difference has been observed due to voltage. It was also substantiated by the outcomes reported in the non-thermal Plasma treatment of chocolate milk (Coutinho et al. 2019), Tiger Nut Milk (Muhammad et al. 2019), plasma processed milk (Kim et al. 2015), Pea protein extract (Bubler et al. 2015), guava flavoured whey beverage (GFWB) (M. R. Silveira et al. 2019), High Voltage Atmospheric Cold Plasma (HVACP) treated orange juice (OJ) (Xu et al. 2017), Apple juice treated in DBD ACP (Liao et al. 2018; Xiang et al. 2018), where the pH decreased gradually with the increase in the treatment time, gas flow rate and applied voltage accordingly. But, when compared to thermal treatment (pasteurization, retort, etc.,) of milk drinks and beverages, the pH reduction during cold plasma processing was significantly less. Hence, the CP processed beverages had relatively higher pH value than in the heat-processed samples (M. R. Silveira et al. 2019). This is because, during heat treatment, the lactose present in the milk through various reactions form acids such as formic acid, acetic acid, etc and precipitation of calcium phosphate releasing H^+ ions, which consequently increases the acidity of the beverage (Dursun et al. 2017; Fox et al. 1981). Similarly, a slight significant reduction in pH in the treatment of prebiotic orange juice (OJ) at 20 kV with direct and indirect exposure to plasma compared to untreated samples has been reported by Almeida et al. (2015). In their study, they also stated that there was no effect of treatment time on the decrease in pH and there was no change in pH has been observed between the ozone-treated and control samples (Almeida et al. 2015). This study was in corroboration with the findings of Manoharan et al. (2021) and Kim et al. (2015) in the cold plasma treatment of milk, and Ponraj et al. (2017) in the Argon gas CP treated milk. Also, tomato juice after Cold plasma processing showing statistically no drastic or noteworthy decrease in pH among the treatments but a very narrow reduction was observed between treatment and control (Ali et al. 2021). Whereas, Xu et al. (2017) reported no significant change in the CP processed orange juice at 90kV. These results were supported by the data found in the cold plasma treatment of tender coconut water beverage with ascorbic acid and tomato-based beverage and White grape juice (Chutia and Mahanta, 2021; Mehta et al. 2019; Pankaj et al. 2017b). A similar report for no variations among control and treatments was submitted for the nonthermal processing like Ultra Sonication (US) (Caminiti et al. 2012) and Pulsed Electric Field (PEF) treatment of orange juice (Tiwari et al. 2008), Ultra Violet treatment (UV) of Apple juice (Tiwari et al. 2008), UV & US processed tomato beverage. But in the case of PEF processed TNM, a mild decrease in pH was observed at 20kV/ cm for 300µs. Also, in another study, no decrease or increase in pH of PEF processed orange juice milk beverage (OJMB) has been noted after treatment but a slow decline was observed across storage due to lactic acid formation by microbial growth (Sampedro et al. 2009). This result was in accordance with Rivas et al. (2006), in the PEF treatment of carrot juice which was comparable to the immediate observations after the cold plasma processing of carrot juice with respect to both voltage and processing time (Muhammad Umair et al. 2019).

Conclusion

The developed CAJMB was subjected to cold plasma with varying treatment time and voltage in two different configurations of Dielectric Barrier Discharge plasma (DBD) and was analyzed for physico-chemical quality parameters viz., pH and acidity. The data obtained were compared with the data of the control sample statistically. The observed results showed a slight variation of decrease in pH and increase in acidity due to the reaction between the components present in the food matrix of the sample and plasma reactive species. But, during the storage studies, the quality attributes were within the acceptable level for more days in the treated samples compared to control samples respectively. This exhibits the decontamination effect of plasma species on microbes slowing down their growth in the treated samples. The key finding in this study was that the CAJMB processed in

HVACBD parallel plate plasma system at 35kV for 3min (T_2) revealed promising results among other treatments, keeping pH and acidity within control limits for 9 to 10 days of storage in refrigeration temperature. Whereas, the control sample crossed the acceptable range in 3 to 5 days of storage. Thus, cold plasma shows to be potentially effective in extending the shelf life of milk-based beverages.

Acknowledgement

This research was funded by Tamil Nadu State Council for Science and Technology, Student Project Scheme -2021-2022 (TNSCST SPS). The authors of this article acknowledge the support rendered by the College of Food and Dairy Technology, Koduveli, Chennai – 52 and the authors also thank the Cold Plasma technical support rendered by Dr. Z. H. Sholapurwala, Managing Director, Zeonics Systech Defence and Aerospace Engineers (P) Ltd., Bangalore.

References

Ali M, Cheng JH,Sun DW (2021) Effects of dielectric barrier discharge cold plasma treatments on the degradation of anilazine fungicide and quality of tomato (Lycopersicon esculentum Mill) juice. Int J Food Sci Technol 56: 69–75

Almeida FDL, Cavalcante RS, Cullen PJ, Frias JM, Bourke P, Fernandes FA, Rodrigues S (2015) Effects of atmospheric cold plasma and ozone on prebiotic orange juice. Innovative Food Sci Emerging Technol 32: 127–135

AOAC International Official Methods of Analysis. AOAC International. Gaithersburg, MD, USA, 1999

Bakane PH, Khakare MM, Gajabe MH, Khedkar MB (2016) Standardization of Process for Custard Apple Milk Shake. Int J Environ Agricd Biotechnol 1: 2456-1878

Bourke P, Ziuzina D, Boehm D, Cullen PJ, Keener K (2018) The potential of cold plasma for safe and sustainable food production. Trends in Biotechnol 36: 615–626

Bußler S, Steins V, Ehlbeck J & Schlüter O (2015) Impact of thermal treatment versus cold atmospheric plasma processing on the techno-functional protein properties from Pisum sativum "Salamanca". J Food Eng 167: 166–174

Caminiti IM, Palgan I, Muñoz A, Noci F, Whyte P, Morgan DJ, Cronin DA, Lyng JG (2012) The effect of ultraviolet light on microbial inactivation and quality attributes of apple juice. Food Bioprocess Technol 5: 680–686

Chutia H, Mahanta CL (2021) Influence of cold plasma voltage and time on quality attributes of tender coconut water (Cocos nucifera L.) and degradation kinetics of its blended beverage. J Food Processing Preserv 45: e15372

Coutinho NM, Silveira MR, Fernandes LM, Moraes J, Pimentel TC, Freitas MQ , Rodrigues S (2019) Processing chocolate milk drink by low-pressure cold plasma technology. Food Chem 278: 276–283

Coutinho NM, Silveira MR, Rocha RS, Moraes J, Ferreira MVS, Pimental TC, Monica QF, Marcia CS, Renata SLRC, Fabio SR, Simone OB, Fabiano PM, Sueli PMF, Cruz AG (2018) Cold plasma processing of milk and dairy products. Trends Food Sci Technol 74: 56-68

Dasan BG, Boyaci IH (2018) Effect of cold atmospheric plasma on inactivation of Escherichia coli and physicochemical properties of apple, orange, tomato juices, and sour cherry nectar. Food Bioprocess Technol 11: 334–343

Dursun A, Güler Z, Şekerli YE (2017) Characterization of volatile compounds and organic acids in ultra-high-temperature milk packaged in tetra brik cartons. Int J Food Prop 20: 1511–1521

Eazhumalai G, Ranjitha Gracy TK, Mishra A, Annapure US (2021) Atmospheric pressure nonthermal pin to plate plasma system for the microbial decontamination of oat milk. J Food Process Preserv 00: e16181

Fox PF (1981) Heat-induced changes in milk preceding coagulation. J Dairy Sci 64: 2127–2137

Gupta SA (1976) Sensory evaluation of food, Indian Dairyman 28: 293-295

Helmke A, Hoffmeister D, Berge F, Emmert S, Laspe P, Mertens N, Vioel W, Weltmann KD (2011) Physical and microbiological characterisation of staphylococcus epidermidis inactivation by dielectric barrier discharge plasma. Plasma Processes Polymers 8: 278–286

Illera AE, Chaple S, Sanz MT, Ng S, Lu P, Jones J, Carey E, Bourke P (2019) Effect of cold plasma on polyphenol oxidase inactivation in cloudy apple juice and on the quality parameters of the juice during storage. Food Chem 3: 1–10

Kim H, In H, Park S, Kim K, Choe W, Jo C (2015) Microbial safety and quality attributes of milk following treatment with atmospheric pressure encapsulated dielectric barrier discharge plasma. Food Control. 47: 451–456.

Li R, Xiong YL (2021) Sensitivity of oat protein solubility to changing ionic strength and pH. J Food Sci 86: 78–85

Liao X, Li J, Muhammad AI, Suo Y, Chen S, Ye X, Ding T (2018) Application of a dielectric barrier discharge atmospheric cold Plasma (Dbd-Acp) for Eshcerichia coli inactivation in apple juice. J Food Sci 83: 401–408

Liao X., Liu D, Xiang Q, Ahn J, Chen S, Ye X, Ding T (2017) Inactivation mechanisms of non-thermal plasma on microbes: A review. Food Control. 75: 83–91

Liu F, Sun P, Bai N, Tian Y, Zhou H, Wei S, Zhou Y, Zhang J, Zhu W, Becker K, Fang J (2010) Inactivation of bacteria in an aqueous environment by a direct-current, cold-atmospheric-pressure air plasma microjet. Plasma Processes Polymer 7: 231–236

Liu ZC, Liu DX, Chen C, Li D, Yang AJ, Rong MZ, Chen HL, Kong MG (2015) Physicochemical processes in the indirect interaction between surface air plasma and deionized water. J Physics D: Appl Physics 48: 495201

Manoharan D, Stephen J, Radhakrishnan M (2021) Study on low-pressure plasma system for continuous decontamination of milk and its quality evaluation. J Food Process Preserv 45: e15138.

Mehta D, Sharma N, Bansal V, Sangwan RS and Yadav SK (2019). Impact of ultrasonication, ultraviolet and atmospheric cold plasma processing on quality parameters of tomato-based beverage in comparison with thermal processing. Innovative Food Sci Emerging Technol 52:343–349

Misra NN, Pankaj SK, Segat , Ishikawa K (2016) Cold plasma interactions with enzymes in foods and model systems. Trends in Food Sci Technol 55: 39–47

Montejano MRC, Escandón CMC, Lopez VEE, Canton IC, Velázquez MGN and Arguello GS (2019) Construction of a power electronic source for cold plasma generation. Ingeniería Investigación y Tecnología 10: 1-12

Muhammad AI, Li Y, Liao X, Liu D, Ye X, Chen S, Hu Y, Wang J, Ding T (2019) Effect of dielectric barrier discharge plasma on background microflora and physicochemical properties of tiger nut milk. Food Control 96: 119–127

Muhammad AI, Xiang Q, Liao X, Liu D & Ding T (2018) Understanding the impact of nonthermal plasma on food constituents and microstructure - Areview. Food Bioprocess Technol 1: 463–486.

Muhammad Umair, Saqib Jabbar, Mustapha Muhammad Nasiru, Tayyaba Sultana, Ahmed M. Senan, Faisal Nureldin Awad, Zhuang Hong, Jianhao

Zhang (2019) Exploring the Potential of High-Voltage Electric Field Cold Plasma (HVCP) Using a Dielectric Barrier Discharge (DBD) as a Plasma Source on the Quality Parameters of Carrot Juice. Antibiotics. 8: 235

Pan Y, Cheng JX, Lu X , Sun DW (2019) Assessing the inactivation efficiency of Ar/O2 plasma treatment against listeria monocytogenes cells: Sublethal injury and inactivation kinetics. LWT-Food Sci Technol 111:318–327

Pankaj SK, Keener KM (2017a) Cold plasma: Background, applications and current trends. Curr Opin Food Sci 16: 49- 52

Pankaj SK, Wan Z , Keener KM (2018) Effects of cold plasma on food quality: A review. Foods 7: 4.

Pankaj SK, Wan Z, Colonna W, Keener KM (2017b) Effect of high voltage atmospheric cold plasma on white grape juice quality. J Sci Food Agric 97: 4016–4021

Ponraj SB, Sharp JA, Kanwar JR, Sinclair AJ, Kviz L, Nicholas KR, Dai XJ (2017) Argon gas plasma to decontaminate and extend shelf life of milk. Plasma Processes and Polymers. 14: 1–8

Ramesh Avhad, Vaibhav Patil, Navnath Sarode (2017) Standardization and Physicochemical Properties of Custard Apple Soya Milk Shake. Int J Currt Microbiol Appl Sci 6: 1811-1817

Rivas A, Rodrigo D, Martinez A, Barbosa-Canovas GV, Rodrigo M (2006) Effect of PEF and heat pasteurization on the physical–chemical chemical characteristics of blended orange and carrot juice. Lebensmittel - Wissenschaft and Technologie.(LWT) 39:1163–1170

Sampedro F, Geveke DJ, Fan X, Rodrigo D, Zhang QH (2009) Shelf life study of an orange juice–milk based beverage after PEF and thermal processing. J Food Sci 74:S107-112

Silveira MR, Coutinho NM, Rocha RS, Moraes J, Esmerino EA, Pimentel TC, Freitas MQ, Silva MC., Raices RSL, Senaka Ranadheera C, Borges FO, Fonteles TV, Neto RPC, Tavares MIB, Fernandes FAN, Rodrigues S, Cruz AG (2019) Guava flavored whey-beverage processed by cold plasma: Physical characteristics, thermal behavior and microstructure. Food Res Int 119: 564-570

Solanke SB, Bakane PH, Gawande AB (2019) Technological, Nutritional Approach, Processing and Storage of Custard Apple (Anonna squamosa). Review. Int J Curr Microbiol Appl Sci 8: 2766-2775

Surowsky BO, Schluter, Knorr D (2015) Interactions of non-thermal atmospheric pressure plasma with solid and liquid food systems: A review. Food Eng Rev 7:82–108

Tiwari BK, Muthukumarappan K, O'Donnell CP, Cullen PJ (2008) Kinetics of freshly squeezed orange juice quality changes during ozone processing. J Agric Food Chem 56: 6416–6422

Xiang Q, Liu X, Li J, Liu S, Zhang H, Bai Y (2018) Effects of dielectric barrier discharge plasma on the inactivation of Zygosaccharomyces rouxii and quality of apple juice. Food Chem 254: 201–207

Xu L, Garner AL, Tao B, Keener KM (2017) Microbial inactivation and quality changes in orange juice treated by high voltage atmospheric cold plasma. Food Bioprocess Technol 10: 1778–1791

Yong HI, Kim HJ, Park S, Kim K, Choe W, Yoo SJ, Jo C (2015) Pathogen inactivation and quality changes in sliced cheddar cheese treated using flexible thin layer dielectric barrier discharge plasma. Food Res Int 69: 57-63

Zheng Y, Wu S, Dang J, Wang S, Liu Z, Fang J, Han P, Zhang J (2019) Reduction of phoxim pesticide residues from grapes by atmospheric pressure non-thermal air plasma activated water. J Hazardous Mater 377: 98–105