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Guava flavored whey-beverage processed by cold plasma: Physical characteristics,  
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## Abstract

The present study aimed to compare the physicochemical (pH), physical (rheology parameters and particle size), microstructure (optical microscopy) and thermal properties (differential scanning calorimetry) of guava flavored whey-beverages submitted to cold plasma technology in different processing time (5, 10, and 15 min) and gas flow (10, 20, and 30 mL min<sup>-1</sup>) conditions with a conventional pasteurized product. Whey beverages treated by cold plasma presented higher pH values, lower consistency and lower viscosity, and a flow behavior index similar to Newtonian fluids. Milder cold plasma conditions resulted in whey beverages with higher pH, lower viscosity and consistency, and similar particle distribution and microstructure compared to the pasteurized product. In contrast, more severe processing conditions resulted in a higher particle surface area ([D 3,2]) and smaller particles (~10 µm), due to the decrease in the number of larger particles (1000 µm), cell rupture, the formation of cell fragments, and higher viscosity and consistency. The treatments did not affect the thermal properties (enthalpy and bound water) of any sample.

**Key-words:** cold plasma; guava flavored whey-beverage; physical characteristics; microstructure

## 1.Introduction

Whey, a valuable source of highly valued nutraceutical compounds, is a byproduct of cheese production and has attracted much attention due to its nutritional and functional properties (Chavan, Shraddha, Kumar & Nalawade, 2015; Seyhan et al., 2016). There is a great demand for whey protein consumption due to its potential health benefits, including satiety increase, metabolic regulation and weight loss (Hector et al., 2015; Nilsson, Holst & Björck, 2007; Zafar, Waslien, AlRaefaei, Alrashidi, & AlMahmoud, 2013).

In this context, whey-based fruit beverages have received remarkable attention due to its growing market and high nutritional value. When used as a flavoring agent, guava (*Psidium guajava* L.) provides a pool of vitamins and minerals, high levels of polyphenolic antioxidants, phenolic compounds, carotenoids, flavonoids, triterpenoids, and other biologically active compounds (Blancas-Benitez, Pérez-Jiménez, Montalvo-González, González-Aguilar & Sáyago-Ayerdi, 2018).

In general, whey beverages are conventionally subjected to pasteurization or sterilization treatments to eliminate pathogens and spoilage microorganisms, increasing the shelf life of the product (Porcellato, Aspholm, Skeie, Monshaugen, Brendehaug & Mellegård, 2018). However, those treatments might have negative effects on the food matrix, causing changes in the physicochemical, nutritional, and sensory properties, such as protein denaturation, non-enzymatic browning (Maillard reaction), and nutrients, bioactive compounds, and flavor compounds degradation (Herceg, Kovačević, Kljusurić, Jambrak, Zorić, & Dragović-Uzelac, 2016).

Cold plasma technology has emerged as an interesting alternative to traditional heat treatment (Liao et al., 2017), being defined as an ionized gas made up of neutral molecules, electrons, and positively and negatively charged particles,

with multiple interactions (Pankaj & Keener, 2017). The product subjected to cold plasma processing presents greater preservation of thermosensitive compounds and physicochemical and sensory characteristics, once the processing occurs at room temperature (Coutinho et al., 2018).

Although the ability of cold plasma to decontaminate food has been investigated (Coutinho et al., 2018), the impact on the physicochemical characteristics and the retention of compounds with health benefits have not been extensively studied in dairy products. Some authors have studied cold plasma processing of milk (Korachi et al., 2015), cheese (Yong et al., 2015), milk fat (Saragapani, Keogh, Dunne, Bourke, & Cullen, 2017) and whey protein isolate (Segat, Misra, Cullen & Innocente, 2015).

The main processing parameters involved in the cold plasma technology include gas type, treatment time, gas flow, electric current intensity, among others, and are related to the efficiency in microbial inactivation (Liao et al., 2017). The number of collisions and the possibility of reactive species acting on the microorganisms, and consequently the plasma efficiency, are greater in larger gas flows and times (Liao et al., 2017). However, these parameters can cause changes in the physicochemical characteristics of the cold plasma treated products (Coutinho et al., 2018). Therefore, the objective of this study was to compare the physicochemical (pH), physical (rheology parameters and particle size), microstructure (optical microscopy) and thermal properties (differential scanning calorimetry) of guava flavored whey-beverages submitted to cold plasma technology (400 W using nitrogen gas) in different processing time (5, 10, and 15 min) and gas flow (10, 20, and 30 mL min<sup>-1</sup>) conditions with a conventional pasteurized product (63 °C for 30 min).

## 2. Material and Methods

### *2.1 Processing of guava flavored whey beverage*

The whey beverage was made by mixing pasteurized milk (3% fat, Jaguaribe, Brazil) and whey (whey powder, Alibra, São Paulo, Brazil) reconstituted in water in the proportion of 70/30% v/v, as described by Castro et al. (2013), with adaptations. Then, 16% w/v guava pulp (Pomar da Pulpa, Fortaleza, Brazil), 10% w/v organic crystal sugar (Nativa, Sertãozinho, Brazil) and 0.5% gelatin powder (Royal, Pedreira, Brasil) were added.

After the preparation procedure, part of the beverage was subjected to cold plasma processing in a PlasmaEtch PE-50 Venus (Plasma Etch Inc, USA) apparatus, consisting of an aluminum chamber with a horizontal electrode. The equipment operates with a power supply of 400 W and 50 kHz connected to the mains. Vacuum is generated by a two-stage pump with a capacity of 5 m<sup>3</sup> min<sup>-1</sup>. The gas flow is controlled by electronic valves. The system is fully automated and controlled by the Plasma Etch, Inc. software. For each treatment, 120 mL of beverage were divided into three portions of 50 mL falcon tubes and placed in the equipment chamber. Nine treatments (I-IX) were performed, using nitrogen as working gas at a flow of 10, 20, and 30 mL min<sup>-1</sup> for 5, 10, and 15 min, according to Table 1. The other portion of the beverage was pasteurized at 63 °C for 30 min in a temperature-controlled water bath equipment.

### *2.2 Chemical properties*

#### *2.2.1 Determination of pH*

The pH values were measured in a digital potentiometer calibrated with pH 4 and pH 7 buffer (AOAC, 2012). The analyses were performed in triplicate, at room temperature (25 °C).

## 2.3 Physical properties

### 2.3.1 Particle size distribution

The particle size distribution was determined by the laser diffraction technique in a Mastersizer 2000 (Malvern Instruments Ltd, Malvern, UK) at 25 °C as described by Rojas, Leite, Cristianini, Alvim, and Augusto (2016). The following parameters were used:  $D_{[4,3]}$  = volume diameter or De Brouckere mean diameter, corresponding to the diameter of the sphere having the same volume of particles of the system (Eq. 1), and  $D_{[3,2]}$  = surface diameter or Sauter mean diameter, which corresponds to the mean diameter of particles proportional to the ratio of the surface area to total volume (Eq. 2). The parameter  $D_{[4,3]}$  is influenced by large particles while  $D_{[3,2]}$  is influenced by small particles. The wet dispersion method was used for the analysis, with a refractive index of 1.52.

$$D_{43} = \frac{\sum n_i d_i^4}{\sum n_i d_i^3} \quad (\text{Eq. 1})$$

$$D_{32} = \frac{\sum n_i d_i^3}{\sum n_i d_i^2} \quad (\text{Eq. 2})$$

where,  $d_i$  is the mean particle diameter, and  $n_i$  is the number of particles.

### 2.3.2 Rheological aspects

Steady state flow curves assays were performed in a controlled stress Rheometer DHR3 (TA Instruments, New Castle, DE, USA), with parallel plate geometry (40 mm Peltier Plate Steel 991,905) and a gap of 1 mm. The guava flavored whey beverages were placed in the dish and allowed to rest at  $10 \pm 0.1$  °C for 10 min to recover the structure. The temperature of the tests was maintained with the aid of TEK Physica 150P temperature control system. Two rheological measurements were carried out to obtain the flow curves: the first with ascending shear rate ( $0-300 \text{ s}^{-1}$ ) within 200 s, and the second with descending shear rate ( $300-0 \text{ s}^{-1}$ ) also within 200 s. Data from the first curve were fitted to the model of the power law (Eq. 3) using non-linear regression analysis and STATISTICA 5.0 software (StatSoft, Tulsa, OK, USA) (Balthazar et al., 2017a, Penna, Sivieri & Oliveira, 2001).

$$\sigma = k\dot{\gamma}^n \quad (\text{Eq. 3})$$

where  $\sigma$  represents the shear stress (Pa),  $k$  is the consistency index ( $\text{Pa}\cdot\text{s}^n$ ),  $\dot{\gamma}$  is the shear rate ( $\text{s}^{-1}$ ), and  $n$  represents the consistency index (dimensionless).

#### 2.4. Differential scanning calorimetry analysis (DSC)

The DSC analysis was performed according to Cappato et al. (2018) with some modifications. The products were subjected to the following steps at a constant heating rate ( $10 \text{ }^\circ\text{C min}^{-1}$ ): (1) holding for 1 min at  $-60 \text{ }^\circ\text{C}$ ; (2) heating from  $-60 \text{ }^\circ\text{C}$  to  $-40 \text{ }^\circ\text{C}$ ; (3) cooling from  $-40 \text{ }^\circ\text{C}$  to  $-60 \text{ }^\circ\text{C}$ ; (4) holding for 1 min at  $-60 \text{ }^\circ\text{C}$ ; (5) heating from  $-60 \text{ }^\circ\text{C}$  to  $30 \text{ }^\circ\text{C}$ ; (6) holding for 1 min at  $30 \text{ }^\circ\text{C}$ ; (7) cooling from  $30 \text{ }^\circ\text{C}$



to -60 °C; (8) holding for 1 min at -60 °C; and finally (9) heating from -60 °C to 30 °C. Melting and crystallization behavior was evaluated by DSC, using a PYRIS Diamond DSC equipment (Diamond, Perkin–Elmer, Norwalk, PA), equipped with an intercooler and the software Pyris Manager. Calibration was performed using an indium standard, and 15 mg of sample were sealed into aluminum pans (50 µL, Perkin–Elmer). The percentage of bound water (BW) was calculated by subtracting the beverages moisture (%) from the amount of ice formed per gram of sample (IC) (%), which was the melting enthalpy multiplied by 100 and divided by the latent heat of fusion of pure ice (334 J/g) (Balthazar et al., 2017b).

### 2.5 Microstructure

For each sample, about 20 µL were placed on glass slides, covered by cover slips and observed under an optical microscope (Olympus model BX41, Japan) equipped with a digital camera. The images were taken in quintuplicate, using the 20x and 40x objective (Cappato et al., 2018; Costa et al., 2018).

### 2.6 Microbiological analysis

Mesophilic aerobes, total and thermotolerant coliforms and *Salmonella* sp. were assessed according official methods (Brasil, 2003).

### 2.6 Statistical analysis

All the processing was performed in triplicate, being the analyses were performed in triplicate. The effects of treatments were analyzed by analysis of variance (ANOVA) followed by the Tukey's test ( $p\text{-value} \leq 0.05$ ). Statistical analyses were performed using XLSTAT 2018.2 (Adinsoft, Paris, France).

## 3. Results and Discussion

### 3.1 pH values

Table 1 shows the pH values of guava flavored whey beverages. The pH value of the untreated beverage was 6.04, while the treated ones presented pH values in the range from 5.90 to 6.41, corroborating a previous study with unfermented strawberry flavored whey beverages (Janiaski, Pimentel, Cruz & Prudencio, 2016). The thermally processed beverage had lower pH when compared to the plasma-treated beverages ( $p \leq 0.05$ ). The heat treatment leads to a decrease in pH of dairy products due to the production of organic acids (mainly formic acid) from lactose and precipitation of calcium phosphate (from primary, secondary or organic phosphate) with consequent release of  $H^+$  (Fox 1981).

The application of cold plasma resulted in higher pH values when compared to the untreated or pasteurized products ( $p \leq 0.05$ ). The gas flow rate affected pH values of the beverages, once lower pH values were observed in the samples submitted to higher flow rates ( $p \leq 0.05$ ). There was no effect of treatment times ( $p > 0.05$ ). When nitrogen molecules are present in the gas phase, energetic electrons collide with them along their trajectory and a cascade of linked reactions results in the formation of nitrogen oxides ( $NO_x$ ) and other components (Misra, 2016, Coutinho et al., 2018). Some of the components could have contributed to the increase in the pH of the plasma-treated beverages (Maheux et al., 2015). Higher flow rates lead to the formation of higher concentrations of acidogenic molecules, such as  $NO_x$ , which reacted with water present in food, resulting in nitric acid, which decreases pH of the medium (Yong et al., 2015). Lower pH values are commonly observed in plasma treated products, mainly when nitrogen is used as gas (Misra, 2016). The influence of cold plasma on pH is associated with the intrinsic

characteristics of the products, such as the buffering capacity (Kim et al., 2015). Whey beverages have high buffering capacity, which can explain the highest pH values observed in mild cold plasma processing conditions.

Despite the statistical significance, the pH of the plasma processed beverages was within the expected value. Therefore, the cold plasma resulted in products with a higher pH when compared to the thermal technology, which may be interesting from an industrial point of view, as the consumer expects a low acidic character and a typical fruit flavor in unfermented whey beverages.

## 3.2 Physical Properties

### 3.2.1 Particle size distribution

The values obtained in the analysis of particle size distribution are shown in Table 1 and Figure 1. Differences ( $p \leq 0.05$ ) were found between the plasma-treated and the pasteurized sample, demonstrating that the use of cold plasma altered the physical characteristics of the beverage.

Regarding the  $D[3,2]$  values, which represents the surface area of the particles, the values varied from  $4.617 \pm 0.77$  to  $19.356 \pm 0.36$ , with the lowest value for the pasteurized sample and the highest value for the sample subjected to cold plasma at gas flow of  $20 \text{ mL min}^{-1}$  for 15 min (VI). Milder processing conditions (I-III) resulted in products with lower number of small particles ( $10 \text{ }\mu\text{m}$ ) (Figure 1), larger surface area ( $D [3,2]$ ) and greater number of larger particles ( $1000 \text{ }\mu\text{m}$ ), similar to the pasteurized product ( $p > 0.05$ ). For medium flow rate and time conditions (IV to VI) there was found an increase in the number of small particles ( $10 \text{ }\mu\text{m}$ ) and surface area ( $D [3,2]$ ), with a consequent decrease in the number of larger particles ( $1000 \text{ }\mu\text{m}$ ) when compared to the pasteurized product. Finally, in

more drastic conditions (VIII-IX), there was a decrease in the surface area of the particles, maintenance of a higher number of larger particles (1000  $\mu\text{m}$ ) and a reduction of the smaller particles (10  $\mu\text{m}$ ), comparing to the products subjected to milder and intermediate processing conditions. Concerning the volume diameter ( $D_{[4,3]}$ ), only the plasma-treated samples subjected to conditions I (10  $\text{mL min}^{-1}$  for 5 min) and III (10  $\text{mL min}^{-1}$  for 15 min) presented higher values. Thus, the beverages from these treatments exhibited particles with larger volume diameter (Table 1). Therefore, the results demonstrate that mild cold plasma processing conditions (I-III) resulted in particle distribution similar to that observed for the pasteurized product. In the other conditions (IV-VII) an increase in the particle surface area and the number of small particles (10  $\mu\text{m}$ ) was observed after plasma processing (Table 1), with a consequent decrease in the number of larger particles (1000  $\mu\text{m}$ ). The microstructure of the ingredients presented in the whey beverage, such as guava's cellular material, can be disrupted due to the tension applied to the product, leading to the formation of smaller particles (Amaral et al., 2018). However, under more drastic processing conditions, degradation of these compounds might result in a decrease in the number of these particles (Almeida et al., 2015).

As reported by Janhoj, Frost & Ipsen (2009), the particle size is inversely correlated with the creaminess of dairy products. Therefore products with a higher number of smaller particles are creamier than those with the smallest number of large particles. As the cold plasma treated beverages had a higher number of smaller particles, this processing may be advantageous for the creaminess of the products.

### *3.2.2 Rheology parameters*

Figure 2 shows the typical steady-state flow curves (2a) and the apparent viscosity (2b) of the guava flavored whey beverages. All samples treated with cold plasma showed similar behavior; however, the heat-treated product presented higher values of shear stress and shear stress by shear rate than the plasma treated samples, indicating that the pasteurized product had higher viscosity.

Table 2 exhibits the values of the parameters consistency index ( $k$ ) and flow behavior index ( $n$ ) adjusted to Power law model. The flow curves had a great adjustment with a determination coefficient ( $R^2$ ) varying from 0.9733 to 0.9999. The highest consistency index was achieved by the pasteurized whey beverage ( $86.053 \pm 0.522$  MPa.sn). All plasma treated samples exhibited lower consistency index ( $p \leq 0.05$ ), with values varying from 5.148 to 9.385 MPa.s.

All samples showed pseudoplastic behavior ( $n < 1$ ), with a decrease in apparent viscosity with the increase in shear rate (Fig. 2b), with values varying from 0.664 to 0.949. The lowest value was reached by the pasteurized product while the application of cold plasma increased flow behavior index, reaching values very close to 1, which suggests a more Newtonian-like behavior.

The results indicate that the heat processed product had higher consistency and viscosity, and lower flow behavior. In fact, the use of heat allows a greater interaction between the components present in food products, increasing the molecular mobility and resulting in aggregates formation, consequently an increase in  $k$  and decrease  $n$  values (Amaral et al., 2018).

The results also suggest that cold plasma treatment promoted a significant decrease on the consistency of the samples when compared to the heat-processed sample and has changed the arrangement of the macromolecules responsible for the

flow behavior of the samples. These results agree with those observed for particle size (Figure 1 and Table 1) and microstructure (Figure 3), in which the application of cold plasma resulted in the rupture of aggregates and formation of a great number of small structures, which generated beverages with lower  $k$ , and rheological behavior more similar to Newtonian fluids. Besides, they can be associated with increased pH values (Table 1) of guava flavored whey beverages submitted to cold plasma. According to Rojas et al. (2016), the lower particle size leads to an increase in dispersibility despite the remaining large particles, as observed in the present study, once the smaller particles occupy the spaces between, the larger particles, resulting in a lubricating effect with a consequent reduction in viscosity of the products.

The process parameters influenced the rheological characteristics of the products. In milder conditions, the impact on  $k$  and  $n$  was higher, especially in lower processing times. In more severe conditions, the products presented higher consistency index and lowered flow behavior index, like the pasteurized product. However, marked differences were maintained between the plasma-treated and heat-treated samples. The results of the rheological characteristics corroborate those observed for the microstructure since the formulations with the greatest cell rupture presented higher consistency index and lower flow behavior index (Table 2). Possibly this result is related to the leaching of intracellular material caused by the cell rupture, increasing the consistency of the products. Greater consistency may be interesting for an improvement in consumer acceptance of the products (Cappato et al., 2018). In addition, lower sedimentation of particles from pulp and higher bioaccessibility of compounds with health benefits might be observed (Rojas et al.,

2016). However, greater cell ruptures may result in nutrient losses and more marked alterations in the color of the products (Rojas et al., 2016; Cappato et al., 2018). Non-fermented dairy beverages are characterized as a liquid product. Thus, the decrease in consistency provided by the plasma may not influence the consumers' acceptance of the products. However, further studies should confirm this assumption.

Cold plasma has been shown to be a source of reactive species (Coutinho et al., 2018), which are responsible for many chemical modifications, including enzyme inactivation, protein denaturation and chemical degradation (Bahrami et al., 2016). It is well-known that consistency and flow behavior indexes in milk and dairy products are mostly related to macromolecular interactions, such as protein-protein intermolecular attraction, for example. The results suggest that the application of cold plasma may have promoted a modification on the molecular structure and conformation of the milk proteins, which might be responsible for an increase in the protein network mobility, thus leading to a decrease in the consistency index and an increase in the flow behavior index of the cold plasma-treated samples (Belsito et al., 2017). Denaturation followed by then restructuration of disulfide bonds along with the generation of new intra/inter molecular bonds is the effect of the heat treatment on proteins. On the other hand, the reactive species of cold plasma can change the protein conformation (Ekezie et al., 2018). The different mechanisms on protein molecules corroborate with the different rheology aspects reported in the present study for pasteurized and plasma treated samples.

Although this hypothesis is reasonable, no study has been focused on the effect of cold plasma on the rheological behavior of milk and dairy products, and it is

still unknown how cold plasma can truly affect the molecular structure and conformation of milk proteins. Misra et al. (2015) studied the effect of cold plasma treatment (60–70 kV, 5–10 min) on the rheological properties of soft and hard wheat flour. Unlike the present results, those authors found an increase in the viscous and elastic moduli of strong wheat flour after cold plasma treatment, probably due to oxidation of protein sulfhydryl groups and subsequent disulfide bond formation between cysteine moieties.

### *3.3. Differential scanning calorimetry (DSC)*

The melting temperatures ( $^{\circ}\text{C}$ ), enthalpies ( $\Delta H$ ), and bound water (%) are presented in Table 3. The DSC thermograms showed an endothermic peak (data not shown), corresponding to the melting process (Farah, Silva, Cruz & Calado, 2018), resulting from the energy gain during cold plasma processing. The melting temperature ranged from  $5.28^{\circ}\text{C}$  (pasteurized beverage) to  $11.34^{\circ}\text{C}$  (beverage IX), with an average temperature of  $7.7^{\circ}\text{C}$ . The application of pasteurization or cold plasma treatments ( $T=5.28^{\circ}\text{C}$  against  $8.57\text{--}11.34^{\circ}\text{C}$ , respectively,  $p>0.05$ ) resulted in an increase in the melting temperature of the beverages when compared to untreated beverage ( $T=4^{\circ}\text{C}$ ). There was no significant difference between the enthalpy values (average  $\Delta H$ :  $179.04\text{ J/g}$ ,  $p > 0.05$ ), with minimum and maximum enthalpy values of  $149.6\text{ J/g}$  and  $193.13\text{ J/g}$  for the beverage I and VI, respectively. This result is probably due to the cold plasma and pasteurization procedures, and the capacity of the beverage to absorb energy. The percentage of bound water varied from  $22.37\%$  to  $37.77\%$  for beverages VII and I, respectively; which probably was related to the whey protein and fiber from guava fruit that was the same for all formulations. The bound water represents the amount of water strongly linked to



food molecules, such as protein or fiber (Balthazar et al., 2017b, Soukoulis et al., 2009).

The crystallization and melting behavior of milk fat can help in understanding the composition, physical properties, and functionality, once they are influenced by the intensity of the heat treatment applied (Herrera & Hartel, 2000). DSC can be an interesting approach for evaluating the intensity and the impact of the emerging technologies, such as cold plasma, on the fouling behavior and biochemical modifications of milk proteins. Therefore, our findings suggest that the cold plasma had similar effects on the thermal properties compared to the conventional processing.

### *3.4 Microstructure*

Figure 3 shows the results of the microstructure of guava flavored whey beverages. It is observed that the beverage is basically composed of a serum phase, particles in suspension, and intact plant cells. The serum phase comprises milk, reconstituted whey, gelatin, and the guava pulp compounds, whereas the intracellular compounds are found in plant cells, such as those responsible for the coloring of guava (Cappato et al., 2018).

The application of cold plasma under mild conditions (I and II) resulted in beverages with a microstructure similar to the thermally treated product. However, the cells were somewhat more widespread, probably due to a possible rupture of intracellular structures, providing the dispersion of intracellular content in the cytoplasm (Rojas et al., 2016). In contrast, the other processing time and gas flow conditions resulted in rupture of intact cells and formation of cell fragments. At medium gas flow rates (20 mL min<sup>-1</sup>, IV, V, and VI), there was a cell rupture and

formation of a larger number of small fragments. At higher flow rates (30 mL min<sup>-1</sup>, VII, and VIII) there was a rupture of intact cells and less formation of fragments. Finally, the beverages V and IX showed greater cell rupture. The results of the microstructure corroborate those observed for the particle size distribution (Table 1 and Figure 1).

### 3.5 Microbiological analysis

The untreated guava-flavored whey beverage had 4.2 log CFU/mL of aerobic mesophiles, < 3 log MPN/mL for total and thermotolerant coliforms and no *Salmonella* sp. in 25 mL of product. The pasteurized product presented 2 log CFU/mL of mesophilic bacteria count and for the plasma-treated whey beverages presented counts in the range of 1.32-1.82 log CFU/mL, respectively. The guava-flavored whey beverages treated by pasteurization or cold plasma had < 3 MPN/mL for total and thermotolerant coliforms and no *Salmonella* sp. in 25 mL of product. These results confirm the adequacy of the processing operations, as the counts of aerobic mesophiles were reduced. In addition, the microbial counts are in accordance with the Brazilian Legislation of whey dairy beverages (Brasil, 2005), which establishes values ranged 4.87 to 5.17 log CFU/mL for aerobic mesophilic bacteria counts and absence of total and thermotolerant coliforms.

## 4. Conclusion

The application of the cold plasma processing resulted in guava flavored whey beverages with higher pH values, lower consistency indexes, higher flow behavior indexes, and lower pseudoplastic characteristics when compared to the sample processed by the slow pasteurization (traditional thermal process). If the industry is

interested in products with low viscosity and consistency, the milder plasma processing conditions should be used, resulting in products with particle size distribution and microstructure similar to the pasteurized product, and lower viscosity and consistency values. If the viscosity and consistency are important parameters to the beverage quality, it is advised to use more drastic processing conditions, resulting in an increase in the particle surface area and in the number of smaller particles, with a consequent decrease in larger particles and greater viscosity and consistency. Therefore, plasma treatment can be a useful tool to modify the structure and the rheology of dairy products, allowing the development of new products with distinct characteristics. Future studies should evaluate the shelf life of the products and the sensory acceptance by consumers.

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- ✓ Guava-flavored whey beverage processed using cold plasma;
- ✓ Milder operational conditions increased pH values and resulted in similar microstructure;
- ✓ More severe processing conditions increased the particle surface area; viscosity and consistency
- ✓ No effect on the thermal behavior;

.

**Table 1.** pH and particle size distribution of guava-flavored whey beverages\*

Sample	pH	Particle size distribution			
		D <sub>32</sub>	D <sub>43</sub>	d <sub>10</sub>	d <sub>90</sub>
Pasteurized	5.90 ± 0.01 <sup>e</sup>	4.617 ± 0.77 <sup>e</sup>	167.348 ± 2.65 <sup>b</sup>	1.349 ± 0.15 <sup>e</sup>	58.959
I- 5 min / 10 mL	6.41 ± 0.01 <sup>a</sup>	5.901 ± 0.07 <sup>e</sup>	458.934 ± 2.26 <sup>a</sup>	1.508 ± 0.01 <sup>e</sup>	366.175
II- 10 min / 10 mL	6.39 ± 0.01 <sup>abc</sup>	7.657 ± 0.50 <sup>cde</sup>	96.809 ± 2.30 <sup>b</sup>	3.603 ± 0.34 <sup>cd</sup>	16.130
III- 15 min / 10 mL	6.40 ± 0.1 <sup>ab</sup>	7.013 ± 0.16 <sup>de</sup>	518.825 ± 1.90 <sup>a</sup>	1.694 ± 0.05 <sup>e</sup>	457.70
IV- 5 min / 20 mL	6.36 ± 0.02 <sup>bcd</sup>	10.407 ± 1.85 <sup>bc</sup>	126.605 ± 1.90 <sup>b</sup>	5.969 ± 1.20 <sup>b</sup>	17.882
V- 10 min / 20 mL	6.36 ± 0.02 <sup>bcd</sup>	11.583 ± 0.87 <sup>b</sup>	176.915 ± 2.81 <sup>b</sup>	6.002 ± 0.40 <sup>b</sup>	22.013
VI- 15 min / 20 mL	6.37 ± 0.01 <sup>abcd</sup>	19.356 ± 0.36 <sup>a</sup>	203.988 ± 2.04 <sup>b</sup>	10.034 ± 0.12 <sup>a</sup>	48.587
VII- 5 min / 30 mL	6.34 ± 0.01 <sup>d</sup>	10.969 ± 0.58 <sup>b</sup>	86.264 ± 2.80 <sup>b</sup>	6.415 ± 0.30 <sup>b</sup>	19.960
VIII- 10 min / 30 mL	6.33 ± 0.01 <sup>d</sup>	6.589 ± 1.02 <sup>de</sup>	209.982 ± 2.04 <sup>b</sup>	2.362 ± 0.30 <sup>de</sup>	35.165
IX- 15 min / 30 mL	6.35 ± 0.01 <sup>cd</sup>	9.479 ± 0.45 <sup>bcd</sup>	116.526 ± 2.54 <sup>b</sup>	4.936 ± 0.07 <sup>bc</sup>	23.438

\*Results are presented as the mean ± standard deviation. D<sub>32</sub>, D<sub>43</sub>, d<sub>10</sub>, d<sub>50</sub>, d<sub>90</sub> are expressed in µm.

<sup>a-e</sup> Different letters in the same column denote difference according the Tukey test (p-value < 0.05).

**Table 2.** Rheological characteristics of guava-flavored whey beverages\*

Sample	Consistency index (k)	Flow behaviour index (n)	R <sup>2</sup>
Pasteurized	86.053 ± 0.52 <sup>a</sup>	0.664 ± 0.01 <sup>d</sup>	0.9999
I	5.845 ± 0.05 <sup>d</sup>	0.949 ± 0.01 <sup>a</sup>	0.9999
II	6.937 ± 0.83 <sup>c</sup>	0.896 ± 0.04 <sup>ab</sup>	0.9930
III	6.177 ± 0.76 <sup>c</sup>	0.913 ± 0.04 <sup>a</sup>	0.9928
IV	5.871 ± 0.75 <sup>de</sup>	0.919 ± 0.04 <sup>a</sup>	0.9925
V	9.276 ± 1.68 <sup>b</sup>	0.745 ± 0.06 <sup>c</sup>	0.9733
VI	5.148 ± 0.78 <sup>e</sup>	0.924 ± 0.05 <sup>a</sup>	0.9896
VII	5.429 ± 0.83 <sup>d</sup>	0.923 ± 0.05 <sup>a</sup>	0.9894
VIII	6.551 ± 1.02 <sup>c</sup>	0.843 ± 0.05 <sup>b</sup>	0.9858
IX	9.385 ± 1.27 <sup>b</sup>	0.789 ± 0.05 <sup>c</sup>	0.9880

\*Results are presented as the mean ± standard deviation. Results obtained by Power law model at 25°C. Consistency index is expressed in mPa.s. Flow behavior index is dimensionless. R<sup>2</sup> = coefficient determination. <sup>a-e</sup> Different letters in the same column denote difference according the Tukey test (p < 0.05). Pasteurized, I, II, III, IV, V, VI, VII, VIII, IX = [see Table 1.](#)

**Table 3.** Differential Scanning parameters of melting temperature (°C), enthalpy ( $\Delta H$  J/g), and bound water (%), respectively, of guava-flavored whey beverages submitted to cold plasma processing.

Samples	Temperature	$\Delta H^\circ$	Bound water
Pasteurized	5.28 $\pm$ 0.5 <sup>d</sup>	182.14 $\pm$ 19.5 <sup>a</sup>	27.92 $\pm$ 3.5 <sup>a</sup>
I	8.57 $\pm$ 0.3 <sup>b</sup>	149.60 $\pm$ 15.5 <sup>a</sup>	37.77 $\pm$ 4.5 <sup>a</sup>
II	6.81 $\pm$ 0.3 <sup>c</sup>	175.25 $\pm$ 19.8 <sup>a</sup>	29.87 $\pm$ 3.0 <sup>a</sup>
III	8.67 $\pm$ 0.5 <sup>b</sup>	163.42 $\pm$ 13.7 <sup>a</sup>	33.63 $\pm$ 2.8 <sup>a</sup>
IV	6.15 $\pm$ 0.5 <sup>c</sup>	180.87 $\pm$ 14.3 <sup>a</sup>	28.23 $\pm$ 2.5 <sup>a</sup>
V	7.48 $\pm$ 0.4 <sup>c</sup>	191.56 $\pm$ 18.4 <sup>a</sup>	25.37 $\pm$ 2.3 <sup>a</sup>
VI	7.60 $\pm$ 0.3 <sup>bc</sup>	186.03 $\pm$ 17.3 <sup>a</sup>	26.41 $\pm$ 2.6 <sup>a</sup>
VII	6.92 $\pm$ 0.5 <sup>c</sup>	193.13 $\pm$ 14.4 <sup>a</sup>	22.32 $\pm$ 1.5 <sup>a</sup>
VIII	8.26 $\pm$ 0.5 <sup>b</sup>	187.87 $\pm$ 18.5 <sup>a</sup>	24.09 $\pm$ 3.5 <sup>a</sup>
IX	11.34 $\pm$ 0.5 <sup>a</sup>	180.53 $\pm$ 17.5 <sup>a</sup>	26.43 $\pm$ 2.9 <sup>a</sup>

\* Data are expressed as mean  $\pm$  standard deviation. Analysis performed in triplicate. <sup>a-d</sup> Different letters at the same column indicates significant differences between samples ( $p < 0.05$ ) by Tukey test. Pasteurized, I, II, III, IV, V, VI, VII, VIII, IX = see Table 1.