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Study of strawberry flavored milk under pulsed electric field processing

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ABSTRACT

Few studies exist on flavored milk processed by pulsed electric fields (PEF). The main concern is product stability. This study aimed to analyze the degradation of coloring agent Allura Red in strawberry milk under PEF. Four systems were tested containing Allura Red: two commercial milks and two model systems. PEF conditions were 40 kV/cm, 48 pulses (2.5 μ s), and 55 °C; coloring agent was quantified via RP-HPLC. After processing, only minor changes were observed in color, Allura Red concentration, and pH. During storage (32 d) at refrigerated conditions (4 °C) commercial samples maintained pH above 6. Model systems dropped below pH 6 after 10 days of storage. Color of samples showed important decrease in a^* ; hue angle and chroma changed during storage. HPLC analysis reported a bi-phasic effect in Allura Red concentrations versus time. Concentration changed, reaching a maximum value during the middle of storage, possibly attributed to microbial growth, pH reduction, or interaction of proteins. However, PEF affected the stability of Allura Red in milk when additional ingredients were not added to the product.

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1. Introduction

There are a number of commercially flavored milks such as chocolate, vanilla, banana, orange and strawberry. Chocolate milk is the most popular flavored milk, especially with children (90% like its taste); it has a high protein content and is a good source of vitamins, calcium and other nutrients. Strawberry flavored milk also demonstrates high acceptability with the consumer (Miller, Jarvis, & McBean, 2007). Milk is not only important in maintaining good health, keeping bones strong, and providing nutrients during the development of children, it also one of the main sources of protein in certain populations. For example, in combat rations for soldiers, milk is an important component of the different menus. Combat rations also must be part of a quality lifestyle provided to soldiers, while at the same time, rations should cover all of their nutritional needs. At the department of US Army Natick Soldier Center, food researchers are constantly looking for better products to provide more foods and improved choices on the soldiers' menus. Some of the current milk-based beverages for military rations are dairy shakes (chocolate, vanilla, strawberry and banana) (NSRDEC, 2007). However, the shelf-life of fluid milk products, supplied as an enhancement to group rations, is sometimes limited because of the processing conditions,

shipping and extreme conditions experienced during storage in rugged environments. For feeding purposes, storage life of the product must be as long as possible under the most diverse conditions, but at the same time, it should have good sensory characteristics such as flavor, taste, color and appearance. Conventional food processing operations (e.g. drying, pasteurization, blanching, canning, etc.) have the disadvantage of decreasing a food's overall quality, destroying some vitamins and pigments, and denaturalization of some proteins or volatilizing odors, all of which can affect the final product. Nevertheless, these unit operations must be conducted to inactivate the pathogenic microorganisms in food, to reduce the load of spoilage microorganisms and enzymatic activity, and to extend the shelf-life of the product. Currently, a group of novel emerging technologies such as high hydrostatic pressure, pulsed electric fields, ultrasound, and ultraviolet are being tested on foods to ensure the microbiologically safety and fresh-like characteristics of products. Pulsed electric fields (PEF) technology has shown positive effects on inactivating pathogenic bacteria and reducing the number of spoilage microorganisms in milk (Sobrinho-López, & Martín-Belloso, 2008; Bendicho, Barbosa-Cánovas, & Martín-Belloso, 2002; Odriozola-Serrano, Bendicho-Porta, & Martín-Belloso, 2006; Craven et al., 2008) Also, it may be feasible to extend the shelf-life of milk with PEF, retarding the growth of mesophilic bacteria up to 80 days with only minor effects on quality (Sepúlveda, Góngora-Nieto, Guerrero, & Barbosa-Cánovas, 2005).

Color of food is an important quality parameter that may determine acceptability or rejection by the consumer (Abdullah, Badaruddin, Sayeed, Ali, & Riaz, 2008). Color is important in flavor and

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taste recognition and identification, as well as in food preference (Gifford, & Clydesdale, 1986). Children often prefer beverages and other products with attractive colors rather as opposed to colorless foods. Color of food has a psychological effect on how the product is perceived by the consumer. For example, the degree of sweetness in cherry-flavored drinks was perceived by consumers different because of the presence of red-colored pigments (Gifford, & Clydesdale, 1986). Allura Red AC or FD&C Red No. 40 is a red powder that is soluble in water; it is used as an additive in colored beverages, candy, cereals, confections, gelatins, puddings, ice cream, condiments, dairy products, and more. School children are the main consumers of these products, such as non-alcoholic flavored drinks, confectionery, and desserts including flavored milk products (Tennant, 2008). Allura Red is an organic molecule that can be classified as a monoazo structure ($N=N$); it has been listed for use as in foods since 1971 (Thorngate, 2002). Regulations of this color by the FDA are in accordance with Good Manufacturing Practice and must be certified as a food additive before being used in food products (CFR, 2001). Overdose of this additive has shown some reproductive effects when tested at experimental levels. The TDL_0 (Toxic Dose Low) is the lowest dose over a given period of time capable of producing any toxic effect or carcinogenic, neoplastigenic or teratogenic effects in both animals and humans; for Red No. 40 the TDL_0 is 38,500 mg/kg, as tested in rats via oral ingestion (Lewis, 1989). Hyperactivity has been reported in children as a result of ingestion of this colorant in high concentration (Anonymous, 1984); nevertheless, the reported average daily intake in human beings is 100 mg/kg (Thorngate, 2002), which also represents the maximum amount allowed for this colorant in non-alcoholic beverages (100 µg/ml) in Europe (Al-Degs, 2009). In a detailed study conducted by the Union of European Beverages Association, it was shown that the highest usage of Allura Red in beverages was 21 mg/l (Tennant, 2008).

Some of the analytical techniques used for determination of synthetic colorants in food are thin-layer chromatography, derivative spectrometry, adsorptive voltammetry (Alghamdi, 2005), capillary electrophoresis, reverse-phase liquid chromatography (RPLC) and ion-pair RPLC (Chen, Mou, Hou, Riviello, & Ni, 1998), visible spectrophotometry, and high performance liquid chromatography (Al-Degs, 2009).

The objective of this research was to study the effect of pulsed electric fields in processing strawberry flavored milk, using two commercial brands and two model systems, and to evaluate the physicochemical changes and possible degradation of Allura Red after PEF processing and during refrigerated storage (32 d).

2. Materials and methods

2.1. Milk samples and model systems

Two commercial brands of strawberry flavored milk (A_1 and A_2) were purchased at the local supermarket. Initial characterization of the milks consisted of microbial evaluation (mesophilic load) and assessment of color, pH and concentration of Allura Red. Milk samples were kept at 4 °C until processed but no longer than 48 h. Two model systems for strawberry milk (B_1 and B_2) were prepared using reduced fat unflavored pasteurized milk (2%) (purchased at a local supermarket); then sucrose and Allura Red AC (Sigma-Aldrich® 458848) were added as a coloring agent (80% dye content). Model system 1 (B_1) consisted of milk, sucrose (5.5%), and Allura Red AC (0.01%), using the minimum amount of coloring agent recommended for flavored milk according to the patent (WO/2004/100671). Model system 2 (B_2) consisted of milk, sucrose (5.5%) and Allura Red AC (0.2%) using the maximum allowable concentration for flavored milk according to the above mentioned patent. Even though these concentrations are higher than those used in commercial samples, they were tested to study behavior under PEF in a wide range of concentrations. The

composition of A_1 according to the manufacturing label was as follows: reduced fat milk with vitamin A Palmitate and vitamin D3 added, high fructose corn syrup, sugar, nonfat milk, calcium, carbonate, potassium citrate, artificial flavors, carrageenan, citric acid, salt, Red 40 (Allura Red), and Blue 1. The composition of A_2 was milk, high fructose corn syrup, nonfat milk, natural and artificial flavors, carrageenan, Red 40 (Allura Red), and vitamin D3. The code for each control sample respectively was the following: A_{1C} , A_{2C} , B_{1C} and B_{2C} ; and for PEF processed samples, A_{1P} , A_{2P} , B_{1P} , and B_{2P} . Again, samples were characterized according to microbial load, color, pH and concentration of Allura Red. Samples were kept at 4 °C until used.

2.2. Pulsed electric fields treatment

A pilot plant pulsed electric fields (PEF) system manufactured by Physics International (San Leandro, CA) consisting of a cylindrical concentric-electrodes treatment chamber was used to apply the desired treatments to the milk (Qin et al., 1995). The continuous treatment chamber had a volume of 25 cm³ with a gap between the stainless steel electrodes of 0.6 cm.

Frequency was kept constant at 10 Hz and flow was set up at 490 ml/min. Flow rate of the flavored milk was set up and controlled with a rotary pump (Masterflex 7654-00, Cole Parmer Instruments Co., Chicago, IL). Treatments at peak electric fields of 40 kV/cm were used in combination with 48 pulses (2.5 µs pulse-width each). A refrigerated bath and recirculation system was used to keep the milk temperature at or below 55 °C. Inlet and outlet temperatures of the treatment chamber were monitored with digital in-line thermocouples (Cole-Palmer, Vernon Hills, IL) and registered. The difference between the inlet and outlet temperature of milk during processing was around 45 °C for all samples. Energy consumption, electric field intensity, pulse width, and pulse shape were directly measured with a digital oscilloscope (Hewlett-Packard 54530A, Colorado Springs, CO) connected to the treatment chamber through high voltage probes. These processing conditions were chosen because previous research of unflavored milk showed their effectiveness in inactivating pathogenic bacteria, reducing spoilage loads, and providing longer shelf-life.

2.3. Microbiological analysis

The initial load of microorganisms was evaluated in the flavored and unflavored milks. Plate Count Agar (Difco Becton, Dickinson and Co., Sparks, MD) was used for mesophiles aerobes. Serial dilutions were made with peptone water before pouring samples into dishes. These were incubated at 35 °C for 48 h. For the PEF treatments, the microbial counts were evaluated after 48 pulses. Samples were analyzed in duplicate.

2.3.1. pH

pH was determined by direct immersion in the samples with a potentiometer (Orion Research Inc., Boston, MA). The potentiometer was calibrated previously with two standards (pH 4 and 7). Each sample was measured in triplicate.

2.3.2. Color

Hunter color parameters (L^* , a^* , b^*) were measured in samples after processing and during storage. Lightness to darkness (L^*) (100 to 0), redness(+) to greenness(-) (a^*), and yellowness(+) to blueness (-) (b^*) color parameters were determined using a Minolta CM-2002 spectrophotometer (Minolta Camera Co., Osaka, Japan) in the reflection mode. Twenty ml of each milk sample were poured into sterile plastic bags. A white ceramic plate was used for standardizing the instrument ($L^*=93.4$, $a^*=-0.67$, $b^*=0.78$). Each sample was read in triplicate.

The net color difference was evaluated with the following equation, using the parameters L^* , a^* and b^* and comparing the different treatments with the untreated milk for each system from the beginning to end of storage life.

$$\Delta E^* = \sqrt{(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2}$$

Hue angle (h^*) was determined using the following relationship:

$$h^* = \tan^{-1} \left(\frac{b^*}{a^*} \right)$$

The chroma or saturation index (C^*) was evaluated as follows:

$$C^* = (a^{*2} + b^{*2})^{1/2}$$

2.4. High Performance Liquid Chromatography (HPLC) analysis

2.4.1. Materials

Allura Red and sodium acetate were purchased from Sigma (St. Louis, MO, USA). HPLC grade acetonitrile and water were purchased from J. T. Baker (Phillipsburg, NJ, USA). Commercial samples (A_1 , A_2) and model systems (B_1 , B_2) were used before and after PEF treatments to quantify the concentration of Allura Red.

2.4.2. Allura Red extraction

Stock solutions of Allura Red and kaempferol (internal standard, IS) were prepared in HPLC methanol, around 100 $\mu\text{g}/\text{ml}$, and stored at -20°C protected from the light. A 5 ml aliquot of each sample was collected and mixed with ice-cold HPLC acetonitrile, vortexed for 30 s, and centrifuged at 3500 RPM for 8 min (Lonnerdal, Woodhouse, & Glazier, 1987). The supernatant was transferred to new tubes and any precipitate was discarded. Next, 100 μl of kaempferol was added to each sample, vortexed, and dried to completion under a constant stream of compressed nitrogen gas. Each sample was reconstituted in 500 μl of HPLC acetonitrile, vortexed, and centrifuged, followed by 100 μl injected into the HPLC.

2.4.3. Chromatographic conditions

For quantification of Allura Red, the HPLC system utilized was a Shimadzu HPLC (Kyoto, Japan) consisting of two LC-10ATVP pumps, SIL-10AF auto injector, SPD-M10A VP spectrophotometric diode array detector, and SCL-10A VP system controller. Data collection and integration were accomplished using Shimadzu EZ Start 7.1.1 SP1 software (Kyoto, Japan). The method was adapted from a report by Kirschbaum, Krause, Pfalzgraf, and Brückner (2003); the analytical column was a Phenomenex Luna (2) C_{18} column (250×4.60 mm, i.d. 5 μm particle size, Torrance CA, USA). The mobile phase consisted of 100 mM sodium acetate (NaOAc) (mobile phase A) and 100% acetonitrile (MeCN) (mobile phase B). Separation was carried out at ambient temperature ($25 \pm 1^\circ\text{C}$), and UV detection at 520 nm for Allura Red and 370 nm for kaempferol (internal standard). Standard curves were prepared in reduced fat milk with known concentrations of Allura Red.

2.4.4. Storage life

PEF treated milk samples were collected in sterile plastic bags and stored at 4°C for 40 days for shelf-life studies. Each bag sample was used and discarded after analysis. Every other day, the physicochemical characteristics (pH and color) were evaluated. The microbial loads were poured into Plate Count Agar to count the mesophiles aerobes. Samples were taken from a different bag each day, diluted with peptone water and poured into dishes. Samples were incubated at 35°C for 48 h. Every fifth day the coloring agent concentration was

quantified using HPLC following the methodology described previously.

2.5. Results and discussion

2.5.1. pH

In Fig. 1, the pH behavior of samples is shown for all the PEF treated samples. For commercial and untreated samples (A_{1C} and A_{2C}), pH remained almost constant for one month of storage without important changes. However, PEF treated commercial samples (A_{1P} and A_{2P}) showed a decrease in pH after the first week of storage, changing from 6.7 to 6.3 and remaining at this value for most of the storage period; a final drop in value occurred after day 30 possibly because of the production of lactic acid bacteria. In all cases (treated and untreated), the pH was still within the range of commercial milk after 25 days of storage.

Both model systems showed interesting pH behavior during storage; pH of samples not processed with PEF (B_{1C} and B_{2C}) dropped quickly after 7 days of storage and behavior was very similar between them. Samples treated with PEF (B_{1P} and B_{2P}) showed a slower decrease of pH compared to untreated samples, however, at the end of storage pH was below 5. The reason for this drop could be the growth of lactobacillus and production of lactic acid in the milk, in addition to other possible chemical reactions taking place. In commercial brands there are other ingredients in addition to ones tested in this study that could be providing stability to the system and maintaining pH values during storage.

2.5.2. Color

Immediately after processing, samples did not show any important changes in color, or at least showed similar appearance to those prior to processing. Analytical measurements confirmed that the same Hunter color parameters L^* , a^* and b^* existed between the control samples and respective PEF treated samples after processing. However, during storage at 4°C some samples showed important variations. The three Hunter color parameters were quantified,

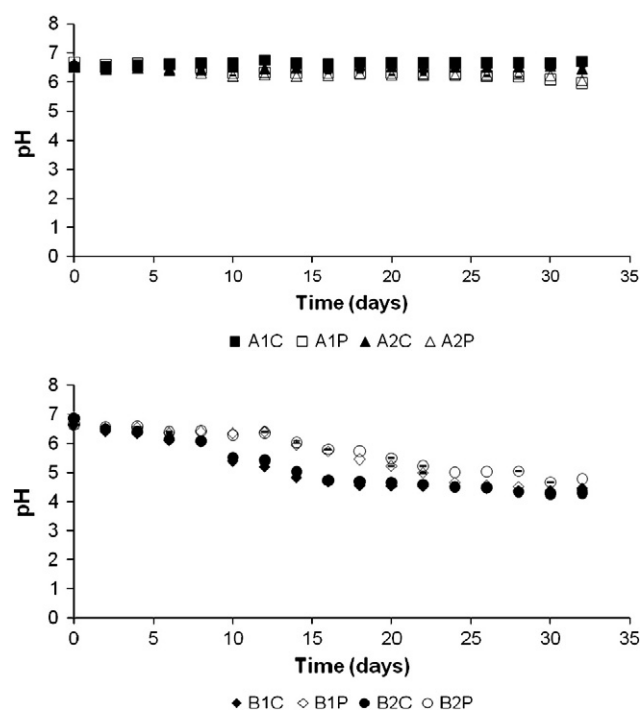


Fig. 1. pH behavior during storage (4°C) of strawberry flavored milk; commercial brands (control: A_{1C} and A_{2C} ; processed A_{1P} and A_{2P}) and model systems (control: B_{1C} and B_{2C} ; processed B_{1P} and B_{2P}).

however a^* , which is of interest because of its meaning, showed changes in the red and green regions. A more positive value means the product is closer to the red region while a negative number means it is closer to the green region. The commercial samples (Fig. 2) retained a constant color according to the a^* value and were pasteurized by Ultra High Temperature (UHT). Abdullah et al. (2008) showed that there was a strong affinity for Allura Red and milk proteins such as caseins; even during heating, color of the dye remained constant because of strong binding with proteins. However, these same samples when treated with PEF lost color after 20 days of storage, with a marked decrease in a^* , dropping below zero. One of the physicochemical mechanisms from which color is produced involves electron transitions between molecular orbitals. Changes in bond conjugation in an organic molecule such as delocalization of electrons or presence of electron donor and acceptor groups affect the absorption of light, causing changes in color (Thorngate, 2002). Here, it is important to mention that the use of pulsed electricity has an observable effect on the stability of the coloring agent Allura Red in flavored milk. Both commercial samples, regardless of the rest of the ingredients, showed this decrease and remained constant until the end of storage (day 32), which could be due the effect of PEF on the proteins of milk. After the formation and binding of dye-protein, the intensity of the electric field could disrupt the stability of this bond, breaking it, with the possible loss of color that was observable in the low concentration of Allura Red in the commercial samples.

Model systems also showed an interesting behavior of a^* parameter; the model with the lowest allowed concentration of Allura Red (B_1) was discolored after 10 days of storage regardless of whether it was pulsed or not. PEF only retarded the loss of color for two days followed by discoloration later. Model system B_2 (with the highest coloring agent concentration) maintained constant color during storage regardless of whether the sample was pulsed or not. This suggests that because the coloring agent is more concentrated,

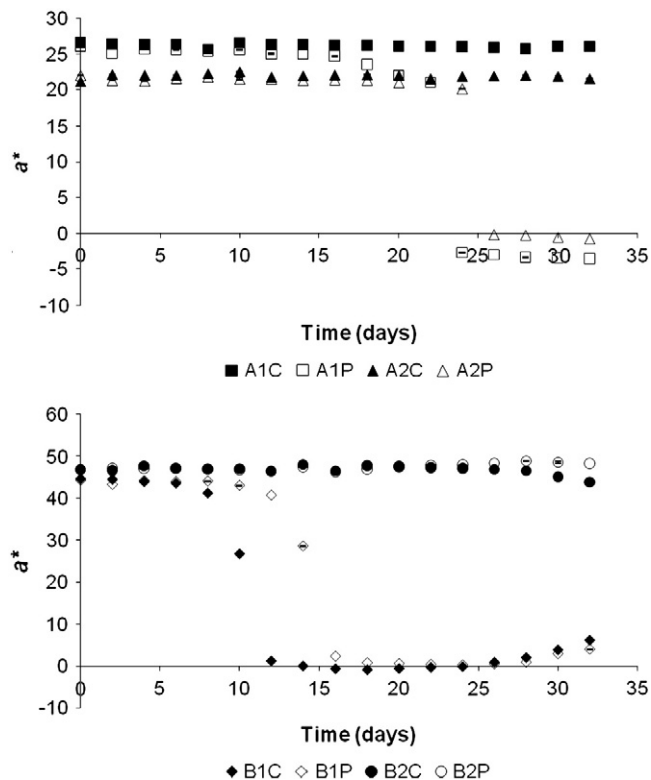


Fig. 2. a^* color parameter behavior during storage (4 °C) of strawberry flavored milk; commercial brands (control: A_{1C} and A_{2C} ; processed A_{1P} and A_{2P}) and model systems (control: B_{1C} and B_{2C} ; processed B_{1P} and B_{2P}).

more stability can be expected after PEF processing and during storage.

2.5.3. Net change of color (ΔE)

To have a better understanding of the changes in color in milk after processing and during storage, some color functions were evaluated based on Hunter parameters. First, the net change in color was calculated for each sample, using as reference the untreated sample (same brand or model system). In Fig. 3, this behavior is shown for the four samples. For the commercial samples (A_1 , A_2), the net change in color was very high after day 20; while model system B_1 showed undefined behavior during storage, with a peak in color after 10 days of storage representing the maximum difference between the treated and untreated samples. Model system B_2 did not show any important changes during storage in ΔE probably because of the high concentration of Allura Red present in the sample.

2.5.4. Hue angle (h^*)

The hue angle is shown in Fig. 4, for commercial untreated samples (A_{1C} and A_{2C}); h^* remained constant during storage, close to 0 °C, which is close to the reddish h^* value. But in PEF processed samples the angle changed to a negative value close to the yellowish region. This change was observed at first sight as loss of color in the milk. Model systems showed similar behavior to ΔE ; the less concentrated sample showed a change in hue angle after 10 days of storage within the hysteresis region from day 15 to day 25. Samples containing the most coloring agent did not change h^* during storage and remained at a constant value, around 27°.

2.5.5. Chroma (C^*)

The saturation index or chroma (C^*) was calculated for the samples, as shown in Fig. 5. This value, which indicates the degree of saturation, purity or intensity of color, showed a constant value for commercial samples during storage. Sample A_1 showed a higher value than sample A_2 the entire time, indicating more purity in the color of the milk. Flavored milk A_1 was a better and more expensive brand of this beverage compared to brand A_2 , which could be a reason to use coloring with higher purity and other stabilizing agents, as mentioned in materials and methods. However, during storage PEF treated samples lost their Allura Red intensity, diminishing the C^* value as observed. Model systems showed similar behavior to the previous functions, i.e., a less concentrated system, less saturation, and faster degradation with or without PEF treatment. But as Allura Red concentration was increased, the stability of the color remained despite the treatment, as observed in Fig. 5.

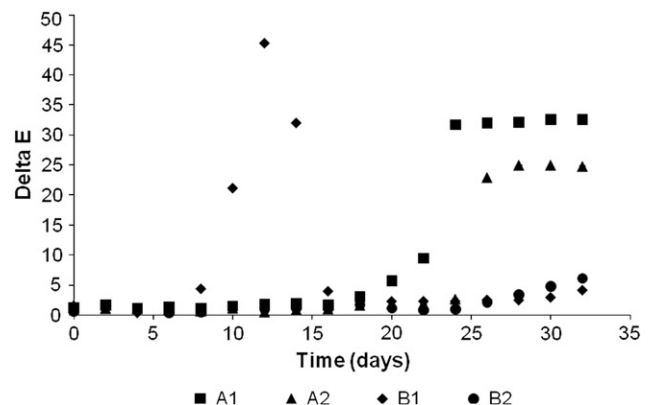


Fig. 3. Net change of color (ΔE) during storage (4 °C) in samples of commercial strawberry flavored milk (A_1 , A_2) and model systems (B_1 , B_2).

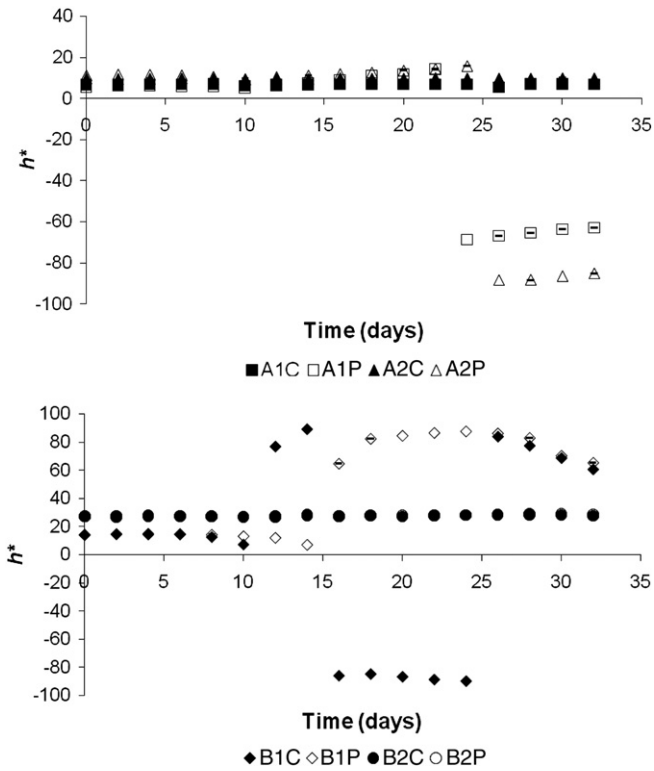


Fig. 4. Hue angle during storage (4 °C) for samples of strawberry flavored milk; commercial brands (control: A_{1C} and A_{2C}; processed A_{1P} and A_{2P}) and model systems (control: B_{1C} and B_{2C}; processed B_{1P} and B_{2P}).

2.5.6. Microbiological counts

Microbiological counts in the commercial milks before processing were too low (<10 cfu/ml) to be detected with conventional methods.

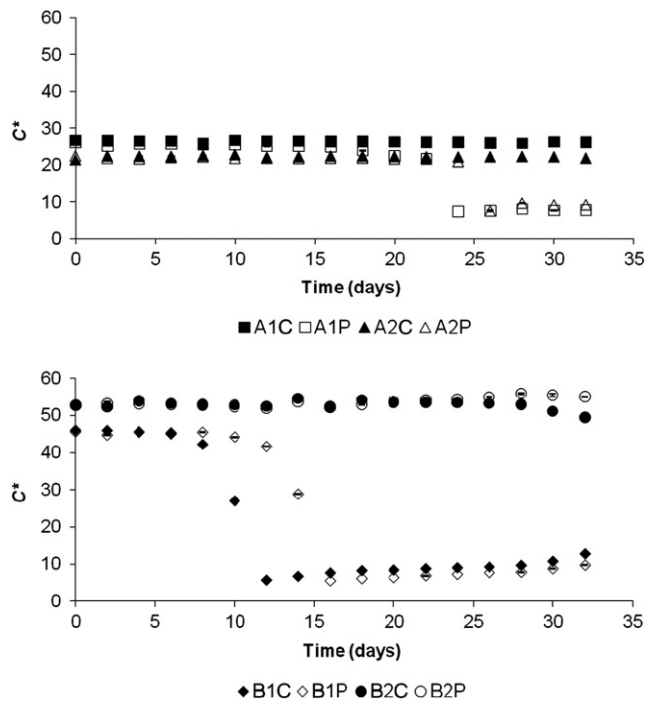


Fig. 5. Chroma or saturation index for samples during storage (4 °C) of strawberry flavored milk; commercial brands (control: A_{1C} and A_{2C}; processed A_{1P} and A_{2P}) and model systems (control: B_{1C} and B_{2C}; processed B_{1P} and B_{2P}).

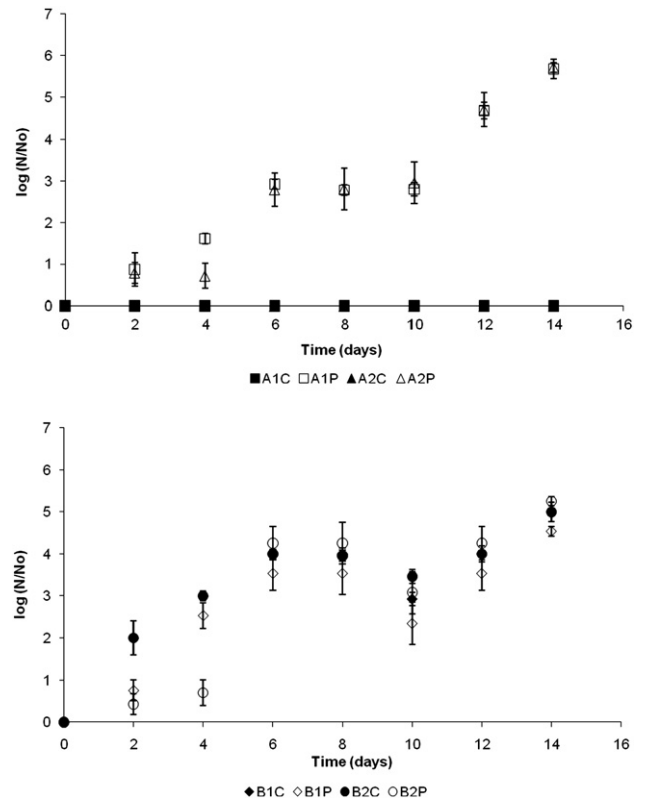


Fig. 6. Microbial growth in samples during storage (4 °C) of strawberry flavored milk; commercial brands (control: A_{1C} and A_{2C}; processed A_{1P} and A_{2P}) and model systems (control: B_{1C} and B_{2C}; processed B_{1P} and B_{2P}).

Model systems showed less than 10 cfu/ml before processing with PEF. The growth of mesophilic bacteria after processing and during storage is shown in Fig. 6. In the first case, the commercial samples A_{1C} and A_{2C} did not show microbial growth throughout storage, at least at a detectable level with conventional methods. Since both samples A_{1C} and A_{2C} were processed with UHT pasteurization, all mesophilic bacteria in the products were eliminated, or at least reduced to very low counts, and therefore could not be detected by pour plating. Commercial samples processed with PEF showed an increase in the bacterial population throughout storage. One reason for this increase in growth could be a possible cross contamination; although the microbial quality of the PEF system and all related materials were tested without evidence of microorganisms, there is the possibility that dormant spores of some thermally resistant microorganisms were activated with the use of PEF. After 12 days of storage, the milks (A_{1P}, A_{2P}) showed almost a 5 log reduction in microbial growth. Model systems (B₁, B₂, control, and processed) showed a similar behavior in microbial growth during storage, increasing to a 5 log reduction after 14 days of storage. In this case, there was no difference between the commercial and model systems because the milk used for the model systems was not UHT pasteurized and contained some bacteria capable of accelerating the growth of more microorganisms. Also, PEF processing conditions were chosen based on those used for milk pasteurization without any added ingredients (just plain milk); it has been shown by others that the presence of other components (i.e. fat and protein) in the product affect the microbial inactivation by PEF and requires the strongest conditions (Sobrinho-López, Raybaudi-Massilia, & Martín-Belloso, 2006). In this research the model systems showed the presence of a coloring agent, plus sucrose; even when pasteurized milk (processed at 72 °C, 15 s) was used in the formulation, pasteurization did not inactivate all the microorganisms in milk and the growth of remaining cells could be stimulated by PEF, showing higher counts (Fig. 6).

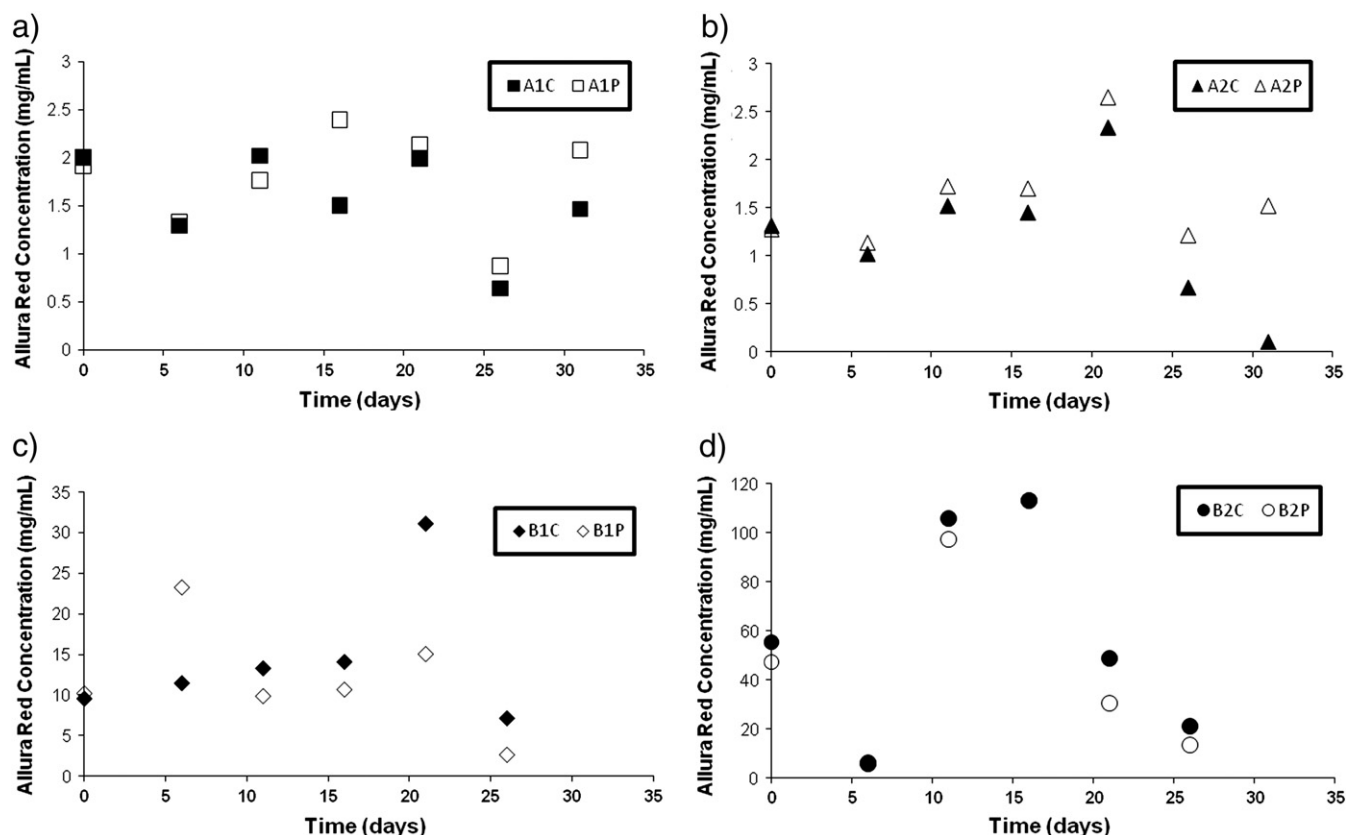


Fig. 7. Allura Red concentration (mg/mL) in samples during storage (4 °C) of strawberry flavored milk; commercial brands: (a) control A_{1C} and processed A_{1P}; (b) control A_{2C} and processed A_{2P}; and model systems: (c) control B_{1C} and processed B_{1P}; (d) control: B_{2C} and processed B_{2P}.

2.5.7. Concentration of Allura Red

In Fig. 7, concentration of Allura Red in all systems is shown during storage. Samples were analyzed the storage period but results only reflect up until day 31 for commercial samples and day 26 for model systems, since after these respective days of storage the HPLC values were not possible to integrate because of the presence and interference of big noisy peaks. These peaks were not present originally and their presence in later days towards the end of storage could be due to the binding of protein with the dye or other chemical reactions. The presence of other dyes such as Sunset Yellow or Tartrazine may cause interference with Allura Red because of similar azo structure (Fig. 8) (Alghamdi, 2005). Even though none of the commercial brands contain any of the above dyes, sample A₁ contains

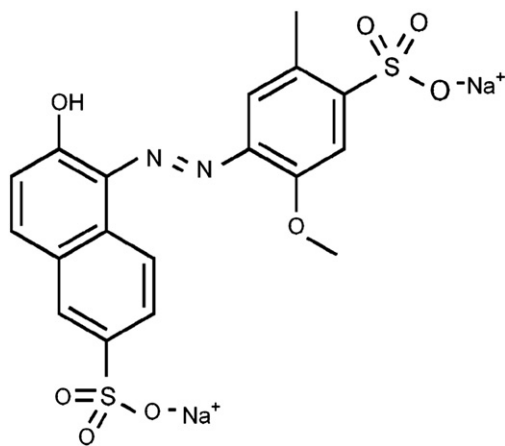


Fig. 8. Chemical structure of Allura Red (FD&C Red No. 40); chemical name, disodium (5Z)-5-[(2-methoxy-5-methyl-4-sulfonatophenyl)hydrazinylidene]-6-oxonaphthalene-2-sulfonate.

the dye Blue 1, which is a charged molecule (Fig. 9); and sample A₂ contains some artificial flavors, which could be interacting with Allura Red. The first part of Fig. 7 shows the behavior of commercial brand A₁; both samples, untreated (A_{1C}) and PEF treated (A_{1P}), showed a constant concentration during storage; the PEF sample even showed a slight increase in concentration. For the commercial sample A₂, concentration of the coloring agent showed a maximum peak of 2.5 mg/mL after 20 days of storage; opposite behavior was observed for the untreated sample (A_{2C}) and the PEF sample (A_{2P}) after day 26, with an important decrease in the A₂ sample at end of storage period.

The model systems showed different behaviors. Model system B₁ had an almost constant concentration of coloring agent during storage with a final drop of coloring agent. Meanwhile, model system B₂ presented the same concentration in the untreated (B_{2C}) and treated (B_{2P}) samples, although concentration did not follow a constant trend. Reports on stability data for FD&C Red No. 40 shows that this dye has very good stability regardless of pH (from 3 to 8); it is stable with light, heat (up to 105 °C), acid (10% acetic acid), and presence of

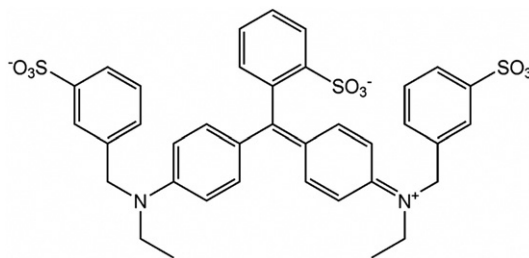


Fig. 9. Chemical structure of Brilliant Blue (FD&C Blue No. 1); chemical name, disodium 2-[[4-[[ethyl-[(3-sulfonatophenyl)methyl]amino]phenyl]-4-[[ethyl-[(3-sulfonatophenyl)methyl]azoniumylidene]cyclohexa-2,5-dien-1-ylidene]methyl]benzenesulfonate.

SO₂; the colorant also shows fair stability in the presence of base (Thorngate, 2002).

So, the changes observed in the coloring agent in these samples are not a result of changes in pH or increase of temperature (in all experiments temperature was kept below 55 °C); one theory that could describe these changes is the possibility of binding between the colorant agent and some other charged molecules in milk because of the presence of strong electric fields attracting particles and forming new matrices; this could thus change the electron configuration of the molecules and scatter or absorb the light in different ways and produce different colors to the human eye. Even though color and concentration of Allura Red are two different aspects, as studied in this research, the intensity of the color is directly correlated with the concentration of the coloring agent. Some samples lost intensity of color and there were some variations in Allura Red concentration during storage (Fig. 7). These facts could be related to chemical reactions taking place during storage as well as the microbial growth observed in samples processed with PEF. The affinity of Allura Red with proteins has been shown in work by Abdullah et al. (2008) in which the dye was attached to casein fractions and the effect enhanced by heat as previously mentioned. Because of the thermal effect in protein denaturation, Allura Red showed improved and constant binding with the casein. Even though PEF is a nonthermal technology, it is clear that during pulsing inside the treatment chamber there is an increase of temperature lasting a few seconds; as a result, milk proteins like casein could be attaching some dye molecules and forming stronger structures that could be broken instantaneously because of the intensity of the electric field applied at the same time inside the chamber. Sample A₁, containing molecule of Blue 1 (Fig. 9), is a good example of the possible interaction between dyes after PEF processing. Blue 1 has some charged groups that could be reacting and experiencing a rearrangement in electron configuration after interaction with pulsed electric fields. The interaction with other components in the food such as sugar, starch and other dyes could also interfere with the detection and quantification of Allura Red (López-de-Alba, Wróbel-Kaczmarczyk, Wróbel, López-Martínez, & Amador Hernández, 1996). However, further research on flavored milks must be conducted to obtain a better explanation of what is happening with the electrons of the molecules and the new complexes and chemical reactions taking place during and after PEF processing. The possibility of formation of new compounds is also high, which should be carefully studied to eliminate any possibility of toxicological reactions. Also, other processing conditions should be studied in more complex systems different from plain milk that could be of interest because of the presence of other macromolecules.

3. Conclusions

Pulsed electric fields technology is a nonthermal method that could offer important energy and cost savings to the dairy industry in the pasteurization of milk. However, because of the interaction of electricity between charged molecules in milk with coloring agents and other additives during PEF treatment, new matrices were formed within the product in the studied PEF processed milk samples, resulting in a lack of color after some days of storage. But the presence of other ingredients and dyes helped retain this color after PEF processing in the commercial samples. The concentration level of

Allura Red also showed better stability of color in samples at higher concentrations regardless of treatment.

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