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Response Surface Methodology (RSM) Optimization of Pulsed Electric Field (PEF) Pasteurization Process of Milk-Date Beverage

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Abstract: Milk beverage with added natural sweetener is well appreciated by consumers as a nutritious and healthy product with unique sensorial quality attributes. However, this product requires a suitable pasteurization method without significant impact on the sensorial and physicochemical quality characteristics of the product. This study optimizes the pulsed electric filed (PEF) conditions for the pasteurization of a milk-date beverage with conserved physicochemical quality properties. The effect of process variables, such as pulse off time (20, 30, and 40 µs), number of pulses (20, 50, and 80), powder ratio (10, 15, 20, and 25% *w*/*w*), storage time (2, 4, and 6 days), and storage temperature (5, 15, and 25 °C) on the responses of total viable count (TVC), color difference (ΔE), pH, and total soluble solids (TSS) was evaluated using the RSM central composite design (CCD). Pulse off time, number of pulses, date powder/milk ratio (*w*/*w*), storage time, and storage temperature greatly impacted the microbial and physical properties of the beverage. The optimal conditions for decreasing the microbiological load and physical change of beverages were a pulse off time of 40 µs, number of pulses of 80, and storage temperature of 5 °C for all powder ratios. These variables gave a safe beverage for up to six days. At optimal conditions, the values of pH, TSS, ΔE, and TVC were 7.51, 15.44 °Brix, 18.01, and 0.138 Log 10 CFU/mL, respectively, for the powder ratio of 10% (*w*/*w*); 7.66, 18.6 °Brix, 21.46, and 0.284 Log 10 CFU/mL, respectively, for the powder ratio of 15% (*w*/*w*); 7.56, 21.52 °Brix, 25.24, and 0.577 Log 10 CFU/mL, respectively, for the powder ratio of 20% (*w*/*w*); and 7.2, 24.2 °Brix, 29.34, and 0.741 Log 10 CFU/mL, respectively, for the powder ratio of 25% (*w*/*w*).

Keywords: date powder; pulsed electric field; total viable count; response surface methodology

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1. Introduction

Palms of date (*Phoenix dactylifera* L.) are very important fruit-producing trees that have great relevance in cultivation in semi-arid and arid regions of the globe because of their low water and nutrient requirements, in addition to their tolerance to high temperature, and thus are considered to be an eco-efficient crop [1]. Date palm fruits are highly nutritious, having numerous health benefits, and consequently, have been used in food and pharmaceutical applications as raw and processed date products [2]. Date powders have been introduced in many types of food, such as pastries, juices, and flavored milk. Hence, there is great importance for the production of date powder using innovative drying techniques and the subsequent utilization of the produced powder as a rich source of nutrients, such as minerals, vitamins, sugars, and fiber, in food and pharmaceutical applications. There is currently a strong trend toward the consumption of high-quality milk-based beverages with natural additives such as date fruit products that are passed via the cold chain and have a comparatively brief shelf life [3,4]. Consumers tend to choose a beverage based on its nutritive value, sensory attributes, and functional properties, such as color, flavor, and aroma. Nowadays, huge consumer demand exists for cocktails or juice blends due to their nutritional properties. Beverages blending with natural sources of fiber and bioactive compounds are one of the best ways to enhance a product's nutritional value [5].

Pasteurized milk is produced by heating and cooling raw milk to completely eliminate pathogenic microorganisms and the overwhelming majority of other microorganisms [6]. During storage, the temperature must be regulated for preventing the growth of bacteria and the production of enzymes that degrade the milk quality [7]. Thermal pasteurization is traditionally used to deactivate microorganisms and expand the storage life of food products [8]. Nevertheless, thermal treatment could cause a negative impact on the sensory and nutrition quality of food products. As consumers demand less-processed products that retain their nutritional value, there is a developing interest in non-thermal food storage and processing methods [9]. Pulsed electric field application is used to pasteurize and sterilize food as one of the non-thermal methods of preserving food. Due to the fact that the PEF procedure does not greatly raise the temperature of the product, microorganism inactivation is also achieved. It has been observed that food properties such as color, flavor, odor, and protein content are well preserved [10]. It is believed that high-voltage impulses inactivate microorganisms by disintegrating the cell membrane and cell wall, thereby increasing the storage stability of food products [11]. The main goal of food preservation is to maintain the food's physical properties while controlling the growth of microorganisms to guarantee product safety and prevent spoilage that renders food undesirable. This investigation aims to optimize the PEF conditions for pasteurizing a milk beverage containing date palm powder and determine the impact of the PEF pasteurization process on the total viable counts and some physical properties of milk date beverage.

2. Materials and Methods

2.1. Prepare the Beverage

The date powder was purchased via the local market of Al-Qassim, Saudi Arabia. The date powder was placed in sterile polythene bags and stored at 5 °C for further use. The beverage was made using four addition ratios of the date powder (10, 15, 20, 25% (*w*/*w*)). The powder was added to low-fat cow's milk and mixed in an electric mixer (type: T2, MACAP, Italy) for two min. The beverage samples were pasteurized using PEF and treated as follows:

Pulsed Electric Field (PEF)

The pulsed electric field (PEF) process is a non-thermal preserving method with minimal energy input. The PEF method treats food in a chamber containing 2 electrodes that emit brief pulses of electricity. A pilot scale pulsed electric field generator model JSV-20-21/02 (Thane, Maharashtra, India) was used for the PEF treatment. The treatment device comprised a chamber containing a 1 L cup. The beverage temperature was at room temperature and tested at 40 kV cm^{-2}; pulse off time of 20, 30, and 40 μs; and No. of pulses of 20, 50, and 80. The pasteurized beverage samples were filled into sterile 50 mL jars, which were stored at 5, 15, and 25 °C for six days immediately following filling. The specimens were determined in triplicate for total viable count (TVC), pH, color change (ΔE), and total soluble solids (TSS) at intervals of 2, 4, and 6 days of storage.

2.2. Optimization of Pasteurization

A number of experiments have been designed to find out the best PEF pasteurization treatment and storage condition of the milk-date beverage. Experiments were carried out by means of a set of variables, namely, PEF amplitude, PEF off time, PEF No. of pulses, powder ratio, storage time, and storage temperature.

2.3. Experimental Design

The RSM was utilized to optimize the pasteurization factors that impact the beverage properties and microbial growth. Using Design expert® 13.0.4.0, the experimental design and statistical analysis were implemented (version13.0.4.0, Stat-Ease Inc., Minneapolis, MN, USA). The experiments were conducted according to a Box–Behnken design into one group of design. A PEF consisting of thirty-one test runs and five replicates at the central point was utilized. The independent factors were PEF pulse off time (X_1) (20, 30, and 40 µs), PEF No. of pulses (X_2) (20, 50, and 80), powder ratio (X_3) (10, 15, 20, and 25% (w/w)), storage time (X_4) (0, 2, 4, and 6 days), and storage temperature (X_5) (5, 15, and 25 °C) at a constant amplitude of 40 kV/cm [12]. The dependent variables were pH, ΔE, TSS, and TVC. Table 1 displays the factors and their values in both coded and natural form.

Table 1. Factors and their coded and actual values used for optimizing pulsed electric field.

Factors	Level (Code)			
Pulse off time, µs (X1)	20 (−1)	30 (0)	40 (1)	
No. of Pulses, (X2)	20 (−1)	50 (0)	80 (1)	
Powder ratio,% (w/w) (X3)	10 (−1)	15 (−0.333)	20 (0.333)	25 (1)
Storage time, day (X4)	0 (−1)	2 (−0.333)	4 (0.333)	6 (1)
Storage temperature, °C (X5)	5 (−1)	15 (0)	25 (1)	

Using experimental data, the second-order polynomial equation was fitted with the following multiple regression equation.

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_i X_j + \sum_{j=1}^{k} \beta_{jj} X_j^2 + \sum\sum_{i<j} \beta_{ij} X_i X_j$$

where β_0, β_j, β_{ij}, and β_{jj} are the coefficients of regression for the intercept, interaction, and square, respectively, and Y is the predicted response. Xi and Xj are independent codes of variables. By using an F test, coefficients were interpreted. Utilizing surface plotting, regression analysis, and analysis of variance (ANOVA), the optimal pasteurization conditions were determined.

2.4. Determination of pH of the Beverage

The pH of the milk-date beverage was measured at 2-day intervals using a pH meter (Jenway, Model 3510, pH Meter, UK) in duplicate.

2.5. Color

The color parameter of the beverage was identified by measuring sample color before and after treatment using a Nix Sensor Ltd. 175 Longwood Road South, Suite 408A Hamilton, ON, Canada. Basic color parameters a* redness, b* (yellowness), and L* (lightness) were determined in triplicate. Using the following equation, the total color difference (ΔE) was computed:

$$\Delta E = \sqrt{(L_0 - L)^2 + (a_0 - a)^2 + (b_0 - b)^2}$$

where the subscript '0' indicates initial color of milk.

2.6. Total Soluble Solids (TSS)

The total soluble solids concentration of the beverage was determined with a refractometer (Model: ATAGO-28E) fitted with a sugar percentage scale and expressed in °Brix.

2.7. Microbial Analysis

Total Viable Count (TVC) of Bacteria

In accordance with the Association of Official Analytical Chemists [13], 1 mL of milk-date beverage samples was mixed with 9 mL of the 0.85% NaCl sterile solution. The samples were shaken carefully. A suitable serial dilution of 10^{-1}–10^{-4} was prepared, and 1 mL of the appropriate dilution was added to 10 mL of sterilized and chilled nutrient agar (45 °C) in sterilized Petri dishes (Oxoid, CM0309). The dishes were left to incubate at 35 °C for 24–48 h, and the TVC was counted and expressed as CFU/mL.

2.8. Statistical Analysis

The data from each sample were collected in triplicate and analyzed statistically. Utilizing SPSS software version 18.0 (SPSS Inc., Chicago, IL, USA), the microbial and physical quality attributes of milk-date beverage were statistically analyzed. Using Design expert, RSM data were examined® 13.0.4.0 (version13.0.4.0, Stat-Ease Inc., Minneapolis, MN, USA). ANOVA was used to examine the relationships of quadratic, interaction, and linear effects of the independent variables (pulse off time, No. of pulses, powder ratio, storage temperature, and storage time) on the responses (TSS, pH, ΔE, and the growth of total viable counts). Using adjusted coefficient of determination (adjusted R^2), adequate precision, coefficient of determination (R^2), and coefficient of variation (CV), the precision and adequacy of response surface methodology models were verified. Acceptable significance levels for the RSM models were $p < 0.001$, $p < 0.01$, and $p < 0.05$ levels.

3. Results and Discussion

3.1. Model Fitting

However, the exposure duration, pulse intensity, number of pulses, and post-treatment storage time and temperature affect the ability of PEF interventions to preserve the quality characteristics of beverages. Each of these variables must be optimized in order to get the greatest advantages of PEF as a beverage-processing technique. Using the RSM model, this investigation optimized PEF pasteurization treatment conditions.

The impacts of pasteurization and conditions of storage on the qualitative characteristics of a milk-date beverage were investigated in this study. Using experimental data, second-order polynomial equation coefficients were derived to determine the significance of model coefficients. Table 2 displays the experimental data's resulting polynomial models with coefficient of variance (CV) and coefficients of multiple determinations (R^2) for total viable count, pH, color difference, and TSS. The results suggested that the regression model could adequately represent the values of the dependent variables, and the error analysis revealed that the lack of fit was not statistically significance with regard to any of the dependent variables. The model's *p*-value for all evaluated parameters indicated that the model was statistically significant ($p > 0.001$) for the measured characteristics. In addition, the *p*-value for the lack of fit ranges between 0.0592 and 0.9998, indicating no statistical significance ($p > 0.05$) and demonstrating that the utilized models adequately explained the experimental data. Additionally, R^2 values for the assessed characteristics range between 0.9703 and 0.9998, whereas adjusted R^2 values range between 0.9108 and 0.9993. Consequently, more than 97% of the total variance of the trait data is accounted for by the polynomial quadratic models generated with Equation (1). Additionally, adjusted R^2 and R^2 values were close to one, showing extremely significant correlations among experimental and predicted values of all evaluated characteristics [14]. Optimal models have an adequacy precision value greater than 4.0 [15]. This research revealed adequacy precision values ranging from 14.17 to 157.47, indicating that the model is competent and is capable of effectively analyzing the data. In addition to the coefficient of variation (CV), which measures the model's precision and validity, it is preferable for it to be less than 10% [16]. In this research, the coefficient of variation (CV) values for the evaluated parameters ranged from 0.9068 to 8.62% (<10%), indicating reproducibility and high accuracy of experimental data. The RSM models used were reproducible and adequate, according to the results of this investigation. They could be utilized to effectively optimize the pulsed electric field pasteurization process conditions and the storing conditions of milk beverages containing date powder in order to preserve their quality characteristics during storage.

Table 2. Regression coefficients for product responses and process factors.

Factors	**pH**	**TSS**	**ΔE**	**TVC**
Intercept				
β0	5.96	19.24	28.72	4.25
Linear				
X1 (β1)	−0.0979 ***	−0.8050 ***	0.3676 ***	0.0015
X2 (β2)	0.2733 ***	0.2113 *	0.2572 ***	−1.12 ***
X3 (β3)	−0.2143 ***	3.09 ***	5.16 ***	−0.2658 ***
X4 (β4)	−0.7028 ***	−0.6860 ***	1.33 ***	−0.3515 ***
X5 (β5)	−0.5358 ***	−0.4153 ***	1.75 ***	−0.6713 ***
Interaction				
X1X2 (β12)	0.0073	0.4245 ***	−0.4555 ***	0.2590 **
X1X3 (β13)	0.0575 *	−0.1732	0.0589	−0.0117
X1X4 (β14)	0.1794 ***	0.5329 **	−0.6862 ***	−1.10 ***
X1X5 (β15)	0.0245	0.2514 *	0.3995 ***	−0.2108 *
X2X3 (β23)	0.0470 *	0.4614 ***	−0.3372 ***	0.9433 ***
X2X4 (β24)	0.1360 ***	−0.0312	−1.10 ***	−1.17 ***
X2X5 (β25)	−0.0891 **	−0.1422	−0.1882 **	−0.0155
X3X4 (β34)	0.0157	0.5526 ***	−1.04 ***	0.1653 *
X3X5 (β35)	0.0612 **	−0.4573 ***	−1.82 ***	0.3917 ***
X4X5 (β45)	−0.5955 ***	−0.4769 **	1.57 ***	−1.26 ***
Quadratic				
$(X1)^2$ (β11)	0.1989 ***	−0.1655	−1.57 ***	−0.7505 ***
$(X2)^2$ (β22)	−0.1084 **	−0.3459 *	0.3255 **	−0.9047 ***
$(X3)^2$ (β33)	−0.2845 ***	−0.2633	0.3732 ***	0.1452
$(X4)^2$ (β44)	0.3025 ***	−0.0251	0.4709 ***	−0.8056 ***
$(X5)^2$ (β55)	0.3047 ***	0.9537 ***	−0.8266 ***	−0.9078 ***
Model F-value *p*-value	366.99 0.0001	128.19 0.0001	2094.62 0.0001	121.32 0.0001
Mean	6.24	19.16	27.16	2.42
C.V.% Adeq. precision R^2	0.9068 68.1428 0.9986	1.54 45.2780 0.9961	0.5161 157.47 0.9998	8.62 35.97 0.9959
Adjusted R^2	0.9959	0.9883	0.9993	0.9877
Std. Dev.	0.0566	0.2955	0.1402	0.2082
F-value (Lack of Fit)	0.2736	0.0154	0.0796	4.62
p-value (Lack of Fit)	0.9094	0.9998	0.9926	0.0592

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

3.2. Impact of PEF Processing and Storage Conditions on pH in Milk-Date Beverage

The results of the independent variables (pulse off time, powder ratio, storage time, and storage temperature) had showed a negative impact on pH, i.e., pH declines with increase in powder ratio, pulse off time, storage time, and storage temperature. The variables exhibited extremely significant ($p < 0.001$) influences on pH (CV = 0.9068, R^2 = 0.9986, and adjusted R^2 = 0.9986), indicating that the model fits exceptionally well (Table 2), while the number of pulses had a positive effect ($p < 0.001$) on pH, suggesting that increasing the number of pulses could increase the pH of milk-date beverages.

The interaction effects of pulse off time and powder ratio and number of pulses and powder ratio had positive ($p < 0.05$) impacts on pH, implying that increased pulse off time with powder ratio and increased number of pulses with powder ratio could elevate the pH of the beverage. Furthermore, assessing the combined effect of storage temperature with powder ratio, pulse off time with storage time, and storage time with number of pulses showed positive ($p < 0.01$; 0.001; 0.001, respectively) effects on the pH. The interactive influence of storage temperature with number of pulses and storage temperature with storage time on pH was negative ($p < 0.001$, 0.01), implying that an increase in these factors would result in a reduction in the pH of the beverage. In quadratic terms, storage temperature, pulse off time, and storage time showed a positive impact ($p < 0.001$) on pH, implying that a rise in these parameters would lead to a rise in beverage pH, whereas the number of pulses and powder ratio had a negative ($p < 0.001$; $p < 0.01$) impact on pH, respectively, demonstrating that increasing the number of pulses and powder ratio would reduce the pH of the beverage.

Using significant terms of coded factors, the following equations were developed to predict the impacts of PEF usage and storage conditions on the pH of the milk-date beverage:

$$\begin{aligned} Y_{pH} = 5.96 &- 0.0979X_1 + 0.2733X_2 - 0.2143X_3 - 0.7028X_4 - 0.5358X_5 + 0.0575X_1X_3 + 0.1794X_1X_4 \\ &+ 0.0471X_2X_3 + 0.136X_2X_4 - 0.0891X_2X_5 + 0.0612X_3X_5 - 0.5955X_4X_5 + 0.1989X_1^2 \\ &- 0.1084X_2^2 - 0.2845X_3^2 + 0.3025X_4^2 + 0.3047X_5^2 \end{aligned} \quad (1)$$

Figure 1 displays the 3D response surface plots of the pH as affected by the PEF treatment and storage conditions. Whereas raising the number of pulses caused the pH to reach its maximum at 80 (Figure 1a,e–g), increasing the pulse off time declined the pH of the beverage to the lowest value at 32 µs, and then raised it at the highest pulse off time (Figure 2a–d). Increasing the powder ratio increased the beverage's pH to the maximum value at 16% (*w*/*w*), and then decreased it at higher powder ratio (Figure 1b,e,h,i). When the storage temperature was increased, the pH of the beverage progressively decreased, achieving its lowest levels at the highest temperature (25 °C) (Figure 1d,g,i,j). Comparable results were observed by [17], who observed that the pH of probiotic soymilk stored at low temperatures (4 °C) for 28 days decreased gradually and slowly. Reduction in pH and increase in titratable acidity at high storage temperatures could be explained by the increased action of microorganisms that use the beverage's nutrients to produce an acid medium. In addition, sucrose fermentation by lactic bacteria is accompanied by a rapid increase in acidity at high temperatures [18]. In contrast, when the temperature is low, the acidity remains stable due to the temperature's inhibiting effect. In fact, the cold inhibits the multiplication of microorganisms [19], prolonging the storage time and decreasing pH to its lowest levels at 6 days. During storage, the pH decreased due to the production of lactic acid by microorganisms. Due to the increase in microbial population, during the storage period, the pH of PEF-treated samples decreased slightly. Furthermore, [20] discovered that the pH of a PEF (25 kV/cm, 280 µs)-treated orange–carrot beverage blend reduced after 8.5 weeks of storage at 12 °C due to microbial deterioration. In addition, they reported a decrease in pH during the last week of their shelf life analysis of orange and carrot juice blends and suggested that microbial proliferation was the cause [20].

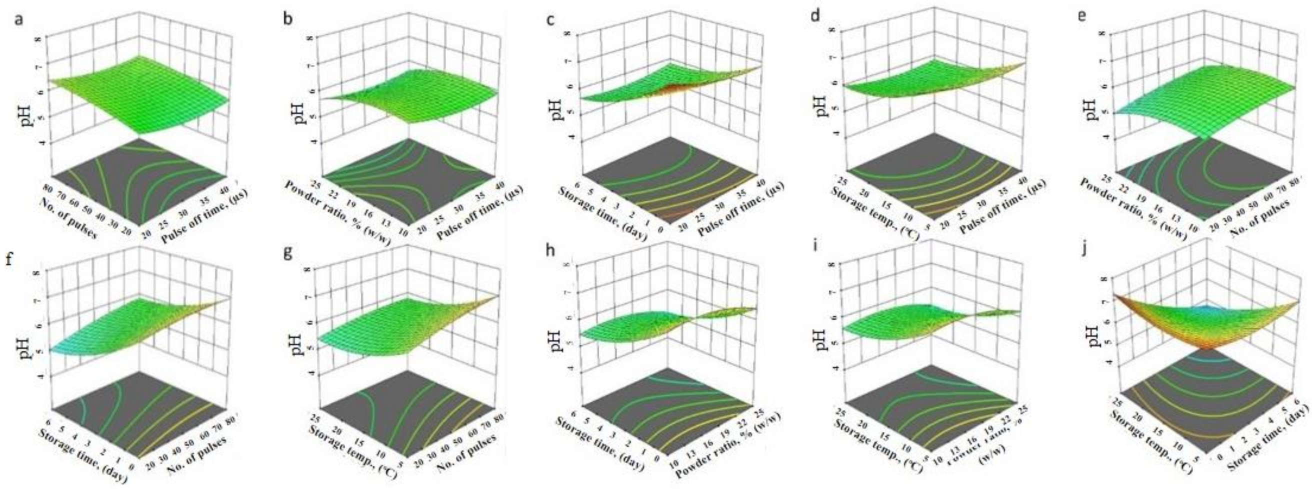

Figure 1. Response surface plots of pH of beverage as a function of pulse off time and No. of pulses (**a**), pulse off time and powder ratio (**b**), pulse off time and storage time (**c**), pulse off time and storage temperature (**d**), No. of pulses and powder ratio (**e**), No. of pulses and storage time (**f**), No. of pulses and storage temperature (**g**), powder ratio and storage time (**h**), powder ratio and storage temperature (**i**), and storage time and storage temperature (**j**).

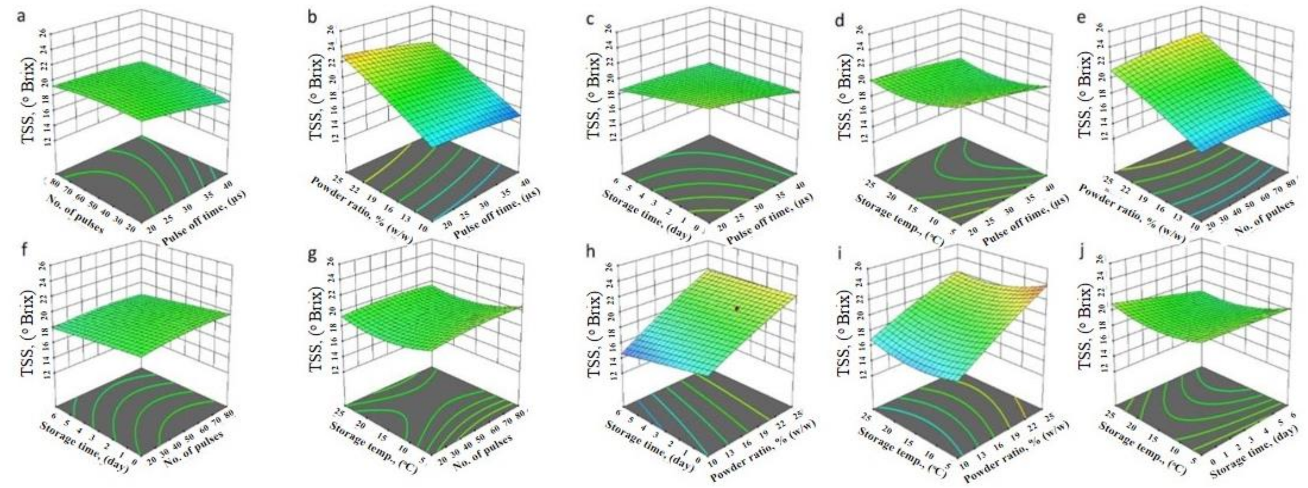

Figure 2. Response surface plots of total soluble solids (TSS) of beverage as a function of pulse off time and No. of pulses (**a**), pulse off time and powder ratio (**b**), pulse off time and storage time (**c**), pulse off time and storage temperature (**d**), No. of pulses and powder ratio (**e**), No. of pulses and storage time (**f**), No. of pulses and storage temperature (**g**), powder ratio and storage time (**h**), powder ratio and storage temperature (**i**), and storage time and storage temperature (**j**).

3.3. Impact of PEF Treatment and Storage Conditions on TSS in Beverage from Milk-Based Date Powder

The independent variables (storage temperature, pulse off time, and storage time) had a negative impact on TSS, i.e., TSS decreased as the independent variables increased. Table 2 demonstrates that these variables had extremely significant ($p < 0.001$) impacts on TSS (adjusted $R^2 = 0.9883$, CV = 1.54, $R^2 = 0.9961$), indicating that the model fits extremely well, whereas the number of pulses and powder ratio had a positive effect ($p < 0.001$, $p < 0.05$, respectively) on TSS, implying that a rise in these variables could lead to a rise in the TSS of the beverage.

The interaction impact of number of pulses with pulse off time, number of pulses with powder ratio, and powder ratio with storage time had showed positive ($p < 0.001$) impacts on TSS, implying that increased levels of these variables could increase the TSS of milk-date beverages, while the interaction effect of pulse off time with storage time and pulse off time with storage temperature had positive ($p < 0.001$, $p < 0.05$, respectively) impacts on TSS. The interactive influence of storage temperature with storage time and storage temperature with powder ratio on TSS were negative ($p < 0.01$), implying that an increase in these factors would result in a decline in TSS of the beverage. In quadratic terms, storage temperature had a positive impact ($p < 0.001$) on TSS, implying that a rise in storage temperature would increase the TSS of the beverage, whereas number of pulses had a negative ($p < 0.05$) impact on the TSS, demonstrating that increasing the number of pulses would decrease the TSS of the beverage.

Using significant terms of coded factors, the following equations were developed to predict the impacts of PEF usage and storage conditions on the TSS of milk-date beverages:

$$\begin{aligned} Y_{TSS} = 19.24 &- 0.805X_1 + 0.2113X_2 + 3.09X_3 - 0.686X_4 - 0.4153X_5 + 0.4245X_1X_2 + 0.5329X_1X_4 \\ &+ 0.2514X_1X_5 + 0.4614X_2X_3 + 0.5526X_3X_4 - 0.4573X_3X_5 - 0.4769X_4X_5 - 0.3459X_2^2 \\ &+ 0.9537X_5^2 \end{aligned} \quad (2)$$

Figure 2 illustrates 3D response surface plots of milk-date-beverage TSS as affected by the storage conditions and PEF treatment. Increasing the number of pulses caused TSS to peak at 50, and then decreased it at a higher number of pulses (Figure 2a–g). Increasing the pulse off time decreased the beverage's TSS to its lowest level at 40 µs (Figure 2a–d). In agreement with these findings, refs. [20,21] observed a small reduction in the TSS values following PEF treatment of orange and orange–carrot juice, respectively. The fermentation of carbohydrates by microorganisms could produce this phenomenon. Increasing the powder ratio brought the beverage's TSS to its utmost level at 25% (*w*/*w*) (Figure 2b,e,h,i). When the temperature of storage was increased, the TSS of the beverage progressively decreased, achieving its lowest values at 18.5 °C and then increasing at a higher storage temperature (25 °C) (Figure 2d,g,i,j). This may be the result of an increase in the total soluble solids (TSS) of a beverage at high storage temperatures. The solubility of sugars in water increases with increasing temperature. As the temperature of the beverage increases, the sugars will dissolve more easily, resulting in a higher TSS, whereas increasing the storage time decreased TSS to the minimum value at 6 days.

3.4. Impact of Storage Conditions and PEF Treatment on ΔE in Beverage from Milk-Based Date Powder

The independent variables (number of pulses, pulse off time, storage temperature, powder ratio, and storage time) showed positive impacts on ΔE, i.e., ΔE increased with an increase in number of pulses, pulse off time, storage temperature, powder ratio, and storage time. Extremely significant ($p < 0.001$) impacts of these variables on ΔE were observed (CV of 0.5161, R^2 of 0.9998, adjusted R^2 of 0.9993), which indicates that the model fits extremely well (Table 2).

Positive ($p < 0.001$) impacts on the ΔE were seen in the interaction between pulse off time with storage temperature as well as between storage temperature and storage time, indicating that combinations of increased pulse off time with storage temperature as well as storage time with storage temperature may increase the ΔE of beverage. The interaction effect of storage temperature with powder ratio, number of pulses with pulse off time, powder ratio with pulse off time, storage time with number of pulses, storage time with pulse off time, and storage time with powder ratio showed negative ($p < 0.001$) impacts on ΔE. The negative effects of the interaction between the number of pulses with the storage time and the pulse time with the storage time indicated a decrease in ΔE, especially at the lowest temperature of 5 °C. The lowest values for ΔE were recorded as 18.01, 21.4, 21.4, and 29 for powder ratios of 10, 15, 20, and 25% (*w*/*w*), respectively, at 80 pulses, 40 µs, and 5 °C, whereas the interaction effect of storage temperature with number of pulses showed negative ($p < 0.01$) impacts on the ΔE. In quadratic terms, storage temperature and pulse off time had a negative effect ($p < 0.001$) on ΔE, indicating that an increase in storage temperature and pulse off time will decrease the ΔE of the beverage. However, number of pulses, powder ratio, and storage time had a positive ($p < 0.01$, $p < 0.001$, $p < 0.001$, respectively) impact on the ΔE, demonstrating that raising these factors could reduce the ΔE of the beverage.

The prediction equations specifying the impacts of PEF usage and storing beverage on ΔE using significant terms of coded factors are as follows:

$$\begin{aligned} Y_E = 28.72 &+ 0.3676X_1 + 0.2572X_2 + 5.16X_3 + 1.33X_4 + 1.75X_5 - 0.4555X_1X_2 - 0.6862X_1X_4 + 0.3995X_1X_5 \\ &- 0.3372X_2X_3 - 1.1X_2X_4 - 0.1882X_2X_5 - 1.04X_3X_4 - 1.82X_3X_5 + 1.57X_4X_5 - 1.57X_1^2 \\ &+ 0.3255X_2^2 + 0.3732X_3^2 - 0.4709X_4^2 - 0.8266X_5^2 \end{aligned} \quad (3)$$

Figure 3 presents the 3D response surface plots of ΔE as influenced by PEF treatment and the storage conditions. The ΔE rose as the number of pulses increased, peaking at 80 pulses and 25 °C (Figure 3g), while the ΔE decreased to its lowest value at 80 pulses and 5 °C (Figure 3g) and at 80 pulses, 6 days, and 5 °C (Figure 3f). In addition, the beverage's ΔE rose with an increase in pulse off time, especially when the number of pulses decreased and the storage temperature decreased. The ΔE reached the maximum level at 30 µs and 25 °C, and then decreased at the pulse off time of 40 µs and 25 °C (Figure 3d), while the ΔE decreased to its lowest value at 40 µs and 5 °C (Figure 3d) and at 40 µs, 6 days, and 5 °C (Figure 3c). The ΔE of the beverage reached its maximum level at 25% (*w*/*w*) with an increase in the powder ratio (Figure 3b,e,h,i). This may be good if some consumers want to increase the percentage of sweetness, which is followed by an increase in the powder ratio and thus an increase in ΔE. When the storage temperature was raised, the beverage's ΔE increased as well, reaching its maximum level at 25 °C and lowest level at 5 °C (Figure 3d,g,I,j); a comparable decrease in lightness was observed at each of the three storage temperatures (5, 15, and 25 °C). However, the lightness value decreased more rapidly at 25 °C. In contrast, at storage temperatures of 5 °C and 15 °C, the lightness value decreased slowly [22], and extending the storage period concurrently led to a rise in ΔE, which peaked at 6 days at 25 °C and had the lowest level at 5 °C [23]. A crucial aspect of a beverage's appeal to consumers is its hue. A decrease in L* and b* values indicated that the beverage color became darker. Interestingly, noticeable increases in a* values indicated an increase in redness [24]. A significant rise in ΔE was seen after 15 days of storage in [25] on fresh carrot juice treated in a high-voltage electrostatic field and then kept at 4 °C, which is broadly consistent with the results of this research.

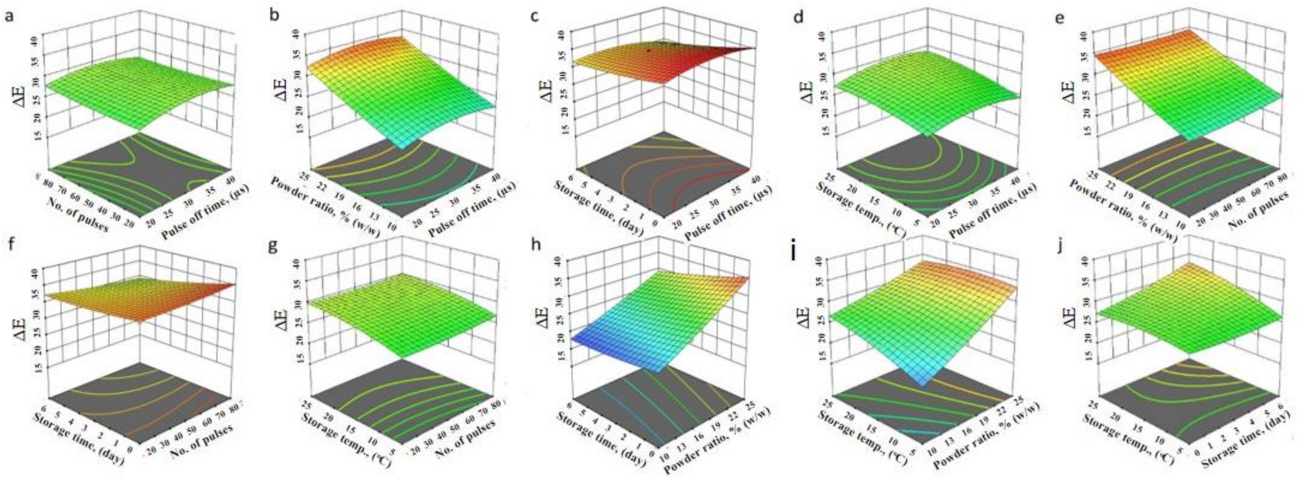

Figure 3. Response surface plots of color difference (ΔE) of beverage as a function of pulse off time and No. of pulses (**a**), pulse off time and powder ratio (**b**), pulse off time and storage time (**c**), pulse off time and storage temperature (**d**), No. of pulses and powder ratio (**e**), No. of pulses and storage time (**f**), No. of pulses and storage temperature (**g**), powder ratio and storage time (**h**), powder ratio and storage temperature (**i**), and storage time and storage temperature (**j**).

3.5. Impact of Storage Conditions and PEF Treatment on TVC in Beverage from Milk-Based Date Powder

The independent variables (number of pulses, storage temperature, powder ratio, and storage time) had a negative impact on TVC, i.e., TVC declines as the number of pulses, storage temperature, powder ratio, and storage time increase. These variables exhibited extremely significant ($p < 0.001$) impacts on TVC (adjusted R^2 of 0.9993, R^2 of 0.9998, and CV of 0.5161), indicating that the model matches well (Table 2).

The interaction effect of number of pulses with powder ratio, powder ratio with storage temperature, pulse off time with number of pulses, and powder ratio with storage time showed positive ($p < 0.001$, $p < 0.001$, $p < 0.01$, $p < 0.05$) impacts on the TVC, indicating that increased levels of these variables may enhance the TVC growth of a beverage. However, the interactive impact of storage time with pulse off time, storage temperature with storage time, storage temperature with pulse off time, and storage time with number of pulses on TVC was negative ($p < 0.001$, $p < 0.05$). In quadratic terms, pulse off time, number of pulses, storage temperature, and storage time had a negative impact ($p < 0.001$) on TVC, showing that an increase in these factors would decrease the milk-date beverage's TVC.

Using significant terms of coded factors, the following equations were developed to predict the impacts of storage conditions and PEF usage on the TVC of the milk-date beverage:

$$\begin{aligned} Y_{TVC} = {} & 4.25 - 1.12X_2 - 0.2658X_3 - 0.3515X_4 - 0.6713X_5 + 0.259X_1X_2 - 1.1X_1X_4 - 0.2108X_1X_5 \\ & + 0.9433X_2X_3 - 1.17X_2X_4 + 0.1653X_3X_4 + 0.3917X_3X_5 - 1.26X_4X_5 - 0.7505X_1^2 \\ & - 0.9047X_2^2 - 0.8056X_4^2 - 0.9078X_5^2 \end{aligned} \quad (4)$$

Figure 4 illustrates the 3D response surface diagrams of the TVC as influenced by conditions of storage and PEF treatment. Increasing the number of pulses decreased the TVC to its lowest value at 80 (Figure 4a,e–g), whereas increasing the pulse off time raised the TVC of the beverage to the highest value at 30 µs, and then reduced it to the minimum level at the pulse off time of 40 µs (Figure 4a–d). Microorganism inactivation is associated with alterations in the cell membrane and its electromechanical instability [26]. The primary impact of an electrical field is an increase in permeability of the membrane due to membrane compression and pore formation. Cell inactivation is believed to be caused by unusual membrane porosity [27]. Increasing the powder ratio decreased the beverage TVC to the minimum level at 25% (*w*/*w*) (Figure 4b,e,h,i), which could be due to increases in the TSS. The TVC of the beverage was gradually increased with the decrease in storage temperature. The beverage TVC was increased to the highest level at 15 °C, and then reduced to the minimum level at the storage temperature of 5 °C (Figure 4d,g,i,j). Low temperatures reduce the fluidity of the cytoplasmic membranes of bacteria, interfering with their transport pathways, and thus help in eliminating a large number of them [28]. However, at 25 °C, the TVC reduction could be due to the total soluble solids (TSS) of a beverage, which can increase at high storage temperatures. The solubility of sugars in water increases with increasing temperature. As the temperature of the beverage increases, the sugars will dissolve more easily, resulting in a higher TSS and a decrease in microbial growth rate. Therefore, the microbial growth rate increases with temperature until the maximal growth temperature is reached [29]. Furthermore, the interactive impact of storage time with pulse off time, storage temperature with storage time, and storage time with number of pulses on TVC was negative ($p < 0.001$; $p < 0.05$), and the TVC values were low and below the safe limits of TVC according to the International Standard No. GSO 984 at 80 pulses, 40 µs, and 5 °C during 6 days. This is because bacteria will continue to grow and multiply over time, and as they do, they will use up the available nutrients in the food. Once the nutrients are depleted, the bacteria will start to die off [30]. This is why it is important to store food in the refrigerator or freezer to slow down the growth of bacteria.

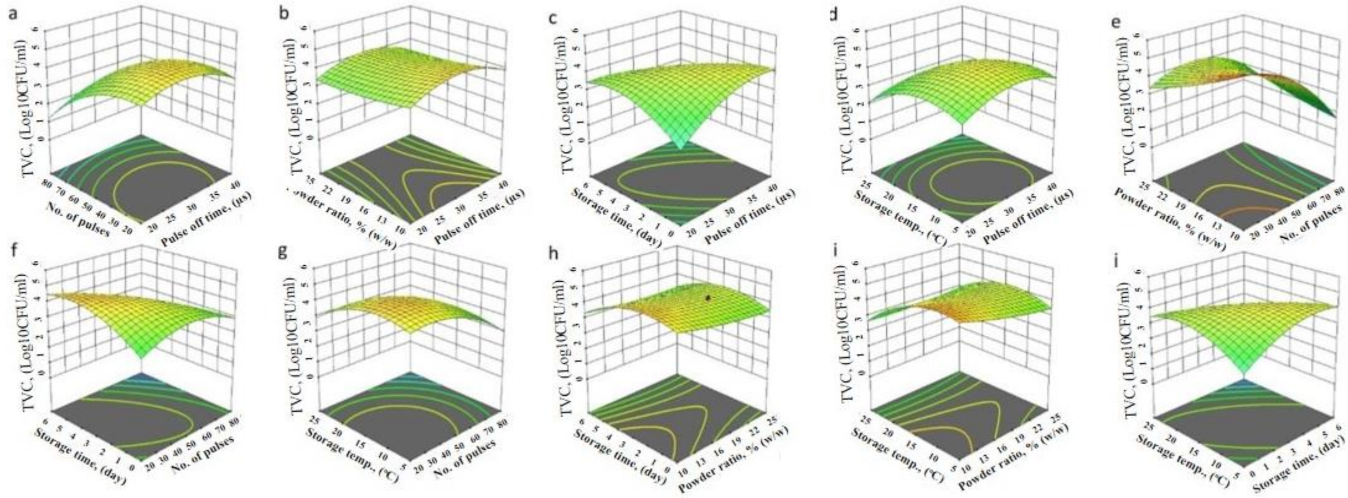

Figure 4. Response surface plots of total viable count (TVC) of beverage as a function of pulse off time and No. of pulses (**a**), pulse off time and powder ratio (**b**), pulse off time and storage time (**c**), pulse off time and storage temperature (**d**), No. of pulses and powder ratio (**e**), No. of pulses and storage time (**f**), No. of pulses and storage temperature (**g**), powder ratio and storage time (**h**), powder ratio and storage temperature (**i**), and storage time and storage temperature (**j**).

3.6. Optimization of PEF Treatment and Conditions of Storage

In this research, efforts were made to optimize the conditions of storage and PEF treatment to preserve the qualitative characteristics of a milk-date beverage without adding any preservatives. The RSM models and generated equations were used to determine the optimal conditions for reducing the physical changes and microbial load of the beverage. On the basis of this information, the optimal storage conditions and PEF treatment to maintain the beverage were pulse off time of 40 µs, number of pulses of 80, storage time of 6 days, and storage temperature of 5 °C for all powder ratios. Under these optimal conditions, the experimental values for the evaluated results matched the predicted values, indicating a high level of desirability.

4. Conclusions

Preserving a milk-based date-powder beverage's qualitative attributes remains the primary objective for consumers and producers. In this research, RSM was used to optimize pulsed electric field treatment and conditions of storage for maintaining the physical and microbiological properties of the beverage. Positive and negative effects of PEF conditions (number of pulses and pulse off time) and subsequent conditions of storage (temperature and time) at various powder ratios were observed on the quality attributes of the beverage. The optimal conditions for decreasing the microbiological load and physical change of beverages were pulse off time of 40 µs, number of pulses of 80, and storage temperature of 5 °C for all powder ratios. These variables gave a safe beverage for up to six days, with TVC values lower than those stipulated in the International Standard No. GSO 984. Overall, PEF can be used to preserve the quality of a milk-date beverage, and the beverage could be kept for up to 6 days after treatment at 5 °C without a substantial decrease in the product's quality attributes.

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