Optimization of spray drying parameters and food additives to reduce glycation using response surface methodology in powdered infant formulas

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Abstract The heat-based spray drying process generating the highest level of advanced glycation end-products (AGEs) in the infant formula processing was set as a control point from which the levels of AGE markers, *N*-carboxymethyllysine, 5-hydroxymethylfurfural, and fluorescence intensity, can be mitigated. We optimized the parameters, including inlet temperature, feeding rate, and aspirator rate during spray drying, and alternatively optimized food additives, including pyridoxine hydrochloride, *DL*- α -tocopheryl acetate, and *L*-carnitine. Using response surface methodology, the optimal condition based on our experimental condition for the inlet temperature, pump rate, and aspirator rate were 148.7 °C, 342.4 mL/h and 28.6 m³/h, respectively, and the optimal conditions of pyridoxine hydrochloride, *DL*- α -tocopheryl acetate and *L*-carnitine were 0.99 mg/100 g dry mass (DM), 8 mg/100 g DM and 20.4 mg/100 g DM, respectively. These results suggest that AGEs can be mitigated by controlling the parameters and optimizing the addition of food additives during the spray-drying process.

Keywords Glycation · Infant formula · Spray drying · Response surface methodology · Advanced glycation end-products

Introduction

Milk-based infant formulas have composition based on human milk but are composed of cow's milk as the main ingredient (sugar and proteins), and are used to replace breastfeeding for infants. Also, minor components, such as vitamins and minerals, are also adjusted to the levels in human milk. Therefore, the powdered ingredients (lactose, whey protein, and caseinate) are reconstituted in water targeting their ratio in human milk. To powder the liquid-reconstituted milk for storage and to ensure microbiological safety, heating is carried out in several processes, including concentration and spray drying. However, this heating process promotes glycation, which is the non-enzymatic reaction between an amino group of milk protein and a carbonyl group of milk sugar, lactose (Birlouez-Aragon et al., 2004). This reaction consists of three stages: early, intermediate, and advanced stage. These occur sequentially or simultaneously depending on the reaction conditions. Milk products can be evaluated using glycation-markers to determine the reaction levels. A modified lysine, lactulosyllysine, of Amadori products is produced at an early stage, and it is measured using ion-exchange chromatography (Henle et al., 1991). Although lactulosyllysine is a major compound as an early stage, furosine, analyzed with reversed-phased HPLC, is a more frequently chosen marker due to the time-efficient method of analysis (van Boekel, 1998). 5-Hydroxymethylfurfural (HMF), a marker for the intermediate stage, is frequently used as a maker for heat-induced damage, and it is measured via

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HPLC (Burton, 1984). The advanced glycation reaction proceeds with the break-down of Amadori products or other related Schiff's base products (Burton, 1984). *N*-carboxymethyllysine (CML) is a compound of advanced stage products, and it is a useful marker for processed milk products, which are advanced glycation end-products (AGEs) under severe conditions (Fenaille et al., 2006). In addition, the fluorescence is indirectly estimated through the accumulation of glycation reaction products (Diez et al., 2008).

The levels of AGEs in human and cow's milk are quite similar, but the AGE levels in milk based-infant formula are 100 times higher (Mericq et al., 2010). Furthermore, infants fed with formula showed a plasma CML levels that were approximately 46% higher than those of breast-fed infants (Šebeková et al., 2008). Serum AGE concentrations are correlated with microvascular complications in type 1 diabetes, meaning that the chance of these microvascular conditions may occur at an early age (Chiarelli et al., 1999). Oxidative stress and inflammation induced after AGE binding to the receptor for AGEs (RAGE) are known to be associated with the pathogenesis of insulin resistance, and the subsequent development of diabetes (Unoki and Yamagishi, 2008) and chronic kidney diseases (Cai et al., 2008).

A response surface methodology (RSM) determines the relationship between independent variables and dependent variables, and it optimizes complicated processes while lessening the experimental trials (Box and Wilson, 1992). Due to the development and improvement in mathematical and statistical techniques, RSM can be used to improve the experimental conditions in the interest of productivity, efficiency, and safety in a positive way (Zhu et al., 2010). In this study, two strategies were employed to mitigate the levels of AGEs in infant formula through RSM. The first is to control the parameters during the spray drying process, including inlet temperature, pump rate, and aspirator rate (drying as flow rate). The second is to apply food additives, including pyridoxine hydrochloride, DL- α -tocopheryl acetate, and L-carnitine.

Materials and methods

Materials

Whey protein concentrates (WPC) and sodium caseinate were supplied by DAVISCO Foods International, INC (Le Sueur, MN, USA). Lactose and soybean oil were purchased from Sigma Aldrich (St. Louis, MO., USA). Analytical standard CML was obtained from Polypeptide laboratories (Strasbourg, France), and HMF standard was purchased from Tokyo Company Industry (Tokyo, Japan). Sodium

dihydrogen phosphate anhydrous was purchased from Kanto Chemical Co. (Tokyo, Japan), sodium phosphate dibasic anhydrous from Samchun Chemical Co. (Seoul, Korea), and *o*-phthalaldehyde from Pickering Laboratories (Mountain View, CA, USA). HPLC-grade methanol and acetonitrile were purchased from Burdick & Jackson (Rutherford, NJ, USA).

Sample preparation and spray drying

The infant formula model system consisted of WPC, sodium caseinate, soybean oil, and lactose with a ratio 1:1:3:7 (weight basis) in water according to practice of a Korean dairy company. The mixture was homogenized and freeze dried, and this mixture was reconstituted with water to 50% total solid content, as set by the company.

Spray drying was performed using a laboratory-scale spray dryer (Buchi Mini Spray Dryer B-290, Flawil, Switzerland). The samples, which were fed using a peristaltic pump through an 0.7 mm diameter nozzle tip were formed a droplet with a co-current flow nozzle system with the same direction as the flow of hot air and the sample, with an automatic nozzle cleaning system. The inlet air temperature, aspirate rate, and pump rate varied from 149 to 199 °C, 24 to 34 m³/h and 342 to 803 mL/h, respectively. The concentrations of pyridoxine hydrochloride, DL- α -tocopheryl acetate, and L-carnitine were from 0.2 to 1.8 mg/100 g dry matter (DM), 4.0 to 14.0 mg/100 g DM, 3.6 to 20.4 mg/100 g DM, respectively.

Fluorescence measurements

The intensity of the fluorescence in the samples was measured at an excitation wavelength of 370 nm and an emission wavelength of 440 nm (Nagaraj and Monnier, 1995) using a Hidex sense microplate spectrofluorimeter (Hidex, Turku, Finland).

Quantification of CML by HPLC analysis

The CML level was measured following the method by Resmini and Pellegrino (1991). The sample (100 mg) was hydrolyzed with 3 mL of 7.9 N HCl at 110 °C for 24 h in a PTFE screw-cap Pyrex tube. The hydrolysis tubes were sealed under a nitrogen atmosphere. The hydrolysates were cooled at 25 °C and were cleaned up with Supelco C₁₈ cartridge (Sigma, Gillingham, UK). The cartridge was pre-wetted with 3 mL of methanol and 3 mL of deionized water (DW), and the hydrolysates were eluted with 3 mL of 3 N HCl. The elution was evaporated using a speed vacuum concentrator (N-Biotec, Seoul, Korea). The dried sample was dissolved in 1 mL of DW and was filtered with

a syringe membrane filter (0.45 µm pore size, Pall Corporation, Ann Arbor, MI, USA).

Quantification of the CML content was conducted on an Agilent 1200 Series system (Agilent, Santa Clara, CA) with FLD 1100A. The column consisted of a ZORBAX Eclipse plus C₁₈ (C₁₈, 4.6 × 250 mm i.d., 5 µm; Agilent, Santa Clara, CA), and the sample derivatization method was based on a Drusch device (Drusch et al., 1999). The *o*-phthalaldehyde-mercaptoethanol (OPA) reagent consisted of 0.40 mM OPA dissolved in 1 mL of HPLC grade methanol, 50 µL of 2-mercaptoethanol, and the reagent was made up to 10 mL with filtered DW. Before injection, 20 µL of the sample were mixed with 20 µL OPA reagent. The temperature of the column oven was 40 °C, and 40 mM sodium phosphate buffer (pH 7.8) (solvent A) and methanol/acetonitrile/water (4.5:4.5:1) (solvent B) were used for the gradient elution. The elution profile 0% B for 2 min, 0–57% B for 16.1 min, 57–100% B for 0.5 min, 100% B for 3.7 min, 100–0% B for 1 min, 0% B for 2.8 min at a flow rate was 2 mL/min. The injection volume was 20 µL and was detected at an excitation wavelength of 340 nm and emission wavelength of 450 nm.

Quantification of the HMF via HPLC analysis

The baseline method was used according to the procedure (van Boekel and Zia-Ur-Rehman, 1987). The sample reconstituted with DW (0.15 g/mL) was added to 500 µL of 0.3 N oxalic acid in a sealed tube. The mixture was then heated in a water bath at 100 °C for exactly 25 min and was cooled at room temperature. After cooling, the mixture was mixed thoroughly with 300 µL of 40% (w/v) TCA for 5 min and centrifuged at 4500 rpm for 15 min. The separated supernatant was filtered using a filter cartridge with a pore size of 0.45 µm, injected into an HPLC system (Agilent, Santa Clara, CA). The chromatographic separations were performed on a YMC-Triart C₁₈ column (250 × 4.6 mm i.d., 5 µm, YMC GmbH, Dinslaken, Germany). The isocratic separation was formed with 0.05% of TFA in water (solvent A) and methanol (solvent B) and was carried out with 12.5% solvent B for 30 min at a flow rate of 1 mL/min. The injection volume was 20 µL, and the detector wavelength was of 280 nm.

Quantification of the total protein content

The protein content was measured using the AOAC official Kjeldahl method (Horwitz, 1980). The total protein was converted from the nitrogen value by a factor of 6.38.

Experimental design

RSM was used to optimize the conditions to mitigate the glycation products in terms of the fluorescence intensity, HMF content, and CML content, which correspond to independent factors. The first design of the RSM is conducted by controlling the spray drying parameters, including the inlet temperature, pump rate, and aspirator rate. The second design of the RSM is controlled by combining the anti-glycation additives of pyridoxine hydrochloride, DL- α -tocopheryl acetate, and L-carnitine. The central composite design with a five-level (− 1.682, − 1, 0, 1, and 1.682), and three-factor (A, B, and C) was used to obtain the optimal conditions. The results of each experiment were analyzed using nonlinear regression models. The Y value was fitted to the polynomial equation.

$$Y = \beta_0 + \beta_1X + \beta_2X_2 + \beta_3X_3 + \beta_4X_1^2 + \beta_5X_2^2 + \beta_6X_3^2 + \beta_7X_1X_2 + \beta_8X_1X_3 + \beta_9X_2X_3$$

β_0 is the constant, β_1 to β_9 are the coefficients, X_1 to X_3 are coded variables, and Y is the dependent variable. The MINITAB statistical software (Version 16.1, Minitab Inc., State College, PA, USA) was used to conduct the statistical analysis of the experimental and prediction data. The correlation coefficients were examined via analysis of variance (ANOVA).

Statistical analysis

The statistical analysis was conducted using the Sigma stat software (Sigma Stats, Jandel Corp, San Rafael, CA) to determine the differences among the groups evaluated using a one-way analysis of variance and Duncan's tests. The values were expressed as mean ± standard deviation (SD).

Results and discussion

Monitoring the CML in the infant formula process

Glycation during food processing is considered as a major challenge (Jaeger et al., 2010). Infant formulas are manufactured with heat-based treatments to ensure bacteriological safety, to lessen the viscosity of the fluid condition for transferability (Birlouez-Aragon et al., 2004), and to extend the shelf-life. Furthermore, the ingredients of milk-based infant formulas including lactose, WPC and caseinate are prone to heat-induced glycation (Palombo et al., 1984). The processing step during the manufacture of powdered infant formulas generally includes spray drying. Since the spray drier has a low thermal efficiency due to a large chamber and large amounts of low-grade waste heat, the inlet drying

gas temperature increases (Chen and Mujumdar, 2009). The physicochemical properties of the dried materials depend on several process variables, including the flow rate of feed liquid, drying gas, and the drying air temperature (Kurozawa et al., 2009), and thus controlling the conditions of these parameters of spray drying can modulate glycation. Since the increase in air temperature from the inlet results in an elevated gradient temperature between the sprayed droplet and the drying air (Telang and Thorat, 2010), the heat damage of the droplet is directly proportional to the inlet temperature. The inlet temperature parameter of the spray dryer has been reported to be a major factor (Birlouez-Aragon et al., 2004).

The CML quantification is used as a marker to determine the progression of glycation (Ames, 2008). Therefore, the extent of glycation can be estimated by monitoring the CML concentration when processing the infant formulas. Figure 1 shows the CML concentration when processing the infant formulas. There were no differences between the milk formulas without heat treatment and pasteurized milk formulas. However, the concentrating process and spray drying processes were found to significantly increase CML level by 132% and 202% ($P < 0.001$), respectively, when compared to the milk formula sample.

Fig. 1 *N*-carboxymethyllysine (CML) concentration of infant formula processing. The values are expressed as mean $\pm$ SD ($n = 3$). Significant differences were determined by Duncan's tests ($P < 0.001$). The processing of infant formulas consists of the following sequential steps: Milk formulas are prepared by wet blending the raw ingredients of powdered milk without heat treatment; pasteurized formulas are UHT-sterilized sample at 120 °C and 5 s; concentrated milk formulas are heat-treated samples standardized to have 50% total solid; heated milk formulas are heat-treated for facilitating sample transfer; spray dried formulas are the final spray-dried product before packaging

Optimization of the processing parameters

Model fitting and statistical analysis of the processing parameters

The fluorescence intensity and CML are used as markers to monitor changes due to glycation in infant formulas according to the heat treatment (Birlouez-Aragon et al., 2004). Additionally, HMF is used to indicate heat-induced damage in infant formulas (Ferrer et al., 2002). In the present study, the inlet temperature (X_1), pump rate (X_2), and aspirator rate (X_3) as the independent variables were assessed to reduce the glycation using the markers, fluorescence intensity (Y_1), HMF content (Y_2), and CML content (Y_3). A total of 17 samples with a central composite design were analyzed to optimize the independent variables. As shown in Supplementary Table 1, these data were calculated as percentage when compared to the controls, which are fixed at 100%. This response was calculated as a second-order polynomial equation (Supplementary Table 2). As shown in Table 1, the coefficient of determination (R^2) values were 0.94 (Y_1), 0.91 (Y_2), and 0.86 (Y_3) indicating that the dependent variables have significance for these models. An ANOVA was used to determine the significance and suitability of the experimental model.

An increase in the aspirator rate results in a larger air flow making contact with the same quantify of droplets which are atomized as particles (Telang and Thorat, 2010). Due to this larger volume of drying air, glycation is expected to be promoted. An increase in the aspirator rate results in a larger air flow making contact with the same quantify of droplets which are atomized as particles (Telang and Thorat, 2010). Due to this larger volume of drying air, glycation is expected to be promoted. The increase in the feeding rate scales up the atomized droplets in the drying chamber (Elversson et al., 2003). The coarser feed droplets reduce the incidence of the exposure from hot dry air, and it was reported that the coarser droplets with the higher feed rates provide less heat damage to probiotic microorganisms due to less exposure (Maa et al., 1997). Also, an increased pump rate reduces the outlet temperature because the feed volume that is to be evaporated increases (Ståhl et al., 2002). In the present study, for the fluorescence intensity (Y_1), based on the *t*-statistic, linear variables X_1 and X_2 showed statistically-significant influence ($P < 0.05$), except X_3 . Also, the linear variables X_1 and X_2 were statistically significant ($P < 0.05$) in the CML content (Y_3). The pump rate (X_2), which is the actual feeding speed of the samples, was related only with the CML content in this study (Table 1). However, the linear variables of the HMF content (Y_2) showed that X_1 and X_3 were statistically significant ($P < 0.05$) except for X_2 . X_1^2 ,

Table 1 Model fitting and ANOVA for response parameters dependent variable^b for the processing parameters

Variance source ^a	DF	Y ₁		Y ₂		Y ₃	
		Sum of squares	P value	Sum of squares	P value	Sum of squares	P value
X ₁	1	164.84	0.047	16,295.5	0	7215.5	0.01
X ₂	1	301.5	0.026	819.8	0.196	4590.4	0.027
X ₃	1	36.87	0.316	6650.1	0.021	292.3	0.329
X ₁ X ₂	1	38.18	0.605	227.1	0.476	999.8	0.107
X ₁ X ₃	1	163.23	0.114	198.1	0.505	5253.7	0.008
X ₂ X ₃	1	633.50	0.085	1291.3	0.116	387.7	0.502
X ₁ ²	1	21.67	0.834	81.6	0.666	3983.7	0.007
X ₂ ²	1	645.47	0.506	3.3	0.931	5232.2	0.017
X ₃ ²	1	0.01	0.684	3522.7	0.021	930.2	0.494
Residual	7	117.96		2809.6		4132.9	
Lack of fit	5	113.03	0.012	2806.5	0.003	4130.6	0.001
Pure error	2	4.93		3.1		1.1	
R ²		0.94		0.91		0.86	
Total	16	2163.64		32,113		39,756	

^aX₁: inlet temperature, X₂: pump rate, X₃: aspirator rate

^bY₁: fluorescence intensity, Y₂: HMF content, Y₃: CML content

the quadratic variable, had a significant influence ($P < 0.01$) in Y₃. X₂² also showed a significant influence ($P < 0.05$) in Y₃. X₃², the quadratic variable, had a significant influence ($P < 0.05$) in Y₂. By observing the coefficients, the anti-glycation effect was mainly influenced by the inlet temperature and pump rate compared to the aspirator rate.

Analysis of RSM for the processing parameters

Three-dimensional graphs of the three responses were developed for a function of the two independent variables among the inlet temperature, pump rate, and aspirator rate in response to their significance. As shown in Fig. 2(A, B), a decrease in the inlet temperature drastically reduced the intensity of the fluorescence, whereas the pump rate and aspirator rate had no significant relation [Fig. 2(C)]. The reduction of the inlet temperature and aspirator rate had an influence on the reduction of the HMF content [Fig. 2(E)]. A decrease in the inlet temperature and aspirator rate similarly reduced the CML content [Fig. 2(H)]. However, the pump rate and aspirator rate had influence on the reduction of the CML content compared to the aspirator rate [Fig. 2(I)].

Optimization of the conditions and verification of the models for the processing parameters

The independent variables of the first model are set to see how much the amount of glycation products increases as the inlet temperature, the pump speed and the aspirator rate for spraying. Therefore, based on the maximum and minimum values of the range that can be set on the scale only under the condition that the powdered sample can be made. In the case of the first model, the center value, 0 of central composite design with a five-level (− 1.682, − 1, 0, 1, and 1.682) was set to 174 °C, 80%, and 80%. The range of inlet temperature was 148.7–199.2 °C including decimal point, and the final optimized inlet temperature of 148.7 °C was the minimum value set (Table 2 and Supplementary Table 1). The optimized pump rate and aspirator rate were 63.1% and 78.1%, respectively as optimal conditions (Table 2). When the unit of % on device is expressed in actual units, optimal values are pump rate, 342.4 mL/h and aspirator rate 28.6 m³/h, respectively which are in the range of 342–803 mL/h and 24–34 m³/h, respectively obtained in the central composite design. For these optimization conditions, fluorescence intensity, HMF content, and CML content were predicted to be 102.58%, 236.07%, and 113.32% of the control values, respectively, with 0.96 of desirability. The linear effects of three independent variables were statistically significant for the CML content ($P < 0.05$). Thus, the CML content can be concluded to be a more sensitive marker to compare the experimental

Fig. 2 Response surface plots on fluorescence intensity for the effect of followed processing parameters. (A) Inlet temperature and pump rate, (B) aspirator rate and inlet temperature, (C) aspirator rate and pump rate, (D) inlet temperature and pump rate, (E) aspirator rate and inlet temperature, (F) aspirator rate and pump rate, (G) inlet

temperature and pump rate, (H) aspirator rate and inlet temperature, (I) aspirator rate and pump rate, (J) pyridoxine hydrochloride and DL- α -tocopheryl acetate, (K) pyridoxine hydrochloride and L-carnitine, and (L) L-carnitine and DL- α -tocopheryl acetate

Table 2 Predicted and experimental values of response variables for the processing parameters

Response variables ^b	Independent variables ^a			Predict value (%)	Experimental value (%)
	X ₁	X ₂	X ₃		
Y ₁	148.7	63.1	78.1	102.58	103.29
Y ₂				236.07	250.36
Y ₃				113.32	121.82

^aX₁: inlet temperature (°C), X₂: pump rate (%), X₃: aspirator rate (%)

^bY₁: fluorescence intensity (% of control), Y₂: HMF content (% of control), Y₃: CML content (% of control)

conditions. The CML content is described as a useful marker to process the milk products (Fenaille et al., 2006).

Optimization of the food additives

Model fitting and statistical analysis for the food additive

Our group has previously reported that these three food additives including pyridoxine hydrochloride, DL- α -tocopheryl acetate, and L-carnitine reduce fluorescence intensity and HMF content in a liquid infant formulas under a sterilization condition via RSM (Jung et al., 2015). Pyridoxine hydrochloride is a dietary supplement for vitamin B₆, and vitamin B₆ can inhibit lipid oxidation and glycation

by inhibiting radical formation in red blood cells exposed to high sugar content (Jain and Lim, 2001). DL- α -tocopheryl acetate, which is fully methylated on an aromatic ring, is the most biologically active of the tocopherols (Burton and Ingold, 1986). The phenolic hydrogens of DL- α -tocopheryl provide antioxidant activity (Burton and Ingold, 1981), and the antioxidant properties of L-carnitine were reported (Gülçin, 2006) to scavenge 1,1-diphenyl-2-picryl-hydrazyl (DPPH) stable free radical, superoxide anion radical, and hydrogen peroxide and possess reducing power, total antioxidant activity, and metal chelating activities. Therefore, these antioxidants can be used to trap electrophiles and scavenge free radicals, which promote the main reaction forming CML (Morales and Jiménez-Pérez,

2001). When sugars conjugate with the amino group of lysine, the unstable Amadori rearrangement products are generated as intermediates, which can be oxidized to form CML (Ames, 2008). The oxidation product of sugars, glyoxal can react with lysine directly to make CML as another route (Ames, 2008). Pyridoxine hydrochloride decreases the yield of CML (Khalifah et al., 1999) and potently inhibits the formation of glycation products in a more effective manner than aminoguanidine (Booth et al., 1996). To ensure an acceptable RSM experiment, the R^2 values of the dependent variable are recommended to be more than 0.75 (Le Man et al., 2010).

A total of 17 samples with a central composite design were analyzed to optimize the independent variables. The second optimization with food additives focuses on how much the concentration of the additive can reduce the amount of the glycation products, so that the difference in the level of the reduced glycation products is more clearly confirmed under the condition that the glycation products are more produced. As shown in Supplementary Table 3, the inlet temperature, which was applied to the second model; 193 °C, pump rate; 24 m³/h and aspirator rate; 719 mL/min is a condition obtained through RSM as a condition that glycation products can be theoretically generated as possible. This response was calculated to have second-order polynomial equations (Supplementary Table 4). As shown in Table 3, the coefficient of determination (R^2) values were 0.77 (Y_1 , fluorescence intensity), 0.77 (Y_2 , HMF content) and 0.87 (Y_3 , CML content)

indicating that dependent variables have a significance for the models. In the CML content (Y_3), based on the t-statistic, linear variables X_1 and X_2 showed statistically-significant influences ($P < 0.05$) except for X_3 . Also, quadratic variables X_1^2 and X_2^2 were statistically significant ($P < 0.05$) in Y_3 . By observing the coefficients, the anti-glycation was affected by pyridoxine hydrochloride and DL- α -tocopheryl acetate compared to L-carnitine. Since the R^2 values of the dependent variables in our present experiment are 0.96 for optimized spray drying parameter and 0.75 for optimized food additives, these variables are predictable in statistics.

Analysis of RSM for the food additives

Three-dimensional graphs of three responses were developed for a function of the two independent variables among pyridoxine hydrochloride, DL- α -tocopheryl acetate, and L-carnitine in response to their significance. When pyridoxine hydrochloride and DL- α -tocopheryl acetate were about 1.25 mg/100 g DM and 10.5 mg/100 g DM respectively, CML content was reduced to 88.8% as a minimum of the CML content Fig. 2(J). As shown in Fig. 2(K, L), the increase in L-carnitine reduced the CML content to 87.4% and 87.2%, respectively.

Table 3 Model fitting and ANOVA for response parameters dependent variable^b for the food additives

Variance source ^a	DF	Y_1		Y_2		Y_3	
		Sum of squares	<i>P</i> value	Sum of squares	<i>P</i> value	Sum of squares	<i>P</i> value
X_1	1	1.76	0.09	23.65	0.42	105.33	0.01
X_2	1	59.35	0.19	138.38	0.08	58.69	0.03
X_3	1	30.20	0.11	15.03	0.52	17.97	0.19
X_1X_2	1	10.76	0.06	8.46	0.62	0.70	0.78
X_1X_3	1	70.80	0.08	145.71	0.07	12.89	0.25
X_2X_3	1	9.56	0.04	308.43	0.02	0.42	0.83
X_1^2	1	3.32	0.28	125.09	0.09	148.37	0.00
X_2^2	1	1.38	0.82	12.04	0.56	96.64	0.03
X_3^2	1	1.60	0.80	0.45	0.91	0.69	0.78
Residual	7	62.86		224.72		58.44	
Lack of fit	5	62.66	0.008	224.47	0.003	57.00	0.06
Pure error	2	0.2		0.24		1.44	
R^2		0.77		0.77		0.87	
Total	16	389.25		997.74		457.89	

^a X_1 : pyridoxine hydrochloride, X_2 : DL- α -tocopheryl acetate, X_3 : L-carnitine

^b Y_1 : fluorescence intensity, Y_2 : HMF content, Y_3 : CML content

Table 4 Predict and experimental values of response variables for the food additives

Response variables ^b	Independent variables ^a			Predict value (%)	Experimental value (%)
	X ₁	X ₂	X ₃		
Y ₁	0.99	8	20.4	89.0	97.5
Y ₂				60.3	57.5
Y ₃				86.9	84.2

^aX₁: pyridoxine hydrochloride (mg/100 g DM), X₂: DL- α -tocopheryl acetate (mg/100 g DM), X₃: L-carnitine (mg/100 g DM)

^bY₁: fluorescence intensity (% of control), Y₂: HMF content (% of control), Y₃: CML content (% of control)

Optimization of conditions and verification of models for the food additives

The concentration of independent variables for the food additives was optimized to reduce the CML content. The optimal concentration was determined by using the desirability, and the optimal concentrations for the food additives were found to be 0.99 mg/100 g DM pyridoxine hydrochloride, 8 mg/100 g DM DL- α -tocopheryl acetate, and 20.4 mg/100 g DM L-carnitine in Table 4. The optimal condition was predicted to have a fluorescence intensity, HMF content, CML content of 89.0%, 60.3% and 86.9% that of control, respectively, with 0.75 of desirability. In this study, the optimization condition of food additives inhibited glycation of the infant formulas, leading to 15.8% reduction in CML content (the experimental value), compared to control.

In conclusion, different experimental conditions were carried out to determine the effect of the operation parameters and the concentration of food additives for spray-dried infant formulas. The present results suggest that AGEs can be efficiently mitigated by controlling the parameters and optimizing the addition of food additives during the spray-drying process. It is required for the pilot-scaled and/or industrial spray dry processes, which have different environmental factors, including the hygroscopicity, moisture content, and yield, remain to test with these optimal conditions.

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Compliance with ethical standards

Conflict of interest None of the authors of this study has any financial interest or conflict with industries or parties.

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