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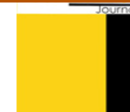

Microbial and quality attributes of thermally processed chili shrimp paste

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Abstract

Chili shrimp paste (CSP) is a favorite condiment in Southeast Asia. Microbial spoilage makes CSP unsuitable for consumption within several days. Thermal treatment was applied to produce microbiologically safe CSP. The effect of heating process on physicochemical and sensorial properties of CSP was studied. Heating at the optimum condition (21.6 min, 80 ˚C) has been shown effective and reliable in controlling microorganisms in CSP. Complete inactivation of peroxidase activities could not be accomplished at the optimal point, and significant reduction of the total phenolic and capsaicinoids contents was observed. Sensorial evaluation indicated that thermally processed CSP was less preferred by panelists when compared to freshly prepared samples of dry weight respectively.

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Introduction

Chili shrimp paste (CSP) is a regional favorite condiment, usually consumed uncooked as a side dish with meal or raw vegetables. It is known as ‘sambal belacan’ in Malaysia. This paste is prepared by crushing the fresh chilies, toasted fermented shrimp paste ‘belacan’, calamansi juice, sugar, and salt (Sobhi *et al.*, 2010). These ingredients contain natural microflora such as mold, yeasts and specific bacteria genera *Bacillus*, *Clostridium*, *Enterobacter*, *Escherichia*, *Micrococcus*, *Proteus*, *Streptococcus*, *Acetobacter*, *Pediococcus*, *Lactobacillus*, *Sarcina*, *Staphylococcus*, Brevibacterium-like, Flavobacterium-like, and Corynebacterium-like bacteria (Steinkraus, 1996; Saraya *et al.*, 2009). Toasting of fermented shrimp paste before mixing with other ingredients can reduce its microbial load. Since the ingredients used contain natural microflora and that CSP is usually consumed uncooked, this paste can be easily spoiled over time. This is supported by the fact that shelf life of CSP is not more than three days when kept in a refrigerator (Passmore, 1991).

Busy lifestyles and changing of consumer food habits resulted in growing demand of ready to eat, safe and convenient foods. This drives a need to explore preservation method for traditional food such as CSP. Currently, there is no standard method for preservation of CSP. Nadia *et al.* (2010) studied the effect of acidity level (pH 3.0-5.0) on sensorial quality of CSP. They found that the preferred level of acidity for CSP is at pH 4.0, which was adjusted using calamansi juice as the natural source of citric acid. Foods with pH of 4.6 or below prevent bacterial spore growth and require only low thermal treatments to reduce microbial load, inactivate enzymes and produce safe, shelf stable products, while retaining the original organoleptic characteristics and nutritive compounds of fresh food (Codex, 1993; Ray, 2004). Thus in this study the heat processing below 100 °C was applied to eliminate the bacteria and inactive the enzymes in CSP.

The fresh properties of CSP influenced by chili as the major ingredient are, (i) color (Ismail and Revathi, 2006), (ii) pungency (capsaicinoids) (Schweiggert *et al.*, 2006) and (iii) phenolic compounds (Tangkanakul *et al.*, 2009; Kumar *et al.*, 2010; Ruanma *et al.*, 2010). These quality attributes were selected as freshness indicators during thermal process optimization. The objectives of the this study were to (i) optimize the time and temperature combination in thermal processing of CSP using the response surface methodology (RSM), and (ii) determine the consumer acceptance of fresh and thermally processed CSP through sensory evaluation.

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Materials and Methods

Sample preparation

Formulation of CSP in this study was based on acceptable sensorial qualities as reported by Nadia *et al*. (2010). Formulation included 55% red chili (*Capsicum annum*), 14% bird's eye chili (*Capsicum frutescence*), 17% fermented shrimp paste ('belacan'), 7% sugar, 2% salt, 4% calamansi juice and 1% food grade citric acid. All ingredients were bought from a wholesale market. The stem of the chilies were removed before washing. Chili and calamansi were soaked in plain water for 10 minutes, then drained and soaked again for 5 minutes and drained for 10 minutes to remove excess water. Calamansi juice was extracted manually by squeezing the fruits. Fermented shrimp paste was diced (1cm×1cm×1cm) and toasted in the oven (ST-2, SALVA, Spain) at 180°C for 25 minutes. Ingredients were ground using a milling machine (Super mass colloider, Masuko Sangyo, Japan) with 120 µm of gap size between two parallel stone plates at 1500 rpm. The ground ingredients were then mixed using a spiral mixer (HS20 Sakura, Good and Well, Malaysia) for 10 minutes. The pH and moisture content of final product were 4.0 and 70% (w/w, wet basis) respectively.

Thermal treatment

Nature of CSP having chunky chili pieces makes it unsuitable to process in a continuous plate or tube heat exchanger. Thus, a batch thermal process was done in a jacketed and steam sealed tank. The paste was heated in an electrical steam jacketed kettle (TDB/6-10, Groen, Illinois) equipped with a stainless steel three vertical blade mixer (80 mm radius, 25 mm height, 1.0 mm blade thickness, 9 mm hub radius) rotating at 130 rpm. The top of the kettle was covered by stainless steel plate and this plate was kept cool by ice to prevent moisture loss during heating. Approximately 3.0 kg of CSP was processed in each batch and after treatment, 10 gram of CSP was hot-filled into multi-layer (pet12/pe20/al7/pe20/ldpe40) (Vempac, Malaysia) sachets with the dimensions of 3.5cm × 7.5cm × 1cm. The sachets were sealed immediately and cooled to reach ambient temperature.

Color measurement

The color of samples was measured using a Hunter Lab machine (Hunter Associate Laboratory Inc., Reston, USA) as described by Sobhi *et al*. (2010).

Peroxidase assay

Peroxidase activity was determined using method from Schweiggert *et al*. (2005) with some modifications to suit CSP. For enzyme extraction, aliquots of 50 g of CSP sample were homogenized in a blender (HGBTWTS3, Waring Commercial, USA) with 150 ml of chilled (4 ± 1 °C) 50 mM citrate-phosphate buffer (pH 6.5) at low speed for 2 min. An aliquot of 2 ml of the homogenates were filtered through a nylon membrane syringe filter (0.45 µm mesh size / 25 mm diameter). The filtered samples were kept in ice until assay. For enzyme activity determination, aliquots of 0.2 ml of enzyme extract were added to 1.3 ml of 50 mM chilled citrate-phosphate buffer containing 12 mM tropolone (Merck, Germany) and 3.3 mM H_2O_2 in test tubes, and then the tubes were vortexes for 10 s in 3000 rpm and the increase in absorption of yellow product was recorded at 418 nm (Molar extinction coefficient = 2075 L mol^{-1} cm^{-1}) every 15 s for 3 min and used for the quantification of enzymatic activity. Enzyme activity was reported as nanokatal (nkat) unit (1 katal is the amount of enzyme that converts 1 mole of substrate per second).

Total phenolic content

An aliquot of 30 g samples were mixed with 60 ml of ethanol (95%) in capped glass bottle. The mixture was homogenized using shaker (M65820, Thermolyne, USA) in 300 rpm for 10 hours in room temperature (25 ± 1 ºC). The mixture was allowed to stand for 1 hour, then 1 ml of supernatant was diluted with ethanol (95%) to 10 ml in test tube and mixed using vortex for 15 s in 3000 rpm. Total phenolic contents were determined with Folin–Ciocalteu reagent according to the method adapted from McDonald *et al*. (2001). An aliquot of 1 ml of diluted extract or 95% ethanol (blank solution) was mixed with 5 ml Folin Ciocalteu reagent (diluted with distilled water by 1:10 ratio). After 5 min standing, 4 ml of aqueous Na_2CO_3 1 M (preheated to 50 ºC) was added and mixed using vortex (3000 rpm) for 15 s. The mixture was kept standing in a dark place for 1 hour. Total phenols were determined colorimetrically at 765 nm using spectrophotometer. The standard curve was prepared using 4, 8, 20, 40, 80, 120 and 160 mg 1^{-1} solutions of gallic acid in ethanol (95%). Total phenol values were expressed as gallic acid equivalents (mg/100g dry mass).

Capsaicinoids content

An aliquot of 10 g samples were mixed with 40 ml of acetonitril (HPLC grade) in capped glass bottle. The mixtures were homogenized using shaker

(M65820, Thermolyne, USA) in 300 rpm for 10 hours in room temperature (25 ± 1 ºC). The mixtures were allowed to stand for 1 hour, then 1 ml of supernatant was filtered (using cellulose membrane syringe filter 0.45 µm mesh size / 25 mm diameter) and diluted with acetonitril (HPLC grade) to 10 ml in test tube. Dilution was mixed using vortex for 15 s in 3000 rpm and injected to HPLC to determine capsaicin, dihydrocapsaicin and nordihydrocapsaicin contents in CSP.

A portion of 20 µl dilution was then injected into Thermo Hypersil Gold C18 (250 × 4.6 mm, Thermo Scientific, USA). The HPLC system (Shidmadzu LC-6A, Japan) was equipped with fluorescence detector and LCsolution software (Version 1.21 SP1). The eluent (isocratic) was a mixture of acetonitrile, deionized water and acetic acid (100:100:1, v/v/v) at a flow rate of 1 ml/min at room temperature. The detector was set at 270 nm excitation and 330 nm emission. Capsaicin and dihydrocapsaicin contents were expressed in mg/100g dry mass and nordihydrocapsaicin in µg/100g dry mass. Quantification of the compounds were done based on individual standard curves.

Microbial analysis

Microbiological evaluation of CSP was carried out immediately after processing. An aliquot of 10 g of samples were aseptically homogenized with 90 ml sterile peptone solution (1.0% w/v neutral peptone) in a glass bottle by manual shaking for 30 seconds. Serial dilutions (1:10) of each homogenized sample were made in the same diluents, and duplicate counting plates were prepared using appropriate dilutions. Total mesophilic bacteria using Plate Count Agar (Merck, Germany), yeast and mould using Dichloran Rose Bengal Chloramphenicol Agar (Oxoid, England) and pathogenic enterobacteriaceae using Eosin Methylene Blue Agar (Merck, Germany) were enumerated using pour-plating technique; 1ml of the dilution was mixed with molten (45 °C) medium and incubated for 5 days at 30°C (yeast an mould) and 2 days at 37°C (mesophil and enterobacteriaceae) prior to counting the colony forming units (CFU).

Statistical design and analysis

Response surface methodology was used to study the effect of two variables (time and temperature) on thermal processing of CSP. The range of time and temperature were 1 to 30 min and 60 to 100 °C, respectively. The range of independent variables was adopted from low heat processing-hot pack studies on semisolid low pH (less than 4.6) food products such as: jam (Tucker, 2008), tomato paste (Sandoval *et al*., 1994), tomato and fruit products (Ray, 2004). Thirteen experiments were performed, according to the central composite design for the study of 2 independent variables, each at 3 levels in axial points. Experiments involved 4 factorial points, 4 axial points and 5 center points in 1 block (Table 1), the center point was repeated 5 times to determine the reproducibility of the experiment. The experiments were repeated twice. The generalized polynomial model proposed for predicting the response variables as function of independent variables is given as:

$$Y = \beta_0 + \beta_{1x1} + \beta_{2x2} + \beta_{11x12} + \beta_{22x22} + \beta_{12x1x2}$$

where Y is response variable, β_0 is offset term, β_1 and β_2 are the regression coefficients for linear effect terms, β_{11} and β_{22} are quadratic effects and β_{12} is interaction effects. In this model, $_{x1}$ and $_{x2}$ are the independent variables (time and temperature). The significance of the equation parameters for each response variable was also assessed by F-ratio at a probability of 0.05. The adequacy of the models were determined using model analysis, lack-of-fit test and coefficient of determination were calculated. The experimental design matrix, data analysis and optimization procedure were performed using the Minitab V. 14 statistical package (Minitab Inc., State College, Pennsylvania).

Optimization and validation procedures

The individual and overall optimization procedures were carried out to obtain the optimal levels of two independent variables ($_{x1}$ and $_{x2}$) leading to the desired response goals. Optimization of the model was performed using the response optimizer in order to obtain the highest color values (lightness, redness and yellowness), total phenolics and capsacinoids content and lowest peroxidase activity and microflora as the goal. The optimum point was validated through one-sample t-test to determine the agreement ($p \leq 0.05$) between experimental and predicted responses values.

Sensory analysis

Consumer acceptance of CSP in term of sensory quality was evaluated by 55 untrained panelists. The panelists were Malaysians who are familiar with CSP and consume it on a daily basis. Panelists were asked to evaluate the samples using a nine-point hedonic scale ranging from "1, dislike extremely" to "9, like extremely". Five different attributes were used to evaluate the quality, including color, flavor, taste, texture and overall acceptability. For every sample, 20 g of CSP was served with a carrier (cucumber

Table 1. Design of experiment (central composite design) and experimental data obtained for the response variables

Run Order	Block	Independent variables		Responses variables											
				Color values			Microbial counts (CFU)			Chemical responses					
		Time (min)	Temperature (°C)	*L*	*a*	*b*	Total Mesophile	Yeast and Mould	*Pathogenic Enterobacteriaceae*	Peroxidase activity (nkat)	Total phenolic content (mg/100g)	Capsaicin (mg/100g)	Dihydrocapsaicin (mg/100g)	Nordihydrocapsaicin (µg/100g)
1	1	4.51	70.13	29.63	23.41	15.78	300	0	68	30.52	181.05	21.42	5.09	33.09
2	1	13.00	100.00	24.55	11.96	7.74	0	0	0	0.55	59.82	3.64	0.92	5.18
3*	1	13.00	82.50	27.91	19.97	13.89	0	0	0	2.59	126.98	14.51	3.54	21.37
4	1	13.00	65.00	30.31	23.05	16.02	264	0	62	80.84	171.43	19.45	4.69	27.89
5	1	25.00	82.50	24.62	18.26	12.36	0	0	0	1.49	91.82	7.29	1.77	11.20
6*	1	13.00	82.50	27.87	19.85	14.00	0	0	0	2.35	128.50	12.69	3.06	20.14
7*	1	13.00	82.50	27.43	20.03	14.02	1	0	0	2.25	131.46	13.58	3.35	20.22
8	1	1.00	82.50	31.73	21.85	14.96	55	0	9	2.85	182.45	20.98	5.01	32.04
9	1	4.51	94.87	27.55	19.99	14.22	0	0	0	1.70	136.15	15.31	3.73	22.76
10	1	21.49	94.87	24.32	11.51	7.82	0	0	0	0.51	55.00	3.14	0.76	5.05
11*	1	13.00	82.50	27.87	19.85	14.00	0	0	0	2.09	129.44	13.21	3.31	20.57
12*	1	13.00	82.50	27.87	19.85	14.00	1	0	0	2.01	128.68	13.92	3.21	20.31
13	1	21.48	70.13	29.57	21.21	14.95	120	0	31	14.38	135.04	15.26	3.82	19.95

*Centre points

Table 2. Estimated regression coefficients, correlation coefficient (R^2), and probability values for responses in the final reduced models

Coefficients	Responses variables									
	Color values			Microbial counts		Chemical responses				
	L	*a*	*b*	Total Mesophile	*Pathogenic Enterobacteriaceae*	Peroxidase activity	Total phenolic content	Capsaicin	Dihydrocapsaicin	Nordihydrocapsaicin
Constant										
b_0	43.2430	-20.4804	-22.9020	4422.52	1005.92	891.956	40.8842	39.1121	8.72593	-0.379746
Linear										
b_1	-0.196487	1.00331	0.934488	-46.3272	-8.00042	-	1.63566	0.625301	0.203967	-0.888630
b_2	-0.156431	1.15088	0.977319	-91.2904	-21.0566	-20.0316	5.32790	-0.223984	-0.0458868	1.38010
Square										
b_{11}	-	-	-	0.275868	-	-	0.0579028	-	-	-
b_{22}	-	-0.00755876	-0.00612635	0.470939	0.109246	0.111839	-0.0430106	-	-	-0.0118751
Interactions										
b_{12}	-	-0.0149762	-0.0132738	0.428571	0.0880952	-	-0.0836667	-0.0143095	-0.00404762	-
R^2	85.3%	96.8%	96.3%	97.9%	95.9%	83.8%	99.2%	96.8%	96.8%	97.8%
R^2 (adjusted)	82.3%	95.3%	94.4%	96.4%	93.8%	80.6%	98.7%	95.8%	95.8%	97.1%
Regression (p-value)	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*	0.000*
Lack of fit (p-value)	0.002*	0.000*	0.000*	0.000*	0.000*	0.000*	0.010*	0.082	0.108	0.011*

Subscripts: 1 = Time; 2 = Temperature (*Significant at 0.05 level)

slices), and three digits random numbers were used for coding the samples. Plain water was provided for rinsing in between samples. Evaluations were held in a sensory laboratory equipped with individual tasting booths in a conducive environment for testing. The average scores for every attribute were calculated and analyzed using Minitab V.14 software (Minitab Inc., State College, Pennsylvania). One-way ANOVA and the Tukey's multiple range tests were used to generate confidence intervals for the differences between the means. Significantly different means ($p \leq 0.05$) are denoted by different superscripts.

Results and Discussion

Statistical analysis

Table 1 shows the experimental design and the results of eleven response variables in every run of the experiments. Yeast and mold were totally eliminated in all runs, indicating the low thermal resistance of these microorganisms towards moderate time-temperature regimes. Since the values of yeast and mold count were zero, these results were not used in data analysis for optimization by RSM.

Tables 2 and 3 show the analyzed results of the response surface design. The statistically non-significant ($p > 0.05$) terms were removed from the initial models to obtain the final reduced model. The estimated regression coefficients for the response variables, along with the corresponding R^2, R^2 (adjusted) and p-value of lack of fit, are shown in Table 2. Each response (Y) was assessed as a function of processing time ($_{x1}$) and temperature ($_{x2}$). The results indicated that the response surface models with high coefficient of determination (R^2) ranging from 83.8% to 99.2% were significantly ($p \leq 0.05$) fitted for all response variables studied. Jogleker and May (1987) also suggested that for a good fit of a model, the R^2 should be at least 80%. However, there was significant ($p \leq 0.05$) lack of fit. This may be

Table 3. Analysis of variance (p-value and F-ratio) for the independent variables in the final reduced models

Variables	Probability values	Main effect		Quadratic effect		Interaction effect
		b_1	b_2	b_{11}	b_{22}	b_{12}
Color value (*L*)	p-value	0.001*	0.000*	-	-	-
	F-ratio	24.63	33.21	-	-	-
Color value (*a*)	p-value	0.000*	0.000*	-	0.005*	0.004*
	F-ratio	49.55	165.46	-	15.12	15.78
Color value (*b*)	p-value	0.000*	0.000*	-	0.004*	0.002*
	F-ratio	38.12	133.49	-	15.98	19.94
Total Mesophile	p-value	0.003*	0.000*	0.036*	0.000*	0.003*
	F-ratio	20.37	192.93	6.73	88.72	19.86
Pathogenic Enterobacteriaceae	p-value	0.021*	0.000*	-	0.000*	0.016*
	F-ratio	8.27	116.58	-	53.00	9.16
Peroxidase activity	p-value	-	0.000*	-	0.001*	-
	F-ratio	-	30.88	-	21.00	-
Total phenolic content	p-value	0.000*	0.000*	0.047*	0.007*	0.006*
	F-ratio	389.43	477.68	5.78	14.42	14.75
Capsaicin	p-value	0.000*	0.000*	-	-	0.033*
	F-ratio	124.06	143.86	-	-	6.31
Dihydrocapsaicin	p-value	0.000*	0.000*	-	-	0.015*
	F-ratio	120.08	146.72	-	-	8.92
Nordihydrocapsaicin	p-value	0.000*	0.000*	-	0.010*	-
	F-ratio	206.84	186.95	-	10.64	-

Subscripts: 1 = Time; 2 = Temperature
*Significant at 0.05 level

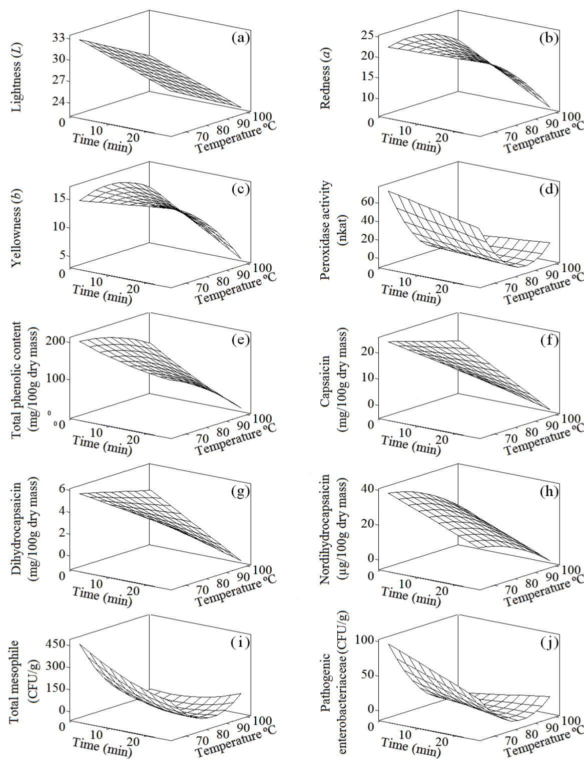

Figure 1. Surface plot of microbial and physicochemical properties in chili shrimp paste versus time and temperature

due to the wide range of independent variables in the study. A model with a significant lack of fit could still be used when the high correlation coefficients are indicating the applicability of the model within the range of variables included, or large amount of data were included in the analysis (Baeza and Pilosof, 2001).

F-ratio and p-value of linear, quadratic and interaction effects of time and temperature on independent variables are given in Table 3. As shown in Tables 2 and 3, the linear effect of processing time and temperature had significant ($p \leq 0.05$) effect on all responses except the effect of time on peroxidase activity.

Color changes

The color of chili is the dominant color in CSP, and has an important role in appearance and acceptance of CSP (Sobhi *et al*., 2010). Figures 1(a), 1(b) and 1(c) show the effect of thermal treatment on lightness, redness and yellowness of CSP. The surface plot of redness and yellowness values exhibited the reduction in a quadratic manner, while lightness values reduced linearly over the range of time and temperature tested. The models parameters in Table 3 show the interaction effect of time and temperature and quadratic effect of temperature significantly ($p \leq 0.05$) reduced redness and yellowness. While, only time and temperature had significant linear effect on lightness reduction. The higher F-ratio value of temperature indicated that temperature had more significant effect on color than time. Ismail and Revathi (2006) applied thermal process to extend the shelf life of chili puree in different time and temperature ranged from 5min to 20 min and 60˚C to 90˚C respectively. They found significant reduction in lightness and redness values of chili puree after subjected to thermal process. They reported that decrease in color intensity of puree is due to the degradation of responsible pigments for color in chili such as: keto-cerotenoids, violoxanthin, lutein and β-carotene which occur during various heating treatments.

Peroxidase activity

Peroxidase is a deteriorative heat resistant enzyme (Schweiggert *et al*., 2005). In chili, it is mainly located in both placental and epidermal cell layers where the capsaicin is concentrated (Bernal *et al*., 1994). Capsaicinoids are responsible for the pungent flavor and hot sensation of chili, which are easily oxidized. Peroxidase is responsible for oxidizing capsaicinoids and carotene which could adversely affect color, taste and aroma of chili products therefore, its inactivation is crucial in CSP processing. Table 3 illustrates the

Table 4. Physicochemical, microbial and sensorial comparison between the fresh and processed chili shrimp paste on optimum point (21.6min - 80˚C), and predicted values from model

	Responses	Fresh CSP	Processed CSP (experimentally)	Predicted by model (Desirability)
Physicochemical and microbial properties	Lightness (*L*)	32.62 ± 0.53	26.29 ± 0.32^a	26.49^a (0.29)
	Redness (*a*)	23.92 ± 0.16	18.86 ± 0.35^a	19.00^a (0.63)
	Yellowness (*b*)	16.71 ± 0.32	13.42 ± 0.27^a	13.32^a (0.67)
	Total mesophilic bacteria (CFU/g)	<5000	2 ± 2^a	1.91^a (0.99)
	Pathogenic enterobacteriaceae (CFU/g)	<100	0	0 (1.0)
	Yeast and mold (CFU/g)	<300	0	0 -
	Peroxidase activity (nkat)	197.08 ± 6.34	5.09 ± 0.51^a	5.19^a (0.94)
	Total phenolic content (mg/100g dry mass)	185.5 ± 0.66	110.16 ± 0.64^a	109.66^a (0.43)
	Capsaicin (mg/100g dry mass)	22.87 ± 0.27	9.68 ± 0.34^a	9.98^a (0.37)
	Dihydrocapsaicin (mg/100g dry mass)	5.75 ± 0.52	2.47 ± 0.22^a	2.47^a (0.39)
	Nordihydrocapsaicin (µg/100g dry mass)	33.17 ± 0.48	14.38 ± 0.55^a	14.85^a (0.35)
Sensorial characteristics	Color	7.54 ± 0.96^a	5.96 ± 1.23^b	-
	Flavor	6.89 ± 1.07^a	5.39 ± 1.59^b	-
	Taste	6.75 ± 1.40^a	5.46 ± 1.69^b	-
	Texture	7.11 ± 1.10^a	5.64 ± 1.34^b	-
	Overall acceptability	7.14 ± 1.01^a	5.71 ± 1.21^b	-

*Means having the same superscripts within the row did not differ significantly ($p \leq 0.05$)

significant effect of temperature (linear and quadratic manners) on enzyme inactivation, while, time showed non-significant effect on enzyme activity. Complete inactivation of peroxidase was not observed even when intensive time-temperature regimes were applied (Table 1). Figure 1(d) also clearly shows that increasing temperature remarkably affected enzyme activation.

Total phenolic content

Chilies are extremely popular for their abundance content of phenolics which is higher than other vegetables commonly recognized as a source of this substance, phenolic compounds possess a wide span of physiological functions such as: antioxidant, anti-carcinogenic, anti-inflammatory, anti-allergenic, anti artherogenic, anti-microbial, anti-thrombotic, cardio-protective and vasodilatory effects (Kumar *et al*., 2010). Thermal treatment causes the reduction of total phenolic content in chilies (Ruanma *et al*., 2010). Since the main ingredients of CSP are chilies, total phenolic content of the paste was significantly affected by thermal processing. Figure 1(e) shows the effect of thermal processing on nonlinear reduction of total phenolic content in CSP. Time and temperature had significant full quadratic effect on total phenolic content reduction of CSP (Table 3). The higher F-ratio value of temperature shows that temperature was more effective than time in reducing amount of phenolic compounds. Thermal processes promote phenolic compound degradation (chemically or enzymatically if the oxidative enzymes have not been inactivated) or can produce chemical changes that affect quality characteristics of phenolic compounds (Vallejo *et al*., 2003).

Capsaicinoids content

The pungent metabolites in the fruits of *Capsicum* species are called capsaicinoids, which are a group of 12 or more alkaloids with a structure of vanillylamide of branched fatty acids with 9–11 carbons, but mostly are present at very low levels and not expected to contribute greatly to overall pungency (Cisneros-Pineda *et al*., 2007). Capsaicin, dihydrocapsaicin and nordihydrocapsaicin are three major pungent principles responsible for more than 90% of the pungency in the capsicum species (Schweiggert *et al*., 2006; Ziino *et al*., 2009). The HPLC separation of these three pungent compounds from CSP is presented in Figure 2. Heating resulted in degradation of the capsaicinoids content (Schweiggert *et al*., 2006). Figures 1(f), 1(g), and 1(h) show similar heat susceptibility of capsaicin, dihydrocapsaicin and nordihydrocapsaicin to thermal processing, where all three components showed nonlinear reduction by increasing time and temperature. Table 3 shows that the time and temperature and their interaction had significant effect on capsaicin and dihydrocapsaicin reduction. For nordihydrocapsaicin reduction the main effect of time and temperature and the quadratic temperature had significant effect. The higher F-ratio value indicated that temperature had more significant effect than time on capsaicin and dihydrocapsaicin reduction. In contrast, nordihydrocapsaicin reduction was significantly influenced by time as compared to temperature.

Microbial content

Thermal processing of CSP using various time-temperature combinations had lethal effect on the tested microorganisms. Yeast and mold were completely eliminated in all samples (Table 1), indicating that these microorganisms are more heat sensitive than total mesophile and pathogenic enterobacteriaceae in CSP. Total mesophile and pathogenic enterobacteriaceae exhibited nonlinear reduction in studied time and temperature ranges (Figures 1(i) and 1(j)). Total mesophile bacteria reduction followed the significant effect of full quadratic model, and pathogenic enterobacteriaceae reduction followed the significant effect of the main independent variable (time and temperature), their

interaction and quadratic temperature. In both total mesophile and pathogenic enterobacteriaceae, effect of temperature was more significant than time for bacteria elimination (Table 3).

Optimization and validation

A numerical optimization was carried out to determine the exact optimum level of time and temperature leading to overall optimized conditions using response optimizer. The overall optimal condition was predicted to be at the combined level of 21.6 min and 80 ˚C. The responses values and their desirability levels in this point are shown in Table 4. The adequacy of the response surface equation was checked by comparison of experimental and predicted values from the reduced response regression models. The experimental and predicted values of responses are shown in Table 4. No significant difference ($p > 0.05$) was observed between experimental and predicted values, thus verifying the adequate fitness of the response equations.

Sensory evaluation

Thermal treatment has been known to cause negative effect on chili products (Ismail and Revathi, 2006; Ruanma *et al*., 2010) which also may affect sensorial attributes. Thus, the sensory acceptability test was conducted on fresh and thermally processed CSP at the optimized conditions (21.6 min and 80˚C) to determine consumer acceptability. Results of sensory evaluation using nine point hedonic scales are presented on Table 4. The mean score values of color, flavor, taste, texture and overall acceptability as freshness indicator for CSP were significantly higher in fresh sample.

Conclusions

The combined level of time (21.6 min) and temperature (80 ˚C) as the optimum point of thermal treatment has been shown effective and reliable in controlling microflora of CSP. Complete inactivation of POD activities could not be accomplished in optimal point and even applying intensive temperature-time regimes. Thermal treatment reduces the total capsaicinoid contents, resulted lower pungency in optimal point. The experimental value agreed with the predicted value within a 95% confidence interval, suggesting a good fit between the models and the experimental data. Sensory analysis revealed the sensorial properties of thermally processed CSP was less preferred by panelist. Therefore, the development of an alternative processing method to produce microbiologically safe product while preserving fresh properties of CSP is suggested.

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