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RESPONSE SURFACE METHODOLOGY FOR STUDYING THE QUALITY CHARACTERISTICS OF COWPEA (*VIGNA UNGUICULATA*)-BASED TEMPEH

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ABSTRACT

Response surface methodology was used to optimize the processing conditions in the preparation of cowpea tempeh. The independent factors studied were boiling time (varying from 5 to 30 min), incubation time (varying from 12 to 48 h) and incubation temperatures (varying from 25 to 50C), whereas the dependent factors were protein content, protein solubility, pH, titratable acidity and total color difference (using L, a and b*). Regression models were generated and adequacy was tested with regression coefficients (R^2) and the lack-of-fit tests. Optimum processing conditions were determined by method of superimposition. There was a strong and significant influence ($P < 0.01$) of the quadratic effect of the incubation time on the protein content of the cowpea tempeh, with similar significance ($P < 0.01$) noted in protein solubility with increasing boiling time. The optimum processing conditions observed for the preparation of cowpea tempeh were boiling time of about 20 min, incubation time of about 28 h and incubation temperature of about 37C.*

PRACTICAL APPLICATIONS

Response surface methodology (RSM), as a statistical tool, has been effectively used in food process applications. This study embraced the use of RSM in the optimization of the processing conditions involved in the preparation of cowpea tempeh. Superimposition of the contour plots developed from the regression models indicated that cowpea with optimum quality character-

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istics should be processed at a boiling time of 20 min, incubation time of 28 h and incubation temperature of 37°C. These conditions could be adopted for the industrial production of cowpea tempeh.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp), also known as black-eye peas, is one of the most commonly utilized legumes in Africa. It is an important crop in some areas of the tropics where it provides more than half the plant protein in human diets. It is a highly nutritious crop with a dry seed protein content of about 25% and protein digestibility higher than that of other legumes (Afoakwa *et al.* 2006). Cowpeas constitute about 52% of the total world output of grain legumes. It is cultivated extensively, mostly in the savannah areas of West Africa, and in Nigeria and Ghana it is referred to as beans. It has been found to contribute up to 80% of the total dietary protein intake in some parts of West Africa. Many varieties of cowpea seeds exist and are known by their different sizes, shapes and especially seed color, which can be either white, red, brown, black, cream or mottled (Sefa-Dedeh *et al.* 2001).

Tempe, also referred to as “tempeh,” is a collective name for a sliceable mass of precooked fungal-fermented beans, cereals or some other food processing byproducts bound together by the mycelium of a living mould (mostly *Rhizopus* spp.) (Nout and Kiers 2005). Tempeh is a widely consumed traditional Indonesian fermented food, which is principally made with soybeans (Astuti 2000). Tempeh is a highly nutritious, easily digestible and delicious product, and as such, it meets an increasing demand from consumer requirements for high quality meat replacers. With its high protein content (40–50%, dry matter) it serves as a tasty protein complement to starchy staple foods such as rice, and it can replace meat or fish (Nout and Kiers 2005). In Indonesia, the estimated consumption ranges from 19 to 34 g/day/person (Hermana *et al.* 1990). Different processing conditions have been reported by various authors in the preparation of tempeh-like products from cowpeas. The different processing conditions reported by the various authors are expected to result in products of varying characteristics and acceptability. There is therefore the need to study how the various processing conditions affect the product quality characteristics through the use of RSM.

Response surface methodology is a statistical–mathematical method that uses quantitative data in an experimental design to determine and simultaneously solve multivariate equations to optimize processes or products (Giovanni 1983). The rationale for the use of RSM models is that it determines the optimum operating conditions for the system as well as the region of the factor space in which the operating specifications are satisfied. It also ensures

simplicity of calculation of the model parameters (Sefa-Dedeh *et al.* 2003). Therefore, this study aimed at studying the physico-chemical characteristics of cowpea tempeh employing the use of RSM.

METHODOLOGY

Materials and Methods

Raw Materials and Sample Preparation. Cowpea seeds (Nigeria cowpea) were obtained from the Crop Research Institute of Ghana in Tafo, Ghana, and stored at 4C (relative humidity 65–100%) prior to the study. Tempeh starter culture (*Rhizopus oligosporus*) was obtained from Germ Cultures, Lakewood, Washington.

Propagation of Fungal Starter Culture. The preparation of the fungal starter culture is a modification of the method reported by Sooriyamoorthy *et al.* (2004). About 100 g of white long-grain rice was cooked with 200 mL of distilled water. The resulting cooked rice was inoculated with 1% *Rhizopus oligosporus* starter culture from after cooling to 35C and incubated at 37C for 48 h for the mould to sporulate, oven-dried at 45C for 24 h and milled using a hammer mill with mesh size 4 (Christy and Morris Ltd., UK).

Preparation of Cowpea Tempeh. Cowpea seeds were dehulled using the method described by Sefa-Dedeh *et al.* (1978). The dehulled beans were soaked in distilled water for 2 h. Fifteen milliliters of vinegar was then added after which the cotyledons were boiled in distilled water for times determined by the central composite rotatable design (CCRD) (5–30 min). The cotyledons were then drained and heating continued with stirring to get rid of excess water from the cotyledons and cooled to 37C. Five milliliters of vinegar was added and stirred continuously to allow effective mixing. One percent of *Rhizopus oligosporus* starter culture obtained from Germ Culture was added to the cotyledons, and the mixture was packed in transparent polyethylene bags perforated with a 2-in. nail and pressed; the perforations were 1 cm apart. The thickness of the packed cotyledons was from 1 to 2 cm. The packed cotyledons were then incubated at predetermined incubation temperatures and times between 25 and 50C and 12 and 48 h, respectively, using the experimental runs generated by the design matrix. The resulting tempeh samples were dried at 50–55C for 24 h and milled into flour with a hammer mill (Christy and Morris Ltd., UK) with mesh number 4.

Experimental Design and Statistical Analysis. In this study, a CCD with three variables at five levels was used (Khuri and Cornell 1987) to

determine the optimum processing conditions in the processing of cowpea tempeh. In the use of a central composite design, an imbedded factorial or fractional factorial design with center points that are augmented with a group of “star points” allows for the estimation of curvature. If the distance from the center of the design space to a factorial point is ± 1 unit for each factor, the distance from the center of the design space to a star point is $\pm\alpha$ with $|\alpha| > 1$ (Singh *et al.* 2007). The CCRD design is able to uniformly predict at all constant distances from the center points. In the case of rotatable designs, the variances and covariances of the estimated coefficients in the fitted model remain unchanged when the design points are rotated about its centers. For this study, the experimental design was generated by Statistica computer software (Statistica 1999 edition, Kent release 5.5 A, 1984–1999 by Stat Soft Inc., Tulsa, OK). Statgraphics Plus 3.0 for Windows (Statistical Graphics Corporation, Herndon, VA) was used for the response graphs, while Minitab 14.3 Statistical software (Minitab Inc., State College, PA) was used for superimposing the graphs. The three variables used in the study were boiling time (X_1 , varying from 5 to 30 min), incubation time (X_2 , varying from 12 to 48 h) and incubation temperature (X_3 , varying from 25 to 50C) (Djurtoft and Jenson 1977, Steinkraus *et al.* 1983; Shurtleff and Aoyagi 1985; Asmah 1987).

The total number of experiments according to the design = $(2)^{\text{number of variables}} + 2 \times \text{number of variables} + \text{central points}$.

The central point, which is annotated as (0, 0 and 0), gives an indication of the replications of the experiment and therefore the number of variables, which means the total number of experiments for each factor will be equal to 20 (i.e., $2^3 + 2 \times 3 + 6$).

The five different levels in each experiment were coded as follows: $-\alpha$, -1 , 0 and 1 , α where $\alpha = 2 \times \text{number of variables}^{1/4} = 2^{3/4} = 1.682$. The independent variable codes were therefore -1.682 (lowest level), 0 (middle level) and 1.682 (highest level). The relationship between the coded and actual values can be obtained using the following formula (for variable X):

$$Z = (X - X^0) / \Delta X \quad (1)$$

where Z is the coded value for the variable, X is the corresponding actual value, X^0 is the actual value in the center of the domain and ΔX is the increment of X corresponding to 1 unit of Z . Twenty sample combinations were generated using the design matrix and variable combinations in experimental runs as shown in Tables 1 and 2.

Statistical Analysis and Optimization. The data collated from the experiments conducted on the various combinations were then subjected to a second-order polynomial regression analysis using least square regression

TABLE 1.
PROCESS VARIABLES AND THEIR LEVELS USED IN THE CENTRAL COMPOSITE
ROTATABLE DESIGN FOR K = 3

Variable	Code	-1.682	-1	0	+1	+1.682
Boiling time (min)	X_1	5.0	10.1	17.5	24.9	30.0
Incubation time (h)	X_2	12.0	19.3	30.0	40.7	48
Incubation temperature (C)	X_3	25.0	30.1	37.5	44.9	50.0

TABLE 2.
DESIGN MATRIX AND VARIABLE CODE COMBINATIONS IN EXPERIMENTAL RUNS

	X_1	X_2	X_3	Boiling time (min)	Incubation time (h)	Incubation temperature (C)
1	-1	-1	-1	10.1	19.3	30.1
2	-1	1	1	10.1	40.7	44.9
3	1	-1	1	24.9	19.3	44.9
4	1	1	-1	24.9	40.7	30.1
5	0	0	0	17.5	30.0	37.5
6	0	0	0	17.5	30.0	37.5
7	-1	-1	1	10.1	19.3	44.9
8	-1	1	-1	10.1	40.7	30.1
9	1	-1	-1	24.9	19.3	30.1
10	1	1	1	24.9	40.7	44.9
11	0	0	0	17.5	30.0	37.5
12	0	0	0	17.5	30.0	37.5
13	1.682	0	0	30.0	30.0	37.5
14	-1.682	0	0	5.0	30.0	37.5
15	0	1.682	0	17.5	48.0	37.5
16	0	-1.682	0	17.5	12.0	37.5
17	0	0	1.682	17.5	30.0	50.0
18	0	0	-1.682	17.5	30.0	25.0
19	0	0	0	17.5	30.0	37.5
20	0	0	0	17.5	30.0	37.5

methodology to obtain the parameters of the mathematical models. The regression analysis was carried out to observe the significant effects of the various process parameters on the various responses measured at a significance level of $P < 0.05$. The relative effect of each process parameter was compared with the β values corresponding to that parameter. The β coefficients are the regression coefficients obtained by first standardizing the process variables to a mean of 0 and an SD of 1. The higher the positive value of β of a parameter, the higher would be the effect of that parameter and vice versa. Table 3 shows the

TABLE 3.
REGRESSION COEFFICIENTS, R^2 VALUES AND LACK OF FIT IN THE MODELS

Variables	Protein	Protein solubility	pH	Acidity	L	a^*	b^*	ΔE
Constant	12.823	-150.189*	5.825**	0.179**	78.407**	-1.176	5.115	43.654**
X_1	0.893	8.596*	-0.113**					-1.378*
X_2	-0.447	-0.744*		-0.010**				-0.599**
X_3		9.315**	-0.028**	0.002			0.919**	
X_1^2	-0.014	-0.064*	0.003**	0.0003*				
X_2^2	0.014*		0.002**	0.0002*				
X_3^2		-0.106*				-0.002**	-0.014**	
$X_1 * X_2$	-0.016		-0.008	0.0002*	-0.028**	0.008**	0.007**	0.062
$X_1 * X_3$		-0.139		-0.0003*	0.010**			
$X_2 * X_3$								
Lack of fit	0.99	0.19	0.12	0.15	0.47	0.14	0.12	0.22
R^2	0.87	0.72	0.95	0.89	0.71	0.82	0.92	0.75

* Significant at $P \leq 0.05$.

** Significant at $P \leq 0.01$.

coefficients of the variables in the models and their contribution to the model's variation and their lack of fit.

The behavior of the system can be described by the following second-order polynomial equation:

$$Y = \beta_o + \sum \beta x_i + \sum \beta_{ii} x_i^2 + \sum \sum \beta_{ij} x_i x_j \quad (2)$$

where Y is the predicted response, β_o is the interception coefficient, β_i is the linear term, β_{ii} are the quadratic terms, β_{ij} are the interaction terms, and x_i and x_j represent the coded levels of the independent variables. Tests for lack of fit and R^2 values were used to determine the adequacy of the models. The response surface and contour plots were generated for different interaction of any two independent variables while holding the value of the third variable at the midpoint. These three-dimensional surfaces could give accurate geometrical representation and provide useful information about the behavior of the system within the experimental design. The optimization of the process was aimed at finding the levels of independent variables that would give the best possible pH, protein content and protein solubility. The optimum processing conditions were determined by superimposing the contour plots for the protein content, protein solubility and the pH of the samples using Minitab 14.13 Statistical software. The optimization criteria used were pH of 5.0–7.0,

protein content of 18–25% and protein solubility of 70–90% (Nout and Kiers 2005).

Analytical Methods

Protein Content Determination. The protein content ($N \times 5.7$) was determined using AOAC (1990)-approved method 920.87 using the Kjeldahl procedure.

Protein Solubility Determination. This was determined as reported by the Malaysian Standard (1997). One and a half (1.5) grams of the sample was weighed into a beaker and 75 mL of 0.2% (0.36 N, pH 12.5) potassium hydroxide was added. The sample was then stirred for 20 min on a magnetic stir plate and centrifuged at 2,700 rpm for 15 min. The supernatant was then filtered through glass wool into a beaker, being careful to avoid transferring. It was centrifuged again and 15 mL supernatant was transferred into two Kjeldahl tubes for duplicate analysis (this gives a 0.3 g aliquot of the original sample); 12.5 mL concentrated sulfuric acid and 2 mL hydrogen peroxide was added to each tube for nitrogen determination by the Kjeldahl method. The total nitrogen of the original sample was also determined. Protein solubility was expressed as the soluble protein fraction (from supernatant) as a percentage of the total protein in the cowpea.

pH and Titratable Acidity Determination. Ten grams of dried flour was mixed with 100 mL distilled water. The mixture was allowed to stand for 15 min, shaken at 5 min intervals and centrifuged at 3,000 rpm for 15 min using a Denley centrifuge (Model BS4402yD; Denley, UK). The supernatant was decanted and its pH was determined using a pH meter (model HM-30S; Tokyo, Japan). Ten-milliliter aliquots (triplicate) were titrated against 0.1 M NaOH using 1% phenolphthalein as indicator. Acidity was calculated as g lactic acid/100 g sample.

Color. The color space parameters L , a^* and b^* of the samples were measured with a Minolta CR-310 Tristimulus Colorimeter (Minolta Co. Ltd., Osaka, Japan). The L represented lightness (with 100 = perfect/brightness to 0 = darkness/blackness); a^* represented the extent of green color (in the range from negative = green to positive = redness); b^* qualified blue in the range from negative = blue to positive = yellow. The color change of the samples was also calculated as follows: $\Delta E = \sqrt{(L^2 + a^{*2} + b^{*2})}$. All measurements were conducted in triplicate and the mean values reported.

RESULTS AND DISCUSSION

Development of Models

Eight regression equations were obtained when Eq. (2) was fitted into experimental data (Table 2). These equations were then tested for adequacy and fitness using ANOVA. Table 3 summarizes the results of the ANOVA. The models developed for the protein content, pH, protein solubility, titratable acidity, L , a^* , b^* and total color change had high R^2 values ranging from 0.71 to 0.95. All the models also had nonsignificant lack-of-fit P values; hence, models could be effectively used to make accurate predictions. The second-order regression models developed for the various factors are as follows:

- (1) Protein content = $12.823 + 0.893 \times \text{boiling time} - 0.447 \times \text{incubation time} - 0.014 \times \text{boiling time}^2 + 0.014 \times \text{incubation time}^2 - 0.016 \times \text{boiling time} \times \text{incubation time}$;
- (2) Protein solubility = $-150.189 + 8.596 \times \text{boiling time} - 0.744 \times \text{incubation time} \times 9.315 \times \text{incubation temperature} - 0.064 \times \text{boiling time}^2 - 0.106 \times \text{incubation temperature}^2 - 0.139 \times \text{boiling time} \times \text{incubation temperature}$;
- (3) pH = $5.825 - 0.113 \times \text{boiling time} - 0.028 \times \text{boiling temperature} + 0.003 \times \text{boiling time}^2 + 0.002 \times \text{incubation time}^2 - 0.008 \times \text{boiling time} \times \text{incubation time}$;
- (4) Acidity = $0.179 - 0.010 \times \text{incubation time} + 0.002 \times \text{incubation temperature} + 0.0003 \times \text{boiling time}^2 + 0.0002 \times \text{incubation time}^2 + 0.0002 \times \text{boiling time} \times \text{incubation time} - 0.003 \times \text{boiling time} \times \text{incubation temperature}$;
- (5) $L = 78.407 - 0.028 \times \text{boiling time} \times \text{incubation time} + 0.010 \times \text{boiling time} \times \text{incubation temperature}$;
- (6) $a^* = -1.176 - 0.002 \times \text{incubation temperature}^2 + 0.008 \times \text{boiling time} \times \text{incubation time}$;
- (7) $b^* = 5.115 + 0.919 \times \text{incubation temperature} - 0.014 \times \text{incubation temperature}^2 + 0.007 \times \text{boiling time} \times \text{incubation time}$;
- (8) Color change (ΔE) = $43.654 - 1.378 \times \text{boiling time} - 0.599 \times \text{incubation time} + 0.062 \times \text{boiling time} \times \text{incubation time}$.

The experimental data (Table 3) showed that the second-order polynomial models showed a good fit with a lack-of-fit P value of 0.99 and R^2 of 87%. The R^2 of a model refers to the proportion of variation in the response attributed to the model rather than random error. For a good fit of a model, an R^2 of at least 60% was used. Malcolmson *et al.* (1993) commented that an R^2 of 80% appears to be excessive for a preliminary study and therefore recommended that an R^2 of 60% can be used. There was a strong and significant influence ($P < 0.001$) of the quadratic effect of the incubation time on the protein content

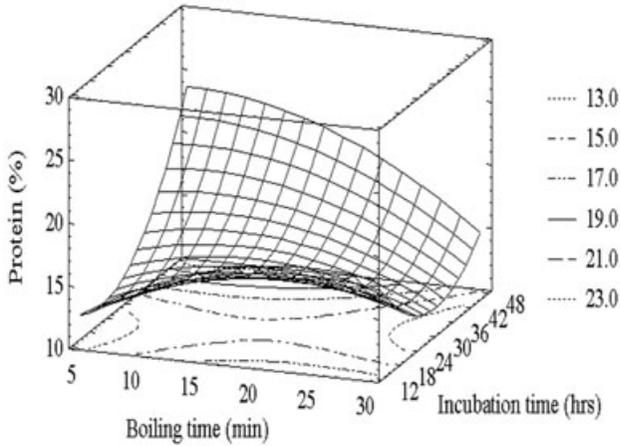


FIG. 1. RESPONSE SURFACE PLOT SHOWING PREDICTED EFFECT OF INCUBATION TIME AND BOILING TIME ON THE PROTEIN CONTENT OF THE COWPEA TEMPEH

of the cowpea tempeh. The incubation temperature was found not to have any effect on the protein content of the cowpea tempeh. This may be due to the fact that protein content was measured as nitrogen ($N \times 5.7$); hence, the apparent protein content was not affected by incubation temperature, as nitrogen is not affected by heat treatment (Pelembé *et al.* 2001). This may suggest that time of fermentation should be controlled more carefully than the fermentation temperature during cowpea fermentation (Ibanoglu and Ibanoglu 2000). Even though the boiling time, incubation time and their various combinations did not have any significant influence on the protein content at $P \leq 0.05$, they had to be kept in the model to obtain a reasonable lack of fit and a nonsignificant lack of fit. The response plot (Fig. 1) shows the predicted effects of boiling time and incubation time on the protein content of the cowpea tempeh. The plot generated shows a curvilinear plot with boiling time. A look at the response plot shows that the protein content of the tempeh reduced at the initial stages of fermentation after which there were increases after about 32 h of fermentation. The reduction in the protein content at the initial stages of fermentation could be attributed to the fact that the proteins in the sample were being hydrolyzed by proteases produced by *Rhizopus oligosporus* as reported by Higasa *et al.* (1996). Higasa *et al.* (1996) again reported that at 46 h of fermentation, hydrolyzed protein in soy tempeh represented 25% of the initial protein. The increase in the protein content of the tempeh samples could be attributed to the apparent increase in the mould biomass (Sparringa and Owens 1999).

Solubility of protein is one of the critical functional attributes required for its use as a food ingredient, as solubility greatly influences other properties, such as emulsification, gelation and foaming (Wang and Kinsella 1976). The digestibility and bioavailability of proteins are functions of its solubility. The model developed to predict the protein solubility had an R^2 of 0.72; thus, it could explain 72% of the variations in the protein solubility. The model also had a nonsignificant lack of fit with P value of 0.19. It was observed from the statistical analysis that only the incubation temperature was significant at 99% level. The quadratic term of the incubation time and the combination of the boiling time and incubation time did not have any effect on the protein solubility of the cowpea tempeh, and hence, were removed from the model. The response plot (Fig. 3) shows a linear plot with incubation time. However, curvilinear plots were observed with boiling time and incubation temperature. The protein solubility was observed to decrease with fermentation. This observation is contrary to the fact that the soluble protein content increases sharply due to the action of protease enzyme produced by mould during fermentation of soybeans into tempeh, as reported by Astuti (2000). The protein solubility was also observed to increase to a maximum as the incubation temperature increases and then reduces. The increase in solubility and digestibility in fermented seeds could be attributed to the proteolytic activity of *Lactobacillus* (Fadda *et al.* 1999). It was postulated that the increase of protein susceptibility to proteolytic enzymes, due to partial protein denaturation and pH during fermentation, led to increased solubility and digestibility (Czarnecki *et al.* 1993).

There was a general increase in the protein solubility with increases in boiling time. However, it was observed that the effect of the boiling time on the protein solubility depended on the temperature of incubation.

The regression model developed for the pH of the cowpea tempeh could explain 95% of the variation in the pH and had a nonsignificant lack of fit with P value of 0.12. The regression coefficients (Table 3) showed that the linear and quadratic terms of boiling time, the linear term of incubation temperature, the quadratic term of incubation time, and the interaction between the boiling time and incubation time had strong and significant influence ($P < 0.01$) on the pH of the tempeh samples. The response plots (Fig. 2) developed show linear curves with incubation temperature. It was observed that changes in incubation temperature did not result in significant changes in pH of the tempeh samples. There were slight decreases in the pH with increases in incubation temperature. The response plots showed that the effect of boiling time on the pH of the tempeh samples depended on the time of incubation.

Titrateable acidity is a measure of the total acid (dissociated and undissociated) produced in the fermentation product. Sefa-Dedeh (1991) and Afoakwa *et al.* (2004) reported that fermentation contribute to the

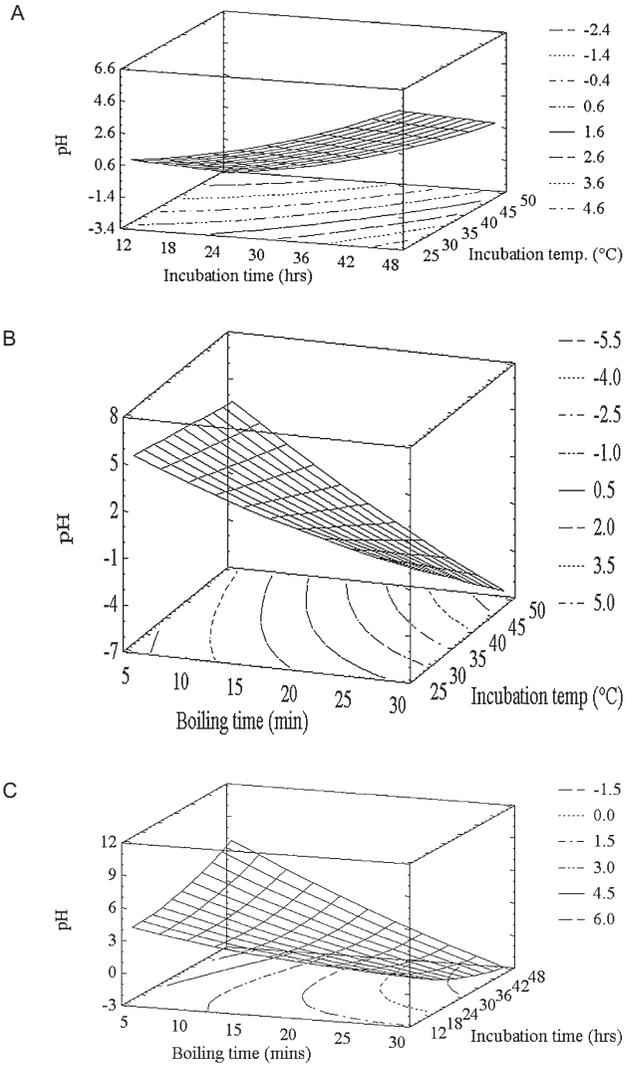


FIG. 2. RESPONSE SURFACE PLOTS SHOWING PREDICTED EFFECTS OF DIFFERENT FACTORS ON THE pH OF THE COWPEA TEMPEH

(A) Predicted effect of incubation time and incubation temperature on the pH of the cowpea tempeh at constant boiling time. (B) Predicted effect of boiling time and incubation temperature on the pH of the cowpea tempeh at constant incubation time. (C) Predicted effect of boiling time and incubation time on the pH of the cowpea tempeh at constant incubation temperature.

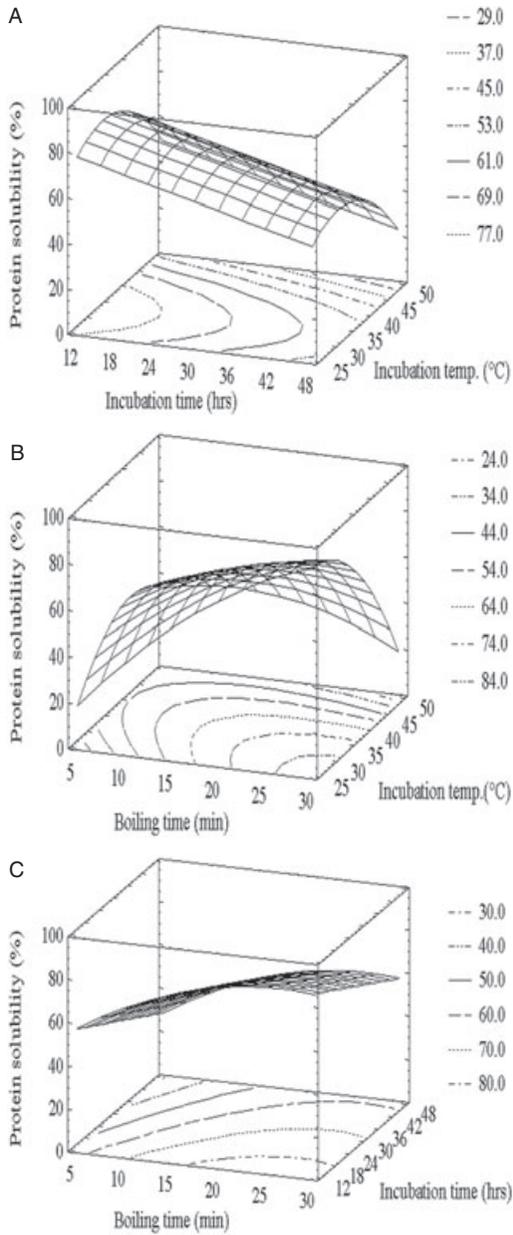


FIG. 3. RESPONSE SURFACE PLOTS SHOWING PREDICTED EFFECTS OF DIFFERENT FACTORS ON THE PROTEIN SOLUBILITY OF THE COWPEA TEMPEH (A) Predicted effect of incubation time and incubation temperature on the protein solubility of the cowpea tempeh. (B) Predicted effect of boiling time and incubation temperature on the protein solubility of the cowpea tempeh at constant incubation time. (C) Predicted effect of boiling time and incubation temperature on the protein solubility of the cowpea tempeh at constant incubation temperature.

development of souring or acids, resulting in improved flavor, color, texture and other product quality attributes in cereal products. Only the linear term of the incubation time was found to have a strong and significant influence ($P < 0.01$) on the acidity of the tempeh samples. The regression model developed could explain 89% of the variations in the acidity of the samples. A nonsignificant lack of fit with P value of 0.15 was obtained for the model. The response plots (Fig. 4) indicated that acidity of the tempeh samples increased with increasing boiling and incubation times. However, changes in the incubation temperature of the tempeh samples did not result in significant changes in the acidity. The increase in the acidity of the tempeh may be attributed to the utilization of protein by the mould, as reported by Xu *et al.* 2005.

Color is one of the most important attributes used to determine the acceptability of foods. This is because no matter how nutritious, flavored or well textured a food is, it is unlikely to be accepted unless it has the right color and appearance (Serna-Saldivar *et al.* 1990). The model developed for the L value of the tempeh samples could explain 71% of the variations in the color. The model had a nonsignificant lack of fit with P value of 0.47. The regression coefficients in Table 3 show that only the interactions between the boiling time and incubation time had significant influence on the L value of the tempeh samples.

The response plots (Fig. 5) indicated that the L value of the samples significantly reduced during the fermentation period. In other words, the samples became darker with increasing fermentation time. Increases in boiling time also resulted in consequential decreases in L value of the samples. Fermentation tended to produce a slightly darker color, which may be attributed to the influence of mycelia color and the drying step (Reyes-Moreno *et al.* 2004). However, changes in the incubation temperature did not result in significant variations in the L value of the samples. The model developed for the total color change of the tempeh could explain 75% of the variations (Table 3), with a nonsignificant lack-of-fit P value of 0.22. The model showed that the linear terms of boiling and incubation times significantly influenced the total color change of the tempeh samples. It was also observed that the interaction between the boiling and incubation times also significantly influenced the total color change. The total color change of the tempeh samples was not affected by the incubation temperature. The response plot (Fig. 6), predicting the effect of incubation time and boiling time on the total color change of the cowpea tempeh, showed that changes in total color of the cowpea tempeh was more significant at the higher incubation temperatures and boiling times.

Determination of the Optimum Processing Conditions

Contour plots for some of the response variables (i.e., pH, protein and protein solubility) were plotted, and these plots were superimposed to obtain

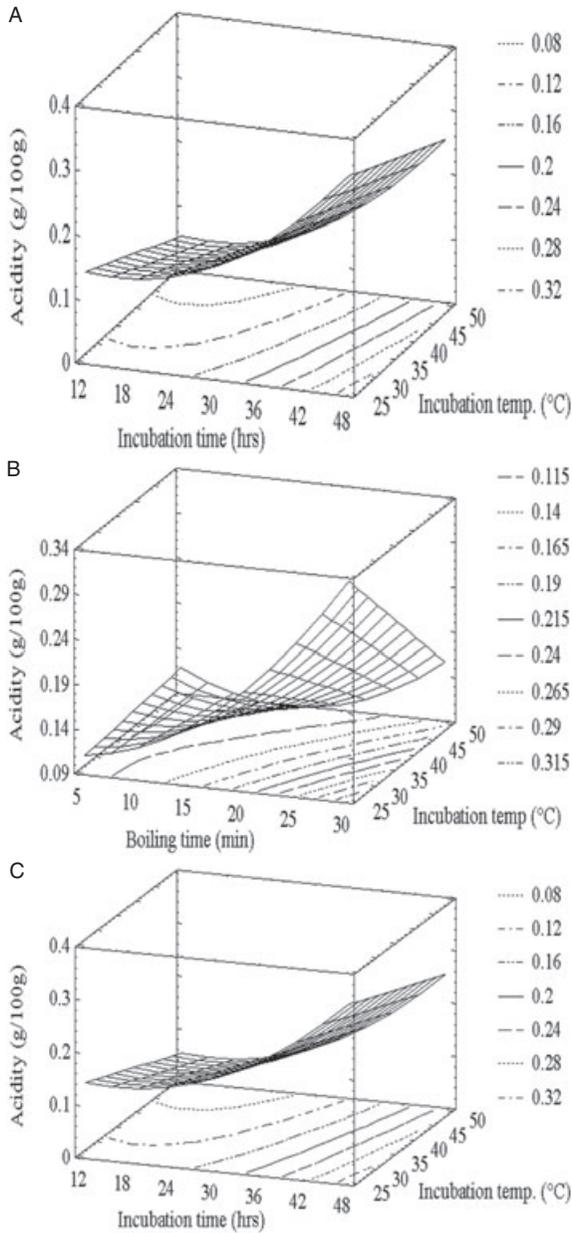


FIG. 4. RESPONSE SURFACE PLOTS SHOWING PREDICTED EFFECTS OF DIFFERENT FACTORS ON THE ACIDITY OF THE COWPEA TEMPEH (A) Predicted effect of incubation time and incubation temperature on the acidity of the cowpea tempeh at constant boiling time. (B) Predicted effect of boiling time and incubation temperature on the acidity of the cowpea tempeh at constant incubation time. (C) Predicted effect of incubation time and boiling time on the acidity of the cowpea tempeh at constant incubation temperature.

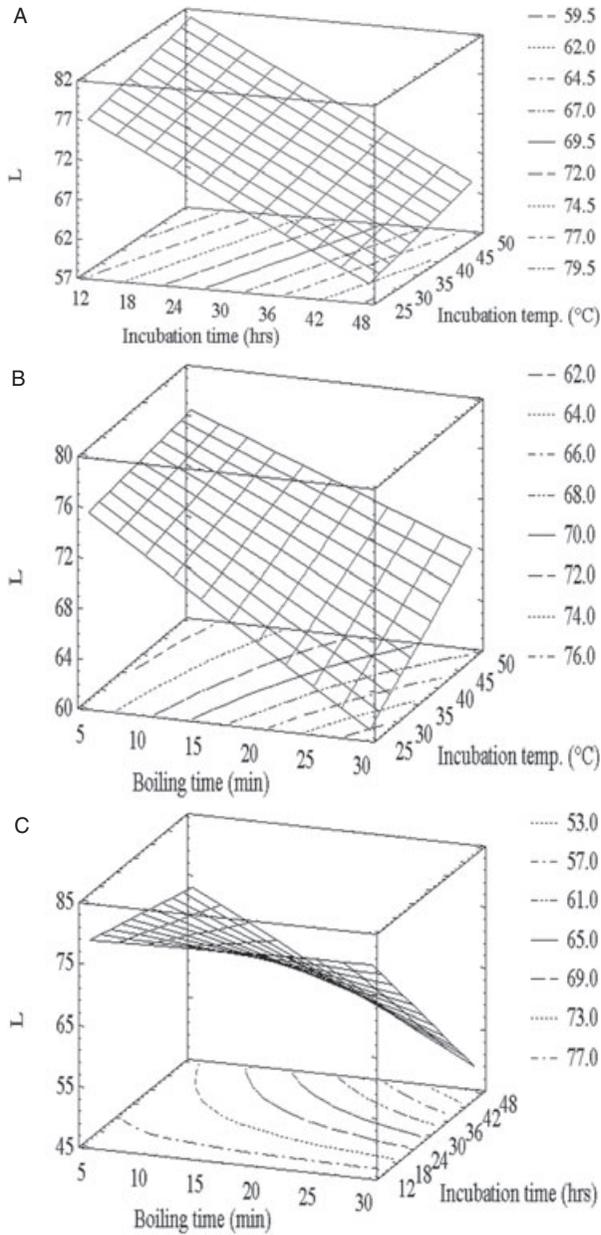


FIG. 5. RESPONSE SURFACE PLOTS SHOWING PREDICTED EFFECTS OF DIFFERENT FACTORS ON THE L VALUE OF THE COWPEA TEMPEH

(A) Predicted effect of incubation time and incubation temperature on the L value of the cowpea tempeh at constant boiling time. (B) Predicted effect of boiling time and incubation temperature on the L value of the cowpea tempeh constant incubation time. (C) Predicted effect of incubation time and boiling time on the L value of the cowpea tempeh at constant incubation temperature.

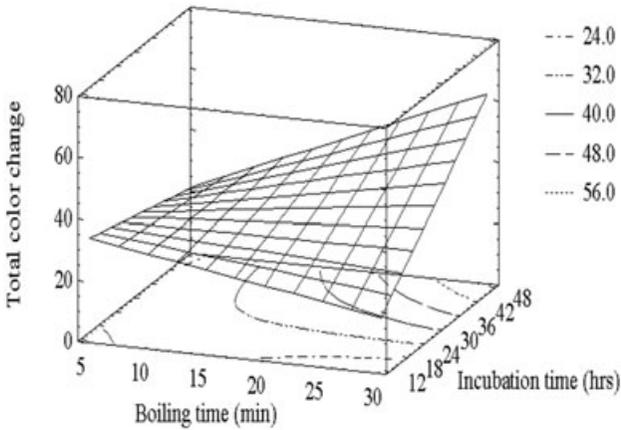


FIG. 6. RESPONSE SURFACE PLOT SHOWING PREDICTED EFFECT OF INCUBATION TIME AND BOILING TIME ON THE TOTAL COLOR CHANGE OF THE COWPEA TEMPEH

contour plots to allow for selection of the best (optimum) combination of the variables studied. The optimization criteria used were pH of 5.0–7.0, protein content of 18%–25% and protein solubility of 70%–90%.

The regions that satisfied these conditions for the cowpea tempeh samples were obtained by superimposing, shown in Fig. 7. A number of combinations of boiling time, incubation time and incubation temperature in the unshaded area was found to meet the criteria set for the process of optimization. The optimum incubation temperature was conveniently set at 37°C since it is the optimum temperature for microbial activity. Temperatures above 40°C have been reported as too high for mould growth (Nout and Kiers 2005). The time of incubation has been reported by Nout and Kiers (2005) to depend on the temperature of incubation. It has also been reported that the fermentation of tempeh is usually complete after 24 h. Based on this observation, the time of incubation for the cowpea tempeh was conveniently set at 28 h when incubated at 37°C. The unshaded regions of Fig. 7 indicates that for an optimum cowpea tempeh, the boiling times of the cotyledons ranged from 17 to 28 min. However, for economical reasons with respect to the amount of energy needed for boiling, the boiling time was set at 20 min.

CONCLUSIONS

Response surface methodology was effectively used in optimizing the processing condition in the processing of cowpea tempeh. Both boiling and

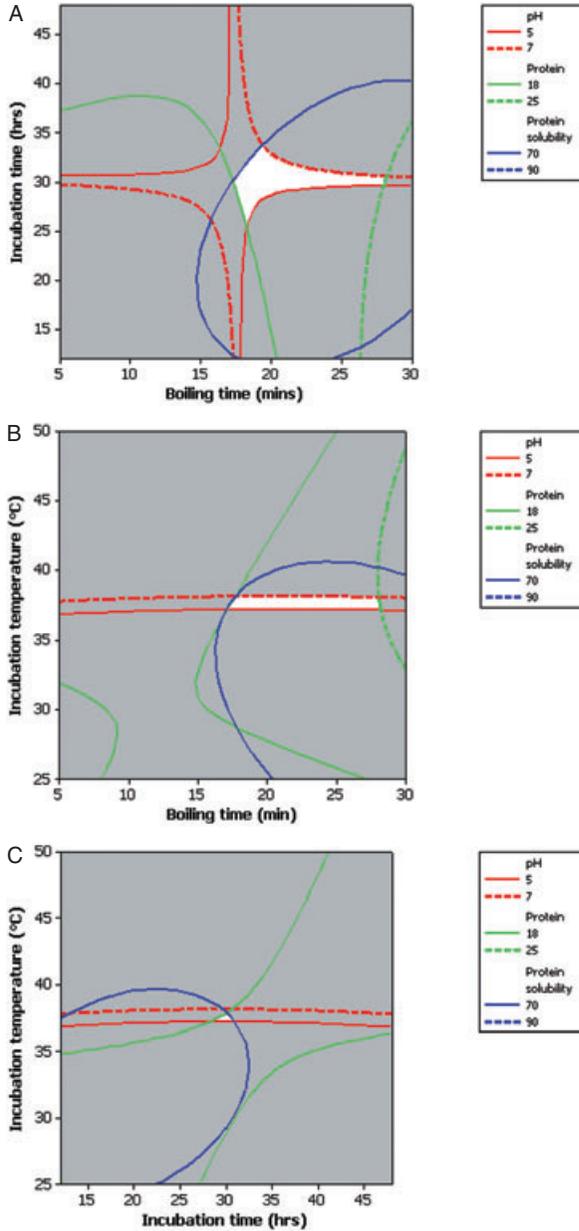


FIG. 7. SUPERIMPOSED CONTOUR PLOTS FOR PROCESSING VARIABLES (A) At incubation temperature of 37.5°C showing optimum region (unshaded region). (B) At fermentation time of 30 h showing optimum region (unshaded region). (C) At boiling time of 17.5 min showing optimum region (unshaded region).

incubation times were identified as influential processing conditions during preparation of cowpea tempeh, effecting significant variations in quality characteristics (protein content, protein solubility, pH, acidity and color changes) of products. Incubation temperature did not significantly influence the protein content, the degree of lightness and the total color change of the products. However, the protein solubility of the cowpea tempeh depended significantly on the temperature at which the cowpeas were incubated. The optimum processing conditions observed for the preparation of cowpea tempeh were boiling time of about 20 min, incubation time of about 28 h and incubation temperature of about 37C.

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REFERENCES

- AFOAKWA, E.O., SEFA-DEDEH, S. and SAKYI-DAWSON, E. 2004. Effects of cowpea fortification dehydration method and storage time on some quality characteristics of maize-based traditional weaning Foods. *Afr. J. Food Agric. Nutr. Dev.* 4, 25–29.
- AFOAKWA, E.O., YENYI, S.E. and SAKYI-DAWSON, E. 2006. Response surface methodology for optimizing the pre-processing conditions during canning of a newly developed and promising cowpea (*Vigna unguiculata*) variety. *J. Food Eng.* 73, 346–357.
- AOAC 1990. *Approved Official Methods of Analysis*, 13th Ed., Association of Official Analytical Chemists, Washington, DC.
- ASMAH, K.B. 1987. *Production and Evaluation of a Fermented Cowpea Food*. BSc Dissertation, Department of Nutrition and Food Science, University of Ghana, Legon, Ghana.
- ASTUTI, M., MELIALA, A., DALAIS, F.S. and WAHLQVIST, M.L. 2000. Tempeh, a nutritious and healthy food from Indonesia. *Asia Pac. J. Clin. Nutr.* 9, 322–325.
- CZARNECKI, Z., GUJSKA, E. and KHAN, K. 1993. Enzyme pretreatment of pinto bean high protein fraction and time of blending corn meal affects extrudate properties. *J. Food Sci.* 58, 1404–1408.
- DJURTOFT, R. and JENSON, J.S. 1977. *Tempeh-like foods produced from broad beans* (*Vicia faba*), *cowpeas* (*Vigna senensis*), *barley* (*Hordeum*

- vulgare), wheat (*Triticum aestivum*) or from mixture thereof. Symposium on Indigenous Fermented Foods, November 21–22, 1977, Bangkok, Thailand.
- FADDA, S., SANZ, Y., VIGNOLO G., ARISTOY M.C., OLIVER, G. and TOLDRA, F. 1999. Hydrolysis of pork muscle sarcoplasmic proteins by *Lactobacillus curvatus* and *Lactobacillus sake*. *Appl. Environ. Microbiol.* *65*, 578–584.
- GIOVANNI, M. 1983. Response surface methodology and product optimization. *Food Technol.* *37*, 41–45.
- HERMANA, H., MAHMUD, M.K.M.S. and KARYADI, D. (Eds) 1990. *Second Asian Symposium on Non-salted Soybean Fermentation*. Nutrition Research and Development Centre, Bogor, Indonesia.
- HIGASA, S., NEGISHI, Y., AOYAGI, Y. and SUGAHARA, T. 1996. Changes in free amino acids of tempe during preparation with velvet beans (*Mucuna pruriens*). *J. Jpn. Soc. Food Sci.* *43*, 188–193.
- IBANOGLU, S. and Ibanoglu, E. 2000. Modelling of natural fermentation in cowpeas using response surface methodology. *J. Food Eng.* *48*, 277–281.
- KHURI, A.I. and CORNELL, J.A. 1987. *Response Surfaces Design And Analysis*, Marcel Dekker, Inc., New York, NY.
- MALAYSIAN STANDARD 1997. *Animal Feeding Stuffs – Determination of Protein Solubility Index, Ics: 65.120*, Department of Standards, Malaysia.
- MALCOLMSON, L.J., MATSUO, P.R. and BALSHAW, R. 1993. Textural optimization of spaghetti using response surface methodology: Effects of drying temperature and durum protein level. *Cereal Chem.* *70*, 417–423.
- NOUT, M.J.R. and KIERS, J.L. 2005. Tempeh fermentation, innovation and functionality: Update into the third millennium. *J. Appl. Microbiol.* *98*, 789–805.
- PELEMBE, L.A.M., ERASMUSW, C. and TAYLOR, J.R.N. 2001. Development of a protein-rich composite sorghum–cowpea instant porridge by extrusion cooking process. *Lebensm. Wiss. Technol.* *35*, 120–127.
- REYES-MORENO, C., CUEVAS-RODRIGUEZ, E.O., MIL AN-CARRILLO J., CARDENAS-VALENZUELA O.G. and BARRON-HOYOS, J. 2004. Solid state fermentation process for producing chickpea (*Cicer arietinum* L) tempeh flour. Physicochemical and nutritional characteristics of the product. *J. Sci. Food Agric.* *84*, 271–278.
- SEFA-DEDEH, S. 1991. *Improvement of cereals and root crop processing in Africa*. 8th World Congress of Food Science and Technology, June 14–18, 1991, Orlando, FL, USA.
- SEFA-DEDEH, S., CORNELIUS, B., SAKYI-DAWSON, E. and AFOAKWA, E.O. 2003. Application of response surface methodology to study the quality characteristics of cowpea-fortified nixtamalized maize. *Innov. Food Sci. Emerg. Technol.* *4*, 109–119.

- SEFA-DEDEH, S., SAKYI-DAWSON, E. and AFOAKWA, E.O. 2001. *Comparative evaluation of cowpea varieties and their performance in a fermented food product*. IFT book of abstracts 44D-13 and paper presented at the annual meeting of the institute of food technologists, June 23–27, 2001, New Orleans, LA, USA.
- SEFA-DEDEH, S., STANLEY, D.W. and VOISEY, P.W. 1978. Effects of soaking time and cooking conditions on texture and microstructure of cowpea (*Vigna unguiculata*). *J. Food Sci.* 43, 1832–1838.
- SERNA-SALDIVAR, S.O., GOMEZ, M.H. and ROONEY, L.W. 1990. Technology, chemistry and nutritive value of alkaline-cooked corn products. In *Advances in cereal science and Technology* (J.J. Skinner, ed) pp. 243–295, American Association of Cereal Chemist, Inc., St. Paul, MN.
- SHURTLEFF, W. and AOYAGI, A. 1985. *The Book of Tempeh*, 2nd Ed., pp. 68–71, Harper and Row Publishers.
- SINGH, B., PANESAR, P.S., GUPTA, A.K. and KENNEDY, J.F. 2007. Optimisation of osmotic dehydration of carrot cubes in sucrose-salt solutions using response surface methodology. *Eur. Food Res. Technol.* 25, 157–165.
- SOORIYAMOORTHY, S.S., SILVA, K.F.S.T. and GUNAWARDHANE M.H.W. & ILLEPERMA, C.K. 2004. Isolation and identification of indigenous *Aspergillus oryzae* for saccharification of rice starch. *Trop. Agric. Res.* 16, 121–127.
- SPARRINGA, R.A. and OWENS, J.D. 1999. Protein utilization during soybean tempeh fermentation. *J. Agric. Food Chem.* 47, 4375–4378.
- STEINKRAUS, K.H., CULLEM, R.E., PEDERSON, C.S., NELLIS, L.F. and GAVITT, B.K. 1983. *Indonesian Tempeh and Related Fermentations: Handbook of Indegenous Fermented Foods, Microbiology Series*, Vol. 9, Marcel Dekker, Inc., New York, NY.
- WANG, J.C. and KINSELLA, J.E. 1976. Functional properties of novel proteins: Alfalfa leaf protein. *J. Food Sci.* 41, 286–289.
- XU, C., HAO, K. PENG, C., CAI, W. and JIN, Z. 2005. Effects of fermentation temperature on the characteristics of Douchi. *Food Technol. Biotechnol.* 43, 307–311.