

Optimization of germination process of cowpea by response surface methodology

N. Wang,^{a*} M. J. Lewis,^a J. G. Brennan^a & A. Westby^b

^aDepartment of Food Science and Technology, University of Reading, Whiteknights, Reading, U.K.

^bNatural Resources Institute, Central Avenue, Chatham Maritime, Chatham, U.K.

(Received 4 August 1995; revised version received 7 May 1996; accepted 7 May 1996)

The effect of soaking time, germination time and temperature on the nutrient content, oligosaccharides and trypsin inhibitor activity in cowpea was evaluated by response surface methodology. All the three processing variables affected nutrient content, oligosaccharides and trypsin inhibitor activity. Optimization was based on conditions that maximized reduction in oligosaccharides and trypsin inhibitor activity and minimized total solids losses. Optimum conditions for germination of cowpea were: soaking time = 8 h, germination time = 52 h and germination temperature = 25°C and under these conditions the predicted values using a polynomial equation for total solids loss, protein, fat and ash contents were 7.71, 23.30, 1.48 and 2.29 (% dry basis), respectively. The trypsin inhibitor level was 4.28 mg/g dry solids. The values for raffinose, stachyose and sucrose were 0.90, 1.96 and 3.61 (% dry basis), respectively. Good agreement was found between experimental values obtained at optimum process conditions and predicted values. Copyright © 1996 Elsevier Science Ltd

INTRODUCTION

Cowpeas (*Vigna unguiculata*) are important in world agriculture with more than 12 million acres produced annually (Fery, 1981). Widely grown in Africa, India and Brazil, cowpeas constitute an important source of protein and carbohydrate for populations of these countries and contain 23–30% and 56–68% of these nutrients, respectively (Bressani, 1985). Although cowpea seeds are typically consumed as a boiled vegetable-alone or in combination dishes (Dovlo *et al.*, 1976), considerable interest has developed in expanding usage of cowpeas in other forms as a low cost, high quality protein supplement and to impart desirable functional properties in products such as baby food, bread, flour, paste and extruded products.

Despite the nutritional potential of cowpeas as an economic source of significant amounts of proteins, calories and some B-vitamins, the utilization of this legume has been limited, due to the presence of certain anti-nutritional factors and undigestible substances (Iyer *et al.*, 1980). Among the former are trypsin inhibitors which reduce nutritional quality of the protein. The latter are the flatulence-producing oligosaccharides,

namely, raffinose, stachyose and verbascose (Reddy *et al.*, 1980) which due to the absence of α -galactosidase in humans, are fermented anaerobically by micro-organisms to produce carbon dioxide, hydrogen and methane.

Attempts to increase the utilization of legumes have employed a wide range of processing techniques such as germination (East *et al.*, 1972; Hsu *et al.*, 1980; Jood *et al.*, 1985), cooking (Iyer *et al.*, 1980) and microbial α -galactosidase treatment (Thananunkul *et al.*, 1976). Of these techniques, the germination process has been studied the most. Germination is also an appropriate low cost, low technology processing option for household level processing in lesser developed countries.

The effect of germination on the nutritive value of grain has been reported by several workers (El-Hag *et al.*, 1978; Price, 1988). During sprouting of dry beans, the storage materials are converted into other forms more usable to both plants and humans. Germination may improve the nutritive value of legumes by inducing the formation of enzymes, including α -galactosidase which eliminates or reduces the anti-nutritional factors (Azhar *et al.*, 1972). Mostafa *et al.* (1987) and Ologhobo and Fetuga (1984) reported that germination reduced trypsin inhibitor activity by 32–41%. Germination can also reduce the amounts of oligosaccharides in legumes. Jood *et al.* (1985) reported that germination for 24 h led to considerable reductions in oligosaccharides in legumes, i.e., 17–70% (raffinose), 35–75% (stachyose)

*Present address: Department of Applied Microbiology & Food Science, University of Saskatchewan, 51 Campus Drive, Saskatoon, SK, Canada S7N 5A8.

and 66–91% (verbascose), respectively. East *et al.* (1972) found that raffinose in soybeans disappeared after 96 h of germination and stachyose disappeared after 144 h of germination. Nnanna & Phillips (1988) reported that germination temperature had an effect on oligosaccharides. The reduction in oligosaccharides was greater at higher temperatures than at lower temperatures. It has been reported that germination of faba beans improves solubility of proteins, water absorption, emulsification capacity and foaming properties (Kumar & Venkatasgmun, 1978). Germinated meal from pea had a better appearance and taste when compared with the raw material and, can be utilized as a nutritional and functional ingredient in food products (Jeffers *et al.*, 1978). However, germination also initiates metabolic processes and rootlet development, which may produce undesirable compositional changes.

There is a lack of information on the interactive effects of soaking time, germination time and temperature on the nutrients and anti-nutritional factors and germination conditions which maximize the reduction of oligosaccharides and anti-nutritional factors while retaining desired nutritional content. Therefore, the aim in this study was to investigate the combined effect of soaking, germination time and temperature on nutrients and anti-nutritional factors and to determine optimum germination conditions for cowpea using response surface methodology (RSM). Optimization of the germination process will allow the maximum nutritional benefit to be gained from this simple, low cost technology.

MATERIALS AND METHODS

Materials

Cowpeas (California blackeyes) were obtained from the local health food store. The moisture content was 8.08% dry basis.

Experimental design

Response surface methodology (RSM), defined by Giovanni, 1983 as a statistical method that uses quantitative data from appropriate experimental designs to determine and simultaneously solve multivariate equations, was used to examine factors affecting cowpea germination.

The germination process was assumed to be a system affected by three independent variables, also called factors, Z_i (in this study, soaking time, germination time and temperature), which were controlled and measured. It was assumed that the dependent variables, also referred to as responses, Y (in this study, solids loss, protein, fat and ash content and anti-nutritional factors), defined the system and they were experimentally determined. Furthermore, a mathematical function, f , was assumed to describe the relationship between responses, Y , and factors, Z_i ,

$$Y = f(Z_1, Z_2, Z_3) \quad (1)$$

Due to the unknown and/or complex form of the function, f , a second degree polynomial equation was assumed to approximate the true function

$$Y = b_0 + \sum_{i=1}^3 b_i X_i + \sum_{i=1}^3 b_{ii} X_i^2 + \sum_{i=1}^2 \sum_{j=i+1}^3 b_{ij} X_i X_j \quad (2)$$

where b_0 , b_i , b_{ii} , and b_{ij} are constant coefficients and X_i s are the coded independent variables linearly related to Z_i . The coding of Z_i into X_i was given by the following equation:

$$X_i = 2(Z_i - Z'_i)/d_i \quad (3)$$

where Z_i is the actual value in original units; Z'_i is the mean of high and low levels of Z_i and d_i is the difference (spacing) between the low and high level on the variable Z_i . After coding, the experimental region extends from -1.682 to 1.682 in terms of X_i (Myers, 1971). The levels of the variables in coded (X_i) and uncoded (Z_i) form are given in Table 1. The experimental design consisting of three-variables at five-levels with 23 runs is shown in Table 2.

Germination procedure

Cowpea seeds were washed with tap water and then soaked in tap water at room temperature for different times (Table 1). Soaked seeds were then spread in a layer on moist paper and placed in plastic trays, which were then incubated at different temperatures for different periods (Table 1). The seeds were washed twice a day with tap water during germination. The germinated seeds were removed and then freeze-dried. The freeze-dried seeds were ground into flour with a coffee grinder.

Table 1. Values of independent process variables and their corresponding levels

Independent variables	Symbol		Levels				
	Coded	Uncoded	-1.682	-1	0	+1	+1.682
Soaking time (h)	X_1	t_1	1	3.2	6.5	9.8	12
Germination time (h)	X_2	t_2	12	24.2	42	59.8	72
Germination temperature (°C)	X_3	T	20	22	25	28	30

Chemical analysis

Proximate composition

Moisture content, crude protein (N \times 6.25), fat (ether extraction) and ash content were estimated by standard methods (AOAC, 1984).

Trypsin inhibitor activity (TIA)

Trypsin inhibitor levels were determined by the method of Smith *et al.* (1980).

Determination of oligosaccharides by HPLC

One gram of cowpea flour was extracted with 20 ml of distilled water for 30 min at 100°C (Kennedy *et al.*, 1985). The suspension was then centrifuged at 5000 *g* for 45 min. The supernatant was decanted and a sample was centrifuged at 5000 *g* for 1 h through Millipore ultrafree filters (Millipore). These filters have a membrane retaining macromolecules of 5000 or greater molecular weight. The clean filtrate was analysed by high performance liquid chromatography (HPLC) without further treatment. 20 μ l samples were analysed by HPLC using a modular system comprised of a 3200 solvent delivery pump (spectra-physics), RI-71 refractive index detector (Shodex), 3390A recording integrator (Hewlett Packard), Reodyne 7125 injector valve (Reodyne, U.S.A.) and an Aminex HPX-87N (300 \times 78 mm) resin-based column in the sodium form (Bio-Rad, Richmond, CA). The mobile phase was 0.015 M Na₂SO₄ and the flow rate was 0.5 ml/min. The column was kept at a constant temperature of 85°C.

RESULTS AND DISCUSSION

The second order polynomial eqn (2) was fitted to the experimental data (Table 2) using SAS (SAS, 1985). The regression coefficients of eqn (2) are given in Table 3. The analysis of variance for the model for the eight response variables (Table 4) indicated that the model was statistically acceptable at the 1% level, possessing no lack of fit. Therefore, the model could be used to predict the eight response variables at different processing conditions.

Effect of process variables on the nutrient content

From the statistical analysis (Table 5), it was found that soaking time, germination time and temperature had an effect on total solids loss, protein, fat and ash contents. Figure 1 shows the interactive effect of soaking time, germination time and temperature on total solids loss. It can be seen from Fig. 1a that there was a sharp increase in total solids loss with increase in germination time. Total solids loss increased gradually as soaking time increased. However, the combination of soaking and germination times had a significant effect on total solids loss. Total solids increased significantly with increasing soaking and germination times. Total solids loss rose steadily as germination temperature increased (Fig. 1b,c). Again, the interactive effect of germination temperature, soaking time and germination time was significant.

Table 2. Experimental data for the three-factor, five-level response surface analysis

Experiment	Factors			Responses							
	X ₁	X ₂	X ₃	Solids loss (% dry wt)	Protein (% dry wt)	Fat (% dry wt)	Ash (% dry wt)	TIA (mg/g)	Raffinose (% dry wt)	Stachyose (% dry wt)	Sucrose (% dry wt)
1	+1	+1	+1	11.48	23.2	1.36	2.04	4.05	0.710	1.41	3.70
2	+1	+1	-1	8.229	23.4	1.50	2.44	4.35	0.868	1.92	3.46
3	+1	-1	+1	4.124	23.3	1.48	2.35	4.60	0.957	3.02	3.10
4	+1	-1	-1	3.880	23.5	1.52	2.65	4.93	1.02	3.29	2.73
5	-1	+1	+1	7.914	23.2	1.44	2.14	4.53	0.825	1.90	3.69
6	-1	+1	-1	5.507	23.7	1.52	2.58	4.81	0.946	2.17	3.70
7	-1	-1	+1	3.393	23.8	1.54	2.80	4.76	1.03	3.26	3.46
8	-1	-1	-1	3.627	24.0	1.56	3.04	5.02	1.05	3.41	3.23
9	+1.682	0	0	6.846	23.4	1.51	2.26	4.43	0.900	2.25	3.25
10	-1.682	0	0	3.524	23.9	1.59	2.74	4.92	1.02	2.75	3.74
11	0	+1.682	0	11.01	23.0	1.39	2.15	4.39	0.733	1.01	3.81
12	0	-1.682	0	3.261	23.6	1.56	2.91	5.22	1.05	3.44	2.92
13	0	0	+1.682	7.322	23.1	1.35	2.14	4.14	0.831	2.36	3.51
14	0	0	-1.682	4.753	23.6	1.53	2.74	4.72	0.986	2.96	3.17
15	0	0	0	5.738	23.5	1.51	2.42	4.47	0.960	2.46	3.52
16	0	0	0	5.693	23.5	1.52	2.40	4.34	0.967	2.51	3.52
17	0	0	0	5.621	23.4	1.54	2.45	4.45	0.963	2.52	3.52
18	0	0	0	5.742	23.4	1.50	2.43	4.44	0.958	2.44	3.54
19	0	0	0	5.986	23.5	1.51	2.44	4.44	0.960	2.38	3.54
20	0	0	0	5.535	23.5	1.52	2.42	4.42	0.964	2.45	3.51
21	0	0	0	5.745	23.4	1.51	2.46	4.46	0.954	2.40	3.52
22	0	0	0	5.894	23.4	1.53	2.43	4.44	0.966	2.45	3.52
23	0	0	0	5.532	23.3	1.52	2.44	4.45	0.953	2.41	3.54

There was a steady reduction in the protein and fat content when soaking time, germination time and temperature increased (Figs 2 and 3). Germination involves the breakdown of seed reserves and their utilization by the growing shoot and root for respiration (Abudu & Akinyele, 1990). Thus, the reduction in protein and fat content may be a result of the effect of germination. Ash content decreased with an increase in soaking time, germination time and temperature (Fig. 4). Similar observations for the composition changes during germination of soybeans were reported by Mostafa *et al.* (1987).

Effect of process variables on the anti-nutritional factors

Effect on trypsin inhibitor activity (TIA)

The mean TIA value for the raw cowpea was 6.99 mg/g dry solid. Kochhar *et al.* (1988) reported TIA values for different varieties of cowpea in the range of 4.6 to 13.9 mg/g. The TIA value in this study falls within the range of published results.

Figure 5 represents the combination effect of soaking time, germination time and temperature on TIA. TIA decreased steadily with increasing soaking time (Fig. 5a). It can also be seen from Fig. 5a that TIA decreased with

Table 3. Regression coefficients of the second order polynomials for eight response variables.

Coefficient	Solids loss (% dry wt)	Protein (% dry wt)	Fat (% dry wt)	Ash (% dry wt)	TIA (mg/g)	Raffinose (% dry wt)	Stachyose (% dry wt)	Sucrose (% dry wt)
b_0	5.723	23.4	1.52	2.43	4.44	0.96	2.44	3.53
b_1	0.942	-0.155	-0.024	-0.138	-0.148	-0.037	-0.142	-0.139
b_2	2.280	-0.150	-0.041	-0.214	-0.218	-0.090	-0.707	0.261
b_3	0.731	-0.131	-0.043	-0.175	-0.158	-0.045	-0.162	0.100
b_{11}	-0.215	0.102	0.012	0.026	0.081	0.001	0.036	-0.011
b_{12}	0.663	0.090	0.000	0.075	-0.084	-0.011	-0.047	0.083
b_{22}	0.474	-0.032	-0.014	0.037	0.127	-0.023	-0.060	-0.058
b_{31}	0.165	0.035	-0.010	-0.003	-0.011	-0.009	-0.047	0.047
b_{32}	0.706	-0.055	-0.020	-0.038	0.001	-0.025	-0.045	-0.052
b_{33}	0.086	-0.017	-0.027	0.005	-0.006	-0.017	0.093	-0.066

Table 4. Analysis of variance for eight response variables.

Source	df	Sum of squares for							
		Solids loss (% dry wt)	Protein (% dry wt)	Fat (% dry wt)	Ash (% dry wt)	TIA (mg/g)	Raffinose (% dry wt)	Stachyose (% dry wt)	Sucrose (% dry wt)
Model	9	102.58 ^a	1.16 ^a	0.078 ^a	1.391 ^a	1.71 ^a	0.176 ^a	7.74 ^a	1.54 ^a
Linear	3	90.41 ^a	0.872 ^a	0.057 ^a	1.302 ^a	1.29 ^a	0.157 ^a	7.47 ^a	1.33 ^a
Quadratic	3	4.45 ^a	0.187 ^a	0.017 ^a	0.033 ^a	0.361 ^a	0.013 ^a	0.217 ^a	0.123 ^a
Cross Product	3	7.72 ^a	0.099 ^a	0.004 ^b	0.056 ^a	0.058 ^a	0.007 ^a	0.051 ^b	0.094 ^a
Residual	13	0.284	0.048	0.003	0.006	0.024	0.0005	0.048	0.003
Lack of fit	5	0.101	0.031	0.002	0.003	0.013	0.0003	0.031	0.002
Pure error	8	0.183	0.017	0.001	0.003	0.011	0.0002	0.017	0.001

^a Significant at 1% level.

^b Significant at 5% level.

Table 5. Analysis of variance for the overall effect of the three process variables on the eight responses.

Process variables	df	Sum of squares for							
		Solids loss (% dry wt)	Protein (% dry wt)	Fat (% dry wt)	Ash (% dry wt)	TIA (mg/g)	Raffinose (% dry wt)	Stachyose (% dry wt)	Sucrose (% dry wt)
Soaking time (h)	4	16.578 ^a	0.568 ^b	0.011 ^b	0.317 ^b	0.463 ^a	0.020 ^a	0.083 ^a	0.337 ^a
Germination time (h)	4	82.077 ^a	0.414 ^a	0.030 ^a	0.701 ^a	0.962 ^a	0.125 ^a	1.73 ^a	1.06 ^a
Germination Temperature (°C)	4	11.630 ^a	0.274 ^b	0.040 ^b	0.430 ^b	0.343 ^a	0.038 ^a	0.133 ^a	0.245 ^a

^a Significant at 1% level.

^b Significant at 5% level.

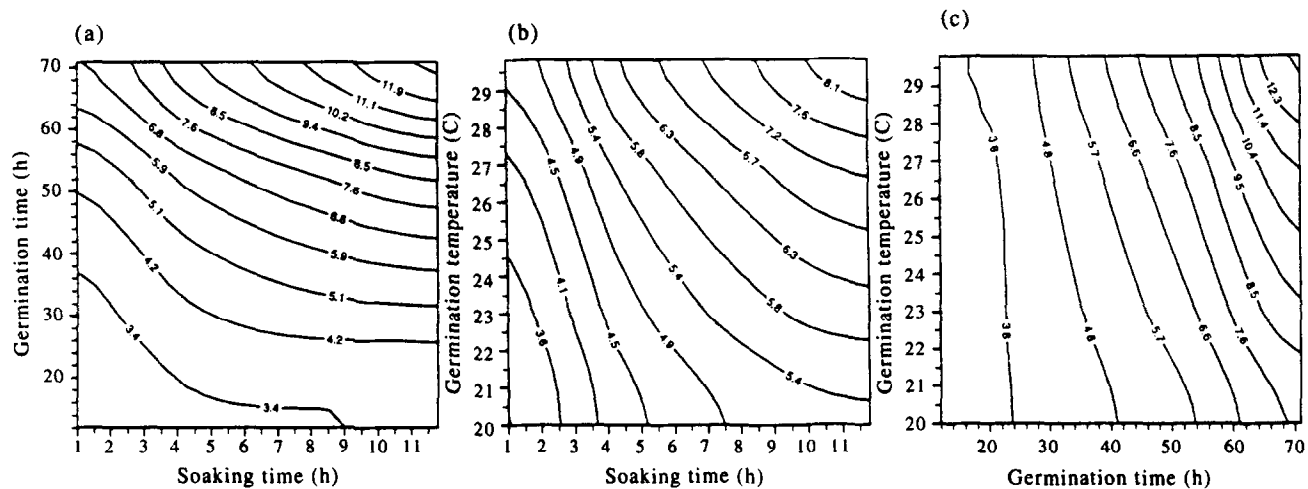


Fig. 1. Contour plots of total solids loss (% dry solid): (a) at germination temperature $T=25^{\circ}\text{C}$; (b) at germination time $t_2=42$ h; (c) at soaking time $t_1=6.5$ h.

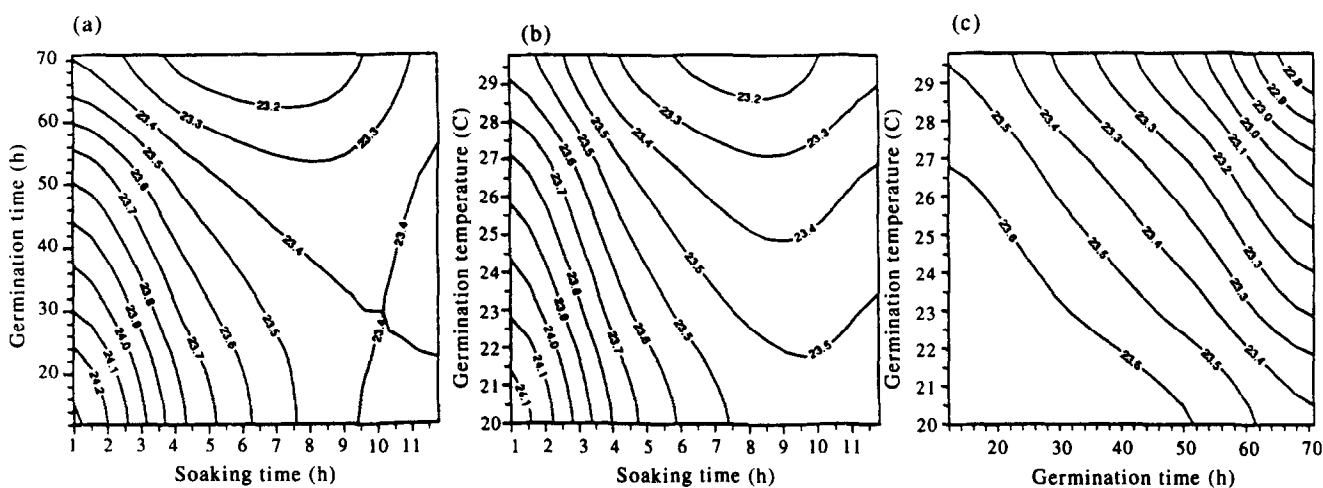


Fig. 2. Contour plots of protein content (% dry solid): (a) at germination temperature $T=25^{\circ}\text{C}$; (b) at germination time $t_2=42$ h; (c) at soaking time $t_1=6.5$ h.

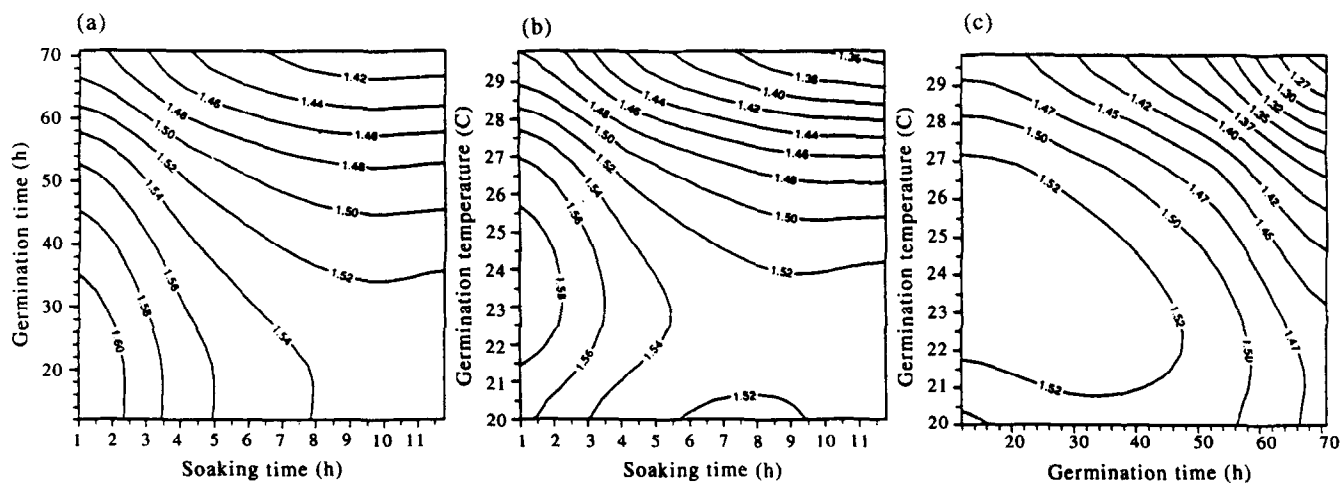


Fig. 3. Contour plots of fat content (% dry solid): (a) at germination temperature $T=25^{\circ}\text{C}$; (b) at germination time $t_2=42$ h; (c) at soaking time $t_1=6.5$ h.

germination time in the early stages and then increased slightly later as germination time rose. Kakade and Evans (1966) studied the effect of soaking and germination on the nutritive value of navy beans. Similar trends were reported. There was a steady decrease in TIA as the germination temperature increased. The decrease in TIA due to the germination process could be attributed to the leaching out of some elements during the daily washing of cowpea seeds (Collins & Sand, 1976). There was about 40% reduction in TIA after soaking 12 h and then germinating for 72 h. Mostafa *et al.* (1987) reported about 30% reduction in TIA after 4 days germination of soybean. In general, as far as the reduction of TIA is concerned, germination may not be an effective method to reduce TIA when compared with other treatments such as water and steam blanching (Wang *et al.*, 1996).

Effect on oligosaccharides

The mean raffinose, stachyose and sucrose contents of the raw cowpea were 1.24, 5.53 and 2.74 (g/100 g dry

basis), respectively. Akpapunam and Markakis (1979) and Onigbinde and Akinyele (1983) investigated oligosaccharide contents of different varieties of cowpeas. They reported raffinose in the range of 1.1–4.12 (g/100 dry basis), stachyose in the range of 1.21–4.84 (g/100 dry basis) and sucrose in the range of 0.36–3.10 (g/100 dry basis), respectively. The results in the present study, except that stachyose was slightly higher than that reported, were within the range of published work.

Figure 6 shows the effect of process variables on the raffinose and stachyose contents. The raffinose and stachyose contents decreased steadily with an increase in soaking time (Fig. 6a,d) while they decreased sharply with increasing germination time. The combination of soaking and germination times significantly reduced the raffinose and stachyose contents. Germination at all temperatures reduced raffinose and stachyose contents (Fig. 6(b,c,e,f)). Similar results were reported in chickpea and pigeon pea by Jood *et al.* (1985).

Figure 7 represents the interactive effect of soaking time, germination time and temperature on sucrose

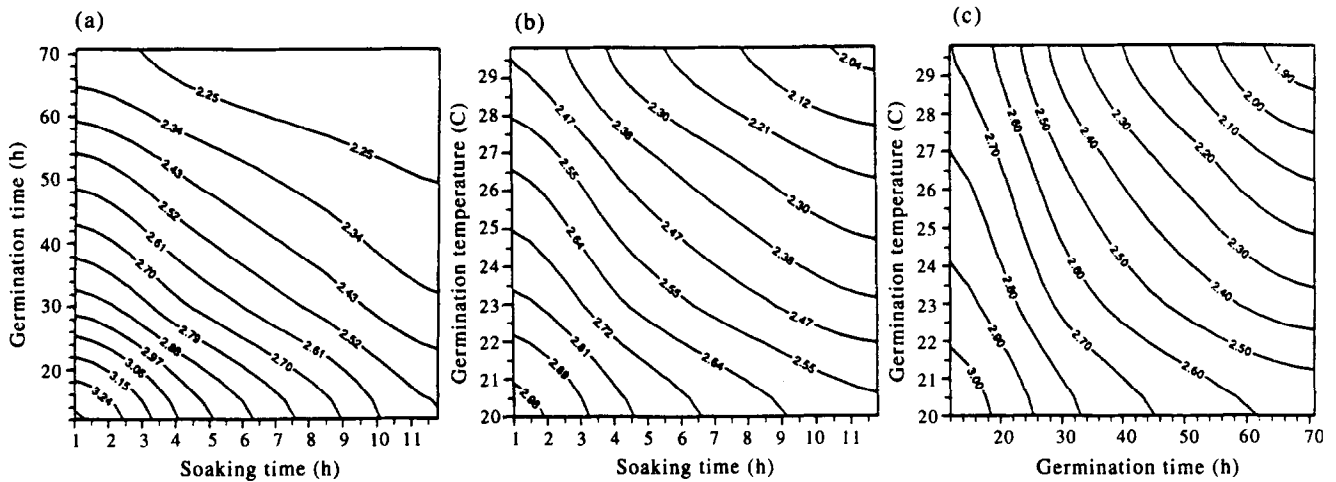


Fig. 4. Contour plots of ash content (% dry solid): (a) at germination temperature $T = 25^{\circ}\text{C}$; (b) at germination time $t_2 = 42$ h; (c) at soaking time $t_1 = 6.5$ h.

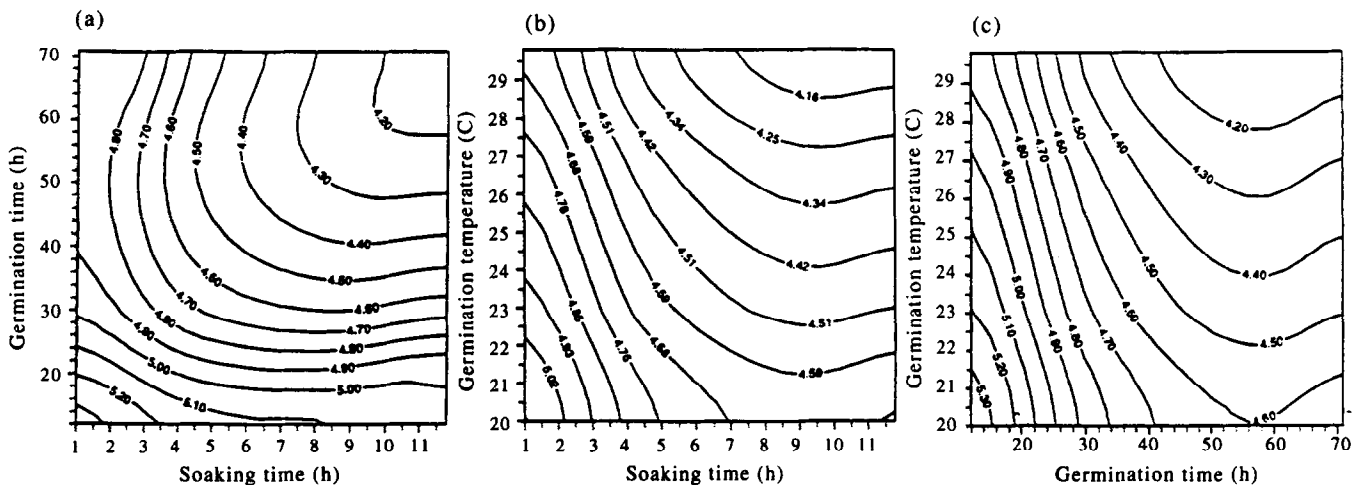


Fig. 5. Contour plots of TIA (mg/g dry solid): (a) at germination temperature $T = 25^{\circ}\text{C}$; (b) at germination time $t_2 = 42$ h; (c) at soaking time $t_1 = 6.5$ h.

content. It can be seen from Fig. 7 that the sucrose content increased as germination time and temperature increased while it decreased with increasing in soaking time. Nnanna & Phillips (1988) reported an increase in sucrose content with increasing germination time.

The increase in sucrose content during germination may be due to the hydrolysis of raffinose and stachyose into sucrose (Labaneiah & Luh, 1981). The combined effect of soaking time-germination time and germination time-germination temperature on sucrose content was to

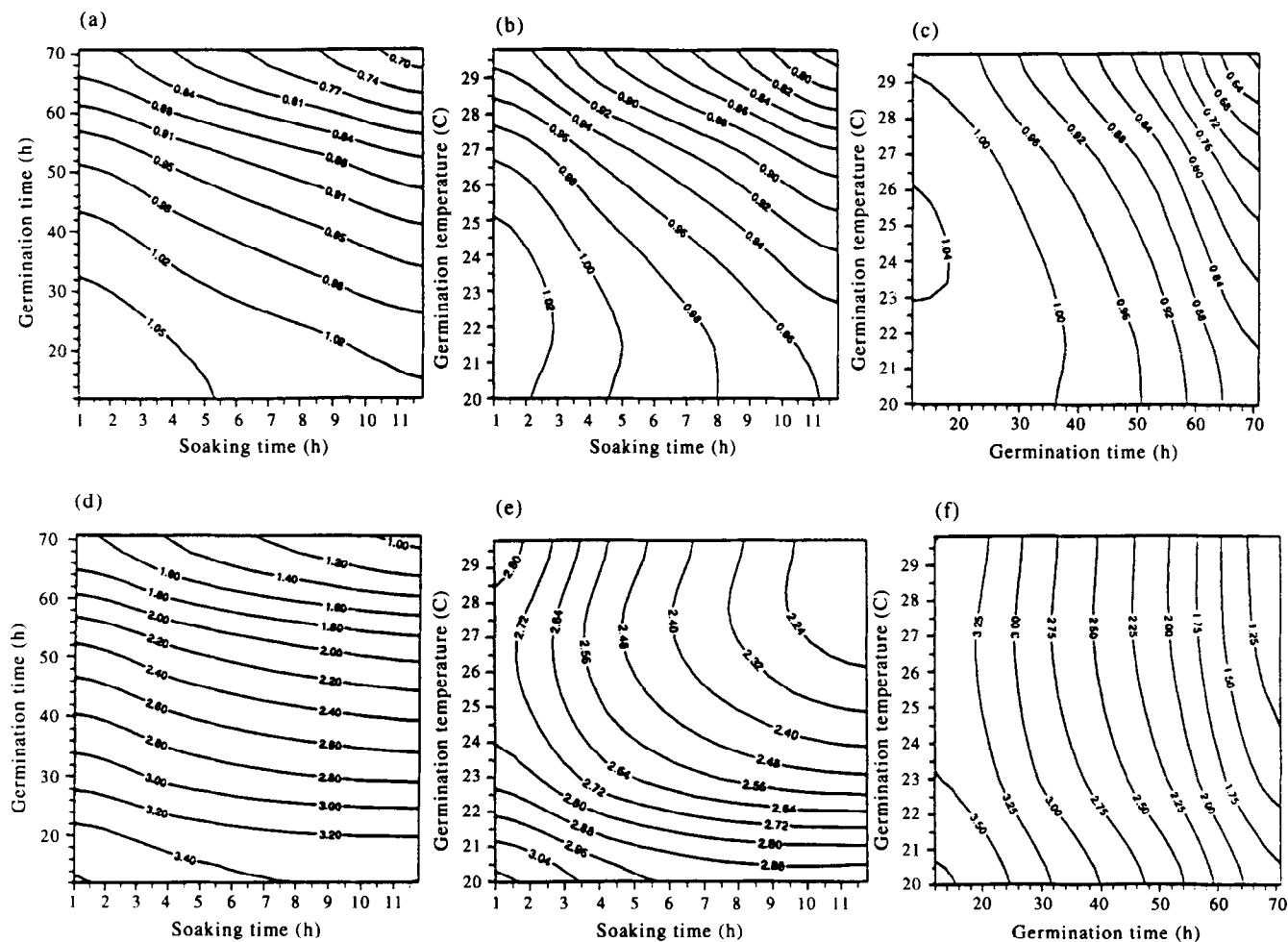


Fig. 6. Contour plots of raffinose and stachyose contents (% dry solid): (a), (d) at germination temperature $T=25^{\circ}\text{C}$; (b), (e) at germination time $t_2=42\text{ h}$; (c), (f) at soaking time $t_1=6.5\text{ h}$.

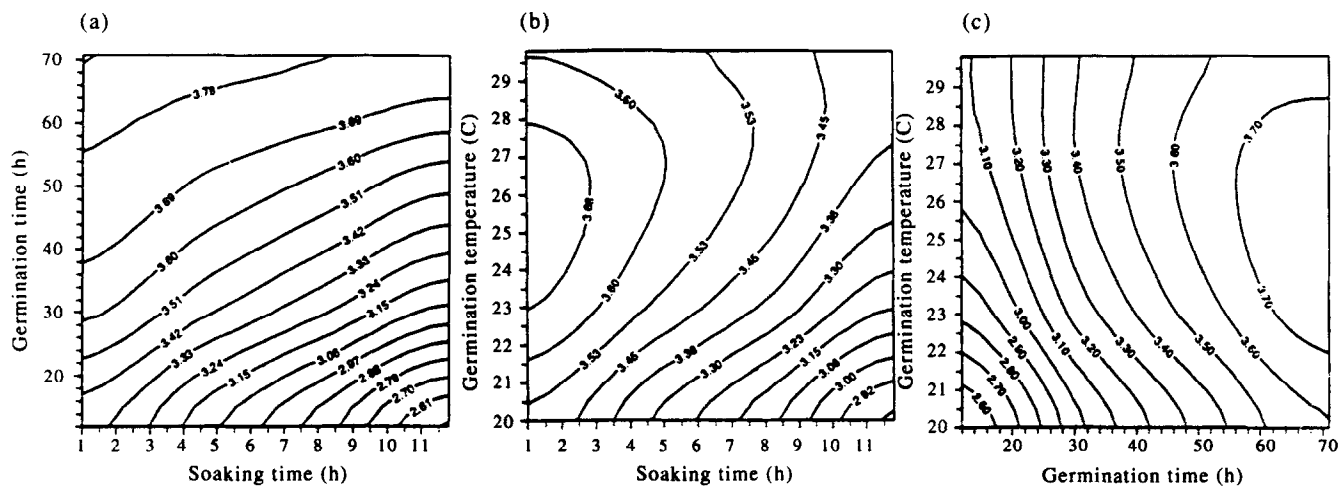


Fig. 7. Contour plots of sucrose content (% dry solid): (a) at germination temperature $T=25^{\circ}\text{C}$; (b) at germination time $t_2=42\text{ h}$; (c) at soaking time $t_1=6.5\text{ h}$.

increase the sucrose content while the combination of soaking time-germination temperature decreased sucrose content steadily. However, the overall effect of these factors was to increase total sucrose content in germinated cowpea seeds.

The germination conditions had a great effect on total solids loss, TIA and oligosaccharides. There was a slight reduction in protein, fat and ash contents. Therefore, the optimization of the germination process was based on maximum reduction of oligosaccharides and TIA and minimal total solids loss.

Localization of optimum conditions

The stationary points were obtained by canonical analysis (Myers, 1971) and the response surfaces around these stationary points were developed. It was found that these stationary points were not only located outside the experimental space but also were saddle points, implying that the analytical method could not be used

to identify the optimum conditions. A graphical method was therefore used (Floros & Chinnan, 1988).

Stachyose was the major oligosaccharide in cowpeas. Germination effectively reduced the level of stachyose with increasing soaking and germination times. However, germination also increased total solids loss. It is necessary to make an acceptable compromise for the optimization of the germination process. In this case the following criteria were used: reduction in stachyose was set at more than 64%, total solids loss less than 8% and TIA level reduced by more than 38%.

The contour plots in Fig. 8 were obtained from the predictive model of stachyose by holding germination temperatures at 20, 25 and 30°C. The shaded regions indicate that the reduction in stachyose content was more than 64%. Contour plots for total solids loss at germination temperatures of 20, 25 and 30°C are shown in Fig. 9. The regions which satisfy both conditions, i.e. the reduction in stachyose content more than 64%, total solids loss less than 8%, were obtained by superimposing

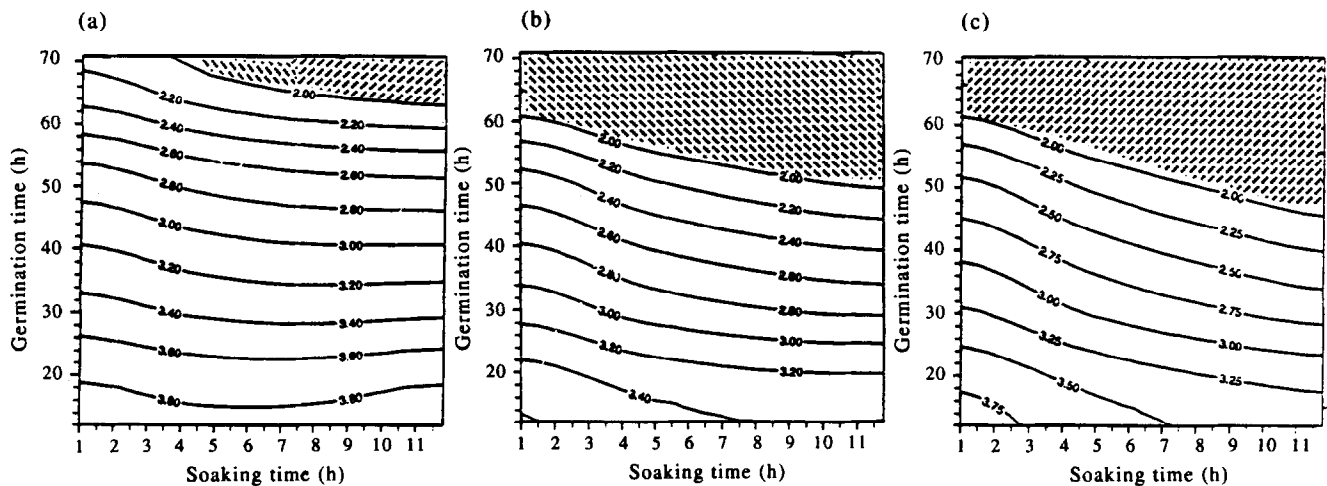


Fig. 8. Contour plots of stachyose content (% dry basis). Shaded area <2%: (a) at germination temperature $T=20^{\circ}\text{C}$; (b) at germination temperature $T=25^{\circ}\text{C}$; (c) at germination temperature $T=30^{\circ}\text{C}$.

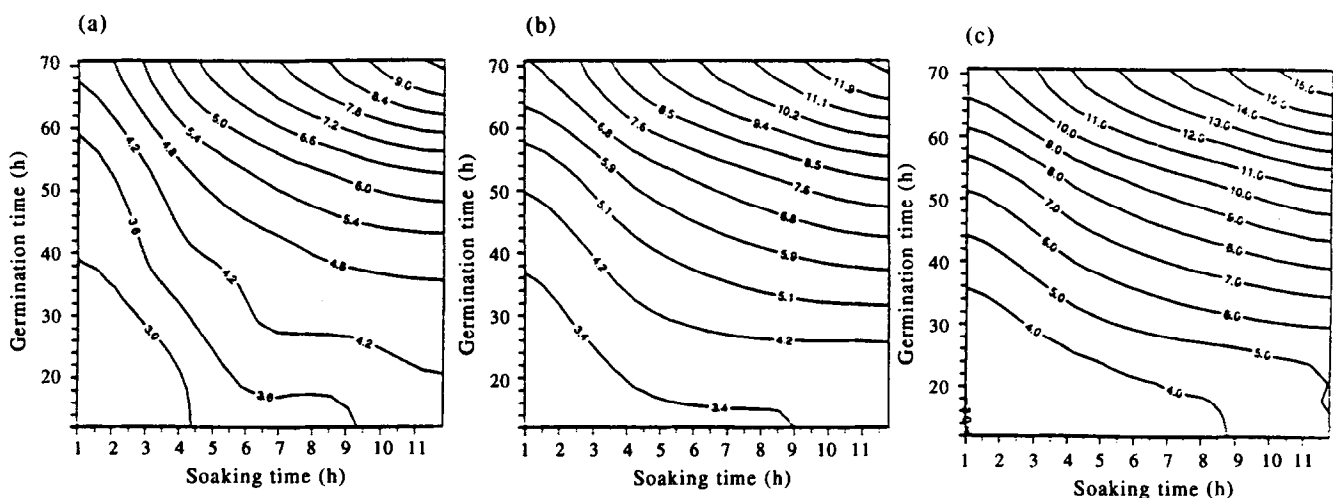


Fig. 9. Contour plots of total solids loss (% dry basis): (a) at germination temperature $T=20^{\circ}\text{C}$; (b) at germination temperature $T=25^{\circ}\text{C}$; (c) at germination temperature $T=30^{\circ}\text{C}$.

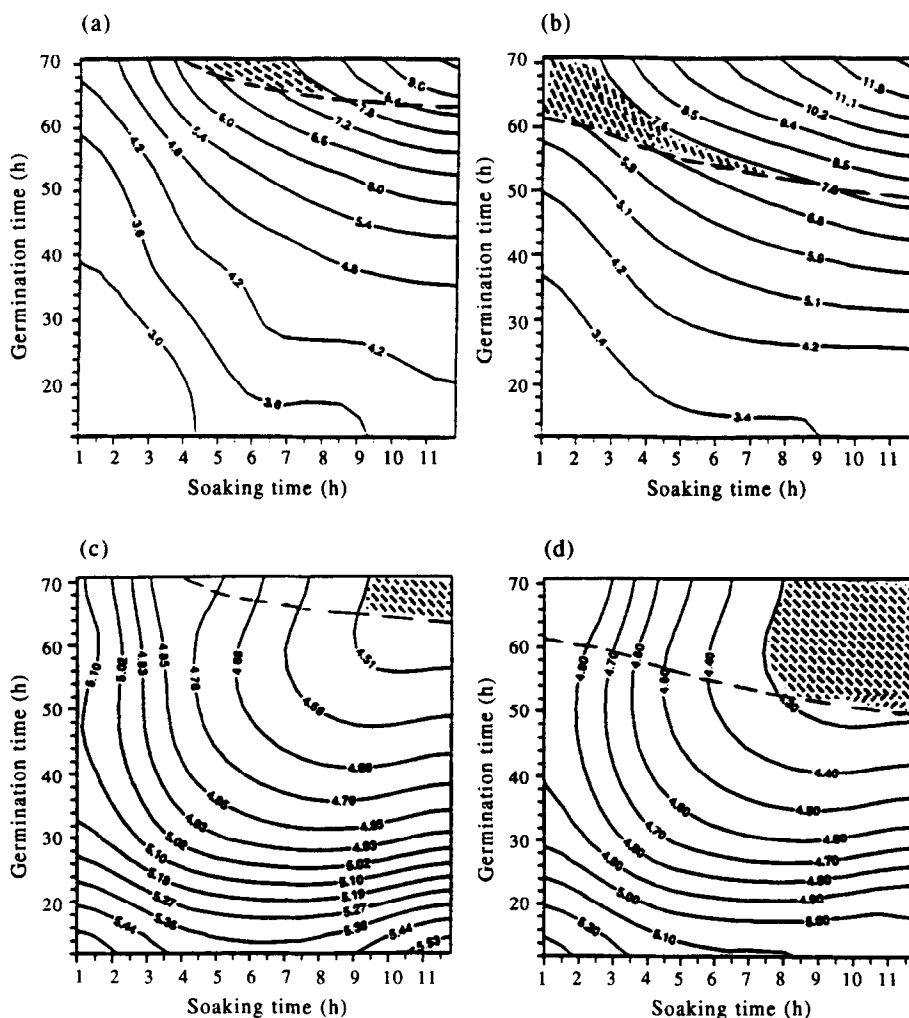


Fig. 10. Optimum regions obtained by superimposing contour plots of stachyose content (<2% dry basis) over total solid losses and TIA. Shaded regions represent. (a) and (b) total solid losses <8%; (c) and (d) TIA <4.3 mg/g dry solid.

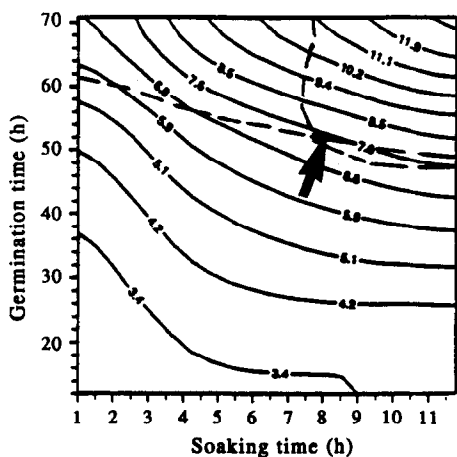


Fig. 11. Optimum region obtained by superimposing contour plots of all three responses. The shaded region (see arrow) represents stachyose <2%, total solid losses <8% and TIA <4.3 mg/g dry solid.

Fig. 8 over Fig. 9 to obtain the shaded areas shown in Fig. 10(a,b). Two regions could be found to meet those conditions. The first region, represented as a shaded area in Fig. 10a, is a superimposition of Fig. 8a over

Fig. 9a. The second region is given in Fig. 10b; it was obtained from Fig. 8b and 9b. Both regions had a reduction in stachyose content more than 64% and total solids loss less than 8%. A similar procedure was followed to obtain an optimum region for a reduction in stachyose content more than 64% and maximum reduction in TIA (more than 38%). Results are shown in Fig. 10(c,d), respectively. The optimum region for the combined effect of all three responses is shown (shaded area) in Fig. 11 which was obtained by superimposing Fig. 10(b,d). There was no overlap by superimposing Fig. 10(a,c). A combination of optimum germination process conditions can be selected from the area shown in Fig. 11. The point at soaking time = 8 h, germination time = 52 h and germination temperature = 25°C could be recommended as the optimum process conditions. The estimated values using eqn (2) for the eight response variables are shown in Table 6.

A verification experiment at the optimum conditions was performed. The results are given in Table 6. The results showed that the experimental and predicted values are reasonably close.

Table 6. Predicted and experimental values for the eight response variables at optimum conditions

	Solids loss (% dry wt)	Protein (% dry wt)	Fat (% dry wt)	Ash (% dry wt)	TIA (mg/g)	Raffinose (% dry wt)	Stachyose (% dry wt)	Sucrose (% dry wt)
Predicted	7.71	23.3	1.48	2.29	4.28	0.90	1.96	3.61
Experimental	7.80 ± 0.045	23.4 ± 0.40	1.45 ± 0.046	2.31 ± 0.065	4.31 ± 0.074	0.88 ± 0.04	1.89 ± 0.042	3.54 ± 0.089

CONCLUSIONS

Response surface methodology was used to evaluate the effects of soaking time, germination time and temperature on total solids loss, protein, fat and ash contents, trypsin inhibitor activity and oligosaccharides and to establish the optimum process variables for the germination cowpea. Predicted models for total solids loss, protein, fat, ash content, TIA and oligosaccharides were developed as functions of soaking time, germination time and temperature. Germination process conditions yielding an optimum process (maximum reduction in stachyose and TIA and minimum total solids loss) were: soaking time, 8 h; germination time, 52 h and germination temperature, 25°C. The predicted values using a polynomial equation for total solids loss, protein, fat and ash contents under these process conditions were 7.71, 23.30, 1.48 and 2.29 (% dry basis), respectively. The trypsin inhibitor activity level was 4.28 mg/g dry solid. The values for raffinose, stachyose and sucrose were 0.90, 1.96 and 3.61 (% dry basis), respectively. Experimental values obtained at the above optimum process conditions were compared with the predicted values and good agreement was found.

From a practical standpoint, the results of this work could be applied at both household and commercial levels.

REFERENCES

- Abudu, I. A. & Akinyele, I. O. (1990). The effect of germination on the oligosaccharides, trypsin inhibitors and nutrient content of cowpea milk. *Food Chem.*, **35**, 161–166.
- Akpapunam, M. A. & Markakis, P. (1979). Oligosaccharides of 13 American cultivars of cowpeas. *J. Fd Sci.*, **44**, 1317–1318, 1321.
- AOAC (1984). *Official Methods of Analysis*, Association of Official Analytical Chemists, Washington, D.C.
- Azhar, S., Srivasta, A. K. & Krishna, M. C. R. (1972). Compositional changes during the germination *Cicer arietinum*. *Phytochem.*, **11**, 3173.
- Bressani, R. (1985). *Nutritive Value of Cowpea in Cowpea Research, Production and Utilization*, Ed. Singh, S. R. & Rachie, K. O., Wiley, Chichester, U.K., 353–359.
- Collins, J. L. & Sand, G. G. (1976). Changes in trypsin inhibitor activity in some soybean varieties during maturation and germination. *J. Fd Sci.*, **41**, 168–172.
- Dovlo, F. E., Williams, C. E. & Zoaka, L. (1976). Cowpeas: Home preparation and use in West Africa. International Development Research Center, Bulletin IDRC-055e, 1–96.
- East, J. W., Nakayama, T. O. M. & Parkman, S. B. (1972). Changes in stachyose, raffinose, sucrose and monosaccharides during germination of soybeans. *Crop Sci.*, **12**, 7.
- El-Hag, N., Hard, N. F. & Morse, R. E. (1978). Influence of sprouting on the digestibility coefficient, trypsin inhibitor and globulin proteins of red kidney bean. *J. Fd Sci.*, **43**, 1874–1875.
- Fery, R. L. (1981). Cowpea production in the United States. *Hortsci.*, **16**, 4.
- Floros, J. D. & Chinnan, M. S. (1988). Seven factors response surface optimization of a double stage lye (NaOH) peeling process for pimento peppers. *J. Fd Sci.*, **53**(2), 631–638.
- Giovanni M. (1983). Response surface methodology and product optimization. *Food Technol.*, **11**, 41–45, 83.
- Hsu, D., Leung, H. K., Finney, P. L. & Moran, M. M. (1980). Effect of germination on nutritive value and baking properties of dry peas, lentils and faba beans. *J. Fd Sci.*, **45**, 87.
- Iyer, V., Salunkhe, D. H., Sathe, S. K. & Rockland, L. B. (1980). Quick-cooking beans (*Phaseolus vulgaris* L.) 1. Investigations on quality. *Qual. Plant Foods Hum. Nutr.*, **30**, 27.
- Jeffers, H. C., Rubenthaler, G. L., Finney, P. C., Anderson, P. D. & Bruinsma, B. L. (1978). Pea: A highly functional fortifier in wheat flour blends. *Bakers Digest*, **52**(1), 36–40.
- Jood, S., Mehta, U., Singh, R. & Bhat, C. M. (1985). Effect of processing on flatulence producing factors in legumes. *J. Agric. Fd Chem.*, **33**, 268.
- Kakade, M. L. & Evans, R. J. (1966). Effect of soaking and germinating on the nutritive value of navy beans. *J. Fd Sci.*, **31**, 781–783.
- Kennedy, I. R., Mwandemele, O. D. & McWhirter, K. S. (1985). Estimation of sucrose, raffinose and stachyose in soybean seeds. *Food Chem.*, **17**, 85–93.
- Kochhar, N., Walker, A. F. & Pike, D. J. (1988). Effect of variety on protein content, amino acid composition and trypsin inhibitor activity of cowpeas. *Food Chem.*, **29**, 65–78.
- Kumar, K. G. & Venkatasgmun, L. V. (1978). Chickpea seed proteins: modification during germination. *Phytochem.*, **17**, 605–607.
- Labaneiah, M. E. O. & Luh, B. S. (1981). Changes of starch, crude fibre and oligosaccharides in germinating dry beans. *Cereal Chem.*, **58**(2), 135–138.
- Mostafa, M. M., Rahma, E. H. & Rady, A. H. (1987). Chemical and nutritional changes in soybean during germination. *Food Chem.*, **23**, 257–275.
- Myers, R. H. (1971). *Response Surface Methodology*, Allyn and Bacon, Boston, MA.
- Nnanna, I. A. & Phillips, R. D. (1988). Changes in oligosaccharide content, enzyme activities and dry matter during controlled germination of cowpeas (*Vigna unguiculata*). *J. Fd Sci.*, **53**(6), 1782–1786.
- Ologhobo, A. D. & Fetuga, B. L. (1984). Effect of processing on the trypsin inhibitor, haemagglutinin, tannic acid and phytic acid contents of seeds of 10 cowpea varieties. *Trop. Agric. (Trinidad)*, **61**(4), 261–264.

- Onigbinde, A. O. & Akinyele, I. O. (1983). Oligosaccharide content of 20 varieties of cowpeas in Nigeria. *J. Fd Sci.*, **48**, 1250–1254.
- Price, T. V. (1988). Seed sprout production for human consumption—a review. *J. Can. Inst. Fd Sci Technol.*, **21**, 57–65.
- Reddy, N. K., Lunke, D. K. & Sharma, R. P. (1980). Flatulence in rats following ingestion of cooked and germinated black gram and a fermented product of black gram and rice blends. *J. Food Sci.*, **45**, 1161.
- SAS (1985). *SAS User's guide: Statistics*, Version 5 edition. SAS Institute Inc., Cary, NC.
- Smith, C., Megen, W. V., Twaalfhoven, L. & Hitchcock, C. (1980). The determination of trypsin inhibitor levels in foodstuffs. *J. Sci. Fd Agric.*, **31**, 321–350.
- Thananunkul, D., Ttanaka, M., Chichester, C. O. & Lee, J. C. (1976). Degradation of raffinose and stachyose in soybean by α -galactosidase within polyacrylamide gel. *J. Fd Sci.*, **41**, 173.
- Wang N., Lewis M. J. Brennan J. G. & Westby A. (1996). Effect of processing methods on nutrients and anti-nutritional factors in cowpea. *Food Chem.*, **58**, 59–68.