ACRYLAMIDE RESULTING FROM HEAT-TIME TREATMENT IN *CAJANUS CAJAN*, A NEGLECTED AND UNDERUTILIZED LEGUME.

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ABSTRACT

The influence that heat-time treatment has on the concentration of acrylamide in roasted *Cajanus cajan* was analysed. The study focussed on optimising the roasting conditions using Response Surface Methodology (RSM) to minimise concentration of acrylamide in roasted *Cajanus cajan*. The raw *Cajanus cajan* was soaked in solvent containing an additive of either citric or phosphoric acid in the range 0.1 – 1.0 (g or mL). The soaked *Cajanus cajan* was then dried and roasted. The roasting conditions used were temperatures from 80 – 120 °C and time of 10 - 60 min. Response Surface Methodology (RSM) analysis of the data showed that the concentration of acrylamide significantly increased as the roasting temperature and roasting time for processing increased ($p<0.05$). Citric acid and phosphoric acid used as additives in soaking the *Cajanus cajan* had no significant effect on acrylamide formation. The optimization of process parameters to give low concentration of acrylamide resulted in roasting temperature of 80 ºC, roasting time of 10 min and 0.1 g of citric acid with the maximum desirability of 0.972. Acrylamide concentration in roasted *Cajanus cajan* produced with optimized condition (80 ºC, 10 min and 0.1 g citric acid) was 1.757 g/kg.

Keywords: Acrylamide, Heat-time treatment, Response Surface Methodology, *Cajanus cajan*

INTRODUCTION

Acrylamide is a water-soluble low-molecular weight compound built up of a reactive ethylenic double bond that is linked with a carboxamide group (Eriksson and Karlsson, 2005). Its presence in food was highlighted in April, 2002 when Swedish scientists reported the compound in carbohydrate-rich foods that were heated to very high temperatures (Graf *et al.*, 2006). The International Agency on Research Cancer (IARC) has classified acrylamide as a probable human carcinogen and exposure to high levels has been found to cause damage to the nervous system (IARC, 1994). Epidemiological studies have also highlighted its association between dietary acrylamide and an increased risk of some types of cancer (Olesen *et al.*, 2008; *Hogervost et al., 2009*).

One of the reasons for processing foods is to produce safe food with best possible sensory properties and minimum content of possibly harmful substances but processing of food at high temperatures has been shown to generate various kinds of cooking toxicants (Skog *et al.*, 1998). Acrylamide has been found in different food products such as potato chips, deep-fat fried foods, crisp bread, biscuits, crackers, and some breakfast cereals (Tareke *et al.*, 2002). Coultate (2009), has reported that prolonged heating of legumes such as soya bean have shown significant loss of amino acids due to Millard reactions; meanwhile acrylamide, has also been identified to be formed as a result of Millard reaction (Pedreschi *et al.*, 2004).

Cajanus cajan is an underutilized legume in Ghana. It is an inexpensive and valuable source of food protein which can serve as a supplement to animal protein. It contains an appreciable amount of protein of about 27% (Abdel-Rahman *et al.,* 2010).Apart from being rich in protein, it also contains reasonable amount of carbohydrate, fibre and low fats. Food legumes such as *Cajanus cajan* is processed and cooked by methods such as soaking, dehulling, boiling, microwaving, fermentation, micronization and roasting depending on the type of food and the region. In Ghana, some adult foods and infant formulas usually have in them legumes such as beans and peas mixed with other cereals. However, roasting, baking and frying are some of the dry heat processing methods that employ high temperature and could produce considerable amount of acrylamide.

It is believed that, antinutrients could successfully be removed or reduced to an appreciable limit by employing certain processing methods such as soaking and roasting (Chi-Fai *et al.*, 1997). Numerous studies and research activities have been developed by various researchers to help understand the reduction of acrylamide levels (Salazar *et al.*, 2012; Graf *et al.*, 2006, Pedreschi *et al.*, 2004 and Cummins *et al.*, 2008). This study concentrates on the use of a model to control processing conditions in order to minimize the amounts of acrylamide that would be formed during roasting of an underutilized legume. The objective of this study was to determine the optimum roasting temperature and time for reducing acrylamide formation in *Cajanus cajan*.

MATERIALS AND METHODS

Sources of Materials

Cajanus cajan was obtained from an outgrower in the Sunyani Metropolis in Ghana and all chemicals used in this research were procured from Sigma Aldrich Company Limited in the United States of America.

Preparation of Materials

Sample was sorted and cleaned to removed dust and foreign materials and kept in clean plastic container prior to treatment.

METHODS

Experimental Design

The effect of four factors: soaking solvent, amount of additive (citric acid/ phosphoric acid), temperature and time on the production of acrylamide was studied. Design Expert (version 9) was used to randomize the factors. Table 1 indicates the specified levels at which each factor was varied during the experiment. A total of thirty four runs were generated to study the acrylamide concentration.

Table 1: Constraint for Treatment Factors

Factors	Level of Variation
Soaking	H_3PO_4 /Citric acid
Amount of additive	0.1 – 1.0 g or mL
Roasting temperature	80 – 120 ^{O}C
Roasting time	10 – 60 min

Soaking Treatment

Fifty grammes of *Cajanus cajan* was soaked in tap water containing an additive of either phosphoric acid or citric acid at concentration between 0.1-1.0g with respect to the experimental runs that were generated (Table 2). It was then kept overnight (10 h) at room temperature. The soaked legumes were then drained, dried in a solar dryer for 3 days and roasted according to the conditions of the experimental runs that were generated (Table 2).

Roasting Treatment

For studies of roasting, the oven was preheated to the desired temperatures and maintained for roasting. The dried samples obtained from soaking treatment were roasted in batches in a Melano-60-oven (Model: DCGML 6/P, Flavel Milano, UK) at a temperature between 80 -120 ^{o}C and time interval between 10-60 min as represented in Table 2. After roasting, the legumes were taken out of the oven and cooled to room temperature. All treated runs were then milled with Panasonic blender (Model No MX-1515P1, Malaysia).

Table 2: Summary of RSM Design for Roasting of *Cajanus cajan* and Their Corresponding Response Value

	Factor 1	**Factor 2**	**Factor 3**	**Factor 4**	**Response 1**
Run	A: amount of additive	B: Roasting time	C: Roasting temp	D: soaking solv	Acrylamide
	g or mL	min	oC		g/kg
1	0.10	60.00	100.00	H_3PO_4	3.615
2	0.55	35.00	100.00	H_3PO_4	6.610
3	0.10	10.00	100.00	H_3PO_4	3.350
4	0.55	35.00	100.00	Citrate	5.522
5	1.00	35.00	80.00	H_3PO_4	3.784
6	1.00	60.00	100.00	Citrate	7.516
7	0.55	35.00	100.00	Citrate	6.707
8	0.10	10.00	100.00	Citrate	4.42
9	0.55	60.00	80.00	Citrate	2.559
10	0.55	35.00	100.00	H_3PO_4	6.677
11	0.55	35.00	100.00	Citrate	3.42
12	1.00	35.00	80.00	Citrate	5.775
13	0.55	10.00	120.00	Citrate	2.612
14	0.55	35.00	100.00	H_3PO_4	6.483
15	0.55	60.00	120.00	Citrate	5.367
16	0.10	35.00	120.00	Citrate	5.902
17	0.55	35.00	100.00	H_3PO_4	2.955
18	1.00	35.00	120.00	Citrate	3.426
19	0.55	10.00	80.00	Citrate	2.159
20	0.55	10.00	80.00	H_3PO_4	2.296
21	0.55	60.00	120.00	H_3PO_4	*
22	1.00	10.00	100.00	H_3PO_4	3.685
23	0.55	35.00	100.00	H_3PO_4	8.368
24	0.55	10.00	120.00	H_3PO_4	5.601
25	0.55	35.00	100.00	Citrate	1.735
26	1.00	10.00	100.00	Citrate	*
27	0.10	60.00	100.00	Citrate	7.228
28	1.00	60.00	100.00	H_3PO_4	5.509
29	1.00	35.00	120.00	H_3PO_4	6.607
30	0.10	35.00	120.00	H_3PO_4	6.147
31	0.55	35.00	100.00	H_3PO_4	7.038
32	0.10	35.00	80.00	Citrate	1.758
33	0.55	60.00	80.00	H_3PO_4	*
34	0.10	35.00	80.00	H_3PO_4	2.189

*= outlier; A= Mass of additive B= Roasting time C= Roasting temperature D= Soaking solvent

Preparation of Defatted *Cajanus cajan* Flour

The flour to solvent ratio used for defatting was 1:10 w/v. The flour of all treated runs were defatted using the cold extraction method by soaking the flours (tied in a cheese cloth) and in hexane (placed in plastic containers). The containers were then covered with their plastic caps and the set up was left for 3 days at room temperature after which it was then solar-dried for 72 h (3days) to expel residual solvent. Defatted *Cajanus cajan* was then packed in polyethylene bags for further analysis .

Determination of Acrylamide

The quantitative analysis of acrylamide was adopted from previous published methods by Zhang and Zhang (2007) and Gokmen *et al.* (2005). A mass of 1.0 g portion of defatted treated *Cajanus cajan* sample was measured into a 50 mL polypropylene tube with cap. A volume of 30mL of 70 % acetonitrile (Zhang and Zhang, 2007) was added and the mixture was vortex briefly to disperse test portion in 30 mL of 70 % acetonitrile. A volume of 500 µL of Carrez I and carrez II that was prepared by diluting 15 g of potassium hexacyanoferrate(II) trihydrate and 30 g of zinc sulphate heptahydrate respectively in water (the volume adjusted to 100 mL) were then added to the suspended solution to precipitate proteins and carbohydrates in order to minimize interferences. Afterwards, the mixture was then shaken for 60 min on shaker (Gallenkamp orbital shaker, London-UK) at 100 rpm without heat as described by Karasek *et al.* (2009) and centrifuge at 3000 rpm for 30 min. The supernatant obtained after centrifugation was then decanted and filtered with a microfiber filter paper and a volume of 2µL was transferred with auto-sampler vial for HPLC analysis.

The analysis of acrylamide was performed on HLPC (microsob-mv 1005) equipped with UV detector using an Atlantis dC18 column (150 ×2.1mm, 5 mm, USA). Six standard acrylamide solutions (100ppm- 600ppm) were prepared in 70 % acetonitrile and its corresponding retention time and peak height measured. The peak area values were calculated and plotted against their respective concentrations to obtain a calibration curve.

Optimization of Processing Conditions for Roasting *Cajanus cajan*

The study is focussing on minimizing the acrylamide content that would be produced during roasting of *Cajanus cajan*. Numerical optimization was carried out to determine the optimum condition for roasting *Cajanus cajan* in order to give low concentration of acrylamide. The optimal condition was obtained using predicted equation determined by RSM. Goals were therefore set by imposing constraints on processing and response factors to obtain optimum conditions desired to process the *Cajanus cajan*. Constraining the factors is very critical for the statistical tool to select the optimum. Therefore the response factor was set to minimum acrylamide whilst the process factors; amount of additive (0.1- 1.0 g or mL) roasting time (10- 60 min), roasting temperature (10- 60 °C) were set in ranges.

Statistical Analysis

The response data for acrylamide obtained from the analysis was loaded and fitted to models using Design Expert (2009). The model that best fit the data was identified by evaluating regression parameters such as regression (R^2), adjusted regression (adj. R^2), predicted regression (pred. R^2), and adequate precision (adeq. precision).

When a model had been selected, Analysis of Variance was calculated to find out how well the model represented the data. P and F–values were also determined to identify the variations between the factors and data obtained. The P-values were tested against $P \leq 0.05$.

RESULTS

Identification of Acrylamide

The determination of acrylamide in samples was based on the peak area and retention time of acrylamide in a chromatographic run. The calibration curve as shown in Figure 1 was obtained by plotting the peak area against concentration of acrylamide. The retention time for acrylamide was 3.08–3.13 min in the acrylamide standard. Once the acrylamide in the sample was identified, the quantification of the

acrylamide was made based on its calibration curve y = bx + a, where a = -0.28848, b = 0.02179, x = acrylamide concentration and y = Area for acrylamide concentration with a correlation coefficient of 0.9951.

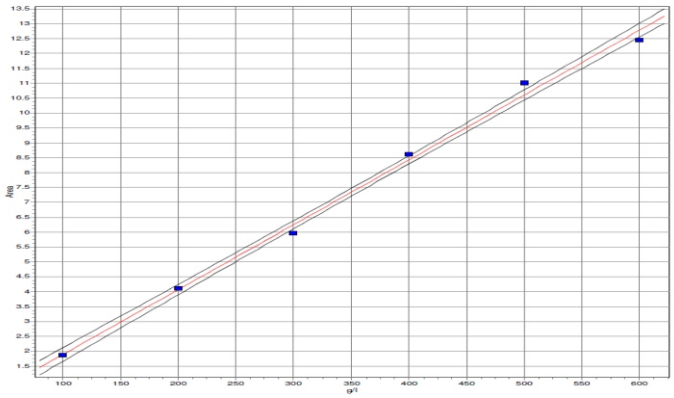

Figure 1: Acrylamide Standard Curve

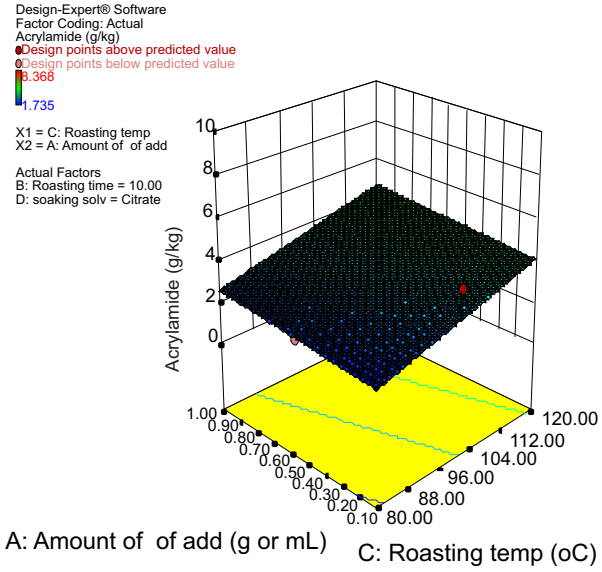

Figure 2a: Three Dimensional Response Surface Plot of Produced Acrylamide and its Relation with Roasting Temperature and Amount of Additive (Citric acid)

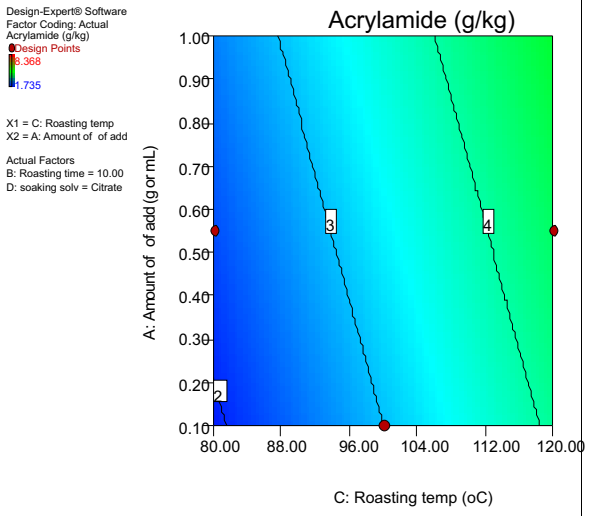

Figure 2b: Contour Plot of Figure 2a

Effects of Treatments on Acrylamide Levels in *Cajanus cajan*

Figure 2(a,b) and 3(a,b) indicate the relationship between amount of additive and roasting temperature and their influence on acrylamide level. From Figure 2a and 2b, when amount of citric acid increased from 0.1-1.0 g, initial acrylamide concentration produced was 2 g/kg and was observed at roasting time of 10 min and roasting temperature of 80 °C. As the roasting temperature increased to 100 °C, acrylamide production also increased to 3 g/kg and at a temperature of 120 °C, acrylamide production increased further to 4g/ kg. However, from Figure 3a and 3b, initial acrylamide concentration of 3 g/kg was observed at 80 °C as amount of phosphoric acid increased from 0.1 – 1.0 mL (Figure 3a and 3b). As the roasting temperature increased to 100 °C, 4 g/kg of acrylamide was produced. A higher level of acrylamide of concentration 5 g/kg was detected at a temperature of 120 °C (Figure 3a and 3b).

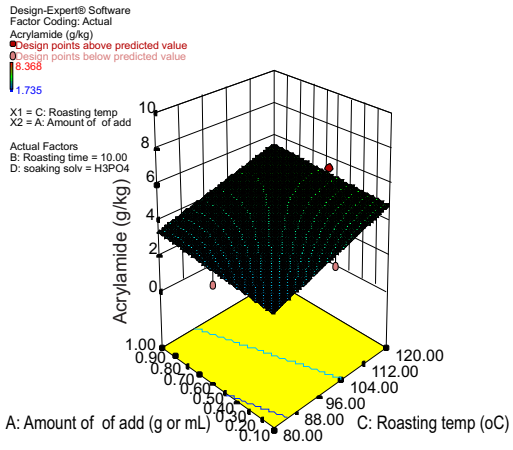

Figure 3a: Three Dimensional Response Surface Plot of Produced Acrylamide and its Relation with Roasting Temperature and Amount of Additive (Phosphoric acid)

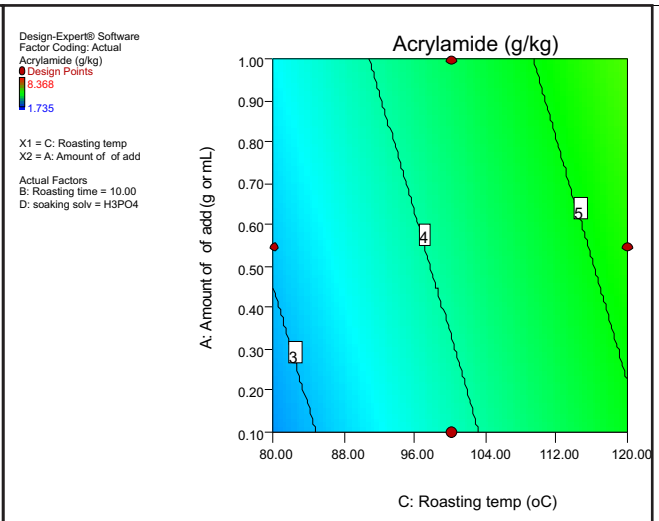

Figure 3b: Contour Plot of Figure 3a

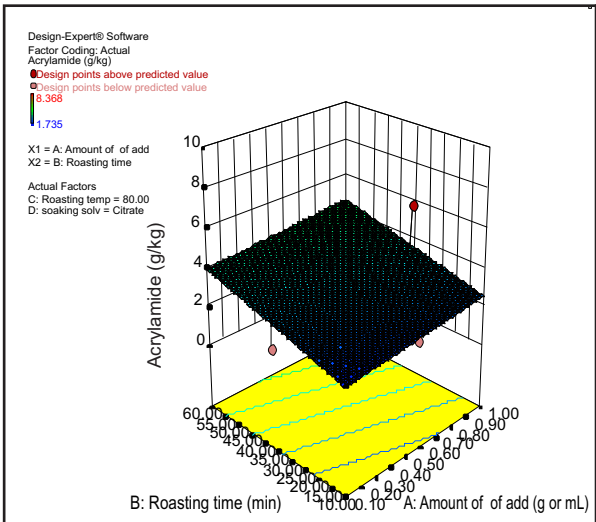

Figure 4a: Three Dimensional Response Surface Plot of Produced Acrylamide and its Relation with Roasting Time and Amount of Additive (Citrate).

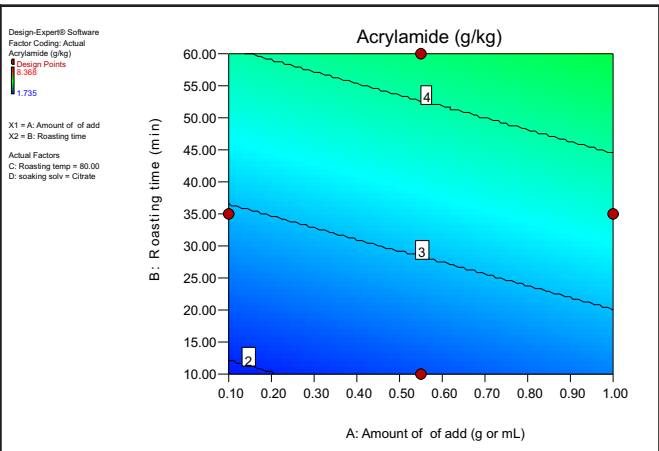

Figure 4b: Contour Plot of Figure 4a

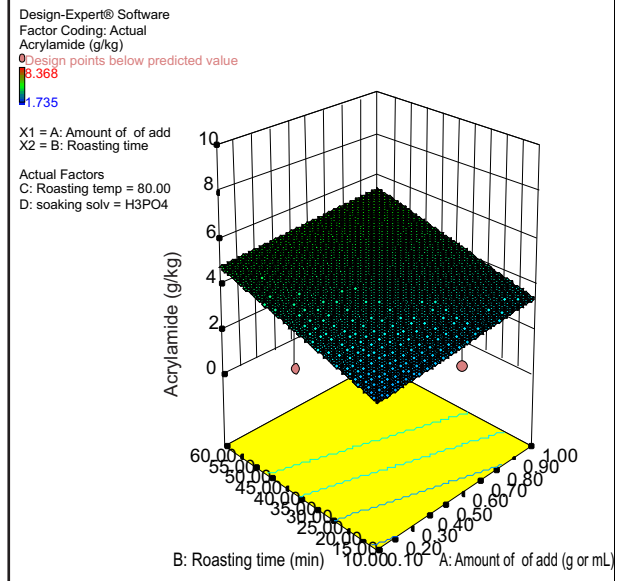

Figure 5a. Three Dimensional Response Surface Plot of Produced Acrylamide and its Relation with Roasting Time and Amount of Additive (Phosphoric acid)

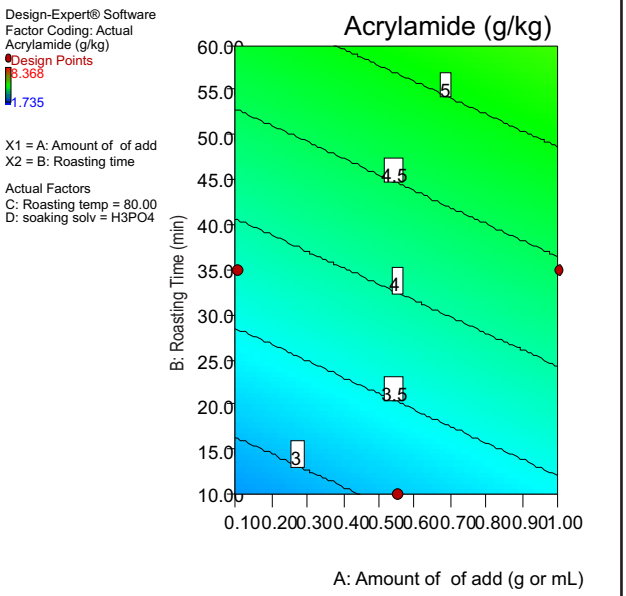

Figure 5b: Contour plot of Figure 5a

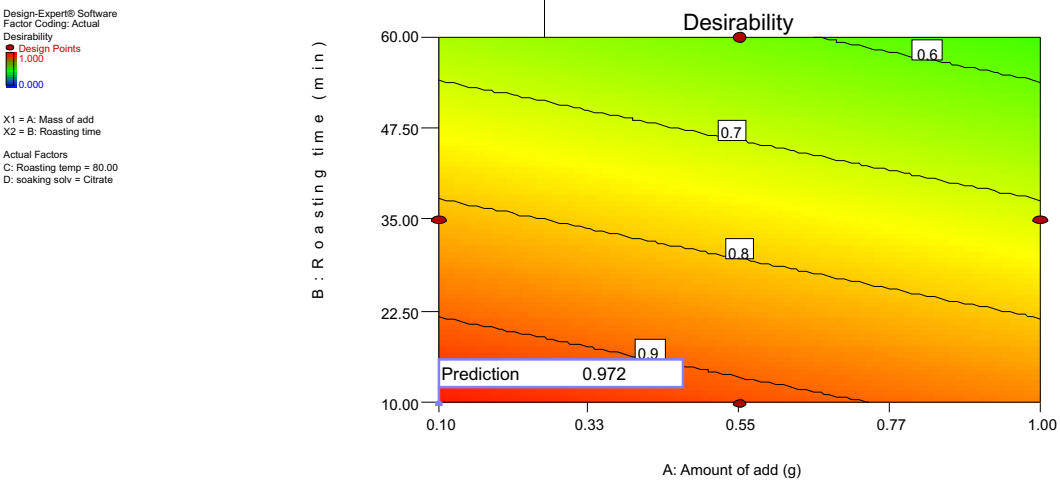

Figure 6: Desirability Graph Showing Optimum Conditions Using Citric Acid as an Additive

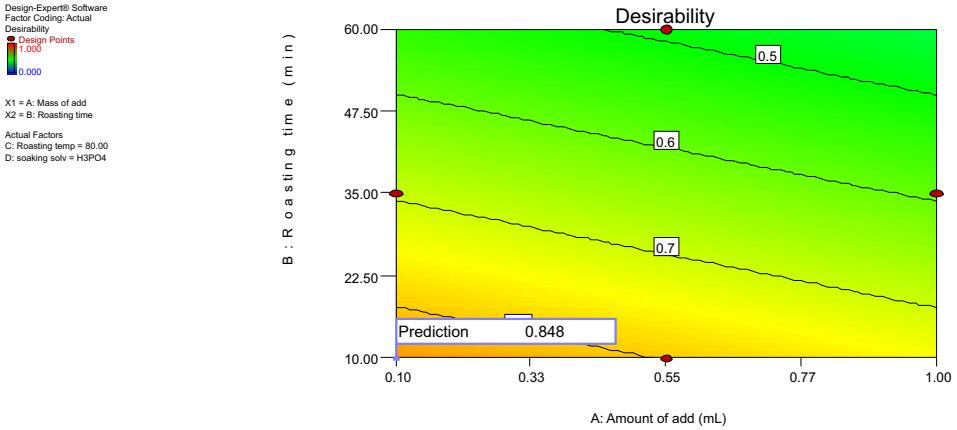

Figure 7: Desirability Graph Showing Optimum Conditions Using with Phosphoric acid as an Additive.

Figure 4(a, b) and 5(a, b) indicate the relationship between roasting time and amount of additive and their influence on acrylamide levels. At roasting temperature of 80 °C, lower acrylamide concentration of 2 g/kg was detected at roasting time of 10 min up to 35 min as amount of citric acid was increased from 0.1 – 1.0 g (Figure 4a and b). After 35 min, acrylamide production increased to 3 g/kg. In addition to this, an increase in acrylamide formation was observed at 4g/kg when roasting time was 60 min. Meanwhile, from Figure 5a and 5b, at temperature of 80 °C, acrylamide concentration of 3 g/kg was detected at roasting time of 10 min as amount of phosphoric acid increased from 0.1 - 0.45 mL (Figure 5a and 5b). After about 28 min, acrylamide production increased to 3.5 g/kg as amount of phosphoric acid increased from 0.1- 1.0 mL up to the 40th minute. Acrylamide concentration then increased to 4 g/ kg after 40 min. Moreover, at a temperature of 80 °C and roasting time of 60 min, 5 g/kg acrylamide concentration was observed when amount of phosphoric acid was increasing from 0.1 – 1.0 mL (Figure 5a and 5b).

From the ANOVA (Table 3), amount of additive on acrylamide concentration was not significant at confidence level 95% as compared to roasting temperature and roasting time. Increasing the amount of the additives from 0.1- 1.0 g or mL did not have a significant increase in acrylamide concentration.

DISCUSSION

Effects of Treatments on Acrylamide Levels in *Cajanus cajan*

It has been reported that the effects of antinutrients may disappear or decrease when legumes are properly soaked and cooked (Onder and Kahraman, 2009). Antinutrients such as acrylamide has been reported to decrease when potato slices were soaked in acidic medium prior to frying (Predreschi *et al.*, 2004). The relationship between temperature, time and amount of additive and the type of soaking solvent on the concentration of acrylamide is shown in Figure 2 (a ,b), 3(a, b), 4(a, b) and 5(a, b).

In this study, roasting temperature of 80 °C was capable of producing low acrylamide level in *Cajanus cajan* using citric acid and H_3PO_4 as additives. The roasting temperature had a significant impact on the acrylamide content of *Cajanus cajan* ($P \leq 0.0289$) as does roasting time ($P \leq 0.04$) as shown in Table 3 and therefore controlling these two factors would have influence on acrylamide formation. This is evident in a report by Zhang *et al.* (2011) that controlling the roasting temperature resulted in low acrylamide levels at all roasting times that he evaluated.

Acrylamide contents of 7.228 g/kg and 8.368 g/kg were produced when *Cajanus cajan* was roasted at 100 °C for approximately 60 min and for 35 min respectively (Table 2). The acrylamide concentration of 7.228 g/ kg was produced when citric acid was used to prepare the soaking solvent whilst 8.368 g/ kg was produced when phosphoric acid was used to prepare the soaking solvent.

From the Table 2, acrylamide concentration of 5.522 g/ Kg that was produced was roasted at 100 °C for 35 min. This clearly indicates that substantial amounts of acrylamide was produced at temperatures lower than 120 °C and this was contrary to the report that 120 °C was required for the formation of acrylamide (Becalski *et al.*, 2003). The trend of acrylamide production observed in this study agreed with those published by several investigators (Gökmen *et al.*, 2005, Erickson, 2005, Mottram, 2002) regarding the relationship between cooking temperature and acrylamide formation in foods.

Figure 4(a, b) and 5(a, b) also indicates that the acrylamide concentration in roasted *Cajanus cajan* could be very high when it remains in the roasting vessel for a long time (Karesek *et al.*, 2009). An increase in roasting time results in increased amounts of acrylamide in roasted *Cajanus cajan* as shown in Figure 4(a,b) and 5(a,b). This confirms a report by Karasek *et al.* (2009) that increasing roasting time up to 60 min resulted in increased amount of acrylamide. Similar trends have been reported by investigators in food models (Stadler *et al.*, 2002; Tareke *et al.*, 2002).

In this study, roasting experiments under different processing conditions showed that acrylamide

increases with time and temperature. Temperature had a much stronger effect on acrylamide formation than time and a similar trend was reported by Amrein *et al.* (2004).

According to Michalak *et al.* (2011), the effect of high-temperature and prolonged time of treatment on acrylamide production in foods decrease due to evaporation of acrylamide. Also, a report by Biedermann *et al.* (2003) showed that elimination of acrylamide due to evaporation occurred at a temperature higher than 120 °C (120 °C - 200 °C). However, at roasting temperatures between 80 °C-120 °C, the trend of formation of acrylamide did not decline which means that temperature for acrylamide to evaporate in roasted *Cajanus cajan* should be higher than 120 °C. Meanwhile, *Cajanus cajan* that were roasted at 120 °C and even at 100 °C for longer times were observed to be either partially or totally burnt.

The optimization solution which was based on minimum level of acrylamide was obtained at a roasting temperature of 80 °C, roasting time of 10 min, amount of additive 0.1 g or mL. At optimum condition (80 °C, 10 min, amount of additive of 0.1 g or mL), predicted acrylamide concentration was lower with citric acid than phosphoric acid (Table 4). The desirability predicted by response surface methodology for using citric acid and H_3PO_4 (phoshoric acid) to soak *Cajanus cajan* were 0.972 (Figure 6) and 0.848 (Figure 7) which indicates that citric acid has the ability to lower acrylamide production than phosphoric acid. This agrees with a report by Low *et al.* (2006) that citric acid has much stronger effect on acrylamide production when they processed foods with citric acid and acetic acid in combination with glycine. From Figure 8, it was evident that minimum acrylamide concentration was produced when citric acid was used to prepare the soaking solvent. This is in agreement with a report by Mestdagh *et al.* (2005) that citric acid has a stronger reducing effect on acrylamide formation.

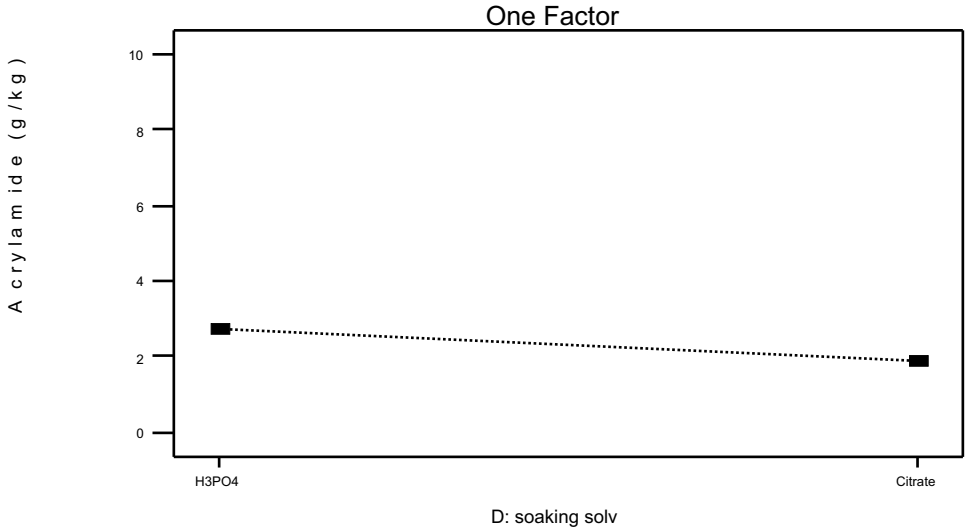

Figure 8: A Plot for the Effect of Soaking Solvent on Acrylamide Formation at Optimum Conditions.

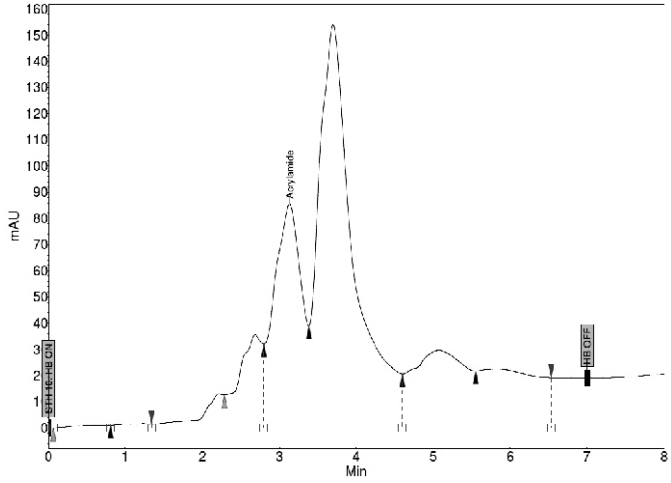

Figure 9 : Chromatogram for produced acrylamide in *Cajanus cajan* processed with optimum condition

Table 3: ANOVA for Response Surface Linear Model for Produced Acrylamide

Source	Sum of Squares	df	Mean Square	F Value	p-value Prob > F	
Model	35.43	4	8.86	2.89	0.0418	significant
A-Mass of add	1.67	1	1.67	0.54	0.4672	
B-Roasting time	13.29	1	13.29	4.34	0.0472	
C-Roasting temp	16.38	1	16.38	5.35	0.0289	
D-soaking solv	5.13	1	5.13	1.67	0.2070	
Residual	79.62	26	3.06			
Lack of Fit	48.73	18	2.71	0.70	0.7483	not significant
Pure Error	30.90	8	3.86			

Table 4: Predicted Acrylamide Level for Both Additives at Optimum Temperature and Time

Type of additive	Optimum condition			Acrylamide level (g/kg)
	Temperature (^{O}C)	Time (min)	Additive (g)	*Cajanus cajan*
Citrate	80	10	0.1	1.918
H_3PO_4	80	10	0.1	2.742

Table 5: Peak Result for Produced Acrylamide in *Cajanus cajan* Processed with Optimum Condition

Time(Min)	Acrylamide Concentration (G/Kg)	Peak Height (Mau)	Peak Area (Mau.Min)	Area (%)
3.13	1.757	85.5	35.3	20.079

By using the optimum condition (80 °C, 10 min and 0.1 g citric acid) for processing *Cajanus cajan*, acrylamide concentration of 1.757 g/kg was produced (Table 5).

Model Fitting

The p-value was used as a tool to check the significance of each of the coefficients, which in turn indicated the pattern of the interactions between the variables. The smaller P-value was more significant to the regression. From Table 3 it was observed that there was a non-significant ($p > 0.05$) lack of fit which validates the model (Ku-Madihah, 2013). The Regression models generated by the RSM for acrylamide production for both soaking solvents were given as:

Acrylamide formation using

$$Citric\ acid = -2.069 + 0.745A + 0.041B + 0.054C$$

Acrylamide formation using

$$H_3PO_4 = -2.069 + 0.745A + 0.041B + 0.054C$$

Where A = Amount of additive, B = Roasting time, C = Roasting temperature and D = Soaking solvent.

The adequacy of the model was determined by using lack of fit test and Coefficient of Determination (R^2). The significance of the equation parameter was assessed by F value at probability ($p > F$) less than 0.05 (Zaibunnisa *et al.*, 2009). The regression model was significant at the considered confidence level (95%) since the regression has $p \leq 0.05$ and F value of 2.89. Lack of fit test has P-value of 0.75 and an F-value of 0.70 (Table 2).

In this study, low R^2 value of 0.3079 and adjusted R^2 of 0.2015 were obtained. This result indicates that the presence of acrylamide was not influenced by soaking solvent and amount of additive. However, roasting time and roasting temperature were the only significant model terms. The predicted R-squared of 0.0410 was in reasonable agreement with adjusted-R-squared 0.2015 with the difference being less than 0.2 as suggested by the Response Surface Methodology.

For a model to be used to navigate the design space there should be adequate signal which is measured as adequate precision showing the signal to noise ratio. A ratio greater than 4 is desired and the adequate precision measured was 6.189 indicating adequate signal.

CONCLUSION

An average intake of acrylamide for the general population has been estimated to be in the range of 0.3-0.8 μg/kg of body weight/day (FAO/WHO, 2005). Acrylamide occurred in roasted *Cajanus cajan* with unexpectedly high levels at different roasting temperatures, additives and roasting times, soaking solvents. Comparably, under the studied roasting conditions, acrylamide concentration of 1.758 - 8.368 g / kg was detected in roasted *Cajanus cajan*. The model with equation; -2.069 + 0.745A + 0.041B + 0.054C gave the optimized condition for roasting *Cajanus cajan* with minimum acrylamide concentration. The optimized conditions using RSM was found at temperature of 80 °C, roasting time of 10 min and 0.1 g of citric acid) with desirability of 0.972. Under these conditions, acrylamide concentration of 1.757 g/kg was observed. The experimental result (1.757 g/kg) was close to the predicted response (1.918 g/kg). From this study, soaking of *Cajanus cajan* in acidic medium before roasting aided in minimising acrylamide production. Further use of citric acid above 1.0 g in minimising acrylamide concentration in *Cajanus cajan* should be investigated to find out whether acrylamide production would be significantly reduced.

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