

Research Article

Effect of Roasting Temperatures and Times on Test Parameters Used in Determination of Adequacy of Soybean Processing

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Abstract: In this study, the effect of roasting temperatures (110, 120 and 130°C) and times (20, 40, 60, 80, 100 and 120 min) respectively on soybean protein quality test parameters was investigated. Results have revealed that urease activity decreased gradually with increasing time at 110°C, suggesting that the test could be a suitable indicator for both under processing and over processing as opposed to 120 and 130°C where there was a sudden drop in urease activity. Results further showed that at 110°C, protein solubility in potassium hydroxide remained high with increasing time while at 120°C, protein solubility decreased inconsistently. On the other hand, protein solubility at 130°C decreased steadily suggesting that the test could be a suitable indicator for both under processing and over processing. It was further observed that at all roasting temperatures, protein dispersibility index decreased gradually with the highest and lowest decreases observed at 130°C and 110°C respectively. Results further showed that at 130°C, protein digestibility and protein dispersibility index tests could yield results that were comparable with urease activity and protein solubility tests unlike at 110 and 120°C. The findings have demonstrated that roasting temperatures and times significantly affected the test parameters used in determining the adequacy of soybean processing. These findings justify the need to carefully consider roasting temperatures for potential applications of processed soybeans in animal feeds processing as well as product development for human consumption.

Keywords: Comparable, digestibility, dispersibility, solubility, soybean protein, urease

INTRODUCTION

Soy, a popular legume is widely consumed in many parts of the world including Asia, America, Europe and Africa. Because it is highly nutritive in various essential nutrients, it is used as a raw material in development of different food products such as tofu, soymilk, tempeh, soy yoghurt, miso, soy sauce and soy bread (Giri and Mangaraj, 2012; Orhevba, 2011; Riaz, 2006). Although the consumption of soy and its products has increased over the years, there is still resistance by considerable proportion of consumers to readily accept these foods in their conventional diet due to many reasons. For example, it has been well documented that soy contains trypsin inhibitor which in addition to its detrimental effects on proteolytic action (Bora, 2014; Jiang *et al.*, 2013; Newkirk, 2010) and lowering of protein quality (Momonoki *et al.*, 2002) it dramatically increases the

size of the pancreas and amount of trypsinogen production (Rocha *et al.*, 2014). In addition, soy products exude beany flavours and astringency that limit acceptance by many consumers from the West (Ogbonna *et al.*, 2013; Silva *et al.*, 2010; Prathap and Ratnavelu 2015).

It is generally well recognized that various techniques such as heat treatment, soaking, germination, fermentation, irradiation, fortification and blending have been previously used in soy processing to improve the bioavailability of essential nutrients as well as improving the acceptability of soy products (Agrahar-Murugkar and Jha, 2010; Anderson, 1992; Bajpai *et al.*, 2005; Žilić *et al.*, 2010; Sowonola *et al.*, 2005). Among the heat treatment techniques, roasting has been reported to offer several advantages such as improvement of colour, shelf life, flavour, digestibility, oil content and reduced anti-nutritional factors (Kavitha

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and Parimalavalli, 2014; Krička *et al.*, 2009; Shin *et al.*, 2013). Roasting has also been reported to have decreased hardness, toughness and average rupture force on soybean (Mridula *et al.*, 2007). However, excessive application of roasting can decrease the biological value of protein in food (Reddy *et al.*, 1993).

Several laboratory tests including urease activity (UA), protein solubility in potassium hydroxide (KOH), Protein Dispersibility Index (PDI) and protein digestibility have been developed in order to monitor soybean protein quality. Although many studies have been conducted to evaluate the suitability of these tests (Araba and Dale, 1990a; Batal *et al.*, 2000; Caprita and Caprita, 2010; Căpriță *et al.*, 2010) most of the studies were conducted under limited processing conditions. It is against this background that a holistic study using different roasting temperatures would be appropriate to provide useful insights in how the properties related to proteins are affected during the roasting process as well as provide new information regarding the relevance of applying the tests at different temperatures. The aim of this study was to investigate the effect of roasting temperatures (110, 120 and 130°C) and times (20, 40, 60, 80, 100 and 120 min) on UA, protein solubility in KOH, PDI and invitro protein digestibility. Specifically, the study identified changes in protein quality at different temperature and time and later on compared results from different test parameters. Additionally, the study established the suitability of each test at different roasting temperatures. Finally, the study established optimum conditions for roasting soybeans for monogastric animal feeding. It is expected that findings from this study would be applied in the development of soy based products such as soy nuts, soymilk and soy flour for human consumption as well as animal feeds.

MATERIALS AND METHODS

Sample collection and preparation: Soybeans harvested in the autumn of 2015 were purchased from the North East region of China and transported in polythene bag. They were stored at 4°C prior to use. The soybeans were sorted out to remove grit and rotten samples. Proximate composition and protein quality of soybeans were determined (Table 1), later, samples weighing 25g each were placed in aluminium pans (7 cm diameter, 3.5cm height). The pans were placed in a forced air oven preheated to 2°C above the required temperatures (110, 120 and 130°C) in order to ensure that the temperature was standardized after it was observed during preliminary work that the temperature was dropping by 2°C once the oven door was opened. Exactly at the last second of roasting time, the oven door was opened and the samples were removed. In order to regulate temperature and prevent interferences

Table 1: Soybean properties before roasting applications

Property	Value
Protein content	36.3±0.03%
Moisture content	9.20±0.05%
Fat content	20.4±0.81%
Carbohydrate content	29.9±1.14%
Ash content	4.20±0.04%
UA	2.50±0.07%
Protein solubility in KOH	89.6±0.71%
PDI	79.9±0.55%
Protein digestibility	59.4±1.27%

due to opening of the oven door, a triplicate of samples of the same roasting temperature and time was placed at a time. The temperature was monitored through a digital thermometer on the oven while roasting time was monitored by means of a stop watch. Before the next triplicate of samples at a different roasting time was placed, the oven was preheated again to above 2°C of the roasting temperature and the sample was treated as the previous sample. After roasting, soybeans were allowed to cool in desiccators for 1 hr, ground through an 80 mesh sieve and stored at 4°C till use.

Chemicals: All chemicals and other reagents used in this study were purchased from Sigma-Aldrich Trading Co., Ltd. and were of analytical grade.

Determination of proximate composition: Crude protein content of raw soybean flour and roasted samples was determined by Micro-Kjeldahl method (Miller and Houghton, 1945). Moisture content in raw soybeans was determined according to the method by Kashaninejad and coworkers as reported previously (Wandkar *et al.*, 2012) with minor modifications. Crude lipid was extracted from a 2 g sample of soybean flour by FOSS SoxtecTM2043 fat extraction system (China) and determined according to AOAC (2000) official method. Ash content was determined by dry-ashing a 5g sample of soybean flour in a muffle furnace set at 550°C overnight (AOAC, 2000). The amount was expressed as a percentage of total weight of original sample. Carbohydrate content was determined by difference method according to AOAC (2000) official method.

Urease Activity (UA): UA was determined according to the method reported previously (AOAC, 1980). A test sample was prepared by adding 0.2 g of soybean flour to a test tube. Ten ml of urea-phosphate buffer solution [30g of urea into 1 L of phosphate buffer (3.403g KH₂PO₄, 4.355g K₂HPO₄), pH 7] was added and the tubes were incubated at 30°C for 30 min. A test blank consisting of 0.2g sample and 10 mL of phosphate buffer only (3.403g KH₂PO₄ and 4.355 g K₂HPO₄ was dissolved and made to the 1 L mark with deionised water) and incubated at 30°C for 30 min. UA

was calculated as the difference in pH between the test sample and blank sample.

Protein solubility in potassium hydroxide (KOH):

The Method as described by Araba and Dale (1990a) with some modifications was used to determine protein solubility. A 1.5 g sample of soybean flour was mixed with 75 mL of 0.2% KOH (0.05 N, pH 12.5) and stirred on a magnetic stir plate for 20 min. The mixture was centrifuged at 2700 rpm, filtered with 8 layers of gauze and 200 mesh sieve to recover the supernatant. The quantity of protein in the supernatant was determined by Bicinchoninic acid method (Smith *et al.*, 1985) using Bovine Serum Albumin as standard protein. Protein solubility was calculated as the percentage of total protein dissolved in the 0.2% of KOH solution.

Protein Dispersibility Index (PDI): PDI was determined according to AOAC (1980) official method with minor modifications. Soybean flour was mixed with deionised water at flour to water ratio 1:15 (w/v). The mixture was stirred in a blender at 8500 rpm for 10 min and centrifuged at 1000g for 10 min. The precipitate was discarded while the supernatant was collected for determination of protein content by Bicinchoninic acid method (Smith *et al.*, 1985) using Bovine Serum Albumin as standard protein. Protein solubility was calculated as the percentage of total protein dispersed in deionized water.

Protein digestibility: Determination of protein digestibility was done in accordance with the method by Akeson and Stahmann (1964). A 1 g sample of soybean flour was added to 50 mL centrifuge tubes containing 1.5 mg pepsin in HCL (15 mL, 0.1M). The mixture was vortexed, incubated in a slow shaking bath at 37°C for 3 h and neutralized with NaOH (7.5 mL, 0.2M). Four mg pancreatin in phosphate buffer (7.5 mL, 0.2M, pH 8.0) was added. One mL toluene was added to prevent microbial growth and the mixture was vortexed and incubated at 37°C for 24 h. Trichloroacetic acid (10 mL, 10%) was added to suspend undigested proteins and the mixture was centrifuged at 5000 g for 20 min under room temperature. The precipitate was discarded while the supernatant was collected to determine protein by Micro-Kjedahl method (Miller and Houghton, 1945). Protein digestibility was expressed as the percentage of total proteins in the supernatant.

Data analysis: Samples were prepared in triplicates and statistical analysis was performed using IBM-SPSS Inc. software (version 20). One-way analysis of variance (ANOVA) was used to determine significant differences between means, with the significance level taken at ($p < 0.05$). Duncan multiple range test was used

Table 2: UA values of soybeans at different roasting temperatures and time

Heating time (min)	Roasting temperatures (°Celsius)		
	110	120	130
20	2.33 ⁱ	0.39 ^d	0.05 ^a
40	2.26 ^h	0.23 ^c	0.04 ^a
60	2.14 ^g	0.16 ^b	0.03 ^a
80	2.10 ^{fg}	0.16 ^b	0.01 ^a
100	2.07 ^f	0.15 ^b	0.01 ^a
120	2.01 ^e	0.15 ^b	0.01 ^a

Means at different temperatures and times with different superscripts differ significantly ($p < 0.05$)

to analyse significant difference in different mean values and differences were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

Urease Activity (UA): UA test is applied to indirectly assess whether anti-nutritional factors have been sufficiently destroyed during processing of soybean products (Van Eys, 2005). Roasting soybeans at 110°C for 120 min (Table 2), resulted in gradual decrease of UA implying that the test had a potential application for monitoring under processing and over processing. Alternatively, more time would be required since according to the results, UA at 120 min of roasting time was significantly high (2.01). On the other hand, UA values decreased suddenly from 2.45 to 0.05 and 0.39 within 20 min at 130 and 120°C roasting temperatures respectively. The pattern of change in UA as a result of roasting at 120 and 130°C is similar to findings previously reported by other authors on soy flakes (Araba and Dale, 1990a) in which the values dropped suddenly to 0.02 in a period of 5 min using 121°C autoclaving treatment and was zero at processing time of 10 min or longer. In another related study, Wiriyapaisong *et al.* (2001) observed that the levels of UA of soybean subjected to inlet temperature in the ranges of 120-150°C was insignificantly changed in the early stages and then it was reduced sharply. The non linear pattern of UA to soybean heat treatment was reported to be attributed to the inconsistency in various previous research works on the minimum value that determine sufficient processing (Batal *et al.*, 2000). However, despite all these inconsistencies, UA value of less than 0.1 units is widely recommended as an indicator of properly heat processed soybean (Newkirk, 2010; Van Eys, 2005). In this study, this level was obtained over a time period of 20 min of roasting soybeans at 130°C.

Protein solubility in potassium hydroxide (KOH):

The protein solubility in KOH test is particularly used to detect over processed soybean meal (Batal *et al.*, 2000; Araba and Dale, 1990a) but the assay has also been reported to be useful for monitoring

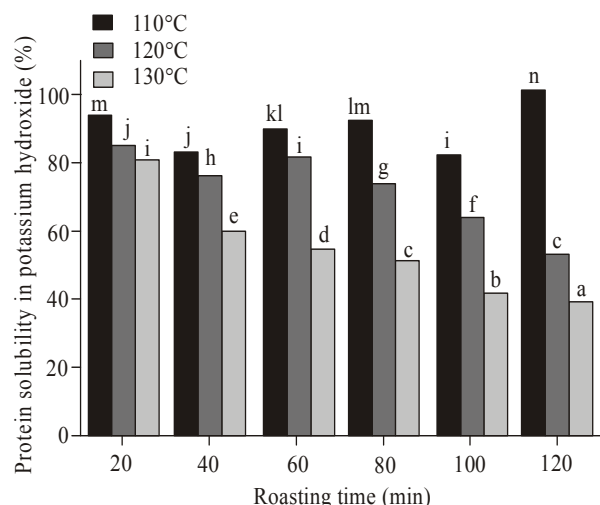


Fig. 1: Protein solubility in KOH. Means at different temperatures and times with different superscripts differ significantly ($p < 0.05$)

under processing (Araba and Dale, 1990b). Soy beans roasted at 110°C were found to have high protein solubility values from 20 to 120 min (Fig. 1) roasting time implying that the treatment was insufficient. On the other hand, with 120°C roasting temperature, the values decreased between 20 and 40 min but later increased between 40 and 60 min. Thereafter, there was a steady decreasing pattern which was maintained up to 120 min. These observations are consistent with findings of other authors (Batal *et al.*, 2000) in which protein solubility of soy flakes subjected to autoclaving decreased between 3, 6 and 9 min and then increased at 12 min. In this study, inconsistencies were observed with short roasting times at 120°C which coincided with high protein solubility suggesting that the test could not be applied for monitoring under processing at that temperature. On the other hand, the steady decreasing pattern of values obtained between 60 and 120 min of roasting time at 120°C coincided with low protein solubility which is in line with recommendations on the use of the test for monitoring over processing as reported previously by other authors (Căpriță *et al.*, 2010). It was further observed that at 130°C roasting temperature, protein solubility decreased steadily with both short and long time roasting times and this is in agreement with previous findings reported on soybean flakes (Araba and Dale, 1990a). The steady decreasing pattern of protein solubility implies that the test could reliably be applied to monitor both under processing and over processing at 130°C roasting temperature. Generally, protein solubility in the range of 78-85% is usually recommended as a standard for adequately processed soybeans meals (Van Eys, 2005; Newkirk, 2010). On the other hand, values between 84 and 89% imply under

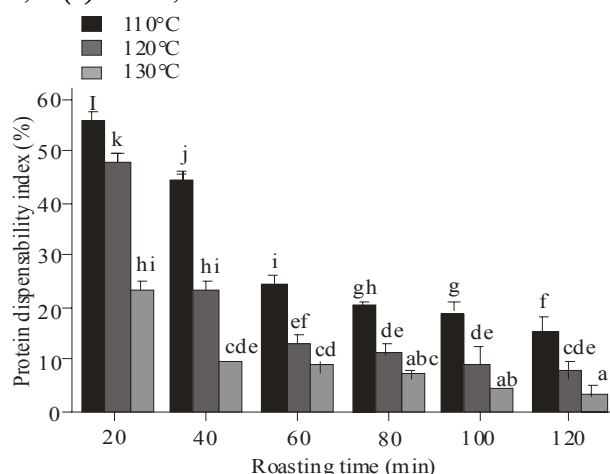


Fig. 2: PDI. Means at different temperatures and times with different superscripts differ significantly ($p < 0.05$)

processing while those below 74% imply over processing. In this study, the optimum protein solubility values (78-85%) were obtained at between 20-40 and 40-60 min of soybean roasting at 130°C and 120°C respectively.

Protein Dispersibility Index (PDI): Results for PDI are presented in Fig. 2. Results showed that PDI decreased steadily with increasing time from 20 to 120 min at all levels of roasting temperatures. PDI was found to decrease the most in soybeans subjected at 130°C and the least in those subjected at 110°C roasting temperature. Although it has been established in previous studies that 45% PDI value usually coincided with high animal performance, the recommended values for the feeds industry fall in the range of 15 to 30% (Van Eys, 2005). In this study, it was found out that soybeans roasted at 130°C took the shortest time (20-40 min) to attain the recommended PDI value followed by soybeans roasted at 120°C (40-60 min) while those roasted at 110°C took the longest time (60-120 min). The consistent trend of PDI exhibiting a decreasing pattern with different roasting temperatures and time which is consistent with previous findings by other authors (Batal *et al.*, 2000; Căpriță *et al.*, 2010; Newkirk, 2010) has justified the reason to recommend it as the most suitable test for monitoring adequacy of soybean processing. However, despite the consistent patterns of PDI observed at different roasting temperatures, the test was a sufficient indicator for monitoring adequacy of soybean processing only at 130°C roasting temperature unlike at 110 and 120°C due to undesirable high levels of UA.

Protein digestibility: Protein digestibility reflects the availability of proteins in a substrate for utilisation by animals. Results showed that at 110°C, protein digestibility was high at both short and long roasting times (Fig. 3). It was further observed that the maximum value (80.2%) obtained at 120 min was even higher than the values reported previously

Table 3: Optimum heat treatment time based on different protein quality test parameters

Quality test parameter	Optimum values	Heat treatment time (min)		
		110°C	120°C	130°C
UA	0.05-0.10	Not applicable	Not applicable	0-20
Protein solubility in KOH	78-84%	Not applicable	60-80	20-40
PDI	40-45%	60-120	40-60	20-40
Protein digestibility	69-80.2%	40-120	40-60	40

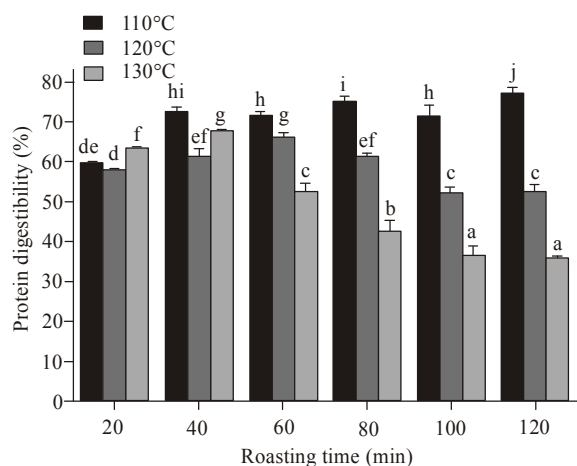


Fig. 3: Protein digestibility. Means at different temperatures and times with different superscripts differ significantly ($p < 0.05$)

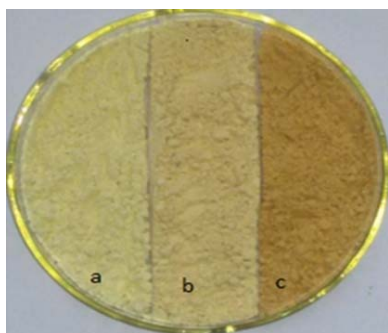


Fig. 4: Soy flour from; a: Under processed; b: Adequately processed and c: Over processed soybeans

by other authors (Han *et al.*, 2007). On the other hand, at 130 and 120°C, protein digestibility increased up to 40 and 60 min respectively, and thereafter, the values decreased consistently to minimum levels at 120 min. The increasing protein digestibility with increasing roasting time in the study can be attributed to unfolding of proteins which promoted cleavage of proteolytic enzymes (Kavitha and Parimalavalli, 2014). Further increase in roasting time at 120 and 130°C roasting temperatures subsequently led to decreased protein digestibility. These observations can be attributed to Maillard reaction which is characterised by binding of protein lysine to sugars and formation of additional cross links involving amino acids and sulphur groups which resist digestion by enzymes (Dills, 1993; Newkirk, 2010). In this study, Maillard reaction

affected soybeans roasted at 130 and 120°C due to the low protein digestibility values observed at 120 min roasting time (37.2 and 54.2%, respectively). Additionally, soy flour obtained from the ground soybeans appeared brown in colour (Fig. 4) which symbolised advanced levels of Maillard reactions (Dills, 1993). Although soybeans roasted at 110°C for 120 min were the most digestible ones, the high values of UA and protein solubility in KOH implied that they were still under processed. This observation was similar to the one observed in soybeans roasted at 120°C in which high protein digestibility (69%) at 60 min coincided with undesirable UA. On the other hand, high protein digestibility value (69.9%) obtained with 40 min of roasting at 130°C coincided with inactivation of UA implying that the test was a sufficient indicator for monitoring adequacy in soybean processing at 130°C. Therefore, at 9.2% moisture content, soybeans roasted at 130°C for 40 min can be deemed to be sufficiently processed since results from all test parameters were comparable (Table 3).

CONCLUSION

This study has revealed that roasting temperature affect the response of soybean protein to test parameters that determine adequacy of soybean processing. At 130°C, the test parameters yielded results that were comparable unlike at 110 and 120°C due to undesirable high UA and the observed inconsistencies of protein solubility in KOH. The gradual decline of UA at 110°C implies that the test could be applied for monitoring both under processing and over processing. This is contrary to observations at roasting temperatures of 120 and 130°C in which UA dropped suddenly suggesting therefore that the test could only be suitable for monitoring under processing. Protein solubility in KOH could be applied to monitor both under processing and over processing at 130°C due to the steady decrease in observed values with increasing time unlike at 120 and 110°C. The findings of this study have shown that optimum conditions for processing soybeans were obtained with roasting soybeans at 130°C for 40 min. From the study findings, it is recommended that the correct use of test parameters should take into consideration processing temperatures for soybeans. Further studies should be conducted to evaluate other properties of soybeans such as proximate composition, anti-nutritional factors, functional and antioxidant

properties as affected by roasting temperatures and time. It is further recommended that new products such as soymilk could be produced from roasted soybeans and evaluated for their nutritive value, sensory properties and consumer acceptability.

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