



ASSESSMENT ON QUALITY OF LEGUMES PROTEIN INFLUENCED BY HEAT TREATMENT

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ABSTRACT

Heat treatment of legumes can reduce anti-nutritional factors and the optimization of protein digestibility, however, over or under heating would damage the amino acids content of legumes therefore, adequate time is necessary to maintain the quality of legumes proteins. But direct analysis of both specifications is difficult in routine operations, therefore, replaced with indirect tests such as urease assay index (UAI), protein solubility in 0.2% potassium hydroxide (PSKOH) and protein dispersibility index (PDI). The UAI is useful to determine if the legumes have been heated enough to reduce the anti-nutritional factors, while PSKOH is a good protein solubility index (PSI) for determining over processing of legumes. The PDI is the best method of evaluating these legume ingredients for both under heating and overheating. So, combining the PDI test with the UAI and PSI could be useful for better monitoring of legumes quality. In this research, five various legume samples commonly used in household are placed in an oven at 120°C for up to 30 minutes; analysis was accomplished every 5 minutes. Adequate processing of black eye bean is (20 minutes), chickpea (10-15 minutes), pea (10 minutes), soybean (15-20 minutes) and kidney bean (5 minutes) which are determined by combining UAI, PSI and PDI.

KEYWORDS: Anti-nutritional factors, protein dispersibility index, urease activity index, protein solubility.

INTRODUCTION

Contrasting animal proteins, legume proteins are known to be inferior quality, due to the deficiency of sulphur comprising amino acids as well as due to other factors like digestibility, presence of essential amino acids, anti-nutritional factors (ANF) etc. The ANF mainly occurs in the uncooked state, foods and feed material prepared from grain legumes.^[1, 2] They can cause negative effects to humans and animal development and performance by impairing intake, uptake or utilization of other foods and feed components or by causing discomfort and stress.^[3] In order for the nutritional value of legumes to be maximized, these ANF needs to be inactivated.^[4, 5] It is reported that ANF is generally eliminated by soaking and subsequently discarding of the liquid and/or by heat treatment at a relatively elevation, temperature.^[6, 7] The major concerns is, the role of heat on legumes if legumes are under- or over-processed, second, either under processing is more detrimental than over processing or vice-versa.^[8]

During the under processing of legumes the ANF are not destroyed and this leads to a reduction in the use of amino acids.^[9] The mild heating (~90°C) increases the nutritional cost of legumes by protein degradation and

revealing new sites for enzymatic hydrolysis as well as inactivating heat labile ANF.^[10-12] Overheating, however, will denature proteins and reduce the availability of amino acids, especially the essential ones.^[6]

Being relatively inexpensive and simple to perform some commonly used tests for determining the protein quality are: urease assay index (UAI), protein solubility in 0.2% potassium hydroxide (KOH) solution (PSKOH) and protein dispersibility index (PDI).^[9, 13] The UAI is based on the release of ammonia from urea by the residual urease enzyme in legumes.^[2, 14] Due to the release of ammonia from urea, the pH of the test solution increases significant because of urease activity.^[22] The damage of the urease done by heating is associated with the destruction of inhibitors of trypsin and lectins. The percentage of protein that is solubilized in a KOH solution is determined by PSKOH.^[2, 14] Another method PDI, simply measure of protein solubility in water with high speed mixing, since the protein solubility of legumes decrease as the heat exposure increases.^[15, 16] Current recommendations for legumes that are considered adequately heat processed are those with UAI, PSKOH and PDI values between 0.05-0.20, 71-85% and 15-40% respectively.^[9, 17, 18]

The UAI is very useful for determining if legumes have been under processed and value “zero” does not necessarily indicate over processing of legumes.^[19] PSKOH is a sensitive index for monitoring over processing of legumes and appears to be a better indicator for over processing.^[20] In marked contrast the PDI index decreased incrementally for the heating times.^[19] The experimental data suggest that PDI is more sensitive than UAI, PSKOH. Therefore combining UAI and PSKOH or UAI and PDI could be useful to monitor legumes quality.^[20]

Since legumes are comparatively low-priced related to meat and its products, therefore, eating more legumes may be an alternative to meat for some.^[9] So in this research some of very common legumes used in household are heat treated and tried to uncover the least amount of ANF content on them by UAI, PSKOH and PDI for assessing legumes quality.

MATERIALS AND METHOD

Samples

Five varieties of leguminous seeds (red kidney beans, black eyed beans, soybeans, chickpeas and peas) were obtained from the local farmers near Kathmandu, Nepal. Their systematic botanical names were identified from Nepal Agriculture Research (NARC), Khumaltar, Latilpur. The collected seeds were cleaned, rinsed with tap water and followed by distilled water and then dried again. The samples were ground to permit the 200 μ sieve. Powered legume samples were heated in a forced air oven at 120°C for varying periods of time: 5, 10, 15, 20, 25 and 30 minutes.

Methods

Protein of raw and heat treated legume seed powder was determined by the Bardford method with bovine serum albumin as standard.^[21] The modified UAI test was conducted as follows: 10 ml buffered urea solution (pH=7. 0) was added to 200 mg finely ground legumes (test sample), and 10 ml phosphate buffered solution was added to 200 mg of the same sample (blank sample). The two solutions were incubated at room temperature for 1 hour. After incubation, the pH is determined rapidly of the solutions and the pH differences of the test and blank is calculated as UAI.^[22]

The PSKOH test is based on the legumes protein solubility in a dilute solution of potassium hydroxide. The modified procedure was followed, which involves incubation of the 20 mg sample with 1 ml 0.2% KOH (wt/Vol; 0.036 N) solution for 30 minutes at room temperature. Following this incubation, the sample was centrifuged for 5 minutes at 6,000 rpm and the supernatant was analysed for the protein concentration by the Bradford method.^[14]

The PDI assay is also based on the solubility of legumes protein in water.^[15] The modified PDI method was used, in which 20 mg of legumes was incubated with 1 ml of distilled water for 30 minutes at room temperature,

following this incubation, diluted legumes were centrifuged for 10 minutes at 8,500 rpm and supernatants were collected ^[23] and analysed for the protein concentration by the Bradford method.^[14]

Statistics

Correlation coefficients (r) to determine correlations between particular parameters were calculated using SPSS version 21 and MS Excel Software (window 7) for plotting graphs.

RESULTS AND DISCUSSION

The primary purpose of the UAI is to decide if legume has been sufficiently heated to destroy trypsin inhibitors and most of the other ANF.^[14] Table 1, shows the variation of UAI (Δ pH), PSKOH and PDI when various legume samples were placed in an oven at 120°C for up to 30 minutes (5, 10, 15, 20, 25 and 30 minutes); analysis were accomplished every 5 minutes.

Table 1: Effect of heat treatment at 120°C on UAI (Δ pH), PSKOH and PDI in various legumes.

Samples	Heating time (minutes)																	
	UAI (Δ pH)						PSKOH						PDI					
	5	10	15	20	25	30	5	10	15	20	25	30	5	10	15	20	25	30
Black eye bean	0.3 ^a	0.1 ^b	0.1 ^b	0.1 ^b	0.1 ^b	0.1 ^b	107.88 ⁱ	98.63 ⁱ	87.84 ⁱ	82.79 ⁱⁱ	60.62 ⁱⁱⁱ	55.48 ⁱⁱⁱ	52.4 ¹	49.7 ¹	47.6 ¹	37.95 ²	30.02 ²	28.51 ²
Chickpea	0.2 ^b	0.1 ^b	0.1 ^b	0.1 ^b	0.1 ^b	0 ^c	80.49 ⁱⁱ	80.03 ⁱⁱ	76.37 ⁱⁱ	64.02 ⁱⁱⁱ	60.82 ⁱⁱⁱ	60.82 ⁱⁱⁱ	40.1 ¹	38.8 ²	37.1 ²	34.1 ²	30.9 ²	20.8 ²
Pea	0.2 ^b	0.1 ^b	0.1 ^b	0.1 ^b	0 ^c	0 ^c	85.21 ⁱ	84.42 ⁱⁱ	68.72 ⁱⁱⁱ	68.72 ⁱⁱⁱ	54.97 ⁱⁱⁱ	51.44 ⁱⁱⁱ	25.7 ²	23.7 ²	23.5 ²	21.8 ²	20.5 ²	16.1 ²
Soybeans	0.3 ^a	0.3 ^a	0.2 ^b	0.1 ^b	0 ^c	0 ^c	98.19 ⁱ	87.60 ⁱ	82.73 ⁱⁱ	75.29 ⁱⁱ	74.14 ⁱⁱ	70.71 ⁱⁱⁱ	51.47 ¹	38.13 ²	36.80 ²	36.27 ²	34.67 ²	26.67 ²
Kidney beans	0.1 ^b	0.1 ^b	0 ^c	0 ^c	0 ^c	0 ^c	75.87 ⁱⁱ	68.37 ⁱⁱⁱ	67.79 ⁱⁱⁱ	64.90 ⁱⁱⁱ	63.17 ⁱⁱⁱ	60.87 ⁱⁱⁱ	39.66 ²	37.97 ²	35.16 ²	33.47 ²	32.91 ²	30.10 ²

Note; ^a: Under processing (>0.20), ^b: Adequately processed (0.05-0.20) and ^c: Over processing (<0.05) of the legumes however zero does not necessarily indicate over processing of legumes. Data were compared with UAI values for determining the degree of legumes processing.^[17]

ⁱ: Under processing (>85), ⁱⁱ: Adequately processed (71-85) and ⁱⁱⁱ: Over processing (<70) of the legumes. Data were compared with PSKOH values for determining the degree of legumes processing.^[9]

¹: Under processing (>40), ²: Adequately processed (15-40) and ³: Over processing (<15) of the legumes. Data were compared with PDI values for determining the degree of legumes processing.^[18]

Results collected in this study indicated that UAI and PSKOH observed a positive connection ($r = 0.510$, $p = .002$), similarly, UAI and PDI also observed a progressive connection ($r = 0.275$) as shown in table 2. Similar kind of positive correlation was observed between PDI and UAI values ($r = 0.9857$).^[14, 20, 23, 26, 27]

Table 2: Correlation of UAI with PSI and PDI in legume samples.

		PSI	PDI
UAI	Pearson Correlation	.510 ^{**}	.275
	Sig. (1-tailed)	.002	.071

^{**}: Correlation is significant at the 0.01 level (1-tailed), total number of sample (N): 30.

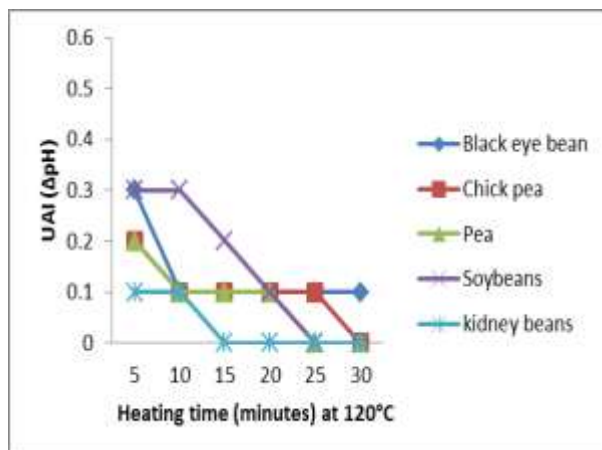


Figure 1: Effect of heat treatment at 120°C on UAI (ΔpH) in various legumes.

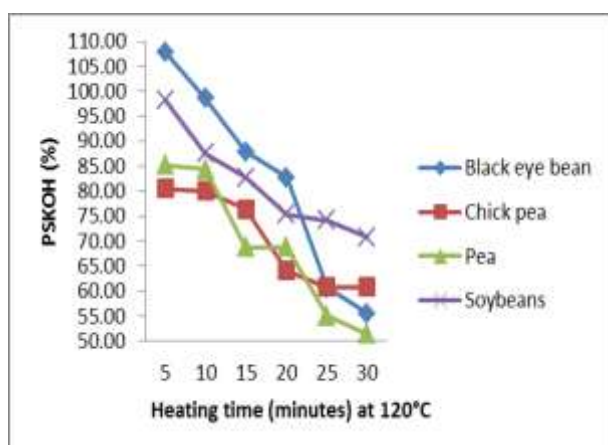


Figure 2: Effect of heat treatment at 120°C on protein solubility in 0.2% KOH (PSKOH) in various legumes.

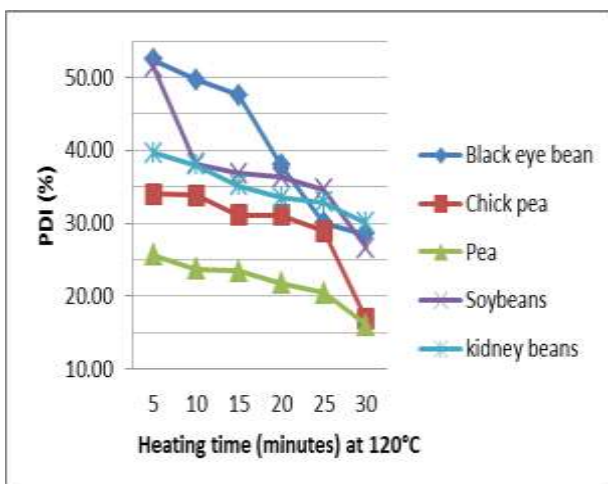


Figure 3: Effect of heat treatment at 120°C on protein dispersibility index (PDI) in various legumes.

Table 3: Adequate processing time (minutes) of various legume samples at 120°C.

Sample	Black eye bean	Chickpea	Pea	Soybeans	Kidney beans
	Time (minutes) at 120°C				
UAI	10-30	5-25	5-20	15-20	5-10
PSKOH	20	5-15	10	15-25	5
PDI	20-30	10-20	5-30	10-30	15-30

Protein quality of legume depends on two parameters, the decrease of anti-nutritional factors and the optimization of protein digestibility.^[2] Direct analysis of both specifications is difficult in routine operations, therefore replaced with indirect tests such as UAI, PSKOH and PDI.^[24] Additional heat treatment decreased PSKOH, and the UAI rapidly approached zero.^[25]

The primary purpose of the UAI is to determine if legume has been sufficiently heated to destroy trypsin inhibitors and most of the other ANF.^[14] The variation of UAI when various legume samples were placed in an oven at 120°C for up to 30 minutes (5, 10, 15, 20, 25 and 30 minutes); analysis were accomplished every 5 minutes. Data compared with standard UAI, PSKOH and PDI values for determining the degree of legumes processing were shown in table 1. This data was found to similar with previous studies done.^[14, 20, 23, 26, 27]

The overall experimental data suggest that PDI is more sensitive and demonstrated more consistent responses to heating of legumes than UAI or PSKOH which is in agreement with earlier studies.^[2,28,29] PDI was reduced by approximately half when raw legumes were heated for 20 minutes. Decreases in PDI were again quite consistent and large. These results also suggest that PDI is a better indicator of minimum adequate heating of legumes than is UAI or PSKOH. Therefore combining UAI and PSKOH or UAI and PDI could be useful to monitor legumes quality.^[20]

By the help of group of tests, done in *in vitro* condition, adequate processing at 120°C for five varieties of legumes has been set. According to UAI, the adequate time for black eye bean (10-30 minutes), chickpea (5-25 minutes), and pea (5-20 minutes), soybean (15-20 minutes) and kidney bean (5-10 minutes) at 120°C was set, as shown in figure 1.

Similarly according to PSKOH, the adequate time for black eye bean (20 minutes), chickpea (5-15 minutes), and pea (10 minutes), soybean (15-25 minutes) and kidney bean (5 minutes) was set, as shown in figure 2. Lastly the adequate time for black eye bean (20-30 minutes), chickpea (10-20 minutes), pea (5-30 minutes), soybean (10-30 minutes) and kidney bean (5-30 minutes) was set for PDI as shown in figure 3. Therefore adequate time was set at 120°C for black eye bean (20 minutes), chickpea (10-15 minutes), pea (10 minutes), soybean (15-20 minutes) and kidney bean (5 minutes) in which protein quality of legumes remains, as shown in table 3.

CONCLUSIONS

Protein quality of legume is linked to both the reduction of anti-nutritional factors, and the optimization of protein digestibility, however, excessive heating would damage amino acids content of legumes therefore, adequate time is necessary to maintain the quality of legumes proteins. But direct analysis of both specifications is difficult in routine operations, therefore replaced with indirect tests such as UAI, PDI and PSKOH. The UAI is useful to determine if the legumes have been heated enough to reduce the anti-nutritional factors, while PSKOH is a good index for determining over processing of legumes. The PDI is the best method of evaluating these legume ingredients for both under heating and overheating. So, combining the PDI test with the UAI and PSI could be useful for better monitoring of legumes quality. In this research also adequate time at 120°C for black eye bean (20 minutes), chickpea is (10-15 minutes), pea (10 minutes), soybean (15-20 minutes) and kidney bean (5 minutes) are determined by combining UAI, PSI and PDI.

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