RESEARCH ARTICLES

EFFECTS OF HYDROLYSING CONDITIONS ON CHEMICAL AND SENSORY PROPERTIES OF HYDROLYSED MUNGBEAN PROTEIN

PANTIPA JANTAWAT, NINNART CHINPRAHAST AND UBONRAT SIRIPATRAWAN

Food Technology Dept., Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand.

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ABSTRACT

Effects of proportion of HCl to protein, strength of acid, time and temperature in the hydrolysing reaction on chemical properties and flavour of mungbean protein hydrolysate were studied. The optimum conditions found were: ratio of protein to acid, 1:3; hydrolysing time, 6 hours; temperature, 120°C and acid strength, 6 M. At these conditions, the highest amino nitrogen, amino nitrogen to total nitrogen ratio and odor scores were accomplished.

INTRODUCTION

Mungbean protein, a by-product separated from the production of vitreous mungbean vermicelli, contains as high as 72 % (dry basis) of this essential nutrient. In addition, mungbean protein is of significant nutritional values since it is composed of all essential amino acids (Thompson, 1977). It was estimated, in Thailand, that approximately 200,000 tons per anum of mungbean seeds were used in the bean vermicelli industry. Mungbean protein which is the by-product from this industry accounted for about 27 % of the mungbean raw material. Therefore, approximately 54,000 tons per annum of this nutrient was sold as ingredient for animal feeds. This protein, if hydrolysed, may yield a flavouring product that has flavour and aroma resemble that of soybean protein hydrolysate and can be used as flavouring ingredients in food products such as soups, sauces, meat, fishery product and ready-to-eat foods.

In order that the hydrolysates would possess desirable properties, the important factors including processing conditions and selection of a raw material itself should carefully be monitored. In general, the good raw material should contain more than 30% protein (dry basis), be cheap and readily available (Grace, 1974). With significantly high content of proteins as mentioned previously, the mungbean by-product is suitable for conversion into protein hydrolysate and, thereby, efficient exploitation of such product can be achieved.

The objectives of this research were to (i) investigate the effects of mungbean protein-hydrochloric acid (HCl) ratio on chemical properties of the hydrolysed product, and (ii) study the influences of temperature, hydrolysing time and acid concentration on chemical and sensory characteristics of the product.

MATERIALS AND METHODS

Mungbean protein by-product was collected from Thai Wa Products Co., Ltd.(Thailand). The product was obtained from the production of vitreous mungbean vermicelli whose process was as follows. Mungbeans were wet-milled using electric attrition mills and filtered to remove debris of seed coat and other impurities. A suspension of starch, protein and other minor constituents was centrifuged to separate the starch. The supernatant was then heated at 80°C to precipitate the proteins. This precipitate was filtered and solar dried until the moisture content reached 3% . The dried precipitate was ground and screened through a sieve of 25 mesh. The protein by-product powder was analysed for its proximate composition (AOAC, 1980) and could readily be used.

Hydrolysation was performed by using 10 g of mungbean protein powder and HCL (6 M) in 250 ml Erlenmeyer flask. Proportions of protein powder to acid were 1:2, 1:3, 1:4, 1:5, 1:6 and 1:7 (g:ml). Each flask was plugged with cotton wool, wrapped with aluminium foil and placed in an autoclave (Sanyo MLS 2400) at 120°C for 2 hr. The hydrolysates were cooled in an open air to an ambient temperature (30±2°C). The pH of each flask was adjusted, using a pH meter (Corning 220), to 5.5 - 6.0 by an addition of 50% (by weight) sodium hydroxide (NaOH) solution which was simultaneously mixed by a magnetic stirrer. The resulting hydrolysates were filtered to remove the precipitates.

Each hydrolysate was analysed for its total nitrogen content(AOAC, 1980); amino nitrogen (AOAC, 1980); ratio of amino nitrogen to total nitrogen and sodium chloride (AOAC, 1980). The experimental design was completely randomized design with two replicates. Analysis of variance (ANOVA) and Duncan's New Multiple Range Test (DNMRT) were performed with significance level accepted at $p \leq 0.05$ (Cochran and Cox, 1985).

Thereafter, the effects of temperature , hydrolysing time and acid concentration on chemical and sensory properties of the hydrolysates were studied. The proportion of protein powder to amount of acid yielding the hydrolysate with suitable characteristics was selected from the previous experiment. Mungbean protein powder was then hydrolysed using the identical procedures as described previously. However, the parameters used in this experiment included temperatures at 120 and 130°C, acid concentrations at 4 and 6 M and hydrolysing times at 2, 4 and 6 hr respectively.

Each hydrolysate was analysed for its total nitrogen, amino nitrogen,and ratio of amino nitrogen to total nitrogen. The experimental design was asymmetric factorial experiment (2x2x3) with 2 replicates. ANOVA and DNMRTwere performed with significance level accepted at $p \leq 0.05$ (Cochran and Cox, 1985).

In addition, the hydrolysates were also evaluated for their aroma using ten panelists. These testers were trained by assessing the aroma of five commercial flavorings, *viz.* HVP, tuna, chicken, meat and squid for ten times. The test used in sensory assessment was scoring test (Larmond, 1982) with a score of 0 representing "with no aroma of HVP" and 10 representing "with extremely good aroma of HVP".

Ten ml of sample was placed in an ampule with a diameter of 2 cm and a capacity of 15 ml. Each sample was coded with three digits random number. The experimental design was factorial randomized complete block experiment. ANOVA and DNMRT were also performed in a similar manner.

RESULT

Composition of Mungbean Protein

Composition of mungbean protein is shown in Table 1. The protein accounted for 78.90% of the total weight. Less than 1% of fat was extracted and the carbohydrate content was 15.08 %.

Table 1 Composition of the mungbean protein powder.

Composition	Means ± S.D.
Moisture	2.97±0.03
Protein	78.90±0.21
Fat	0.90±0.15
Ash	3.93±0.06
Fibre	0.30±0.07
Carbohydrate (by difference)	15.08±0.11

Effects of Proportion of Protein to Acid

The total nitrogen content of the hydrolysates decreased as quantity of acid increased ($p \leq 0.05$). The amino nitrogen level and amino nitrogen to total nitrogen ratio increased as protein to acid ratio of the reactants increased from 1:2 to 1:4 ($p \leq 0.05$). Accumulation of sodium chloride was also observed ($p \leq 0.05$) as proportion of acid increased (Table 2).

Table 2 Total nitrogen, amino nitrogen, amino nitrogen:total nitrogen and NaCl contents of mungbean protein hydrolysates as affected by various ratios of protein powder to acid quantity.

Protein:	Percentage (wt./vol.) ± S.D.			
HCl (g:ml)	Total N	Amino N	Amino N:Total N	NaCl
1:2	3.80^{a}±0.07	1.84^{b}±0.03	59.74^{c}±0.36	14.61^{f}±0.23
1:3	2.82^{b}±0.03	1.93^{a}±0.06	68.01^{ab}±0.23	16.17^{e}±0.52
1:4	2.78^{b}±0.04	1.94^{a}±0.04	69.44^{ab}±0.94	17.73^{d}±0.47
1:5	2.51^{c}±0.04	1.78^{b}±0.04	71.03^{a}±0.50	20.85^{c}±0.29
1:6	2.16^{d}±0.06	1.47^{c}±0.03	68.02^{ab}±0.30	21.57^{b}±0.47
1:7	2.05^{d}±0.04	1.36^{d}±0.04	66.11^{b}±0.47	22.28^{a}±0.33

a, b, c .... Values with different superscripts in the same column are significantly different ($p \leq 0.05$).

Effects of Temperature, Hydrolysing Time and Acid Concentration

Effects of temperature, time and acid concentration on the extent of hydrolysation and aroma of the hydrolysate were studied (Tables 3-7). Increasing the temperature, time and acid concentration resulted in decreasing total nitrogen (Tables 4, 5 and 6).

Table 3 Total nitrogen, amino nitrogen and amino nitrogen:total nitrogen contents as affected by temperature, hydrolysing time and acid concentration.

Temp. (°C)	Hydrolysing Time (hr)	Acid Conc. (M)	Percentage (wt./vol.) ± S.D. Total N	Amino N	Amino N:Total N
120	2	4	3.10±0.03	1.85^{d}±0.02	59.69^{g}±1.05
		6	2.92±0.04	1.92^{cd}±0.06	65.80^{e}± 1.03
	4	4	3.03±0.05	1.93^{cd}±0.03	63.54^{f}±0.17
		6	2.86±0.03	1.98^{bc}±0.02	69.31^{bc}±0.24
	6	4	2.91±0.06	1.99^{bc}±0.06	68.34^{d}±0.13
		6	2.85±0.04	2.10^{a}±0.04	72.87^{a}±0.29
130	2	4	3.05±0.05	1.94^{c}±0.04	63.63^{f}±0.64
		6	2.90±0.04	2.00^{bc}±0.02	68.95^{c}±0.99
	4	4	2.99±0.03	1.99^{bc}±0.06	66.47^{e}±0.62
		6	2.82±0.03	2.01^{b}±0.04	71.44^{ab}±0.79
	6	4	2.88±0.03	2.06^{ab}±0.03	71.38^{ab}±0.48
		6	2.73±0.03	1.89^{cd}±0.03	69.89^{b}±0.45

a, b, c .... Values with different superscripts in the same column are significantly different ($p \leq 0.05$).

The highest amino nitrogen and amino nitrogen to total nitrogen ratio were accomplished at 6 hours hydrolysation, with 6 M HCl, at 120°C and 6 hours hydrolysation, with 4 M HCl, at 130°C.

Table 4 Total nitrogen contents of mungbean protein hydrolysates as affected by different temperatures.

Temperature (°C)	Total N (wt./vol.) ± S.D.
120	2.94^{a}±0.11
130	2.89^{b}±0.12

a,b Values with different superscripts are significantly different ($p \leq 0.05$).

Table 5 Total nitrogen contents of mungbean protein hydrolysates as affected by different hydrolysing times.

Hydrolysing Time (hr)	Total N (wt./vol.) ± S.D.
2	$2.99^{a}\pm0.09$
4	$2.92^{b}\pm0.11$
6	$2.84^{c}\pm0.08$

a, b, c Values with different superscripts are significantly different ($p \leq 0.05$).

Table 6 Total nitrogen contents of mungbean protein hydrolysates as affected by different acid concentrations.

Acid Concentration (M)	Total N (wt./vol.) ± S.D.
4	$2.99^{a}\pm0.08$
6	$2.84^{b}\pm0.07$

a,b Values with different superscripts are significantly different ($p \leq 0.05$).

The highest odor score for the hydrolysate was obtained at 6 hours hydrolysation, with 6 M HCl, at 120°C (Table 7).

Table 7 Sensory scores for the aroma of the hydrolysed mungbean proteins as affected by temperature, hydrolysing time and acid concentration.

Temperature (°C)	Hydrolysing Time (hr)	Acid Conc. (M)	Means ± S.D.
120	2	4	$4.55^{e}\pm1.18$
		6	$6.20^{cd}\pm1.09$
	4	4	$5.97^{cd}\pm1.06$
		6	$7.00^{bc}\pm1.37$
	6	4	$6.88^{bc}\pm0.78$
		6	$8.17^{a}\pm1.17$
130	2	4	$6.06^{cd}\pm1.63$
		6	$7.02^{bc}\pm0.99$
	4	4	$6.55^{c}\pm0.80$
		6	$7.45^{b}\pm0.65$
	6	4	$7.55^{b}\pm0.97$
		6	$5.63^{d}\pm1.03$

a, b, c .... Values with different superscripts are significantly different ($P \leq 0.05$).

When correlating the odor scores with the total nitrogen, the amino nitrogen and the amino nitrogen to the total nitrogen ratio, significant correlations ($p \leq 0.05$) were observed between the odor score and the amino nitrogen, and the odor score and amino nitrogen to total nitrogen ratio (Table 8).

Table 8 Correlation coefficients between odor score and total nitrogen, amino nitrogen and amino nitrogen to total nitrogen ratio.

	Correlation coefficient
odor score and total nitrogen	0.57
odor score and amino nitrogen	0.94*
odor score and amino nitrogen to total nitrogen ratio	0.94*

* significant ($p \leq 0.05$).

DISCUSSION

Proteins are essential ingredients in the preparation of flavouring compounds since they are precursors of peptides and amino acids which will react with carbohydrates to form volatile substances *via* Maillard and Strecker degradation reactions. The low fat level in raw material is essential for the production of protein hydrolysate since saponification can occur during the neutralisation process of the acid hydrolysation. Besides, fat can interfere with protein hydrolysis since it can form complex molecules with protein and result in less available protein molecules for reaction. Carbohydrates are also essential ingredient in flavour development. Carbohydrates of high molecular weight (*i.e.* starches) are broken down into compounds such as 5-hydroxy furfural, hydroxy methyl furfural and other components which play an important role in the amino acid-carbohydrate reaction known as Maillard reaction (Fennema, 1985).

Effects of Proportion of Protein to Acid

Hydrolysing process depends on many factors which can influence the flavour of hydrolysate. The proportion of acid to protein was one of such factors. In this experiment, the extent of the hydrolysing reaction as affected by the proportion of HCl used was expressed in terms of total nitrogen, amino nitrogen and ratio of amino nitrogen to total nitrogen. The total nitrogen content of the hydrolysates decreased as the proportion of acid used in the reaction increased (Table 2). This result indicated that further hydrolysis reaction produces more free amino acids. These acids then reacted with the available carbohydrates *via* Maillard and followed by Strecker degradation reactions to form volatile substances such as aldehydes and humin or melanoidin precipitates (Manley and Fagerson, 1970; May, 1974). Simultaneously, as the humin formed and accumulated, losses of nitrogen of some amino acids such as tryptophan, tyrosine, cystine, arginine, lysine and histidine took place. As a result, lowering of total nitrogen in the hydrolysate was observed.

As the proportion of acid increased from 1:2 to 1:4 (protein:acid), the amino nitrogen level and amino nitrogen to total nitrogen ratio increased. But further increasing of the proportion of acid to protein resulted in decreases of both amino nitrogen and amino nitrogen to total nitrogen ratio. This result revealed that, at lower proportion of HCl, more proteins were broken down into free amino acids and the accumulation of the amino acids resulted. At too high concentrations of HCl, deamination of some amino acids could occur and resulted in decreasing of the total amino nitrogen level as well as amino nitrogen to total nitrogen ratio.

Accumulation of sodium chloride was observed as the proportion of the HCl used in hydrolysing reaction increased. Large excess of HCl needed equal proportion of sodium hydroxide to neutralise and the high salt level would result. This condition had to be avoided since the resultant hydrolysate will be too salty.

Effects of Temperature, Hydrolysing Time and Acid Concentration

Decrease of total nitrogens of the hydrolysates as temperature, time and acid concentration increased was attributed to losses of nitrogen in humin precipitation. Manley and Fagerson (1970) explained that in the later state of the acid hydrolysation of plant protein, the free amino acids, especially tryptophan, released during hydrolysation could react with aldose sugars to produce humin precipitates.

Change of amino nitrogen level and amino nitrogen to total nitrogen ratio as affected by acid concentration, hydrolysing time and temperature indicated that when lower temperature (120°C) was used in the acid hydrolysing reaction, higher concentration of acid was needed in order that the same degree of hydrolysation could be obtained comparing to when higher temperature (130°C) was used.

The highest odor score for the hydrolysate was obtained at 6 hours hydrolysation, with 6 M HCl, at 120°C (Table 7). This was also the same product that had the highest level of amino nitrogen and amino nitrogen to total nitrogen ratio. When correlating the odor scores with the total nitrogen, the amino nitrogen and the amino nitrogen to the total nitrogen ratio, significant correlations ($p \leq 0.05$) were observed between the odor score and the amino nitrogen, and the odor score and amino nitrogen to total nitrogen ratio (Table 8). Yokotsuka (1962) recommended that the amino nitrogen to total nitrogen ratio should be used as an indicator to the flavour quality of soy sauce. Pham and Del Rosario (1983) reported that increasing amino nitrogen resulted in an increase of odor score of soy bean hydrolysate. Manley and Fagerson (1970) mentioned that the acid fraction of the hydrolysate attributed to its meat-like aroma. Production of these acids was *via* the Maillard type reaction of carbohydrate and free amino acids followed by Strecker degradation to produce aldehydes which are then oxidised to acids.

The experimental results also indicated that the less the destruction of the amino acids the better the flavour of the hydrolysate. The decreasing quantity of the amino nitrogen after 6 hours hydrolysation with 6 M HCl at 130°C (Table 3) indicated that some amino acids released during hydrolysing reaction were destructed at this severe condition. The lower odor score was also observed in this particular sample. May (1974) stated that in a complete acid hydrolysation of plant protein, the less destruction of amino acid the better would be the flavour of the hydrolysate. Ammonia which was one of the final products from amino acid destruction was attributed to the low odor quality of the protein hydrolysates.

CONCLUSION

From all experimental results, it can be concluded that the optimum conditions for the acid hydrolysation of mungbean protein were: ratio of protein to acid, 1:3; hydrolysation time, 6 hours; temperature, 120°C and acid concentration, 6 M. At these conditions, the highest amino nitrogen, amino nitrogen to total nitrogen ratio and odor score were observed.

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บทคัดย่อ

ได้ศึกษาผลของอัตราส่วนกรดไฮโดรคลอริคต่อโปรตีน ความเข้มข้นกรด เวลาและอุณหภูมิที่ใช้ในการไฮโดรไลซ์โปรตีนถั่วเขียวต่อคุณภาพทางเคมีและประสาทสัมผัสของผลิตภัณฑ์ที่ได้ ภาวะเหมาะสมที่สรุปได้คือ อัตราส่วนโปรตีนต่อกรด 1 : 3 เวลาที่ใช้ในการดำเนินปฏิกิริยา 6 ชั่วโมง อุณหภูมิ 120 ° C และความเข้มข้นกรด 6 M ที่ภาวะนี้ ผลิตภัณฑ์ที่ได้มีปริมาณอมิโนไนโตรเจน อะมิโน-ไนโตรเจนต่อไนโตรเจนทั้งหมดและคะแนนกลิ่นสูงที่สุด