

Extraction of Bran Protein Using Enzymes and Polysaccharide Precipitation

Sudarat Jiamyangyuen, Tipawan Thongsook, Riantong Singanusong, Chanida Saengtubtim

I. INTRODUCTION

Abstract—Rice bran is normally used as a raw material for rice bran oil production or sold as feed with a low price. Conventionally, the protein in defatted rice bran was extracted using alkaline extraction and acid precipitation, which involves in chemical usage and lowering some nutritious component. This study was conducted in order to extract of rice bran protein concentrate (RBPC) from defatted rice bran using enzymes and employing polysaccharides in a precipitating step. The properties of RBPC obtained will be compared to those of a control sample extracted using a conventional method. The results showed that extraction of protein from rice bran using enzymes exhibited the higher protein recovery compared to that extraction with alkaline. The extraction conditions using alcalase 2% (v/w) at 50 C, pH 9.5 gave the highest protein (2.44%) and yield (32.09%) in extracted solution compared to other enzymes. Rice bran protein concentrate powder prepared by a precipitation step using alginate (protein in solution: alginate 1:0.016) exhibited the highest protein (27.55%) and yield (6.84%). Precipitation using alginate was better than that of acid. RBPC extracted with alkaline (ALK) or enzyme alcalase (ALC), then precipitated with alginate (AL) (samples RBP-ALK-AL and RBP-ALC-AL) yielded the precipitation rate of 75% and 91.30%, respectively. Therefore, protein precipitation using alginate was then selected. Amino acid profile of control sample, and sample precipitated with alginate, as compared to casein and soy protein isolated, showed that control sample showed the highest content among all sample. Functional property study of RBP showed that the highest nitrogen solubility occurred in pH 8-10. There was no statically significant between emulsion capacity and emulsion stability of control and sample precipitated by alginate. However, control sample showed a higher of foaming capacity and foaming stability compared to those of sample precipitated with alginate. The finding was successful in terms of minimizing chemicals used in extraction and precipitation steps in preparation of rice bran protein concentrate. This research involves in a production of value-added product in which the double amount of protein (28%) compared to original amount (14%) contained in rice bran could be beneficial in terms of adding to food products e.g. healthy drink with high protein and fiber. In addition, the basic knowledge of functional property of rice bran protein concentrate was obtained, which can be used to appropriately select the application of this value-added product from rice bran.

Keywords—Alginate, carrageenan, rice bran, rice bran protein.

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RICE bran is an under-utilized milling by-product of rough rice. At the present, a large amount of rice bran has been discarded and used as animal feed. Several studies reported a nutritional quality of rice bran, which composes of protein, fiber, vitamins, and minerals. The protein found in rice bran is reported approximately 12-15%. Rice is a staple food as well as exported commodity of Thailand. Rice bran, a 10.5% constituent of rice grain, is considered as an under-utilized by-product of rough rice. At the present, a large amount of rice bran has been discarded and used as animal feed. The preparation of protein concentrate from rice bran is an alternative approach to prepare protein concentrate from a valuable by-product. Rice bran protein concentrate is suitable for those who are allergic of protein from other sources e.g. milk, wheat. In addition to its hypoallergenic property, rice bran protein also contains good quantity of lysine. Thus it may act as a suitable ingredient for infant food formulations while adding variety to the restricted diets of children with food allergies

Several researches have involved in attempt to increase utilization of rice bran as human foods. For the protein aspect, most of the literature available is focused on preparation and functional property study of rice bran protein. The commonly used preparation method of extracting rice bran protein is solvent extraction. The solvent extraction of protein from rice bran employs alkaline condition and then precipitate at the isoelectric point at pH 4.5 [1], which results in reduction of some nutrition in rice bran. In addition, the formation of compounds called lysinoalanine could also occur in such extraction.

Alkali extraction has been developed for the recovery of rice bran protein. However, the yield was only about 40% by using acid precipitation [2]. One alternative is the use of polysaccharides for protein precipitation such as the previous works by [3] in which carboxymethylcellulose and other anionic polysaccharides were employed to improve the precipitation of milk and soy whey proteins. Reference [4] has proposed an alternative way of protein precipitation using carrageenan and alginate. They reported that both alginate and carrageenan were found to be effective in precipitating rice bran protein. At pH 3.5, the maximum amount of protein precipitated were 95% for a carrageenan-to-protein ratio of 2:1, and 93% for an alginate-to-protein ratio of 1:1. However, change of temperature (20–55 °C) did not have a significant effect ($p>0.05$) on the amount of protein precipitated. Therefore, to further study about using alginate and carrageenan in rice bran protein precipitation, this study aimed to increase value of defatted rice bran as obtained after

extracting of rice bran oil. The objectives of this study were to compare extraction of protein from defatted rice bran using enzymes together with precipitation step using polysaccharides (alginate and carrageenan) to those used in a conventional method. Besides producing a value-added product, the output of this research was to obtain rice bran protein concentrate which can be used as nutraceutical food ingredient in different food products.

II. MATERIAL AND METHOD

A. Material

Rice bran was freshly obtained after solvent extraction of rice bran oil from a rice bran oil production factory in Singburi province, Thailand. Enzyme used in this study was alcalase (novozymes®), a mixture of enzymes mainly proteases, with optimum pH, temperature, and digesting time of 9.5, 50°C, and 120 min., respectively.

B. Methods

1. Preparation of Rice Bran Extract

The procedures of alkaline extraction and acid precipitation employed were the modification of [5] Alkaline extraction followed by isoelectric precipitation.

Enzymatic extraction was used to prepare rice bran extract at the optimum conditions (pH 9.5, 50 C, and 120 min. digesting time).

Precipitation by carrageenan or alginate (modification from [4] was performed by varying ratio of protein: polysaccharide (1:1, 1:1.5, and 1:2) and the percent protein precipitation was measured.

2. Precipitation Using Polysaccharides

The process was a modification from that reported by [4]. The clear fraction obtained from alkaline and enzyme extraction was subjected to pH adjustment using 0.1M HCl until the pH reached 3.5. Carrageenan or alginate was added in the varying protein: polysaccharide ratio (1:1, 1:1.5, and 1:2) at the selected conditions (55 °C, 30 min and centrifuge at 10,000g, 25 °C for 15 min.).

3. Nitrogen Solubility

Nitrogen solubility was determined by the method of [5]. Sample (1 g each) was dispersed in deionized water. The pH was adjusted from 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0 using either HCl 0.1 N or NaOH 0.1 N. Sample was then shaken at 250 rpm for 30 min. at room temperature and then centrifuged at 5000 g 15 min. The nitrogen content (NS) of the supernatants was determined by the Kjeldahl method and percent nitrogen solubility was calculated as in [1].

$$\% \text{ NS} = \frac{\text{Nitrogen in the supernatant (mg)}}{\text{Total nitrogen in a sample (mg)}} \times 100 \quad (1)$$

4. Foaming Property

Foaming property was determined by the method of [6]. For foaming capacity, sample (1.5 g) was dispersed in deionized water then adjusted pH to 7.0. The volume was then adjusted

to 50 ml. The solution was transferred to a blender and mixed with the highest speed for 3 min. and then transfer to a graduate cylinder. From the initial volume was previously measured, the foaming capacity was calculated as in [2].

$$\text{Foaming capacity (\%)} = \frac{\text{Volume after mixing} - \text{initial volume}}{\text{Initial volume}} \times 100 \quad (2)$$

For foaming stability, the time that takes for volume of foam to decrease to half of initial volume was measured. The foaming stability was calculated as in [3].

$$\text{Foaming stability (\%)} = \frac{2t \times 50}{V_m} \quad (3)$$

where t is the time taken for volume of foam decreased to half of initial volume, V_m is the maximum volume, and 50 is the initial volume (ml).

5. Emulsifying Property

Emulsifying property was determined according to the method described by [7]. Approximately 0.5 g. of sample was weighted. Add deionized water to volume of 50 ml and then adjust pH to 7.0. Transfer 25 ml into a centrifuge tube and add 25 ml of soy bean oil. The mixture was transferred to a vortex mixer to form emulsion. The fixed quantity of emulsion (50 ml, VT) in a tube was leaf in a sonicator for 30 s and centrifuged at 2,500 g for 30 min. The volume of emulsified fraction (VF1) was recorded. The tube containing the oil in water emulsified fraction was heated in a water bath at 70°C for 45 min and cooled to room temperature. Upon cooling, the tube was centrifuged again and the volume of the remaining emulsified fraction (VF2) was recorded. EC and ES were reported as in [4] and [5], respectively.

$$\text{Emulsion capacity(\%)} = \text{VF1/VT} \times 100 \quad (4)$$

$$\text{Emulsion stability(\%)} = \frac{\text{VF2}}{\text{VT}} \times 100 \quad (5)$$

III. RESULTS AND DISCUSSION

A. Extraction of Protein by Alkaline (ALK)

In order to select the appropriate ratio of protein and polysaccharides used in RBPC extracted by alkaline, the different ratio or either alginate or carrageenan was studied and results shown in Fig. 1. It was found that the optimum ratio of protein with both carrageenan and alginate was 1:1.5, resulting in protein precipitation of 56.03% and 75.00%, respectively. Therefore, this ratio was selected to prepare RBPC extracted with alkaline and compare precipitating agent (acid and polysaccharides) and the results are shown in Table I. It is generally known that the average isoelectric point was reported to be about pH 4–5.5 [2], [8], [9]. In this study, the experiment was carried out below pH 4 so that protein will have a net positive charge. Two anionic polysaccharides (alginate and carrageenan) were applied as a precipitating agent. From the table, it was found that RBP-ALK-AL

exhibited the highest protein content (22.42%) as well as the percent yield (4.39%).

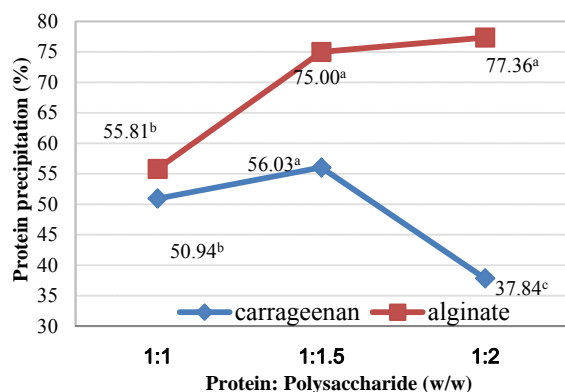


Fig. 1 Percent protein precipitation using alginate or carrageenan of rice bran protein concentrate extracted by acid

TABLE I
PROTEIN CONTENT AND PERCENT YIELD OF RICE BRAN EXTRACTED BY ALKALINE (ALK) AND PRECIPITATED BY ACID OR POLYSACCHARIDES

Sample	Protein content (g/100 g Protein powder)	Percent yield (g/100g rice bran)
RBP-ALK-ACID	19.40 ^b ±1.00	1.48 ^b ±0.01
RBP-ALK-CAR (protein: carrageenan, 1:1.5)	ND	0.46 ^c ±0.04
RBP-ALK-AL (protein: alginate, 1:1.5)	22.42 ^a ±0.25	4.39 ^a ±0.30

Different letters in column indicate significant difference ($p \leq 0.05$)

B. Extraction of Protein by Enzyme Alcalase (ALC)

Similar to the previous study, the optimum ratio between protein and carrageenan or alginate was conducted and results shown in Figs. 2, 3, respectively.

It was found that optimum protein: carrageenan and protein: alginate and were 1:0.25 and 1:0.016, respectively. When using higher protein: polysaccharide ratio, it showed that protein precipitation decreased, which could due to the insufficient amount of protein to bind with polysaccharide molecules. Protein content and percent yield of rice bran extracted by enzyme alcalase and precipitated by acid or polysaccharides are shown in Table II. As in the case of extraction by alkaline, a similar trend was found in which RBP-ALC-AL exhibited the highest protein content (27.55%) as well as the percent yield (6.84%) In the interaction of

protein and polysaccharide, [10] have reported that parameters such as pH, ionic strength, conformation, charge density and concentration of the biopolymers play significant roles during precipitation.

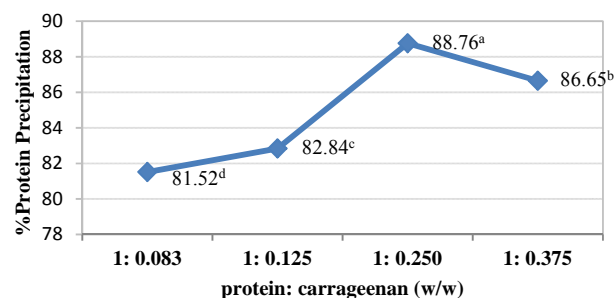


Fig. 2 Percent protein precipitation using carrageenan of rice bran protein concentrate extracted by enzyme alcalase

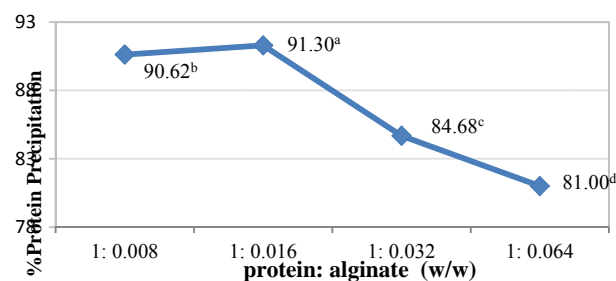


Fig. 3 Percent protein precipitation using alginate of rice bran protein concentrate extracted by enzyme alcalase

TABLE II
PROTEIN CONTENT AND PERCENT YIELD OF RICE BRAN EXTRACTED BY ENZYME ALCALASE (ALC) AND PRECIPITATED BY ACID OR POLYSACCHARIDES

Sample	Protein content (g/100 g Protein powder)	Percent yield (g/100g rice bran)
RBP-ALC-ACID	25.05 ^b ±0.23	5.54 ^a ±0.99
RBP-ALC-CAR (protein: carrageenan, 1:0.25)	23.40 ^a ±1.66	3.56 ^b ±0.55
RBP-ALC-AL (protein: alginate, 1:0.016)	27.55 ^a ±0.53	6.84 ^a ±0.56

Different letters in column indicate significant difference ($p \leq 0.05$)

TABLE III
CHEMICAL COMPOSITIONS OF RICE BRAN EXTRACTED BY ALKALINE OR ENZYME ALCALASE AND PRECIPITATED BY ACID OR ALGINATE

Samples	Chemical composition (%)					
	Protein	Moisture	Ash	Fiber	Fat	Carbohydrate
Rice bran	14.83±0.18	6.98±0.52	10.37±0.07	8.98±0.25	0.74±0.01	58.07±2.85
RBP-ALK-ACID	20.54 ^d ±1.86	7.06 ^a ±1.11	3.48 ^c ±0.43	1.07 ^a ±0.47	11.75 ^a ±0.50	56.06 ^{bc} ±0.34
RBP-ALK-AL	22.42 ^c ±0.25	6.30 ^{ab} ±0.09	5.97 ^a ±0.14	0.67 ^{ab} ±0.22	5.46 ^b ±0.32	59.16 ^a ±0.41
RBP-ALC-ACID	25.05 ^b ±0.23	6.02 ^{ab} ±0.22	4.48 ^b ±0.86	0.45 ^c ±0.01	10.35 ^b ±0.31	53.36 ^c ±1.12
RBP-ALC-AL	27.55 ^a ±0.53	5.49 ^b ±0.06	5.68 ^a ±0.05	0.68 ^{ab} ±0.19	1.76 ^d ±0.58	58.81 ^{ab} ±0.36

Different letters in column indicate significant difference ($p \leq 0.05$)

C. Properties of Selected RBPC

From the results obtained previously in Tables I, II and Figs. 1-3 as to compare the extraction and precipitation method, the four samples of RBP were selected, including RBP-ALK-ACID, RBP-ALK-AL, RBP-ALC-ACID and RBP-ALC-AL. Those selected samples were then analyzed for chemical composition (Table III), amino acid profile (Fig. 4), nitrogen solubility (Fig. 5) and emulsifying and foaming properties (Fig. 6, 7).

From Table III, it was found that original protein content in rice bran (14.83%) increased approximately two folds in RBP-ALC-AL (27.55%) The range of moisture, ash, fiber, fat, and carbohydrate in four samples was reported as 5-7%, 3-6%, 0.5-1%, 1-12%, and 53-59%, respectively. Protein content in samples: RBP-ALK-ACID 20.54% RBP-ALK-AL 22.42% RBP-ALC-ACID 25.05% RBP-ALC-AL 27.55%. From the table, it was found that precipitation using acid yielded higher amount of amino acid compared to those found when alginate was used as a precipitating agent. Reference [11] studied protein extraction from rice bran using phytase and xylanase and precipitated by acid, resulting in 90.72% protein content and 74.60% yield, which were much higher than those found in this study. This could be due to different rice bran properties as well as the defatting step prior to protein extraction. The raw material used in this study was obtained from a rice bran oil extraction plant in which a solvent extraction and high temperature were involved in the process. These conditions could potentially affect the properties of defatted rice bran e.g. composition and arrangement of molecules, which resulted in less protein content and yield obtained in this study.

Amino acid profiles of different rice bran protein concentrate samples are shown in Fig. 4. It should be noted that amino acid content shown were based on different protein content in samples (as mentioned in remarks). From the figure, it was found that RBPC obtained in this study contained fewer amount of amino acids (except proline) compared to those of commercially available casein and soy protein isolate. However some essential amino acids; for examples threonine valine tyrosine histidine and arginine, were found in RBPC obtained in this study.

The result of nitrogen solubility (Fig. 5) showed that the highest nitrogen solubility occurred in pH 8-10 and the minimum solubility occurred at pH 4. It is generally known that pH 4.5 is a protein isoelectric point (pI) indicating that protein has minimal solubility at this point. Results of emulsion capacity and emulsion stability (Fig. 6) were found in the same trend, in which RBPC extracted by enzyme alcalase showed higher emulsion capacity and stability compare to those extracted by alkaline. Surface hydrophobicity was reported as a factor associated with emulsion properties. References [12] and [13] reported that rice bran protein concentrate exhibited lower emulsion capacity and stability compare to Bovine Serum Albumin (BSA) due to lower hydrophobicity value.

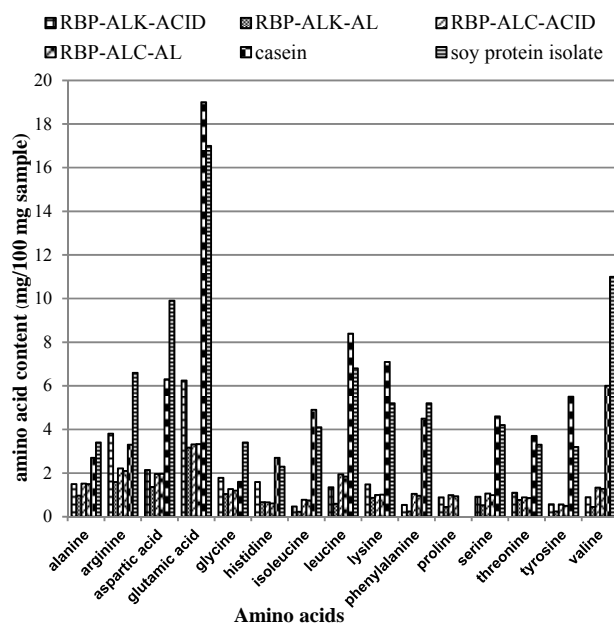


Fig. 4 Amino acid profiles of RBPC concentrate extracted by alkaline or enzyme alcalase and precipitated by acid or alginate compared to those of casein and soy protein isolate. Data for casein and soy protein isolated obtained from Standard Tables of Amino Acid Compositions of Food in Japan [14]

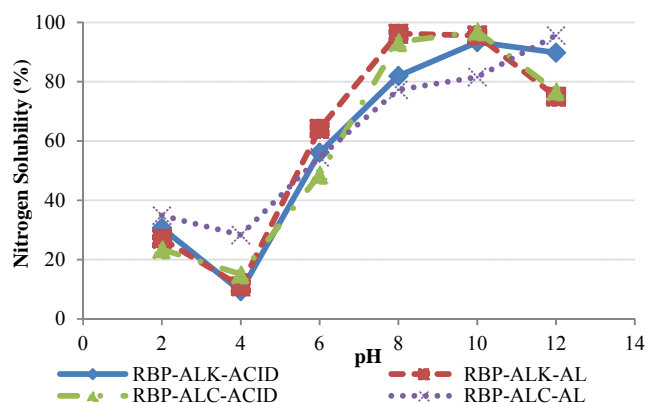


Fig. 5 Percent nitrogen solubility of rice bran protein concentrate at different pH

It was also noted that extraction using enzyme alcalase resulted in samples that show higher solubility as well as emulsion capacity. References [15] and [16] reported that solubility is well correlated with emulsion capacity. This could be explained by the fact than enzyme hydrolyzed bonds of protein to smaller fractions, resulting in better solubility and emulsion capacity [11].

Fig. 7 shows that RBP-ALK-ACID exhibited the highest foaming capacity (43.06%), followed by RBP-ALK-AL, RBP-ALC-ACID and RBP-ALC-AL ($p > 0.05$) (32.74%, 17.04% and 13.43%), respectively. The same trend was also found for foaming stability, in which RBPC extracted by alkaline had the higher foaming stability compared to those extracted by alcalase. This is due to the small molecular weight of proteins

hydrolyzed by enzyme can form a thin layer and easily degraded surrounded gas bubbles.

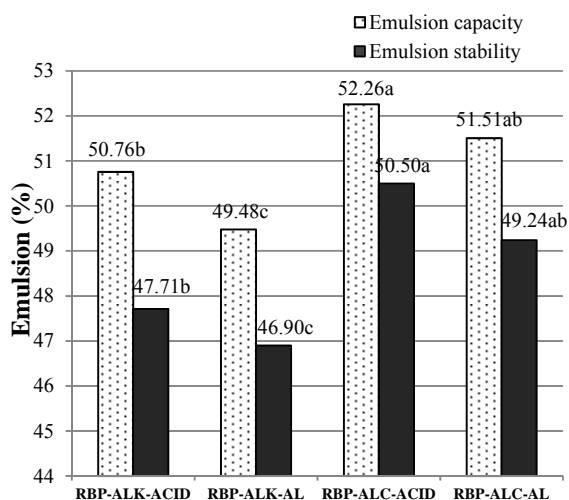


Fig. 6 Emulsion capacity and stability of rice bran protein concentrate

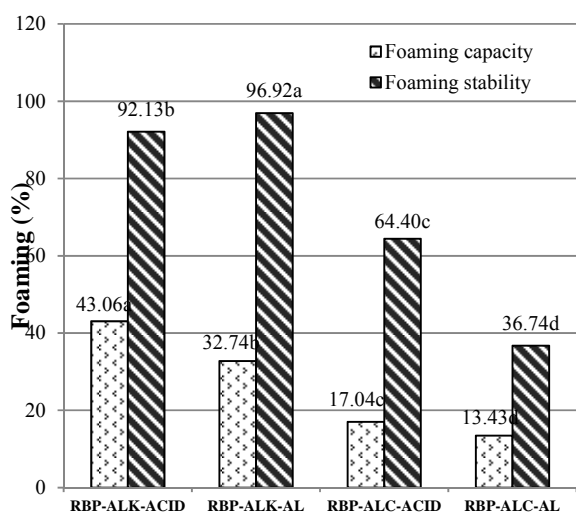


Fig. 7 Foaming capacity and stability of rice bran protein concentrate

It was reported that protein-polysaccharide complex obtained by using polysaccharide as a precipitating agent may be used in food formulation as emulsifiers and foam stabilizers, as well as applications as microencapsulation of drug and new biomaterial in medicine grafts, food protection and food packaging [4], [16].

IV. CONCLUSION

Extraction of protein from defatted rice bran, a by-product of rice bran oil manufacturing process was made possible, which can increase value-added to the raw material. Two polysaccharides including carrageenan and alginate that are commonly used in food products as gelling agents, thickeners and stabilizers were employed as the agents for the precipitation of protein.

Extraction using enzymes and polysaccharides can produce

rice bran protein concentrate with limited amount of chemicals involved in the process. As they are normally used ingredients in food, enzymes and polysaccharides used can help minimizing chemicals compared to a conventional method in which alkaline and acid are used. However, as a high cost of enzymes as well as low yield, which could be partially due to quality of a raw material itself which was a result of solvent and high heat treatment used in step of oil extraction from rice bran, it is recommended to conduct further study to solve problems and determine break-even point of production should be considered in the case of up-scale production in an industrial level.

ACKNOWLEDGMENT

The authors greatly appreciated the Agricultural Research Development Agency (Public Organization) for the financial support of this research.

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