EXTRACTABILITY OF COCONUT PROTEIN AND CHANGES IN ITS GEL FORMING PROPERTIES ON HEATING

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Abstract: Fresh mature coconut kernels contain about 4 percent protein. The protein efficiency ratio, biological value, net protein utilization and digestability values suggest a high nutritional quality for coconut protein. The water extract of coconut referred to as coconut milk is used in the preparation of daily meals in Sri Lanka. Incorporation of coconut milk into soya milk in the preparation of gels like tofu enhance the calorific value of the final product and improves the nutritive value, acceptability and palatability. However, the conditions of extraction of coconut milk to obtain appropriate concentration of protein and functional properties suitable for gel formation needs investigation. In this study, the extractability of protein into the coconut milk was tested by determining the total protein by Kjeldhal procedure; changes of coconut protein on heating was determined by SDS polyacrylamide gel electrophoresis and ultracentrifugal technique and the textural properties of the gel were determined using a Tensipresser. The extractability of coconut protein was seen to be higher (15.5 mg/100 ml) with a solution of 0.2 M sodium chloride at an extraction ratio of 1:1 at room temperature, than with water (9.5 mg/100 ml) under the same conditions. The gel forming ability of coconut proteins was seen to be highest around the isoelectric point at a temperature of 80°C. An increase in turbidity of protein solutions was also observed under the above conditions. A change in the protein components 1.5 S and 7 S was observed at 80°C, at which the gel formation properties were seen to be the best.

1. Introduction

Coconut is the fruit of the palm *Cocos nucifera* Linn.. It is an economically important member of the Palmae and is grown over a wide geographical area. The fresh white meat which is the endosperm or the kernel containing 35 - 40 percent oil and 4 percent protein is used as it is, or is extracted to obtain coconut milk in the preparation of daily meals in Sri Lanka. It forms one of the main sources of protein in the Sri Lankan diet.

Studies have indicated that the protein efficiency ratio of coconut protein is comparable to that of casein.¹ The protein efficiency ratio, biological value, net protein utilization and digestability values suggest good nutritional quality of coconut protein.² Incorporation of coconut milk with soy milk in the preparation of gels, like

in tofu, would enhance the calorific value of the final product, improves the nutritive value and increases the acceptability and palatability.

The gel forming ability of coconut milk depends on the concentration and the functional properties of coconut protein. Therefore, suitable conditions of extraction and the effect of temperature on the changes of protein is a pre-requisite in the development of such products.

Previous attempts on the extraction of coconut protein has been made using defatted meal.³ These results indicate that the functional properties and solubility characteristics of coconut protein isolates change with the method of extraction. The studies also indicate that coconut protein displays a pH dependant nitrogen solubility profile in salt solutions showing minimal solubility at acidic pH values (pH 3), with a sharp rise in solubility with a maximum at neutrality. The point of minimum solubility has been found at pH 3.9^{3,4} in citrate phosphate buffer.^{5,6} The nitrogen solubility increases with increasing acidity and alkalinity. The possibility of the presence of two main protein fractions soluble at pH 8.0 - 8.5 and at 10.5 - 11.0 is also suggestive from the studies of Samson Khaund.²

Coconut meal protein, according to Osbourne classification, has shown that 90 percent of the protein would be albumins and globulins of which 61.9 percent globulin is reported to be extracted in IM NaCl at pH 7.0. The studies on nitrogen solubility index of heated coconut meal suggest that fresh coconut meals can tolerate up to 100°C air temperature and coconut layer temperature of 78° - 83°C without significant loss of protein solubility. The protein solubility of coconut meal upon heating with hot air at 120°C is shown to decrease significantly.

A chromatographic separation of coconut protein by Sephadex G-200, has shown the presence of two major protein components having approximate molecular weights of 1.5×10^5 and 2.4×10^4 respectively.⁷ The heat coagulation of the large molecular weight component has been observed to be maximum at neutral pH, low salt concentration and high temperature.

The low molecular weight proteins were found to be quite soluble and heat stable. Overlapping protein peaks corresponding to molecular weight 15000 and 23000 daltons has been reported from acid extracts of copra meal on Sephadex G-200 chromatography.⁸

Various conditions such as the use of completely ripe nuts,⁹ the effect of amount and temperature of water^{10,11,12,13,14,15} affecting the extraction of coconut milk from fresh coconut has been reported by many authors. However, the conditions of extraction of coconut milk with an appropriate concentration of protein and functional properties suitable for gel formation still remains to be investigated. In this study, the extractability and changes of coconut milk protein on heating and their subsequent behaviour in the formation and properties of gels were examined.

2. Materials and Methods

2.1 Materials

The raw, mature coconuts used in this study were imported to Japan from Phillipines.

The coconuts were plucked in Phillipines and air freighted to Japan. The experiments commenced after 7 days of plucking at which stage they were green in colour and contained water in the inner cavity.

2.2 Preparation of Samples

Whole extract: The nuts were cracked and the water discarded. The coconut kernel was removed from the shell, pared and homogenised with water, or with a solution of sodium chloride in the required proportion of 1:0.5, 1:1 and 1:2. The homogenisation was carried out for one minute at low speed and another minute at high speed using a Waring blender. The resulting slurry was filtered through a cloth to obtain milk.

Supernatant 1: The whole extract was centrifuged at 10,000 rpm for 10 minutes, to remove the cream layer. The resulting supernatant is referred to as supernatant 1.

Supernatant 2: The supernatant 1, was heated and centrifuged at 10,000 rpm for 10 minutes to obtain supernatant 2.

Precipitate : The precipitate obtained on centrifuging supernatant 1, after heating is referred to as the precipitate.

Heating: All samples were heated in 10 ml aliquots, for 10 minutes immersed in a water bath equilibrated at the specific temperatures of 60°C, 70°C, 80°C, 90°C and 100°C. The samples were cooled in ice.

Preparation of gels: The gels were prepared in test tubes using 10 ml aliquots of whole extract, in the ratio 1:1 parts weight by volume of kernel with water or with salt. Freshly prepared glucono delta lactone (G.D.L) was used as the coagulating agent at varying concentrations of 0.1 to 1.0 percent. Precautions were taken to prevent hydrolysis of G.D.L. before heating. With added G.D.L., all the samples were shaken and left immersed in a water bath maintained at 80°C for one hour, and cooled immediately in ice.

2.3 Methods of Analysis

2.3.1 Polyacrylamide Gel Electrophoresis

The method of Kitamura *et al.*¹⁶ was modified as follows. A gel containing 10 per cent acrylamide was prepared from a stock solution of 30 per cent by weight of acrylamide and 0.8 per cent by weight of N-N bis methylene acrylamide. The final concentration in the separation gel was as follows; 0.070 M phosphate buffer (pH 7.0) and 0.12

per cent SDS. The gels were polymerised chemically by the addition of 45 μ l of tetramethyl ethylenediamine (TEMED) and ammonium peroxydisulphate. One mm thick slab gels of total length 12 cm. were prepared. The samples for electrophoresis was prepared with a final concentration of 5M urea, 2 per cent SDS and 2 per cent 2-mercaptoethanol and the samples were left overnight at room temperature. A sample volume of 20 μ l was used for electrophoresis. The electrophoresis was carried out at constant voltage with a current of 100 mA.

2.3.2 Ultracentrifugal Analysis

The samples were prepared by adding phosphate buffer and mercaptoethanol to make the pH, ionic strength and concentration of mercaptoethanol in the final solution as 7.6, 0.05 and 0.01 respectively. The analysis was carried out at 55,000 rpm at 20°C, using the Hitachi UCA-1A ultra-centrifuge. A 12 cm single sector cell and a single sector plus 1 wedge cell were used. The pictures were taken at an exposure interval of 9 seconds. The changing pattern of the composition of protein solution was determined calculating the sedimentation value (S20W).¹⁷

2.3.3 Determination of Total Protein

The total protein in the samples was determined by Kjeldhal procedure using M.C.I.-TN-01-C analyser (Mitsubishi Chemical Ind).

2.3.4 Analysis of Gels for Determining Textural Properties

The gels were analysed for the pH and gel hardness. The elasticity was measured using the Tensipresser (Model TP 501), at a bite speed of 40, multiplication 5, range 0.5 and chart speed of 1500 mm/min. The water holding capacity was measured as the percentage gel remained after discarding the whey on centrifugation at 1,500 rpm for 15 min.

3. Results and Discussions

3.1 The Effect of Ratio of Extraction of Coconut Milk on Protein Extractability

The total protein extracted (mg/ml x volume) was found to be significantly higher in 0.1 M sodium chloride at the ratio of 1:1 with no significant difference between 1:1 and 1:2 in the water extract (Figure 1).

Figure 1: Effect of ratio of kernel to water/0.1M sodium chloride in extraction of coconut milk on protein concentration (●—● 1:0.5 H₂O, ○—○ 1:1 H₂O, □—□ 1:2 H₂O, ▲—▲ 1:1 0.1M NaCl. RT = 20°C.

3.2 Effect of Sodium Chloride on the Extractability and Solubility of Protein at Different Temperatures

The supernatant 2, obtained with water, 0.1M NaCl, 0.2M NaCl and with 0.4 M NaCl in the ratio of 1:1 at room temperature, was used for analysis. A significant increase in soluble protein extracted was seen with 0.2 M NaCl and 0.4 M NaCl. The turbidity of the supernatant of water extract and salt extract increased with increase in temperature up to about 80°C, the turbidity being high in the salt extract. An increase in turbidity was observed simultaneously with decrease in protein solubility (Figure 2). Complete denaturation of the proteins in the water extract was observed at 80°C, where as in the salt extract, denaturation was incomplete even at 90°C. The coagulum began to appear on heating at 80°C and increased with rapid decrease in protein solubility in the salt extract and to a lesser extent in the water extract with increase in temperature.

Figure 2: Effect of sodium chloride on the extractability and solubility of proteins. (●---● 0.1M NaCl, □---□ 0.2M NaCl, ▲---▲ 0.4M NaCl, ○---○ H₂O extract). RT = 20°C.

3.3 Effect of Heat on Changes of Protein as Determined by SDS-PAGE Electrophoresis

The supernatant 2 and the precipitate of water extract and 0.1 M NaCl extract (1:1) were used for electrophoresis. On comparing the electrophoretic patterns of the unheated protein of the water extract (A) and salt extract (B) of supernatant 2, three main bands were seen in both extracts at room temperature (Figure 3). The concentration of high molecular weight component was higher in the salt extract. The changes of protein in the supernatant 2 of water extract on heating occurred in three stages. A significant change, from the native state was observed around 80°C (Figure 3). The high molecular weight component disappear at 90°C, with the appearance of two new components of low molecular weight and a simultaneous increase in the high molecular weight component in the precipitate. At 100°C, the newly formed high

molecular weight component disappeared in the supernatant 2, with a significant increase of the same in the precipitate.

Changes of protein in supernatant 2 of salt extract (0.1 M NaCl) on heating, was not significant until 90°C. At 90°C a decrease in the high molecular weight component with simultaneous appearance of a low molecular weight component in the supernatant 2, and of high molecular weight component in the precipitate occurred. The complete disappearance of the high molecular weight component was seen at 100°C (Figure 3 B, supernatant 2).

However, on comparing the changes of protein in the water extract with that of the salt extract the high molecular weight component in the water extract disappeared at 90°C, where as it remained at 90°C in the salt extract, but disappeared at 100°C.

The cream layer obtained from coconut milk by separating the supernatant 1, showed a slight increase in the high molecular weight component on SDS-PAGE electrophoresis at temperatures beyond 80°C (Figure 3). On comparing the cream layer proteins in the water extract with that of the salt extract using the SDS-PAGE electrophoretic pattern, the concentration of protein in the cream layer in salt extract appeared to be lower than that of the water extract (Figure 3). This is in contrast to the supernatant.

3.4 Effect of Temperature on the Changes of Protein in the Water Extract and Salt Extract as Determined by the Ultracentrifugal Analysis

The ultracentrifugal analytical patterns of the water extract (A) and 0.1 M NaCl extract (B) at room temperature and after heating to 80°C and 100°C are shown in Figure 4. The protein components of 1.5 S and 7 S were seen in the ultracentrifugal patterns of both extracts at room temperature and at 80°C. However a higher concentration of 7S proteins was evidenced with a larger peak in the 0.1 M NaCl extract, at room temperature. Both 1.5 S and 7 S proteins were stable at 80°C. A change in the pattern was observed on heating at 100°C. With the complete disappearance of the 7 S component, in the water extract and its slight presence in the 0.1M NaCl extract. In contrast, the 1.5 S proteins were stable even at 100°C.

3.5 Gel Formation Characteristics of coconut Milk

All the gels prepared using the whole extract of water separated in to a cream phase and to an aqueous phase. Around pH 4.5, the turbidity of the aqueous phase increased. On cooling the gels, the hardness of the cream layer increased due to solidification of fat. In order to prevent the discrepancies resulting from creaming, the gel formation ability of the water extract was measured by the water holding capacity.

Figure 3: SDS - PAGE Electrophoretic patterns of supernatant 2, precipitate and cream layer, of water extract (A) and 0.1M NaCl extract (B).

RT (20°C)

Top- 80°C , Bottom- 100°C

(A)

RT (20°C)

Top 80°C , Bottom- 100°C

(B)

Figure 4: The Effect of heating on the ultracentrifugal patterns of coconut protein in supernatant 2 of, (A) water extract at RT, 80°C and 100°C , (B) 0.1M NaCl extract at RT, 80°C , 100°C .

RT = 20°C

- (a) 1.5 S component
- (b) 7 S component

3.5.1 Effect of Temperature of Coagulation and pH on Water Holding Capacity

Figure 5 shows the water holding capacity of gels prepared with whole extract of water, as a function of gel pH at different temperatures. The water holding capacity of the gels was highest around the isoelectric point of coconut protein (around pH 4.0). A significant increase in the water holding capacity occurred at temperatures around 80°C to 100°C.

3.5.2 Effect of Temperature of Coagulation and pH, on Gel Forming Characteristics of Salt Extract

Gels were prepared using the whole extract of 0.1 M NaCl at 80°C. In contrast to the gels prepared using the whole extract of water, the gels were homogenous without evident creaming. The hardness and elasticity was found to be highest around pH 3.8 (Figure 6), with a steep decrease observed beyond. The water holding capacity of the gels were maximum around pH 4.0 to 4.2 (Figure 5).

The extractability of coconut protein was higher in salt solution, when compared with water (Figure 1, 2). This may be expected, as major storage protein in coconuts is globulin which is soluble in salt solutions. The optimum protein extractability was seen with a concentration of sodium chloride around 0.2 M at room temperature, with no significant difference being observed at 0.4 M. A decrease in protein solubility occurred rapidly at higher temperatures.

The thermocoagulation of proteins is caused by intermolecular interactions. Heating causes denaturation of protein, followed by protein-protein interactions. The phenomenon of coagulation may be described as observed visually as follows:-

The original protein solution (native protein) which changed to a turbid solution (denatured protein) was converted into a coagulum on further heating. Since the coagulum or the insoluble aggregate was formed with increase in turbidity during heating, it seems likely that coconut protein contain many hydrophobic amino acids. The hydrophobic groups exposed during heating, largely contribute to net work formation for hydrophobic interactions and is favoured by rise in temperature.¹⁸ This phenomenon occurred to a greater extent in the salt extract as compared to the water extract. This may be due to sodium chloride, having a greater affinity for water, depleting interactions between the amino acids, and causing a change in the native state of the protein molecules thus exposing the hydrophobic residues. Amino acid residues containing S-S bonds and SH groups being mostly hydrophobic, this situation is appropriately met as the temperature is increased.

The coagulum started forming above 70°C in the water extract and 80°C in the salt extract (Figure 3) due to ionic strength, which delayed the protein denaturation.¹⁹

Figure 5: Water holding capacity as a function of pH of gels prepared using whole extract of water and 0.1M NaCl at different coagulating temperatures. Whole extract of water.
 □---□ 40°C, △---△ 60°C, ○---○ 80°C, ○---○ 100°C. Whole extract of 0.1M. NaCl 80°C.

Figure 6: Hardness and elasticity as a function of pH of gels prepared using the whole extract of 0.1M NaCl 80°C. △---△ Hardness ○---○ Elasticity

The amount of protein that remained in the cream layer of the salt extract (Figure 3) was less than that of the water extract, indicating that the salt soluble proteins in the cream layer was extracted into the supernatant. The stability of the high molecular weight component in the salt extract at 90°C, may be attributed to the high ionic strength of solvent. This change in composition of coconut protein of supernatant 2, on heating to 100°C, with the disappearance of high molecular weight component as evidenced in SDS-PAGE electrophoretic patterns was substantiated by ultracentrifugal analytical pattern (Figure 4), which showed disappearance of 7 S protein in the sample heated to 100°C in the water extract and its almost total disappearance in the salt extract. The ultracentrifugal patterns of water and salt extracts at room temperature, revealed the presence of two major protein components of sedimentation rates 1.5 S and 7 S. This is in agreement with the previous studies.⁷

Moreover, the gel formation ability was also seen highest around pH 4.0 - 4.5, which is the isoelectric point of coconut proteins at which the interactions between proteins are high (Figures 5, 6). The fact that the water holding capacity of the whole-extract of water was high at 100°C, at pH values beyond the isoelectric point (Figure 6) may be explained as due to the fat-protein interactions.

The gel prepared using whole extract of 0.1 M NaCl, had a higher water holding capacity than the water extract (Figure 5). This revealed that the interactions between proteins were enhanced in the presence of sodium chloride, resulting in a better gel net work formation.

In conclusion, the highest extractability of coconut proteins under the conditions of study was seen with a solution of 0.2 M sodium chloride at an extraction ratio of 1:1 with kernel at room temperature. The gel forming ability of coconut proteins was highest around the isoelectric point at a temperature of 80°C (Figures 5, 6). It was also evidenced that the turbidity of the protein solutions being high at the above conditions, the reactivity of proteins was at a maximum resulting in the formation of coagulum. Moreover, both 1.5 S and 7S proteins changed partly at 80°C, around which the gel formation properties appeared best. This indicated a close relation between the changes of protein and gel formation.

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