Chapter 5

Coconut Protein Powder
Chapter 5A

Production of Coconut Protein Powder and its Characterization
5A.1. Introduction

Coconut, *Cocos nucifera*, is one of the most economically important palm species and is cultivated mainly for the endosperm. The fresh coconut kernel will have about 37% oil and 4% (w/w) protein. In literature, there are several methods for oil and protein extraction from coconut meal. However, the conditions employed affect the quality of the product. Hagenmaier et al. (1973) has reported that it has been possible to recover good quality oil as well as food grade protein without denaturation, especially with developments in several wet processing methods.

Expelling is used to produce coconut oil traditionally from copra, the dehydrated coconut. However, in recent times, a superior product, virgin coconut oil (VCO) has been gaining popularity. Wet processing without any thermal treatment was employed for the development of a process for the production of VCO from fresh coconut in order to retain the flavour and nutrients in VCO (Raghavendra et al., 2009). Three main steps are involved in wet processing of coconut for virgin oil production: (i) Expelling of coconut milk (emulsion), (ii) Fractionation of coconut milk into cream and skim milk and (iii) Breaking of the cream emulsion to recover oil. Different methods based on thermal, pH, chilling, enzyme treatment and also combination of enzyme treatment followed by chilling and thawing have been employed for effective destabilization of the coconut milk emulsion. In order to gain insight into the destabilization mechanism and to examine the efficacy of the different methods employed, creaming behaviour and microstructure analysis have been studied (Raghavendra and Raghavarao, 2010, 2011). The coconut skim milk that eventually remains on centrifugation of the whole coconut milk for
the separation of VCO, is a byproduct. Aseptic packaging or spray drying can be employed for further processing for value addition (Hagenmaier et al., 1975).

During wet processing for VCO production, two principal protein fractions are obtained. The coconut skim milk is the first one which contains 70% of the total protein while the insoluble protein or ‘protein solid’ being the second one containing 21% of the total protein (Hagenmaier et al., 1973). Isolation and concentration of these proteins has been carried out by many methods as described in section 4A.1. The coconut protein, besides being highly nutritious (Sreenivasan and Rajasekharan, 1967), has excellent functional properties such as foaming and emulsifying capacities (Onsaard et al., 2005). Furthermore, coconut protein isolates, like soy proteins or cheese whey proteins, as a result of some functional properties that they possess may also find added value.

The major byproducts of the virgin coconut oil industry are coconut skim milk, insoluble protein and coconut residue (left after extraction of coconut milk). An attempt was made to impart value addition to coconut skim milk and insoluble protein which otherwise cause environmental pollution on disposal.

Due to the renewability of raw material and variety of sources (especially legumes, cereals and oilseeds), the protein from vegetable origin is an attractive alternative to animal protein for food and cosmetics applications. Growing potential is shown for other non-food sources. However, surplus of vegetable protein is not there and at the moment most of the production is catered for the food industry (Moure et al., 2006). Currently, the coconut protein obtained during wet processing is either discarded or used as animal
feed. As the short time of heat contact and high rate of evaporation (at wet bulb temperature) gives a high quality product, the spray drying is one the most widely used commercial dehydration methods. Further, spray drying offers scope for continuous operation for the production of powders with precise specifications (Souza and Oliveira, 2006).

Accordingly, obtaining coconut protein powder (CPP) from coconut wet processing waste is the main focus of the present work. The physico-chemical and functional properties of the powder obtained after spray drying are evaluated.

5A.2. Materials and methods

5A.2.1. Materials

Mature coconuts (10-12 months) were procured fresh from local market. Skimmed milk powder, defatted soybean powder and refined soybean oil were procured from local departmental stores. Gallic acid and Folin Ciocalteau phenol reagent was purchased from Sigma Aldrich, St. Louis, USA and Sisco Research Laboratory Pvt. Ltd., Mumbai, India, respectively. Commercial grade enzyme aspartic protease [EC 3.4.23] (Activity: 2500 tyrosine units/g) of was purchased from Kaypeeyes Biotech Private Ltd., Mysore, India. All other chemicals of analytical grade were obtained from Merck chemicals, Mumbai, India.

5A.2.2. Preparation of coconut skim milk

Mature coconuts, freshly procured, were subjected to preprocessing operations of deshelling, paring (removal of testa) and removal of coconut water (manually). The white coconut kernels were grated using rotary wedge
cutter (Krauss Maffei, Germany). Coconut milk from these fresh coconut gratings was expelled using screw press. The coconut milk was subjected to enzymatic treatment using protease with 100 tyrosine units/liter of coconut milk) for about 2 h in order to achieve effective and faster destabilization of the coconut milk emulsion. Three fractions, namely, cream, coconut skim milk and solid protein were obtained by subjecting the enzyme treated milk to centrifugation (Model: SAOG 2016, Westfalia separator, Germany) at 7000 rpm. Subsequently, skim milk and solid protein was thoroughly mixed in the ratio 8:2 v/w and homogenized. Hand held refractometer (Erma, Japan) was used to measure the total soluble solids and expresses as ° Brix. Prior to the measurement, the instrument was adjusted to 0° using distilled water.

5A.2.3. Spray drying

The mixture of coconut skim milk and solid protein (without any additives) was thoroughly mixed and homogenised and fed into a spray dryer (Model: BE1216, Bowen, USA). The mixture was fed at a flowrate of 75 ml/min by a peristaltic pump and atomized into small droplets through a nozzle having 2 mm diameter at 3 bar air pressure in a co-current air flow system at room temperature (25 ± 2°C). The inlet air temperature and outlet air temperatures were set at 130 ± 2°C and 100 ± 2°C, respectively. Through a cyclone, the powder was collected and samples were taken for the purpose of analysis. The process flowchart for the preparation of Coconut Protein Powder (CPP) is shown in Figure 5A.1.
5A.2.4. Methods of measurement

5A.2.4.1. Proximate analysis

AOAC standard methods were employed for proximate analyses for moisture, ash, fat and protein (N X 6.25) contents of the CPP as described in section 4A.2.4 and expressed as % (w/w). Total carbohydrate content was calculated by difference.

5A.2.4.2. Total soluble solids

Total soluble solids were estimated according to the procedure described in AOAC standard method (AOAC, 2007) with some modifications. CPP powder (2.5 g) was dispersed in distilled water (100 ml) and mixed thoroughly using a magnetic stirrer for 2 h at room temperature (25 ± 2°C). The solution was then filtered through Whatman paper (No.1). 50 ml of the filtrate was dried in a hot air oven at 105 ± 2°C until constant weight was obtained. Total soluble solids are expressed as percentage of powder soluble in water under specified conditions.

5A.2.4.3. Protein solubility

The modified method described by Tang (2007) was used to determine the protein solubility (PS). Aqueous solutions of CPP (0.1%, w/v) were prepared by dissolving it in distilled water, 0.1M NaCl or 1M NaCl solutions. The solutions were stirred for 15 min and the pH was then adjusted from 3.0 to 10.0 with HCl or NaOH of high normality to limit dilution and stirred with magnetic stirrer for 1 h. Samples were centrifuged at 8000 g for 15 min at 25 ± 2°C and the protein content of the supernatant was determined by the Bradford method (Bradford, 1976) using bovine serum albumin as a standard.
The following equation was used for the calculation of percent protein solubility:

\[
PS (\%) = \left( \frac{\text{protein content of supernatent}}{\text{total protein content}} \right) \times 100. \quad \text{(5B.1)}
\]

The protein content of the solution (0.1%, w/v) in 0.1 N NaOH was used as the total protein content (or 100% protein solubility).

5A.2.4.4. Bulk density

The method described by Kwon et al. (1996b) was used to determine the bulk density of CPP. Powder was freely loaded up to the 100 ml mark in a 100 ml graduated cylinder and the weight was recorded. The bulk density was calculated as the ratio of mass to volume and is expressed as kg/m³.

5A.2.4.5. Water and fat absorption

Water holding capacity (WHC) of CPP was estimated according to the method described by Quinn and Paton (1979) and expressed as ml of H₂O absorbed/g of sample. 5 g sample was weighed in a transparent 50 ml centrifuge tube and the tube with the sample was weighed again. Small amounts of distilled water were added intermittently while stirring using spatula to form a paste. The sample was centrifuged at 2000 X g for 10 min. The small amount of supernatant was discarded and the weight was recorded. The weight difference per gram of dry sample is taken as the water hydration capacity (WHC). If no supernatant appeared on centrifugation, more water was added and centrifuged again. This procedure was repeated until the supernatant appeared.

Fat absorption capacity (FAC) was determined by the method described by Wang and Kinsella (1976) and expressed as oil bound (ml)/g sample. 1 g of
sample was added to 50 ml centrifuge tube. 10 ml of soybean oil was added and the contents were thoroughly mixed for 1 min using a vortex mixer. The sample was then centrifuged at 2000 X g for 10 min and the clear oil above the pellet was poured off. The weight was recorded and the difference in weight per gram of sample was given as fat absorbance capacity (FAC).

5A.2.4.6. Emulsifying properties

Emulsifying properties of sample powders were studied using method as described by Pearce and Kinsella (1978) as described in section 4A.2.6.1.

5A.2.4.7. Dispersibility

Dispersibility of the powder was determined according to A/S Niro Atomizer (1978) with some modifications. CPP (1 g) of sample was added to distilled water (10 ml) and mixed for 30 s manually using a teaspoon at room temperature (25 ± 2°C) until the powder was completely dispersed leaving no lumps at the bottom of the beaker. The solution was then passed through a 150 micron sieve. 1 ml of the filtrate was dried in hot air oven at 105 ± 1°C for 4 h. The quantity of powder passing through the sieve and being dispersed was found by the determination of total solids in the filtrate and dispersibility was estimated using the following equation:

\[
\text{Dispersibility (\%)} = \frac{(10 + a) \times TS}{a \times \frac{100 - b}{100}} \tag{5A.2}
\]

where ‘a’ is the amount of sample being used, ‘b’ is the moisture content of the powder and TS are the total solids of the filtrate.
5A.2.4.8. Wettability

Measurement of the wettability of CPP was carried out as per to the procedure described in A/S Niro Atomizer (1978) with minor modifications. Distilled water (100 ml) was poured in a beaker (250 ml) at room temperature (25 ± 2°C). A glass funnel was held on a ring stand above the beaker. The distance was adjusted to 5 cm between the bottom of funnel and the water surface. For closing the passage, a test tube was placed inside the funnel and 0.1 g powder sample was placed in the funnel around the test tube. The powder was allowed to fall at once into the beaker by lifting the test tube. The stop watch was started simultaneously in order to record the time for the powder to be become completely wetted. The complete wetting was assessed visually as the state when all the powder particles penetrated the surface of water.

5A.2.4.9. Polyphenol content

5 g of CPP was mixed for 1h in 40 ml methanol at room temperature (25 ± 2°C). The slurry was centrifuged at room temperature (25 ± 2°C) at 3000 g for 10 min. The supernatant was collected and the volume was made to 50 ml in volumetric flask using methanol. Total polyphenol content was determined by Folin-Ciocalteau colorimetric method as described in section 4B.2.5.4.

5A.2.5. Statistical analysis

The physico-chemical analysis as well as the functional property measurements were carried out in triplicate. The results are expressed as mean ± standard deviations. Significant differences between means (defined
at p < 0.05) were determined by one-way analysis of variance (ANOVA) using Microsoft Excel 2007.

5A.3. Results and discussion

The coconut skim milk was successfully spray dried and in the following sections, the physicochemical characterization and the functional properties are presented.

5A.3.1. Physicochemical characterization

The results of proximate analysis of CPP are presented in Table 5A.1. It can be seen from the table that the protein content of CPP was high (33%, w/w) and the fat content low (3%, w/w). The ash content (12%, w/w) signifies high concentration of minerals, as reported by Hagenmaier et al. (1974). Ultrafiltration can be employed to increase the protein content while decreasing the carbohydrate and ash contents. Higher protein content was reported by Kwon et al. (1996b) and Jinapong et al. (2008) in coconut protein concentrate and soy milk protein using ultrafiltration.

Carbohydrates and proteins are generally employed as wall materials for microencapsulation during spray drying. Proteins have an amphiphilic character that offers physicochemical and functional properties required to encapsulate hydrophobic core materials. Sodium caseinate, soy protein isolate, and whey protein concentrates and isolates are some of the protein compounds which have good microencapsulating properties. The results of the proximate analysis of CPP indicate that it can also be considered as a suitable wall material for microencapsulation of food ingredients during spray-drying.
CPP was found to have 57.01 ± 0.47% total soluble solids. It is well known that during spray drying proteins undergo thermal denaturation. Spray drying was shown to affect the solubility of α-lactalbumin and β-lactoglobulin (up to 40%) during dehydration of whey proteins at high temperatures (100-120°C) (Anandharamakrishnan et al., 2008). Mixing of the insoluble protein precipitate with coconut skim milk prior to spray drying could be also the reason for low solubility.

5A.3.2. Protein solubility

From Figure 5A.2, it can be observed that the protein solubility (PS) of CPP was minimum at pH 4.0 and increased below and above it except in 1M NaCl solution, where in minimum PS was observed at pH 3.0. The solubility depends on whether the proteins are in their native or denatured state but denaturation alone is not enough to cause a measurable loss of solubility, as aggregation of proteins contributes more significantly to the loss of solubility. The pH significantly influences the aggregation of proteins as it affects the net surface charge of the protein molecules and in turn the electrostatic repulsive forces between molecules. The net surface charge is zero at the isoelectric point (pH of 4.0 for the present case) and electrostatic repulsion forces are at a minimum, facilitating the aggregation. The greater the deviation of pH from the isoelectric point, the greater are the repulsive forces and aggregation is less likely to occur (Pelegrine and Gasparetto, 2005). Protein concentrates can be prepared by isoelectric precipitation by employing such methods. However, the PS increased at pH above 7.0 (protein in distilled water), where dissociation of the protein aggregates occurs due to increased net negative charge on the protein. An increase in PS was observed with an increase in
the ionic strength of the solvent from 0.1M to 1M. When proteins are suspended in an electrolyte solution, repulsive electrostatic forces come into play. Aggregation is prevented by repulsive forces between proteins facilitating dissolution.

5A.3.3. Functional properties

Table 5A.2 provides the comparison of functional properties of coconut protein powder (CPP), defatted soybean powder (DSP) and skimmed milk powder (SMP). The bulk density of the CPP, which is commercially and functionally important property of powders, was determined to be 350 kg/m$^3$. This value is lower as compared to that of DSP and SMP (Table 5A.2). Kwon et al. (1996b) reported the bulk densities of coconut protein concentrate, sodium caseinate and soy protein concentrate to be 520 kg/m$^3$, 420 kg/m$^3$ and 430 kg/m$^3$, respectively. Interaction between the protein product and water resulting in some water holding within the product is denoted by water hydration capacity of proteins. CPP has very low water hydration capacity (0.44 ml/g) as compared to that of DSP (1.73 ml/g) and SMP (0.74 ml/g). The water retention capacity, in food applications is related with the ability to retain water against gravity and includes bound, hydrodynamic, capillary and physically entrapped water contents. The quantity of water associated with proteins is closely related with its amino acids profile and increases with the number of charged residues, conformation, hydrophobicity, pH, temperature, ionic strength and protein concentration. The native structure of proteins is a consequence of the interactions of amino acids with water and some functional properties can be interpreted as a result of the protein–water interactions thermodynamically favourable (wettability, water retention and
solubility) or unfavourable (foaming and emulsification). Other properties reflecting the interaction of the protein polymers with water are viscosity, gelation and coagulation (Damodaran, 1997).

Fat absorption and emulsifying properties can be of importance while incorporating the proteins in mixed systems (water and oil). The fat absorption capacity (FAC) of CPP (0.91 ml/g) and SMP (0.84 ml/g) were not significantly different from each other, however, are higher than that of DSP (0.62 ml/g). Oil absorption was mainly attributed to the physical entrapment of oil and to the number of nonpolar side chains of proteins (of sample) that bind the fatty acids in the oil (Al-Kahtani and Abou-Arab, 1993). CPP was found to have the highest emulsifying activity index (EAI) (40 m²/g) followed by SMP (33 m²/g). EAI indicates the area of interface (aqueous and oil) stabilized per unit weight of protein. As indicated by the emulsifying stability index (ESI) the emulsions were found to be more stable although EAI value was low for DSP, as compared to that of CPP and SMP. CPP was found to have better emulsifying properties than SMP and hence can be possibly used as an ingredient in emulsified foods.

The value of ‘dispersibility’ of CPP was estimated to be 93.36 ± 0.28% (w/w) indicating good dispersibility. It is higher than that of DSP and lower than that of SMP. Dispersibility is an important property of instant powders. It is the ability of the powder to disperse in water by gentle stirring. It is necessary that the powder is wettable in order to obtain good dispersibility of a given powder. Wettability (a measure for the ability of a powder to be wetted by water at a given temperature) of the spray dried CPP was found to be higher (60.21 ± 4.75 s) than SMP (39.32 ± 2.6 s) and lower than the wettability of DSP (78.89
± 8.83 s). Wettability depends on the surfaces of particles, at which the gaseous phase at the surface of the solid phase is replaced by a liquid phase. It is beneficial to have the value of wettability in the range 30-60 s, as it facilitates subsequent dispersion of the powder into water.

5A.3.4. Polyphenol content

Polyphenols such as condensed tannins are regarded as antinutritional factors found in many plant products, except for soy isoflavones and tea tannins. Polyphenolics may form complexes with minerals and amino acids thereby reducing the nutritional value of the isolated protein and cause astringency due to their ability to precipitate the proteins in the mouth. Phenolic compounds, on oxidation can cause the development of dark colour in oilseed protein products (Naczk and Shahidi, 1997). Polyphenol content of CPP was found to be 0.72 mg/g GAE (Gallic acid equivalents). This value is much lower than the polyphenol content of many oil seed protein products and no further processing would be required for the removal of polyphenols.

5A.4. Conclusions

Coconut protein powder (CPP), a value added product, could be successfully recovered from coconut skim milk and insoluble protein which are wet processing wastes obtained during the production of virgin coconut oil. Spray drying could be used successfully for the dehydration of CPP while retaining the functional properties. The protein content was high (33%) and fat content low (3%) in CPP and it was found to have better emulsifying properties (EAI 40 m^2/g) than skimmed milk powder (EAI 33 m^2/g) and defatted soybean powder (EAI 17 m^2/g). These results open perspectives for its use as a natural and multifunctional dietary food additive or supplement.
Table 5A.1: Proximate analysis of coconut protein powder

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Parameters</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protein</td>
<td>33.10 ± 1.2</td>
</tr>
<tr>
<td>2</td>
<td>Fat</td>
<td>3.0 ± 0.61</td>
</tr>
<tr>
<td>3</td>
<td>Moisture</td>
<td>2.7 ± 0.3</td>
</tr>
<tr>
<td>4</td>
<td>Carbohydrate (by wt. difference)</td>
<td>48.37 ± 2.3</td>
</tr>
<tr>
<td>5</td>
<td>Ash</td>
<td>12.83 ± 0.91</td>
</tr>
</tbody>
</table>

Values are averages ± SD form three replicate analysis
Table 5A.2: Functional properties of coconut protein, defatted soybean and skimmed milk powders

<table>
<thead>
<tr>
<th>Product#</th>
<th>Bulk Density (kg/m³)</th>
<th>WHC* (ml/g)</th>
<th>FAC* (ml/g)</th>
<th>EAI* (m²/g)</th>
<th>ESI* (min)</th>
<th>Wettability (s)</th>
<th>Dispersibility (% w/w)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CPP</td>
<td>350 ± 10ᵃ</td>
<td>0.44 ± 0.01ᵃ</td>
<td>0.91 ± 0.07ᵃ</td>
<td>39.98 ± 1.50ᵃ</td>
<td>36.7 ± 4.93ᵃ</td>
<td>60.21 ± 4.75ᵃ</td>
<td>93.36 ± 0.28ᵃ</td>
</tr>
<tr>
<td>DSP</td>
<td>415 ± 12ᵇ</td>
<td>1.73 ± 0.12ᵇ</td>
<td>0.62 ± 0.07ᵃ</td>
<td>17.36 ± 1.02ᵇ</td>
<td>112.85 ± 12.05ᵃ</td>
<td>78.89 ± 8.83ᵇ</td>
<td>90.68 ± 0.26ᵇ</td>
</tr>
<tr>
<td>SMP</td>
<td>506± 0.3ᶜ</td>
<td>0.74 ± 0.01ᶜ</td>
<td>0.84 ± 0.06ᵇ</td>
<td>33.00 ± 1.43ᶜ</td>
<td>22.31 ± 2.49ᵇ</td>
<td>39.32 ± 2.6ᶜ</td>
<td>96.06 ± 0.15ᶜ</td>
</tr>
</tbody>
</table>

Values are averages ± SD form three replicate analysis

ᵃᵇᶜ Values in columns followed by same superscript letters are not significantly different (P ≤ 0.05).

*WHC- Water holding capacity
*FAC- Fat absorption capacity
*EAI- Emulsifying activity index
*ESI- Emulsifying stability index

#Product:
CPP- Coconut Protein Powder
DSP- Defatted Soybean Powder
SMP- Skimmed Milk Powder
Figure 5A.1: Flow diagram for preparation of coconut protein powder
Figure 5A.2: Protein solubility profile of coconut protein powder
Chapter 5B

Storage Studies and Quality Evaluation of Coconut Protein Powder
5B.1. Introduction

Eating is one of the great pleasures of life with the organoleptic properties being the primary factors influencing food choice. Hence it has become essential to systematize the relatively subjective discipline of sensory analysis for the fast growing food industry developing it into sensory science. It is a discipline that exploits the human senses (smell, sight, sound, touch and taste) in characterizing a product. Sensory scientists have developed sensory analysis as a formalized, structured and codified testing methodology, and they pursue development of new methods while refining the existing ones. Sensory analysis can position a product, establish the worth of a commodity or even its very acceptability by the consumer (Meilgaard et al., 1999). The shelf-life of many food products, especially the microbiologically stable foods, whose shelf life is defined by the changes in their sensory properties is determined by sensory quality evaluation during storage (Hough, 2006). As legislation in many countries demands some form of ‘sell by’ or ‘use by’ labelling, the food manufacturers are under increasing pressure to introduce attractive new products into retail outlets with minimum delay. While this is feasible for ‘short shelf life’ products, the knowledge of the sensory characteristics over the intended shelf life period is required for the introduction of ‘long shelf life’ products into the market. Evaluation of shelf life at ambient conditions is ideal. However, the running of the test is time consuming, especially for dehydrated products with a shelf life of several months. Consequently, in order to circumvent this problem accelerated shelf life procedures are often attempted. As the accelerated conditions such as high temperature as well as humidity,
enhance deteriorative reactions can considerably reduce the duration of the test (Larsen et al., 2005).

As mere instrumental technique does not reflect the sensory profile, a judicious blend of sensory and instrumental analysis is essential to obtain fair quality assessment of food (Hariom et al., 2006). A multisensor system, known as Electronic Nose (E-nose) was designed at the beginning of 1980’s for aroma analysis (Gardner and Bartlett, 1994). E-nose comprises of array of sensors and a neural network for data processing. Similar approaches were implemented for liquid sensing eventually leading to the development of Electronic Tongue (E-tongue) (Toko, 1998). Recently, a novel sensing system (comprising of both, E-tongue and E-nose) has been developed for simultaneous analysis of taste and aroma/flavour liquids and gaseous phases respectively (Cole et al., 2011). In spite of such intensive studies a correlation between chemical composition of food and its flavour is highly complicated and yet to be established (Rudnitskaya et al., 2002).

A process has been developed for the production of VCO from fresh coconuts employing wet processing (Marina et al., 2009a, 2009b; Raghavendra and Raghavarao, 2010, 2011; Raghavendra et al., 2009) as the product is gaining popularity due to its health benefits. Coconut residue (left after extraction of coconut milk), coconut skim milk (CSM) and insoluble protein are the major byproducts of wet processing. Coconut spent residue finds application, besides several other ones as a dietary fiber, since it was found to have the highest water-holding and swelling capacities when compared to any other dietary fibers (Raghavendra et al., 2004, 2006). Coconut skim milk and insoluble protein create environmental pollution problems on disposal. Coconut protein
powder (CPP), a value-added product, was successfully obtained from coconut skim milk and insoluble protein. CPP was demonstrated as a substitute for cow milk for the preparation of a highly acceptable dessert such as Kheer (Naik et al., 2012). The main focus in the present work is to evaluate the product quality of CPP at different storage time and conditions by both sensory and instrumental analysis while establishing its shelf life.

5B.2. Materials and Methods

5B.2.1. Materials

Fresh and mature coconuts (10-12 months) were purchased from the local market. Enzyme aspartic protease [EC 3.4.23] (Activity: 2500 tyrosine units/g) of commercial grade was procured from Kaypeeyes Biotech Private Ltd., Mysore, India. All other chemicals of analytical grade were obtained from Merck chemicals, Mumbai, India.

5B.2.2. Preparation of Coconut Protein Powder

Coconut protein powder was prepared as described in section 5A.2.2 with minor variations as described below. Fresh and mature coconuts were subjected to manual deshelling, paring and removal of water. Pared coconuts were disintegrated using rotary wedge cutter (Krauss Maffei, Germany) and coconut milk was expelled using screw press. The coconut milk was subjected to protease treatment (i.e. 100 Tyrosine units/liter of coconut milk) for 2h in order to carry out effective destabilization of the coconut milk emulsion. Enzyme treated milk was subjected to centrifugation (Model: SAOG 2016, Westfalia separator, Germany) at 7000 rpm to obtain cream, coconut skim milk and insoluble protein. Coconut skim milk was homogenised along with the
entire insoluble protein and the slurry was passed through vibratory filter to remove coarse particles. The slurry at ambient temperature \((25 \pm 2^\circ C)\) was fed into a spray dryer (Model: BE1216, Bowen, USA) by a peristaltic pump at a flow rate of 75 ml/min. Nozzle type atomizer (2 mm diameter) was employed at 3 bar air pressure in a co-current mode air flow system. The inlet air temperature was set at \(130 \pm 2^\circ C\) and the outlet air temperature was about \(100 \pm 2^\circ C\). The powder was collected through a cyclone. The mass balance flow chart is presented in Figure 5B.1.

5B.2.3. Packaging and storage of coconut protein powder

The packaging material used in this study was metallised polyester of 12 \(\mu\) thickness. Pouches (15 X 10 cm) were made by sealing the corners. The properties of the film such as low permeability to light, moisture and oxygen, higher toughness, the ability to be heat sealed, and a lower density at a lower cost than an aluminium foil were considered as selection criteria for packaging material. CPP (50 g) was packaged in metalized polyester pouches and stored at three different storage conditions: Control (4\(^\circ\)C, Refrigerated); Ambient (27\(^\circ\)C, 65% RH) and Accelerated (38\(^\circ\)C, 90% RH) conditions. Samples were withdrawn periodically at designated intervals of 15 days for accelerated and 30 days for ambient condition. Withdrawn samples were tested for sensory quality aspects in the powder form (as it is) and also in the product (Kheer) form.

5B.2.4. Preparation of Kheer

20 g of fine noodles (extruded product called vermicelli) was added to 150 g water and allowed to cook for 10 min on medium flame. 20 g sugar was added and stirred until the sugar got completely dissolved. 12 g CPP was mixed with
50 g water to form thin slurry without lumps, separately, and this was then added to the cooked noodles and stirred (conventionally, cow milk is added at this point). The flame was put off and a pinch of cardamom powder was added to enhance the flavour. The *Kheer* was cooled down to room temperature before it was served to the panellists for sensory evaluation.

Conventional product (*Kheer*) was also prepared employing cow skim milk powder for the purpose of comparison.

**5B.2.5. Methods of measurement**

**5B.2.5.1 Proximate analysis**

Proximate analyses for moisture, ash, fat and protein (N X 6.25) contents of the CPP were carried out by using AOAC standard methods as described in section 4B.2.4. Total carbohydrate content was calculated by difference.

**5B.2.5.2 Powder flowability and cohesiveness**

Flowability and cohesiveness of the powder were evaluated in terms of Carr Index and Hausner ratio as described in section 4B.2.5.7. The criteria for classification of flowability and cohesiveness of powder according to Carr (1965) and Hausner (1967) are presented in Table 5B.2 and Table 5B.3, respectively.

**5B.2.5.3 Functional properties**

Water hydration capacity (WHC) and Fat absorption capacity (FAC) were determined as described in section 5A.2.4.5. Emulsifying properties of sample powders were studied using method as described by Pearce and Kinsella (1978) as described in section 4A.2.6.1.
5B.2.6. Sensory analysis

Sensory analysis for CPP and product (Kheer) form was carried out as follows. A group of 12 panelists aged 25-50 years were trained for Quantitative Descriptive Analysis (QDA). The members of the panel were drawn from scientific staff familiar with sensory analysis and who had earlier experience in sensory evaluation of food products. Evaluations were conducted under white fluorescent light, with the booth area maintained at temperature 22 ± 2°C and RH 50 ± 5% as per American Society for Testing and Materials (ASTM) standards. A suitable score card was developed using “Free-Choice Profiling” method for selecting suitable terminology. The typical attributes for the CPP and Kheer samples were identified in the preliminary session to trace the quality changes in the stored samples and used for developing the score card.

The impact making attributes were Body, Milky, Coconut like aroma, Nutty like aroma, Starch like aroma, Coconut oily, Sweetness, and Rancid. Samples were presented in glass beakers coded with 3-digit random numbers to the panelists. A glass of water was also presented to cleanse the palate in between the samples.

Quantitative Descriptive Analysis (QDA) was used to assess the quality of samples (Stone and Sidel, 1998). Panelists were asked to mark on a scale of 0-15 cm to indicate the intensity of each attribute listed on the score card. The scale was anchored at 1.25 cm on either end, representing ‘Recognition Threshold’ and ‘Saturation Threshold’, respectively. The scores given for all the attributes for each sample were tabulated. Next, the mean value was calculated for each attribute of a sample, representing the panel's judgment about the sensory quality of the product.
Pilot consumer acceptance study was carried out for coconut protein powder and *Kheer* prepared with coconut milk powder among 50 panelists. Seven point hedonic scale ranging from ‘like very much’ to ‘dislike very much’ with ‘neither like nor dislike’ as midpoint was used for the study.

**5B.2.7. Instrumental Analysis**

**5B.2.7.1 Electronic nose**

Alpha MOS electronic nose (**α**-Fox model 4000) is composed a manual sampler, an array of gas sensors, and an advanced multivariate chemometric software. The sensor array consists of 3 metal oxide sensor chambers and 18 resistive chemical sensors, where each sensor has a specific sensitivity and selectivity characteristics. Sensors were selected based on their discrimination power between samples. They measure volatile organic compounds (VOC) in headspace over the samples. The coconut protein powder (1 g) was filled in vials after each withdrawal. Experimental conditions employed for analyses were: acquisition time (s): 120, acquisition period (s): 0.5, delay (s): 100, start injection (s): 0, injection time (s): 60, headspace generation time (s): 120 and zero air flow (ml/min): 150, sample temperature: room temperature (25 ± 2°C). Analysis was performed in triplicates, each vial analyzed five time thus by making 15 replicates from each sample lot. The sensor response was generated automatically through data acquisition and data was analyzed by a complete in-built software package provided by the manufacturer.

**5B.2.7.2 Electronic tongue**

E-tongue analyses were performed using **α**-Astree electronic tongue (Alpha MOS, France), whose sensor array comprising of seven potentiometric
chemical sensors with varying sensitivity. Each sensor is composed of an organic coating sensitive to the sample and a transducer, which can convert the response of the membrane into signals. The working principle is based on the changes in the voltage (mV) intensity between the chemical sensor and the reference electrode (Ag/AgCl). Samples were prepared by dissolving 5 g of CPP in 100 ml double distilled water, out of which 80 ml was taken in E-tongue beakers for analysis. All samples were analysed in triplicates. Experimental conditions employed for analyses (auto sampler method) were: acquisition time: 120 s, acquisition period: 1.0 s, delay: 0 s, stirring rate: 1, sample temperature: Room Temperature (25 ± 2°C). At the end, the sensor response values were subjected to DFA analysis through the inbuilt multivariate software provided by the manufacturer.

5B.2.8. Statistical analysis

Sensory data was statistically analysed by Duncan’s Multiple Range Test to establish the significant difference between samples (Duncan, 1955). Significance was tested at a probability level of $p \leq 0.05$.

5B.3. Results and Discussion

5B.3.1. Proximate analysis

Spray drying of 15 kg of coconut skim milk homogenised with insoluble protein yielded 1.18 kg CPP. This powder was visually observed to be off-white in colour. CPP was observed to have high content of carbohydrate (~49%, w/w), protein (~31%, w/w) and ash (~10%, w/w) while low content of fat (~7%, w/w) and moisture (~3%, w/w) as shown in Table 5B.1. The proximate analysis of CPP is comparable to that reported by Naik et al. (2012) except for the fat
content. The difference is due to the higher quantity of coconut skim milk added to insoluble protein in the present study.

**5B.3.2. Flowability and cohesiveness**

The bulk density of CPP was found to be $415.57 \pm 7.43 \text{ kg/m}^3$ while the tapped density was $577.18 \pm 10.32 \text{ kg/m}^3$. The fair flowability of the CPP was indicated by Carr index of 28 as shown in Table 5B.2. Hausner ratio was 1.39, which implies intermediate cohesiveness of the powder (Table 5B.3).

**5B.3.3. Effect of storage conditions on moisture content and functional properties**

The moisture content of CPP samples stored at refrigerated, ambient and accelerated conditions increased gradually with storage time. CPP stored at different conditions exhibited marginal moisture uptake (by 0.74% w/w for control, 0.76% w/w for ambient and 1.26% w/w for accelerated condition). CPP stored at control and ambient conditions had practically no difference in moisture content throughout the storage period. CPP stored at accelerated conditions was found to have the highest moisture content compared to CPP stored at control and ambient conditions. The low moisture uptake by CPP is attributed to packaging material i.e. metallized films, which is plastic containing a thin layer of aluminium metal having improved barrier properties to moisture, oils, air, and odours. The highly reflective surface of the aluminium is also attractive to consumers (Fellows and Axtell, 2002). Extension of shelf-life, retention of beneficial effects of processing, maintenance of the quality and safety of food and slow down the product deterioration is possible by appropriate food packaging (Marsh and Bugusu, 2007). Other food packaging
materials include glass, metals (aluminium foils and laminates, tinplate, and tin-free steel), paper and paperboards and plastics. Today’s food packages use combination of several materials in order to make use of each material’s functional or aesthetic properties.

The storage time was shown to have marginal effect on water hydration capacity as well as fat absorption capacity as shown in Figures 5B.3A and 5B.3B. This effect can be attributed to the increase in moisture content of CPP. The storage period as well as the storage conditions did not affect the emulsifying properties of CPP as observed in Figures 5B.3C and 5B.3D.

5B.3.4. Sensory evaluation

The ASTM International (2005) defines sensory shelf life (SSL) as “the time period during which the products' sensory characteristics and performance are as intended by the manufacturer”. The product is consumable or usable during this period, providing the end-user with the intended sensory characteristics, performance and benefits”. The sensory evaluation revealed that there were no significant changes in the attributes of the CPP in powder and product form (Figures 5B.4 and 5B.5). The rancidity of CPP gradually increased with respect to time at accelerated conditions. The overall quality was almost constant for powder stored at control and ambient conditions but the quality of the powder began to deteriorate when stored at accelerated conditions after 30 days (Figure 5B.6A). Similar was the case with Kheer prepared using this powder as seen in Figure 5B.6B.

The pilot consumer acceptance study revealed that 40% of the population have rated coconut protein powder as ‘like very much’, 20% as ‘like moderately’, another 20% as ‘like slightly’, 12% as ‘neither like nor dislike’ and 8% has
indicated ‘dislike slightly’ as shown in Figure 5B.7. In case of Kheer prepared using coconut protein powder, the study showed that 52% of the population rated the product as ‘like very much’, 28% rated as ‘like moderately’, 12% as ‘like slightly’ and 8% rated as ‘neither like nor dislike’ (Figure 5B.7). Since most of the ratings have fallen on the “like category” it may be concluded that CPP as well as Kheer made using CPP have high acceptability.

5B.3.5. E-nose and E-tongue analysis

The principal component analysis of volatile compounds indicated that principle axis 1 (PC 1) has accounted for 82.13% of the variance, while principal axis 2 (PC 2) accounted the remaining 13.45% of the variance in the data matrix. The aroma patterns indicate that there is a change volatile compounds but not significant (Figure 5B.8). This may be attributed to the storage variables like storage days (0, 15, 30, 45 and 60 days) and temperature (control, ambient and accelerated conditions). Similarly, e-nose with metal oxide sensors was used to determine shelf life of milk at ambient or refrigerated conditions and also to detect the bacterial growth in milk (Labreche et al., 2005). Using PCA, the e-nose could distinguish the difference among the milk flavorings and between the natural and enzyme-induced milk flavorings which otherwise were not that distinguishable in sensory tests (Wang et al., 2010).

Non-volatile compounds were analysed by E-tongue with seven sensors. The sensor responses were subjected into discriminant function analysis (DFA) analysis. Figure 9, indicated that the DF axis 1 has accounted for 61.02% variance, while DF2 accounted for 38.98% variance of the data matrix. There were three clusters were formed based on the non-volatile compounds present in the three samples. The difference in clusters can be attributed to the storage
conditions like storage days and temperature (Figure 5B.9). Further the e-nose and e-tongue profiles provide the nature of volatile and non-volatile compounds present in the samples in a short span of time, respectively and these analyses are complementary to the sensory analysis performed by using human panels.

5B.3.6. Sensory analysis of Kheer

Sensory evaluation of Kheer (prepared using cow skim milk powder) and Kheer prepared with CPP are presented in Figure 5B.10. It was observed that the Kheers were similar in attributes such as starch-like and sweet taste but Kheer made using CPP had a stronger nutty and coconut-like flavour. Attributes such as colour, texture and milky aroma were rated slightly lower for Kheer made from CPP compared than that of cow skim milk powder. The overall quality rating was found to be ~9 out of 15 for Kheer made using CPP which is slightly lower than ~11 out of 15 for Kheer made using cow skim milk powder, indicating high acceptability of both the products. This indicates suitability of CPP to be used as a cow skim milk powder substitute for preparation of typical product such as Kheer.

5B.4. Conclusion

Coconut Protein powder (CPP), obtained by spray drying coconut skim milk and insoluble protein (byproducts of VCO process) was shown to have good flow characteristics. Functional properties of CPP did not change much with respect to storage period as well as conditions of storage. QDA of CPP and product (Kheer) showed no significant differences in attributes between samples during the storage period of two months. E-nose analysis revealed that samples were not much different with respect to aroma pattern matching.
The consumer acceptance ratings have fallen on the “like category” hence the product was considered acceptable. Studies indicate suitability of CPP to be incorporated in typical products such as Kheer as a cow milk powder substitute. Results indicate that the coconut protein powder has shelf life of approximately 6 months under ambient conditions when metalized aluminium is used as the packaging material.
Table 5B.1: Proximate analysis of coconut protein powder

<table>
<thead>
<tr>
<th>SI no.</th>
<th>Parameter</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Protein</td>
<td>30.94 ± 2.44</td>
</tr>
<tr>
<td>2</td>
<td>Fat</td>
<td>7.22 ± 0.14</td>
</tr>
<tr>
<td>3</td>
<td>Carbohydrate (by difference)</td>
<td>49.22 ± 3.23</td>
</tr>
<tr>
<td>4</td>
<td>Moisture</td>
<td>2.62 ± 0.05</td>
</tr>
<tr>
<td>5</td>
<td>Ash</td>
<td>10 ± 0.6</td>
</tr>
</tbody>
</table>
Table 5B.2: Classification of powder flowability based on Carr Index (CI)

<table>
<thead>
<tr>
<th>CI (%)</th>
<th>Flowability</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 15</td>
<td>Very good</td>
</tr>
<tr>
<td>15-20</td>
<td>Good</td>
</tr>
<tr>
<td>20-35</td>
<td>Fair</td>
</tr>
<tr>
<td>35-45</td>
<td>Bad</td>
</tr>
<tr>
<td>&gt; 45</td>
<td>Very bad</td>
</tr>
</tbody>
</table>
Table 5B.3: Classification of powder cohesiveness based on Hausner Ratio (HR)

<table>
<thead>
<tr>
<th>HR</th>
<th>Cohesiveness</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 1.2</td>
<td>Low</td>
</tr>
<tr>
<td>1.2-1.4</td>
<td>Intermediate</td>
</tr>
<tr>
<td>&gt; 1.4</td>
<td>High</td>
</tr>
</tbody>
</table>
Figure 5B.1: Mass balance flow chart for preparation of coconut protein powder
Figure 5B.2: Effect of storage on moisture content (wet basis) of coconut protein powder stored under different conditions
Figure 5B.3: Effect of storage on functional properties of coconut protein powder stored under different conditions: A- Water Hydration Capacity, B- Fat Absorbance Capacity, C- Emulsification Activity Index and D- Emulsion Stability Index
Figure 5B.4: Sensory profilogram of coconut protein powder stored under different conditions: A – Refrigerated [4°C], B – Ambient [27°C, 65% RH] and C – Accelerated [38°C, 90% RH]
Figure 5B.5: Sensory profilogram of Kheer prepared from coconut protein powder stored under different conditions: A – Refrigerated [4°C], B – Ambient [27°C, 65% RH] and C – Accelerated [38°C, 90% RH]
Figure 5B.6: Overall quality of coconut protein powder (A) and *Kheer* prepared from coconut protein powder (B) during storage at different conditions.
Figure 5B.7: Pilot consumer acceptance study of CPP and *Kheer* prepared from CPP
Figure 5B.8: E-Nose profile of Coconut Protein Powder (CPP) samples stored at different conditions. 1-Milk Powder (cow skimmed milk); 2-CPP Day 0; 3-CPP Day 15 Control, 4-CPP Day 15 Accelerated, 5-CPP Day 30 Control, 6-CPP Day 30 Ambient, 7-CPP Day 30 Accelerated, 8-CPP Day 45 Control, 9-CPP Day 45 Accelerated, 10-CPP Day 60 Control, 11-CPP Day 60 Ambient, 12-CPP Day 60 Accelerated
Figure 5B.9: E-Tongue profile coconut protein powder (CPP) samples stored at different conditions
Figure 5B.10: Sensory analysis of *Kheer* prepared using CPP and cow skim milk