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Chemical Composition, Physicochemical And Functional Properties Of Custard Apple (Annona Squmosa) Seed Flours And Protein Isolate

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Abstract : ***Proximate and mineral compositions, together with functional properties, of custard apple seed flour & protein isolate (Annona squmosa) were studied. Also, physicochemical characteristics of the seed flour were determined. The seed flour contained higher percentages of Total Carbohydrates, Fat crude protein, total ash and moisture. The most abundant mineral was Sodium. Protein solubility studies showed that both seed flour and protein isolate were soluble at acidic and basic pH regions, indicating that they may be useful in formulating acid foods, such as meat and milk analogue products and protein-rich beverages. The minimum solubility of defatted custard apple seed flour (DCASF) was found to be 10.87 % at pH 4, while the solubility was 77.36% at pH 2. The solubility of defatted custard apple seed flour increased with increase in pH 5 to 12 and the maximum solubility of 94.02% was obtained at pH 12.***

Index Terms: Protein isolate, defatted meal, lyophilization

I. INTRODUCTION

Custard apple belonging to *Annonaceae* family. The *Annonaceae* family contains a considerable number of plants of economic significance because of their edible fruits. These crops represent the fruits of tropical America, Australia, Africa, Malaysia and India (in India the custard apple is one of them) with a very sharp and short season, lasting for about 3 months a year. Custard apple has three varieties *Annona squamosa, Annona cherimola and Annona reticulata.* This fruit is popularly known as sweet sop. Custard apple (*Annona squamosa)* is popularly called as sitaphal in South India and sharifa in North India. It is a heart shaped fruit weighing about 150 g, with a very bumpy skin. When ripe, pulp is creamy, very sweet and pleasantly flavoured. It is cultivated in most of the south India including Andhra Pradesh, Karnataka, Tamilnadu and Maharashtra. The production of custard apple in Maharashtra is around 20,497 MT from 4,990 ha of area **(Sontakke, 2003)**. Storage of the fresh fruits of custard apple has limitations, since it is perishable. Custard apple is a tropical fruit which shows very short storage life at room temperature due to its fast ripening. It presents climacteric behaviour, high respiration rate and ethylene production is fast, which resulted in fruit softening reducing the fruit quality and commercialization (**Benassi *et al.*, 2003).**

The seeds are so hard that they may be swallowed whole with no ill effects but the kernels are very toxic. Ancorine is an alkaloid extracted from custard apple seeds, which has insecticidal properties. The custard apple seeds contain 25.5% oil used in soap and paint industries. The seed cake can be used as green manure for agriculture. The seeds, leaves and young fruits are insecticidal and leaf juice used to kills lic **(Morton, 1987).** The total amount of the essential amino acids (phenylalanine, leucine, valine, threonine, isoleucine, methionine, tyrosine, histidine, argnine, cystine, and lysine) found in *Annona* squamosa seed. The percentage of sulphur–containing amino acids (methionine and cystine) in *Annona* squamosa seed was 0.106 g/100g protein. The amino acids content of A. squamosa seeds showed a high difference when compared with egg, sesame and broad bean amino acids **(Mariod *et al*., 2010).**
Based on end use requirements, various extractions, isolation and fractionation procedures are followed. Generally, the extraction of protein rich material in alkaline solution followed by isoelectric precipitation is commonly followed for food applications **(Sathe, Deshpande, & Salunkhe, 1984).** Response surface methodology (RSM) is a statistical technique that helps us in getting information with less cost and short time. This technique relates input and output parameters **(Montgomery, 1984).** Its use leads to rapid and efficient development of new/improved products or processes. To optimize, extraction of protein isolate from custard apple seed using response surface methodology.

II. MATERIALS AND METHODS

Collection and preparation of sample:
Custard apple fruit was collected from local market of Sailu, Dist-Parbhani (Maharashtra). Well matured, fully eye opened fruit, slightly yellow and green in colour was selected which was free from blemishes and mechanical injuries. Then the seed of custard apple were separated from fruit pulp manually by splitting the fruit.

Preparation of custard apple seed flour
Whole fruit (matured) where procured from market with then removing seed from fruit. Seeds were then properly cleaned and dried. Then cleaned seeds were soaked in water for overnight (12 hrs) at room temp to facilitate manual dehulling of seeds. After dehulling, seeds were splited and dried at 65˚C until constant moisture content was attained. Drying was followed by grinding in grinder, sieving through 60 mesh size sieve. Finally prepared custard apple seed flour was packed in air tight polythene bag and stored at refrigerated condition until it was used for further processing is the protein isolate preparation.

Preparation of defatted seed meal

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Seeds were dehulled, ground using hammer mill (M/S Narang Scientific Works, India), extracted with n-hexane three times (n-hexane ratio: flour 10:1, v/w), desolventized and ground again to pass through 72 mesh sieve to obtain fine powder, termed as deoiled meal and was stored at 20 1C till use. The experiment was carried out in duplicate.

Preparation of custard apple seed protein isolate (CASPI)

Custard apple seed protein isolate (CASPI) was prepared by using alkali method (Liu, 1997) and process for preparation of custard apple seed protein isolate is given in (Figure 3.3) for the preparation of CASPI 15 g defatted custard apple seed flour weighed and transferred in to clean and dry conical flask. To this 100 ml water was added and mixed. The pH was set at 9.0, with 1N NaOH, and kept in water bath-cum shaker (200 rpm, 60º C) for 30 min. The slurry was centrifuged at 3000 rpm for 15 min. The supernatant was separated and precipitated at pH 4.5 by using 1N HCl, and again centrifuged at 5000 rpm for 10 min. Protein curd and whey was obtained, whey was discarded. Protein curd was washed (water), freezed and then dried at 60ºC for 12 hrs. The final product, CASPI was stored in glass bottles till further use.

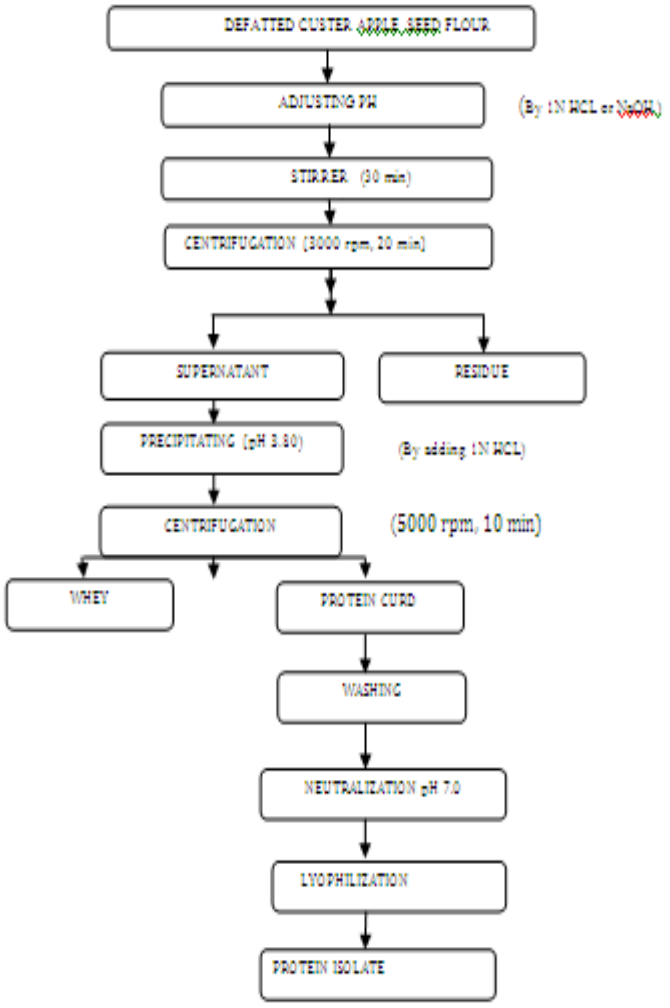

Chart 1 Preparation of custard apple seed protein isolate

Proximate analysis

Moisture, crude fat, crude protein, crude fiber and ash content of watermelon seed, kernel and meal were determined following standard method of analysis **(AOAC, 1990).** Carbohydrate was determined by subtracting all constituents from hundred.

Mineral determination (A.O.A.C 2003).

Wet digestion of sample:

Take1 gm of ground dried plant sample was taken and placed it in a small beaker. Add 10 Ml of concentrated HNO3 and allow to stand overnight. Heat carefully on a hot plate until the production of red NO2 fumes has ceased. Cool the beaker and add a small amount (2.4 ml) of 70% HClO4. Heat again and allow to evaporate to a small volume. Transfer the sample to a 50-ml flask and dilute to volume with distilled water.

Preparation of standard solutions in parts per million

ppm is a term used in chemistry to denote a very, very low concentration of a solution. One gram in 1000 ml is 1000 ppm and one thousandth of a gram (0.001g) in 1000 ml is one ppm. One thousand of a gram is one milligram and 1000 ml is one liter, so that 1 ppm = 1 mg per liter = mg/Liter. PPM is derived from the fact that the density of water is taken as 1kg/L = 1,000,000 mg/L, and 1mg/L is 1mg/1,000,000mg or one part in one million.2, 4, 6, 10, 15 ppm solutions are prepared by using molecular weight of required mineral (Ca,Zn,Fe,Na).

Analysis method

After digesting the sample 2,4,6,10,15 ppm solution prepared by using required reagents. All the samples including standards were taken in to the 100ml beaker and estimated by using atomic absorption spectrophotometer. The reading was noted down in ppm and it was converted to the milligrams.

III. RESULTS AND DISCESSION

Proximate analysis

The proximate analysis of seeds, of custard apple contained 18% and 90 % crude protein, respectively. Seeds showed very high content of fibre due to fibrous seed coat. studies show that seeds contained 2.96% moisture, 22% fat,11%crude fibre, 2% ash and 48.04% carbohydrate.

Table 1 Proximate analysis of custard apple seed flour

Component (%)	Custard apple (%)	Bitter melon seed (%)
Moisture	2.9±0.28	6.7%
Total Carbohydrates	48.18±0.61	23.2%
Protein	18.05±0.27	11.8%
Fat	22±0.5	19.0%
Ash	2±0.24	3.6%
Crude Fiber	11±0.26	34.8%

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Values are means ± standard deviation of triplicate determination

Physical properties of Custard apple seed protein isolate

The bulk density of the custard apple seed protein isolate was found to be 0.31 g/cm^3 which was similar to the bulk density of the soy protein isolate 0.31 g/cm^{3}as reported by **(Dench, 1982)**. The change in bulk density might result from moisture absorption, chemical reaction or mechanical attribute. **(Peleg and Bagley, 1983).**

Tap density and True density

Tap density and true density of custard apple seed protein isolate were 0.13±0.005 g/cm^3 and 0.82±0.05 g/cm^3 respectively. The true density is the density of solid material excluding the volume of any open and closed pore. Depending on the molecular arrangement of the material, the true density can be equal to the theoretical density of the material and therefore be indicative of how close the material is to a crystalline state or the proportion of a binary mixture.

Porosity

The porosity is a measure of the voids between the solid particles of a material. Pore space can be filled with fluids including gas and water. Air filled porosity allows gases to move within material. For gases to move throughout material, pores must be continuous. The porosity of custard apple seed protein isolate was (0.62±0.05%).

Table 2: Physical properties of Custard apple seed protein isolate

Parameter	Content
Bulk density(g/cm3)	0.13±0.02
Tap Density(g/cm3)	0.31±0.005
True density(g/cm3)	0.82±0.05
Porosity (%)	0.62±0.05

Values are means ± standard deviation of triplicate determination

Mineral content of custard apple seed flour

Mineral composition of custard apple seed flour was shown in below Table 4.6. Results shown that custard apple seed flour was high in sodium, calcium, and iron but low in zinc. The sodium content of custard apple seed flour was (87.52±0.43 mg/100gm) which is higher than that of watermelon (Citrullus vulgaris) seeds (10.52±1.18). Calcium content was found in this seed flour was (15.26±0.50 mg/100 gm).Which is higher than of watermelon (Citrullus vulgaris) seeds (8.11±1.15mg/100gm). Iron content of custard apple seed flour was (4.96±0.5003 mg/100gm) which is higher than watermelon (Citrullus vulgaris) seeds (0.63±0.02mg/100gm). Custard apple seed flour having zinc content (0.070±0.02mg/100gm) which is lower than watermelon seeds flour (1.27±0.01mg/100gm) (**Lakshmi and Kaul, 2011).**

Table 3: Mineral content in custard apple seed flour

Parameter	Content (mg/100gm)
Calcium	15.26 ±0.50
Iron	04.95±0.50
Sodium	87.52±0.43
Zinc	0.07±0.02

Values are means ± standard deviation of triplicate determination

Functional properties

The nitrogen solubility of custard apple seed protein isolate at different pH levels ranges between 2 and 12. The minimum solubility of defatted custard apple seed flour (DCASF) was found to be 10.87 % at pH 4, while the solubility was 77.36% at pH 2. The solubility of defatted custard apple seed flour increased with increase in pH 5 to 12 and the maximum solubility of 94.02% was obtained at pH 12. The solubility of custard apple seed protein isolate (CASPI) in water at different pH showed the same U-shaped pattern, which are typical and similar to the profiles reported for peanut proteins **(Monteiro and Prakash, 1994)**. The minimum solubility as observed at the pH range of 4-5 indicated that custard apple seed proteins are acidic in nature and that the isoelectric point of custard apple seed proteins was in the range of pH 4.0-4.5. This was confirmed by the highest protein content and yield obtained from the isoelectric precipitation method.

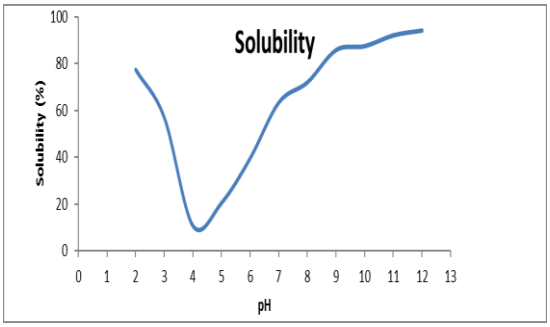

Chart 2 Solubility and pH

Water absorption capacity (WAC):

Water absorption capacity is an important processing parameter and has implications for viscosity, bulking and consistency of products, as well as in baking applications. Water absorption capacity of custard apple seed protein was 2.27 g/g which was significantly lower than physic nut (3.22g/g) (Donlaporn *et al.*, 2011). This result indicates that custard apple seed protein isolate had good water binding capacity possibly due to the interactions between polar amino acid residues of protein and molecules of water.

Oil absorption capacity (OAC)

The ability of flours to absorb and retain water and oil may help improve binding of the structure, enhance flavor retention,

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improve mouth feel and reduce moisture and fat losses of extended meat products **(Mc Watters and Heaton, 1979)**. Oil retention has been attributed to the physical entrapment of the lipid by the protein (**Padmashree *et al.*, 1987**). OAC is the ability of the protein to physically bind fat by capillary attraction and it is of great importance, since fats act as flavor retainer and also increases the mouth feel of the foods, especially bread and other baked foods **(Kinsella, 1976)**. OAC of custard apple seed protein isolate was (2.42 g/g) of protein observed. Donlaporn *et al.,* (2011) reported that oil absorption capacity of physic nut protein isolate was (1.86 g/g) proteins which lower than that of custard apple seed protein isolate (2.42g/g). But oil absorption capacity of soy protein isolate was higher than custard apple seed proteins isolate i.e (3.29g/g).

Foaming capacity and stability

Foam capacity and stability of proteins depend on the type of protein, degree of denaturation, pH, temperature, and whipping methods. Foams are used to improve texture, consistency and appearance of foods (Akubor, 2007). Foaming properties are dependent on the proteins and some other components, such as carbohydrates which are present in the flour (Sreerama *et al*., 2012). Foaming properties of custard apple seed protein isolate was 15% which was higher than foaming properties of physic nut protein isolate (13%). The higher values seem to be due to increasing solubility and increased net charges of protein isolate where the hydrophobic interactions are weak and the flexibility of protein is increased. This caused might have increasing protein diffusion to the interface of air-water for encapsulating air particles and enhancing the foam formation. Foaming stability is important since the usefulness of whipping agents depends on their ability to maintain the whip as long as possible (Lin *et al*., 1974).

Emulsifying activity and stability

The ability of proteins to act as emulsifiers varies with the molecular properties of proteins, the main factors affecting the properties of emulsions being molar mass, hydrophobicity, conformation stability, charge and physico-chemical factors as pH, ionic strength and temperature **(Kinsella, 1984)**. According **to Kato and Nakai (1980)**, the emulsifying properties show a good correlation with protein surface of hydrophobic residues which is unstable in the oil–water interface. The presence of salts and the pH value influence the emulsion stability **(Tsaliki *et al*., 2004).** Denaturation could improve the emulsifying properties of proteins due to increased hydrophobic surface and flexibility **(Dickinson and Hong, 1994; Kilara and Sharkasi, 1986; Raymundo, Franco, Gallegos, Empis, and Sousa, (1998)**. Emulsion stability is influenced by several physical interdependent processes, cream formation, flocculation or aggregation and coalescence **(Damodaran, 1997).** Emulsion activity of custard apple seed protein isolate was (63.6%) which is higher than that of rosa mosqueta (53.4%) **(Moure *et al.*, 2001).**

Least gelation concentration (LGC) of custard apple seed protein isolate

A gel can be defined as an intermediate state between solid and liquid. In food systems the liquid is water and the molecular net is formed by proteins, polysaccharides or by a mixture of both Proteins are more efficient gelling agents than carbohydrates because large molecules are capable of forming crosslinks in three dimensions. Gelation is favoured by the protein size, since large molecules form extensive networks by crosslinking in three dimensions, and by the flexibility and ability of the proteins to denature **(Oakenfull *et al.*, 1997)**. According to Shimada and Matsushita 1980 those containing hydrophilic. amino acids form transparent ones. Rheological measurements are useful to obtain information on the nature of the gel. The ability of proteins to form gels is traditionally measured by the least gelation concentration (LGC). The least concentration endpoint (LCE) which is defined as the lowest protein concentration at which gel remained in the inverted tube was used as an index of gelation capacity. The lower the LCE, the better the gelating ability of the protein ingredient. LGC of 12% (w/v) for custard apple seed protein isolate observed in this study was lower than that of lupin seed protein 14% (w/v).

Table 4: Least gelation concentration of custard apple seed protein isolate

Concentration %	Custard apple seed protein isolate	
	Gelation	Appearance
2	-	Liquid
4	-	Liquid
6	-	Viscous
8	-	Viscous
10	-	Viscous
12	-	Viscous
14	+	Gel
16	+	Firm Gel
18	+	Solid Gel
20	+	Very Solid Gel

IV. Conclusion

Custard apple seed flour and protein isolate are rich in important food properties compared to some other oil seeds and nuts. In view of the assessed functional performances, the use of Custard apple seed flour and protein isolate in model food systems may be suggested.

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