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Physico-chemical and functional properties of cowpea protein isolate as affected by the dehydration technique

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ABSTRACT

Cowpea protein isolate (CPI) was extracted through salt assisted extraction technique and dehydrated by freeze drying, spray drying and vacuum drying. The effect of dehydration technique for preparation of cowpea protein isolate powder on the nutritive and functional properties was investigated. The x-ray crystallography, phase transition analysis and surface morphology established the variation in the agglomerates and nature of the particles formed upon dehydration. Freeze-dried and spray dried powders displayed least gelation capacity at 8% compared to 12% for vacuum dried isolates besides higher gel strength. The crossover point of storage and loss modulus for freeze-dried CPI was observed much earlier than for the spray and vacuum dried CPI. The emulsion capacity and creaming stability indices for freeze dried CPI were higher. Vacuum dried samples however showed highest emulsion activity index (94.88 m^2/g) and good foaming ability (88.89%). Thus depending on the functional attribute intended for end-use, the most economic drying method can be considered as reasonable option for converting the protein isolates into powders.

Key words: Cowpea protein isolate, Dehydration, Emulsifying, Functional, Rheology, SEM.

INTRODUCTION

Cowpeas (*Vigna unguiculata*) have an annual global production of 12.5 million tons (Huang *et al*., 2012), grow well in a diverse range of conditions and environments and contain only moderate levels of bioactive/anti-nutritional factors. It has potential of becoming an industrial crop. Proteins serve as an important ingredient in developing foods for all segments of population, however the functionality of proteins also assists in texture designing of foods. The functional attributes of proteins like gelation, foaming, emulsification, thickening also drive the incorporation of isolated proteins in various foods like mayonnaise, baked foods and beverages. However the manner of converting the isolated proteins into powders also determines their functional properties (Swanson, 1990). Understanding the composition of proteins, their processing methods and their effects on the functional properties shall provide better insights for promoting the application of cowpea protein isolates in new food formulations. Numerous studies have been conducted on soy proteins in this subject area (Hua, *et al*., 2005; Hu *et al*., 2010). However some people exhibit allergic reactions to soy proteins with soy being included in the FAOs list of eight common allergens (Hall *et al.*, 2003). Considering a need to develop sources of concentrated plant proteins other than soybeans, the nutritive and functional properties of cowpea protein isolate were investigated. The composition, amino acid profile and bioactive/anti-nutritional factor content of whole meal, processed meal and protein concentrate prepared from cowpea have been reported by Olivera-Castillo *et al.* (2007).

It is a common practice to dehydrate protein isolates to enable their storage and use as ingredients in various food formulations. However the technique employed for dehydration of proteins affects their functional properties (Joshi *et al.*, 2011). The major problem during dehydration is the formation of irreversible insoluble aggregates due to partial denaturation of protein during dehydration (Vojdani, 2006). The commercial production of protein isolate involves spray drying while most of the researchers have used freeze dried isolate powders for characterization of protein isolates (Kaur and Singh, 2007). Vacuum drying is a low cost drying method and is carried out at low temperature far below the protein denaturation temperature. Therefore, vacuum drying could be explored as one of the preferred methods of drying cowpea protein isolates for commercial purposes. This study aims to evaluate the impact of dehydration of cowpea protein isolates prepared by drying freeze drying, spray drying and vacuum drying on their functionality in terms of solubility, gelation, emulsification and foaming characteristics.

MATERIALS AND METHODS

Cowpea (organic produce) was purchased from local store in New Delhi. Chemicals for pH adjustment and buffers were procured from Merck.

Salt assisted protein isolate preparation: The cotyledons were passed through a hammer mill (Savco, India) and

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defatted using hexane. Protein extraction was done using a modified version of the isoelectric method (Rangel *et al*., 2004). Briefly, proteins were extracted from flour twice by suspending them in Tris-HCl buffer (50 mM, pH 8.5) containing 200 mM sodium chloride and centrifuging at 10000 g for 20 min (Thermofisher RC 6 plus, USA). The proteins were precipitated from the pooled supernatant by lowering the pH to 4.3 and centrifugation for 15 min. The resulting pellet was collected, suspended in minimum amount of distilled water and pH adjusted to 7.0.

Drying of protein isolates: *Freeze drying*: Protein isolates were first frozen for 24 h at -18°C. These samples were then freeze dried (Labconco, USA). Freeze dried CPI powder was free flowing without any lumps.

Spray Drying: Spray drying of CPI was done in a bench-top spray dryer (Labultima, USA). The outlet and inlet temperatures were maintained at 85° and 180°C respectively. The flow rate of the feed solution was 6.5 mL/ min.

Vacuum drying: The neutralized CPI solution was vacuum dried in vacuum dryer (Alcon, Lucknow, India) at 60°C, 85 kPa for 72 h in stainless steel trays. The dried CPI was collected and powdered in a grinder.

All the three dried CPI powders were passed through BSS 40 mesh sieve to obtain fine powders.

X-ray diffraction crystallography: The X-ray diffraction (XRD) pattern of the CPI powders was measured using a Philips (PW 1710, Netherlands) diffractometer with CoKa< 1 radiation. Diffractograms were taken between 5° and 55°(2q) at the rate of 1.2°/ min with step size of 0.05° (2θ).

Phase transition analysis: Determination of softening temperature, analogous to glass transition temperature (Karkle, *et al*., 2012) was done using Phase transition analyser (PTA 240, Wenger, Sabetha, USA). 1.25 grams of CPI powder was inserted in the compression chamber with a closed die underneath where it was subjected to a linear increase in temperature from 30°-140 °C at heating rate of 5 °C/ min at fixed pressure of 100 bars.

Zeta potential: Zeta potential was studied using zetasizer (SZ-100, Horiba, Japan) in milliQ water set at pH 1 to 11.0 at increments of 1.0. Clear disposable zeta potential cuvettes were used in these measurements. Three replicate measurements were made for each sample.

Colour: Colour of the powders was measured using HunterLab colorimeter (V 670, Jasco, Japan).

Surface morphology: Surface morphology (SEM) was studied by viewing under scanning electron microscope (Zeiss, Evoma-10, Germany). The CPI powders were deposited onto aluminium stubs using double-sided adhesive carbon conductive tape and were coated with a thin gold layer with the help of gold sputter. An accelerating potential of 15 kV was used during micrography.

Solubility: Nitrogen solubility index was determined by means of 1% dispersion solution of freeze dried and spray dried CPI in phosphate buffer at pH 7.0 (20+/-2°C). However for vacuum dried CPI, a 5% dispersion was used to enable the quantification in the measuring range of the instrument. The suspensions were stirred in magnetic stirrer for 2 to 60 min followed by centrifugation at 700 g for 5 min. 1.5 mL of the supernatant was then used for nitrogen estimation using Kjeltech instrument (UDK 152, Welp, Italy). Solubility was expressed as percentage of total protein (% N×5.6) content of the sample.

Water/ Oil absorbing capacity was determined (Tsaliki *et al*., 2004).

Rheological properties: Rheological studies were done using dynamic rheometer (MCR 52, Anton paar, Austria) according to Joshi *et al*. (2011). Briefly, 25% w/v dispersions of the CPI were made in phosphate buffer, pH 7.0 and tested using parallel plate geometry (50 mm diameter) with a gap of 0.5 mm between the plates.

Least Gelation Concentration (LGC) was determined using CPI dispersions of 6 to16% (w/v) in 2% increment levels in phosphate buffer at pH 7.0 (Joshi *et al*., 2011).

Foaming properties: Foam expansion and stability was determined using one gram of CPI whipped with 50 ml (B) phosphate buffer (pH 7.0) for 5 min in a mixer at 500 rpm and pouring into 250mL graduated cylinder (Intarasirisawat *et al*., 2012). Foam expansion was calculated according to the following equation (Sathe and Salunkhe, 1981).

$$Foam\ stability\ (\%) = \frac{A_{30min} - B}{A_{0min} - B} \times 100$$

$$Foam\ expansion\ (\%) = \frac{A - B}{B} \times 100$$

where, A_t is the volume at specified time interval after whipping and B is the volume before whipping.

Texture profile analysis: To determine the mechanical properties of the gels formed, texture profile analysis of the CPI gel samples was performed using TA.XT Plus texture analyser (Stable Microsystems Ltd., Crawley, UK). For the TPA experiment, protein gels were prepared by heating supernatant of 25% (w/v) CPI dispersion at 90°C for 30 min. (centrifuged at 8000 g for 15 min.) in aluminium foil covered test tubes. After cooling the gels and overnight storage in refrigerator (5-7 °C), the gels were subjected to the texture analysis with a cylindrical probe of 25 mm diameter by compressing twice to 30% of the original height at constant speed of 0.3 mm/s. TPA parameters hardness, cohesiveness, adhesiveness, gumminess and springiness were calculated using definitions of Bourne (2002). Each experiment was performed in triplicate.

Emulsifying properties: Emulsion capacity, Emulsifying activity index (EAI), Emulsion stability index (ESI) and Creaming stability were determined as described by Karaca *et al*. (2011). Sample absorbance was measured at 500 nm using a UV-vis spectrophotometer (Jasco-V670, Japan). EAI and ESI values were calculated using the following equations:

$$EAI\ (m^2/g) = \frac{2 \times 2.303 \times A_o \times N}{c \times \varphi \times 10000}$$

$$ESI(\text{min}) = \frac{A_o}{\Delta A} \times t$$

where A_o is the absorbance of the diluted emulsion immediately after homogenization, *N* is the dilution factor (× 150), *c* is the weight of protein per volume (g/mL), φ is the oil volume fraction of the emulsion, Δ*A* is the change in absorbance between 0 and 10 min (*A0-A10*) and *t* is the time interval (10 min).

Statistical analysis: All experiments were conducted in triplicates. Results are presented as mean ± standard deviation. Means comparisons were performed by one-way analysis of variance (ANOVA) followed by Duncan's test ($p \leq 0.05$). Statistical analyses were run using the computer SPSS 17.0 software (SPSS Institute Inc., Cary, NC, USA).

RESULTS AND DISCUSSION

Physical properties of CPI: The yield of protein isolate from cowpea was 12%. The protein content of cowpea protein isolate was estimated to be 89.14% (db) with 12.48%. nitrogen. Rangel *et al*. (2004) established the conversion factor for nitrogen to protein for CPI as 6.00 based on their true protein contents.The colour of freeze dried CPI measured in terms of L, a, b values was 56.79, 5.26, 20.44, respectively while the corresponding values for vacuum dried CPI were 85.10, 1.62 and 23.65 (Table 1). Spray dried CPI was lighter in colour with L value of 53.75 and b value 18.65. These variations are expected as no heat is encountered during dehydration by the freeze drying compared to spray and vacuum drying. Maillard browning during prolonged dehydration at 40°C and higher bulk density can explain higher L value of vacuum dried CPI . The spray dried CPI was more charged, dusty and less compact (bulk density 560.7 kg/m_3) thus allowing more number of reflecting surfaces for light. The bulk density of the CPI varied from 428.5 for freeze dried CPI to 882.0 in vacuum dried CPI (Table 1).

Sanchez-Vioque *et al*. (1999) reported that isolates with high water and fat absorption are suitable for preparation of cheese, bakery and meat products whereas isolates with good emulsion capacity are suitable for products such as frankfurters or creams. The water absorption capacity of the CPI was found to be 5.13, 4.64 and 3.23 g/ g (wb) for freeze dried, spay dried and vacuum dried samples. The corresponding CPIs' oil absorption capacity of CPI was

Table 1: Physicochemical properties of freeze dried, spray dried and vacuum dried CPI(T_s °C)

	CIE colour			Bulk density (kg/m³)	Water absorption g/ g CPI	Oil absorption g/ g CPI	Protein (%), db	Solubility[A] %	Least gelation concentration (%), w/v	Softening temperature (T_s °C)
	L	a	b							
Freeze dried CPI	56.79±0.52^a	5.26±0.89^a	20.44±0.21^a	428.5±0.31^a	5.13±0.11^a	6.35±0.21^a	89.14±0.07^a	58.21±0.22^a	8^a	56.1±0.20^a
Spray dried CPI	53.75±0.85^b	2.86±0.23^b	18.65±0.41^b	560.74±0.12^b	4.64±0.92^b	2.52±0.63^b	88.57±0.89^a	58.06±0.15^a	8^a	58.6±0.15^b
Vacuum dried CPI	85.10±0.42^c	1.6±0.05^b	23.65±0.03^a	882.35±0.12^a	3.23±0.34^c	3.22±0.16^c	90.21±0.32^a	50.64±0.24^b	12^b	66.3±0.12^c

All data are mean of three replicates

Means with different superscript lowercase letters within the same column are significantly different ($p \leq 0.05$)

[A] Solubility of CPI indicated as percent nitrogen soluble in 60 min at pH 7.0

found to be 6.35, 2.52 and 3.22 g/ g (wb), respectively. The water absorption capacity of CPI was found higher than those reported for the isolates from great northern bean (2.81 g/g (Sathe and Salunkhe, 1981); fenugreek 1.8 g/ g (Nazar *et al.*, 2007), lupin seed 2.09 to 2.19 g/ g (El Adawy *et al.*, 2001). Oil absorption of the CPI was also higher than fenugreek protein isolate (1.56 g/ g) and lupin seed isolates (2.71-2.9 g/ g) (El Adawy *et al.*, 2001; Nazar *et al.*, 2007). However these variations may aslo be accounted for the different methods used to prepare the isolates and pH of the isolates obtained. Thus we can infer excellent use of the cowpea protein isolate for cheese, bakery and meat products as extenders and for retarding staling in baked foods.

Zeta potential and Iso-electric point: As can be seen from Fig. 1, at pH values 3.4 to 11, CPI was negatively charged. These strong negative potentials have a bearing on the ability of CPI to form stable suspensions and emulsions due to intermolecular repulsions. The zeta potential value was zero at pH 3.4 thus indicating this to be the isoelectric point of CPI. Another point to be noted in this regard is that the reported zeta potential is concentration dependent, thus the reported iso-electric point values may vary from other cited values e.g., 4.4 (Zayas, 1997) and even 6.0 (Chel-Guerrero *et al.*, 2011). The method of preparation of isolates might also account for these deviations.

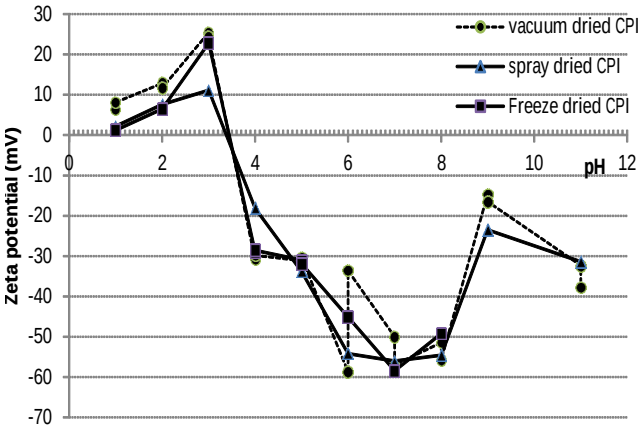

Fig 1: Dependence of CPI zeta potential on ph

X-ray crystallography and SEM: The CPI samples showed three characteristic peaks around 10° (small intensity), 32° and 45° (high intensity). Three diffraction peaks have also been reported by Wang *et al.* (2006) for soy protein isolate at 8.5°, 19.5° and 24.5°. As can be seen from Fig. 2, the intensity of peaks for vacuum dried CPI is much higher as compared to the freeze dried CPI powder. This may be because of the fact that CPI might have partially crystalized during vacuum drying. This fact is further substantiated by the SEM profiles of the CPI powders (Fig 3). During freeze drying, proteins aggregate as a consequence of freeze concentration, adsorption to ice/solvent interfaces or water removal (Chang *et al.*, 1996). The micrographs of freeze dried CPI show an open structure like ribbons/ flakes (Fig 3a), as these proteins have not been subjected to thermal denaturation but only concentration. Spray drying involves transforming a fluid, pumpable medium into a dry-powdered or particle form in a one-step process. This is achieved by atomizing the fluid into a drying chamber, where the liquid droplets pass through a hot-air stream. Although evaporation keeps product temperature to a minimum, little high-temperature deterioration occurs (Masters, 2002). However, shear stress and surface-dependent degradation on atomizing a liquid feed may occur (Maa and Hsu, 1997). In the present study, globular nature of spray dried CPI was found (Fig. 3b) due to denaturation of surface proteins during the pumping action and contact of its droplets with air at high temperature in the spray drying chamber. On the other hand, the vacuum dried samples were found to be highly crystalline (Fig. 3c) because of long duration dehydration at 40°C. Under vacuum condition, the water is transferred in a rapid way from the inside of the sample and evaporated from the surface, which could cause crystallization and a number of burning spots inside the matrix in a very short time (Hu *et al.*, 2013).This explains the delay in solubilisation and decreased functionality of the vacuum dried CPI as evidenced and reported subsequently in this manuscript.

Solubility kinetics: The effect of mixing time on the protein solubility of CPI powders is shown in Fig. 4. For freeze dried and spray dried CPI, 1% dispersion was stirred on magnetic stirrer for desired time intervals (2-60 min.), whereas for

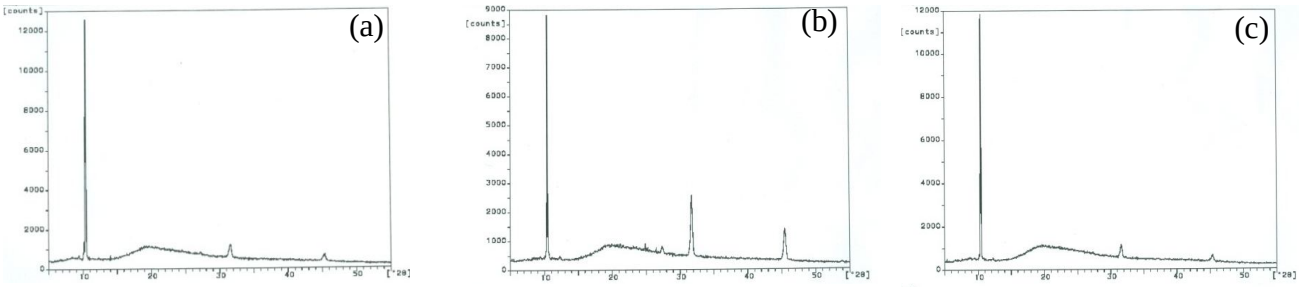

Fig 2: X ray diffraction pattern of (a) Freeze dried CPI (b) Spray dried CPI and (c) Vacuum dried CPI

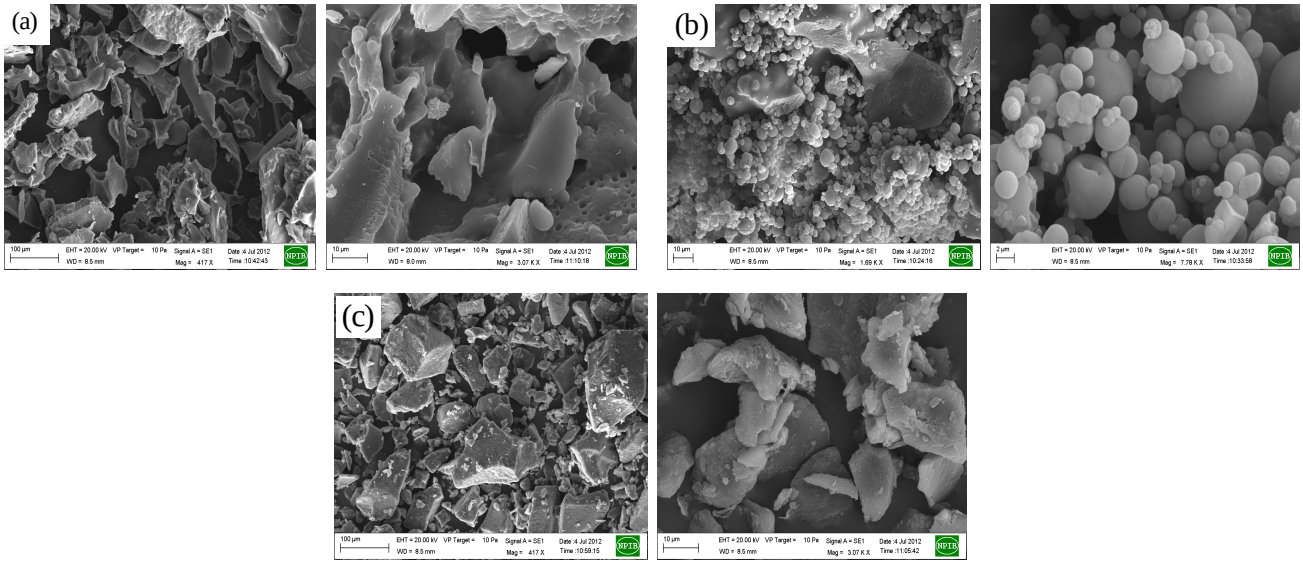

Fig 3: Surface electron micrographs of (a) Freeze dried CPI (b) spray dried, and (c) Vacuum dried CPI

vacuum dried samples 5% dispersion was used. 56-57% protein from freeze dried and spray dried CPI was solubilized in phosphate buffer at pH 7.0 in 30 min. (Fig. 4), however only 36.5% protein from vacuum dried PI could be dissolved in 40 min. Upon increasing the duration of stirring to 60 min however 50.64% protein was solubilized.

The lower rate of solubility of vacuum dried samples is attributed mainly to the crystalline structure formed during drying. In contrast the flaky/ ribbon textured freeze dried powders and spray dried CPI are much easier to hydrate and solubilize. The decreased solubility of the proteins heat denatured during dehydration can be because of exposure of previously buried non-polar protein sites, leading to increased hydrophobicity (Zayas, 1997). Similar results have been reported by Soetrisno (1991) for pea protein isolate who attributed lower solubility of drum dried sodium proteinate proteins than the freeze dried and spray dried counterparts to protein denaturation. The scope for modification of CPI by acetylation, succinylation or fermentation exists to improve upon the solubility of these powders which in turn will affect their interactions with food components and thereby their functionality.

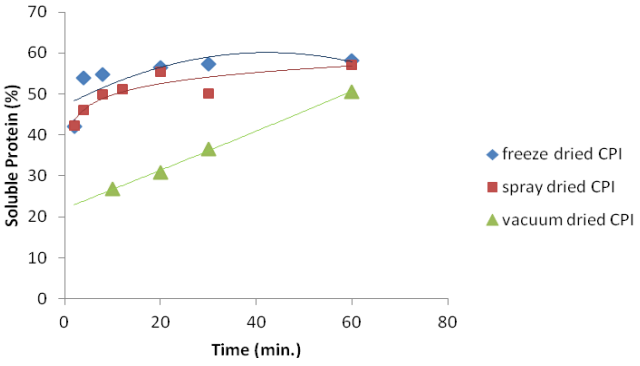

Fig 4: Solubility kinetics of CPI gels

Gelation: Protein gels are important in food products such as sausage and yoghurts. Concentrated forms of seed storage proteins such as soy protein isolates have widely been employed in more sophisticated applications; like generation of gel structures that give the same texture but with reduced lipid and animal protein content in the final product. Freeze-dried and spray dried powders displayed least gelation capacity at 8% as compared to 12% for vacuum dried isolates. This difference is also due to lower rate of solubility of the crystalline vacuum dried CPI in water. This range of least gelation concentration compares favourably to Mwasaru *et al*. (1999) who found 6-10% concentration for gelation of CPI obtained by precipitation at varying pH levels. Texture profile analysis of the gels obtained from CPI are presented in Table 2. All the textural attributes for freeze dried CPI gels were comparable to spray dried CPI and much higher than vacuum dried CPI gels. Thus for gelation purposes, spray dried CPI are as good as freeze dried CPI, whereas softer and less chewy (0.459 N) gels can be obtained from vacuum dried CPI as compared to freeze dried and spray dried CPI (0.55-0.59 N). The higher hardness of the spray dried CPI (2.3 N) and freeze dried (2.2 N) is also attributable

Table 2: Texture profile analysis of CPI gels

Parameter	Freeze dried CPI	Spray dried CPI	Vacuum dried CPI
Hardness (N)	2.215±0.049^{a}	2.347±0.122^{b}	1.871±0.010^{c}
Cohesiveness	0.501±0.016^{a}	0.505±0.042^{a}	0.495±0.011^{a}
Springiness	2.137±0.052^{a}	2.772±0.03^{b}	1.761±0.012^{c}
Gumminess (N)	1.109±.011^{a}	1.185±0.15^{b}	0.926±0.026^{c}
Chewiness (N)	0.555±0.023^{a}	0.598±0.173^{b}	0.459±0.024^{c}

All data are mean of three replicates
Means with different superscript lowercase letters within the same column are significantly different ($p \leq 0.05$)

to the higher solubility of the proteins in the buffer which enables formation of more number of inter and intra-molecular protein bonds in the network.

Rheology: The process of extraction of protein isolates involves suspension of pulse flours in alkaline suspension followed by precipitation, which leads to proteins principally constituted by the globulin fraction (Sanchez-Viouque *et al.*, 1999). Gelation of these globular proteins upon heating is a multistage process involving unfolding of the native molecules to expose interaction sites, intermolecular interaction of these exposed interaction sites on protein chains or aggregation of unfolded molecules, and agglomeration of these aggregates to form a network (Clark, *et al.*, 2001). The dehydration mechanism and the intensity and duration of heat impacted by the proteins definitely have a role in determining the interaction and nature of bonds formed. Thus the nature of interactions and bonding, electrostatic and hydrophobic interactions govern the gelation behaviour and viscoelastic properties of these globular proteins (Clark *et al*., 2001). Lower viscosity of protein suspension before heating is desirable during pumping and piping, and higher viscosity and gel formation after heating is desirable for the thickening of soup, and production of sausage and meat analogue. The gel formation of 25% CPI gels' supernatants was studied by monitoring the storage modulus (G'), loss modulus (G'') and loss tangent during temperature sweep from 5° to 90 °C followed by holding at 90°C and cooling to 25°C. The storage and loss moduli for the vacuum dried CPI at 25°C (during heating) was 0 and 2.29Pa indicating liquid like behaviour, while the torque was recorded to be 47.3. On the other hand, the corresponding moduli for spray dried CPI were 29.3 and 19.7, indicating semi-solid like behaviour. The torque exerted by the spray dried CPI at 25°C was 74.6 µNm while freeze dried CPI exerted a torque of 92 µNm. The corresponding storage and loss moduli for the freeze dried CPI were 173 Pa and 109 Pa. The vast differences in the storage and loss moduli for the CPI dehydrated by three methods indicate the variation in solubilisation and subsequently gel formation and gel strength.

This variation may be because of the fact that the rate of solubility of vacuum dried CPI is much less as compared to freeze dried samples. Gelation of proteins for which aggregation and unfolding of protein chains are pre-requisite occurs at thermal transition above the denaturation temperature of the protein (Shand *et al*., 2007). Figure 5 presents the rheogram of CPI gels. As can be seen from the rheogram, the cross-over point for G' and G" of freeze dried gels had arrived at less than 20 min at approximately 40°C whereas the vacuum dried CPI solidified only after the dispersion reached 60°C. Owing to crystalline nature of the vacuum dried CPI, unfolding of protein network and formation of protein-protein bonds for gel formation started

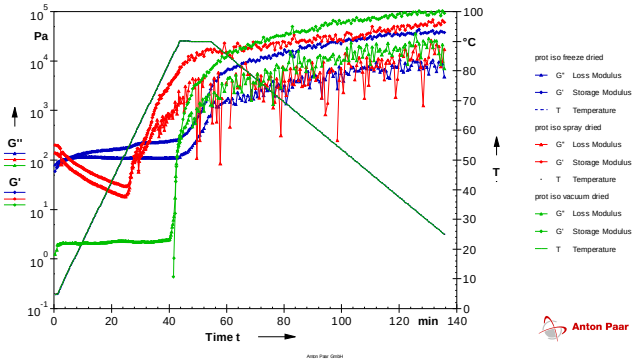

Fig 5: Rheograph of the CPI during heating to 90°C followed by cooling

at higher temperature as seen in Fig. 5. This is further substantiated by the phase transition analyzer data which showed softening temperature (T_s) (analogous to glass transition temperature) of 56.3°C and 58.6°C for freeze dried and spray dried CPI and 66.3°C for vacuum dried CPI (Table 1).

During cooling stage, the torque exerted by the gels was also found to vary. The freeze dried CPI exerted a torque of 99 µNm in 53.3 min. at 90 °C with storage and loss moduli of 1850 and 619 Pa, respectively. In rheogram of vacuum dried CPI, the torque of 99 µNm was observed earlier in 47.3 min (90°C) with 1710 and 803 Pa as storage and loss moduli respectively. The spray dried CPI exerted the same torque even earlier (36.7 min. at 76°C) with storage and loss moduli of 2020 and 462 Pa, respectively. Thus, once the network formation has occurred, the gel strength of the cooled gels from CPI obtained from the three drying methods were similar with final torque of 99-100 µNm exerted at 25°C, though gelation ability of spray dried CPI can be rated better.

Emulsifying properties: Proteins facilitate the formation of smaller oil droplets in emulsions during homogenization by lowering the interfacial tension at oil–water interface. They also increase the stability of the droplets against aggregation by increasing repulsive colloidal interactions (Kim *et al*., 2002). Emulsion capacity of the freeze dried CPI was higher (7 mL). This is also attributed to its higher oil absorption capacity (11.7 g) compared to 6-7 g for spray and vacuum dried CPI. Abu *et al*. (2006) have reported a statistically significant positive correlation between oil absorption capacity and emulsifying properties of cowpea protein concentrates. The ability of emulsification can be measured in terms of emulsion activity index (EAI) which is a measure of available interfacial area that can be stabilized per unit amount of protein and is estimated from the turbidity of a diluted emulsion (Pearce and Kinsella, 1978). Emulsion stability index (ESI) provides a measure of the stability of the same diluted emulsion over a defined time period (Yust *et al*., 2010). Both emulsion activity index and stability are

Table 3: The emulsifying properties of CPI

	Emulsion capacity (mL)	Emulsifying activity index (m^2/g)	Emulsion stability index (h.)	Creaming stability index (%)
Freeze dried CPI	7[a]	39.54±2.52[a]	3656.1±26.85[a]	67.71±3.24[a]
Spray dried CPI	6[b]	101.83±5.82[b]	65.96±3.72[b]	65.08±1.96[b]
Vacuum dried CPI	5[c]	40.14±2.23[a]	98.25±6.21[c]	65.15±1.24[b]

All data are mean of three replicates

Means with different superscript lowercase letters within the same column are significantly different ($p \leq 0.05$)

important parameters for potential use of CPI as ingredient for foaming applications. The freeze dried and vacuum dried CPIs' EAI though comparable (39.75 and 40.14 m^2/g, respectively) vary much in terms of stability with freeze dried CPI having ESI of 3566 h compared to ESI of vacuum dried CPI with 98.25 h stability. For spray dried CPI, even though the EAI of spray dried CPI is higher (Table 3), the stability is lower (66 h). Karaca *et al* (2011) have reported EAI of 39.7–40.5 m^2/g for flaxseed protein isolates and 25.1 m^2/g for canola protein isolates and 55.0 m^2/g for whey protein isolate,. Thus, as compared to EAI of protein isolates obtained from oilseeds, the CPI perform equally well. These values are in agreement to Mwasaru *et al*. (2002), who reported EAI of 40-43 m^2/g for cowpea isolates. The creaming stability of all the three dehydrated CPI were comparable (~65%). Thus we can infer that the vacuum dried and spray dried CPI perform equally well in terms of emulsion formation and creaming ability, though freeze dried CPI show much better performance in emulsifying.

Foaming properties: Surface-activity of proteins is an essential attribute. Lowering the surface tension of the phases is important during the formation of emulsions and foams, but the decrease need not translate into stable films. Proteins enable the fluid interfaces to resist tangential stresses from the adjoining flowing liquids (Lucassen and Benjamins, 1999). Foam expansion was found highest (86 %) for freeze dried CPI however, the foam stability was quite low (25.5 %). The vacuum dried CPI showed poor foam expansion (56%) but good stability (71%). Spray dried CPI performed well in both the expansion (80%) and stability aspects (75%). Thus for foaming application, spray dried CPI showed most promising results. This can also be attributed to the more charged nature of the spray dried CPI and the globular nature of the proteins as visualized by micrographs which enhanced the interfacial tension for foam formation and stability. These globular proteins contain disulphide bridges, tertiary structure, and preserve their globular molecular shape even after adsorption on an interface and thus aid in better foam stability.

CONCLUSION

The good water and oil absorption ability makes cowpea protein isolate a ingredient in designing foods with better nutrition. The amino acids score of CPI was found good with essential: total amino acids ratio of 52-58%. The physicochemical properties of CPI vary extensively with drying technique employed. Therefore it is important to consider the drying technique used to powder the samples while investigating functionality of proteins. The freeze drying technique for dehydration of cowpea protein isolate resulted in better emulsifying properties as compared to spray and vacuum dried samples. The gelation ability of freeze dried and spray dried CPI was better than vacuum dried CPI. However, upon cooling, the gels obtained were almost similar in texture irrespective of drying technique used. Foambility of spray dried proteins was found better. Thus depending on the functional property intended for CPI application, the drying technique may be chosen accordingly.

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CONFLICT OF INTEREST

The authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge or beliefs) in the subject matter or materials discussed in this manuscript.

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