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Review

Protein co-precipitates: A review of their preparation and functional properties

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ABSTRACT

Advances in protein co-precipitation technology over the past two decades have made it possible to commercially produce different types of proteins from mixtures of raw materials. Incorporation of protein co-precipitates improves the functional (e.g. appearance, texture, and stability) and nutritional characteristics of many food products. Increasing world population, increasing demand for and cost of protein-rich foods, and the continuing need to improve the nutritional and functional properties of protein ingredients have contributed to greater research into blends or composites as food ingredients. Protein co-precipitates have a range of biological, physical, chemical, functional, sensory and nutritional properties giving the potential application as ingredients in the food industry, though relatively little published information is available on this subject. There is limited information about the use of protein co-precipitates by the food industry when developing products for different groups of potential consumers. The aim of this review is to evaluate the current status of protein co-precipitate research as a potential way of improving utilization of protein rich raw materials (e.g. dairy protein), oil seed meals (e.g. sesame, soybean, flaxseed and canola) and by-products (e.g. brewing yeast). By blending proteins from different sources, protein co-precipitates are a way of overcoming deficiencies in essential amino acid contents found in proteins from a single source, which giving ingredients with good functional properties and desirable sensory characteristics.

Keywords: Co-precipitate; Soybean; Protein; Milk; Whey; Plant

Contents

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1. Introduction

The term protein co-precipitates was first used by Everette (1952) to describe a product produced by the acidification and heating of a combination of casein and whey proteins. Initially the term was confined to co-precipitates produced from milk, though the use of the term broadened to include mixtures of milk proteins with proteins from other sources (Thompson, 1977), and also mixtures of proteins with polysaccharides (Goncalves and Bourgeois, 1986a; Goncalves et al., 1986; Zaleska et al., 2001). Co-precipitates are produced by isoelectric coagulation, with precipitation occurring as a result of the simultaneous effects of acid and heat combination, or acid, heat and precipitating agents such as $CaCl_2$ (Babella, 1984).

Milk proteins have a good nutritional profile and functional properties, but are also expensive and are not available in sufficient quantities. Whey proteins, including α-lactalbumin and β-lactoglobulin, are recognized for their high nutritional value (Forsum, 1975; Loewnstein and Paulraj, 1971; Qi and Onwulata, 2011). Plant proteins are cheaper than animal proteins (Pinthong et al., 1980; Bautista-Teruel et al., 2003), but are deficient in some essential amino acids which limits their nutritional quality. For example, rice and wheat proteins are deficient in lysine, and soy proteins have low levels of the sulfur containing amino acids. Casein and whey protein co-precipitates contain relative high concentrations of lysine, and can be useful for complementing plant proteins, mainly those of cereals (Babella, 1984).

Protein co-precipitates may be an alternative and more economical method for production of high protein extruded foods with desirable functional characteristics (Hagan et al., 1986). Protein co-precipitates utilization can help overcome the pollution problems of cheese whey disposal (Kebary, 1993).

The combination of a wide range of physical and functional properties and superior nutritive properties allows co-precipitates to be used as an ingredient in a variety of food products either by contributing necessary functional properties to the ingredient mix or for nutritional purposes.

This review will look at the production of three main categories of protein co-precipitates: dairy co-precipitates (casein and whey); plant protein co-precipitates (made from a blend of two or more plant proteins; and dairy protein and plant protein co-precipitates. For each of the three categories of co-precipitate, the processes used for co-precipitate preparation, co-precipitate functional properties and product applications will be reviewed.

Several processes have been developed for the manufacture of protein co-precipitates. An example of the utilization of protein co-precipitates in meat products, the effect of co-precipitates on the quality and yield of boiled sausages (Salavatulina et al., 1983); it is be possible to replace the meat in comminuted foods with mixtures of other proteins such as blood, lupin, sunflower, casein whey and leaf protein concentrate (Skurray and Osborne, 1976).

2. Types of protein co-precipitates

2.1. *Milk protein co-precipitates*

The preparation of milk protein co-precipitates has a long industrial history, with a process for preparing milk protein co-precipitates first being patented in 1952 (Southward and Goldman, 1975).

Milk protein co-precipitates are produced by the precipitation of casein and whey proteins using a combination of heat treatments and addition of acid with or without addition calcium salts (Al-Saadi and Deeth, 2011).

The function of heat treatment in the preparation of protein co-precipitate is to denature whey proteins and may cause an interaction between whey and casein proteins including casein and β-lactoglobulin through disulfide bond formation (Jang and Swaisgood, 1990). When whey proteins are denatured in the presence of casein, the exposed sulfhydryl groups of the whey proteins react preferentially with the casein (Creamer et al., 1978).

Milk co-precipitates are stabilized through a combination of mechanisms. Ionic strength, particularly calcium, plays an important role in preparation of milk protein co-precipitates (Modler, 1985a,b). The three main functions of ionic strength are electrostatic shielding, ion–hydrophobic interaction, and cross-linking of anionic molecules through forming bridge between whey proteins and casein proteins (Kinsella et al., 1989; Wang and Damodaran, 1991). When whey proteins are heat denatured in the presence of casein, the exposed whey sulfhydryl groups react preferentially with the casein (Creamer et al., 1978). This means it is possible for the quantity of denatured milk protein co-precipitates to reach 98–100% of the total proteins (Babella, 1984).

The composition of milk protein co-precipitates is affected by the extent of washing of the precipitated materials (Buchanan et al., 1965). The calcium content of milk protein co-precipitate is determined mainly by the pH of the co-precipitation (Muller et al., 1967).

2.1.1. *Preparation*

Milk protein co-precipitates have been prepared by heating milk or a mixture of milk proteins (whey protein and casein) to above 60 °C and holding at that temperature (typically for

5–20 min). After heat treatment, the protein complex can be precipitated directly or it can be cooled and then precipitated with either acid, divalent ions such as calcium or other ions that affect the solubility of the protein complex (Kosaric and Ng, 1983).

The optimum conditions to prepare milk co-precipitate from sheep milk were 0.2% calcium chloride at pH 6.5, combined with heating at 85–95 °C for 15 min, resulting in 97.5% protein present being recovered as co-precipitate proteins (Al-Saadi and Deeth, 2011).

2.1.2. *Physical properties*

The general physical properties of milk protein co-precipitates may be regarded in much the same way as the physical properties of the individual proteins when examined alone or dispersed or dissolved in water. These properties include pH, solubility (in water, alkali, or acid), density, particle size, viscosity and color of solution.

The pH and composition of milk protein co-precipitates are affected to some extent by the number of washes in water during the preparation of the product (Southward and Goldman, 1975; Buchanan et al., 1965). In contrast, the calcium content of milk protein co-precipitate is determined mainly by the pH during co-precipitation (Muller et al., 1967).

The viscosity of solutions of soluble milk protein co-precipitate in water at different concentrations, temperature and shear rates were measured by Hayes et al. (1969a,b). The addition of calcium at different levels to milk co-precipitates led to an increase in the viscosity and whiteness of solutions.

The whiteness of milk protein co-precipitates was affected by pH, calcium content, calcium chloride and polyphosphate content of the co-precipitates (Smith and Snow, 1968; Hayes et al., 1969a).

The bulk density of milk protein co-precipitates can vary markedly depending on the method of manufacture. Granular, insoluble co-precipitates have a density of approximately 0.6 g/ml, depending on particle size. The density of spray dried soluble co-precipitates can be much lower (Southward and Goldman, 1975). Granular co-precipitates are comparatively coarse and must be ground before they can be used in food products.

2.1.3. *Nutritional properties*

The nutritional value of milk protein co-precipitates has been assessed from its amino acid composition. Because they contain a substantial portion of whey proteins, milk protein co-precipitates are rich in the sulfur containing amino acids, cysteine and cystine (Southward and Goldman, 1975). This means that milk protein co-precipitates have a high nutritional value when compared to casein and other reference proteins (Muller et al., 1966; Resmini et al., 1971).

2.1.4. *Functional properties*

Functional properties of proteins refer to the ability of protein to perform a specific function (e.g. solubility, emulsifying capacity, foaming characteristics, water holding capacity, gelling capacity) in a food product. The nature of desirable functional properties, and their magnitude, depends on the specific food and the type of processes used in its preparation (Poure-El, 1981). For example, solubility is important in milk beverages, emulsification is important in processed cheese, stability and viscosity are important in fermented milk, and foaming is vital for ice cream (Kinsella, 1982). In such mixed protein gels, filler effects play an important role. The microparticulation of whey protein or mixtures of whey protein and casein is emerging as a novel technique to create protein–aqueous systems with a fat-like appearance and mouthfeel (Kosaric and Ng, 1983). The solubility of sheep milk protein co-precipitate increased below pH 5 (Al-Saadi and Deeth, 2011).

An important property of protein gels is their water holding capacity (WHC) (Stanley and Yada, 1992). WHC is a physical property, and is the ability of a gel to prevent water from being released from the three dimensional structure of the gel (Hermansson, 1979, 1986). In protein co-precipitates, WHC is affected by both calcium content and temperature during preparation. WHC of milk protein co-precipitates was measured by Thomas et al. (1974).

An emulsion is a system in which the droplets of a liquid are dispersed in another liquid; it can be either oil in water or water in oil (Dickinson, 1988). Milk protein co-precipitates were found to improve the emulsifying and water binding capacity of the meat when 20% of the meat protein were replaced by the co-precipitates. When higher quantities of the co-precipitates were employed, both emulsion stability and water binding capacity of the meat decreased (Beuchat et al., 1975; Thomas et al., 1974). The emulsion activity index (EAI) of sheep milk co-precipitate increased as pH increased above 6.0 (Al-Saadi and Deeth, 2011).

Foams are dispersions of gas bubbles (air) in a continuous liquid or semi-solid phase (Eisnera et al., 2007). Foaming capacity of milk protein co-precipitates reached a minimum at pH 4.5–5.0, then increased either in acidic or alkaline conditions, as a result of increased protein solubility (De Witt, 1989). Foaming stability for milk protein co-precipitates decreased by increasing protein concentration due to stronger protein interaction at high concentration (Kosaric and Ng, 1983). Milk protein co-precipitates exhibited better foaming properties when compared with other proteins, except soybean proteins (Kosaric and Ng, 1983).

Ramshaw and Dunstone (1970) found that the development of "gluey" off-flavors in low calcium co-precipitates was inhibited by the addition of 0.01–0.05% sodium metabisulphate. Milk protein co-precipitate flavor stability was improved by heating them in solution to encourage the formation of volatile off-flavor components that could be removed during drying.

2.1.5. *Evaluation in food products*

The baked loaves produced from the low absorption milk protein co-precipitates have been shown to be more acceptable than those produced from higher absorption soluble co-precipitates (Muller et al., 1970). Insoluble and dispersed milk protein co-precipitates have also been considered as suitable ingredients for the fortification of breakfast cereals (Muller et al., 1970). Milk protein co-precipitates prepared with calcium (0.5–0.8%) appeared to be the most soluble, and 1.0–1.5% calcium the least soluble (Kosaric and Ng, 1983).

Montigny (1983) described a process for the precipitation of casein and or whey protein by combined application of acidification and heating to produce a co-precipitate suitable as a pre-cheese for cheese manufacture.

Yoghurts fortified with caseinates showed higher viscosity and syneresis index compared to yoghurts prepared from skim milk fortified with co-precipitate (Guzman-Gonzalez et al., 2000). The milk co-precipitate was prepared by mixing milk with 0.2% of $CaCl_2$, heating at 90 °C and adjusting to pH 5.9 (Eswarapragada et al., 2010).

Dairy protein co-precipitates have been evaluated as an ingredient in meat products, primarily sausages and nuggets or patties. Milk co-precipitate was prepared from fresh skim milk using heat and salt (Eswarapragada et al., 2010). This co-precipitate was used replaced to fat at levels of at 0–2% to pork sausages. When added to sausages, dairy protein co-precipitates increased the fat binding potential and shear force, and decreased the water activity and color intensity (Eswarapragada et al., 2010). Sensory evaluation showed a progressive increase in the flavor, juiciness and overall acceptability scores of sausages containing up to 1% dairy protein co-precipitate (Eswarapragada et al., 2010).

The incorporation of milk co-precipitates in meat improved the nutritional and functional properties of meat products (Kumar et al., 2004). Low-fat ground pork patties containing 7% milk co-precipitate had lower peroxide value and better sensory and microbiological properties compared to high-fat ground pork patties (Kumar and Sharma, 2003).

The optimum level of milk co-precipitate addition to buffalo meat blocks was found to be 10% (Kumar et al., 2004). At this level, the meat blocks containing co-precipitates had a better cooking yield, sensory score, hardness/firmness properties, and shrinkage and moisture retention as compared to the original meat block formulation (Kumar et al., 2004).

Adding of milk co-precipitate to chicken steaks improved emulsion stability and cooking yield and at 20% improved the juiciness, color and consumer acceptability as compared to the original chicken steak formulation (Bhoyar et al., 1998).

The incorporation of milk co-precipitates into mutton nuggets and patties had no effect on sensory properties, cooking yield, emulsion stability, protein quantity and moisture content compared to control samples, but it affected flavor, juiciness, pH, frying loss and shear force compared to control (Rao et al., 1997). Patties containing milk co-precipitates had higher moisture contents and improved sensory characteristics ratings for appearance, flavor and texture (Thomas et al., 1978).

Table 1 – Proximate composition, functional properties and nutritional value of bean–chickpea–sesame protein co-precipitates.

Propreties	Protein co-precipitites
Proximate composition	
Moisture (%)	5.76
Crude protein (%) (total N. X 6.25)	91.2
Ether extract (%)	‘0.51
Ash (%)	4.22
Carbohydrate (%)	4.02
Functional properties	
Water absorption (%)	232
Fat absorption (%)	74.0
Emulsifying capacity (ml oil/100 g flour)	79.0
Foaming capacity (%)	220
Nutritional value	
Tannis (mg/g)	0.81
Phytic acid (mg/g)	7.69
Trypsin inhibitor (units/mg)	4.01
Chymotrypsin inhibitor (units/mg)	2.6
In vitro protein digestibility (%)	89.1
Amino acids (g/16 g N)	
Isoleucine	4.29
Leucine	8.35
Lysine	5.11
Methionine	1.62
Cysteine	0.58
Phenylalanine	4.73
Tyrosine	2.54
Threonine	3.11
Valine	4.58
Arginine	8.91
Histidine	2.81
Aspartic acid	9.46
Glutamic acid	15.3
Glycine	5.29
Serine	4.03

Source: Youssef et al. (1995).

2.2. *Plant protein co-precipitates*

Seeds are a source of low cost, edible vegetable proteins for supplementing diets (Mattil, 1971; Bautista-Teruel et al., 2003), and it has been shown that plant proteins can be used to prepare new nutritionally balanced food, equal in nutritive value to protein diet from animal sources (Moharram and Abu-Foul, 1992; Abo-foul et al., 1995).

2.2.1. *Preparation*

Processes to produce co-precipitates from various combinations of plant proteins have been developed. The conditions used for plant co-precipitates vary depending on source. A soybean and peanut (1:1, w/w) co-precipitate was produced by mixing in water with a ratio 1:18 and stirring for 1 h, followed by precipitation of curd by adjusting the pH to 4.5 with 1 N HCl (Hagan et al., 1986). The washed curd was finally adjusted to pH 7.0 with 1 N NaOH and spray dried.

Various co-precipitates have also been prepared from two proteins blends of cotton seed, soybean and peanut flour (1:1, w/w). Notably, the protein co-precipitation stage was performed by acidification of the protein extract to pH 2.5, which is lower than the range (pH 4.5–5.0) typically used for co-precipitation (Berardi and Cherry, 1981). After co-precipitation the mixture was adjusted to pH 5.0, and lyophilized (Berardi and Cherry, 1981).

2.2.2. *Chemical composition*

There are recognized advantages in the forming co-precipitates from plant flours and isolates (Dendy et al., 1975; Tsen, 1976). Plant protein co-precipitates have shown higher nutritional value, and superior functional properties, lower level of anti-nutritional factors, higher in vitro protein digestibility and higher levels of essential amino acids, than those of the individual protein isolates (Youssef et al., 1995) (Table 1).

Fernandez-Quintela et al. (1993) reported that the conditions used during the preparation of the protein co-precipitates resulted in the removal of most of the natural anti-nutritional factors in plant protein sources.

2.2.3. *Functional properties*

Extrusion processing has been employed extensively for the production of texturized plant proteins for use as meat extenders. Use of a twin screw extruder to texturize a co-precipitated soy bean and peanut proteins resulted in the formation of a highly moist, less structurally rigid and moderately expanded product compared to individually textured soybean and peanut protein concentrates (Hagan et al., 1986).

2.3. *Milk–plant protein co-precipitates*

A soy cheese–whey protein co-precipitate was prepared from a concentrated mixture of defatted soy flour and cottage cheese whey (Loewnstein and Paulraj, 1971). Plant–why protein co-precipitates prepared by acid-heat processing have also seen reported (Morr, 1978).

2.3.1. *Preparation*

2.3.1.1. Whey and plant protein mixtures. Whey–plant protein co-precipitate formation by acid and heat precipitation have used different combinations of raw materials, including defatted soy flour and cottage cheese whey (Loewnstein and Paulraj, 1971), soybean–whey and cottonseed–whey (Thompson, 1978), however the co-precipitation conditions of temperature (95–98 °C), pH 4.6–4.7) and time (15–30 min) have been similar.

A more recent investigation of whey–plant protein co-preciptate production modified this approach by incorporating a pH 11 extraction stage (Alu'datt et al., 2012). In this process, soybean and whey co-precipitates were prepared from defatted soy flour and whey powder (Alu'datt et al., 2012). The co-precipitates were formed by suspending the powders in distilled water, adjusting to pH 11 and holding at 40 °C for 60 min to extract the protein. The protein extract was precipitated by adjusting to the isoelectric point (pH 4.6), and either heating at 95 °C for 30 min followed by cooling at 4 °C, or by cooling directly to 4 °C. The co-precipitate was recovered by centrifugation followed by freeze drying. The maximum yield of soybean protein–whey protein co-precipitates was obtained by extraction at 40 °C, and precipitation at pH 4.5 and 98 °C (Alu'datt et al., 2012).

The yield of whey–soy proteins co-precipitates was increased by the addition of 0.5% calcium ethylenediamine and sodium hexametaphosphate compared to the 0% level (Alu'datt et al., 2012). Precipitation pH affects protein yield, with maximum yield occurring at pH 4.5 (34%), with lower yields at lower pH (pH 3.5, 25% yield; pH 4.0, 30%) and higher pH (pH 5.0, 32%; pH 6.0, 24%). Temperature also affected yield, recovery increasing from 25 to 35% as the precipitation temperature was increased from 60 to 98 °C (Alu'datt et al., 2012). Rapeseed–cheese whey protein co-precipitates prepared by hexametaphosphate, polyacrylic acid and ferric polyphosphate were used (Thompson et al., 1979, 1984).

2.3.1.2. Casein–wheat germ protein mixtures. Fayed (1987) prepared protein co-precipitates from wheat germ protein solution (3.5% protein, pH 9) and skim milk (pH 6.6) with a volume ratio of 30:70, respectively. The blend mixture was pH 6.7–6.8. The blended mixture was adjusted to pH 9 by addition of 2 M NaOH with stirring. Centrifugation (3000 × *g* for 10 min) gave no protein precipitate. However, protein co-precipitate was obtained by adjusting the pH to 4.6 using HCl (2 N).

2.3.2. *Functional properties*

Table 2 shows the essential amino composition of protein isolate, concentrates and co-precipitates from rapeseed proteins and cheese whey protein. Tables 3 and 4 show some functional properties of milk–plant protein co-precipitates.

Milk–plant protein co-precipitates from whey and bean protein have been reported to show improved functional properties compared to the bean and whey proteins individually (Kebary, 1993). Protein solubility is important for the application of proteins in beverages, infant formula, texturized meats, and sauce, and as an index of protein changes during food processing (Kebary, 1993). Protein solubility of whey–bean protein co-precipitates reaches a minimum around pH 4.5–5, the region of isoelectric points of the proteins (De Witt, 1989). Whey milk and bean protein co-precipitates carry negative and positive changes above and below the isoelectric point respectively, and water molecules can interact with these charges to enhance the solubility (Kebary, 1993). In general proteins showed higher solubility at alkaline pH (7–10) than at acid pH (2–5), while the minimum solubility is observed at around pH 4.5 (Fayed, 1997).

The formation and stabilization of emulsions is critical for many applications such as chopped, cakes, salad dressings, coffee whiteners, and comminuted meats. The emulsion capacity of milk and bean co-precipitates was reported to be a minimum around pH 4.5–5.0, then increased in both sides of this pH range. Emulsion properties were also affected by NaCl (Kebary, 1993).

The foaming capacity of a whey and bean protein co-precipitate increased as the NaCl concentration was increased to 0.4 M; above this concentration foaming capacity decreased (Kebary, 1993). The whey and bean protein co-precipitate showed the highest foaming stability at pH 10.0 with decrease in foaming stability with decreasing to pH 4.0 (Kebary, 1993).

A soybean–whey co-precipitate showed improved gelation properties compared with gels made from the individual whey and bean proteins (Catsimpoolas and Meyer, 1970). The whey and bean protein co-precipitates also showed improved water and oil absorption (Kebary, 1993).

Variation in the processing conditions to improve protein extraction (with or without holding at 40 °C) and precipitation (immediate cooling to 4 °C, or heating to 95 °C followed by cooling to 4 °C) of soybean–whey co-precipitates gave co-precipitates with a range of functional properties depending on the processing they had received (Alu'datt et al., 2012). Co-precipitation by immediate cooling 4 °C gave much higher gel strengths and WHCs than co-precipitates formed by holding at 95 °C followed by 4 °C. Extraction at 40 °C prior to co-precipitation had no benefit, producing either a marginal increase or decrease in WHC and gel strength.

Soybean–whey and cottonseed–whey co-precipitates had higher solubility, yields and protein scores, lighter color, lower water and fat absorption and emulsifying capacities when compared with individual protein precipitates formed from cottonseed, soybean and whey (Thompson, 1978). In contrast, the whipping properties of soybean–whey and cottonseed–whey proteins co-precipitates isolate was lower and higher in soybean–whey and cottonseed–whey proteins co-precipitates concentrate as compared individual protein precipitates of cottonseed, soybean and whey (Thompson, 1978).

The cottonseed whey protein co-precipitate had lower nitrogen yield than the individual protein precipitate of cottonseed and cheese whey proteins. The cottonseed whey protein co-precipitate had a higher protein score and protein efficiency ratio than the cottonseed and whey protein precipitate.

Protein composite blends were formed from potato and whey proteins. The emulsification and foaming properties of the potato–whey composite blends improved as the pH increased and the potato protein content decreased (Jackman and Yada, 1988). Composite blends were also prepared with different proportions of pea and whey proteins (Jackman and Yada, 1989).

Table 2 – Essential amino acids composition for protein isolates, concentrates and co-precipitates from cheese whey protein and rapeseed flour, isolate or concentrate.

Essential amino acids as (% of total)

Products	Lys	Thre	Met	Val	Ileu	Leu	Tyr Phe
Rapeseed flour (RF)	15.1	11.3	8.9	14.5	12.2	20.8	17.2
Rapeseed isolate (RI)	10.4	123.2	6.0	14.8	12.4	20.7	22.5
Rapeseed isolate whey co-precipitate (RIW)	17.0	12.4	8.4	12.3	10.7	21.7	17.5
Rapeseed isolate whey co-precipitate (theoretical) (RIW)	14.0	12.3	7.3	13.4	11.4	21.2	19.6
Rapeseed concentrate (RC)	14.9	13.1	8.6	13.6	9.0	20.2	20.9
Rapeseed concentrate whey co-precipitate (RCW)	17.1	12.5	7.9	12.8	8.9	22.8	18.2
Whey (W)	19.6	10.7	9.2	11.0	9.7	21.9	14.9

Source: Thompson (1977).

Table 3 – Functional properties for protein isolates, concentrates and co-precipitates from rapeseed proteins and cheese whey protein.

Products	Color Y_{CIE}	pH of 10% dispersion	Nitrogen solubility (%)		Water absorption (%)	Fat absorption (%)	Emulsifying capacity (ml oil/20 ml sample)	Whipping capacity (%)
			H_2O%	0.2% NaOH				
RF	75.8	5.5	48.8	100	174.5	146.2	34.5	73.7
RI	29.8	7.3	9.2	100	273.0	105.5	13.0	37.0
RIW	32.8	7.3	8.8	100	362.3	121.3	20.5	25.4
RC	54.7	7.3	6.4	100	405.6	198.0	13.5	13.2
RCW	58.8	7.3	10.7	100	239.9	127.0	17.5	35.6
WC	82.9	7.2	8.0	100	255.9	153.9	22.0	32.0

Source: Thompson (1977).

For abbreviations, see Table 2.

The functional properties (solubility, heat stability, emulsion properties and foaming capacity) of the pea–whey composite blends increased as the pH increased and the pea protein level decreased.

Various levels of pea and cheese whey proteins prepared at low and high heat treatments. The results revealed that the blend of pea and cheese whey proteins at low and high temperate had superior functional and baking properties as compared to either of whey or pea (Patel et al., 1981; Patel and Grant, 1982).

A protein co-precipitate was prepared from cheese whey and soy protein by papain addition. The cheese whey and soy protein co-precipitates had more solubility as compared to either soybean or whey proteins (Pallavicini and Trentin, 1987).

Rapeseed–cheese whey protein co-precipitates formed by the addition of polyacrylic acid had high protein content, solubility, fat absorption and emulsifying and whipping capacities, and a light color (Thompson et al., 1984). Thompson et al. (1984) suggested that rapeseed–cheese whey co-precipitates prepared with polyacrylic acid had potential as egg white substitute or beverages (Thompson et al., 1984).

2.4. *Other co-precipitates*

Whey proteins have been co-precipitated with other proteins such as blood, egg and yeast proteins. Maximum yield was obtained when acid in conjunction with low levels of $CaCl_2$ was used (Mathur and Shahani, 1977; Hill et al., 1982).

A skim milk and porcine blood plasma co-precipitate prepared by adjusting to pH 9.5, heating to 70 °C for 3 min, cooling to 68 °C, adjusting pH to 3.5 for 5 min and final pH adjustment to 4.7 (De Haast et al., 1987). Whey–blood protein co-precipitates was found to be a poor protein source because the heat treatment required for its preparations destabilized the whey proteins causing a decrease in the protein efficiency ratio and the biological value of the blood proteins (Young, 1980). The solubility, viscosity and emulsifying capacity of the skim milk and porcine blood plasma co-precipitates were superior as compared to individual skim milk and blood plasma protein.

Chemical precipitation agents have been used to prepare egg protein–whey protein and blood protein–whey protein

Table 4 – Water absorption, oil absorption and gelation ability of whey proteins, bean proteins and whey–bean protein co-precipitates.

Functional properties	Bean protein	Whey protein bean protein co-precipitates	Whey protein
Water absorption (g H_2O/100 g sample)	275.98	190.69	147.78
Oil absorption (ml oil/100 g sample)	107.6	99.6	82.6
Protein concentration to form gel (% protein)	7.0	4.0	6.5

Source: Kebary (1993).

co-precipitates (Schmidt and Illingworth, 1978). Whey–egg protein co-precipitate showed a higher WHC than proteins from whey powder and egg powder individually (Kosaric and Ng, 1983).

Protein–polysaccharide co-precipitates were produced from bovine plasma and anionic polysaccharides (e.g. sodium alginate, sodium alginate, λ-carrageenan and xanthan) over a range conditions (pH, ionic strength, protein:polysaccharide ratio) (Goncalves and Bourgeois, 1986a; Goncalves et al., 1986). The maximum co-precipitate yield was obtained at pH 3.5–4.5 and a protein to polysaccharide ratio of 6:1 (Goncalves and Bourgeois, 1986a; Goncalves et al., 1986). Goncalves et al. (1986) reported that lower shear rate range in protein co-precipitates with anionic polysaccharides as compared to plasma protein is due to the appearance of a yield stress associated with aggregation phenomena between macromolecules.

3. Summary and future research demands

Protein co-precipitates are a valuable food ingredient as they allow enhancement of the chemical, physical, functional, nutritional, biological, nutraceutical and pharmaceutical properties. Protein co-precipitates may also have positive effects on allergenicity of food proteins. Several processes have been developed to prepare of protein co-precipitates, though it may be possible to find new and novel approach to prepare protein co-precipitates with highest protein yield in order to be used as a commercial product in food industry. Co-precipitated products can be directly used for supplementing and or enrichment of low quantity and poor quality food sources, also co-precipitate products can be used in different areas of food and nutrition as beverage. Several workers indicated the potential uses for co-precipitates in the bread baking industry either for the purpose of protein fortification or as a replacement for skim milk powder. They have also be evaluated in a variety of meat products. Co-precipitates can be used for the preparation of a variety of protein enriched or high protein dried, fried or puffed snack foods.

The following major aspects require further attention in order to develop protein co-precipitates as ingredients in high quality, nutritious and safe food products.

1. Understand the mechanism of protein–protein interactions for protein co-precipitates including, hydrophobic, ionic, London and disulfide interactions.
2. Optimization the protein yield of protein co-precipitates including extraction and precipitation conditions.
3. Utilization of protein co-precipitates to protein based by-products of the food industry such as brewing yeast, whey proteins, and oilseeds industry.
4. Preparation of protein co-precipitates using more than two sources of food proteins.
5. Evaluation the effect of fortification of protein co-precipitates on nutritional, biological including in vivo and in vitro study of chronic diseases such as diabetic and angiotensin converting enzymes.
6. Evaluation the effect of protein co-precipitates on allerginicity of allergic food proteins.
7. Evaluation the effect of protein co-precipitates functional properties of food products.
8. Study the effect of protein co-precipitates on the sensory properties of food products.

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