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Functional Properties of Co-Precipitate Mixtures of Casein and Flaxseed Protein Isolate.

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Abstract

Flaxseed protein isolate containing 59.60 ± 0.1% protein was obtained from flaxseeds. The protein recovery yield was 30.53%. The protein isolate had a very light brownish color, and no off-smell, was observed. Three co-precipitate mixtures were obtained by blending cow's fresh skim milk (pH 6.6) with a solution of flaxseed protein isolate (3.5% protein, pH 9) at different volume-to-volume ratios of,90:10,80:20and 70:30fresh skim milk: flax seed protein solution the co-precipitates mixtures were co-precipitate at pH 4.5. The three co-precipitated mixtures were termed Cn-FPI-10, Cn-FPI-20, and Cn-FPI-30. The protein contents of three precipitate mixtures powders were 70 ± 2.28%, 68.5 ± 2.62%, and 67.6 ± 2.29% for Cn-FPI-10, Cn-FPI-20, and Cn-FPI-30, respectively. The protein co-precipitate mixture powders had a yellow to very light brown color and a smell Resembles that of skim milk powders.

At neutral pH (6–7), the co-precipitate mixtures had solubilities ranging from 58.21% to 70.76%. The three co-precipitate mixtures had water-holding capacity comparable to that of casein, whereas the oil-holding capacity was less than that of casein and higher than that of flaxseed protein. Emulsion capacity (EC) of the co-precipitate was comparable to that (EC) of casein. Foam overrun (%) of the obtained co-precipitates was intermediate between that of casein and flaxseed protein, and its stability was higher than that of flaxseed protein. The co-precipitate mixtures showed good gelling properties comparable to those of casein. The co-precipitate revealed viscosity higher than that of flaxseed protein at the same protein solution concentrations.

Conclusively: it can be concluded that the. The co-precipitate mixtures showed good gelling properties comparable to those of casein. The co-precipitate revealed viscosity higher than that of flaxseed protein at the same protein solution concentrations.

Key words: Flaxseed protein- Isolation –Casein analysis

1. Introduction

Skim milk powders and casein derivatives provided the food and dairy industry with a reliable and stable source of milk proteins, which have been widely incorporated into various dairy and processed food products as functional ingredients **(Hinderink et al, 2021,Suther et al,2017,Bautista-Ternal et al,2003).**In many of developing countries, the dairy industry has been facing a shortage in milk supply and consequently a limited availability of milk proteins **(Hofi,2011, jonas,1975**) (**Alves and Tavares, 2019;Pinthong et al,1980)**. Therefore, the search for novel, high-quality, inexpensive protein sources remains an important objective to overcome the shortage of animal proteins in developing countries **(Hussain et al.2008, Kotecha-Majchrzak et al, 2020).**

Due to the insufficient supply of milk protein powders, there has been increasing interest in vegetable proteins **(Lin et al., 2017).** Plant proteins are abundant, economical, and readily available **(Sharma et al., 2021).**

Oil seed proteins are an attractive alternative. Flaxseed (*Linum usitatissimum L.*) is one of the major oil crops worldwide **(Peng et al., 2022)**. It contains a relatively high amount of protein, ranging between 18–30% **(Tang et al., 2020; Kajla et al., 2015).** Vegetable proteins are increasingly used to partially replace animal proteins in dairy products, which reduces costs and enhances functional properties **(Alves and Tavares, 2019).** As a result, a range of double-protein dairy products, where plant proteins substitute part of the animal protein content, have emerged in the market **(Hu et al., 2022).** Plant–dairy protein mixtures, especially combinations of soy protein and dairy protein, are receiving growing attention in the food industry **(Hu et al., 2022; Hinderink et al., 2021).**

Protein co-precipitates represent one type of such mixtures and are considered a cost-effective method for generating multifunctional protein blends **(Aludatt et al., 2012).** Casein and corn germ protein co-precipitates were previously studied for their functionality **(Fayed,1995)**. Additionally, investigated interactions between casein and rice glutelin. (**Hu et al, 2020)**. Accordingly, the dairy industry is actively exploring co-precipitated mixtures of dairy and plant proteins to obtain enhanced functionality and versatility **(Hu et al., 2022; Racikos et al ,2010, Lin et al., 2017)**.

When developing novel protein sources for the food industry, functional properties—such as solubility, gelation, emulsification, and foaming—are key indicators of potential use **(Boye et al., 2010; Foegeding and Davis, 2011).**

Therefore, the aims of the present study were:

1. To obtain a protein isolate from flaxseeds.
2. To use the obtained flaxseed protein isolate in the production of co-precipitate mixtures with casein.
3. To characterize the chemical and sensory properties of the obtained protein isolate and protein co-precipitates.
4. And To evaluate the functional properties of the obtained co-precipitate protein mixtures by comparing them with casein and flaxseed protein isolate, each in single form.

Materials and Methods

Materials

Whole flaxseeds (*Linum usitatissimum L.*) Giza11 was obtained from the Fiber Crops Institute, Agricultural Research Center, Egypt.

Fresh cow's skim milk (pH 6.7) was obtained from the commercial market.

The chemicals and reagents used were of analytical and food-grade quality, obtained from El-Gomhouria Company, Egypt.

Methods

1. **Flaxseed Mucilage Extraction**

Mucilage present in the outer coat of whole raw flaxseeds was extracted using a hot water method adapted from **Safdar et al,2019** with slight modifications. Specifically, 100 g of flaxseed were added to distilled water preheated to 90 °C, maintaining a seed-to-water ratio of 1:20 (w/v), and stirred continuously for 3 hours. The mixture was filtered through a double-layered cheesecloth, and the remaining seeds were manually pressed to recover the insoluble gum fraction. The demucilaged seeds were subsequently dried in a hot-air oven at 50 °C for 24 hours.

2. **Defatting Flaxseed Powder**

Defatted flaxseed powder was prepared based on the methods described by **Tirgar et al,2017 and Amza et al, 2011**, with slight modifications. Demucilaged flaxseed powder was suspended in hexane at a powder to solvent ratio of 1:5 (w/v), and the mixture was stirred for 6 hours, with the hexane being replaced every 3 hours. The solvent was then separated by centrifugation at 3000 × g for 15 minutes. The resulting

defatted powder was left to stand overnight at room temperature (approximately 30 °C) to allow complete evaporation of any residual solvent.

3. **Flaxseed Protein Isolate Preparation**

Flaxseed protein isolate (FPI) was extracted following the method described by **(Silva et al, 2013 and Zhao et al, 2015)**, with slight modifications. Specifically, flaxseed powder was dispersed in distilled water at a 1:10 (w/w) ratio, and the pH of the dispersion was adjusted and maintained at pH11 using 1 N NaOH solution. The mixture was stirred at 30 °C for 60 minutes. After extraction, the mixture was centrifuged at 3500 × g for 20 minutes to separate the supernatant containing the soluble proteins. The pH of the supernatant was then adjusted to 4.5 using 2 N HCl solution to precipitate the flaxseed proteins. The resulting precipitate was recovered by another centrifugation step (3500 × g, 20 minutes), followed by freeze-drying. The isolated FPI was stored in hermetically sealed containers for further use.

Protein Recovery Yield

The percentage of flaxseed protein recovery yield in the current study was calculated using the following equation which was put by **Sharma and Saini,2021**

$$\% \text{ Protein Recovery Yield} = \left(\frac{w3}{w4}\right) \times 100$$

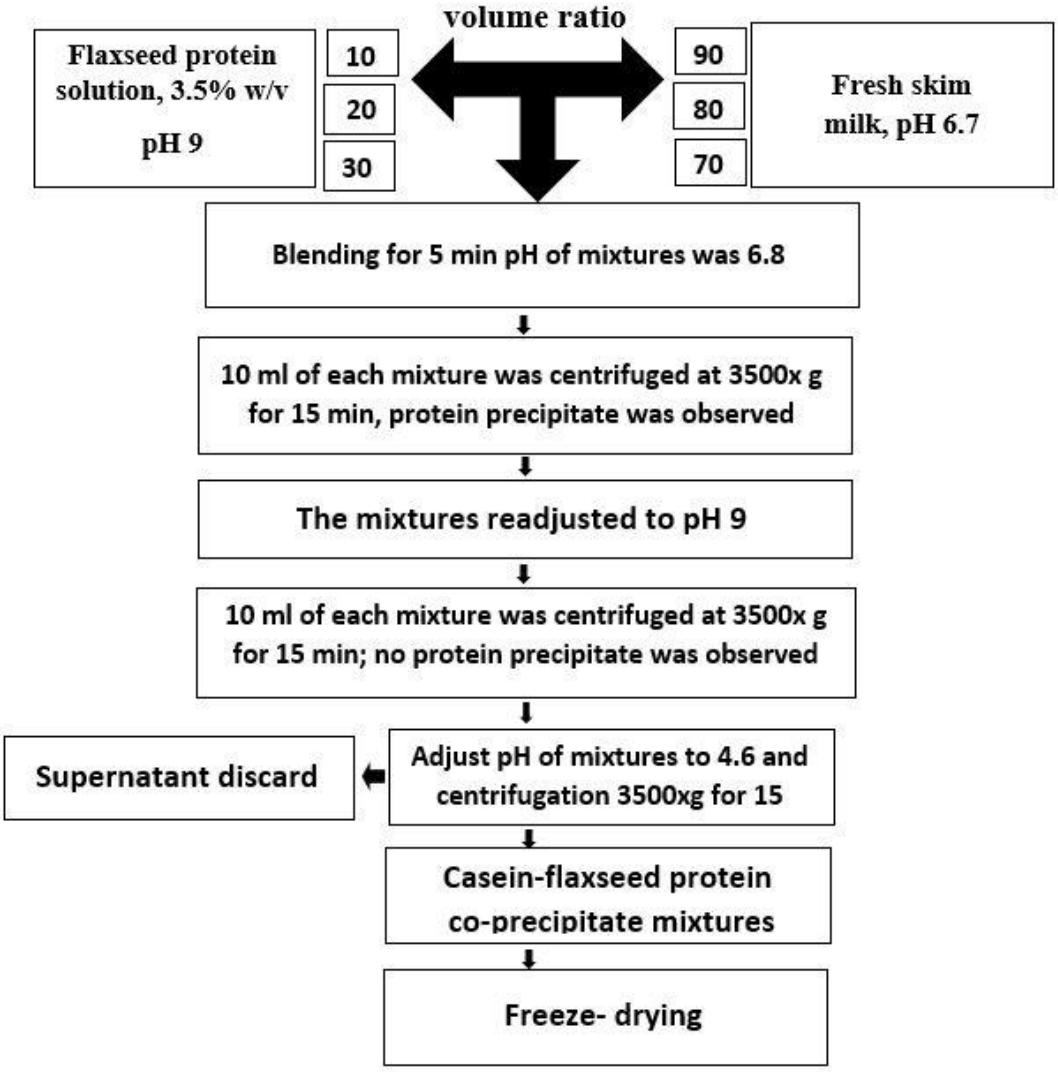
where: W3 is the amount of protein (in grams) in isolated Flaxseed protein, and W4 The amount of protein (grams) in whole demucilaged and defatted, flaxseed powder used in the determination **(Sharma and Saini, 2021)**.

4. Preparation of Co-Precipitate Mixtures of Casein and Flaxseed Protein Isolate

An outline of the method used to prepare co-precipitate mixtures of casein and flaxseed protein isolate is shown in Fig. (1), based on the previous method described by **Fayed (1995).** Flaxseed protein isolate (FPI) solution (3.5% protein, at pH 9) was blended with fresh liquid skim milk (pH 6.7) at volume ratios of 90:10, 80:20, and 70:30 (skim milk protein solution). The blended protein mixtures were stirred for 5min. The pH of all protein mixtures was then measured. Ten milliliters of each mixture were centrifuged at 3500 × g for 15min; protein precipitate was observed. Therefore, the pH of the mixtures was readjusted to pH 9 while stirring. Then, 10 mL of each mixture was centrifuged again at 3500 × g for 15 min; no protein precipitate was observed. The mixtures of proteins were then stirred for 10 min. The protein mixtures were co-precipitated by adjusting the pH of the mixtures to 4.5 using 2M HCl.

The co-precipitated proteins were separated by centrifugation at 3500 × g for 15 min. Then, the casein–flaxseed protein co-precipitate mixtures were freeze-dried. The obtained protein mixtures were designated as Cn-FPI-10, Cn-FPI-20, and Cn-FPI-30 for the mixtures prepared at volume ratios of 90:10, 80:20, and 70:30 (skim milk: flaxseed protein isolate solution), respectively.

A control sample of fresh skim milk was treated as above without the addition of flaxseed protein solution to precipitate acid casein, which was termed (Cn) Fig (1).



Fig(1): flow digram for prodcution co-precipitate mixtures of casein ad flaxseed protein bassed on the method previously described by **(Fayed,1995)**

Proximate Chemical Composition Analysis

Chemical composition of whole flaxseed, demucilaged flaxseed, defatted flaxseed, flaxseed protein isolate and casein–flaxseed protein isolate mixtures was determined for dry matter, protein, lipids, ash, and fiber according to **ISO-5933-1 (2002).** Crude fat was determined according to the method described in the Official Journal of the European Union **(EN), 2009, L54/37**, Volume 52, and crude fiber was determined according to the methods described in the Official Journal of the European Union **(EN), 2009, L54/40**, **Volume 52**.

Determination of Cyanide

Alkaline picrate reagent was prepared by a modification of the method described by **Williams and Edwards (1980)** as follows: Test tubes with 2mL of 2% KOH and 1mL of picric acid: Na_2CO_3:H_2O (1:5:200 v/w/v) were prepared. Standard absorbance curves were made with 3 Whatman No 1 papers each with a dimension of 8×1cm. The papers were dipped into the alkaline picrate solution for 15 minutes. The picrate impregnated papers were removed from the solution and used immediately for cyanide determination.

Cyanide solutions containing (50-200µgKCN/mL) were each prepared in glass bottles. The cyanide was acidified with 20% HCl solution heated to 800°C and immediately sealed with 3 picrate impregnated

papers. The system was incubated at room temperature (28±20C) for 24 h. The red-colored complex formed was eluted with 50% ethanol solution for 30 minutes. The eluate absorbance was measured at 510 nm using a Spectrum lab 23A spectrophotometer.

Preparation of samples and cyanide analysis:

Whatman number 1 filter papers (8 x 1cm) were dipped into the alkaline picrate solution and drained free of excess liquid just before use. The filter paper strips were prepared under identical conditions. The samples (10g per sample) were loaded into glass bottles and acidified with 15 mL of hot 20% HCl solution. The bottles were sealed with 3 picrate impregnated strips suspended above the acidified samples as the bottles were sealed.

The system was left at room temperature (28±2°C) for 24h. The red-colored picrate paper strips were removed from the bottles and rinsed in 5mL of 50% ethanol solution for 30 minutes and the absorbance of the solution measured at 510 nm using a Spectrumlab23A spectrophotometer. Cyanide levels of the samples were extrapolated from the standard curve.

Functional Properties Measurements

Protein Solubility Measurements

1- Protein solubility

The protein solubility was performed depending on previous method described by **Morr et al. (1985)**, with minor modifications. Briefly, protein powder was dispersed in distilled water at a concentration of 1% (w/v). The pH of the protein dispersion was adjusted to values ranging from 2 to 10. ranged from 2 to 10. The dispersions were stirred for 60 min while maintaining the pH at specific studied values. Supernatant was separated by centrifugation at 3500 × 5 for 30 min. The protein content in supernatant was determined by Kjeldahl method.

The prtein solubility was calculated by dividing the total protein content in the supernatant by the total protein in the used protein powder.

2- Determination of Water Holding Capacity (WHC) and Oil Holding Capacity (OHC)

The water holding capacity (WHC) and oil holding capacity (OHC) were determined using the method of **Tomotake et al. (2002),** with some modifications.

One gram of protein sample was weighed into a pre-weighed centrifuge tube, and a 10 ml of distilled water was added. The mixture was gently shaken for 1 minute and allowed to stand for 30 minutes, then centrifuged at 3000 × g for 30 minutes The supernatant was decanted, and the tube containing the sediment was weighed again. WHC was calculated as the amount of water held by one gram of protein powder. Oil holding capacity (OHC) was determined using the same procedure, except for 10 ml of sunflower oil was used instead of distilled water. The OHC was expressed as the amount of oil held by one gram of protein powder.

3- Emulsion Capacity [EC] measurements

Emulsifying capacity (EC) of FPI, Cn, Cn-FPI-10, Cn-FPI-20 and Cn-FPI-30 were determined using the method of **Huffman et al. (1975)** with some modifications. Protein solutions at protein concentrations of 0.2, 0.4, 0.6, 0.8 and 1% protein in solution were prepared by adjusting the protein dispersion pH to 5.5 while stirring for 1 h. Protein solutions (100 ml) were mixed in a food mixer at the highest speed while corn oil, containing 40 mg/l of Sudan III dye as an indicator, was added continuously from a burette at a constant rate. The end-point of emulsification capacity was determined by carefully observing the change in emulsion viscosity and separation of the continuous and dispersed phases at the time of emulsion break, as well as the change in colour of the oil phase from pink to orange. EC was ml of emulsified oil per 100 ml of protein solution.

4- Foaming Capacity (FC) and Foam Stability (FS)

FC and FS were examined at protein concentrations rang from (1to5%w/v). A 100 ml protein solution of each concentration at pH 9 was prepared by solubilizing the protein for 30 min using 0.2 M NaOH or HCl solutions. The protein solution was whipped for 5 minutes in a kitchen-type mixer (Tornado model Bl400/2-400w/Egypt) at high speed. The resulting foam was poured into a 250 ml graduated cylinder. Total foam volume was recorded, and foam capacity was expressed as the percent increase in volume, as mentioned by **Martinez-Flores et al. (2006).**

To determine foam stability (FS), after whipping, the foam volume was recorded at time intervals 0, 10, 20, 30,40, 50, and 60 minutes, and foam decay with time was taken as foam stability (**Johls and Ennis,1981).**

5- Viscosity

Effect of protein concentrations on viscosity of studied proteins

Solutions of protein powders at protein concentrations ranged from 1 to 5% protein (w/v) were prepared in distilled water while adjusting the pH to 10 for 1h. The viscosity of the protein solutions was measured.

Viscosity measurements

Viscosity measurements were done using Ostwald's Viscometer. Time of Flow and density of protein were determined. Viscosity was calculated using equation used by **Park and Robinson. (1984).**

Solution density was measured with a 25-ml pycnometer

6- Gelling Properties

Gel formation and measurements were carried out depending on previous methods used by **Pour-El and Swenson. (1976).**

Effect of Protein Concentration on Gel Formation

Each studied protein powder sample was suspended in distilled water at specific concentrations: 2, 4, 6, 8, 10, 12, and 14% protein (w/v). The suspensions were solubilized using 0.2 M NaOH to adjust the pH to 9. Then, 3 ml of the protein solutions were transferred to heating tubes (1×15 cm), which were stoppered and placed in a water bath at 90°C for 30 min. The tubes were removed, checked visually for gel formation immediately, and then plunged into ice baths before being stored at 4°C for 12 h. After cooling, the tubes were rechecked visually for gel strength after cooling. Tubes were reheated at 90°C for 30 minutes and checked to determine if gelation was a reversible process.

The gel strength was determined qualitatively as mentioned by **Pour-El and Swenson**. **(1976)**. Gel strength was indicated by a number from 0 to 5 depending on the ease with which the gel could be shaken to break up, as follows:

0 = No gel (liquid)

1 = Very weak gel (viscous)

2 = Weak gel (clumpy)

3 = Medium gel (clumps stick together)

4 = Good gel (hard to break up)

5 = Strong gel (withstands shaking)

Statistical Analysis

All the data generated in the experiment were statistically analyzed as completely random design according to **Snedecor and Cochran. (1982)**. using the Linear Model Program of SPSS (2014) as The following model

Yijk = μ + Ti + eij. Where: Yijk = Observation for each dependent variable, μ = Overall mean, Ti = Treatments effect (i = 1, 2, and 12), eij = Random error. The differences among treatments means were compared using Duncan's Multiple-Range Test Procedure **Duncan, (1957).**

Results and Discussion

1. **Chemical Composition of Flaxseed and Flaxseed Protein Isolate at Various Processing Stages**

Table (1) shows the chemical composition of flaxseed and flaxseed protein isolate obtained from the present study at various processing stages.

As indicated, the contents of protein, fat, insoluble and soluble fibers, moisture, ash and Cyanogenic glucosides of whole flaxseed were 22.80±0.1%, 29.17±0.37%, 21.4±0.49%, 17.08±0.08%, 5.65±0.04%, 3.88±0.022% and 137.3±1.5 ppm, respectively.

Whole flaxseed contains a high level of protein, 22.80 ± 0.01% as shown in Table (1), this makes flaxseed a good source of vegetable protein. These results agreed with protein content of flaxseed obtained by other workers **Martinez-Flores et al., 2006; Gutierrez et al., 2010).**

Flaxseed protein isolate obtained from our study contained 59.60 ± 1% protein and 12.58 ± 0.01% soluble fiber. This result reflected that the method which was used in the current study for removal of the soluble fiber did not completely remove all the gum. Since the extraction method and its conditions such as temperature, length of extraction time, and pH are factors affecting gum extraction **(Lorence et al., 2022; Rocha et al., 2021).**

A significant reduction ($P<0.05$) in cyanogenic glycosides was observed in flaxseed protein isolate: 91.5 ± 2.47 ppm, compared with whole flaxseed (137.3 ± 1.51 ppm). This reduction may be due to the Hot water used in soluble fiber extraction and n-hexane which was used in oil extraction. Solvent and thermal treatment of foods containing cyanogenic glycosides have been shown to be effective in removing cyanogenic compounds **Yamashita et al. (2007).**

Recovery Yield of Flaxseed Protein

The percentage of flaxseed protein recovery yield in the current study was 30.53%. This yield reflects the effect of processing conditions during demucilaging, defatting, and alkaline/isoelectric precipitation of flaxseed protein. **Gutierrez et al. (2010)** reported that the use of isoelectric precipitation for protein extraction at recovery gives a larger protein yield, i.e., 48%.

Sensory evaluation of flaxseed and flaxseed protein isolate at various processing stages is presented in Table (2). Extraction of the oil in flaxseed using n-hexane led to the removal of off-flavors in the demucilaged-defatted flaxseed. The color changed from dark brown in demucilaged flaxseed to light green to silvery color in demucilaged-defatted flaxseed, and no off-flavor was observed. The obtained flaxseed protein isolate had very light brown color and no off-smell was observed. **Rackis and Honig (1979)** studied the effect of various solvent mixtures on improving the sensory quality of soy flakes, and they observed that the solvent mixture significantly improved the flavor score.

Table (1) chemical composition of flaxseed and flaxseed protein isolate at various processing stages.

Processing step	Protein%	Fat%	Un sol. Fiber%	Sol. Fiber%	Moisture%	Ash%	Cyanogenic glycosides (ppm)
Whole flax seeds	**22.80 ± 0.01^{d}**	**29.17 ± 0.37^{b}**	**21.41 ± 0.49^{b}**	**17.08 ± 0.08^{a}**	**5.65 ± 0.04^{b}**	**3.88 ± 0.022^{c}**	137.3 ± 1.51^{a}

Demucilaged flaxseed	**27.43 ± 0.06^{c}**	**40.93 ± 0.03^{a}**	**21.76 ± 0.29^{b}**	**2.73 ± 0.19^{d}**	**3.36 ± 0.08^{c}**	**3.22 ± 0.006^{d}**	141.6 ± 2.40^{a}
Demucilaged/defatted flaxseed powder	**43.38 ± 0.04^{b}**	**1.34 ± 0.01^{d}**	**24.42 ± 0.01^{a}**	**15.79 ± 0.04^{b}**	**10.02 ± 0.01^{a}**	**5.04 ± 0.003^{b}**	111.3 ± 2.06^{b}
Flax seed isolate	**59.60 ± 0.10^{a}**	**3.28 ± 0.02^{c}**	**10.64 ± 0.01^{c}**	**12.58 ± 0.01^{c}**	**2.10 ± 0.10^{d}**	**11.80 ± 0.015^{a}**	91.5 ± 2.47^{c}
Sig.	**	**	**	**	**	**	**

a ,b,c Means within the same column with different superscripts, differ significantly at ($P < 0.05$). ** = Highly significant ($P < .01$) .

Table (2): Sensory Evaluation of Flaxseed and Flaxseed Protein Isolate at Various Processing Stages

Items	Color	Smell
Whole Flaxseed	**Brown to dark green**	**Noticeable smell**
Demucilaged Flaxseed	**Dark brown**	**No off-smell noticeable**
Demucilaged/Defatted Flaxseed	**Light silver color**	**No smell**
Flaxseed Protein Isolate	**Very light brown**	**No off-smell**

[3] Co-precipitate mixtures of Casein and Flaxseed Protein Isolate

Three co-precipitate mixtures were obtained by blending fresh skim milk (pH 6.6) with a solution of flaxseed protein isolate at 3.5% protein, pH and volume ratios of 90:10, 80:20 and 70:30 of fresh skim milk:

Flaxseed protein Solution. Protein mixtures were Co-precipitated at isoelectric pH of 4.5. The three mixtures termed as Cn-FPI-10, Cn-FPI-20 and Cn-FPI-30 as previously described in Materials and Methods Section.

The Chemical Composition of the three Co-precipitate mixtures, Casein, and FPI are presented in Table (3). The protein content of the three mixtures powders were, 70±2.28%, 68.5±2.62% and 67.6±2.59% for, Cn-FPI-10, Cn-FPI-20 and Cn-FPI-30, respectiveley.The Sensory evaluation color and smell,of the obtained protein mixtures powders had very light brown to yellow color and smell resembled the smell of milk powders.

In the present study, the existence of mixed proteins (casein in micelle form in fresh skim milk) and flaxseed protein in solution form may be facilitate blending process and uniform distribution of both casein and flaxseed proteins. Also, this may facilitat protein-protein interaction during mixing and isoelectric co-precipitation of both proteins. **Bengoechea et al, (2010),** reported that, the proteins can form a fine network structure at pH values far above or below their isoelectric point and tend to aggregate at isoelectric-pH. Many research efforts have so far focused on production of dairy-plant protein blends **(Grosberger et al, 2021; Kim et al, 2020; Hinderink et al, 2020; Hu et al, 2022).**These blends collected both good functionality of dairy proteins and low price and availability of vegetable proteins **(Hinderink et al, 2021 ;Pinthong et al, 1980; Nichols and cheryan, 1982)**.

Table (3): Chemical Composition of the Co-precipitate Mixtures (Cn-FPI-10), (Cn-FPI-20) and (Cn-FPI-30), as well as Acid Casein (Cn) and Flaxseed Protein Isolate (FPI)

a ,b,c Means within the same column with different superscripts, differ significantly at (P < 0.05). .** = Highly significant (P < .01) .

Chemical composition%	Treatments				
	CN	FPI	CN-FPI-10	CN-FPI-20	CN-FPI-30
Protein	71.1±2.72	59.6±2.28	70.0±2.68	68.5±2.62	67.6±2.59
FAT	12.56±0.01	3.28±0.094	11.4±0.05	10.80±0.002	10.27±0.03
Soluble fiber	0.13±0.009	12.58±0.04	0.04±0.003	0.09±0.006	0.49±0.036
Moisture	8.59±0.15	12.58±0.04	7.24±0.13	7.90±0.14	7.47±0.13
Ash	3.55±0.035	11.79±0.11	3.92±0.038	3.54±0.034	3.56±0.035
Sig.	**	**	**	**	**

2- **Functional properties of the co-precipitate mixtures**

Nitrogen Solubility

Nitrogen solubility of the co-precipitate mixtures of casein and flaxseed protein isolate, (Cn-FPI-10), (Cn-FPI-20), and (Cn-FPI-30) compared with the solubilityies of casein (Cn) and flaxseed protein isolate (FPI), is presented in Fig. (2).At pH 2, the co-precipitate mixtures (Cn-FPI-10), (Cn-FPI-20), and (Cn-FPI-30) had solubilities of 26.7%, 29.1%, and 23.6%, respectively, whereas Cn and FPI had solubilities of 10.2% and 38.06%, respectively. At neutral pH (6–7), the protein co-precipitate mixtures had solubilities ranging from 58.21% to 70.76%. At the alkaline side of the solubility curve (pH 8–10), the protein co-precipitate mixtures had solubility comparable to the solubility of casein, e.g., mixtures had solubility at pH 9 that ranged from 80.91% to 84.88%, compared to casein's 82.83%. Similar trends were observed by **Fayed (1997)**, who found that co-precipitate mixtures of casein and fenugreek protein showed higher solubility at pH (6–9) than at acidic pH (2–5), while the minimum solubility is observed at around pH 4.5.Also, casein and fenugreek protein co-precipitate mixture had nitrogen solubility >83% at pH 7 **(Fayed, 1997).**

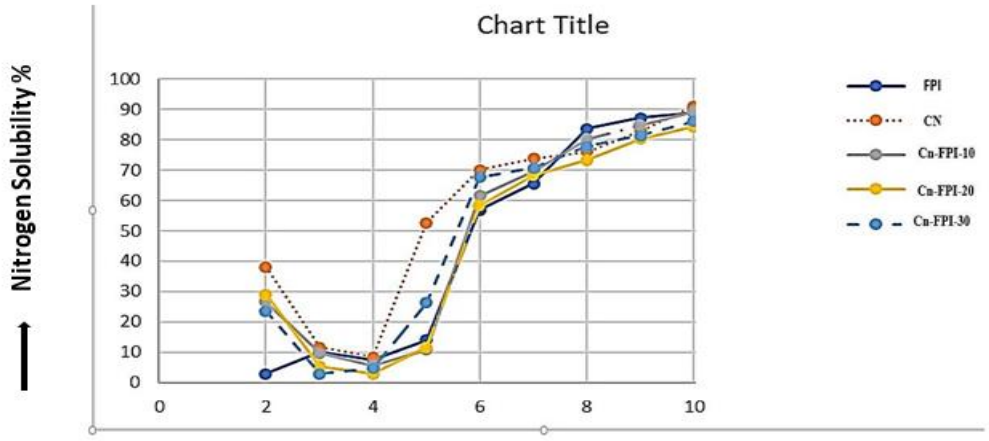


Fig (2): Nitrogen solubility of FPI, Cn, Casein-FPI and Co-precipitate mixtures as function of pH.

Water Holding Capacity (WHC) and Oil Holding Capacity (OHC)

The three co-precipitate mixtures had WHC comparable to that of casein it was: 2.15 ± 0.04, 2.29 ± 0.04 and 2.23 ± 0.057 g water/g protein for the Cn-FPI-10, Cn-FPI-20, and Cn-FPI-30, respectively (Table 4), whereas WHC for casein was 2.67 ± 0.035 g water/g. On the other side, OHC of the three co-precipitate mixtures were higher than that of casein but less than that of FPI. Casein have a high proportion of hydrophilic and hydrophobic residues compared to plant protein; therefore, mixing casein with FPI may improve both the WHC and OHC of the co-precipitate mixtures of proteins.

Table (4): Water Holding Capacity (WHC) and Oil Holding Capacity (OHC)

Studied proteins	WHC g water/g protein	OHC g oil/g protein
FPI	**1.37 ± 0.038c**	**1.66 ± 0.03c**
CN	**2.67 ± 0.035a**	**2.13 ± 0.09a**
CN-FPI-10	**2.15 ± 0.040b**	**1.68 ± 0.04c**
CN-FPI-20	**2.29 ± 0.047b**	**1.94 ± 0.08ab**
CN-FPI-30	**2.23 ± 0.057b**	**1.86 ± 0.07bc**
Sig	**	**

of FPI, Cn, Cn-FPI-10, Cn-FPI-20, and Cn-FPI-30

a,b,c Means within the same column with different superscripts, differ significantly at (P < 0.05). . = Highly significant (P < .01) .**

Emulsifying Capacity (EC)

Table (5) shows the effect of different protein concentrations on Emulsifying Capacity. All the studied proteins showed an increase in EC with increasing protein concentration.

The co-precipitate mixtures obtained in this study had EC comparable to the EC of casein. For example, at a protein concentration of 1%, the EC of the co-precipitated proteins ranged from 111–138 ml, compared with that og that casein (130 ml), and was higher than the EC of FPI (Table 5). Casein, with an open random coil structure, involves conformational realignment when emulsifying, resulting in acting as a rapid changes compared to globulin protein **(Kim et al., 2020).**

Table (5): Emulsifying Capacity (EC) at different protein concertation of FPI, CN, CN-FPI- 10, CN-FPI-20, CN-FPI- 30

Emulsified (ml) of oil the studies proteins ml oil/ 100 ml protein solution					
Percent of protein in 100ml solution	**CN**	**FPI**	**CN-FPI- 10**	**CN-FPI- 20**	**CN-FPI- 30**
0.2%	50 ± 1.53^{a}	26 ± 1.00^{d}	35 ± 0.58^{c}	38 ± 1.00^{bc}	41 ±1.00^{b}
0.4%	72 ± 1.16^{a}	33 ± 1.00^{e}	47 ± 1.16^{d}	54 ± 0.58^{c}	58 ± 1.16^{b}
0.6%	95 ± 1.00^{a}	45 ±1.16^{d}	70 ± 1.53^{b}	62 ± 1.53^{c}	70 ± 1.00^{b}
0.8%	112 ± 7.81^{a}	73 ±1.16^{c}	92 ± 1.00^{b}	73 ± 1.00^{c}	92 ± 1.00^{b}
1%	130 ± 2.52^{b}	105 ±1.53^{d}	111 ± .100^{c}	125 ± 1.53^{b}	138 ± 1.53^{a}

Sig.	**	**	**	**	**

a,b,c Means within the same column with different superscripts, differ significantly at (P < 0.05). .** = Highly significant (P < .01) .

Foaming Properties – Foaming Capacity

Table (6) shows foam overrun of all studied proteins at different protein concentrations. The foam overrun increased with increasing protein concentration (from 1–5%) of whipped protein solutions. **Marinova et al. (2009)** reported that casein and whey protein solutions showed great changed in formability with the concentration of protein, pH and salt.

The co-precipitate mixture of casein–flaxseed protein had foam overrun intermediate between FPI and Cn, i.e., the foam overrun was 110 ± 1.53; 125 ± 1.25 and 140 ± 1.15% for Cn-FPI-10%, Cn-FPI-20%, and Cn-FPI-30%, respectively.

Table (6): Foam overrun (%) of FPI, Cn, Cn-FPI-10, CN-FPI-20 and Cn-FPI-30 at different protein concentrations

Foam over run (%) of studied proteins						
Protein Concentration%	**FPI**	**CN**	**CN-FPI-10**	**CN-FPI-20**	**CN-FPI-30**	**Sig.**
1	75 ± 1.73^{c}	75 ± 1.53^{c}	55 ± 1.15^{d}	100 ± 1.00^{a}	95 ± 1.15^{b}	**
2	105 ± 2.31^{b}	100 ± 1.53^{b}	70 ± 1.53^{c}	115 ± 1.15^{a}	115 ± 1.53^{a}	**
3	150 ± 3.06^{a}	115 ± 2.31^{b}	80 ± 3.21^{c}	120 ± 1.00^{b}	120 ± 1.53^{b}	**
4	160 ± 2.65^{a}	135 ± 1.53^{b}	95 ± 2.08^{d}	120 ± 1.73^{c}	135 ± 1.15^{b}	**
5	180 ± 2.00^{a}	140 ± 2.52^{b}	110 ± 1.53^{d}	125 ± 1.15^{c}	140 ± 1.15^{b}	**

a,b,c Means within the same column with different superscripts, differ significantly at (P<0.05). .** = Highly significant (P < .01) .

Foam Stability

Table (7) indicates foam stability (foam volume over time, in minutes) of all studied proteins at different protein concentrations. The highest foam stability was observed for FPI. Mixing FPI with casein led to improved stability of the co-precipitate mixtures of casein and FPI . This foaming effect was more obvious with mixture of Cn-FPI-30, which contained a higher percent of FPI. **Hinderink et al. (2020)** investigated the interfacial properties of sodium caseinate (SC), pea protein isolate (PPI), and their mixed blends (SC–PPI) at the air–water interface. They found that blending SC with PPI gave a stronger interfacial layer compared to SC alone.

Table (7): Foam Stability (Foam Volume Over Time) of FPI, Cn, Cn-FPI-10, Cn-FPI-20, and Cn-FPI-30 at Different Protein Concentrations

Volume of foam(ml) over time (min)								
Volume of Foam Over Time minutes	**Measurement protein connect ration %**	**0 min**	**10 min**	**20 min**	**30 min**	**40 min**	**50 min**	**60 min**
Studied proteins	**1 %**	175	165	150	120	105	100	100

FPI		175	110	100	100	100	100	100
CN		155	130	120	105	100	100	100
CN-FPI-10		200	120	110	105	100	100	100
CN-FPI-20		195	155	145	130	115	110	110
CN-FPI-30		205	200	195	170	155	110	100
FPI	**2%**	200	12o	100	100	100	100	100
CN		170	120	105	100	100	100	100
CN-FPI-10		215	150	125	120	110	110	110
CN-FPI-20		215	130	120	115	115	110	110
CN-FPI-30		250	245	235	210	200	195	185
FPI	**3%**	215	135	100	100	100	100	100
CN		180	100	100	100	100	100	100
CN-FPI-10		220	130	100	100	100	100	100
CN-FPI-20		220	180	135	130	120	115	115
CN-FPI-30		260	255	205	200	195	195	190
FPI	**4%**	195	100	100	100	100	100	100
CN		195	100	100	100	100	100	100
CN-FPI-10		220	135	105	100	100	100	100
CN-FPI-20		235	200	180	150	135	125	125ml
CN-FPI-30		280	265	260	200	195	195	195
FPI		240	200	120	100	100	100	100
CN	**5%**	210	150	135	115	115	110	110
CN-FPI-10		225	155	110	100	-	-	-
CN-FPI-20		240	180	150	140	140	135	130
CN-FPI-30								

Gelation of the Studied Proteins

Table (8) indicated the effect of protein concentrations on gelation phenomenon of all studied proteins. The least protein concentration that led to gel formation was at 6% for FPI, whereas casein did not form gel at the same protein concentration (Table 8). Gels obtained by casein dispersion were low in strength and need high protein concentration (e.g., 12%) to show strong gels compared with FPI, which formed strong irreversible gel (thermo-set gel) at protein concentration of 10%. We concluded that mixing casein with flaxseed protein isolate (FPI) enhances the gelation properties.

Co-precipitate mixtures of Cn-FPI showed good gelling properties at protein concentrations as low as 8%, and the protein mixture had strong irreversible thermo-set gel, especially with Cn-FPI-20 and Cn-FPI-30 mixtures.

We can conclude that mixing FPI with casein led to obtaining protein mixtures with improved gelling properties. **Lawhon and Cater (1971)** demonstrated that protein isolate from cottonseed formed strong gel, and gel strength increased with protein concentration from 6% to 10%.

Table (8): Effect of Protein Concentrations on Gel Formation by the Studied Proteins (FPI, Cn, Cn, Cn-FPI-10, Cn-FPI-20, and Cn-FPI-30)

Protein Concentration%	FPI			CN			CN-FPI-10			CN-FPI-20			CN-FPI-30		
	Gel strength when(Heating-cooling-reheating)														
	H	**C**	**RH**	**H**	**C**	**RH**	**H**	**C**	**RH**	**H**	**C**	**RH**	**H**	**C**	**RH**
2	**1**	**2**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**1**	**1**	**0**	**1**	**1**	**0**
4	**2**	**2**	**0**	**0**	**1**	**0**	**1**	**1**	**0**	**2**	**2**	**1**	**2**	**2**	**1**
6	**3**	**3**	**1**	**1**	**1**	**1**	**2**	**2**	**1**	**2**	**3**	**2**	**3**	**3**	**2**
8	**4**	**4**	**3**	**2**	**3**	**2**	**3**	**3**	**2**	**3**	**3**	**3**	**3**	**4**	**3**
10	**5**	**5**	**4**	**3**	**4**	**3**	**3**	**4**	**3**	**3**	**4**	**3**	**4**	**5**	**4**
12	**5**	**5**	**4**	**4**	**4**	**4**	**4**	**4**	**3**	**4**	**5**	**4**	**5**	**5**	**5**
14	**5**	**5**	**5**	**4**	**4**	**4**	**4**	**5**	**4**	**5**	**5**	**5**	**5**	**5**	**5**

H = Heating ; C = Cooling ; RH=Reheating (0)= no-gel ; (1) = very weak gel; ; (2) = week gel; (3)= Medium gel ; (4) = good gel (5) = strong gel

Viscosity of the Studied Proteins

Table (9) illustrates the effect of protein concentrations on the viscosity of protein solutions of the studied proteins. For all studied protein solutions, the viscosity increased with increasing protein concentration from 1% to 5% protein in solution. This effect was obvious with the co-precipitate mixtures when the viscosities were, 1.33± 0.05; 1.28±0.04,1.62 ±0.06 and 1.21± 0.05 cp for FPI, Cn, Cn-FPI-10, Cn.FPI-20 and Cn-FPI-30/ respectively. These Viscosities significantly increased ($P<0.05$) ± 4.40± 0.04; 4.57± 0.04; 4.64± 0.03 and 4.86± 0.03 cp at a protein Concentration of 5% for Cn, Cn-FPI-10, Cn-FPI-20 and Cn-FPI-30, respectively.

Augustin et al,(2011) reported that solutions of caseinates exhibit very high viscosity, and that viscosity increases exponentially. with increasing concentration of cascinate. Caseinate solutions exhibit Newtonian behavior at low protein concentration- and pseudo plastic behavior at high Concentrations, and are thixotropic at high shear rates **Augusting et al, (2011)**. Also, **Towler, (1974)** reported that, there is a linear relationship between casein concentration and the apparent viscosity.

Table (9): Effect of Protein Concentrations on Viscosity of FPI, Cn, Cn-FPI-10, Cn-FPI-20, and Cn-FPI-30

Impact of Protein Concentrations on Viscosity(CP) Changes at 25°C.					
protein concentration%	**FPI**	**CN**	**CN-FPI-10**	**CN-FPI-20**	**CN-FPI-30**
1	1.05± 0.04^{c}	1.33± 0.05^{b}	1.28± 0.04^{b}	1.62± 0.06^{a}	1.21± 0.05^{b}
2	1.39± 0.05^{d}	1.48± 0.04^{cd}	1.73 ±0.02^{a}	1.63± 0.03^{ab}	1.55±0.06^{bc}
3	1.73± 0.04^{c}	1.62± 0.04^{c}	2.37± 0.04^{a}	1.73± 0.04^{c}	2.01± 0.05^{b}
4	2.09± 0.06^{c}	2.87± 0.04^{a}	2.52± 0.16^{b}	2.24± 0.03^{c}	2.72± 0.04^{ab}
5	2.77± 0.04^{c}	4.40± 0.15^{b}	4.57± 0.04^{b}	4.64± 0.03^{ab}	4.86± 0.03^{a}
Sig.	**	**	**	**	**

a, b, c Means within the same column with different superscripts, differ significantly at ($P < 0.05$). . ** = Highly significant ($P < .01$).

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