



Research article

Protein isolate precipitation using acid and salt on a by-product of mung bean starch extraction

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Abstract

This research investigated using salt and acid precipitation to extract protein from mung bean wastewater after starch extraction. However, there has been no published report regarding which precipitation treatment was better both in terms of quality and quantity. Thus, the objective of this study was to examine the influence of salt and acid addition on the yield, color, proximate analysis, mineral content and specific functional properties of mung bean protein isolate obtained from mung bean wastewater. Three types of salt (MgSO_4 , CaCl_2 and $(\text{NH}_4)_2\text{SO}_4$), and two types of acid (citric acid and hydrochloric acid) were used to precipitate protein from mung bean wastewater. The results revealed that precipitating protein with hydrochloric acid at pH 4.5 resulted in a significant ($p < 0.05$) increase in the weight of protein isolate compared to salt precipitation. Citric acid provided a brighter color, contained up to 80.33% protein and had higher functional properties. However, MgSO_4 at 10 g/L showed comparable color, with up to 78.61% of protein, higher amounts of mineral compounds and equal functional characteristics. Citric acid and MgSO_4 could be alternative protein precipitation agents since both are inexpensive, readily available and capable of precipitating the mung bean protein of a considerably higher quality.

Introduction

Mung bean (*Vigna radiata* L.) is a species in the legume family that is rich in carbohydrates (62%), especially starch (48%; Shi et al., 2016) and protein (24%; Dahiya et al., 2015; Shi et al., 2016). It also contains many macronutrients,

such as vitamins, minerals and essential amino acids (Dahiya et al., 2015; Zhong et al., 2012).

Starch is the major substance produced from mung bean (Wang et al., 2011), which is the best raw material for glass noodle or vermicelli production. In the extraction process of mung bean starch, while starch is the primary product, by-products include crude fiber and mung bean meal (Sonklin et al., 2021) and protein-rich waste solution (Sirikulchayanont et al., 2007).

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Crude fiber is usually used as animal feed, while the protein-rich waste solution, which contains 1.5% of protein, is customarily disposed of as wastewater (Sirikulchayanont et al., 2007). Another by-product of starch extraction is mung bean meal, which is generally thrown away despite having around 70% protein on a dry basis (Sonklin et al., 2021). Enzymatic hydrolyses of mung bean meal have been reported producing bioactive peptides, which serve as the primary substance for flavor enhancers and perform as antioxidants (Chunkao et al., 2020; Sonklin et al., 2018), anti-hypertensives (Sonklin et al., 2020), anti-allergy agents (Budseekoad et al., 2019) and antitumor agents (Sonklin et al., 2021).

Approximately 600 t/day of protein-rich wastewater is disposed of by the mung bean starch industries in Thailand (Sirikulchayanont et al., 2007). Protein separation treatment is required before wastewater treatment. As this protein is more valuable for other food and feed usage, any protein recovery treatment would contribute to recycling and the sustainable bio-economy of mung bean processing.

Protein recovery from mung bean starch processing effluent could be accomplished by various techniques, including isoelectric deposition, centrifugation followed by freeze and spray drying, and membrane filtration (Srinuworn et al., 2016). Protein precipitation using acids and isoelectric point methods are used by some processors in the mung bean starch industry; this method is considered cheaper and effective for precipitating protein from wastewater, with the mung bean protein usually utilized as animal feed (Sirikulchayanont et al., 2007). Recently, there has been increasing use of plant-based protein to replace animal protein and it is claimed to be sustainable and eco-friendly, with the nutritional content of mung bean protein also being reported (Brishti et al., 2020).

There are several factors that affect the protein precipitation process, such as temperature (heating), pH and the presence of salts and acids (Shrestha et al., 2021). The aggregation of protein molecules occurs as they approach their isoelectric points at which they are precipitated. The pH of the solution at which the net charge of a protein becomes zero is known as the isoelectric point. The addition of divalent cations, such as Ca^{2+} and/or Mg^{2+} , into protein has been reported to promote protein aggregation. Ammonium sulphate, $(\text{NH}_4)_2\text{SO}_4$, is usually used in protein aggregation, because of its high solubility which produces pure ingredients and solutions with very high ionic strength and a low price (Duong-Ly and Gabelli, 2014).

However, there is still no literature that specifically reports the type of salt or acid that is suitable for precipitating protein in mung bean starch wastewater, both in terms of quality and

quantity. Therefore, the aim of the research was to identify the influence of salt and acid addition on the protein isolate from mung bean wastewater precipitation, in particular, on the yield, color, proximate analysis, mineral content and some functional qualities.

Materials and Methods

Peeled mung beans (Raithip brand, Thailand) were obtained from supermarkets in Thailand. The chemicals used were MgSO_4 , CaCl_2 (Thermo Fisher Scientific), $(\text{NH}_4)_2\text{SO}_4$ (Merck) and citric acid, hydrochloric acid and NaOH purchased from Sigma Co. (USA).

Preparation of mung bean protein isolate from mung bean wastewater

The process was started by washing the peeled mung beans because the extracted protein was intended for further use in plant-based foods. The peeled beans were soaked overnight and then re-washed and ground with the addition of water (mung bean:water = 1:3 weight per volume). The ground mung beans were centrifuged to separate the fiber from the starch and protein solution. Centrifugation was carried out twice, after which the solution was left standing for 30 min until the starch had accumulated as sediment and the solution was poured out as protein-rich mung bean wastewater.

The protein precipitation process was initiated by heating the protein-rich mung bean wastewater to 80°C and then adding salt (MgSO_4 , CaCl_2 , or $(\text{NH}_4)_2\text{SO}_4$). Acid precipitation was accomplished by heating the solution to 80°C followed by acid addition (citric acid or hydrochloric acid) and adjusting the pH to 4.5. Neutralization for acid precipitation was carried out by NaOH addition to achieve pH 7.0. The fresh protein produced was then dried at 40°C in a tray drier until the moisture content was less than 12%. The optimum yield observations on protein resultant for each salt and acid were first conducted. The optimum yield was used as a preliminary step for further experiments. The resultant protein was then ground and stored at -4°C prior to analysis.

Yield

The optimum yields of protein precipitation due to salt or acid additions were determined by measuring the weight and moisture content of the resultant dry protein isolate and the mung bean seeds before extraction. Calculations were done on

a dry basis at several salt concentration and pH levels. The salts used were MgSO_4 , CaCl_2 , and $(\text{NH}_4)_2\text{SO}_4$ at concentrations of 5.0 g/L, 7.5 g/L, 10.0 g/L, 12.5 g/L or 15.0 g/L. The two types of acid (citric acid and hydrochloric acid) were used with pH values of 3.8, 4.5, 5.6 or 7.0.

The mung bean protein yield as a percentage dry basis (% db) was determined using Equation 1:

$$\text{Yield} = [\text{Protein weight after drying} / \text{Weight of mung bean}] \times 100 \quad (1)$$

where all weights were in grams on a dry basis (db).

Afterward, the findings of the optimum yield tests for each salt and acid were utilized as the basis for additional observations. Precipitation with one salt concentration and one point acid pH, respectively, were utilized to conduct further study.

Color parameters

The colors of the mung bean protein samples were determined instrumentally using a Miniscan XE colorimeter (Hunter Ultra Scan Pro, USA) at ambient temperature. A sample was ground and packed into plastic and the sample color was examined. The colorimeter was calibrated using white and black standards. The lightness of the product was assessed using L^* values (0 for darkness to 100 for whiteness). The a^* values represented the redness level for positive values and the greenness level for negative values. The b^* values represented the yellowness level for positive values and the blueness level for negative values.

Proximate analysis

On a dry basis, the moisture, ash, crude protein, crude fat, and crude fiber contents of the mung bean proteins were evaluated using AOAC methods (AOAC International, 2016). The moisture content was determined using the oven drying procedure (AOAC method 925.10). The dry ashing technique according to AOAC method 923.03 (AOAC International, 2016) was used to evaluate the ash content. The total nitrogen content of mung bean protein was measured using the Kjeldahl method according to AOAC method 991.20 (AOAC International, 2016), whereas the crude protein content was determined using a 6.25 conversion factor. The crude fat concentration was calculated using Soxhlet equipment in accordance with the protocol in AOAC method 922.06 (AOAC International, 2016).

The crude fiber was assessed using the residues remaining after acid and alkaline hydrolysis in compliance with the procedure in AOAC method 978.10 (AOAC International, 2016).

Mineral compound

The mineral compounds of the mung bean protein, such as calcium, magnesium, and iron content, were measured using an atomic absorption spectrophotometer according to AOAC method 984.27 (AOAC International, 2016).

Functional properties

Water Holding Capacity

The procedure of Du et al. (2018) was used to assess the water holding capacity (WHC) of the mung bean protein isolate. Briefly, the mixture was maintained for 80 min and then centrifuged at $4,000 \times g$ after addition of 0.5 g (W) of protein samples into 10 mL (V_1) distilled water. The free water was discharged and the volume (V_2) was measured. The WHC in grams per milliliter was determined using Equation 2:

$$\text{WHC} = (V_1 - V_2) / W \quad (2)$$

Oil Absorption Capacity

The oil absorption capacity (OAC) of the mung bean protein isolate was evaluated using a modified Du et al. (2018) technique. In brief, 0.5 g (W) of protein samples was dissolved in 5 g (W_1) of soybean oil and swirled uniformly for 30 min using static settling. The mixture was then centrifuged at $4,000 \times g$ for 20 min, with mixing every 5 min. The free oil was dislodged and the amount of free oil (W_2) measured. The OAC was defined as the weight of oil adsorbed per gram of sample and was calculated using Equation 3:

$$\text{OAC} = (W_1 - W_2) / W \times 100 \quad (3)$$

Foaming properties

The foaming characteristics were calculated using a modified version of Kaushik et al. (2016). A sample of 50 mL solution of the protein isolate (20 mg/mL) was spread in a graduated cylinder. The solution was homogenized for 1 min at 10,000 rpm using an homogenizer (T25 Ultra-turrax, USA). A 100 mL graduated cylinder was used to record the volume before and after homogenization. The percentages of foaming capacity (FC) and foaming stability (FS) of the protein solution were measured using Equations 4 and 5, respectively:

$$\text{Foaming capacity} = (V_2 - V_1) / V_1 \times 100 \quad (4)$$

$$\text{Foaming stability} = (V_3 - V_2) / V_2 \times 100 \quad (5)$$

where V_1 is the pre-homogenization protein solution volume, V_2 is the protein solution after homogenization and V_3 is the protein solution foam volume remaining 10 min after homogenization at room temperature.

Statistical analysis

One-way ANOVA with a 95% confidence interval was used to evaluate the influence of salt and acid on the mung bean protein quality. Duncan's multiple range tests were used after the ANOVA, with significant differences determined at the $p < 0.05$ level. Three replications were used for all of the treatments in the experiment. Results were presented as mean \pm SD.

Results and Discussion

Yield

There were different maximum yields of protein precipitation from each salt used. The yields of mung bean protein isolate that precipitated using salt are shown in Fig. 1 and illustrate the salting in and salting out phenomena. At low salt concentration, the protein solution's ionic strength grew stronger; then, the protein solubility also increased and prevented aggregations. However, at high salt concentration, the protein solubility decreased, salt ions were bound to water molecules and

disrupted the hydrolytic layer of the protein, and the protein precipitated (Duong-Ly and Gabelli, 2014). The maximum yield concentration was 10 g/L for magnesium sulfate (MgSO_4) and calcium chloride (CaCl_2), and was 7.5 g/L for ammonium sulfate ($[\text{NH}_4]_2\text{SO}_4$).

Of the three types of salt, magnesium sulfate provided the highest yield (21.09 g/100 g mung bean, db). This was possibly because the salting out ability followed the Hofmeister series (Duong-Ly and Gabelli, 2014). The Hofmeister series for anions is $\text{PO}_4^{3-} > \text{SO}_4^{2-} > \text{CH}_3\text{COO}^- > \text{Cl}^- > \text{Br}^- > \text{ClO}_4^- > \text{I}^- > \text{SCN}^-$, while the Hofmeister series for cations is $\text{NH}_4^+ > \text{Rb}^+ > \text{K}^+ > \text{Na}^+ > \text{Li}^+ > \text{Mg}^{2+} > \text{Ca}^{2+} > \text{Ba}^{2+}$ (Duong-Ly and Gabelli, 2014). However, the pattern did not follow the Hofmeister series completely due to preferential hydration tending to increase in the order NH_4^+ , Ca^{2+} and Mg^{2+} . The behavior of the ions in water was controlled by their water affinity. MgSO_4 provided a higher protein isolate result (21.09 g/100 g mung bean) compared to the other salt precipitation agents.

The protein precipitation yields of mung bean protein isolate using acid are illustrated in Fig. 2. The maximum yield of protein precipitation for both the citric and hydrochloric acids, was at pH 4.5 which was the isoelectric point for both acids.

The amount of protein isolate precipitated using hydrochloric acid (22.03 g/100 g mung bean, db) was slightly greater than from those using citric acid (21.02 g/100 g mung bean, db). Citric acid is a weak acid, so its electronegativity is also weak, while hydrochloric acid is a strong acid, so its electronegativity is also stronger (Kusumah et al., 2020). Thus, it could attract electrons more effectively so that hydrochloric acid could precipitate more protein than citric acid.

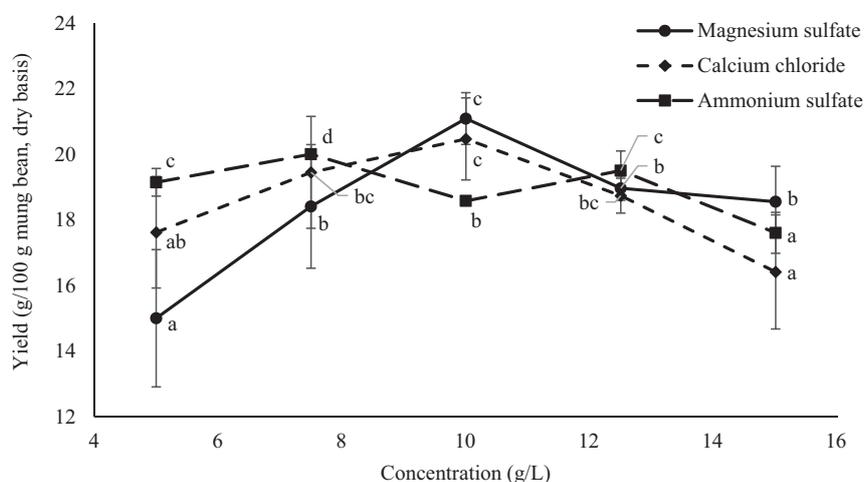


Fig. 1 Mean yield of mung bean protein isolate precipitated using salts at different concentrations, where error bars represent SD. Different lowercase letters on each line indicate significant ($p < 0.05$) difference of mean yield obtained from different concentrations of each salt.

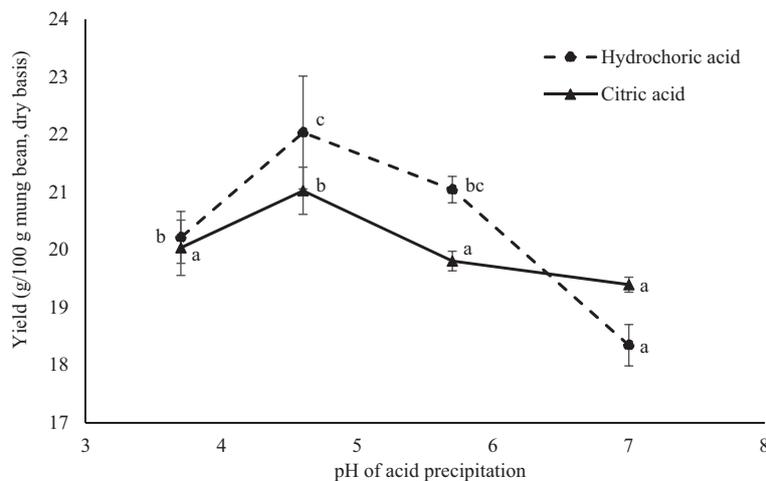


Fig. 2 Mean yield of mung bean protein isolate precipitated using acid with different pH, where error bars represent SD. Different lowercase letters on each line indicate significant ($p < 0.05$) difference of mean yield obtained from different pH of each acid.

Protein precipitation using acid produced significantly higher yields than salt precipitation, CaCl_2 and $(\text{NH}_4)_2\text{SO}_4$, as illustrated in Fig. 3. Thus, based on quantity, acid precipitation was more effective than salt precipitation at extracting protein from mung bean wastewater.

Color

The color values of mung bean protein isolate in fresh and dried forms are shown in Table 1. Regarding the fresh protein isolate, hydrochloric acid provided the lowest lightness (82.84 ± 1.06), but the highest redness (3.18 ± 0.38) and yellowness (23.76 ± 1.67) levels. This was probably related to its strong

electronegativity, so that it could also aggregate some color components.

For the dried protein isolate, both the lightness and redness levels were not significantly different, while for yellowness, MgSO_4 had the lowest (23.96 ± 0.19) level and CaCl_2 had the highest (26.16 ± 1.69) level. Color was influenced by the presence of amino acids or amines which reacted in a browning reaction during the drying process. The complicated reaction between the proteins' carbonyls and amines happened extemporaneously during the thermal process and produced the browning color on the product, defined as the Maillard reaction (Liu et al., 2014).

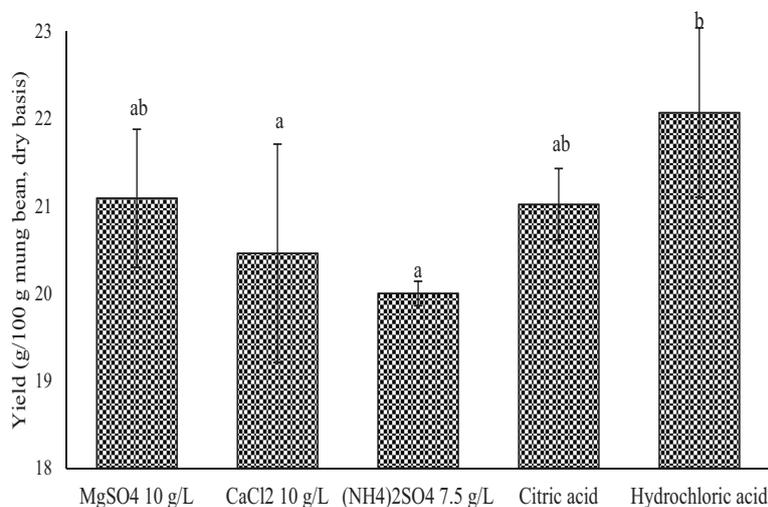


Fig. 3 Mean yield of mung bean protein isolate from salt and acid precipitation, where error bars represent SD and different lowercase letters above columns indicate significant ($p < 0.05$) differences.

Table 1 Color parameters of fresh and dried mung bean protein isolate

Precipitating agent	Fresh			Dried		
	L*	a*	b*	L*	a*	b*
MgSO ₄ 10g/L	84.93 ± 1.83 ^b	0.68 ± 0.19 ^a	19.29 ± 1.35 ^b	79.37 ± 1.57 ^a	3.83 ± 0.55 ^a	23.96 ± 0.19 ^a
CaCl ₂ 10 g/L	85.06 ± 0.54 ^b	1.18 ± 0.14 ^b	23.39 ± 0.82 ^c	78.04 ± 1.84 ^a	4.12 ± 0.93 ^a	26.16 ± 1.69 ^b
(NH ₄) ₂ SO ₄ 7.5 g/L	84.32 ± 0.26 ^{ab}	1.36 ± 0.16 ^b	20.47 ± 0.82 ^b	79.45 ± 1.78 ^a	3.66 ± 0.78 ^a	24.85 ± 0.33 ^{ab}
Citric acid	86.15 ± 0.35 ^b	0.98 ± 0.12 ^{ab}	15.93 ± 0.86 ^a	79.25 ± 1.69 ^a	4.03 ± 0.18 ^a	24.32 ± 0.80 ^a
Hydrochloric acid	82.84 ± 1.06 ^a	3.18 ± 0.38 ^c	23.76 ± 1.67 ^c	79.41 ± 0.86 ^a	3.73 ± 0.27 ^a	24.71 ± 0.85 ^{ab}

Values (mean±SD) within a column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Proximate analyses

Measurement of the proximate contents of protein isolate from mung bean wastewater was required to determine the quality of the protein isolate produced. The proximate results of the mung bean protein isolate are shown in Table 2 for the same drying time. The moisture content of mung bean protein isolate was in the range 7.87–10.44%, which was under the 12% maximum moisture content for long-term storage.

The ash content was in the range 3.86–7.49% db. The amounts of protein isolate that precipitated using salt were significantly higher than those precipitated using acid. A high ash content in food might be associated with a high mineral content (Agoreyo et al., 2011), and could potentially provide micro and macro elements (Adiamo et al., 2016). This indicated that protein precipitation using salt provided a higher mineral content.

The protein content was in the range 47.22–80.33% db. These results were in line with the protein concentrates of chickpeas that were in the range 70.4–75.3% (Singh et al., 2008), peanuts in the range 79.1–82.4% (Hojilla-Evangelista et al., 2018), groundnuts with 72.8% (Ocheme et al., 2018) and kariya seeds with 68.2% (Adiamo et al., 2016). The crude protein contents of the mung bean protein isolates that were precipitated with acid were relatively higher than for those deposited with salt, except for MgSO₄. This showed that

precipitation using the isoelectric point method was able to precipitate more protein molecules. Magnesium ions were able to bind water more strongly, so that more proteins were deposited.

The same phenomena also occurred for crude fat. The crude fat content was in the range 3.29–5.89% db.

The protein precipitation resulting from wastewater in the starch extraction process of mung bean provided protein isolates with a low crude fiber content (< 0.01) for all precipitation treatments. This was possible because the crude fiber had been separated from the mung bean pulp after the mung bean seed milling processes, at which stage, the wastewater contained no crude fiber.

Mineral contents

Minerals (both macro and micro) perform critical functions in the human body, especially as enzyme cofactors (Gharibzahedi and Jafari, 2017). Macro minerals are needed in larger quantities, with concentrations of more than 50 mg/kg, such as calcium (Ca) and magnesium (Mg), whereas micro minerals are needed in smaller amounts for the human body, such as iron (Fe) (Gharibzahedi and Jafari, 2017). The calcium, magnesium, and iron contents in the protein isolates are shown in Table 3.

Table 2 Moisture, ash, crude protein, crude fat and crude fiber contents of mung bean protein isolate

Precipitating agents	Moisture (g/100 g db)	Ash (g/100 g db)	Protein (g/100 g db)	Fat (g/100 g db)	Fiber (g/100 g db)
MgSO ₄ 10g/L	8.56 ± 0.19 ^b	7.49 ± 0.35 ^d	78.61 ± 1.46 ^{bc}	5.60 ± 0.50 ^c	< 0.1
CaCl ₂ 10 g/L	7.87 ± 0.16 ^a	6.89 ± 0.21 ^c	47.22 ± 2.83 ^a	4.16 ± 0.19 ^b	< 0.1
(NH ₄) ₂ SO ₄ 7.5 g/L	7.93 ± 0.34 ^a	5.03 ± 0.43 ^b	50.59 ± 2.33 ^a	3.29 ± 0.34 ^a	< 0.1
Citric acid	10.44 ± 0.22 ^c	3.86 ± 0.12 ^a	80.33 ± 3.36 ^c	5.41 ± 0.36 ^c	< 0.1
Hydrochloric acid	10.16 ± 0.05 ^c	4.06 ± 0.21 ^a	75.50 ± 1.61 ^b	5.89 ± 0.11 ^c	< 0.1

db = dry basis

Values (mean±SD) within a column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Table 3 Mineral contents of mung bean protein isolate

Precipitating agent	Calcium (mg/100 g db)	Magnesium (mg/100 g, db)	Iron (mg/100 g, db)
MgSO ₄ 10g/L	33.45 ± 1.12 ^c	106.54 ± 4.03 ^c	2.07 ± 0.18 ^b
CaCl ₂ 10 g/L	229.16 ± 4.00 ^d	19.92 ± 2.42 ^b	1.67 ± 0.13 ^a
(NH ₄) ₂ SO ₄ 7.5 g/L	29.26 ± 1.18 ^b	12.16 ± 1.20 ^a	1.56 ± 0.22 ^a
Citric acid	24.57 ± 1.61 ^a	16.42 ± 2.00 ^{ab}	2.25 ± 0.07 ^b
Hydrochloric acid	28.16 ± 1.45 ^{ab}	13.39 ± 1.08 ^a	2.17 ± 0.22 ^b

db = dry basis

Values (mean±SD) within a column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Calcium

The mineral content is associated with the salt used to precipitated the protein. At high salt concentrations, salt ions are bound to water molecules, disrupting the hydrolytic layer of the protein and excess salt ions can bind to the protein molecules (Duong-Ly and Gabelli, 2014). Salt ions could also bind to the amino or carboxyl groups in protein, so that the mineral content can increase inside the protein, especially for magnesium and calcium. In the current study, the calcium content was in the range 24.57–229.16 mg/100 g, db. The highest calcium content was in the protein isolate which was deposited using CaCl₂, and this significantly increased the calcium content in the protein isolate, so that it was similar to the calcium content of legume flour (28.78 mg/100 g), legume protein isolate (33.05 mg/100 g) and chickpea flour (200 mg/100 g) (Landi et al., 2021) and chickpea (60.4 mg/100 g), lentil (55.0 mg/100 g) and lupine (12.8–28.4 mg/100 g), according to Grela et al. (2017).

Calcium is a critical nutrient that is required for neuromuscular function, several enzyme-mediated activities, immune system health, blood clotting, and blood pressure management, as well as providing rigidity to the skeleton via its phosphate salts (Cline, 2012; Gharibzahedi and Jafari, 2017). Calcium allowances of 700 mg/d are advised for newborns, 400 mg/d for infants aged 7–12 mth, 500 mg/d for children aged 1–3 yr, 600 mg/d for children aged 4–6 yr, 700 mg/d for children aged 7–9 yr, 1,300 mg/d for adolescents aged 10–18 yr, 1,000 mg/d for females aged 19 yr to menopause, 1,300 mg/d for females post-menopause, 1,200 mg/d for females during pregnancy, 1,000 mg/d for females during lactation, 1,000 mg/d for males aged 19–65 yr and 1,300 mg/d for males aged 65+ yr, based on Western European, USA and Canadian data (WHO, 2005).

Calcium deficiency raises the risk of osteoporosis (Cline, 2012; Fang and Li, 2014; Kumssa et al., 2015) and increases the risk of rickets in premature neonates and of hypocalcemia in infants (Khadilkar et al., 2017).

Magnesium

The magnesium content of the mung bean protein isolates was in the range 12.16–106.54 mg/100 g, db. The highest magnesium content was in the protein isolate deposited using MgSO₄ and this significantly increased the magnesium content in the protein isolate, though it was lower than the magnesium content of legume flour (153.52 mg/100 g), protein isolate (150.37 mg/100 g), chickpeas (8.6 mg/100 g), lentils (17.1 mg/100 g), common beans (14.7 mg/100 g), peas (11.5 mg/100 g) and lupines (12.7–28.2 mg/100 g), according to Grela et al. (2017).

Magnesium works as a cofactor of several enzymes including energy metabolism, protein synthesis, muscle contraction, the immune system, RNA and DNA synthesis and the preservation of nerve tissue and the cell membrane electrical potential (Gharibzahedi and Jafari, 2017). The importance of this element in controlling potassium flows and its participation in the metabolism of calcium is of special relevance with regard to the pathogenic implications of magnesium shortage.

Recommended levels of magnesium for humans are 26 mg/d for milk-fed infants, 36 mg/d for infants aged 0–6 mth, 54 mg/d for infants aged 7–12 mth, 60 mg/d for children aged 1–3 yr, 76 mg/d for children aged 4–6 yr, 100 mg/d for children aged 7–9 yr, 200 mg/d for female adolescents aged 10–18 yr, 220 mg/d for female adults aged 19–65 yr, 260 mg/d for male adults aged 19–65 yr, 190 mg/d for females aged 65+ yr and 224 mg/d for male aged 65+ (WHO, 2005).

Magnesium deficiency raises the risk of coronary heart disease (Glasdam et al., 2016) type 2 diabetes (Kostov, 2019), muscular and nerve function disorders (Glasdam et al., 2016; Rajizadeh et al., 2017), drives cardiovascular disease (DiNicolantonio et al., 2018) and increases inflammation (Nielsen, 2018). Stress and depression are caused by magnesium inadequacy (Rajizadeh et al., 2017), which activates the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system. Stress-induced HPA activation has been associated to increased body fat accumulation (Rayssiguier et al., 2010). Oxidative stress helps raise the blood pressure (Rayssiguier et al., 2010).

Iron

The iron content of the mung bean protein isolate was in the range 1.67–2.25 mg/100 g, db. The highest iron content was in the protein isolate deposited by acid or MgSO₄. It seemed that acid or MgSO₄ could extract iron better than the other two salt agents. However, the iron content was lower than in legume flour (10.46 mg/100 g), legume protein isolate (19.08 mg/100 g), peas (495.8 – 512.6 mg/100 g) and chickpeas (390.8 mg/100 g), according to Grela et al. (2017).

Iron has several critical biological activities. It functions as a transport medium for electric ions inside the cells, an energy source for metabolism and as an integral element of essential enzyme systems in diverse tissues, such as a transporter of oxygen in the lungs by red blood cell hemoglobin (Gharibzahedi and Jafari, 2017).

The greatest frequencies of iron deficiency have been in youngsters, children, adolescents and women of reproductive age, and especially in pregnant women (Means, 2020). In poor nations, however, particularly during weaning, the iron situation is crucial, as iron in the diet is very important for the appropriate development of the brain and other tissues, such as muscles, that are distinguished early in life, with the iron requirement for pregnant women being 1.040 mg (WHO, 2005).

The recommended nutritional intakes of iron, based on variable dietary bio-disposability of iron are 6.2–18.6 mg/d for children aged 0.5–1 yr, 3.9–11.6 mg/d for children aged 1–3 yr, 4.2–12.6 mg/d for children aged 4–6 yr, 5.9–17.8 mg/d for children aged 7–10 yr, 9.7–29.2 mg/d for males aged 11–14 yr, 12.5–37.6 mg/d for males aged 15–17 yr, 9.1–27.4 mg/d for males aged 18+ yr, 9.3–28 mg/d for females aged 11–14 yr, 21.8–65.4 mg/d for females aged 15–17 yr, 19.6–58.8 mg/d for females aged 18+ yr, 7.5–22.6 mg/d for post-menopausal females and 10–30 mg/d for lactating females (WHO, 2005).

Lacking iron can lead to anemia, weakness and lethargy (Camaschella, 2015). Iron deficiency during pregnancy can trigger several conditions, including increased risk of sepsis, maternal mortality, low birth weight, and stunted fetal growth

(Abbaspour et al., 2014). Acute iron insufficiency is linked to increased cardiovascular disease risk (Lapice et al., 2013; Von Haehling et al., 2015).

Functional properties

Water holding capacity

The water holding capacity (WHC) indicates the competence of protein to bind water and to contribute to the juiciness and texture of foods and can be impacted by numerous various variables, including the amino acid content, protein structure and the surface polarity-to-hydrophobicity ratio (Du et al., 2018). The WHC values of the mung bean protein from salt and acid precipitation are presented in Table 4 and were in the range 2.71–3.24 ml/g. This range was consistent with the WHC of mung bean protein isolate (Brishti et al., 2017; Du et al., 2018), and mung bean protein concentrate reported by Sirikulchayanont et al. (2007). In the current study, the highest WHC was for mung bean protein from both acid precipitation and MgSO₄ precipitation, while the lowest was from CaCl₂. One possible cause for the increased WHC in acid protein precipitation may have been the amino acid composition and other polar categories that could interact with water molecules to improve protein hydration.

Oil absorption capacity

The capability to absorb oil is demonstrated by the lateral chains of nonpolar proteins to bind oil. The oil absorption capacity (OAC) is essential as a measure of oil protein absorption, reflecting the protein hydrophobicity (Xu et al., 2014). In the current study, the OAC was in the range 1.73–2.06 g/g, which was lower than the OAC of mung bean protein isolate reported by Sirikulchayanont et al. (2007) and in other mung bean protein isolate samples (Du et al., 2018). This might have been due to the amino acid composition, the oil particle or oil droplet size or the density of the protein. Low-density protein powder and a tiny particle size can adsorb and trap more oil than a high-density protein (Haque et al., 2016).

Table 4 Functional characteristics of mung bean protein isolate

Precipitating agent	Water holding capacity	Oil absorption capacity	Foaming capacity	Foaming stability
MgSO ₄ 10g/L	3.17 ± 0.21 ^b	1.73 ± 0.46 ^a	61.67 ± 12.58 ^b	37.90 ± 4.81 ^c
CaCl ₂ 10 g/L	2.71 ± 0.12 ^a	1.86 ± 0.23 ^a	30.00 ± 5.00 ^a	49.93 ± 2.51 ^d
(NH ₄) ₂ SO ₄ 7.5 g/L	2.90 ± 0.12 ^{ab}	2.06 ± 0.30 ^a	27.50 ± 2.50 ^a	21.55 ± 1.55 ^a
Citric acid	3.24 ± 0.10 ^b	1.73 ± 0.46 ^a	62.50 ± 12.50 ^b	38.10 ± 4.80 ^c
Hydrochloric acid	3.18 ± 0.41 ^b	1.93 ± 0.12 ^a	42.50 ± 7.50 ^a	29.61 ± 3.70 ^b

Values (mean±SD) within a column superscripted with different lowercase letters are significantly ($p < 0.05$) different.

Foaming properties

The functional characteristics of protein must be determined as they are used in foam food systems, especially where food where aeration and overflow occur, such as in whipped toppings, baked meals and mixtures of ice cream (Shevkani et al., 2015). Foamability is determined by several factors, including hydrophobicity at the air-liquid interface, the flexibility of protein complexes and protein solubility (Yi-Shen et al., 2018).

Foam capacity (FC) quantifies a protein to stabilize due to its interfacial area per unit weight or concentration, and it is connected to molecular flexibility, density change and hydrophobicity (Yi-Shen et al., 2018). In the current study, the FC was in the range 27.5–62.5%, (Table 4), in line with the FC of mung bean protein isolate reported by Du et al. (2018), and lower than the 89.66% reported by Brishti et al. (2017). The FC of the mung bean protein isolates were lower than those of *Pisum sativum* L., kidney bean and pea protein isolates (Shevkani et al., 2015). These decreased FC levels might have been caused by the increased hydrophobic amino acid levels. On the other hand, the strength of the protein film and its gas permeability impact the stability of the production of foam (Du et al., 2018).

Foam stability is mostly determined by the efficacy of the protein layer and its gas permeability (Du et al., 2018). The FS of the mung bean protein isolate was in the range 21.55–49.93% after standing for 10 min (Table 4). This result was lower than the 78% reported by Brishti et al. (2017). The performance of whipping agents is dependent on their capacity to keep the whip as long as feasible; the protein may create a viscous and gel-like cohesive layer with great flexibility, resulting in excellent foam stability (Yi-Shen et al., 2018). Furthermore, protein with optimal intermolecular connections and the ability to build a cohesive continuous network might generate stable foams at the air-liquid interface (Brishti et al., 2017).

Practical application

Protein precipitation of mung bean wastewater using hydrochloric acid provided a significantly higher yield (22.07 ± 0.45 g/100 g mung bean db) of protein isolates than salt precipitation, CaCl_2 or $(\text{NH}_4)_2\text{SO}_4$. However, compared to hydrochloric acid and the two types of precipitating salts used, citric acid generated the highest protein content, (80.33 ± 3.36 g/100 g mung bean db), which was not significantly different from MgSO_4 , which produced 78.61 g/100 g db of protein

content.

Protein precipitation, particularly with MgSO_4 and CaCl_2 , resulted in isolates with a greater mineral concentration than precipitation using ammonium sulfate, citric acid or hydrochloric acid. This was most likely due to mineral ion deposition on the protein molecules during the MgSO_4 and CaCl_2 precipitation. Similarly, when CaCl_2 is used to precipitate protein molecules, Ca ions bind to the precipitated protein compounds, leading to a higher mineral content in the protein isolate compared to precipitation with citric or hydrochloric acid (Wang et al., 2015). Magnesium ion (65% recovery) and protein (34.7% recovery) were regained from tofu whey wastewater, with the magnesium ions collected during this process suitable for reuse in further tofu coagulation (Chua and Liu, 2019).

Citric acid had higher functional properties and brighter color for the resultant protein isolate and equivalent to using MgSO_4 , compared to hydrochloric acid, calcium chloride and ammonium sulfate. Therefore, citric acid and MgSO_4 as protein precipitants for mung bean wastewater are applicable in the mung bean starch and glass noodle industries. Apart from contributing to mitigation of the environmental impact of mung bean wastewater, these two components are simple, affordable, inexpensive and can produce protein with high quality.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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