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Effect of pH on Protein Extraction from Mahaleb Kernels and Functional Properties of Resulting Protein Concentrate

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Abstract:
The aims of this research were to examine the effect of pH on extraction of proteins from mahaleb (*Prunus mahaleb* L.) kernels, and to investigate the functional properties of resulting protein concentrate. The optimum pH values for the protein extraction and precipitation were determined as 10.0 and 4.5, respectively. The protein concentrate containing 92.73% dry matter, 6.29% ash, 6.02% carbohydrate, 1.42% fat and 73.11% protein was produced by using these extraction and precipitation pH values. Water-holding capacity, oil-holding capacity and the least gelling concentration of the protein concentrate were 2.81 g water/g, 1.66 g oil/g and 12%, respectively. Moreover, emulsifying activity and stability indices, foaming capacity and stability of protein concentrate were 27.21 m^2/g, 81.05 min, 43.75% and 71.33% (after 30 min), respectively. The functional and chemical properties of the protein concentrate indicate that it may find application as functional ingredient for various food products.

Keywords: mahaleb kernel, protein concentrate, chemical properties, functional properties
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1 Introduction

Prunus mahaleb L., also known as the mahaleb cherry or St Lucie cherry is a species of cherry tree, which is native to the Mediterranean region, Iran and parts of central Asia [1, 2]. In Turkey, it grows naturally and is locally known as Idris, Wild Cherry, Stone Cherry, Endirez, Keniro, Fragrant Cherry, Melhem, Endülüs and Meltem [3, 4]. Use of mahaleb kernels in the cuisine is limited to a few countries such as Turkey, Sudan, Greece and Armenia [5, 6]. The mahaleb kernels are also used for the production of both traditional lotion and antidiarrheal drug in Sudan [6]. In Turkey, the dried fruits are used as spice, the kernels are used to give flavour to muffins or cookies, and the branches are used for the production of tobacco pipes [7]. The kernels are also used as a tonic or an antidiabetic in traditional medicines [8]. Jelly, pestil (dried fruit pulp) and candies are made from the fruits [9]. Since the oil from the kernels is rich in alpha-eleostearic acid (9-cis, 11-trans, 13-trans-octadecatrienoic acid), it is used in the paint industry. The tree bark is pleasantly fragrant due to its coumarin content. Mahaleb kernels contain significant levels of fat (30.9%) and protein (28.0%) [10]. In the kernels, there are coumarin, herniarin (7-methoxycoumarin), dihydrocoumarin and amigdaline (mandelonitrile-β-gentiobioside) in low amounts. The kernel has a soft texture, pleasant aroma, but a bitter taste [11, 12].

Functional properties are important not only in food processing and food product development but also important in facilitating the food production steps such as slicing of processed meat products and mechanically transporting of biscuit dough [13, 14]. Proteins are important food components that influence and determine the functional qualities of foods by carrying the flavours or forming films, foams, emulsions, gels and doughs. The functional properties of proteins are affected by internal factors such as the composition and three-dimensional structure of the proteins, environmental factors such as the composition of the food or model system, and the isolation methods [15–17]. Therefore, the functional properties of the proteins may vary depending on the source.

Because of the growing consumers' concerns, the food industry is persistently searching for protein ingredients to substitute those originated from animal sources (e.g. gelatine, casein, whey proteins and ovalbumin).

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Therefore, research on the plant proteins has become a chief issue to be addressed [18]. Mahaleb kernel could be a valuable source of proteins, for use as both functional food ingredients and nutritional supplements. Thus, understanding the functional and molecular properties of mahaleb kernel proteins (MKPs) is important. There are some researches on the composition of mahaleb kernels in the literature. However, to the best of the researchers' knowledge, no research on protein isolation from mahaleb kernels and functional properties of the isolated proteins is available in the published literature. For this reason, in this study, the effect of pH on extraction of proteins from mahaleb kernels, and some chemical and functional properties of resulting protein concentrate were examined.

2 Materials and methods

2.1 Materials

The mahaleb (*Prunus mahaleb* L.) kernels used in the study were obtained from a local company in the city of Tokat, Turkey. Samples were stored in plastic bags at 4°C until use. Sodium caseinate containing 13.5–16.0% nitrogen was purchased from Sigma-Aldrich (St Louis, MO, USA). All the chemicals and reagents used were of analytical grade and used without further treatment.

2.2 Extraction of MKP

MKP concentrate (MKPC) was prepared by alkaline extraction followed by isoelectric precipitation. The kernels were ground ($\leq$ 1 mm) with a coffee grinder (Bosch MKM 600, Munich, Germany). Then, the kernel flour was defatted four times (1 h each) using hexane in a 1:6 flour to solvent ratio. The defatted flour was left in a fume hood overnight to remove hexane residues, and then transferred to a sealed glass jar and stored at room temperature until use. For protein extraction, the defatted flour was dispersed in distilled water (1:20, w/v) and the pH of the slurry was adjusted to 1.0–12.0 with 2 N HCl or 2 N NaOH using a pH meter (InoLab WTW pH 720, Weilheim, Germany). The resulting slurry was stirred for 150 min at room temperature meanwhile the pH of the slurry was kept constant by readjusting every 30 min, if needed. Then, the slurry was centrifuged for 15 min at 3,000 g and the supernatant was collected and analysed for protein content to calculate the protein extraction yield (the amount of protein in supernatant*100/the amount of protein in the sample) and to determine optimum extraction pH. After that, the pH of the supernatant was adjusted to 4.5 with 2 N HCl, allowed to stand for 15 min, and further centrifuged for 10 min at 4,000 g. The precipitated proteins were resuspended in distilled water and its pH was adjusted to 7.0 and then dried at 50°C for 12–18 h with an airflow oven (Memmert 100–800, Schwabach, Germany) and stored at −18°C until use.

2.3 Proximate composition

The dry matter and ash contents of mahaleb kernel flour, defatted flour and protein concentrate were determined by gravimetric method [19]. Ankom extractor (Ankom XT10 Extractor, NJ, USA) was used to analyse the fat content of the mahaleb kernel flour (MKF), defatted MKF (DMKF) and protein concentrate. The total carbohydrate content of the samples was determined according to the phenol sulphuric acid method [20]. The micro-Kjeldahl method was used to analyse the nitrogen content of the samples [19]. The value of 6.25 was used as protein conversion factor.

2.4 Colour parameters

The CIE Lab parameters (L*, a*, b*) of the MKF, defatted flour and protein concentrate were directly read by means of a colorimeter (Minolta, CR-300, Osaka, Japan) calibrated with a white tile (L* = 96.97, a* = 0.16, b* = 1.86) as reference. In this coordinate system, the L* value is a measure of lightness, ranging from 0 (black) to 100 (white); the a* value ranges from green (−) to red (+) and the b* value ranges from blue (−) to yellow (+) [21].

2.5 Sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE)

The electrophoretic profiles of the proteins from the mahaleb kernels and the protein concentrate were determined according to the method described by Laemmli [22]. The sample equivalent to 5 mg of protein was dissolved in 1 mL of sample buffer (3.8 mL of distilled water, 1 mL of 0.5 M Tris-HCl pH 6.8 buffer, 0.80 mL of glycerol, 1.6 mL of 10% (w/v) SDS, 0.8 mL of β-mercaptoethanol, 0.4 mL of 0.05% (w/v) bromophenol blue) and then heated at 95ºC for 5 min. After cooling, a 10 μL aliquot was loaded to 1 mm thick gels (4% stacking; 12% separating). A mixture of standard proteins (6.5–200 kDa, catalogue number S8445, Sigma-Aldrich, St Louis, MO, USA) was used as molecular weight marker and the gels were stained with Coomassie Brilliant Blue G-250 and de-stained with 10% acetic acid. For stacking gel 25 mA and for separating gel 35 mA currents were employed.

2.6 Protein solubility

Protein solubility of the MKPC was determined as described by Beuchat [23]. Dispersions containing 5% (w/v) MKPC were prepared and the pH of dispersions was adjusted to 1.0–12.0 using either 1 N HCl or 1 N NaOH. The dispersions were stirred at room temperature for 90 min after initial pH adjustment. The pH was checked at 30 and 60 min, and if needed readjusted to the stipulated value. Then, the dispersions were centrifuged at 4,000 g for 30 min. Protein content in the supernatant was determined by micro-Kjeldahl method [19] and the protein solubility was calculated as:

$$\text{Protein solubility } (\%) = \frac{W_1 \times 100}{W_0}$$

where W_1 was the amount of protein in the supernatant (g), W_0 was the amount of protein in the sample (g).

2.7 Water-holding capacity (WHC) and oil-holding capacity (OHC)

Water and oil holding capacities of MKPC were determined by using the method outlined by Naczk [24]. For the WHC, 0.5 g MKPC was dispersed in 4 mL of distilled water in a test tube. The dispersion (pH 7.0) was stirred for 30 s every 10 min and held up for 70 min, and then centrifuged at 25°C for 15 min at 2,000 g. The supernatant was drained for 10 min at 45° angle. The gain in weight was recorded as WHC (g water/g sample).

In the determination of OHC, 0.5 g MKPC was dispersed in 3 mL of commercial sunflower oil in a test tube. The dispersion was stirred for 30 s every 5 min and held up for 30 min, and then centrifuged at 25°C for 25 min at 1,600 g. The supernatant was drained for 5 min at 45° angle. The gain in weight was recorded as OHC (g oil/g sample). Sodium caseinate was used as a reference for WHC and OHC tests.

2.8 Foaming capacity and foaming stability

The foaming capacity and foaming stability of MKPC were determined using the method of Moure [25]. About 0.5 g of MKPC was dispersed in distilled water, the pH was adjusted to 7.0, and the volume was made up to 40 mL (1.25 % w/v) with distilled water. The dispersion was homogenized with Ultra Turrax (T18 basic, IKA-Werke GmbH & Co. KG, Staufen, Germany) at 20,000 rpm for 2 min at 25ºC and then instantly transferred to a measuring cylinder, and the total volume and volume of liquid phase were recorded. Foaming capacity was calculated as follows:

$$\text{Foaming capacity } (\%) = \frac{(V_1 - V_2) \times 100}{V_2}$$

where V_1 was the total volume after homogenization, V_2 was the total volume before homogenization.

The foaming stability was calculated by the following equation by measuring the change in foam volume after 0, 10, 30, 60, 90 and 120 min of storage.

$$\text{Foaming stability } (\%) = \frac{V_t \times 100}{V_k}$$

where V_t was the volume of foam at time t, V_k was the foam volume at 0 min after homogenization.

2.9 Emulsifying activity and stability indices

Emulsifying activity (EAI) and emulsifying stability indices (ESI) were determined according to the method of Pearce and Kinsella [26]. For emulsion formation, 6.6 mL of commercial sunflower oil was added to 20 mL of MKPC dispersions (0.1% protein, w/v, pH 7.0) and homogenized by using Ultra Turrax, (T18 basic, IKA-Werke GmbH & Co. KG, Staufen, Germany) at 20,000 rpm for 1 min. Fifty microliters of emulsion (by avoiding the foam layer) were removed carefully from the emulsions, immediately mixed with 4.95 mL of 0.1% (w/v) SDS solution (1:100 dilution) and vortexed for 10 s, and the absorbance of the mixture (A_0) was measured at 500 nm. Ten minutes later, another 50 µL of emulsion were removed from the emulsions as mentioned above, immediately mixed with 4.95 mL of 0.1% SDS solution and vortexed for 10 s, and the absorbance of the mixture (A_{10}) was determined at 500 nm. EAI and ESI were calculated using the following equations:

$$\text{EAI } (m^2/\text{g}) = \frac{2 \text{ x } 2.303 \text{ x } A_0 \text{ x N}}{c \text{ x } \varphi \text{ x } 10,000}$$

$$\text{ESI (min)} = \frac{A_0 \text{ x t}}{A_0 - A_{10}}$$

where, A_0 was the absorbance of the diluted emulsion immediately after homogenization, N was the dilution factor (100), c was the protein concentration of protein dispersion (0.001 g/mL), φ was the oil volume fraction of emulsion (6.6/26.6 = 0.248), A_{10} was the absorbance at 10 min after homogenization, t was the time interval, 10 min.

2.10 The least gelation concentration

The least gelling concentration (LGC) of the samples was measured according to the method described by Coffman and Garcia [27]. Five millilitre of aqueous dispersions (2–14 % w/v, pH 7.0) of MKPC were placed in a boiling water bath for 1 h, followed by rapid cooling to 4°C in an ice bath, and then held up for 2 h. Gel formation was evaluated by inverting the tubes containing the treated dispersions. The least gelation concentration was then determined as the concentration at which the sample from the inverted tube did not fall or slip.

2.11 Statistical analysis

Three independent determinations were used to obtain mean values and standard deviations. One-way analysis of variance and Tukey tests were used for the statistical evaluation and comparison of the data in the Minitab program (Minitab release 12.1, 1998, Minitab Inc., State College, PA, USA) at $p < 0.05$ significance level.

3 Results and discussion

3.1 Proximate composition

Proximate compositions of mahaleb kernel flour and defatted mahaleb kernel flour were presented in Table 1. After lipid extraction, the fat content of the kernel flour was significantly reduced from 32.47% to 1.59%. After extraction, similar fat content was reported for broad bean [15], hyacinth bean [28] and chickpea [29]. However, lower extraction efficiency was obtained with defatted cherry kernel that contained 9% fat [30]. The removal of lipid caused an increase in protein and total carbohydrate content of defatted mahaleb kernel flour.

Table 1: Proximate composition of mahaleb kernel flour (MKF), defatted mahaleb kernel flour (DMKF), and mahaleb kernel protein concentrate (MKPC).

Parameters (%)	MKF	DMKF	MKPC
Dry matter	94.32 ± 0.76	92.46 ± 0.50	92.73 ± 0.65
Protein	31.28 ± 0.43	41.48 ± 0.27	73.11 ± 0.80
Fat	32.47 ± 1.74	1.59 ± 0.10	1.42 ± 0.09
Total carbohydrate	15.07 ± 1.73	19.20 ± 0.55	6.02 ± 0.35
Ash	ND	ND	6.29 ± 0.12

Note: All the data are expressed as mean ± standard deviations and are the mean of three replicates (n).
ND: Not determined.

3.2 Extraction conditions of proteins from mahaleb kernel

Various techniques such as extraction in alkaline conditions followed by isoelectric precipitation [16], ultrafiltration or reverse osmosis [31], extraction with organic solvents and neutral salts [32, 33] are used for the isolation of proteins. The extraction in alkaline conditions followed by isoelectric precipitation technique is more advantageous than the other techniques due to cheaper chemicals used and simpler equipment needed [34]. Therefore, the alkaline extraction was chosen in this study. In order to determine the optimum pH for the protein extraction from mahaleb kernel, DMKF samples were dispersed in distilled water (5% w/v), and the pH of the dispersions were adjusted from 1.0 to 12.0. After mixing and centrifugation, the yield of protein extraction was determined (Figure 1). The yield of protein extraction from mahaleb kernel was high at pH 1.0 (88.77%), 11.0 (87.40%) and 12.0 (90.60%), and there were no statistically significant differences ($p > 0.05$) among these extraction pHs. The yield of protein extraction was minimum at pH 4.0 (37.42%); therefore, the isoelectric point of the MKPs was assumed to be pH 4.0. However, interestingly there were no significant differences ($p > 0.05$) among the extraction pHs 3.0, 4.0, 5.0, 6.0 and 7.0. The proteins extracted at these pHs are mainly proteins with higher isoelectric point [35] and small polypeptides [28]. The extraction pHs of too high or too low may result in a structural damage in proteins [36]; therefore, the extraction pH should be determined by considering both a higher extraction yield and a minimum structural damage. Although the highest yield of protein extraction was obtained at pH 12.0 (90.60%), due to the above-mentioned reasons, the optimum pH value for protein extraction was determined to be pH 10.0. At this pH, the yield of protein extraction was 77.60%.

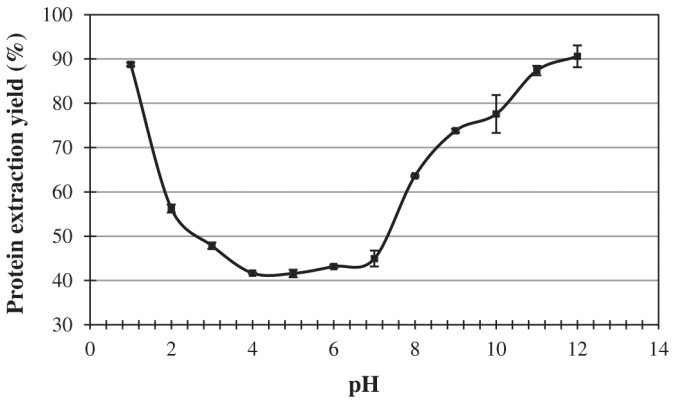

Figure 1: Effect of pH on protein extraction from defatted mahaleb kernel flour.

3.3 Production and composition of MKPC

MKPC was produced by extracting the protein at pH 10.0 and precipitating at pH 4.5 at room temperature. The isoelectric precipitation was performed at pH 4.5 instead of pH 4.0 due to the formation of a firmer precipitate as observed after centrifugation. The product obtained by this way was regarded as protein concentrate because its protein content in the dry matter was less than 90%. The proximate composition of the MKPC produced under these conditions was presented in Table 1. The dry matter, protein, fat, carbohydrate and ash content of the MKPC were 92.73%, 73.11%, 1.42%, 6.02% and 6.29%, respectively. The proximate composition of MKPC, except for protein content was parallel to the results reported for pea protein isolate [31], barley protein concentrate [15], sour cherry kernel protein concentrate [30], kidney bean protein isolate [37] and chickpea protein concentrate [38].

While 77.60 % of the MKPs were solubilized at pH 10.0, only 29.36% of the proteins were recovered in MKPC by precipitating at pH 4.5, indicating a poor protein recovery rate (16.67 g of MKPC was produced from 100 g of DMKF). In order to increase the protein recovery rate, higher extraction temperatures (such as 60°C), multi-stage extraction and membrane techniques such as ultrafiltration can be used. Additionally, enzymes such as pectinase, cellulases, phytase and xylanase can assist to increase the extractability of proteins bound to cellular components and phytate. Studies on the protein extraction from different sources showed that the recovery rates were varied significantly. While Kaushik [39] found a yield of 20.09% for flaxseed protein concentrate, a higher yield of 37–40% for hyacinth bean protein isolate [28] and 35.56% for sour cherry kernel protein concentrate [30] were reported.

3.4 SDS-PAGE

SDS-PAGE analysis was performed to determine molecular weight distribution of the proteins recovered in MKPC and the electrophoretogram generated was presented in Figure 2. As can be seen from the electrophoretogram, the molecular weights of proteins in MKPC varied from 14 to 66 kDa under reducing and denaturing conditions. Almost all of the protein bands detected in the defatted mahaleb kernel flour were also detected in MKPC, indicating that all the kernel proteins were transferred to MKPC but not quantitatively. However, some of the minor bands between 29 and 36 kDa in the defatted mahaleb kernel flour were not detected in MKPC. This may be due to the fact that these protein bands were not extracted at pH 10.0 or remain soluble at pH 4.5. A similar situation was also observed for the sour cherry kernel proteins [30].

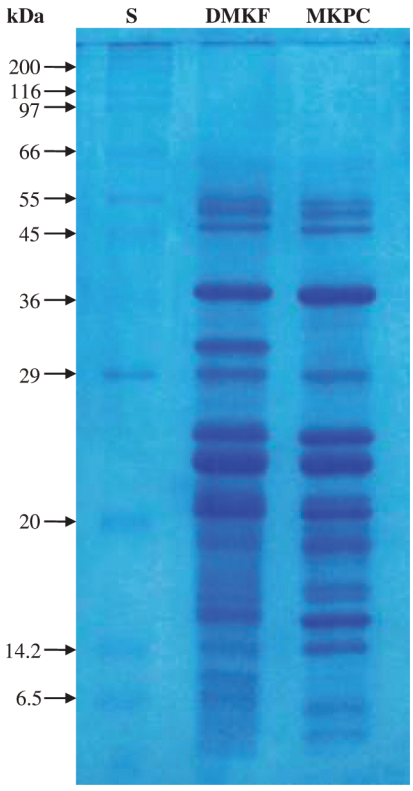

Figure 2: SDS-PAGE electrophoresis of mahaleb kernel products. S: Molecular weight standard, Myosin (200 kDa), β-galactosidase (116 kDa), phosphorylase b (97 kDa), bovine serum albumin (66 kDa), glutamic dehydrogenase (55 kDa), ovalbumin (45 kDa), glyceraldehyde-3 phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20 kDa), α-lactoalbumin (14.2 kDa), aprotinin (6.5 kDa). DMKF: Defatted mahaleb kernel flour, MKPC: Mahaleb kernel protein concentrate.

3.5 Colour properties

The colour parameters (CIE L*, a* and b*) of the MKF, DMKF and MKPC were given in Table 2. The L* and a* values of all samples were significantly different from each other ($p < 0.05$); however, there were no significant differences between DMKF and MKPC for b* values ($p > 0.05$). The reason why DMKF and MKPC had lower b* values than MKF may be the removal of lipid and lipid soluble pigments. Among the three samples, DMKF

had the highest value of L* (87.14 ± 0.21) and the lowest value of a* (1.37 ± 0.05), indicating the highest lightness and the lowest redness compared to the other samples. There was a significant increase ($p < 0.05$) in the L* values of DMKF (87.14 ± 0.21) compared to MKF (79.37 ± 0.60). This may be due to the removal of lipid and lipid soluble pigments. On the other hand, there was a significant decrease ($p < 0.05$) in the L* values of MKPC (55.59 ± 0.84), indicating the darkness in the product. This may be due to the Maillard type browning reactions that occur during drying at 50°C. Similar result (a lower L* value) was reported for the chickpea protein concentrates because of longer drying time at 40°C [38]. Some of the colour values reported for protein isolates or concentrates in the literature were as follows: L*: 62.8, a*: 5.0 and b*: 22.1 for pea protein isolate [31], L*: 90.32, a*: 1.76, b*: 3.36 for the hyacinth bean protein isolate [28], L*: 58.63–61.33, a*: 1.88–2.21, b*: 22.46–24.95 for the chickpea protein isolates [29], L*: 55.43, a*: 5.67, b*: 23.71 for sour cherry kernel protein concentrate [30] and L*: 55.5–64.28, a*: 4.20–6.10, b*: 27.37–30.35 for chickpea protein concentrate [38].

Table 2: Colour parameters of mahaleb kernel flour (MKF), defatted mahaleb kernel flour (DMKF), and mahaleb kernel protein concentrate (MKPC).

Samples	L*	a*	b*
MKF	79.37 ± 0.60[a]	1.78 ± 0.17[a]	17.08 ± 0.99[a]
DMKF	87.14 ± 0.21[b]	1.37 ± 0.05[b]	9.11 ± 0.13[b]
MKPC	55.59 ± 0.84[c]	4.61 ± 0.12[c]	8.58 ± 0.12[b]

Note: All the data are expressed as mean ± standard deviations and are the mean of three replicates (n).
L* ranges from 0 (black) to 100 (white), a* value ranges from green (−) to red (+), b* value ranges from blue (−) to yellow (+).
[a,b,c]Means followed by different letters within the same column represent significant differences ($p < 0.05$).

3.6 Protein solubility

Protein solubility is a crucial factor for food systems because it affects other functional properties such as emulsifying and foaming capacity, and serves as a useful indicator of denaturation and interactions of proteins. The solubility of a protein depends on such factors as pH, ionic strength, temperature, concentration and the presence of other molecules. Information on the solubility of proteins is important for extracting and purifying proteins from natural resources [40].

The protein solubilities of MKPC between pH 1.0–12.0 were presented in Figure 3. As can be seen from the Figure 3, the solubility was clearly pH dependent. The maximum solubility (95.97%) was observed at pH 12.0 and it was significantly different from the solubilities at other pHs ($p < 0.05$). Greater protein solubility at this pH is likely associated with increased negative charge and electrostatic repulsion [41]. The lowest solubility was at pH 6.0 (16.71%), and the solubility at this pH was not different considerably from pH 1.0, 2.0, 3.0, 4.0, 5.0, 7.0, 8.0, 9.0 and 10.0 ($p > 0.05$). It was interesting that the protein solubility was similarly poor at a wide pH range (1.0–10.0). The possible reason for this observation was protein denaturation, which may take place primarily in the course of drying at 50°C. The majority of food proteins are acidic and their minimum solubility is in the range of pH 4.0–5.0, and their maximum solubility is in alkaline pHs [42]. The solubility profiles of the protein isolates and concentrates in the other studies were different from MKPC in the pH range of 1.0–12.0 [15, 18, 30, 32, 43].

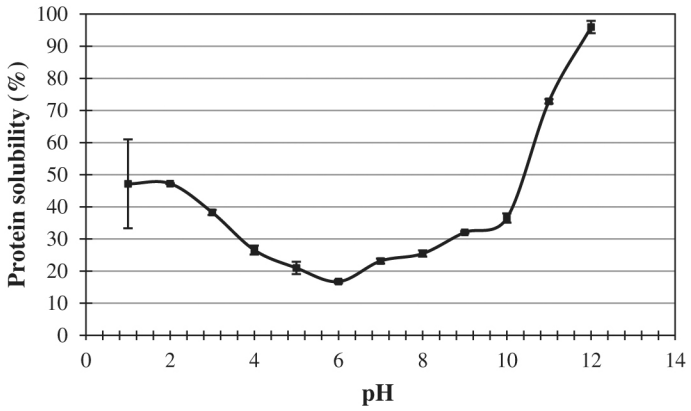

Figure 3: Solubility of mahaleb kernel protein concentrate at different pHs.

3.7 Water-holding capacity

The WHC is regarded as an indicator of the ability of a protein to physically hold water against gravity [44]. WHC is a critical functional property for highly viscous foodstuffs such as soups, doughs and bakery products. Proteins in these products retain water without dissolving, and increase viscosity and give consistency and structure [45]. The water-holding ability of the protein molecule is a function of size, shape, hydrophilic and hydrophobic interactions. Furthermore, WHC is affected by lipid and carbohydrate content, and the properties of amino acid residues on the surface [46].

The WHC of MKPC was 2.81 ± 0.02 g/g (281%). Under the same conditions, sodium caseinate used as reference protein dissolved in water and so no results were obtained. The WHC of MKPC was higher than those of pea protein isolate (1.7 g/g) [15], bean protein isolate (1.8 g/g) [15], soy protein isolate (1.3 g/g) [15], flaxseed protein concentrate (2.7 g/g) [47], bayberry kernel protein isolate (2.2 g/g) [48], peanut protein concentrate (1.15 g/g) [49], cashew protein concentrate (1.74 g/g) [14], apricot kernel protein concentrate (1.40 g/g) [50] and sour cherry kernel protein concentrate (2.42 g/g) [30]. However, it was lower than those of various rice bran protein concentrates (3.87–5.60 g/g) [51] and kidney bean protein isolates (5.34–5.85 g/g) [37].

3.8 Oil-holding capacity

The interaction between protein and lipids determines the sensory qualities of many foods. These interactions are influenced by pH, ionic strength, temperature and other variables in the system. Proteins with low solubility and high hydrophobicity can hold oil in large quantities. Furthermore, protein isolate/concentrate with smaller particle size and lower density can hold more oil. It has been reported that carbohydrates, which may be present in protein concentrate, have no significant effect on oil holding [40].

The OHC of MKPC was 1.66 ± 0.01 g/g (166%) being lower than that of sodium caseinate (2.00 ± 0.04 g/g) ($p < 0.05$). High OHC indicates the presence of many hydrophobic groups on the surface of the protein molecule [28, 29]. According to the results, there may be less hydrophobic groups on the surface of MKPC molecules than those of casein molecules. The OHC of MKPC was higher than pea protein isolate (1.20 g/g) [15], soy protein isolate (1.10 g/g) [15], flaxseed protein concentrate (1.18 g/g) [47] and apricot kernel protein concentrate (1.40 g/g) [50]. However, it was lower than almond protein isolate (2.93 g/g) [52], hyacinth bean protein isolate (2.54 g/g) [28], bayberry kernel protein isolate (1.80 g/g) [48], peanut protein isolate (2.00 g/g) [49], cashew protein concentrate (3.32 g/g) [14], sour cherry kernel protein concentrate (1.73 g/g) [30] and chickpea protein concentrate (1.91–2.77 g/g) [38].

3.9 Foaming capacity and stability

Foam formation and properties are influenced by the type of protein, preparation method, composition, solubility, concentration, pH, the presence of salts and hydrophobic interactions. Moreover, molecular elasticity, surface hydrophobicity, charge distribution and hydrodynamic properties affect foam formation and stability [53]. Foaming capacity depends on the diffusion of soluble proteins toward the air-water interface, rapid conformational change and rearrangement at the interface [54]. Therefore, flexible protein molecules have a good foaming capacity. On the other hand, globular proteins, whose surface denaturation is very difficult, have low foaming capacity. The foaming capacity of MKPC (43.75%) at pH 7.0 (Table 3) was found to be significantly lower than that of sodium caseinate used as a standard protein (87.50%) ($p < 0.05$). This may be partly because of the poor protein solubility of MKPC at pH 7.0. Additionally, this result indicated that the proteins in MKPC was likely less flexible than sodium caseinate. The foaming capacity of MKPC (43.75%) was lower than those of pea protein isolate (143%) [31], almond protein isolate (120%) [52], bayberry kernel protein isolate (47.4%) [48] and peanut protein concentrate (50%) [49]. However, it was higher than those of pea protein isolate (15%) [15], soy protein isolate (22%) [15], rice bran protein concentrate (5.2–8.7%) [51], cashew protein isolate (40%) [14], apricot kernel protein concentrate (21%) [50] and sour cherry kernel protein concentrate (35.0%) [30].

Table 3: Foaming capacities and stabilities of mahaleb kernel protein concentrate (MKPC) and sodium caseinate.

Properties (%)	MKPC	Sodium Caseinate
Foaming Capacity	43.75 ± 8.84[a]	87.50 ± 0.00[b]
Foaming Stability at 10 min	80.00 ± 28.28[a]	83.62 ± 5.12[a]
Foaming Stability at 30 min	71.33 ± 21.68[a]	70.38 ± 17.82[a]
Foaming Stability at 60 min	58.67 ± 20.74[a]	50.71 ± 24.57[a]

Foaming Stability at 90 min	53.33 ± 18.86[a]	34.66 ± 8.16[a]
Foaming Stability at 120 min	48.00 ± 16.97[a]	21.75 ± 0.67[a]

Note: All the data are expressed as mean ± standard deviations and are the mean of three replicates (n).
[a,b]Means followed by different letters within the same line represent significant differences ($p < 0.05$).

Foaming stability is an important property since the usefulness of a foaming agent depends on its ability to maintain gas bubble for as long as possible [55]. High foaming stability often requires a protein having proper surface-active properties and adequate intermolecular interactions at the interface. The rise in the protein concentration of the continuous phase results in an increase in the protein–protein interaction that leads to an increase in viscosity. This facilitates the formation of an adhesive multi-layer protein film around each gas bubble. The foams with this layer resist to liquid drainage and bubble coalescence [29].

Time-dependent (0–120 min) changes in the foaming stability of MKPC were presented in Table 3. MKPC had a lower stability than sodium caseinate after storage at room temperature for 10 min but had a higher stability after 30 min. However, no statistically significant difference was found between the values ($p > 0.05$). The observed lower foaming stability of MKPC after storage at room temperature for 10 min might be due to the lack of formation of a thick, cohesive and viscoelastic film around gas bubbles that prevented the foams from collapsing [56]. Fernandez-Quintela [15] documented that pea protein isolate and soy protein isolate had a foaming stability of 94% and 93%, respectively. Some of the foaming stabilities reported in the literature were as follows: 98% for almond protein isolate [52], 56% for bayberry kernel protein isolate [48], 55% for cashew protein isolate [14] and 71.8% for sour cherry kernel protein concentrate [30].

3.10 Emulsifying activity and stability indices

Surface hydrophobicity and concentration are the most important characteristics affecting the emulsifying ability of a protein. The EAI reflects the ability of a protein to adsorb rapidly to the water/lipid interface during emulsion formation. The EAI values of MKPC and sodium caseinate at pH 7.0 were 27.21 ± 2.50 and 176.44 ± 2.63 m^2/g, respectively. Sodium caseinate was used as a standard protein due to its good emulsifying properties. As seen, the EAI value of MKPC was significantly lower than that of sodium caseinate ($p < 0.05$), indicating that the proteins in MKPC probably did not adsorb to the water/lipid interface as rapidly as sodium caseinate. Inferior EAI value of MKPC might arise from its poor solubility at pH 7.0 since solubility affects the ability of a protein molecule to diffuse and adsorb rapidly at the interface. The EAI value (27.21 m^2/g) of MKPC was lower than those of soy protein isolate (115.9 m^2/g) [57], hyacinth bean protein isolate (534 m^2/g) [28], sour cherry kernel protein concentrate (38.91 m^2/g) [30], chickpea protein concentrates (312.54–410.05 m^2/g) [38] and flaxseed protein isolate (375 m^2/g) [39]. However, it was higher than that of oat bran protein concentrate (with heat treatment 18.90 m^2/g, without heat treatment 20.04 m^2/g) [58].

The ESI is a measure of the ability of a protein to form a stable emulsion for a certain period [28]. Several factors influence the emulsifying properties of proteins such as protein source, concentration, solubility, pH, temperature, and equipment and methods used for forming emulsion. The emulsifying properties of protein concentrates are generally parallel to the water solubility profiles [16]. The ESI value was found to be 1,187.5 ± 17.70 min for sodium caseinate, and 81.05 ± 1.49 min for MKPC. It was obvious that MKPC had significantly lower ESI value than sodium caseinate ($p < 0.05$). From this result, it was inferred that not all kinds of protein molecules have the same capability to reduce the interfacial tension and form a protective layer around the oil droplet. The ESI values of MKPC (81.05 min) was higher than those of peanut protein concentrate (19.18 min) [49] and sour cherry kernel protein concentrate (37.49 min) [30]. However, the values found for hyacinth bean protein isolate (2.7 h) [28], chickpea protein concentrate (124.93–164.19 min) and flaxseed protein isolate (180 h) [39] were much higher than the result of the present study.

3.11 Least gelling concentration

The gelling properties of proteins are particularly important in emulsion meat products such as salami and sausage [44]. The gelling capacities of the proteins depend mainly on protein concentration, ionic strength, pH, and the amount of sulfhydryl and hydrophobic groups. In addition, interactions of proteins with carbohydrates and lipids also affect their ability to form a gel. Hydrogen bonds and ionic interactions are responsible for the stabilization of the gel [40]. Physical, chemical or enzymatic applications can be used to form protein gels [18]. The LGC is a measure of the gel forming ability of a protein; the lower the LGC, the better the gelling capacity. LGC of MKPC at pH 7.0 was determined to be 12% (Table 4). The values reported by other researchers were mostly higher than our result, indicating that the protein content is not the only factor influencing LGC. Some of

the reported LGC values were as follows: 18% for pea protein isolate [15], 14–18% for chickpea protein isolate [29], 14–16% for chickpea protein concentrate [38], 16% for soybean protein isolate [15], 14% for broad bean protein isolate [15], 8% for sour cherry kernel protein concentrate [30] and 8% for rosehip seed protein isolate [25].

Table 4: The least gelling concentration of mahaleb kernel protein concentrate (MKPC).

MKPC Concentration (%)	Gelling Behavior[1]
2	– – –
4	– – –
6	– – –
8	– – –
10	– – –
11	**– – +**
12	**+ + +** (LGC)
14	**+ + +**

Note: [1]Result of three replicates.
–: No gel or weak gel; + : Firm gel.
LGC: The least gelling concentration.

3.12 Conclusions

The optimum pH values for the extraction and precipitation of proteins from the mahaleb kernels were determined as 10.0 and 4.5, respectively. Under these conditions, 29.36% of the proteins in the mahaleb kernels were recovered in MKPC. The maximum solubility of MKPC was observed at pH 12.0 (95.97%) whereas the minimum solubility was at pH 6.0 (16.71%). The WHC of MKPC was found to be 2.81 g water/g. Due to this feature, MKPC can be used in meat, dairy or bakery products. The OHC and the foaming capacity of MKPC were 1.66 g oil/g and 43.75%, respectively. This foaming capacity was reasonable compared to the reported foaming capacities. Emulsifying activity and stability indices of MKPC were determined as 27.21 m^2/g and 81.05 min, respectively. MKPC have a substantial potency as a food additive since its LGC was 12% being reasonably low. The molecular weights of proteins in MKPC ranged from 14 to 66 kDa. Almost all of the protein bands detected in the defatted mahaleb kernel flour were also detected in MKPC. The functional properties of MKPC indicate that it could contribute desirable attributes to some food products. Therefore, it is concluded that MKPC has the potential to be used in the food industry as functional food ingredient. Further studies are required to determine the effect of various protein extraction techniques on functional properties and yield of MKPC.

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