J Food Sci Technol (June 2019) 56(6):3023–3032
https://doi.org/10.1007/s13197-019-03785-8

ORIGINAL ARTICLE

Effect of pH on protein extraction from sour cherry kernels and functional properties of resulting protein concentrate

Mehtap Çelik[1] · Melih Güzel[2] · Metin Yildirim[3]

Revised: 26 March 2019 / Accepted: 12 April 2019 / Published online: 26 April 2019

Abstract The aims of this research were to examine the effect of pH on extraction of proteins from sour cherry (*Prunus cerasus* L.) kernels, and to investigate the functional properties of the resulting protein concentrate. The optimum pH values for the protein extraction and isoelectric precipitation were determined as 10.0 and 4.5, respectively. The protein concentrate contained 4.03 ± 0.16% moisture, 3.31 ± 0.17% ash, 2.94 ± 0.36% carbohydrate, 1.93 ± 0.16% lipid, and 80.48 ± 2.38% protein. Water holding capacity, oil holding capacity and the least gelling concentration of the protein concentrate were 2.42 ± 0.09 g water/g, 1.73 ± 0.17 g oil/g and 8%, respectively. Results showed that emulsifying activity and stability indices, foaming capacity and stability of protein concentrate were 38.91 ± 2.50 m^2/g, 37.49 ± 2.41 min, 35.00 ± 3.54% and 71.80 ± 7.25% (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 Sour cherry kernel · Protein concentrate · Chemical properties · Functional properties

✉ Metin Yildirim
metin.yildirim@ohu.edu.tr

[1] Department of Food Engineering, Faculty of Engineering, Hitit University, 19030 Çorum, Turkey

[2] Department of Food Processing, Şiran Mustafa Beyaz Vocational School, Gümüşhane University, 29700 Gümüşhane, Turkey

[3] Department of Food Engineering, Faculty of Engineering, Niğde Ömer Halisdemir University, 51240 Niğde, Turkey

Introduction

Because of the growing consumers' concerns, the food industry is persistently searching for healthier and inexpensive protein ingredients to substitute those originated from animal sources (e.g., gelatine, casein, whey proteins, and ovalbumin). Therefore, research on the plant proteins has become a chief issue to be addressed (Adebowale et al. 2011).

Cherry belongs to the genus Prunus of the Rosaceae family. Sour (tart) cherry (*Prunus cerasus* L.) has a higher acid/sugar ratio than sweet cherry (*Prunus avium* L.). Therefore, sour cherry is mostly used for the production of juice, concentrate, jam, puree, marmalade or pie filling while sweet cherry is generally consumed fresh (Toydemir et al. 2013; Yılmaz et al. 2018). Worldwide total sour cherry production is about 1.38 million tons in 2016 (FAOSTAT 2017), approximately 85% of which is processed into various food products (Toydemir et al. 2013), creating a high amount of seed as a waste material that causes an important disposal issue for the food industry (Yılmaz and Gökmen 2013). Currently, large amounts of seeds are discarded at the processing plants. For example, 15,000–20,000 tons of sour cherry seeds were obtained in the United States for the year 2014, mainly from juice processing factories (Korlesky et al. 2016). The absence of alternatives for reusing these wastes makes further investigations really important. Sour cherry kernels comprise 25.3–31.7% protein, 9.5–30.3% dietary fibre and 17.0–41.9% oil (Korlesky et al. 2016; Yılmaz and Gökmen 2013; Bak et al. 2010; Kamel and Kakuda 1992; Lazos 1991). García et al. (2015) has also showed the presence of antioxidant and antihypertensive peptides in sour cherry kernel proteins.

Functional properties of proteins are those physicochemical properties which determine their behaviour in food systems during preparation, processing, and storage. Solubility, emulsifying, foaming, water and oil holding, and gelation are a few examples of important functional properties of proteins. Therefore, proteins are used as a functional ingredient in food products to form certain sensory characteristics and/or to improve nutritional quality (Kinsella 1981; Damodaran 1997). Research on the functional properties of food proteins obtained from different sources is an essential in order to understand better their roles in food systems.

The sour cherry kernel could be a valuable source of proteins for use as functional food ingredients. At present, to the best of the researchers' knowledge, no information on the functional properties of these proteins is available. Thus, extracting the proteins from sour cherry kernels and understanding the functional properties of the resulting protein concentrate is crucial for food use. Therefore, the specific objectives of the present study were to: (1) investigate the extractability of sour cherry kernel proteins from defatted meal as a function of pH, and (2) determine the selected functional properties and chemical compositions of the resulting protein concentrate.

Materials and methods

Materials

The sour cherry kernels 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.

Extraction of proteins

Sour cherry kernel protein concentrate (SCKPC) 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 sour cherry kernel flour (SCKF) was defatted four times (1 h each) using n-hexane in a 1:6 flour to solvent ratio at room temperature. The defatted sour cherry kernel flour (DSCKF) was left in a fume hood for 12 h to remove hexane residues, and then transferred to a sealed glass jar and stored at room temperature until use. For protein extraction, the DSCKF was dispersed in distilled water (5%, 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 filtered through a coarse filter paper (Whatman Grade 1) and the filtrate 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 filtrate was adjusted to 4.5 with 2 N HCl, allowed to stand for 15 min, and further filtered through Whatman Grade 1 filter paper. The precipitated proteins were re-suspended in distilled water and its pH was adjusted to 7.0 and then dried at 50 °C for 12–18 h with an air flow oven (Memmert 100-800, Schwabach, Germany) and stored at − 18 °C until use.

Proximate composition

The dry matter and ash contents of sour cherry kernel flour, defatted flour and protein concentrate were determined by gravimetric method (AOAC 1997). Ankom extractor (Ankom XT10 Extractor, Macedon, NY, USA) was used to analyse the lipid content. The total carbohydrate content of the samples was determined according to the phenol sulphuric acid method (Geater and Fehr 2000). The micro-Kjeldahl method was used to analyse the nitrogen content of the samples (AOAC 1997). The value of 6.25 was used as protein conversion factor.

Colour parameters

The CIE Lab parameters (L*, a*, b*) of the sour cherry kernel flour, defatted flour and protein concentrate which were spread with a thickness of 1 cm over glass petri dishes were directly read from 3 different points via a colorimeter (Minolta, CR-300, Osaka, Japan) calibrated by means of a white tile (L* = 96.97, a* = 0.16, b* = 1.86) as reference. In this coordinate system, the a* value varies from green (−) to red (+), the b* value varies from blue (−) to yellow (+) and the L* value is a measure of lightness, ranging from 0 (black) to 100 (white).

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

The electrophoretic profiles of the proteins from DSCKF and SCKPC were determined according to the method described by Laemmli (1970). 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 95 °C 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 (Consort E815, Turnhout, Belgium) were employed.

Protein solubility

Protein solubility of the sour cherry kernel protein concentrate was determined as described by Beuchat et al. (1975) with slight modifications. Dispersions containing 5% (w/v) SCKPC 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 $4000\times g$ for 30 min. Protein content in the supernatant was determined by micro-Kjeldahl method (AOAC 1997) 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).

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

Water and oil holding capacities of SCKPC were determined by using the method outlined by Naczk et al. (1985). For the water holding capacity, 0.5 g SCKPC 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 room temperature for 15 min at $2000\times g$. 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 oil holding capacity, 0.5 g SCKPC was dispersed in 3 mL of commercial sunflower oil in a test tube. The dispersion was stirred for 30 s every 5 min and allowed to stand for 30 min, and then centrifuged at room temperature for 25 min at $1600\times g$. The supernatant was drained for 5 min at 45° angle. The gain in weight was recorded as oil holding capacity (g oil/g sample). Sodium caseinate was used as a reference for WHC and OHC tests.

Foaming capacity and foaming stability

The foaming capacity and foaming stability of SCKPC were determined using the method of Moure et al. (2001) with slight modifications. 0.5 g SCKPC 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 room temperature and then instantly transferred to a measuring cylinder, and the total volume and volume of liquid phase were recorded. Foaming capacity was calculated as:

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

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.

Emulsifying activity and stability indices

Emulsifying activity (EAI) and emulsifying stability indices (ESI) were determined according to the method of Pearce and Kinsella (1978). For emulsion formation, 6.6 mL of commercial sunflower oil was added to 20 mL of SCKPC 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 versus 0.1% SDS as blank using a spectrophotometer (Perkin Elmer UV/Vis spectrophotometer, Lambda EZ 201, Waltham, MA, USA). 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:

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

$$\mathrm{ESI(min)} = \frac{A_0 \times \mathrm{t}}{A_0 - A_{10}}$$

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.

The least gelation concentration

The least gelling concentration (LGC) of the samples was measured according to the method described by Coffman and Garcia (1977). 5 mL of aqueous dispersions (2–14% w/v, pH 7.0) of SCKPC 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.

Statistical analysis

Three independent determinations were used to obtain mean values and standard deviations. One-way analysis of variance and Duncan 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.

Results and discussion

Proximate composition

Moisture, protein, lipid, and total carbohydrate contents of SCKF and DSCKF are shown in Table 1. The proximate composition of SCKF was similar to those reported in the literature. The moisture content of sour cherry kernel was recorded as 3.91% (Yılmaz and Gökmen 2013). The protein content of sour cherry kernel was found to be 25.3% by Lazos (1991) and 37.80% by Kamel and Kakuda (1992). The reported lipid content of sour cherry kernel was 26.0% (Lazos 1991), 32.0–36.0% (Bak et al. 2010), 41.9% (Kamel and Kakuda 1992). Lazos (1991) and Yılmaz and Gökmen (2013) reported that the total carbohydrate content of sour cherry kernel was 34.5% and 46.6%, respectively.

After extraction, the lipid content of the kernel flour was reduced from 34.75 ± 0.68% to 9.00 ± 0.28%, indicating lower lipid extraction efficiency. The reason for this result could be the insufficient solvation of polar lipids such as phospholipids and free fatty acids by hexane (Makri et al. 2005), the extraction of lipids without drying or roasting the kernel and performing the extraction at room temperature. Higher extraction efficiency was reported for broad bean (Fernandez-Quintela et al. 1997), hyacinth bean (Subagio 2006), and chickpea (Kaur and Singh 2007). The removal of lipid caused an increase in protein and total carbohydrate content of defatted sour cherry kernel flour (Table 1).

Table 1 Proximate composition of sour cherry kernel flour (SCKF), defatted sour cherry kernel flour (DSCKF), and sour cherry kernel protein concentrate (SCKPC)

Parameters (%)	SCKF	DSCKF	SCKPC
Moisture	4.64 ± 0.04	6.94 ± 0.35	4.03 ± 0.16
Protein	32.21 ± 0.83	42.07 ± 0.09	80.48 ± 2.38
Lipid	34.75 ± 0.68	9.00 ± 0.28	1.93 ± 0.16
Total carbohydrate	10.16 ± 0.73	16.28 ± 1.63	2.94 ± 0.36
Ash	ND	ND	3.31 ± 0.17

All the data are expressed as mean ± standard deviations and are the mean of three replicates (n)

ND Not determined

Extraction conditions of proteins

There are various techniques such as ultrafiltration or reverse osmosis, extraction with organic solvents or neutral salts for protein isolation, but the extraction in alkaline conditions followed by isoelectric precipitation is more advantageous due to cheaper chemicals used and simpler equipment needed. Therefore, the alkaline extraction was chosen in this study. In order to determine the optimum pH for the protein extraction from sour cherry kernel, DSCKF 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 filtration, the yield of protein extraction was determined (Fig. 1). The yield of protein extraction from sour cherry kernel was high at pH 1.0 (75.3 ± 4.60%), 2.0 (78.9 ± 4.80%), 9.0 (67.8 ± 2.60%), 10.0 (74.1 ± 2.50%), 11.0 (70.5 ± 0.60) and 12.0 (76.9 ± 0.70), and there were no statistically significant differences ($p > 0.05$) among these extraction pHs. The yield of protein extraction was minimal at pH 4.0 (31.9 ± 2.30%); therefore, the isoelectric point of the sour cherry kernel proteins was assumed to be pH 4.0. However, there were no significant differences ($p > 0.05$) among the extraction pHs 4.0 (31.9 ± 2.30%), 5.0 (34.9 ± 1.16%), and 6.0 (38.7 ± 2.71%). The proteins extracted at these pHs are mainly proteins with higher isoelectric point (Makri et al. 2005) and small polypeptides

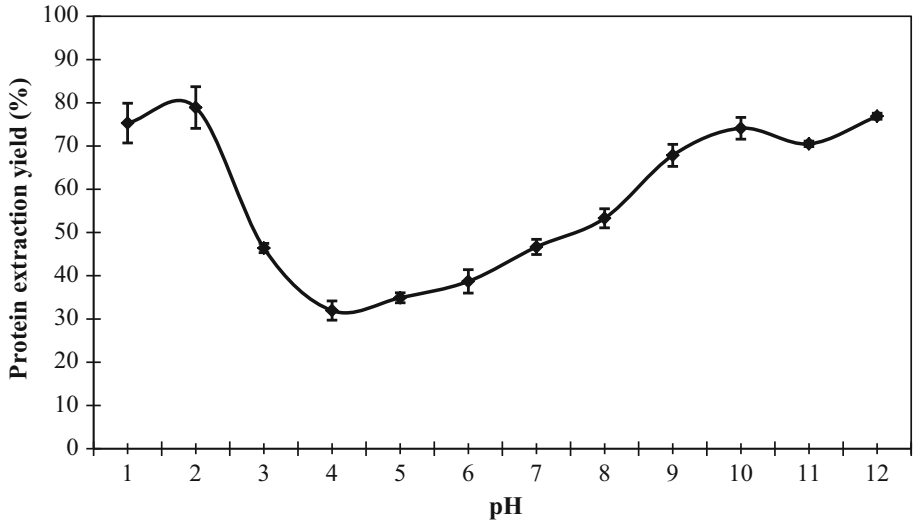

Fig. 1 Effect of pH on protein extraction from defatted sour cherry kernel flour

(Subagio 2006). The extraction pHs of too high or too low may result in a structural damage in proteins; therefore, although a higher yield of protein extraction was obtained at pH 12.0 (76.9%), the optimum pH value for protein extraction was assumed to be pH 10.0.

Production and composition of SCKPC

Sour cherry kernel protein concentrate was produced by extracting the proteins at pH 10.0 and precipitating at pH 4.5 at room temperature. While the minimum solubility was observed at pH 4.0, the isoelectric precipitation was performed at pH 4.5 due to the formation of a firmer precipitate. The protein rich product obtained by this way was regarded as protein concentrate because its protein content in the dry matter (83.86%) was less than 90%. The proximate composition of SCKPC (Table 1), except for protein content was parallel to the results reported for barley protein concentrate (Fernandez-Quintela et al. 1997) and chickpea protein concentrate (Ghribi et al. 2015).

While 74.1 ± 2.50% of the sour cherry kernel proteins were solubilized at pH 10.0, only 35.56 ± 0.52% of the proteins were recovered in SCKPC by precipitating at pH 4.5, indicating a poor protein recovery rate (18.59 g of SCKPC was produced from 100 g of DSCKF). In order to increase the protein recovery rate, higher extraction temperatures, multi-stage extraction and membrane filtration techniques 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 et al. (2016) found a yield of 20.09% for flaxseed protein concentrate; Subagio (2006) reported a higher yield of 37–40% for hyacinth bean protein isolate.

SDS-PAGE

SDS-PAGE analysis was performed to determine apparent molecular weight distribution of the proteins recovered in SCKPC and the electrophoretogram generated is presented in Fig. 2. As can be seen from the electrophoretogram, apparent molecular weights of proteins in SCKPC varied from 14 to 66 kDa under reducing and denaturing conditions. More specifically, five major protein bands (sub-units) with approximate apparent molecular weights of 45, 36, 29, 23, and 16 kDa were observed.

Almost all of the proteins present in DSCKF were also present in SCKPC, indicating that practically all the kernel protein sub-units were transferred to SCKPC. Additionally, the electrophoretogram showed that no significant protein degradation was occurred during protein concentrate production. However, some of the minor bands between 29–36 kDa in DSCKF were absent in SCKPC. 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.

Colour properties

Among the colour parameters (CIE L*, a* and b*), the L* and b* values of SCKF (69.73 ± 1.08 and 27.73 ± 0.15), DSCKF (84.19 ± 1.94 and 15.80 ± 0.71) and SCKPC (55.43 ± 0.96 and 23.71 ± 0.50) were considerably different from each other ($p < 0.05$); however, there were no significant differences between a* values of SCKF (5.11 ± 0.18) and SCKPC (5.67 ± 0.21) ($p > 0.05$). DSCKF had the highest L* value and the lowest

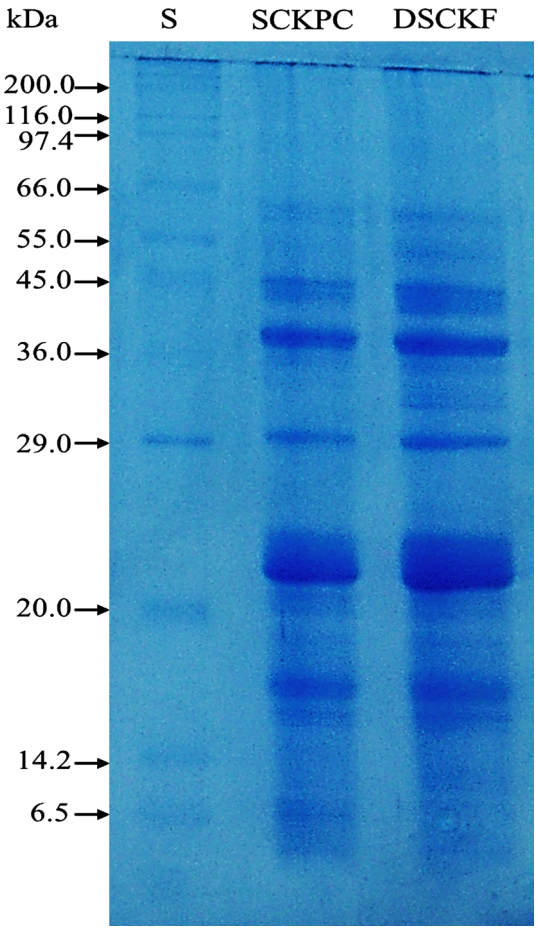

Fig. 2 SDS-PAGE electrophoresis of sour cherry kernel products. *S* Molecular weight standard, *DSCKF* defatted sour cherry kernel flour, *SCKPC* sour cherry kernel protein concentrate

a*(1.56 ± 0.47) and b* values, indicating the highest lightness and the lowest redness and yellowness compared to the other samples. There was a significant increase ($p < 0.05$) in the L* value of DSCKF compared to SCKF, resulting probably due to the removal of lipid and lipid soluble pigments. On the other hand, the lowest L* value ($p < 0.05$) was obtained for SCKPC, indicating that the Maillard type browning reactions occurred probably during drying at 50 °C. Similar result (a lower L* value) was reported for chickpea protein concentrates (Ghribi et al. 2015) because of longer drying time at 40 °C. L*, a* and b* values in the range of 62.9 to 77.6, 2.7 to 7.8 and 16.3 to 19.4, respectively were reported for cowpea protein isolates (Shevkani et al. 2015).

Protein solubility

Protein solubility is a crucial factor for food applications because it affects the other functional properties such as emulsifying and foaming capacity, and serves as a useful indicator of denaturation and interactions of proteins. Additionally, information on the solubility of proteins is important for extracting and purifying proteins from natural resources. The solubility of a protein depends on such factors as pH, ionic strength, temperature, concentration and the presence of other molecules (Damodaran 1997). As can be seen from Fig. 3, the solubility of SCKPC was clearly pH dependent. The maximum solubility (92.96 ± 1.66%) was observed at pH 12.0 and it was similar to the solubilities at pH 2.0 (85.52 ± 2.81%), 9.0 (86.26 ± 1.26%), 10.0 (90.15 ± 1.87%) and 11.0 (90.70 ± 2.20%) ($p > 0.05$). Greater protein solubilities at these pHs were likely associated with increased positive or negative charges resulting in electrostatic repulsion (Moure et al. 2001). The lowest solubility was at pH 5.0 (12.41 ± 1.23%), and the solubility at this pH was considerably different from solubilities at other pHs ($p < 0.05$). As is the case in the present study, the majority of food proteins are acidic and their minimum solubilities are in the range of pH 4.0 to 5.0, and their maximum solubilities are in alkaline pHs (Damodaran 1997). Shevkani et al. (2015) reported that cowpea protein isolates showed the lowest protein solubility at pH 4.0–5.0, while high protein solubility in acidic and alkaline pH.

The protein solubility of SCKPC in water at different pHs showed a U-shaped curve (Fig. 3), similar to cashew nut protein isolate (Ogunwolu et al. 2009), walnut protein isolate and concentrate (Mao and Hua 2012), and cowpea protein isolates (Shevkani et al. 2015).

Water holding capacity

The water holding capacity is regarded as an indicator of the ability of a protein to physically hold water against gravity (Kinsella 1981). WHC is a crucial functional property for highly viscous foodstuffs such as soups, doughs, gravies, sauces, and bakery products. The water-holding ability of the protein molecule is a function of size, shape, hydrophilic and hydrophobic interactions. Furthermore, water holding capacity is affected by lipid and carbohydrate content, and the properties of amino acid residues on the surface (Damodaran 1997).

The WHC of SCKPC was 2.42 ± 0.09 g water/g (242%), being higher than those of pea protein isolate (1.7 g/g), broad bean protein isolate (1.8 g/g), soy protein isolate (1.3 g/g) (Fernandez-Quintela et al. 1997), bayberry kernel protein isolate (2.2 g/g) (Cheng et al. 2009), cashew protein concentrate (1.74 g/g) (Ogunwolu et al. 2009), and apricot kernel protein concentrate (1.40 g/g) (Sharma et al. 2010). However, it was lower than those of flaxseed protein concentrate (2.70 g/g) (Martinez-Flores et al. 2006), and various rice bran protein concentrates (3.87–5.60 g/g) (Chandi and Sogi 2007). Water holding capacity is a useful indication of whether protein concentrate or isolates can be

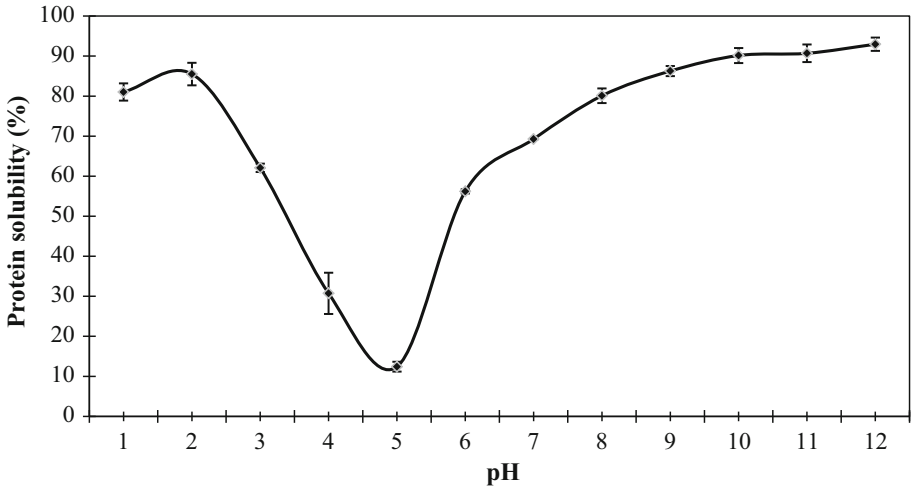

Fig. 3 Solubility of sour cherry kernel protein concentrate at different pHs

incorporated into aqueous food formulations. Higher WHC of SCKPC suggests its appropriateness for improving the viscosity of food formulations. Hence, it could be useful in extension of shelf life of meat products through the reduction of moisture loss.

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 the other variables in the system. Proteins with low solubility and high hydrophobicity can hold oil in large quantities (Damodaran 1997). High oil holding is essential for some food systems such as sausages, cake batters, mayonnaise and salad dressings (Chandi and Sogi 2007).

The oil holding capacity of SCKPC was 1.73 ± 0.17 g oil/g (173%), being lower than that of sodium caseinate (2.00 ± 0.04 g/g), but there were no significant difference ($p > 0.05$). High OHC may indicate the presence of large amounts of hydrophobic groups on the surface of the protein molecule (Subagio 2006; Kaur and Singh 2007). According to these results, there may be similar amount of hydrophobic groups on the surface of sour cherry proteins and casein molecules.

The OHC of SCKPC was higher than that reported for soy protein isolate (1.10 g/g) (Fernandez-Quintela et al. 1997), flaxseed protein concentrate (1.18 g/g) (Martinez-Flores et al. 2006), and apricot kernel protein concentrate (1.40 g/g) (Sharma et al. 2010). However, this value was lower than that reported for hyacinth bean protein isolate (2.54 g/g) (Subagio 2006), bayberry kernel protein isolate (1.80 g/g) (Cheng et al. 2009), peanut protein isolate (2.00 g/g) (Wu et al. 2009), cashew protein concentrate (3.32 g/g) (Ogunwolu et al. 2009), and chickpea protein concentrate (1.91–2.77 g/g) (Ghribi et al. 2015). The oil holding capacity may determine whether the protein concentrate or isolate can perform well as meat extenders or analogues. As the SCKPC demonstrated satisfactory oil holding capacity, it could find application in sausages and cake batters.

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 (Kinsella 1981). Foaming capacity depends on the diffusion of soluble proteins toward the air–water interface, rapid conformational change and rearrangement at the interface (Damodaran 1997). 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 SCKPC at pH 7.0 was found to be significantly lower than that of sodium caseinate used as a standard protein ($p < 0.05$) (Table 2). This result indicated that the proteins in SCKPC was likely less flexible than sodium caseinate. The foaming capacity of SCKPC (35.00 ± 3.54%) was lower than those of bayberry kernel protein isolate (47.4%) (Cheng et al. 2009), cowpea protein isolates (82–93%) (Shevkani et al. 2015), and peanut protein concentrate (50%) (Wu et al. 2009). However, it was higher than those of chickpea protein isolate (30.4%) (Kaur and Singh 2007), soy protein isolate (22.0%) (Fernandez-Quintela et al. 1997), rice bran protein concentrate (5.2–8.7%) (Chandi and Sogi 2007), and apricot kernel protein concentrate (21.0%) (Sharma et al. 2010).

Foaming stability is an important property since the usefulness of a foaming agent depends on its ability to

Table 2 Foaming capacities and stabilities of sour cherry kernel protein concentrate and sodium caseinate

Properties (%)	Sour cherry protein concentrate	Sodium caseinate
Foaming capacity	35.00 ± 3.54[a]	87.50 ± 0.00[b]
Foaming stability at 10 min	71.80 ± 7.25[a]	83.62 ± 5.12[a]
Foaming stability at 30 min	71.80 ± 7.25[a]	70.38 ± 17.82[a]
Foaming stability at 60 min	61.28 ± 11.24[a]	50.71 ± 24.57[a]
Foaming stability at 90 min	54.10 ± 10.52[a]	34.66 ± 8.16[b]
Foaming stability at 120 min	46.92 ± 9.79[a]	21.75 ± 0.67[b]

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 ($p < 0.05$)

maintain gas bubble for as long as possible (Kinsella 1981). 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 (Kaur and Singh 2007).

Time-dependent (0–120 min) changes in the foaming stability of SCKPC are presented in Table 2. SCKPC had a lower stability than sodium caseinate after storage at room temperature for 10 min, but had a higher stability after 30 min, although no statistically significant difference existed between the values ($p > 0.05$). The observed lower foaming stability of SCKPC after storage at room temperature for 10 min might be due to the fact that the ability of SCKPC was limited to form a thick, cohesive and viscoelastic film around gas bubbles (Damodaran 1997). Foaming stability of SCKPC (71.80 ± 7.25%) was higher than those of cashew protein isolate (55%) (Ogunwolu et al. 2009) and bayberry kernel protein isolate (56%) (Cheng et al. 2009), and lower than those of soy protein isolate (%93) (Fernandez-Quintela et al. 1997).

Emulsifying activity and stability indices

Surface hydrophobicity and concentration are the most important characteristics affecting the emulsifying ability of a protein. Also, a higher hydrophobic amino acid content in the protein molecule favour emulsification (Damodaran 1997; Subagio 2006).

EAI reflects the ability of a protein to adsorb rapidly to the water/lipid interface during emulsion formation. The EAI values of SCKPC and sodium caseinate at pH 7.0 were 38.91 ± 2.50 m^2/g 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 SCKPC was significantly lower than that of sodium caseinate ($p < 0.05$), indicating that the proteins in SCKPC probably did not adsorb to the water/lipid interface as rapidly as sodium caseinate. The EAI value of SCKPC (38.91 ± 2.50 m^2/g) was much lower than those of hyacinth bean protein isolate (534 m^2/g) (Subagio 2006), chickpea protein concentrates (312.54–410.05 m^2/g) (Ghribi et al. 2015) and flaxseed protein isolate (375 m^2/g) (Kaushik et al. 2016). However, it was higher than those of oat bran protein concentrates (with heat treatment 18.90 m^2/g, without heat treatment 20.04 m^2/g) (Guan et al. 2007), groundnut protein concentrate (5.43 m^2/g) (Jain et al. 2015), and cowpea protein isolates (7.7–8.9 m^2/g) (Shevkani et al. 2015).

ESI is a measure of the ability of a protein to form a stable emulsion for a certain period (Subagio 2006). Several factors influence the emulsifying properties of proteins such as protein source, concentration, structural and surface characteristics, 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 (Damodaran 1997; Shevkani et al. 2015).

The ESI value was found to be 1187.50 ± 17.70 min for sodium caseinate, and 37.49 ± 2.41 min for SCKPC. It was obvious that SCKPC had significantly lower ESI value than sodium caseinate ($p < 0.05$). From this result, it was inferred that the proteins in SCKPC had a limited capacity to reduce the interfacial tension and to form a protective layer around the oil droplet. The ESI values of SCKPC (37.49 ± 2.41 min) was higher than that of peanut protein concentrate (19.18 min) (Wu et al. 2009) and groundnut protein isolate (28.62 min) (Jain et al. 2015). However, the values found for hyacinth bean protein isolate (2.7 h) (Subagio 2006) and flaxseed protein isolate (180 h) (Kaushik et al. 2016) were much higher than the result of the present study.

The least gelling concentration

The gelling properties of proteins are particularly important in emulsion meat products such as salami and sausage (Kinsella 1981). 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 (Damodaran 1997). Physical, chemical or enzymatic applications can be used to form protein gels. The least gelling concentration is a measure of the gel forming ability of a protein; the lower the LGC, the better the gelling capacity. LGC of SCKPC at pH 7.0 was determined to be 8%. The values reported by the other researchers were mostly higher than the result of the present study, indicating that the protein content is not the only factor influencing LGC.

The least gelling capacity of SCKPC was much lower than that reported for soybean protein isolate (16%) (Fernandez-Quintela et al. 1997), for chickpea protein isolate (14–18%) (Kaur and Singh 2007), and for chickpea protein concentrate (14–16%) (Ghribi et al. 2015). On the other hand, this value was similar to that reported for rosehip seed protein isolate (8%) (Moure et al. 2001) and for Bambara groundnut protein isolate (8%) (Adebowale et al. 2011). As the SCKPC demonstrated superior gelling properties, it could find application in emulsion meat products such as salami and sausage.

Conclusion

In this study, sour cherry kernel protein concentrate was prepared by employing alkaline extraction and isoelectric precipitation, and its functional properties were determined. The optimum pH values for the extraction and precipitation of proteins from the sour cherry kernels were 10.0 and 4.5, respectively. Under these conditions, 35.56% of the proteins in the sour cherry kernels were recovered in protein concentrate. The maximum solubility of SCKPC was observed at pH 12.0 (92.96%) whereas the minimum solubility was at pH 5.0 (12.41%). The water holding capacity of SCKPC was found to be 2.42 g water/g. Due to this feature, SCKPC can be used in meat, dairy or bakery products. The oil holding capacity and the foaming capacity of SCKPC were 1.73 g oil/g and 35.00%, respectively. This oil holding capacity was reasonable. Emulsifying activity and stability indices of SCKPC were determined as 38.91 m^2/g and 37.49 min, respectively. SCKPC have a substantial potency as a food additive since its least gelling concentration was 8% being significantly lower than most of the reported values. The apparent molecular weights of proteins in SCKPC ranged from 14 to 66 kDa. The functional properties of SCKPC indicate that it could contribute desirable attributes to many food products. Therefore, SCKPC 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 sour cherry kernel proteins.

Acknowledgements The study was partially supported by Tokat Gaziosmanpaşa University Scientific Research Projects Unit (Project No.: 2009/59).

References

Adebowale YA, Schwarzenbolz U, Henle T (2011) Protein Isolates from Bambara Groundnut (*Voandzeia subterranean* L.): chemical characterization and functional properties. Int J Food Prop 14:758–775

AOAC (1997) Official methods of analysis of AOAC international, 16th edn. AOAC International Publ, Arlington

Bak I, Lekli I, Juhasz B, Varga E, Varga B, Gesztelyi R, Szendrei L, Tosaki A (2010) Isolation and analysis of bioactive constituents of sour cherry (*Prunus cerasus*) seed kernel: an emerging functional food. J Med Food 13:905–910

Beuchat LR, Cherry JP, Quinn MR (1975) Physicochemical properties of peanut flour as affected by proteolysis. J Agric Food Chem 23:616–620

Chandi GK, Sogi DS (2007) Functional properties of rice bran protein concentrates. J Food Eng 79:592–597

Cheng J, Zhou S, Wu D, Chen J, Liu D, Ye X (2009) Bayberry (*Myrica rubra* Sieb. et Zucc.) kernel: a new protein source. Food Chem 112:469–473

Coffman CW, Garcia VV (1977) Functional properties and amino acid content of a protein isolate from mung bean flour. J Food Technol 12:473–484

Damodaran S (1997) Food proteins: an overview. In: Damodaran S, Paraf A (eds) Food proteins and their applications. Marcel Dekker, New York, pp 1–21

FAOSTAT (2017) Food and agricultural commodities production, countries by commodity. http://www.fao.org/faostat/en/#data/QC. Accessed 14 Dec 2018

Fernandez-Quintela A, Macarulla MT, Del Barrio AS, Martinez JA (1997) Composition and functional properties of protein isolates obtained from commercial legumes grown in Northern Spain. Plant Foods Hum Nutr 51:331–342

García MC, Endermann J, González-García E, Marina ML (2015) HPLC-Q-TOF-MS identification of antioxidant and antihypertensive peptides recovered from cherry (*Prunus cerasus* L.) subproducts. J Agric Food Chem 63:1514–1520

Geater CW, Fehr WR (2000) Association of total sugar content with other seed traits of diverse soybean cultivars. Crop Sci 40:1555–1558

Ghribi AM, Gafsi IM, Blecker C, Danthine S, Attia H, Besbes S (2015) Effect of drying methods on physico-chemical and functional properties of chickpea protein concentrates. J Food Eng 165:179–188

Guan X, Yao H, Chen Z, Shan L, Zhang M (2007) Some functional properties of oat bran protein concentrate modified by trypsin. Food Chem 101:163–170

Jain A, Prakash M, Radha C (2015) Extraction and evaluation of functional properties of groundnut protein concentrate. J Food Sci Technol 52:6655–6662

Kamel BS, Kakuda Y (1992) Characterization of the seed oil and meal from apricot, cherry, nectarine, peach and plum. J Am Oil Chem Soc 69:492–494

Kaur M, Singh N (2007) Characterization of protein isolates from different Indian chickpea (*Cicer arietinum* L.) cultivars. Food Chem 102:366–374

Kaushik P, Dowling K, McKnight S, Barrow CJ, Wang B, Adhikari B (2016) Preparation, characterization and functional properties of flax seed protein isolate. Food Chem 197:212–220

Kinsella JE (1981) Functional properties of proteins: possible relationships between structure and function in foams. Food Chem 7:273–288

Korlesky NM, Stolp LJ, Kodali DR, Goldschmidt R, Byrdwell WC (2016) Extraction and characterization of Montmorency sour cherry (*Prunus cerasus* L.) pit oil. J Am Oil Chem Soc 93:995–1005

Laemmli UK (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 227:680–685

Lazos ES (1991) Composition and oil characteristics of apricot, peach and cherry kernel. Grasas Aceites 42:127–131

Makri E, Papalamprou E, Doxastakis G (2005) Study of functional properties of seed storage proteins from indigenous European legume crops (lupine, pea, broad bean) in admixture with polysaccharides. Food Hydrocoll 19:583–594

Mao X, Hua Y (2012) Composition, structure and functional properties of protein concentrates and isolates produced from walnut (*Juglans regia* L.). Int J Mol Sci 13:1561–1581

Martinez-Flores HE, Barrera ES, Garnica-Romo MG, Penagos CJC, Saavedra JP, Macazaga-Alvarez R (2006) Functional characteristics of protein flaxseed concentrate obtained applying a response surface methodology. J Food Sci 71:C495–C498

Moure A, Rua M, Sineiro J, Dominguez H (2001) Extraction and functionality of membrane-concentrated protein from defatted *Rosa rubiginosa* seeds. Food Chem 74:327–339

Naczk M, Diosady LL, Rubin LJ (1985) Functional properties of canola meals produced by a two-phase solvent extraction system. J Food Sci 50:1685–1692

Ogunwolu SO, Henshaw FO, Mock HP, Santros A, Awonorin SO (2009) Functional properties of protein concentrates and isolates produced from cashew (*Anacardium occidentale* L.) nut. Food Chem 115:852–858

Pearce KN, Kinsella JE (1978) Emulsifying properties of proteins: evaluation of a turbidimetric technique. J Agric Food Chem 26:716–723

Sharma PC, Tilakratne BMKS, Gupta JA (2010) Utilization of wild apricot kernel press cake for extraction of protein isolate. J Food Sci Technol 47:682–685

Shevkani K, Kaur A, Kumar S, Singh N (2015) Cowpea protein isolates: functional properties and application in gluten-free rice muffins. LWT-Food Sci Technol 63:927–933

Subagio A (2006) Characterization of hyacinth bean (*Lablab purpureus* L. Sweet) seeds from Indonesia and their protein isolate. Food Chem 95:65–70

Toydemir G, Capanoglu E, Kamiloglu S, Boyacioglu D, De Vos RC, Hall RD, Beekwilder J (2013) Changes in sour cherry (*Prunus cerasus* L.) antioxidants during nectar processing and in vitro gastrointestinal digestion. J Funct Foods 5:1402–1413

Wu H, Wang Q, Ma T, Ren J (2009) Comparative studies on the functional properties of various protein concentrate preparations of peanut protein. Food Res Int 42:343–348

Yılmaz C, Gökmen V (2013) Compositional characteristics of sour cherry kernel and its oil as influenced by different extraction and roasting conditions. Ind Crops Prod 49:130–135

Yılmaz FM, Görgüç A, Karaaslan M, Vardin H, Bilek SE, Uygun Ö, Bircan C (2018) Sour cherry by-products: compositions, functional properties and recovery potentials—a review. Crit Rev Food Sci Nutr. https://doi.org/10.1080/10408398.2018.1496901