Research Paper / Araştırma Makalesi

Production and Characterization of Plant Protein Concentrates from Shells of Kidney Bean, Pea and Cowpea and Their Effects on Freezing and Freeze Drying of Kiwi Puree

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ABSTRACT

In this study, protein concentrates (PCs) were obtained from the shells of kidney bean (KPC), pea (PPC) and cowpea (CPC) by using alkaline extraction followed by isoelectric precipitation and freeze drying. Among PCs, CPC had significantly the highest protein (41.22%) and the lowest dry matter (93.52%) contents. The protein content of KPC was 19.20% while PPC had a content of 25.48%. The ash content of CPC was the lowest (0.005%). Considering the color values of PCs, the highest L* and a* color values were 44.25 and 0.36 for KPC, respectively and the highest b* value was 0.39 for CPC. The highest total color change (ΔE) was calculated as 30.23 for PPC. Among functional properties, KPC had the highest water (2.26 g/g) and oil holding capacity (3.60 g/g) values. PPC had the highest emulsion capacity (54.28%), stability (51.43%), and foaming capacity (47.63%) values. CPC showed the highest solubility value (99.23%). Based on the results of differential scanning calorimetry (DSC) analysis, CPC displayed a lower denaturation temperature (T_d) and heat of transition (ΔH). With the addition of the PCs (in 0, 1, 2, 4, and 6% of total weight), the duration for the freezing of kiwi puree with a 6% PC (KPC, PPC, and CPC) was the lowest. On the other hand, a clear effect of adding protein to kiwi puree on behavior of freeze drying was not observed.

Keywords: Kidney bean, Pea, Cowpea, Protein concentrate, Freeze drying

Barbunya, Bezelye ve Börülce Kabuklarından Bitkisel Protein Konsantresi Üretimi ve Karakterizasyonu ile Kivi Püresinin Donması ve Dondurularak Kurutulması Üzerine Etkileri

ÖZ

Bu çalışmada, barbunya, bezelye ve börülce kabuklarından izoelektrik noktada çöktürme yöntemi kullanılarak protein konsantreleri elde edilmiştir. Elde edilen protein konsantreleri arasında, börülce kabuğu protein konsantresi en yüksek protein ve en düşük kurumadde içeriğine sahiptir (sırasıyla %41.22 ve 93.52). Barbunya kabuğu protein konsantresinin protein değeri %19.20 ve bezelye kabuğu protein konsantresinin ise %25.48'dir. Börülce kabuğu protein konsantresinin kül değeri en düşük olarak bulunmuştur (%0.005). Renk değerleri dikkate alındığında, en yüksek L* değeri 44.25 olarak barbunya kabuğu protein konsantresi için, en yüksek a* değeri 0.36 olarak barbunya kabuğu protein konsantresi için ve en yüksek b* değeri 0.39 olarak bezelye kabuğu protein konsantresi için ölçülmüştür. En yüksek toplam renk değişim değeri (ΔE) 30.23 olarak bezelye kabuğu protein konsantresi için hesaplanmıştır. Fonksiyonel özellikler incelendiğinde ise, barbunya kabuğu protein konsantresi en yüksek su ve yağ tutma kapasitesine (sırasıyla 2.26g/g ve 3.60 g/g), bezelye kabuğu protein konsantresi en yüksek emülsiyon kapasitesi ve stabilitesine (sırasıyla %54.28 ve %51.43) ve köpük oluşturma kapasitesine (%47.63) sahiptir. Börülce kabuğu protein konsantresi en yüksek çözünürlük değerini göstermiştir (%99.23). Diferansiyel taramalı kalorimetri (DSC) analizine göre, börülce kabuğu protein konsantresi en düşük denatürasyon sıcaklığı (T_d) ve geçiş ısısı (ΔH)

değerine sahiptir. Protein konsantrelerinin eklenmesiyle (toplam ağırlığın %0, 1, 2, 4, 6 oranında), %6 oranında protein konsantresi (barbunya, bezelye ve börülce kabuğu protein konsantresi) eklenen kivi pürelerinin donma faz süresi, diğer oranlara göre daha kısa bulunmuştur. Diğer taraftan, kivi püresine protein konsantresi eklemenin dondurarak kurutma davranışı üzerine net bir etkisi gözlenmemiştir.

Anahtar Kelimeler: Barbunya, Bezelye, Börülce, Protein konsantresi, Dondurarak kurutma

INTRODUCTION

Food waste is a serious problem with growing human population and industralization in the recent years. It causes economic losses, environmental problems, sanitary conformities and social implications [1]. Food wastes can be recovered as a cheap source of valuable components by the possibilities of the current technology such as production of biofuels, recovery of them as protein, phenols, antioxidants, dietary fibers, pectin and flavonoids [2].

Legumes are known as the second most important source of human food after cereals. They are a cheap source of proteins and other nutrients such as minerals, starch, vitamins, polyphenols, and dietary fibre [3]. They are also serious food waste sources in industry. For example, approximately half of a pea is shell. This loss can not be ignored in industry.

Plant based proteins have a great importance in human nutrition because of the consumption less than necessary. Hence, studies have increased about plant based proteins in time. Due to anxiety about deficiency of animal protein sources with growing world population, there has been a continuous search for legumes as new protein sources to utilization as both nutritional supplements and functional food ingredients [4]. Plant proteins which are obtained particularly from grain legumes have been used as ingredients in food products for many years in food industry because of their valuable amino acid contents. Protein concentrates which are obtained from legumes are used as ingredients in food systems such as in cake mix, pudding, sausage [5]. In the literature, many studies have given that protein concentrates are obtained from different legumes such as cowpea [5], barley [6], lentil [3], chickpea [7], and kidney bean [8].

Kidney bean *(Phaseolus vulgaris L.)* is the most grown and consumed one among the legumes [9]. It can be used in crude ingredient of canned, dry, and fresh consumption in the food systems [10]. It is a good source of proteins. It includes 20-30% protein which has valuable amino acid composition [11]. Pea *(Pisum sativum)* is a legume of increasing significance in the world markets [12]. It is rich in protein content and shows miscellaneous functionalities [13]. It contains approximately 25% protein which is higher in levels of lysine and tryptophan [14]. The cowpea *(Vigna unguiculata L. Walp.)* is a widely consumed and nutritious legume. It is durable to high temperatures and drought [15], and has a rich nutritious profile. A mature cowpea includes 24.8% protein content [16].

Freezing and freeze drying are important methods for the long-term preservation of foods but they have some disadvantages such as long and expensive processes [17]. Because of this reason, the use of several drying agents is a crucial point in freeze drying and it is aimed to reduce the total time and energy loss in the studies.

The objective of this study is to have a contribution to waste management by obtaining protein concentrate from the shells of kidney bean, pea, and cowpea and investigate the effect of protein concentrate on the freezing and freeze drying of kiwi puree by adding the obtained protein concentrates to the kiwi puree.

MATERIALS and METHODS

Materials

Kidney beans, peas, and cowpeas as the raw materials were purchased from local markets in Izmir (Turkey). The shells were separated from grains, washed and dried in a conventional oven at 70°C for 1 hour to be half dried. After that, they were stored at -24°C until utilized for protein extraction. All the chemicals are the high quality (Sigma-Aldrich); the DSC pans (TA, Tzero Pan) and the dialysis sacks (Sigma-Aldrich) were used.

Preparation of Protein Concentrates

The shells of kidney bean, pea, and cowpea (500 g) were blended using a home type blender (Tefal Smart, MB450141, Turkey) with the addition of 0.05 M Tris buffer solution (1000 mL) which has a pH of 8.5 at 25°C. For this purpose, 4.36 g of Trizma Base and 2.21 *g* of Trizma HCI were dissolved in 1000 mL of distilled water. The resulting blend was centrifuged at 3500 *g* for 30 min (Hettich, Universal 320R, Germany) to ensure required phase separation for protein isolation. The liquid phase was taken and brought to a pH 4.5 with the addition of HCI and NaOH according to the initial pH and centrifugation process was repeated. The precipitate was gathered and solubilized again in around 5-10 mL in the buffer solution. The obtained solution was put into dialysis sacks (Sigma-Aldrich) which have a pore size of 12000 Da and width of 35 mm and dialyzed in deionized water for 6 hours to purify. Then, the solution was concentrated with the addition of polyethylene glycol (5%, v/v). Lastly, the freeze drying process was performed in a pilot scale freeze dryer (Armfield, FT 33 Vacuum Freeze Drier, England), under vacuum (13.33 Pa absolute pressure) at -48°C condenser temperature for 9 hours to obtain the protein concentrates.

Preparation of Kiwi Puree for Freezing and Freeze Drying

Fresh kiwi fruits were purchased from a local market in Izmir (Turkey). They were peeled, crushed, and pureed with a home type blender (Tefal Smart, MB450141, Turkey). The obtained protein concentrates from the shells of the kidney bean, pea, and cowpea were added to the kiwi puree at 0, 1, 2, 4, and 6% proportions (w:w). Samples were frozen at -24°C in a freezer (Vestel, Turkey) by placing 16 g into a glass test tube (diameter is 16 mm, height is 100 mm). Thermocouples were placed in the bottom of the test tube. Freezing graphs as temperature versus time were plotted using the data obtained with a datalogger (PP222, USB Data Logger, Pico Technology). The control sample did not include any protein concentrates. The initial temperature was 20°C for all samples.

The obtained protein concentrates from the shells of the kidney bean, pea, and cowpea were added to the kiwi puree at 0, 1, 2, 4, and 6% ratios (w:w). Samples were frozen in a freezer (Vestel, Turkey) in a layer of 3 mm in the petri dishes at - 24°C for 24 hours, then, freeze dried in a pilot scale freeze dryer (Armfield, FT 33 Vacuum Freeze Drier, England) under vacuum (13.33 Pa absolute pressure) at -48°C condenser temperature for 9 hours. After drying, the powder products were obtained.

Determination of Protein, Dry matter, and Ash Contents

The protein (N*6.25) and dry matter contents of the KPC, PPC, and CPC were determined according to the AOAC method [18]. The ash contents of the samples were determined using a standard method [19].

Measurement of Colour Values

The colour parameters (L*, a*, and b*) of the KPC, PPC, and CPC were measured using a Minolta CR-400 Colorimeter, Japan. The device was calibrated with white standard plate before all the measurements. The results were expressed as the average of six measurements in accordance with the CIE Lab. System. The L* value measures the lightness value which ranges between 0 and 100, the a* value symbolizes color ranging from red (+) to green (-) and the b* value also represents color ranging from yellow (+) to blue (-). The total color change value (ΔE) was calculated using Equation 1. In the Equation (1), the L*, a*, and b* values were measured on the protein concentrates and the L_0^*, a_0^*, and b_0^* values were measured on the shells.

$$\Delta E = \sqrt{(L*-L_0*)^2 + (a*-a_0*)^2 + (b*-b_0*)^2} \tag{1}$$

Determination of Thermal Properties

Thermal properties of KPC, PPC, and CPC were determined with DSC (Perkin Elmer DSC-8000) system in Food Engineering Department of Ege University.

Approximately 0.1 mg sample were placed into aluminium pans. The pans were hermetically sealed. The DSC was calibrated (indium) and an empty aluminium pan was used as reference. Pans were heated at a rate of 5°C/min from -20°C to 120°C. Denaturation temperature (T_d) and enthalpy change (ΔH) were calculated using the software of device.

Determination of Functional Properties

Determination of Solubility Values

Solubility value of KPC, PPC, and CPC were determined according to Cano-Chauca et al. [20]. For this purpose, 1 g of sample was added to 100 mL of distilled water. It was mixed with magnetic stirrer for 5 min and centrifuged at 3000 rpm for 5 min. Then, 25 mL of the solution was taken, dried at 105°C in the oven for 5 hours and the result as the weight of dry sample of the concentrate was given as percentage compared to total sample.

Determination of Water and Oil Holding Capacity (WHC and OHC) Values

WHC value was measured by using the method described by Rodríguez-Ambriz et al. [21]. For this purpose, 100 mg of each protein sample were mixed with 1000 µL of distilled water by using a magnetic stirrer in a 50 mL beaker. The protein suspension was then centrifuged at 1800 g for 20 minutes at 20°C. The supernatant was decanted, and the tube was drained at 45° angle for 10 min. The water holding capacity value was expressed as water (g) retained by protein sample divided by the amount of (g) protein sample.

The method described by Lin and Zayas [22] was used to determined of OHC value. For this purpose, 100 mg of protein sample was mixed with a vortex in 1000 µL of sunflower oil for 30 s. The emulsion was incubated at room temperature (about 20°C) for 30 min, and then centrifuged at 13,600 g for 10 min at 25°C. The supernatant was decanted and drained at a 45° angle for 20 min. The oil holding capacity value was expressed as oil (g) retained by protein sample divided by the amount of (g) protein sample.

Determination of Emulsion Capacity (EC) and Stability (ES) Values

The emulsion capacity (EC) and emulsion stability (ES) of KPC, PPC, and CPC were determined by using the method of Wu [23]. For this purpose, 0.5 g of each protein sample was dissolved in 10 mL of distilled water. pH was adjusted to 7 with 1 N HCl and 1 N NaOH and 10 ml of sunflower oil was added. The sample was mixed with magnetic stirrer for 1 min. Then, it was centrifuged for 15 min at 3000 g. Emulsion layer (mL) and total tube volume (mL) were recorded from centrifuge tube. EC values were calculated according to the Equation (2). After that, the tube containing the emulsion has been waited at 80°C for 30 min in water bath. Then, fast cooling was done under running water

for 5 minutes. Then, the sample was centrifuged for 5 minutes at 3000x*g*. The remaining emulsion layer (mL) and total volume (mL) were also recorded and ES values were calculated according to the Equation (3).

EC=100*(Emulsion layer (ml)/ Total volume (mL)   (2)

ES=100*(Remaining Emulsion layer (mL)/Total volme (mL))   (3)

Determination of Foaming Capacity (FC) Values

FC was determined according to the method of Sathe and Salunkhe [24]. For this purpose, 2 g of each protein sample were mixed with 100 mL of distilled water in a 250 mL beaker for 5 min at high speed. The sample was rested 4 hours. The total volume was recorded and calculated according to the Equation (4).

FC=((volume after whipping- volume before whipping)/(volume before whipping))*100   (4)

Determination of Moisture Ratio (MR) Values

KPC, PPC, and CPC were added to the kiwi puree at 0, 1, 2, 4, 6% proportions. The samples were freeze dried in a pilot scale freeze dryer (Armfield, FT 33 Vacuum Freeze Drier, England). Mass losses were taken at 1 hour interval. Moisture ratio (MR) of samples was calculated according to the Equation (5).

$$MR = \frac{M_t - M_e}{M_0 - M_e} \qquad (5)$$

Where the M_t, M_0, and M_e are the moisture contents at any time, initial, and equilibrium moisture contents (kg water / kg dry matter), respectively.

Statistical Analysis

The obtained results were expressed as mean values ± standard error. The results were tested with the SPSS 20.0 package program (SPSS Inc., USA) at 95% confidence interval with an ANOVA test.

RESULTS and DISCUSSION

Protein, Ash, and Dry Matter Contents

The protein, ash and dry matter content values of the KPC, PPC, and CPC are shown in Table 1. The protein content values of KPC, PPC, and CPC were found as 19.20% (db), 25.48% (db), and 41.22% (db), respectively. Evaluating the results, CPC demonstrated the highest protein content value among the samples evaluated. The protein content value was comparable to that reported for cowpea protein concentrates which are obtained with the same method from cowpea kernels as 92% (db) [25]. When the results are compared with the results in the literature, it can be stated that a high amount of protein content also exist in the shells of cowpea. When comparing the results of KPC and PPC with the results given by Shevkani et al. [8], protein contents of the protein concentrates from the kernel of kidney bean and pea were determined as 85.3%(db) and 92.8%(db). These results show that, with the same method of protein concentration technique almost a quarter portion of the proteins can be obtained from the shells where in fact they are usually discarded as waste. When ash content values were analyzed, the values of KPC, PPC, and CPC were observed as 0.011%, 0.008%, and 0.005%, respectively. The lowest ash content value was found for CPC. When the ash content values of the protein concentrates obtained with the same method were examined in the literature, the results were given as 4.5%, 3.8% and 4.4% for protein concentrates from the kernel of kidney bean, pea [8] and cowpea [25]. The low ash content demonstrates that the isolation efficiency is high, and it is removed from the unwanted additives. Therefore, the low ash content is expressed a positive impact [26]. The isolation efficiency of the protein concentrates from the shells is higher than that of kernels.

Table 1. Chemical composition and color values of protein concentrates from the shells of kidney bean (KPC), pea (PPC), and cowpea (CPC).

Parameter	KPC	PPC	CPC
Protein% (db)	19.20±0.13[a]	25.48±0.16[b]	41.22±0.45[c]
Ash% (db)	0.011±0.001[c]	0.008±0.000[b]	0.005±0.001[a]
Dry matter (%)	94.02±0.20[b]	94.69±0.14[c]	93.52±0.24[a]
CIE Color L*	44.25±0.02[c]	42.10±0.48[b]	36.35±0.26[a]
CIE Color a*	0.36±0.00[c]	0.34±0.00[a]	0.35±0.00[b]
CIE Color b*	0.37±0.00[a]	0.37±0.00[a]	0.39±0.00[b]
Total Color Change (ΔE)	24.74	30.23	21.97

Values are mean ± SD. Means with similar superscript in a column did not differ significantly (P > 0.05). db: dry matter basis

When the dry matter content values were examined, the values of KPC, PPC, and CPC were found as 94.02%, 94.69%, and 93.52%, respectively. PPC showed the highest dry matter content value among the samples evaluated. These results are comparable to the results given as 94.0% for protein concentrate from cowpea by Frota et al. [25], in the literature. These results show

that, with the same isoelectric precipitation method and freeze drying of the protein concentrates, similar results can be obtained.

Color Values

The measured color values of KPC, PPC, and CPC are also given in Table 1. The L_0^* values of the shells of kidney bean, pea, and cowpea were determined as 19.51, 53.42, and 14.38, respectively. The L^* values (brightness) for KPC, PPC, and CPC were measured as 44.25, 42.10, and 36.35, respectively. According to these, the brightness value of KPC was the highest among the samples evaluated while the highest value was measured for shell of pea. The results show that the protein concentration process increased the brightness value of the KPC and CPC and decreased the brightness value of the PPC.

The a_0^* values of the shells of kidney bean, pea, and cowpea were measured as 0.37, -3.82, and 0.36, respectively. The a^* values of KPC, PPC, and CPC were observed as 0.36, 0.34, and 0.35, respectively. It can be stated that, the protein concentration process did not significantly influenced the greenness values of KPC and CPC, but diminished the greenness value of the PPC as can be understood by the increase in a^* value.

The b_0^* values of the shells of kidney bean, pea, and cowpea were measured as 0.32, 28.09, and 0.43, respectively. The b^* values of KPC, PPC, and CPC were observed as 0.37, 0.37, and 0.39, respectively. According to these results, the effect of the protein concentration process on the yellowness value is as follows: the value increased for KPC, reduced for CPC and dramatically reduced for PPC.

While calculating the ΔE values, the color values of the shells were assumed as reference value. The ΔE values of KPC, PPC, and CPC were computed as 24.74, 30.23, and 21.97, respectively. If the total color change value (ΔE) is more than 2, people can be perceived this change visually [27]. The ΔE values of the protein concentrates in this study are more than 2, thus, this change can be perceived visually. It can be stated that, the effect of the method for obtaining the protein concentrate impressed the color values of the samples of PPC at higher amounts, then, KPC and CPC.

Thermal Properties

Differential scanning calorimetry (DSC) was used to observe the changes in thermal stability of the protein concentrates. The thermal stability of the proteins represents their resistance to aggregation in response to heating [5]. In this study, the protein concentrates were heated from -20°C to 120°C at heating rate of 5°C/min. and the DSC thermograms are obtained. DSC thermograms are given in Figure 1. According to these thermograms, denaturation temperature of KPC, PPC, and CPC were found as 50.26°C, 50.65°C, and 44.96°C, respectively. Among the evaluated samples, CPC has the lowest denaturation temperature. The denaturation temperatures were comparable to that reported for kidney bean, pea, and cowpea protein concentrates which are obtained with the same method

from kernels as 91.0°C and 83.8°C [8], and 87.29°C [5], respectively. The thermal stability of the proteins depends on polar and non-polar components. If non-polar components are in higher proportions, thermal stability will be higher (T_d is higher) [28]. Therefore, it can be stated that, non-polar components of the protein concentrates from kernels are higher when comparable with literature and the results of this study. The transition heat or enthalpy change (ΔH) provides information about the proportion of denatured protein during process [29]. ΔH of KPC, PPC and CPC were found as 71.04 J/g, 65.15 J/g, and 37.14 J/g, respectively. According to these results, proportion of denatured protein of CPC was the lowest among the evaluated samples.

Functional Properties

Functional properties of the proteins are a considerable issue in food processing. It is known that the proteins increase the solubility, water and oil holding capacity, emulsion capacity and stability, and foam capacity of the products when added [30]. The protein concentrate is selected according to which functional property of the food is to be developed. For example; while high water and oil holding capacity are preferred in sausages, bread and cakes, high emulsification and foaming property is preferred for salad dressings, soups, candies, frozen desserts and cakes [31;32].

Protein solubility values of KPC, PPC, and CPC were calculated as 99.22%, 99.13%, and 99.23%, respectively (Table 2). The solubility values of all protein concentrates were similar and were not significantly different from each other (P>0.05). When the DSC thermograms of the protein concentrates were examined, the denaturation temperature of CPC found as the lowest one. Therefore, non-polar components of CPC were the lowest. It can be stated that, polar components of CPC were the highest and solubility value in water of CPC was found the highest value. When the percentages of the solubility values of samples were considered, it can be stated that, the protein concentrates which have high solubility values can be used in most of the food formulations.

WHC/OHC is capability of the proteins to hold water/oil against gravity [8]. Water holding capacity values of the KPC, PPC, and CPC were observed as 2.26 g/g, 1.08 g/g, and 1.85 g/g, respectively (Table 2). The KPC showed the highest water holding capacity value among the samples evaluated. Evaluating the results obtained from KPC, and PPC, these values can be compared with the results given by Shevkani et al. [8], Fernández-Quintela et al. [33], and Mune and Sogi [34] where the WHC value of the protein concentrates from kernels of kidney bean, pea and cowpea as 2.6 g/g, 1.7 g/g, and 2.45 g/g, respectively. These results show that WHC values of the protein concentrates from kernels and shells of kidney bean, pea, and cowpea were similar when the same method of protein concentration technique was used.
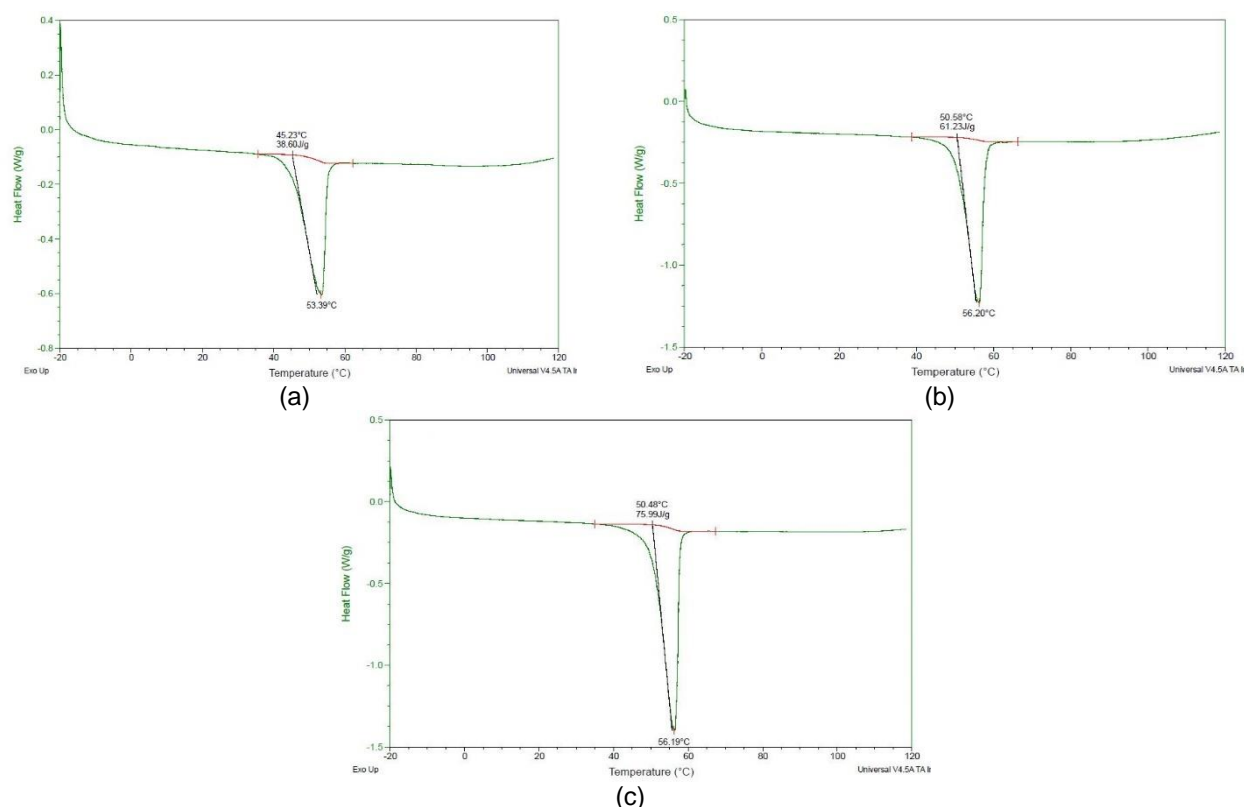
(a)

(b)

(c)

Figure 1. DSC thermograms of the protein concentrates (a) KPC (b) PPC (c) CPC

Table 2. Functional properties of the protein concentrates from the shells of kidney bean (KPC), pea (PPC), and cowpea (CPC).

Parameter	KPC	PPC	CPC
Solubility (%)	99.22±0.07[a]	99.13±0.19[a]	99.23±0,14[a]
WHC (g water/ g protein)	2.26±0.02[c]	1.08±0.01[a]	1.85±0,06[b]
OHC (g oil/ g protein)	3.60±0.02[a]	3.47±0.03[a]	3.37±0,28[a]
EC (%)	51.43±0.00[a]	54.28±0.00[b]	45.71±0,00[c]
ES (%)	45.71±0.00[a]	51.43±0.00[b]	45.71±0,00[c]
FC (%)	27.87±0.65[b]	47.63±0.55[c]	19.88±0,55[a]

Values are mean ± SD. Means with similar superscript in a column did not differ significantly (P>0.05). WHC, water holding capacity; OHC, oil holding capacity; EC, emulsion activity; ES, emulsion stability; FC, foaming capacity.

Oil holding capacity value of the KPC, PPC, and CPC were determined as 3.60 g/g, 3.47 g/g, and 3.37 g/g, respectively (Table 2). The KPC showed the highest oil holding capacity value among the samples evaluated. The oil holding capacity value was comparable to that reported for kidney bean protein concentrates which are obtained with the same method from kernels as 5.8 g/g [8]. OHC value of the protein concentrates from kernels is equal almost one and half times of protein concentrates obtained from shells. The OHC value of PPC is comparable to the result given as 1.2 g/g for pea protein concentrate from kernels by Fernández-Quintela et al. [33]. It shows that OHC value of the protein concentrate from shells is higher than from kernels. The result for CPC is also comparable to the results given as 1.95 g/g for cowpea protein concentrate from kernels by Mune and Sogi [34]. It also shows that OHC value of the protein concentrate from shells is higher than from kernels.

Emulsion capacity and stability value of KPC, PPC, and CPC were found as 51.43% and 45.71%, 54.28% and 51.43%, 45.71% and 45.71%, respectively (Table 2). PPC has the highest emulsion activity and stability value among the samples evaluated.

Foaming capacity values of KPC, PPC, and CPC were observed as 27.87%, 47.63%, and 19.88%, respectively (Table 2). PPC has the highest foaming capacity value among the samples evaluated. Evaluating the results obtained from KPC and PPC, these values can be compared with the results given by Shevkani et al. [8] where the foaming capacity value of the kernel of kidney bean and pea protein concentrates as 103% and 110%. These results show that with the same method of protein concentration technique while almost a quarter portion of the value can be obtained from the shells of kidney bean, a half portion of the value can be obtained from the shells of pea.

Effect of Concentrates on Freezing Behavior of Kiwi Puree

In this part, the effect of the protein concentrates on the freezing behavior of kiwi puree were determined. The test tubes were placed in the tube holder therefore the test tube has no contact with any surface in the freezer. The measurements were obtained from bottom of the test tube. The data hava been recorded as temperature versus time. In order to make a standard interpretation, the value of the temperature difference (ΔT) for the freezing period was decided as 0.6°C. That means, the freezing phase considered during the time where the temperature difference was below 0.6°C.

In the first part, temperature measurements were obtained at different amounts (1%, 2%, 4%, and 6%) for KPC. According to these measurements, the freezing duration for the kiwi puree with 1% proportion of KPC was found as 20 min, with 2% proportion of KPC was found as 15 min and with 4 and 6% proportions of KPC were found as 10 min. In the second part, temperature measurements were obtained at different amounts (1%, 2%, 4%, and 6%) for PPC. According to the results, the freezing duration for the kiwi puree with 1% proportion of PPC was observed as 10 min, with 2% proportion of PPC was observed as 5 min. However, the freezing duration for the kiwi puree with 4%, and 6% proportions of PPC were could not detected since the value of the temperature difference (ΔT) was above 0.6°C. It shows that the duration of freezing was assumed to be very rapid. In the third part, temperature measurements were obtained at different amounts (1%, 2%, 4%, and 6%) for CPC. The freezing duration for the kiwi puree with the 1, 2, 4, and 6% proportions of CPC were also could not detected since the value of temperature difference (ΔT) was above 0.6°. It also shows that the duration of freezing was assumed to be very rapid.

Effect of Concentrates on Freeze Drying of Kiwi Puree

The drying behaviour of the freeze drying process of kiwi puree was observed from the mass loss in samples. Mass loss was performed gravimetrically at 1 hour interval by using a scales with 0.01 precision. Samples were dried until reaching the constant weight and the total drying time was determined as 9 hours. According to the literature, similar results were given by Calıskan et al. [35] for freeze drying of kiwi puree as 9 hours. The moisture ratio (MR) were calculated of the samples. The freeze drying behaviour of plain kiwi puree is given in Figure 2 (a). It was taken as reference to examine the behavior of the freeze drying of added protein to kiwi puree.

In the first part of the experiments, the drying curves of the addition of KPC to the kiwi puree were obtained and are shown in Figure 2 (b). According to this figure, it can be stated that, the firstly, moisture ratio reduced and then became constant with time. The value of the moisture ratio for the kiwi puree with 2%, 4% and 6% proportions of KPC was found as lower compared to 1% proportion of KPC.

In the second part of the experiments, the drying curves of the addition of PPC to the kiwi puree were obtained and are shown in Figure 2 (c). According to this figure, it can be stated that, the firstly, moisture ratio reduced and then became constant with time. The value of the moisture ratio for the kiwi puree with 4% proportion of PPC was found as lower compared to the other proportions.

In the third part of the experiments, the drying curves of the addition of CPC to the kiwi puree were obtained and are shown in Figure 2 (d). According to this figure, firstly, moisture ratio reduced and then became constant with time. The value of the moisture ratio for the kiwi puree with 2% proportion of CPC was found as lower compared to the others.

Functional Properties of Powders of Kiwi Puree Samples

Fresh kiwi fruits have very short shelf-life because of their perishable feature. Hence, to extend their shelf life apply different drying process in the food industry such as freeze drying process. After freeze drying process, the product is form of powder and this form can be utilized as in pudding, instant tea, and dry mixture formulations. In this study, KPC, PPC, and CPC were added to the fresh kiwi puree and freeze dried. Then, functional properties (water and oil holding capacity, emulsion capacity and stability and foaming capacity) of the samples were examined and given in Table 3. WHC values of the kiwi purees increased as the protein proportion increased for KPC, PPC, and CPC. When the samples were evaluated among themselves, the water holding capacity values of the samples that was prepared at 6% proportion KPC, PPC and CPC were the highest according to other proportions. When the protein concentrates were evaluated, water holding capacity value of PPC was the highest, then KPC, and CPC, generally.

Evaluating the results of OHC values, the OHC values of the kiwi purees increased as the protein proportion increased for all protein concentrates. Comparing the results obtained, the oil holding capacity values of the samples that was prepared at 6% proportion KPC, PPC and CPC were the highest according to other proportions. According to the results, oil holding capacity value of CPC was the highest, then KPC, and PPC, generally.

Emulsion capacity and stability values of kiwi purees were observed. Comparing the results obtained, the emulsion capacity and stability values of the samples that was prepared at 6% proportion KPC, PPC and CPC were the highest according to the other proportions. According to the results, emulsion capacity and stability value of KPC was the highest, then CPC, and PPC, generally.

Foaming capacity values were not determined since the plain kiwi puree and samples that prepared with protein concentrates did not exhibit any foam features.
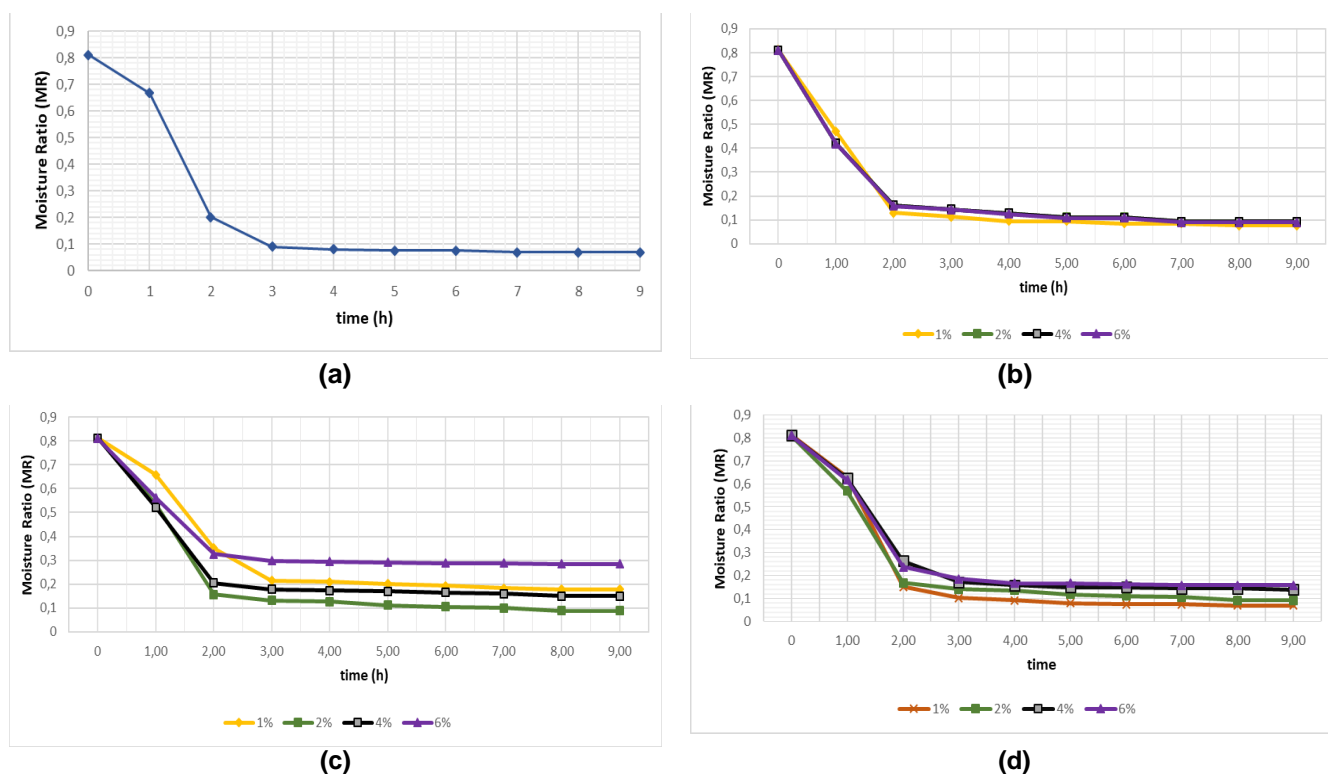
Figure 2. Variation of moisture ratio (MR) with time of freeze drying of the plain kiwi puree and addition of protein concentrates to the kiwi puree (a) kiwi puree, (b) KPC, (c) PPC and (d) CPC

Table 3. Functional properties of the kiwi puree with addition of KPC, PPC, and CPC at 1, 2, 4, 6% proportions

Kiwi puree		WHC (g/g)	OHC (g/g)	EC (%)	ES (%)
		2.19 ± 0.03^{ayn}	3.78 ± 0.06^{aym}	161.03 ± 0.87^{btn}	80.00 ± 0.00^{azn}
KPC	1%	2.24 ± 0.03^{a}	4.18 ± 0.05^{c}	133.41 ± 0.81^{a}	79.71 ± 0.72^{a}
	2%	2.55 ± 0.02^{b}	4.46 ± 0.21^{b}	200.93 ± 0.85^{c}	80.28 ± 0.62^{a}
	4%	2.60 ± 0.05^{a}	4.74 ± 0.03^{e}	200.88 ± 0.64^{c}	90.80 ± 0.77^{b}
	6%	$2.83\pm0,01^{b}$	5.87 ± 0.03^{d}	200.92 ± 0.53^{c}	90.83 ± 0.82^{b}
PPC	1%	1.94 ± 0.10^{z}	3.56 ± 0.03^{x}	111.37 ± 0.55^{x}	55.45 ± 0.75^{x}
	2%	2.62 ± 0.09^{z}	4.69 ± 0.08^{t}	114.60 ± 0.76^{y}	57.97 ± 0.75^{y}
	4%	2.72 ± 0.11^{t}	5.42 ± 0.05^{t}	133.92 ± 0.81^{z}	80.42 ± 0.89^{t}
	6%	2.98 ± 0.12^{gx}	5.48 ± 0.03^{z}	160.85 ± 0.86^{t}	83.90 ± 0.66^{z}
CPC	1%	1.58 ± 0.03^{l}	4.52 ± 0.11^{k}	114.28 ± 0.74^{m}	57.05 ± 0.85^{m}
	2%	2.32 ± 0.06^{l}	5.04 ± 0.15^{l}	160.87 ± 0.80^{n}	80.79 ± 0.72^{n}
	4%	2.63 ± 0.12^{m}	5.64 ± 0.13^{l}	160.65 ± 0.74^{n}	80.03 ± 0.80^{n}
	6%	2.70 ± 0.05^{k}	5.70 ± 0.24^{n}	200.92 ± 0.87^{k}	89.76 ± 0.63^{k}

Values are mean ± SD. Means with similar superscript in a column did not differ significantly (P>0.05). a-e Different letters in the same row indicate significant difference between averages of KPC at P<0.05. x-t Different letters in the same row indicate significant difference between averages of PPC at P<0.05. k-n Different letters in the same row indicate significant difference between averages of CPC at P<0.05.

CONCLUSION

In this study, protein concentrates from the shells of kidney bean, pea, and cowpea were obtained by using the method of isoelectric precipitation and freeze drying. The protein content values of the KPC, PPC, and CPC were found as 19.20% (db), 25.48% (db), and 41.22% (db), respectively. Depending on the results, CPC showed the highest protein content value and the lowest ash content value among the samples evaluated. When the dry matter values of the KPC, PPC, and CPC were examined, the values were observed as 94.02%,

94.69%, and 93.52%, respectively. On the comparison of the color values, KPC had the highest brightnes value and the lowest greenness value (44.25 and 0.36, respectively. While the brightness value of KPC and CPC increased and that of PPC decreased with the protein concentration process. The yellowness value for CPC was the highest value among the samples evaluated. The total color change values (ΔE) were computed as 24.74, 30.23, and 21.97 for KPC, PPC, and CPC, respectively. It means that the change on colour values for all of them can be observed easily. In the food industry, large amounts of wastes occur and

the shells of the selected legumes are one of these wastes. The wastes are usually sold as fertilizer or animal feed. Depending on all the given results, it can be stated clearly that, the high amounts of protein in the waste shells contributed in the scope of sustainability awareness. The freezing duration of the kiwi puree with 4% and 6% proportions of KPC were observed as 10 minutes which was the shortest. There was a reduction in the duration of freezing with the increased protein concentrate, therefore, the protein concentrates can be used to decrease the duration of the freezing phase of foods. The addition of protein to kiwi puree did not show a clear effect on freeze drying behavior. Considering the functional properties of the samples containing the protein concentrates, the samples where 6% proportion was added had the highest water holding capacity value

(2.98 g/g) for PPC, where 6% propotion was added had the highest oil holding capacity value (5.70 g/g) for CPC, and where 2% and 6% proportions were added had the highest emulsion capacity and stability value (200.93% and 90.83%, respectively) for KPC. For the future studies, different methods can be performed to increase the protein yield during the concentration process, such as ultrafiltration, microwave supported extraction, and combined systems and also different drying methods.

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