Effect of alkaline electrolyzed water on physicochemical and structural properties of apricot protein isolate

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Abstract In this current study, comparative study between the effect of electrolyzed water and ultrapure water on the extraction of apricot protein was conducted. The results revealed that under the condition of same pH (pH = 9.5), the extraction efficiency of electrolyzed water on apricot protein was superior to that of ultrapure water. Moreover, apricot protein (EAP) extracted by electrolyzed water displayed preferable foaming capacity and emulsion stability. The foaming capacity and emulsion stability of EAP were 11.17% and 36.33 min, for UAP, only 4.75% and 23.88 min, respectively. Meanwhile, compared to UAP, the secondary structure of EAP was more orderly, in which the orderly structures of α -helix and β -sheet were 7.5 and 60.2%, while the disorderly structures of β -turn and random coil were 8.4 and 23.8%. This work provided a novel extraction strategy, which could improve the extraction rate and minimize the destruction of the structure and functional properties of apricot protein.

Keywords Apricot · Protein · Extraction rate

Introduction

The protein content of apricot is about 22.4–29.3%, which has become an indispensable member among the four leading nuts (including walnut, apricot, chestnut and cashew nut) (Roncero et al., 2016). Because of its outstanding emulsifying property, foaming property, gelation property as well as superior physiological functions in reducing blood lipid, blood pressure, cholesterol and anti-tumor, etc., apricot has recently gained more popularity among people and will certainly have greater potential application prospects in the fields of food and medicine (Junming, 2010).

At present, alkali-solution and acid-isolation extraction is still the major extraction method of apricot protein. This method has been extensively applied to industry because of its simplicity and convenience. However, under high alkaline conditions, protein molecules were susceptible to degradation and even hydrolysis, the hydrogen bonding and van der Waals forces were also easy to be broken, even to lead to the deterioration of their functional properties. Moreover, traditional methods of extraction consumed higher dosage of acid and alkali, while electrolyzed water extraction could significantly reduce the lye dosage and was also eco-friendly (Jiang et al., 2011).

Electrolyzed water, also known as electrolytic ion water or oxidation potential water, was obtained by inserting electrodes to both ends of the pure water solution (Shaposhnik and Kesore, 1997). When aqueous solution was subjected to an external electric field, the charged substance will move to the opposite charged electrode. Since the H^+ and OH^- were generated during the electrolysis to change the pH value of the solution, the pH value changed depending on the time of electrolysis and electrolyte. Electrolyzed water was generally divided into strong

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electrolyzed water (including strongly acid electrolyzed water and strongly alkaline electrolyzed water) and weak electrolyzed water (including slightly acidic electrolyzed water and alkalescence electrolyzed water). Alkaline electrolyzed water contained higher negative potential and held stronger reduction ability (Tao et al., 2003). According to these distinguished features of alkaline electrolyzed water, it was in favor of entering the intercellular space, and possessed strong penetration and certain dissolution characteristics as well as the extraction function (Wang et al., 2012). Besides, alkaline electrolyzed water could be restored to ordinary water and therefore less environmental pollution would be caused (Pei, 2011). Toge and other researchers found that the utilization of alkaline electrolyzed water could effectively improve the extraction rate of marine fish oil (Toge and Miyashita, 2002). Compared to alkali isolation and acid-isolation, the sunflower seeds protein was extracted via alkaline electrolyzed water method, which helped to increase the extraction rate by 10.4% (Aider et al., 2012).

Here, we investigated and contrasted the different extraction effects on apricot protein processed by EAP and UAP. We mainly focused on the influence of these two methods on the extraction rate, structural and functional properties of apricot protein. The extraction method of EAP on apricot protein structure and function was further discussed. This work could provide technical support for the efficient green extraction and application of nuts protein, and promote the development and utilization of apricot and other nuts protein resources.

Materials and methods

Materials

Apricot was obtained from 10th company horticultural field of 9th Regiment, 1st division of Xinjiang Construction Corps (Xinjiang, China). Electrolyzed water (pH = 8) was purchased from Shenzhen Runzheng Electrolysis Technology Co., Ltd. (Shenzhen, China). Bovine serum albumin was obtained from America sigma Co., Ltd. Other reagents were all purchased from Sinopharm Chemical Reagent Co., Ltd.

Sample preparation

The shell of the apricot kernel was removed then grind to powder and pass through 80-mesh sieve. 5 g apricot kernel powder was degreased by petroleum ether (30–60 °C) at a feed ratio of 1:30 g/mL, after suction filtration, the residual petroleum ether was evaporated in fume hood (the degreasing process repeated once). The petroleum ether

was recovered at the same time. Then the degreased powder was added to electrolyzed water and ultrapure water, respectively, and well mixed according to the solid–liquid ratio of 1:30 g/mL. The pH value of the mixed liquor was adjusted to 8.0, 8.5, 9.0, 9.5 and 10.0, respectively, using 1.0 mol/L NaOH, and then magnetically stirring in 35 °C water bath for 3 h. Subsequently, the extracted liquor was centrifuged at 8000 g force for 15 min (Multifuge X1R, Thermo, America), then the supernatant was taken and the residue was washed twice with the electrolyzed water and centrifuged for another 15 min. The protein content of the above supernatant was measured via modified coomassie brilliant blue. Took bovine serum albumin as the standard and drew a standard curve, then established the regression equation as the following:

$$Y = 0.4486X + 0.0199 \quad (R^2 = 0.9984)$$

where X represented protein concentration(mg/mL), Y represented absorbance.

$$\text{Extraction rate} = (\text{content of apricot protein isolate} / \text{content of protein isolate in degreased apricot powder}) \times 100\%$$

Experimental samples were prepared at the optimal pH (pH = 9.5). The pH value of the protein solution was adjusted to 7.0 and dialysis (MW Cut-off: 8000–14,000 Da) 3 days with water changed every 2 h. Apricot protein powder obtained by freeze drying was stored for use in refrigerator at 4 °C.

Analysis of basic components in apricots

Moisture, ash, protein, fat and carbohydrate contents were measured according to AOAC official methods (AOAC, 2005). Protein content was determined by the Kjeldahl's method using a conversion factor ($N\% \times 6.25$) for all samples.

Total carbohydrate content was calculated as by difference (100% minus percent of total fat, protein, moisture and ash values).

Effect of extraction methods on apricot protein components

The amino acid composition was determined by automatic amino acid analyzer (L-8900, Hitachi, Japan) with reference to AOAC960.47 (AOCO, 1960). The analysis conditions were as follows: (1) the analysis period was 53 min; (2) the separation column: the eluent flow rate was 0.4 mL/min and the column temperature was 70 °C; (3) the reaction column: the flow rate of ninhydrin and ninhydrin

buffer solution was 0.35 mL/min and the column temperature was 135 °C.

SDS-PAGE analysis was carried out by SDS gel electrophoresis analysis electrophoresis (DYY-8C, Liuyi, Beijing, China). The concentration of the concentrated gel was 5% and the concentration of the separated gel was 12%.

Effect of extraction methods on apricot protein functional properties

Soluble properties analysis

The solubility was determined by the methods of Bera, Mukherjee and Guo (Bera and Mukherjee, 1989; Guo et al., 2005). Protein samples and 0.05 mol/L sodium phosphate (pH7.0) buffer were mixed to form the sample solution (1%). The protein solution was stirred for 1 h at room temperature and centrifuged at 8000 g force for 20 min. The content of protein in supernatant was determined by Bradford method (Bradford, 1976).

Investigation of emulsifying properties

The emulsifying activity (EA) and emulsifying stability (ES) was investigated based on Pearce and Kinsella's method (Pearce and Kinsella, 1978). 5 mL soybean oil and 15 mL of 0.1% (w/v) protein solution were homogenized in a mechanical homogenizer (IKA, Jintan Fuhua Instruments Co., Ltd.) at 8000 g force for 1 min to produce the emulsion. The 50 µL portions of emulsion were pipetted from the bottom of the container at 0 and 10 min after homogenizing and mixed with 5 mL of 0.1% SDS. The absorbance of the emulsion was measured with a UV spectrophotometer at 500 nm to obtain EA and ES. EA and ES were calculated as follows:

$$EA = (2 \times 2.303 \times A_0 \times DF) / [\rho \times \Phi \times (1 - \theta) \times 10,000]$$

$$ES = A_0 \times (A_0 - A_{10}) \times t$$

where DF is dilution factor (DF = 101), ρ is protein concentration (g/mL), Φ is optical path ($\Phi = 0.01$), θ is the fraction of the oil phase ($\theta = 0.25$ m), A_0 is the absorbance of the sample at 0 min, A_{10} is the absorbance of the sample at 10 min.

Foaming capacity and stability

The foaming capacity (FC) and stability (FS) were determined according to the method of Bandyopadhyay et al. (2008) with slight modification.

80 mL of 1% (w/v) protein solution (pH = 7.0) was dispersed with a mechanical homogenizer at 8000 g for 1 min. FC was calculated from the following equation:

$$FC = (V_0 - 80) \div 80 \times 100\%$$

where V_0 is foam volume (mL). FS was expressed by the ratio of the final foam volume at 30 min and the foam volume after whipping at room temperature.

Effect of extraction methods on physicochemical properties of apricot protein

Particle size distribution

The protein suspensions (1% w/w) were prepared in the same way as for the solubility analysis, the suspensions were filtered with a 0.45 µm diameter filter. The particle size of the filtrates was quantified with a Malvern Zeta sizer (Nano-ZS, Malvern, Britain), according to the method of Zhou et al. (2015). The z-averaged hydrodynamic diameter (z-average) in nm was recorded. Data were collected at 20 °C using a material refractive index of 1.45, a dispersant refractive index of 1.330 and a measurement angle of 173° (backscatter). For each sample, three measurements were performed. Measurements were performed in duplicate for isolates obtained in duplicate.

Surface hydrophobicity

Surface hydrophobicity was determined by 8-anilino-1-naphthalenesulfonic acid (ANS) probe via fluorescence spectroscopy (Nakai et al., 1980). Protein solution was diluted to 0.00125, 0.0025, 0.005, 0.01, 0.02 mg/mL, respectively, by 0.01 mol/mL PBS. Then 20 µL of the ANS solution (8 mmol/L was dissolved in 0.01 mol/L PBS) was added to 4 mL diluted protein solution and shook well before use. Fluorescence intensity was recorded at excitation and emission wave lengths of 390 and 470 nm. Both Excitation and emission wavelength held the slit widths of 2.5 nm. The surface hydrophobicity was identified by the initial ramp rate between the fluorescence intensity and protein concentration curve.

Sulphydryl and disulfide Bond

According to Beveridge, Toma and Nakai's method (Beveridge et al., 1974), 0.5 mL sample solution (1%, pH = 7.0) was mixed with the solution containing 2.5 mL of 8 mol/L urea Tris-Gly buffer (pH = 8.0) and 0.02 mL of 4 mg/mL DTNB, reacting for 30 min at 25 °C. Then the molar concentration of free sulphydryl groups was quantified by a UV spectrophotometer at 412 nm.

Effect of extraction methods on structural of apricot protein

Fourier transform infrared spectrometer analysis (FT-IR)

The dried sample was mixed with KBr by the ratio of 1:100 to 1:200 in the polishing grinding to a particle size of 2–3 μm , and pressed into a tablet. FT-IR spectrum analyses were carried out via ATAVAR FT-IR spectrometer (Nexus470, Mettler, USA). Absorbance intensity was obtained in the wave number range of 4000–400 cm^{-1} . A total of 128 scans was measured and averaged. The KBr spectrum was taken as background. After background correction, all spectra were baseline corrected, and then spectra were obtained.

Circular dichroism analysis (CD)

CD measurement was performed by a CD spectrometer (J-1500, JASCO, Japan) with 0.1 cm quartz cell in the wavelength range of 190–250 nm. Data were generated on such condition of the resolution of 0.1 nm, the response time of 1 s, and the mean of 3 scans.

Scanning electron microscopy (SEM)

Micrographs were recorded by a scanning electron microscope (S-3400 N, Hitachi, Tokyo, Japan). The sample was deposited on a double-sided conductive adhesive and covered with gold of 10 nm. Scanning electron microscopy was performed in a 15.0 kV secondary electron mode.

Data statistics and analysis

Data were presented as the mean $\pm$ SD (three replicates). Difference between means was compared by Tukey's test ($p < 0.05$). Statistical analysis was performed based on SPSS (Version 19.0). The difference between the means with the same superscript letter in the same row was significant at 95% confidence interval. “*”, “**” and “***” Correlation are significant at the 5%, 1% and 1% probability level, respectively.

Results and discussion

Analysis of nutritional components of apricot

According to AOAC official methods, it manifested that the content of total sugar was about $19.24\% \pm 0.23\%$, the content of crude protein reached to a higher level of $23.2\% \pm 0.021\%$, the content of moisture was about $5.16\% \pm 0.02\%$, the content of ash was about

$2.19\% \pm 0.02\%$ and the content of crude fat even reached to a surprising level of $49.93\% \pm 1.8\%$. Such data indicated that apricot protein was an important plant protein resource and oil crop.

Effect of extraction methods on extraction rate of apricot protein

The extraction rate of apricot protein under different pH value was shown in Fig. 1. Compared to ultrapure water, electrolyzed water was easier to penetrate into the inter-cellular space and had a higher infiltration capacity, because it contained higher negative potential and held stronger reduction ability. When pH was set at 8.0 or 8.5, the extraction rate of electrolyzed water was significantly higher than that of ultrapure water ($p < 0.05$). With the increase of pH value (more than 8.5), there was no significant difference in the extraction rate of protein between the electrolyzed water and the ultrapure water. Perhaps the reason for such phenomenon was that the addition of OH[−] facilitates the breaking of the hydrogen bond during the adjustment of the pH. Hydrogen ions, fragmented from carbon atoms or sulfate radicals which could increase the surface charge of protein, promoted electrostatic repulsion between each other and hydration between proteins, then the dissolution rate could be eventually increased. Because of the drastic changes in acid–alkali environmental conditions, the effect of electrolyzed water on the extraction of apricot protein from ultrapure water was significantly weakened.

Furthermore, under higher pH value condition, polyphenols in apricot were easily oxidized to quinones, whereas Michael addition reaction easily occurred between quinones and sulfhydryl or amino groups (Venkatachalam et al., 2008), resulting in significant browning

Fig. 1 Effect of extraction methods on the extraction rate of apricot protein

phenomenon, especially, when $\text{pH} > 10$, the color of the extract was significantly brown (Li et al., 2016). Therefore, in order to avoid serious browning reaction, the pH value of this experiment was set to 9.5.

Effect of extraction methods on the components of apricot protein

According to Fig. 2, the molecular weight of almond protein ranged from 20 to 66 kDa, mainly concentrating between 36 to 50 kDa and 20 to 25 kDa. There wasn't distinct difference in molecular weight between EAP and UAP.

From Table 1, it indicated that the purity of EAP was higher than that of UAP, in which the contents of Cys, Val, Met, Tyr, His and Pro in EAP were obviously greater than that in UAP. The key reason was that Cys was one of the most sensitive amino acids for oxidative modification and the disulfide bond was formed among the Cys protein polypeptide chains (Park and Raines, 2001). From the data concerning the disulfide bond in “Effect of extraction methods on physical and chemical properties of apricot protein” section, it could be seen that the content of disulfide bond in EAP was higher than that in UAP, which also proved the higher content of Cys in EAP than in UAP on a reverse side.

Effect of extraction methods on functional properties of apricot protein

Emulsifying effect was mainly dependent on lowering tension at the oil–water interfaces and controlling diffusion and aggregation of oil droplets by forming adsorption

Fig. 2 Analysis of gel electrophoresis between EAP and UAP; Lanes: 1 molecular weight marker (MWM), 2 and 3 demonstrated the apricot protein molecular weight distribution processed by electrolyzed water. 4 and 5 demonstrated the apricot protein molecular weight distribution processed by ultrapure water

Table 1 Amino acid composition of EAP and UAP

Amino acid	Sample	
	UAP	EAP
Asp	6.78 ± 0.06	6.78 ± 0.05
Thr	1.64 ± 0.02	1.71 ± 0.03
Ser	2.55 ± 0.03	2.48 ± 0.04
Glu	14.78 ± 0.12	14.71 ± 0.13
Gly	2.71 ± 0.02	2.75 ± 0.01
Ala	2.69 ± 0.02	2.69 ± 0.01
Cys	1.61 ± 0.03**	2.23 ± 0.04**
Val	3.17 ± 0.03**	3.52 ± 0.02**
Met	0.77 ± 0.04**	1.00 ± 0.05**
Ile	2.27 ± 0.02	2.41 ± 0.02
Leu	3.97 ± 0.01	3.95 ± 0.05
Tyr	2.76 ± 0.09*	3.29 ± 0.18*
Phe	3.61 ± 0.25	3.89 ± 0.41
Lys	1.76 ± 0.38	1.84 ± 0.23
His	1.68 ± 0.05	1.87 ± 0.09
Arg	6.19 ± 0.05	6.11 ± 0.03
Pro	28.9 ± 0.30**	30.07 ± 0.32**
Content (%)	87.5 ± 0.58	91.31 ± 1.36

“**” “***” and “****” Correlation are significant at the 5%, 1% and 1% probability level, respectively

layer. The EA and ES were shown in Fig. 3A. The EA properties of EAP was slightly lower than that of UAP, while its ES was significantly higher than that of UAP, the EA values were 2904.85 and 3328.60 m²/g, respectively, while the time for ES was 36.33 and 23.88 min, respectively. This might be because of the action of electrolytes in electrolyzed water, the apricot protein polypeptide chain broke down during the extraction process, making it easy to diffuse, thereby increasing its solubility and enhancing the protein-lipid interaction (Bandyopadhyay et al., 2008).

These protein indicators of solubility, EA and FC were generally taken to evaluate the protein functional properties. The protein solubility, to a large extent, could characterize the hydration of protein and also simply reflect the degree of aggregation and denaturation of apricot protein. As shown in Fig. 3B, EAP and UAP had a solubility of 10.82 and 12.55 mg/mL, respectively. This phenomenon might be caused by the addition of lye in the extraction process, which helped to extend the tertiary structure of the protein. Protein molecule structure was easily to become loose and unfold in high alkaline solution, thus improving the solubility (Jiang et al., 2009).

The protein foaming property included FC and FS, the former being the amount of foam produced under certain conditions and the latter being the stability of the resulting foam. As shown in Fig. 3C, the FC of EAP was 11.17% and the FS was 44.26%. The FC and FS of UAP was 4.75

Fig. 3 (A) demonstrated the emulsifying activity and emulsifying stability of EAP and UAP. (B) Demonstrated the solubility of EAP and UAP. (C) Demonstrated the foaming and foaming stability of EAP and UAP. (D) Demonstrated the surface hydrophobicity of EAP

and UAP. (E) Demonstrated the content of sulfhydryl and disulfide bonds of EAP and UAP. (F) Demonstrated the particle size of EAP and UAP

and 54.16%, respectively. Such result was probably caused by the disruption of disulfide bonds in UAP, which led to the decrease in the cohesion and elasticity among protein molecules, thereby reducing the foaming of the protein (Panyam and Kilara, 1996, Tang et al., 2003).

Effect of extraction methods on physical and chemical properties of apricot protein

The surface hydrophobicity (H_o) of protein played an important role in evaluating the physical and chemical properties and conformational changes of protein. It not only expressed the ability of protein molecules to interact with each other, but also performed an important index reflecting hydrophobic amino acids on protein surface and

was closely related to the functional properties of protein (Khalid et al., 2003). Besides, the H_o of protein was also a vital indicator of the number of hydrophobic groups attached to the external polar environment, further affecting the functional properties of protein through its effect on intermolecular interactions. As shown in Fig. 3D, the H_o of UAP was higher than that of EAP, and it was probably the alkali liquor that caused the degradation of the peptide chains in protein molecules during the extraction process. More carboxyl and amino groups were exposed, which caused the increase of the hydrophobicity of protein and the decrease of its hydrophobicity at the same time.

Disulfide bond was the covalent bond formed between the two Cys of the polypeptide chain and was an important indicator which evaluated the spatial structure and

functional properties of protein. Figure 3E revealed that the content of free thiol in EAP was significantly higher than that in UAP and the content of disulfide bond in EAP was slightly lower than that in UAP. Specifically, the content of free thiol in EAP and UAP was 5.65 and 4.87 $\mu\text{mol/g}$, respectively, and the content of disulfide bond was 2.19 and 2.31 $\mu\text{mol/g}$, respectively.

The Zetasizer was used to measure the soluble part of the sample. The results reflected the degree of aggregation of apricot protein to some extent. Figure 3F showed that the distribution of particle size in EAP ranged from 13.5 to 18.17 nm and from 141.8 to 190.1 nm. The distribution of particle size in UAP ranged from 21.4 to 37.8 nm and from 190.1 to 342.0 nm. Probably, the alkaline solution was added to the ultrapure water during the extraction process caused the degradation of the peptide chain of apricot protein, leading to the expansion of molecular structure of apricot protein. Then it strengthened the interaction between the exposed hydrophobic groups. Besides, since the content of hydrophobicity in UAP was higher than that in EAP, soluble aggregates were more easily formed in UAP, resulting in the increase of average particle size.

Effect of extraction methods on apricot protein structure

FT-IR and CD were effective techniques widely used in structural analysis of food protein. By FT-IR and CD spectra, the proportion of α -helix, β -sheet, β -turn and random coil in protein secondary structure could be accurately analyzed (Mine, 1997; Shen et al., 2006). As could be seen from Fig. 4A, a strong absorption peak appeared at 1600–1700 cm^{-1} in the amide I band. The secondary structure of apricot protein was calculated and displayed in Fig. 4A by fitting the peak area of the Gaussian curve via

pick fit 4.0 software. It manifested that compared to secondary structures of UAP, the α -helix structure in EAP slightly decreased, the content of β -sheet structure increased, and the α -helix and β -sheet structure were more orderly, β -turn and random coil structure were more disorderly. Such similar phenomenon was also seen in Fig. 4B: the proportion of α -helix in EAP was less than that in UAP, and the sum of the order of α -sheet and β -sheet in EAP was larger than that in UAP. The reduction of helical content might be due to the fact that alkaline electrolyzed water transformed the apricot protein from a tightly ordered structure into a loosely disordered structure, allowing the protein molecules to stretch to some extent. Such experimental result was generally consistent with the findings of Shen et al. (2006).

Scanning electron microscope

From Fig. 5A and B, it could be seen that the structure of EAP was uniform and the state was more dispersed under the 100 times, while the structure of UAP was aggregated into clusters with uneven dispersion. Figure 5C and D clearly showed the phenomenon: under 2000 times condition, EAP powder particles were smaller with more uniform size, while UAP powder particles were larger with different sizes and shapes. The SEM results further demonstrated the effect of alkaline electrolyzed water on the structural and functional properties of apricot protein.

In this work, we applied electrolyzed water to extract apricot protein, comparatively studied the differences between EAP and UAP in the following aspects such as the extraction rate, functional properties, physicochemical and structural properties. Results illustrated that under the conditions of pH value ranging from 8.5 to 10, the extraction rate of apricot protein extracted by electrolyzed

Fig. 4 Effect of extraction methods on apricot protein structure. (A) Demonstrated the Fourier transform infrared spectroscopy of EAP and UAP and the secondary structure tables of EAP and UAP.

(B) demonstrated the CD of EAP and UAP and also the secondary structure tables of EAP and UAP

Fig. 5 SEM images of EAP and UAP, (A) demonstrated the 100 times electron microscope of EAP. (B) Demonstrated the 100 times electron microscope of UAP. (C) Demonstrated the 2000 times

electron microscope of EAP. (D) Demonstrated the 2000 times electron microscope of UAP

water was 1.7–8.3% higher than that of ultrapure water. Meanwhile, the amino acids content in EAP was higher, the emulsifying stability was preferable and the foaming property was more excellent, which was 2.5 times that of UAP. The experimental results also manifested that the free thiol content in EAP was significantly higher than that in UAP, while the disulfide bond revealed opposite trend. Moreover, the surface hydrophobicity of EAP was higher than that of UAP, and the distribution of particle size in EAP was smaller than that of UAP. Besides, the ordered structure in EAP such as α -helix and β -sheet was more than that in UAP, the structure of EAP electron microscope was more orderly than that of UAP, and the appearance and shape of EAP were more evenly dispersed than that of UAP. Therefore, in future research and production, the use of electrolysis method would be directly applied to adjust the pH value of the extraction solvent to further reduce the harm on the environment. It will certainly play a prominent role in the promotion of industrial production.

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References

- Aider M, Gnatko E, Benali M, Plutakhin G, Kastyuchik A. Electro-activated aqueous solutions: Theory and application in the food industry and biotechnology. *Innov. Food Sci. Emerg.* 15:38–49 (2012)
- AOAC. Official Methods of Analysis. 15th ed. Analysis of Association of Official Analytical Chemists. Arlington, VA, USA (2005)
- AOAC. Official Methods of Analysis. 16th ed. Method 960.47. Amino Acids in Vitamin Preparations Microbiological Methods. Arlington, VA, USA (1960)
- Bandyopadhyay K, Misra G, Ghosh S. Preparation and characterisation of protein hydrolysates from Indian defatted rice bran meal. *J. Oleo Sci.* 57: 47–52 (2008)
- Bera MB, Mukherjee RK. Solubility, emulsifying, and foaming properties of rice bran protein concentrates. *J. Food Sci.* 54: 142–145 (1989)

- Beveridge T, Toma SJ, Nakai S. Determination of SH and S-S groups in some food proteins using Ellman's reagent. *J. Food Sci.* 39: 49–51 (1974)
- Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal. Biochem.* 72: 248–254 (1976)
- Guo RR, Pan SY, Wang KX. Comparing research on functionality of rice protein extracted by alkali and enzyme. *Food Sci. Technol.* 26: 173–174 (2005)
- Jiang J, Chen J, Xiong YL. Structural and emulsifying properties of soy protein isolate subjected to acid and alkaline pH-shifting processes. *J. Agric. Food Chem.* 57: 7576–7583 (2009)
- Jiang L, Lin T, Zhao XH, Gao MX. Extraction of protein from rice dreg using purifying and enzyme treating. *J. Food Sci.* 36: 179–186 (2011)
- Junming LWJX. Research advance in methods of extraction and technologies of separation and purification for protein. *Adv. Fine Petrochem.* 11: 52–58 (2010)
- Khalid E, Babiker E, Tinay AE. Solubility and functional properties of sesame seed proteins as influenced by pH and/or salt concentration. *Food Chem.* 82: 361–366 (2003)
- Li Y, Jongberg S, Andersen ML, Davies MJ, Lund MN. Quinone-induced protein modifications: kinetic preference for reaction of 1,2-benzoquinones with thiol groups in proteins. *Free Radic. Biol. Med.* 97: 148–157 (2016)
- Mine Y. Effect of dry heat and mild alkaline treatment on functional properties of egg white proteins. *J. Agric. Food Chem.* 45: 2924–2928 (1997)
- Nakai S, Ho L, Helbig N, Kato A, Tung MA. Relationship between hydrophobicity and emulsifying properties of some plant proteins. *Can. Inst. Food Sci. Technol. J.* 13: 23–27 (1980)
- Panyam D, Kilara A. Enhancing the functionality of food proteins by enzymatic modification. *Trends Food Sci. Technol.* 7: 120–125 (1996)
- Park C, Raines RT. Adjacent cysteine residues as a redox switch. *Protein Eng.* 14: 939–942 (2001)
- Pearce KN, Kinsella JE. Emulsifying properties of proteins: evaluation of a turbidimetric technique. *J. Agric. Food Chem.* 26: 716–723 (1978)
- Pei H. Strong electrolytic water and its application in food industry. *J. Chin. Inst. Food Sci. Technol.* 9: 268–271 (2011)
- Roncero J, Álvarez-Ortí M, Pardo-Giménez A, Gómez R, Rabadán A, Pardo J. Almond virgin oil: parameters of regulated physicochemical quality and stability. *Riv. Ital. Sostanze Grasse.* 93: 237–243 (2016)
- Shaposhnik VA, Kesore K. An early history of electrodialysis with permselective membranes. *J. Membr. Sci.* 136: 35–39 (1997)
- Shen Q, Huang B, Shao J, Peng Q, Ma L, Gu L. Mechanism discussion of interaction between enzyme and several compounds with circular dichroism method. *J. Sun Yat-sen Univ.* 45: 62–64 (2006)
- Tang S, Hettiarachchy N, Horax R, Eswaranandam S. Physicochemical properties and functionality of rice bran protein hydrolyzate prepared from heat-stabilized defatted rice bran with the aid of enzymes. *J. Food Sci.* 68: 152–157 (2003)
- Tao GS, Hu ZY, Han ZT, Zhang JL, Zhang HH. An experimental research into the effect of alkaline electrolyzed water on blood lipid. *Chin. J. Geriatr. Care* 1: 18–20 (2003)
- Toge Y, Miyashita K. Lipid extraction with electrolyzed cathode water from marine products. *J. Oleo Sci.* 52: 1–6 (2002)
- Venkatachalam M, Roux KH, Sathe SK. Biochemical characterization of soluble proteins in pecan [*Carya illinoensis* (Wangenh.) K. Koch]. *J. Agric. Food hem.* 56: 8103–8110 (2008)
- Wang XJ, Feng H, Li ZY. Effects of electrolyzed water pretreatment on hydrolysis of corn stove and switchgrass. *J Chem Eng Chin Univ.* 26: 174–179 (2012)
- Zhou C, Qi W, Neil LE, Carpenter JF. Concomitant Raman spectroscopy and dynamic light scattering for characterization of therapeutic proteins at high concentrations. *Anal. Biochem.* 472: 7–20 (2015)