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Effects of Enzyme Treatments and Ultrasonification on Extraction Yields of Lipids and Protein from Soybean by Aqueous Process

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Abstract

Lipids and protein were extracted simultaneously from soybean flour by aqeous processing. Extraction yields of lipids and protein were 62 and 68%, respectively, when 120-150 mesh full-fat soybean flour was dispersed in six times of water (w/w) at 40℃ and pH 8. Supplementary treatment for the higher extraction yields such as proteolytic enzymes treatment improved extraction yields of lipids and protein up to 86 and 89%, respectively. Ultrasonification also improved extraction yields of lipids and protein up to 90%. Red and yellow colors of aqeous-extracted soybean oil were slightly darker than those of hexane-extracted oil, but were much lighter in colors than those of Folch-extracted oil.
Key words: aqeous process, soybean, lipids, protein, protease, ultrasonification, extraction yield.

Introduction

During the last three decades, the aqueous processing of oleaginous materials has emerged as a significant alternative to conventional oil extraction process such as pressing and organic solvent extraction[1]. Conventional oil extraction of oilseed generally requires a huge amount of thermal energy to heat oilseeds for improving extractability and to strip extraction solvent from miscella[1]. In contrast, it is well known that the drastic thermal treatment of oilseeds deteriorates quality of extracted oil and proteinous materials[1].

It has been demonstrated that oil and protein can be extracted and separated simultaneously from oilseeds by dispersing oilseed flour in water followed by agitation and centrifugal separation[2]. At the centrifugal separation step, oilseed dispersion was separated into three portions, i.e., fibrous solid as precipitate, protein solution layer, and emulsion layer which contained oil[2]. The extraction process using water as an extraction solvent, so-called aqueous extraction, has successfully been applied to soybean[3-5], sunflower[6], peanut[7,8], palm kernel[9] and coconut[10,11] for simultaneous recovery of oil and/or protein.

The aqueous extraction of oilseed has several advantage such as less energy, less toxicity and less solvent explosion possibility than conventional hexane extraction process[11].

However, the aqeous extraction process is reported to show lower extraction yield than conventional solvent extraction method[11]. To improve the yield of aqueous extraction, several supplementary treatments such as enzymatic hydrolysis and ultrasonification were examined[10,12,13]. Extraction yield of coconut oil increased substantially by combined treatment of polygalacturonase, α-amylase and protease in aqueous system[10]. Oil recovery from avocado seed was improved by pectinase, α-amylase, cellulase and protease[12]. Recently it is reported that ultrasonification resulted higher protein recovery from defatted soybean meal than that from simple shaking method[13].

Soybean oil, one of the most popular vegetable oils used worldwide, has been traditionally extracted by hexane followed by refining process to obtain refined, bleached and deodorized (RBD) soybean oil[1]. It has already found that aqueous extraction method is applicable to soybean[3,4]. However, the utilization of supplementary treatments to improve extraction yield of oil and protein from soybean in aqueous extraction system has not yet been reported, and they were generally confined to the extraction of oil with limited studies on the extraction of protein.

The objectives of the paper are to optimize the extraction conditions of aqueous extraction of oil and

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protein from soybean, and to investigate the effects of enzyme treatments and ultrasonification on extraction yield of oil and protein from soybean.

Materials and Methods

Materials

Full-fat soybean flour was obtained from a local market. Alcalase (0.6 Anson unit/g, alkaline protease of *Bacillus lichenthinous*) and Neutrase (0.5 Anson unit/g, neutral protease of *Bacillus subtilis*) were purchased from Novo Company (Denmark). Protease from *Aspergillus oryzae* (3.7 unit/mg) and papain (2.9 unit/mg) were obtained from Sigma (U.S.A.). All reagents used were of analytical grade unless otherwise specified.

Extraction of oil and protein from soybean flour

Soybean flour was dispersed in distilled water at the desired ratios with continuous stirring to prevent agglomeration, and pH and temperature were adjusted as desired[5]. After one hour vigorous stirring by a Waring blender, the dispersion was separated into three layers (solid, aqueous and emulsion layers) by a laboratory bench-top batch centrifuge at 10,000 g for 15 min. Solid fraction was dried in a vacuum oven, and aqueous and emulsion fractions were lyophilzed for further analyses. To test the effects of enzymes, soybean flour was dispersed in distilled water (soybean flour : water, 1 : 6, w/w) at 40℃, and then certain amount of enzyme was added[10,12]. Solution pH was adjusted to 8.0 with 1 N-NaOH solution. After one hour stirring at 40℃, the solution was applied to centrifuge for separation in the same manner as mentioned as above. For the sonification study, 50 m*l* of soybean flour dispersion was sonicated with a ultrasonicator (Flsher, Model 300) at the relative output of 0.7 for 5, 10 and 15 minutes[13]. Temperature and pH of dispersion were 40℃ and 8.0, respectively. Sonicated soybean dispersion was then stirred vigorously for one hour followed by centrifugal separation and drying as mentioned as above.

Analytical methods

Contents of moisture, crude fat and crude protein in soybean flour and solution were determined according to AOAC method[14]. Oil recovery was calculated as the difference of crude fat content of soybean flour and solid precipitate from centrifugal separation[3,4] and/or gravimetric determination of separated oil. Protein recovery was calculated from the crude protein content in the water layer. Extraction yield of oil and protein were calculated as percentages of recovered oil and protein against crude fat and protein contents of original soybean flour, respectively. Color of oil was measured by a Lovibond Tintometer using 1 inch light path sample cell.

Results and Discussion

Effect of extraction conditions on extraction yields of oil and protein

Effects of extraction process parameters such as pH, flour-to-water ratio (dilution ratio), temperature and flour particle size, on extraction yields of oil and protein are shown in Table 1. Commercial full-fat soybean flour contained 4% moisture, 25% crude fat, and 38% crude protein. Soybean oil and protein were extracted more in alkaline pH than in acidic pH, and the maximum oil and protein were recovered at pH 12 among pHs tested. pH higher than 7 caused more solubilization of oil in water through saponification of oil, and this was also found in the aqueous extraction of peanut[7]. It is well known that soybean protein is solubilized more in alkaline pH than in acidic pH[3,4]. However, further experiments were carried out at pH 8 to avoid oil loss due to excess saponification.

Table 1. Effects of processing parameters on oil and protein yields in aqueous extraction of soybean[a]

	pH								
	4	5	6	7	8	9	10	11	12
Oil yield (%)	1	1	2	23	62	63	63	68	89
Protein yield (%)	1	2	1	66	68	69	68	82	97

	Dilution ratio (water : soybean)				
	4	6	8	10	12
Oil yield (%)	50	62	55	49	48
Protein yield (%)	62	68	67	66	67

	Extraction temperature (℃)			
	20	40	60	80
Oil yield (%)	61	62	62	60
Protein yield (%)	62	68	68	65

	Particle size (mesh)			
	>32	32-48	48-60	60-150
Oil yield (%)	2	15	63	62
Protein yield (%)	10	58	58	68

[a]Extraction was conducted at pH 8.0, dilution ratio 1 : 6, temperature 40℃, particle size 120-150 mesh. All values are the means of triplicates

Table 2. Effects of proteolytic enzymes treatment on oil and protein yields in aqueous extraction of soybean[a]

	Alcalase concentration (%, w/w)				
	0	0.05	0.1	0.15	0.2
Oil yield (%)	62	69	85	85	84
Protein yield (%)	68	75	84	85	84
	Protease concentration (%, w/w)				
	0	0.05	0.1	0.15	0.2
Oil yield (%)	62	80	81	86	86
Protein yield (%)	68	83	86	89	89

[a]Extraction was conducted at pH 8.0, dilution ratio 1 : 6, temperature 40℃, particle size 120-150 mesh with proteolytic enzymes treatments. All values are the means of triplicates

Extraction yield of oil increased gradually as dilution ratio increased up to 1 : 6, and decreased thereafter (Table 1). Extraction yield of protein, however, was not affected by the dilution ratio. Dilution ratio of one part of soybean flour to six part of water was selected for further experiments.

The change in extraction temperature did not affect the oil and protein extraction yields between 20 and 80℃. Extraction temperature was maintained at 40℃ afterwards for the prevention of possible oil oxidation at higher temperature and for easy temperature control.

The smaller the particle size of soybean flour was, the more oil and protein were extracted (Table 1). Extraction yields of oil and protein, however, were not improved with smaller particle size than 60 mesh. Since particle size of commercial soybean flour ranged from 120 to 150 mesh, commercial soybean flour was used directly for the experiments.

Extraction yields of oil and protein were 62 and 68%, respectively, under the conditions of pH 8, dilution ratio of six and 40℃ when soybean flour of 120-150 mesh size was used.

Effects of proteolytic enzymes on extraction yields of oil and protein

To improve the extraction yields of oil and protein from soybean flour, four kinds of proteolytic enzymes such as Alcalase, protease, Neutrase and papain were tested. Based upon preliminary tests, Neutrase and papain were omitted from further experiments since they did not improve extraction yields. Effects of amount of Alcalase and protease added to soybean flour dispersion on extraction yields of oil and protein are shown in Table 2. Extraction yields of oil and protein increased linearly as amount of Alcalase increased up to 0.1% (w/w). However notable effects was not found when Alcalse was added more than 0.1%. Extraction yields of oil and protein were 85 and 84%, respectively, when 0.1% of Alcalase was added. Extraction yields of oil and protein were 86 and 89%, respectively, when 0.15 (w/w) of protease was added (Table 2). It was found that protease possessed slightly better effect than Alcalase of *B. lichenthionous* in extraction of protein. It is reported that uses of proteolytic enzymes in aqueous extraction of oil and protein improved extraction yields of oil and protein through hydrolysis of structural fibrous protein in which fat globules are embedded[12].

Table 3. Effect of ultrasonification time on oil and protein yields in aqueous extraction of soybean[a]

	Ultrasonification time (min)			
	0	5	10	15
Oil yield (%)	62	85	89	90
Protein yield (%)	68	87	90	90

[a]Extraction was conducted at pH 8.0, dilution ratio 1 : 6, temperature 40℃, particle size 120-150 mesh with ultrasonification. All values are the means of triploicates

Effect of ultrasonification on extraction yields of oil and protein

Extraction yields of oil and protein were 89 and 90%, respectively, after the ten minutes of ultrasonification (Table 3). It is reported that higher protein concentraction solution was obtained from defatted soybean meal with ultrasonification compared to simple shaking extraction method since ultrasonification induced cell rupture which made oil and protein be released more readily from oilseed crops[13].

Color of soybean oil obtained by aqueous extraction

Lovibond units of red yellow colors of aquous-extracted oil are 2.0 and 40, respectively, which were slightly darker than hexane-extracted oil showing 1.5 red and 30 yellow unit. Folch-extracted oil was the darkest among three samples showing the Lovibond color of 7.0 red and 29 yellow unit. However, the coloring materials in each oils were not identified and, therefore, the relationships between the extraction solvents and oil colors were not determined.

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Aqueous Process를 이용한 대두유와 대두단백의 추출중 효소와 초음파처리가 추출율에 미치는 영향

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Aqueous process를 이용하여 전지 대두분에서 대두유와 대두단백을 동시에 추출하였다. 효소처리나 초음파 처리를 하지 않은 aqueous extraction의 경우 최적 추출조건은 pH 8, 6배의 회석률, 40℃, 120-150 mesh의 크기로 나타났으며 62%의 유지와 68%의 단백질이 추출되었다. 효소처리를 하였을 경우에는 유지는 최고 86%, 단백질은 최고 89%가 추출되었고, 초음파 처리를 하였을 경우에는 유지와 단백질은 모두 90%가 추출되었다. Aqueous extraction으로 얻어진 대두유는 hexane으로 추출한 대두유보다는 Lovibond의 붉은색과 노랑색이 약간 짙었으나 Folch 용매로 추출한 것 보다는 두 가지 색도가 모두 매우 약했다.