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물-에탄올 혼합액을 이용한 백색 제인의 생산

김강성†
용인대학교 식품영양학과

Production of White Zein Using Aqueous Ethanol

Kang Sung Kim†
Department of Food Science and Nutrition, Yongin University

Abstract

Solubility profiles of zein and carotenoid in aqueous ethanol were studied. Zein showed minimum turbidity at the aqueous ethanol concentration of 87 ~92%, indicating least aggregations between protein molecules. Solubilitiesof zein and carotenoid increased linearly with the content of yellow zein up to 20% in the aqueous ethanol range of 60-95% tested. At room temperature of 20℃, zein showed maximum solubility in broad ethanol concentration ranges of 60-95%, while that for carotenoid was somewhat narrower with ethanol concentration range of 85-95%. However, at incubation temperature of -20℃, solubilities of both carotenoid and zein were lowered, with dramatic reduction being exhibited at aqueous ethanol concentration of 60% for both compounds, while substantial reduction in solubility was shown at 95% ethanol by zein only. Zein was practically insoluble in absolute ethanol, regardless of temperature range tested, while carotenoid remained largely soluble, though there was pronounced decrease in solubility at the subfreezing temperature.

Key word: carotenoid, ethanol, maize, zein

I. Introduction

Maize is one on the most important cash crops of the world, in terms of amount produced and possible applications as industrial raw materials (Shukla R and Cheryan M 2001). Currently, besides being extracted for frying oil, most of maize is directly consumed as food material for both human and domesticated animal (Greg L 2013). However, sky rocketing price hike of fossil fuel has prompted new uses of maize as the raw material for the manufacture of renewable fuel source: ethanol. More than 20% of maize produced is used to produce ethanol every year (Weijie X et al 2007).This trend is expected to accelerate as petroleum reserves around the world continue to dwindle and the interest and search for environmentally safe energy sourcescontinue to escalate.

The major byproduct of ethanol production is distillers dried grain with solubles (DDGS), which is mostly used as animal feed (Tohreripour T et al 2010, Severinghaus J 2006). Annual production of the maize byproduct is estimated to exceed 10 million tons and thus ways to increase economic values for the DDGS is much in need. Zein, which is classified as a prolaminehaving high proline content, is the major component of maize byproducts (Xu W et al 2007). Future for zein as industrial raw material is bright because 1) zein is being produced in large quantities from corn processing industries as byproducts, 2)unlike other proteins, zein can be readily isolated in high purity after relatively simple processes, 3) scale up of purifying process in uncomplicatedas it does not involve costly and time consuming column chromatography, 4) zein, unlike petroleum derived resins, is biodegradable and thus is environmentally

†Corresponding author: Kang Sung Kim, Department of Food Science and Nutrition, Yongin University
Tel: +82-31-8020-2758
Fax: +82-31-8020-2779
E-mail: kss@yongin.ac.kr

friendly, 5) zein is renewable biomaterial, and 6) zein has highly versatile physicochemical properties and thus has numerous possible industrial uses such as fiber, adhesives, coatings, and binders, etc (Shukla R and Cheryan M 2001, Wang Q et al 2004). However, there are certain drawbacks for wider industrial applications of zein, such as high production cost, as compared to comparable synthetic resins, and the difficulties in removing color pigments from the final zein isolate (Sessa DJ et al 2003).

Industrially produced zein is usually yellow in color, due to carotenoid contamination (Shukla R and Cheryan M 2001). Maize carotenoids are β-carotene and xanthophylls which include lutein and zeaxanthin. Carotenoids are thought to be associated with hydrophobic residues of zein and both materials are co-isolated during aqueous alcohol extraction process. Several workers have tried to produce white zein devoid of carotenoid contamination: Sessa DJ and Palmquist DE (2009) decolorized maize zein using activated carbons and zeolites. Subcritical propane and supercritical fluid extraction with carbon dioxide, ultrafiltration/diafiltration, column chromatography on Sephadex LH-60 were also tested by the same research group with similar results (Sessa DJ et al 2003). Solvents for white zein production were undertaken with considerable success (Carter R and Reck DR 1970, Takahashi H and Norimasa Y 1994). However, the limitations of such process are high costs of solvents and complication of the process involved. Also pilot size exclusion chromatography was successfully used for separation of xanthophylls from zein (Kale A et al 2007).

To date, systematic reports on solubilities of both zein and carotenoid in aqueous ethanol are lacking. Thus, objectives of the present study were to reassess parameters that affect solubilities of zein and carotenoids in aqueous ethanol, the most commonly used solvents for extracting zein, so that white zein could be industrially produced without undergoing complicated and expensive purifying processes.

Materials and Methods

Materials

Yellow zein was purchased from Freeman Industries (Tuckahoe, NY, USA). In this paper, the term 'yellow zein' specifically refers to the commercially available zein with carotenoid contamination, while 'zein' refers to the prolamine itself. On dry basis the sample had 93.14% protein, 1.90% lipid. 0.11% crude fiber and 1.53% ash. The aqueous ethanol solutions used in the investigation were v/v basis. All other chemicals and solvents in this study were certified A.C.S. grade.

Determination of concentration of zein and carotenoid

Protein concentration wasdetermined using modified Bradford method. Briefly, to 200 μL of protein samples, 5 ml dye solution (Biorad, Hercules, CA) was mixed and let stand at room temperature for 10 min,before assaying absorbance at 595 nm. Carotenoid concentration was determined using method developed by Sessa DJ et al (2003) with slight modification. Since xanthophyll and lutein, the major pigments present in maize, showed slightly different absorbance maxima at respectively (Moros EE et al 2002), the yellow pigments were quantified by reading absorbance at 448 nm (Genesys 10UV-Vis Spectrophotometer, Thermo Fisher Scientific Inc., USA).

Turbidity

Turbidity profile of zein was investigated at different aqueous ethanol concentrations. 20 mL of 0.3, 1, and 2% (w/v) zein solutions with aqueous ethanol as solvents were vigorously stirred at room temperature for 2hr, and turbidity of the solutions were investigated by reading absorbance at 500 nm. For samples that gave spectrophotometric readings of more than 1, dilution with corresponding ethanol solutions was made prior to spectrophotometric reading.

Concentration dependent solubilities of zein and caroteinoid

Concentration dependent solubilities of zein and caroteinod in aqueous ethanol of different percentages wereinvestigated by formulating sample solutions of 0-20% (w/v). 20 mL samplesolutions were vigorously mixed at room temperature for 2 hr before being centrifuged at 10,000xg for 1 hr, at room temperature. The supernatant was carefully sieved with membrane filter with pore size of 0.45 μm (GD/X disposable syringe filter, Whatman, Kent, UK). Protein concentrations of the filtrate were determined using modified Bradford method as mentioned above. Carotenoid concentration of the solution was investigated by reading absorbance at 448 nm. Proper dilution of sample solutions was made whenever absorbance exceeded 1.0.

Temperature dependent solubilities of zein and carotenoid

Temperature (-20, 1, 20℃)dependent solubilities of zein and carotenoid in aqueous ethanol solutions of different percentages were investigated at sample concentration of 5%. To ensure that the sample solutions reached respective

temperatures, 20 mL solutions were incubated at the test temperatures for 5hr before analysis. Since both zein and carotenoid exhibited greatest solubility change in the aqueous ethanol concentrations of 60-95% at -20℃, time dependent change in solubilities of both compounds were also investigated.

Results and Discussions

Turbidity of yellow zein in aqueous ethanol

Turbidity of yellow zein at room temperature was tested in aqueous ethanol solution of different concentrations (Fig. 1). At low yellow zein concentration of 0.3%, the solution was relatively clear regardless of aqueous ethanol ranges tested. However, above 1%, the solutions became turbid with the profile similar to that of the pH dependent solubility curves of protein. Turbidity value was the lowest at the aqueous ethanol concentration of 87-92%, indicating minimum aggregations between protein molecules at the aqueous ethanol concentration range. Since zein is particularly rich in hydrophobic amino acid residues such as leucine, proline, and alanine (Paulis JW 1981), but deficient in basic and acidic amino acids, maximum solubility is exhibited at around ethanol concentration of 90%. According to Kim S and Xu J (2008), the aggregation number of zein molecules at the ethanol content of 90% is less than 10 units, as compared to 10,000 units for ethanol concentration of 70%. At ethanol concentration below 87%, the aggregation forces between the protein molecules is speculated to be driven by intermolecular hydrophobic interactions, with hydrophilic residues exposed to the solvent and hydrophobic moieties contacting each other to form micelle structure. As the solvent became more hydrophobic above ethanol concentration of 92%, phase inversion of zein molecules is known to occur with the orientation of amino acid residues in the protein revered: hydrophobic moieties faces solvent while the hydrophilic moieties are at the interior, incontact with each other. This view of structural inversion of zein aggregate at different concentrations of ethanol was discussed by Yamada K et al (1996) who observed an odd behavior of zein when films were prepared from two different types from two different types of solvent systems and also by Kim S and Xu J (2008) who formulated series of intricate experiments to show selective interaction of zeinmolecules with hydrophilic and hydrophobic particles to prove such structural inversion indeed occur at respective aqueous ethanol concentrations.

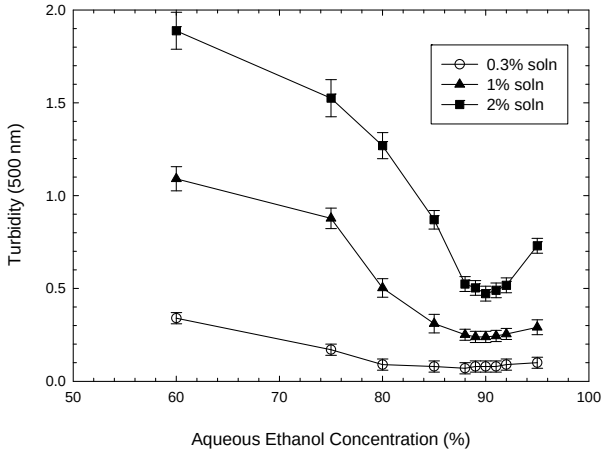

Fig. 1. Turbidity profile of yellow zein in different concentrations of aqueous ethanol at room temperature

Solubilities of zein and caroteinoids in aqueous ethanol

2% yellow zein solutions were formulated using aqueous ethanol of different percentages as solvents. After rigorous mixing, the solutions were centrifuged at 10,000 x g for 30 min and the supernatant thus obtained were filtered with protein filters to ensure clarity of the filtrate. The solutions thus obtained were scanned at 350-520 nm regions, as carotenoids in general absorb blue light (Rodroguez-Amaya DB 1942). The typical visible spectrum of carotenoid chromophore was obtained as in Fig. 2. Scanning spectrum of the solutions showed maximum absorbance peaks at 400, 420, 448 and 469 nm, inferring that the yellow zein solutions contained a mixture of carotenoids (Sessa DJ et al 2003, Moros EE et al 2002). Since the prolamine zein does not absorb wavelength greater than 300 nm, absorbance at 448 nm was used for quantifying carotenoidconcentration. All the measurement was done within 2 hr of sample preparation to avoid discrepancies in chromatographic reading due to oxidation of carotenoid. The results showed that carotenoid was most soluble at the aqueous ethanol concentrations of 85-100% but barely so in aqueous ethanol of less than 40%.

Concentration dependent solubilities of zein and carotenoid were investigated by formulating 0-20% yellow zein solutions in aqueous ethanol of 60, 85, 90, and 95percentages, though the results are not shown here. The results show that the solubilities of carotenoid and zein increased linearly with content of yellow zein up to20% in the aqueous ethanol solvents tested. Testing for solubility of the protein and the pigment above 20% was not feasible as the solution became excessively viscous and gel formation (Evans CD et al 1943, Evans CD and Manley RH 1943). Solubilities of carotenoid and zein at different percentages of aqueous ethanol were so presented in Fig.3 and 4. At room temperature of 20℃, zein

showed maximum solubility in broad ethanol concentration ranges of 60-95%, while that for carotenoid was somewhat narrower with ethanol concentration range of 85-95%. Decrease of solubilities at 60% aqueous ethanol for carotenoid was much more pronounced (about 50% decrease) than that of zein (less than 10% decrease) when compared to the maximum solubilities of both compounds at 95% aqueous ethanol. This phenomenon could be accounted by the differences in two intrinsic properties of the compounds: flexibility and hydrophobicity/ hydrophilicity of the macromolecules. Carotenoid and zein are both water insoluble in nature, but the degree of molecular hydrophobicity exhibited by both compounds differ significantly: Carotenoids are highly conjugated compounds with lipophilic characteristic (Mercadante AZ et al 1999), while zein, though basically hydrophobic, has some hydrophilic characteristic considering the amino acid composition of the protein (Shukla R and Cheryan M 2001). Also, in terms of flexibility, zein is considered to be more flexible than carotenoid (Kim S and Xu J. 2008) and thus the protein is able to change conformation according to environmental conditions (Wang Q et al 2004). As the result zein maintains shapes that induce maximum solubility in solvents with different polarity, while inflexible carotenoid becomes insoluble at reduced ethanol contents. Fig. 5 and 6 show that solubility of carotenoid in absolute ethanol was approximately 80% of that at 95% aqueous ethanol, while zein is barely soluble. Cook RB et al (1972) and Shulman ML (1996) used solubility difference at absolute ethanol of zein and carotenoid to purify white zein.

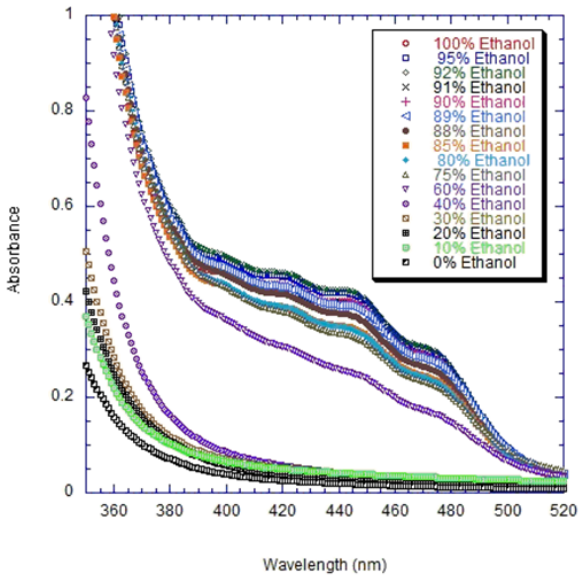

Fig. 2. Scanning chromatograms of carotenoids from 2% yellow zein solubilized in different concentrations of aqueous ethanol

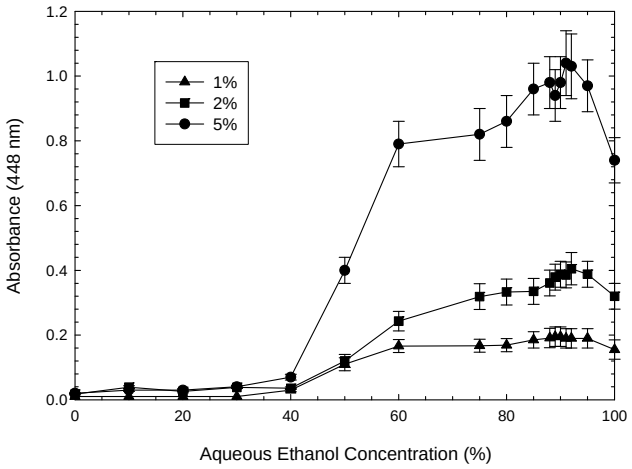

Fig. 3. Solubility profile of carotenoids in different concentrations of aqueous ethanol

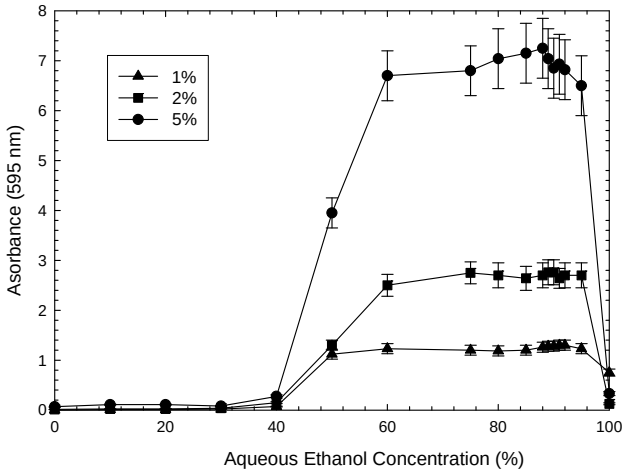

Fig. 4. Solubility profile of zein in different concentrations of aqueous ethanol

Temperature dependence solubilities of zein and carotenoid

The effect of temperature on the solubilities of both carotenoids and zeinin aqueous ethanol of various concentrations were also examined, and the solubility of zein was found to be much more sensitive to shifts in temperature than that of carotenoids (Fig.5 and 6). When incubated -20℃, the solubilities of both zein and carotenoids were decreased, with dramatic reductions observed for both compounds at an ethanol concentration of 60%. A substantial reduction in the solubility of zein, but not carotenoids, was also observed in the 95% ethanol solution at this temperature. Moreover, zein was effectively insoluble in absolute ethanol regardless of temperature, while carotenoids remained largely

soluble. Since most of carotenoid molecule is assumed to be exposed to surrounding solvent, it is highly plausible that the temperature sensitivity of carotenoid is due to a cooperative intermolecular association enhanced by intermolecular bonding, much similar to solidification process of fats at low temperature (Cook RB et al 1979). Aggregation of zein at low temperature is more complicated, however,due to structural complexity of the molecule. Aggregation of protein molecules in cold temperature, called cryoprecipitation, has been known to food protein chemist for some time. Though the mechanisms of the process have not been fully understood, they are speculated to occur in stepwise process: Initial nucleation phase during which small sized particles are generated and flocculation phase where the particles grow by colliding into each other. Factors that affect cryoprecipitability of proteins include concentration (Basha SM and PancholySK 1982) and pH (Miller RE et al 1975) of the solvent. It is highly plausible that marginally soluble macromolecules such as zein would lose its solubility by the small shifts in environmental conditions such as lowering of temperature which induce a shift in conformationthat favor precipitation. Since cryoprecipitation occur in certain proteins only, the overall molecular integrity and conformation are important for the process to occur (Saha A et al 1969). Recovery of zein at low temperature has been occasionally attempted by some researchers (Carter R and Reck DR 1970, Melcher U and Fraij B 1980). The results show that solubility zeinis much more sensitive to the shift in solvent temperature than carotenoid.

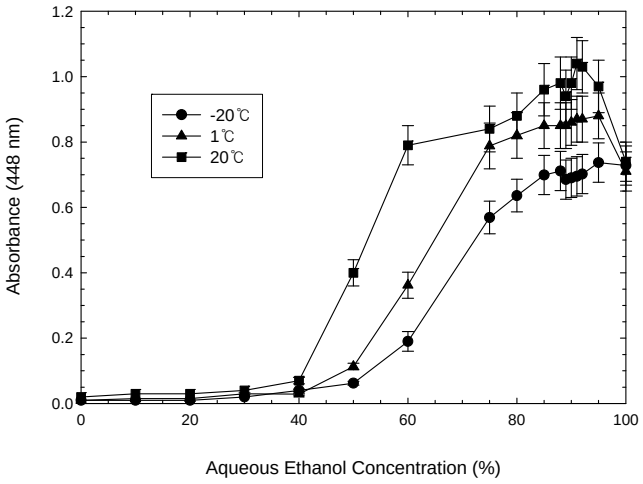

Fig. 5. Temperature-dependent solubility profile of carotenoidsin different concentrations of aqueous ethanol

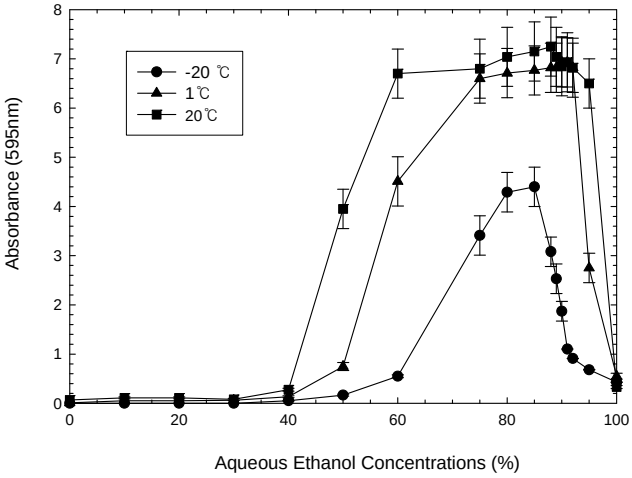

Fig. 6. Temperature-dependent solubility profile of zein in different concentrations of aqueous ethanol

Conclusion

The solubility behaviors of zein and carotenoids in aqueous ethanol were therefore investigated in this work. Zeinwas found to exhibit reduced solubility in 95-100% ethanol solutions, especially at low temperature, while carotenoids remained soluble; these characteristics may provide a method for separation of these 2 compounds. Along with consecutive uses of processes as ultrafiltration with membranes with an appropriate molecular weight cut-off, the results reported herein may lead to a further approach for the purification of white zein.

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