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Urea-cysteine based extraction of densely crosslinked proteins from sorghum distillers grains with high yield and quality

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ABSTRACT

A urea-cysteine based system is developed to extract densely crosslinked sorghum proteins from sorghum distillers grains with high efficiency and quality. Due to the high crosslinking degrees, sorghum proteins are expected to possess better tensile properties and aqueous stabilities than common proteins, thus become a promising candidate to replace the unsustainable petroleum products. However, due to the long-standing difficulty in dissolution of densely crosslinked proteins, to extract sorghum proteins with high efficiency and quality for satisfactory properties in end products has not been achieved by previous methods In this paper, a urea-cysteine based system is used to replace the most widely used ethanol based system. The urea-cysteine based system endows sorghum proteins with high purity and yield, which are 14% and 42% higher than those from the ethanol based system. Based on amine and thiol group analysis, the urea-cysteine based system provides sorghum proteins with preserved peptide backbones, full breakage of disulfide bonds for linear structures and sufficient thiol groups for re-oxidation. Due to such unique structures and thus regular arrangement of protein molecules during film casting, sorghum protein films from the urea-cysteine based system have crystallinity 21% higher than those from the ethanol based system. Tensile properties and water-vapor-barrier properties of sorghum protein films are improved by 27% and 28% compared to the ethanol based system. Sorghum protein films derived from sorghum distillers grains are first reported and their tensile properties are better than those of all sorghum protein films developed previously. Quantified effects of preservation of protein peptide backbones and recovery of disulfide bonds on tensile strength are also compared. With the unique structures, sorghum proteins from the urea-cysteine based system have great potential to achieve excellent properties in various applications, such as films and fibers, which account for a total market share of over $100 billion worldwide.

1. Introduction

Considering the finite and unsustainable nature of petroleum resources, interest has been aroused in exploitation of renewable and naturally derived alternatives, such as plant proteins. As natural plant proteins with high crosslinking degrees, sorghum proteins are expected to possess good tensile properties and aqueous stabilities due to their sufficient intra and intermolecular disulfide bonds (Belton et al., 2006; Taylor et al., 2013). These unique properties along with complete biodegradability make sorghum proteins a sustainable replacement to the environmentally unfriendly petroleum based materials on the market.

Currently, over 65% of sorghum crops worldwide are used for stock feeding and ethanol production (Staggenborg, 2016; Wang et al., 2008). Sorghums are drought tolerant plants that can easily adapt to arid climates. Due to their easy cultivation and high demands of human on clean production, utilization of sorghums in the bio-fuel industry keeps on expanding. As the major coproduct from fermentation of sorghum grains, sorghum distillers grains are abundant, safe, cheap and readily available biomass which are rich in proteins (30–40% on average) (Wang et al., 2009). However, extraction of sorghum proteins from sorghum distillers grains is difficult and has rarely been reported. Such difficulty is attributed to protein denaturation during distillation of dry-grind ethanol after fermentation and drying (Anderson et al., 2012). Studies showed that ethanol could increase the non-covalent interactions among protein molecules (Lin et al., 2004). Thus, it becomes more difficult to break intermolecular interactions in sorghum proteins in sorghum distillers grains than those in original grains.

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Table 1
Purity and yield of sorghum proteins extracted by different systems.

Extraction solvent	Raw materials	Yield (%)	Purity (%)	Reference
70% ethanol	Original grains	54.3	74.6	(Taylor et al., 2005a)
	Sorghum distillers grains	56.8	94.9	(Wang et al., 2009)
55% isopropanol	Original grains	55.3	73.1	(Taylor et al., 2005a)
60% *tert*-butyl alcohol	Original grains	32.0	82.4	(Gao et al., 2005)
Glacial acetic acid		59.3	68.0	(Taylor et al., 2005a, 2005b)
	Sorghum distillers grains	44.1	98.9	(Wang et al., 2009)
Aqueous buffer	Original grains	74.3	Not reported	(Hamaker, 1995)
	Sorghum distillers grains	Not reported		

So far, three systems have been used to extract sorghum proteins, as summarized in Table 1. Few improvements have been made to these systems since 2009. The first system is based on glacial acetic acid under reducing conditions (Taylor et al., 2005a). Protein yield from sorghum distillers grains using this system was reported to be 44.1%, which is significantly lower than that of the most widely used ethanol based system (Wang et al., 2009). Such low extraction efficiency was mainly because non-covalent interactions among protein molecules were not fully interrupted by glacial acid. The second system is alcohol based and includes the most widely used ethanol based system. Reducing agent and alkali have been included in this system to enhance protein yield. Other alcohols, such as isopropanol and *tert*-butyl alcohol, have also been used as solvents. Although the alcohol based systems at alkaline pH provided relatively high protein yield and purity of sorghum proteins, they are low in reducing efficiency (Wolfram and Underwood, 1966) and cause damages to peptide backbones due to the high alkalinity used based on our studies. The third system is based on aqueous solutions under alkaline conditions (Hamaker, 1995). It was reported to be about 40% more efficient than other systems in terms of protein yield due to efficient breakage of electrostatic interactions. However, if the Hamaker solvent system is used at high alkalinity, peptide backbones of proteins could be subjected to undesirable damages. In addition, β-mercaptoethanol, the reducing agent used in this system, is considered toxic to human bodies and not suitable for large-scale production. Although the three systems have considered the breakage of disulfide linkages and electrostatic interactions in sorghum proteins, they still lack effective interruption of non-covalent interactions, such as hydrophobic interactions and hydrogen bonds. Due to the fact that proteins in distillers grains have increased non-covalent interactions among their molecules as discussed previously, extraction of sorghum proteins from sorghum distillers grains needs further improvement.

Only sorghum protein films derived from sorghum grains and extracted by alcohol based systems have been reported for their tensile properties, which are important aspects to evaluate the quality of extracted proteins. Unfortunately, these films are not good enough for practical uses. Over 40 wt% of plasticizers have to be used before the film breaking strength could achieve 1.6–6.0 MPa and the breaking elongation could reach 15%–100% (Buffo et al., 1997; Gao et al., 2006; Taylor et al., 2005a). Experimental errors as large as 40%–60% were observed (Taylor et al., 2005a, 2005b) due to moisture absorbance induced by these plasticizers. It can be inferred that the quality of sorghum proteins extracted still remains a problem to date.

The poor tensile properties of current available sorghum protein films are mainly attributed to the lack of preserved peptide backbones, full breakage of disulfide bonds for linear structures and sufficient thiol groups for re-oxidation in the extracted proteins based on our studies. However, it is difficult to meet such requirements with common solvents because of the stable network structure of original sorghum proteins. The folded conformation of sorghum proteins is stabilized by hydrophobic interactions (about 60% hydrophobic side groups), disulfide bonds (about 3% cysteine level), hydrogen bonds and electrostatic interactions (Belton et al., 2006; Taylor et al., 2013). Thus, unfolding, swelling and controlled degradation are all important to obtain sorghum protein films with reliable properties. This fact has been proved by many studies (Xu et al., 2014; Xu and Yang, 2014; Zhao et al., 2014). Therefore, for sorghum proteins, especially for those in sorghum distillers grains, swelling agent, reducing agent and alkali are used in this study to break non-covalent interactions, disulfide bonds and electrostatic interactions among protein molecules, respectively.

In this research, a urea-cysteine based system is used to extract sorghum proteins from sorghum distillers grains. Compared to the ethanol based system, sorghum proteins are possessed with higher purity and yield with the urea-cysteine based system. End group analysis is used to study the hydrolysis degree of protein peptide backbones, while thiol group analysis is used to investigate the breakage of disulfide linkages and thus the linearity of protein molecules under different extraction conditions. Sorghum protein films derived from sorghum distillers grains are first reported in this paper and their tensile properties are better than those of other sorghum proteins films developed previously. The effect of molecular structures of proteins on crystallinities, tensile properties and water vapor barrier properties has been studied. Quantified effects of preservation of peptide backbones and recovery of disulfide bonds on tensile strength are also compared. With the unique structures, sorghum proteins from the urea-cysteine based system could achieve excellent properties in various applications, such as films, fibers and nanoparticles.

2. Experimental

2.1. Materials

Sorghum distillers grains were provided by an ethanol producer in Kansas, United States. Urea was purchased from Oak Chemical, Inc. West Columbia, SC. Cysteine, sodium sulfate, sodium metabisulfite, ethanol and formic acid were purchased from EMD Chemicals Inc. Gibbstown, NJ. Sodium hydroxide and hydrochloric acid were purchased from VWR international, Bristol, CT. NY. 5,5'-Dithiobis-(2-nitrobenzoic acid) (DTNB) was purchased from G-Biosciences, St. Louis, MO. All reagents were of AR grade and used as received.

2.2. Extraction of sorghum proteins

In the urea-cysteine based system, sorghum distillers grains were first dispersed in 8M urea solution at a weight ratio of 1:7.5. This weight ratio was selected to completely immerse sorghum distillers grains with minimal amount of chemicals. There was no significant change in protein yield when weight ratio increased from 1:7.5 to 1:10. Urea was used since it is a widely used and environmentally friendly swelling agent for proteins. Previous studies have also used urea solutions to solubilize cereal proteins (Bean et al., 2000; Elkhalifa et al., 2006; Field et al., 1983; Taylor and Schussler, 1984). A concentration of 8M was selected in this study because such concentration of urea solution can dissolve most of the proteins to certain levels (Bennion and Daggett, 2003). Cysteine and alkali were used to break disulfide bonds and electrostatic interactions in sorghum proteins. Using alkali was