

## Research Article

### Components Analysis and Flour Preparation of Tofu Whey

<sup>1</sup>Wei Yang, <sup>1</sup>Changzhong Liu, <sup>2</sup>Xiang Xu, <sup>1</sup>Fangfang Kang, <sup>1</sup>Yaping Chen, <sup>1</sup>Min Zhou,  
<sup>1</sup>Shuaitong Wang and <sup>1</sup>Bo Li

<sup>1</sup>Henan Institute of Science and Technology, Xinxiang 453003,

<sup>2</sup>Institute of Apicultural Research, Chinese Academy of Agricultural Sciences, Beijing 100093, China

**Abstract:** The aim of this study is to analyze the composition of tofu whey and spray-dry it into flour, so as to provide the basis for recycling this by-product of tofu manufacture. The results showed that tofu whey contain 3.19% of total solids, including 3.12% of soluble solids; 1.19% of carbohydrate, including 0.45% of reducing sugar, 0.26% of oligosaccharide and 0.48% of polysaccharide; 0.47% of protein; 0.49% of crude fat; 0.54% of total ash, including 0.048% of calcium, 0.042% of magnesium, 0.032% of sulfate ion and 0.060% of chloride ion; 0.20% of saponin and 0.04% of isoflavone. Tofu whey can be prepared into flour by the spray-drying method. The whey flour contained 5.55% of moisture, 23.60% of protein, 18.22% of polysaccharide, 13.67 % of oligosaccharide, 3.86% of saponin, 0.98% of isoflavone and 1.2% of calcium. This study suggested that tofu whey is rich in functional or nutritional ingredients and is worth recycling. Spray-drying whey into flour is a potential way for treatment of tofu whey, especially for those small and medium-sized factories.

**Keywords:** Components analysis, flour preparation, spray drying, tofu whey

## INTRODUCTION

Soybean is one of the most important legumes and the consumption of soy foods is increasing around the world due to its nutritional value and potential health benefits to humans. Tofu (bean curd) is a traditional oriental food since two thousand years ago. During the formation of tofu, a large number of whey wastewater is extruded from tofu jelly. About 4 kg of tofu whey are produced from 1 kg of soybean processing during tofu manufacture. Huge amount of tofu whey are generated everyday from thousands of tofu factories in China, which brings a significant disposal problem.

There are some reports on the utilization of tofu whey, which can be divided into two types. One type is to extract protein, oligosaccharide, isoflavone from tofu whey by the methods of membrane separation, freeze concentration, or foam fractionation (Zhao *et al.*, 2006; Benedetti *et al.*, 2015; Belén *et al.*, 2013; Li *et al.*, 2014; Liu *et al.*, 2015). Another is to utilize tofu whey to produce kojic acid, gamma-aminobutyric acid, astaxanthin, lactic acid, vitamin B12 and biohydrogen, or using tofu whey as the cultivation medium of microalgae and *Lactobacillus* (Hongyang *et al.*, 2011; Ounis *et al.*, 2008; Xiao *et al.*, 2015; Dianursanti *et al.*, 2014; Zheng *et al.*, 2010; Lay *et al.*, 2013; Yu *et al.*, 2015; Liu and Liang, 2014). However, for complicated process, expensive equipment, or high treatment cost,

few of these technologies were put into application. Furthermore, some treatments will generate a lot of new wastewater when recycling tofu whey. Therefore, most of tofu whey is still treated as wastewater in many factories, which have aggravated burden of the industry on sewage treatment and also wastage of this resource (Penas *et al.*, 2006).

Tofu whey contains significant amounts of proteins, polysaccharides and low molar mass substances, such as isoflavones, saponins, oligosaccharides and phytic acids (Li *et al.*, 2014; Penas *et al.*, 2006). Previous studies have reported most phenolic and isoflavones in soybean migrate to the tofu whey when preparing soybean products, such as tofu. Thus, value-added and full utilization of this soybean by-product is of interest to the soybean industry.

To our knowledge, very few studies have been carried out on the principal component analysis of tofu whey and its flour. The flour obtained by spray drying tofu whey may also be beneficial toward waste treatment. Once the principal components of tofu whey and flour have been determined, it is expected to provide a new insight into the separation of bioactive materials and lay a foundation for the utilization of tofu whey. The objective of this study was to determine the principal components of tofu whey and its flour prepared by spray drying and explore the recycling way of tofu whey.

**Corresponding Author:** Bo Li, School of Food Science, Henan Institute of Science and Technology, Hualan Road, Xinxiang 453003, China

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## MATERIALS AND METHODS

**Materials:** The tofu whey was supplied by Zhengzhou New Agricultural Energy Food Industry Co., Ltd. (Zhengzhou, China). All reagents were of analytical grade.

**Preparation of flour:** The fresh tofu whey was directly spray-dried using a spray dryer (MDR.P-5, Wuxi Modern Atomizing Dry Equipment Co., Ltd., China). Work operating conditions were set at: inlet air temperature was 200°C, outlet air temperature was 95-100°C controlled by feed rate and frequency of atomizer was 70 Hz. The dried flour was sealed in polyethylene bag.

**Determination of total solids content:** Total solids content was measured by oven drying at 95-100°C according to the method of AOAC 934.01. To ensure higher accuracy of the results, most of the water is removed by freeze-drying. Soluble solids contents were determined by Abbe refractometer (2WA-J, Shanghai Optical Instrument Factory, China).

**Determination of carbohydrate content:** The total carbohydrate was determined according to the phenol-sulfuric acid method at 490 nm (Dubois *et al.*, 1956). The content of total carbohydrate was calculated on the basis of the calibration curve of glucose. Reducing sugar content was determined by the Fehling reagent method. Oligosaccharide content was calculated by the difference value of reducing sugar content before and after the hydrolysis of sample at 68-70°C for 15 min.

**Determination of protein content:** Protein content was estimated by the Kjeldahl method.

**Determination of crude fat content:** Crude fat contents of air dried and freeze dried samples were analyzed by the Soxhlet extraction method. Crude fat content of tofu whey was also determined by the Rose-Gottlieb method.

**Determination of ash and mineral elements content:** The ash content and mineral elements content was determined by the method of combustion (AOAC 923.03) and inductively coupled plasma atomic emission spectrometry (ICP), respectively. Barium sulfate turbidity was used for determining the  $\text{SO}_4^{2-}$  content. The chloride content was determined by silver nitrate precipitation method.

**Determination of soybean saponin content:** Freeze-dried flour (0.1 g) and spray-dried flour (0.1 g) of tofu whey were dissolved in 50 mL ethanol, respectively, thus an extract crude solution was obtained. 0.4 mL of

5% vanillin in acetic acid solution and 1.6 mL perchloric acid solution were added and the tube placed in a water bath at 70°C for 15 min. The tube was then cooled in tap water and 4 mL ethyl acetate solution added and the absorbency was read at 560 nm in a 722 N visible spectrophotometer (Shanghai Jinghua Scientific Instrument Co., Ltd, China). The saponin content of samples was calculated with reference to an oleanolic acid standard.

**Determination of soy isoflavone content:** Freeze-dried flour and spray-dried flour of tofu whey were transferred to the Soxhlet extraction apparatus for reflux-extraction in methanol for 10 h at 85°C and the absorbency was read at 260 nm in a spectrophotometer. The soy isoflavone content of samples was calculated with reference to a daidzein standard.

**Measurement of the pH value:** pH measurement of tofu whey was carried out with a pH-3C digital pH-meter (Shanghai REX Instrument Factory, China) with a combined glass-calomel electrode. Spray-dried flour was diluted 30-fold with deionized water before measurement.

## RESULTS AND DISCUSSION

**Total solids content:** Tofu whey contains various organic substances, such as protein, carbohydrate and lipid. In principle one could use thermal treatment as drying method, but unfortunately this can cause damage to heat sensitive components or complex chemical reactions, so that alternative drying method, freeze-drying method, a process operated at low temperature, is used for drying on products. The average total solids content obtained in this study was around 3.03%, which was lower than that obtained by the air-drying method (3.19 %). The soluble solids content obtained by the Abbe refractometer was 3.12%, similarly to the average total solids content. Because the refraction method is simple and fast, it could replace the air-drying method to measure the total solids content in factory.

**Ash and mineral elements content:** Tofu whey contained 0.54% of ash. Inductively coupled plasma atomic emission spectrometry indicated that tofu whey contained some amounts of both macrominerals (3.6-483  $\mu\text{g}/\text{mL}$ ; Na, K, Ca, Mg) and trace elements (0.05-2.8  $\mu\text{g}/\text{mL}$ ; Fe, Zn, Mn, Cu, Cr). The mineral elements content in tofu whey were shown in Table 1.

The contents of magnesium and calcium in tofu whey are 0.048% and 0.042 %, respectively, which were the highest point of mineral elements in tofu whey. Prabhakaran *et al.* (2006) reported that the coagulation of soymilk is the most important step in the production process of tofu. There are different types of coagulants used on industrial scale, such as bittern (a

Table 1: Mineral elements content in tofu whey ( $\mu\text{g/mL}$ )

Mineral elements	Ca	Mg	Na	K	Zn	Cu	Fe	Mn	Cr
Content	483	421	4.2	3.6	2.8	1.8	0.8	0.6	0.05

salt extracted from sea water composed primarily of magnesium chloride and calcium chloride) and gypsum (a soft sulfate mineral composed of calcium sulfate). The contents of sulfate and chloride in tofu whey are 0.032 and 0.06%, respectively, which indicated that gypsum and bittern were used as coagulation in this tofu process. The magnesium, calcium, sulfate and chloride present in the tofu whey is probably an excess of coagulant from the coagulation process. According to Chai *et al.* (1999), the divalent cations added to soy milk as coagulant requires the whey further treatment before discharging. Ounis *et al.* (2008) demonstrated the feasibility to recover the salts of the tofu whey and use these as growth medium to microorganisms in fermentation processes.

**pH value:** The values of pH for the tofu whey (4.58) and for the flour rehydration solution (4.74), do not show significant differences between them. Ounis *et al.* (2008) reported that some soy globulins show low solubility in the pH range 4.5-5.5. So, it should be possible to recover these proteins easily.

**Carbohydrate content:** Soybean soluble polysaccharide, the by-product obtained during the production of tofu and soybean protein, has been suggested as a functional food ingredient in many different industrial applications for its various functions, such as dispersion, stabilization, emulsification and adhesion (Maeda, 2000). Among many colorimetric methods for carbohydrate analysis, the phenol-sulfuric acid method is the easiest, most reliable and widely used method for its sensitivity and simplicity. The total carbohydrate content in tofu whey is 1.19%, about one-third of total solids of tofu whey. Reducing sugar content was 0.45% and oligosaccharide content was 0.26%, so the polysaccharide content was 0.48%. The oligosaccharides in tofu whey were mainly stachyose, raffinose and sucrose. Stachyose and raffinose are functional oligosaccharides with the bioactivities such as antitumor, improving the gastrointestinal environment and regulating blood lipid.

**Protein content:** Protein is a necessary component of the diet of humans and other mammals. Production of vegetable protein is gaining increasing commercial importance due to the consumer preference for vegetable sources of food and cosmetic ingredients. In the present study, protein content in tofu whey was 0.47%. Some researchers reported that the content of protein from tofu whey ranged between 0.40 and 0.65%. This difference might be due to geographic

distribution since the protein content of crops varies slightly with the soil, climatic conditions and crop cultivars of an area.

**Crude fat content:** Seed lipids, a diverse group of biological substances made up primarily of non-polar compounds and more polar compounds, have attracted much attention because of their biological properties and effects. The content of crude fat was 0.50 and 0.49% for air-dried sample and freeze-dried sample, respectively, determined by Soxhlet extraction method, higher than the content (0.41%) determined by Roesse-Gottlieb extraction method. Therefore, the Soxhlet extraction may be advised as a convenient automated method for fat extraction from tofu whey. The result showed that fat content and protein content of tofu whey were almost similar. This might be because in tofu jelly the fat is kept in the protein network. When the tofu jelly is broken and pressed to form tofu, the retained substances in the network is extruded into tofu whey due to some protein networks are destroyed and compressed.

**Soybean saponin and isoflavone content:** The presence of saponin in tofu whey is of great importance because of their increasing use as a source of medicines or nutraceuticals (Tarade *et al.*, 2006). Some previous studies reported that the content of saponin in soybean meal is about 0.43-0.67% (Bureau *et al.*, 1998; Ireland *et al.*, 1986). In the present study, the content of saponin in tofu whey is around 0.2%, indicating that a considerable portion of saponin remains in tofu whey.

Isoflavones are a group of naturally occurring hetero cyclic phenols, called phytoestrogens, found mainly in soybean. Oldoni *et al.* (2011) reported that a dietary consumption of foods and food additives containing isoflavone phytoestrogens have been associated with several beneficial properties to human health, such as prevention of coronary heart disease and osteoporosis and reduction of menopausal symptoms. According to Wang and Murphy (1996), the loss of isoflavones in tofu whey during the tofu processing reaches 44%. In the present study, the content of isoflavones in tofu whey is around 0.04%. Sudyani *et al.* (2007) reported that tofu whey has a representative content of genistin and daidzin.

Kao *et al.* (2004) reported that differences in the content of isoflavones could be credited to the type of coagulant used in the preparation of tofu, which is responsible by interactions between proteins and isoflavones. Already, Speroni *et al.* (2010) affirmed that the final isoflavone content in a given soybean-derived product depends on the association/release of isoflavones and proteins during each step of production.

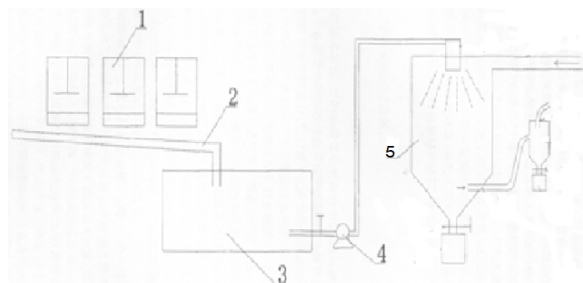


Fig. 1: Schematic diagram of spraying dry facility of tofu whey in factory; (1): Tofu compression facilities; (2): Collecting tank; (3): Storage tank; (4): Pump; (5): Spray dryer

Table 2: Chemical components of tofu whey and its flour (%)

Items	Tofu whey	Flour
Total solids	3.19	--
Moisture	--	5.55
Total sugar	1.19	45.33
Reducing sugar	0.45	13.44
Oligosaccharide	0.26	13.67
Polysaccharide	0.48	18.22
Protein	0.47	23.60
Crude fat	0.49	14.30
Total ash	0.54	18.68
Ca	0.048	1.20
Mg	0.042	1.02
SO <sub>4</sub> <sup>2-</sup>	0.032	1.17
Cl <sup>-</sup>	0.060	2.04
Saponin	0.20	3.86
Isoflavone	0.04	0.98

According to these authors during the process of tofu the various isoflavones may establish different kinds of interactions with proteins because of their diverse polarity and hydrophobicity as well as their ability to form hydrogen bonds.

**Preparation of tofu whey flour:** The analytical results show tofu whey contains appreciable quantity of protein, fat, polysaccharide, oligosaccharide, calcium, saponin and isoflavone, suggesting that tofu whey has high nutrition and health care value. The whey treatment of industrial tofu production by current technologies is problematic, although many trials of its use have been done. Tofu whey is easily spoilage and difficult for storage and transportation. Those whey treatments such as complex process, long operation period or high cost are difficult to be accepted by tofu industries. Therefore, it is still necessary to develop new recycling method for tofu whey.

Based on the properties of tofu whey, we developed a method of spray-drying tofu whey to prepare flour simultaneously with the tofu production. The schematic diagram was shown in Fig. 1. A collecting tank is installed below the facilities of tofu compression to collect the whey extruded from tofu jelly. The whey is transported to a storage tank, thus to a centrifugal spray dryer without any pretreatment. The dried whey flour is easy to store and transport. The flour could be used as food additives or sent to a

conditional factory for further treatment. This method is simple and suitable for many tofu industries. Spray drying is a promising alternative for treatment of whey to recover the soluble solids retained in the whey from the tofu industry, reducing its volume and preventing environmental pollution.

**Component analysis of tofu whey flour:** The components content of tofu whey flour was shown in Table 2. The result suggests the whey flour is a good source of saponin, isoflavone protein, carbohydrates, mainly oligosaccharides, glycoproteins, proteoglycans and glycolipids, as well as and magnesium.

## CONCLUSION

The components of tofu whey and flour were investigated in this study in order to establish the foundation for their nutraceutical potential. The results showed that tofu whey contains many nutrients, such as protein, lipids, oligosaccharides, polysaccharides, saponin, isoflavone and calcium *et al.* Spray drying method is appropriate for treatment of tofu whey, especially for those small and medium-sized factories. Of course, it should be pointed out that tofu whey flour contains sulfate, chloride and magnesium, which could affect the taste and quality of foods when it is added into some foods. Therefore, how to use the whey flour in a rational way is our futural work.

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