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Fermented Soybean Products: Some Methods, Antioxidants Compound Extraction and their Scavenging Activity

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Abstract: Antioxidant compounds in food such as phenolic compounds played various roles as health promoting factors (e.g., cancer and cardiovascular disease), antimicrobial agents; flavor active compounds, colorants precursors and colloidal stability affecting factors as well as chelating agents. Fermented foods such as soy products have been in existence for thousands of years and received attention as sources of many effective antioxidants. This review discusses about the most commonly used fermented soybean food antioxidants, methods of their preparation and description of their scavenging activity/antiradical property.

Key words: Fermented soybean products, antioxidants, isolation, scavenging activity, free radicals

INTRODUCTION

Soybean (*Glycine max* L. Merr.) is one of the most widely grown crops in the world and it is rich in proteins (40-50%), lipids (20-30%) and carbohydrates (26-30%) on dry basis (Gibbs *et al*., 2004). Consequently, it has been generally considered as a good source of protein for humans and animals among cereals and other legume species even though soy protein is limiting in methionine (Liu, 1997).

When soybean is fermented, typical of functional properties from flavor and texture points of view are created. In the past few years, there has been increasing interest in research about antioxidants, such as phenolic compounds and antioxidant peptides, since they can protect the human body from free radicals and retard the progress of many chronic diseases such as cancer and cardiovascular disease (Göktürk *et al*., 2007; Rajapakse *et al*., 2005).

Free radicals are atoms or molecules with an unpaired electron in the outer orbit. They are the byproducts of many normal reactions within the body that include energy generation, breakdown of lipids and proteins, the catecholamine response and the inflammatory process (Ikeda and Long, 1990). This electron imbalance makes them highly reactive to be able to oxidize wide range of food components such as lipids, proteins, vitamins, DNA and carbohydrates as well as colorants. This eventually causes disruption of cell membranes, leading to the release of the cell contents and death (Hu *et al*., 2004). The unpaired electron may also take an electron from another molecule to join with another molecule, or completely disengage itself and reattach itself to another molecule, thus producing more free radicals. Normally, free radicals are neutralized by enzymatic activity or by natural antioxidants. Thus, the generation of free radicals poses no problem as long as there remains a balance between oxygen radical production and eradication (Hu *et al*., 2004).

Fermented foods such as soy products have been in existence for thousands of years and have received attention as sources of many effective antioxidants. Fermented foods have thus become very

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important to human beings worldwide. Between 20 to 40% of our food supply is from fermented food (Campbell-Platt, 1994).

Concentrations of phenolic compound were reported to increase in soybean (McCue and Shetty, 2003), after fermentation, soybean and soybean products, containing various amounts of phenolic compounds have shown to possess antioxidative ability. According to a phytochemical database (USDA, 2003), the number of different antioxidants in some plants can reach up to 40 (soybean 42). Several traditional fermented soybean foods, including miso, natto, tempeh, sufu and douchi, have been proven to possess many advantageous properties such as free radical scavenging ability, reducing ability and ion-chelating ability (Berghofer *et al*., 1998; Gibbs *et al*., 2004; Santiago *et al*., 1992).

Base on these premises, the aim of this study was to indicate the antioxidant activities in the fermented soybean products. The study also discusses the methods of fermentation, antioxidant extraction and its evaluation.

MATERIALS AND METHODS

Methodologies of Fermentation of Soybean Products

Fermentation is one of the oldest techniques in food manufacture and preservation that contributes directly to many advantageous properties of products by biochemical modification of microorganisms (Je *et al*., 2005).

Soybean (*Glycine max* L. Merrill) and different soybean products are known to contain phenolic compounds (Ren *et al*., 2006). Activities of these compounds in soybean were reported to increase after fermentation, for example, during the production of miso, natto, tempeh, furu and rice koji (Berghofer *et al*., 1998; Esaki *et al*., 1997; Ren *et al*., 2006; Yen *et al*., 2003). It was suggested that the increased antioxidative activity was due to the liberation of lipophilic aglycones of isoflavones glucosides such as daidzein and genistein by the catalytic action of â-glucosidase during the production of miso and tempe and due to significant increase in the formation of a water-soluble antioxidative fraction, not the free aglycone during the production of natto (Esaki *et al*., 1994; Murakami *et al*., 1984).

Methods of producing fermented soybean products, Qin and Ding (2007) reported the production method of douchiba a kind of traditional Chinese soy-fermented appetizer (Fig. 1). Han *et al*. (2001)

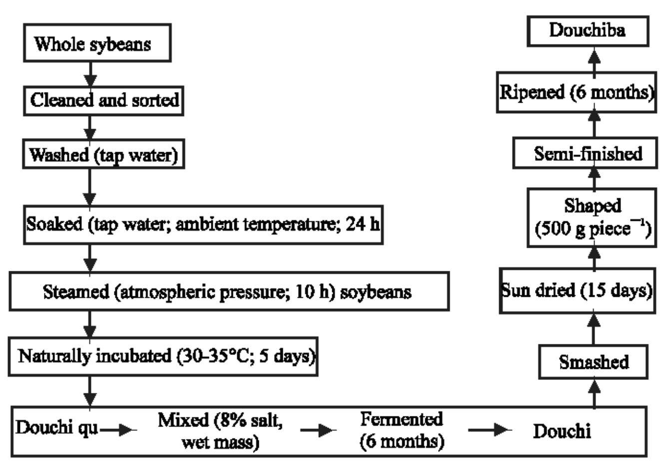

Fig. 1: Schematic representation of douchiba preparation (Qin and Ding, 2007)

also reported the manufacturing process of sufu or furu both traditional and innovated commercial process following 4 main steps: (1) preparation of tofu, (2) preparation of pehtze, (3) salting, (4) ripening. Lin *et al*. (2006) studied the antioxidant activity of fermented soybean koji prepared with various filamentous fungi (e.g., *Aspergillus oryzae*, *Aspergillus sojae*, *Aspergillus awamori*, *Actinomucor taiwanensis* and *Rhizopus oligostorus*) and the procedure was as follow: whole soybeans were washed and the soaked in distilled water that was six times the soybean weight at room temperature overnight. After decanting the water, soybeans were cooked in an autoclave (121°C, 15 min) and then cooled. Solid f ermentation was performed by evenly spraying 1.0 spore suspension into the streamed soybean substrate (50 g). After mixing thoroughly, the inoculated soybean substrate was placed on a round screen with 60 mesh. They were then incubated for 3 days at 30°C and 95% RH (Relative Humidity). During the cultivation period, the soybean was stirred and mixed after 17 and 25 h of cultivation to accelerate the release of fermentation heat.

Soy sauce is a dark, brown and salty liquid used for the seasoning of oriental food. Soybeans, wheat, salt and water are the main components of soy sauce. Its production involves fermentation (*Aspergillus oryzae* or *A. sojae* and other related microorganisms) of carbohydrates to alcohol and lactic acid and proteins are converted to peptides and amino acids (Fukushima, 1981). Wang *et al*. (2007a) has reported many health benefits associated with consumption of soy sauce including; antioxidant activity *in vitro* and some antioxidant activity *in vivo*. In one aspect these methods of processing fermented soybean comprising culturing and combining microorganisms with excellent enzymes activities is known that by isolating and selecting microorganisms, such as *Bacillus subtilis* and *Aspergillus oryzae*, for their ability to produce protease and amylase, the method helps to improve digestibility of fermented soybean products (Lee *et al*., 2008a).

PROCEDURES OF FERMENTED SOYBEAN ANTIOXIDANT EXTRACTION

The extraction procedure is determined by the types of antioxidant compounds to be extracted. Selection of a suitable extraction procedure can increase the antioxidant concentration relative to the food material (Suhaj, 2006). For polyphenols and other antioxidants in plant materials three principal extraction techniques may be used, they are extraction using solvents, solid-phase extraction and supercritical extraction. The extraction can also be performed in a soxhlet apparatus, thus combining percolation and immersion techniques.

Several extraction techniques have been patented using solvents with different polarities, such as petrol ether, toluene, acetone, ethanol, methanol, ethyl acetate and water; in addition, supercritical CO_2-extraction and medium-chain triglycerides as carrier in a mechanical extraction process have been applied (Schwarz *et al*., 2001). Methanol is the solvent most commonly employed. It has also been indicated that aqueous methanol, due to its polarity, extract polyphenols linked to polar fibrous matrices more effectively, while acetone water mixtures are more useful for extracting polyphenols from proteic matrices, since they appear to degrade the polyphenol-protein complexes. Microwave assisted extraction is a new extraction technique that uses microwave energy to heat the solvents and the sample to increase the mass transfer rate of the solutes from the sample matrix into the solvent. The microwave-assisted solvent extraction takes 10-30 min, whereas the other methods can take hours or days to complete. The amount of solvent used in microwave extraction is also considerably less than the amount used in the other extraction process or higher extraction introduced for phenolic compound extraction. In this technique high temperature and high pressure are used to accelerate the extraction (Shah *et al*., 2002). It was illustrated that combination of microwave irradiation and solid state chemistry in the synthesis of methionine complexes of iron (II) was very promising in the terms of

Table 1: Extraction procedure of antioxidant from some fermented soybean products

Fermented soy product	Fermentation organism	Extraction procedure	References
Chungkukjang (Korean)	*Bacillus megaterium*	Methanol extract using *B. megaterium* was a suitable fermentation strain to promote the antioxidant and free radicalscavenging activities	Shon *et al.* (2007)
Tempeh (Indonesian)	*Rhizopus oligostorus* or Aspergillus oryzae	The peptides were extracted either by water or 70% ethanol	Handoyo and Morita (2006)
Miso (Japanese)	Aspergillus oryzae	70% ethanol extracts and hot water extracts	Matsuo (2006)
Sufu/ furu (Chinese)	*Actinomucor* sp., *Mucor* sp. and *Rhizopus* sp.	water extracts and 50% ethanol extracts	Wang *et al.* (2004)
Douchi (Chinese)	*Aspergillus egypticus*	Ethanol extract showed significant antioxidant activities	Wang *et al.* (2007b)
Koji (Korean)	*Aspergillus* sp. and *Rhizopus* sp.	The methanol extract in soybean koji showed higher antioxidant activities.	Lin *et al.* (2006)
Okara (Meitauza) (Chinese)	*Bacillus subtilis*	Water extracts showed increased of peptides content after 12 h fermentation.	Zhu *et al.* (2008)
Chungkukjang CKJ (Korean)	*Bacillus* sp.	CKJ methanol extracts and its constituents, genistein and daidzein, showed significant antioxidant activities *in vitro*	Kim *et al.* (2008)
Thua-Nao (Thailand)	*Bacillus* sp.	The fermentation resulted in extensive hydrolysis of protein to amino acids	Petchkongkaew *et al.* (2008)
Kinema (Indian)	*Bacillus subtilis*	Methanolic extracts of kinema showed significant antioxidant activities	Moktan *et al.* (2008)
Soy Iru (Nigeria)	*Bacillus sphaericus*	The fermentation promoted nutritional composition	Jeff-Agboola and Oguntuase (2006)

mildness of reaction conditions, yields and time of reactions. It also has all the advantages devoted to solvent-free reactions, namely, environmentally friendly conditions, high chemoselectivity and absence of volatile and hazardous solvents (Wang *et al.*, 2008).

The results obtained in the study of *Monascus* fermented soybeans scavenging activity using hot and cold water extraction was found to be very effective extraction procedure, whereas for both cold and hot water extract, soybeans showed higher scavenging abilities on DPPH (1,1-diphenyl-2-picrylhydrazyl) radicals; obviously, hot water extracts showed better scavenging DPPH abilities than cold water extracts. However, the high scavenging ability of cold water and hot water extract from Monascus fermented soybeans might be attributed to the presence of isoflavones (Lee *et al.*, 2004). In addition, McCue and Shetty (2005) reported that bioprocessing of soymilk by Kefir cultures containing *Streptococcus lactis*, *Streptococcus cremoris*, *Streptococcus diacetylactis*, *Lactobacillus plantarum*, *Lactobacillus casei*, *Saccharomyces fragilis* and *Leuconostoc crenoris* could increase phenolic contents and thus enrich the scavenging abilities on DPPH radicals (Lee *et al.*, 2008a). It is yet to compare at the industrial level that water extraction procedure would probably be more environmental friendly than the other solvents used. A summary of extraction procedures and strains used in the fermentation of some soybean products are shown in Table 1.

ANTIOXIDANT / ANTIRADICAL ACTIVITY OF FERMENTED SOYBEAN EXTRACT

Soybean contains a variety of biologically active compounds, such as isoflavones, saponins, oligosaccharides and trypsin inhibitors. Isoflavones are found in small amounts in a number of legumes, grains and vegetables, but soybeans are by far the most concentrated source of isoflavones in the human diet. The glucoside forms (genistin, daidzin and glycitin) are the main components and small amount of these three compounds exist as aglycones in the soybean (Chi *et al*., 2002; Fletcher, 2003; Munro *et al*., 2003; Isanga and Zhang, 2008).

A number of studies have been devoted to assess the antioxidant potential of soy protein fractions as well as the isolation and structural characterization of the most active peptides (Gibbs *et al*., 2004; Chiang *et al*., 1999), which contained 5 to 16 amino acid residues (Chen *et al*., 1996). The amino acid sequence affects the antioxidant power. The antioxidant properties of soy protein hydrolyzates have been ascribed as the cooperative effect a number of properties (Chen *et al*., 1998), including their ability to scavenge free radicals, to act as metal-ion chelator, oxygen quencher or hydrogen donor and to the possibility of preventing the penetration of lipid oxidation initiators by forming a membrane around oil droplets (Penã-Ramos and Xiong, 2002). Wei *et al*. (1993), Record *et al*. (1995) and Ruiz-Larrea *et al*. (1997) have shown that genistein is the most potent compound among isoflavones with an effective scavenging activity of free radicals as well as a strong inhibitor of hydrogen peroxide formation, not only *in vivo* but also *in vitro*. Oxidative stress is one of the causative factors of many diseases such as atherosclerosis (Young and McEneny, 2001). The imbalance between production of free radicals and antioxidant level leads to oxidative stress. The findings of Gu *et al*. (2008) suggested that oxidative stress may occur in digestive organs of mice because of intake diets with high protein caused an imbalance between the production of Reactive Oxygen Species (ROS) and the capacity of the antioxidant defense system. Cysteamine acts as an antioxidant for its sulfhydryl in relation to effectively scavenging free radicals (e.g., hydroxyl radical), the study of oxidative stress in mice fed-soybean protein was lower than that in mice fed-casein (Zhu *et al*., 2005; Yang *et al*., 2006, 2008).

The most commonly used antioxidant methods are ABTS.+ (3-ethylbenzthiazoline -6-sulfonic acid) and DPPH. Both of them are characterized by excellent reproducibility under certain assay conditions, but they also show significant differences in their response to antioxidants. The DPPH free radical (DPPH.) does not require any special preparation, while the ABTS radical cation ($ABTS.^{+}$) must be generated by enzymes or chemical reactions (Arnao, 2000). Another important difference is that DPPH can only be dissolved in organic media, especially in ethanol, while $ABTS.^{+}$ can be dissolved in aqueous and organic media, in which the antioxidant activity can be measured, due to the hydrophilic and lipophilic nature of the compounds in samples and is being an important limitation for DPPH when interpreting the role of hydrophilic antioxidants. Both radicals show similar bi-phase kinetic reactions with many antioxidants. However, the Ferric Reducing Antioxidant Power (FRAP) method is based on the reduction of a ferroin analogue, the Fe^{3+} complex of tripyridyltriazine Fe $(TPTZ)^{3+}$ to the intensely blue-coloured Fe^{2+} complex Fe $(TPTZ)^{2+}$ by antioxidants in acidic medium. However, the reducing capacity does not necessarily reflect antioxidant activity, as has been described by Wong *et al*. (2006) and Katalinic *et al*. (2006). A review of antioxidant active extracts and their scavenging activity of some fermented soybean products are given in Table 2. The Oxygen Radical Absorption Capacity (ORAC) assay is said to be more relevant because it utilizes a biologically relevant radical source (Prior *et al*., 2003). These techniques have shown different results among crop species and across laboratories. Awika *et al*. (2003) observed high correlation between ABTS, DPPH and ORAC among sorghum and its products. While, Lin *et al*. (2006), Lee *et al*. (2008a), Wang *et al*. (2007a), Moktan *et al*. (2008) and Zhu *et al*. (2008), reported no correlation of antioxidant activity between the DPPH, ABTS and FRAP techniques among soybean and soybean products samples. However, significant difference by employing ABTS and DPPH scavenging assay were reported by Lissi *et al*. (1999) and Wang *et al*. (1998), found that some compounds, which have ABTS radical scavenging activity may not show DPPH radical scavenging.

Table 2: Antioxidant active extracts from some fermented soybean products

Soy (name and strain)	Antioxidant extract	Antioxidant activity/scavenging activity	References
Soybean koji (*Rhizopus* sp.)	The methanol extract content ranged from 6.70 to 16. 80% and also varied with starter organism.	The methanol extract of koji prepared with *Asp. Awamori*, at IC_{50}a of 2.40 mg mL^{-1}, exhibited the highest scavenging effect, which was 5.2 fold than that of non fermented soybean extract for DPPHc-free radicals	Lin *et al.* (2006)
Chungkukjang (CKJ) *Bacillus subtilis*	1.28 mg g^{-1} (dry basis) total daidzein and genistein content	CKJ extract exhibited significant free radical scavenging effect against DPPH (14.9-26.6% inhibition) at 150-450 µg mL^{-1}	Kim *et al.* (2008)
Monascus fermented soybean	Cold/hot water extract varied from 25.7±1.78 to 39.60±0.45 (g/100 g dry basis)	Cold/hot extracts (EC_{50}[b] values) showed descending antioxidant activities from soybean to *Monascus* fermented soybean	Lee *et al.* (2008b)
Douchi (*Aspergillus egypticus*)	50% ethanol extract	The antioxidative properties of the 50% ethanol extracts during doutchi prefermentation processing showed a significant increased (p<0.05), through the postfermentation	Wang *et al.* (2007a, b)
Natto (Japanese) *Bacillus subtilis*	Diluted oxidized plasma sample	Water extract, natto water-soluble Fractions and low molecular-weight viscous substance showed inhibitory effect on the oxidation of Low-Density Lipoprotein (LDL) *in vitro* and natto fractions led to inhibition of LDL *in vivo*	Iwai *et al.* (2002)
Kinema (*Bacillus subtilis*)	15.4±0.5 g/100 g (dry basis) methanol extract	Kinema enhanced significantly (p< 0.05) free radical scavenging activity, reducing power, metal-chelating ability and lipid peroxidation inhibitory activity compared to soybean	Moktan *et al.* (2008)
Okara (Meitauza) (*Bacillus subtilis*)	4.4 mg mL^{-1} water extract after fermentation	The water extract in the okora showed antioxidant activity enhancement after fermentation	Zhu *et al.* (2008)
Soybean meal (*Bacillus subtilis*)	10 mg mL^{-1} (TCAd, 0.4M)	The soybean peptides after purification by ultrafiltration and gel chromatography exhibited strong scavenging activity, ranging from 49 to 96% at concentration 10 mg mL^{-1}	Yu *et al.* (2008)
Miso (*Aspergillus oryzae*)	70% ethanol extract and aqueous extract	Aqueous extract showed the highest antioxidant activities ranged from 44.6 to 159.0 while 70% ethanol extract ranged from 7.2 to 25.1 at IC_{50} (mg miso mL^{-1})	Matsuo (2006)

[a]IC_{50}: the half-inhibition concentration [b] EC_{50} value: an effective concentration at which the antioxidant activity is 50%; [c]1,1-diphenyl-2-picrylhydrazyl; [d]Trichloroacetic acid

CONCLUSION

The different methods of fermentation process of soybean contribute to the increase of the biological activities that offers opportunity to the novel food value and also various procedures of extracting the antioxidant are shown for their scavenging activity test. In most cases the studies suggest that the fermented soybean products extracts, their mixtures, isolates and concentrates are with useful nutritional antioxidants effects; it is likely that many of them their structure-activity relationship, possible mechanisms and the essential biofactors contributing to the enhancement of the antioxidant activities during the fermentation remain to be investigated.

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