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Review

Production of bioactive peptides during soybean fermentation and their potential health benefits

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ABSTRACT

Background: Fermented soybean products are consumed in many Asian countries and are one of the potential sources of bioactive peptides. Soybean is fermented using bacteria (*Bacillus subtilis* and lactic acid bacteria) and fungi (*Mucor* spp., *Aspergillus* spp. and *Rhizopus* spp.), resulting in different types of fermented products.

Scope and approach: This review article is focused on production of bioactive peptides in fermented soybean products and their role in prevention and treatment of several metabolic diseases. Studies on novel bioactive peptides having specific health benefits can lead to their application in the development of functional foods and pharmaceuticals with the aim replace synthetic drugs that have several side effects.

Key findings and conclusions: Peptides in fermented soybean products are either released by the hydrolysis of soybean proteins during fermentation or produced by the microorganisms associated with fermentation. During soybean fermentation specific bioactive peptides are produced as a result of hydrolysis of soybean proteins (Glycinin and β-conglycinin). Individual microbial strains contribute in the formation of specific bioactive peptides with respective health benefits depending on the sequence and composition of amino acids. Such bioactive peptides may act like regulatory compounds and exhibit bioactive properties such as anti-hypertensive, antimicrobial, antioxidant, anti-diabetic and anticancer activities. Studies in future, on application of specific strains for soybean fermentation can lead in to the formation of novel bioactive peptides with potential health benefits.

1. Introduction

Soybean is the most acknowledged source of plant protein, which also contributes to a wide range of health benefits. Apart from proteins, soybean contains basic nutritive constituents, such as lipids, vitamins, minerals, free sugar and contains isoflavones, flavanoids, saponins and peptides that are of therapeutic value (Kim et al., 2006; Wang, Neal, Mark, & Elvira, 2008a). Soybean is consumed in two forms, unfermented (roasted and fried soybeans, soy powder, soy butter, soybean oil etc.) and fermented (soy sauce, cheese, pickle, yogurt etc.). Fermentation has been used since ancient times and the basic idea behind the process was to preserve the perishable food materials specially where there was scarcity of such foods. In the present scenario fermentation is applied to improve bioactive components responsible for health benefits (Cho, Kim, Chang, & Jae, 2011; Rai & Jeyaram, 2015; Sourabh et al., 2015) and reduction of antinutritional factors (Difo, Onyike, Ameh, Ndidi, & Njoku, 2014; Egounlety & Aworh, 2003; Rai & Appaiah, 2014). During fermentation, complex organic compounds are broken down into smaller molecules by microorganisms, which exert various physiological functions beyond their nutritional properties. Fermentation of soybean with different microorganisms improves the biofunctional properties due to the increase in free isoflavones and peptides (Cho et al., 2011; Sanjukta, Rai, Muhammed, Jeyaram, & Talukdar, 2015; Zhang, Tatsumi, Ding, & Li, 2006). Fermentation can also result in reduction of antinutritional components such as proteinase-inhibitors, phytic acid, urease, oxalic acids (Egounlety & Aworh, 2003; Reddy & Pierson, 1994). Soybean fermentation using specific microorganisms has shown to reduce components responsible for immunoreactivity (Frias, Song, Martínez-villaluenga, Gonzalez de Mejia, & Vidal-valverde, 2008; Song, Frias, Martinez-Villaluenga, Vidal-Valdeverde, & Gonzalez de Mejia, 2008a). Fermented soybean products have showed

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negligible human IgE immunoreactivity, which may be due to the hydrolysis of immunoreactive soybean proteins during fermentation (Song, Martinez-Villaluenga, & Gonzalez de Mejia, 2008b).

Fermented soybean products are very popular in many Asian countries. The popular soybean products include *natto, miso, tofuyo* (Japan), *douchi, sufu, doubanjiang* (China), *cheonggukjang, doenjang, kanjang, meju* (Korea), *tempeh* (Indonesia), *thua-nao* (Thialand), *kinema, hawaijar, tungrymbai* (India). Soybean fermentation results in release of peptides by the action of proteolytic enzymes produced by the microorganisms involved during fermentation (Rai & Jeyaram, 2015; Sanjukta et al., 2015). Bioactive peptides are inactive within the sequence of parent protein and are released on enzymatic hydrolysis during fermentation and gastrointestinal digestion. These peptides are chains of 2–20 amino acids and their activity depends on chain length, amino acid composition and sequence of amino acids. Peptides improve the functional properties of the fermented foods and can act as natural alternative to various synthetic drugs. In fermented soybean products, peptide mediated therapeutic values differ with specific microbes involved in the fermentation process and the soybean variety used for fermentation. Bioactive peptides in fermented soybean have been studied for various therapeutic properties such as antioxidant (Sanjukta et al., 2015; Watanabe, Fujimoto, & Aoki, 2007), antihypertensive (Gibbs, Zoygman, Masse, & Mulligan, 2004; Zhang et al., 2006), anti-tumor (Jung, Park, & Park, 2006), antidiabetic (Kwon et al., 2011) and are also known to prevent atherosclerosis (Tsai, Chu, Lee, & Pan, 2009). Epidemiological evidences for both lower incidences of cancer and mortality rates in populations consuming high levels of soybean products are garnering attention towards peptides, as protein is one of the major components of soybean (Spector, Anthony, Alexander, & Arab, 2003). During the last two decades, researchers have laid a lot of emphasis on bioactive peptides in fermented soybean products. Thus, the current review article is focused on bioactive peptides in fermented soybeans products and their potential health benefits.

2. Characteristics of soybean protein

Soybean seeds are relatively inexpensive source of dietary protein for human consumption, having protein content of 35–40% on a dry weight basis (Mujoo, Trinh, & Ng, 2003). The proteomic studies of the soybean protein have given the basic information about the major storage proteins in soybean, Glycinin (11S globulin) and β-conglycinin (7S globulin) fractions, accounting approximately 40% and 30%, respectively of the total protein (Utsumi, 1992; Utsumi, Matsuma, & Mori, 1997). As glycinin and β-conglycinin constitute 65–85% of the total soy proteins they are the precursors of most of the isolated bioactive peptides (Yang, Mau, Ko, & Huang, 2000). Glycinin, a hexamer, is composed of five major subunits (G1, G2, G3, G4 and G5), each contains an acidic (MW~ 35,000) and a basic polypeptide chain (MW~ 20,000) linked to each other through a disulfide bond (Golubovic, Hateren, Ottens, Witkamp, & Wielen, 2005; Mujoo et al., 2003). Molecular heterogenicity is exhibited by the glycinin fraction due to polymorphism in the subunit composition, which probably may be due to the difference in the amino acid sequences among the different cultivars (Utsumi et al., 1997). β-conglycinin is a trimer with molecular weight of 150–200 kDa and belongs to a class of glycosylated proteins (Hou & Chang, 2004) with four subunits, α (MW~68,000), α' (MW~72,000), β (MW~52,000), γ (MW~52,000) that are associated with each other by hydrophobic interactions (Mujoo et al., 2003). The 11S polypeptide has five genetic variants, which are divided into group-I and group-II based on the homology of their subunit sequences (Nielsen, 1985; Staswick, Hermodson, & Nielsen, 1981). Heterogeneity in the 11S fraction has also been reported among soybean varieties (Kitamura, Toyokawa, & Harada, 1980; Mori, Utsumi, Inaba, Kitamura, & Harada, 1981) and within a single variety of soybean (Utsumi, Inaba, & Mori, 1981). However, the genomics studies of these protein fractions reveal that the genes of both the globulins (7S and 11S) are derived from a common ancestral gene (Gibbs, Strongin, & McPherson, 1989; Plietz, Drescher, & Damaschun, 1987). From the literature, it is known that both environment and genetics influence the change in the composition and content of soybean storage proteins (Mujoo et al., 2003; Murphy & Resurreccion, 1984). Since, composition and content of soybean storage proteins vary among different varieties, the probability of formation of peptides with different functional properties also increases. The minor proteins/glycoproteins in soybean include trypsin inhibitors, lipoxygenases, lectins and α-amylases (Liu, 1997). Apart from these proteins, potential bioactive peptides have also been reported in raw soybean (Hernandez-Ledesma, Hsieh, & de Lumen, 2009a). Lunasin, a 43 amino acid peptide, which has been isolated from different soybean varieties, has been reported to exhibit antioxidant, anticancer and anti-inflammatory properties (Gonzalez de Mejia, Vásconez, de Lumen, & Nelson, 2004; Hernandez-Ledesma, Hsieh, & de Lumen, 2009b; Lule, Garg, Pophaly, Hitesh, & Tomar, 2015).

3. Impact of fermentation on soybean protein

Soybean proteins may not be highly digestible because of the presence of high amount of enzyme inhibitors such as proteinase and trypsin inhibitors. Fermentation is an eco-friendly process, which improves the digestibility of soybean protein. During fermentation, the proteolytic enzymes produced by the microbial populations, hydrolyzes the proteins into peptides and free amino acids (Fig. 1). Free amino acids contents have been reported to increase in several fermented soybean products such as *thua nao* (Dajanta, Chukeatirote, & Apichartsrangkoon, 2011), *sufu* (Han, Rombouts, & Nout, 2001), *kinema* (Nikkuni et al., 1995), *cheonggukjang* (Hong et al., 2008) and *natto* (Nikkuni et al., 1995). Free amino acid content of soybean fermented with proteolytic strains of *Bacillus subtilis* was found to increase 10–20 folds (Sanjukta et al., 2015). Efficiency of fermentation with respect to functionality of the hydrolyzed products differs with specific cultures used for the fermentation process (Ibe, Yoshida, & Kumada, 2006; Rai et al., 2011). A difference in extent of soybean protein hydrolysis was found in soybean fermented with different strains of the same species. Soybean fermented with *B. subtilis* MTCC5480 resulted in higher degree of protein hydrolysis and free amino acids in comparison with soybean fermented with *B. subtilis* MTCC1747

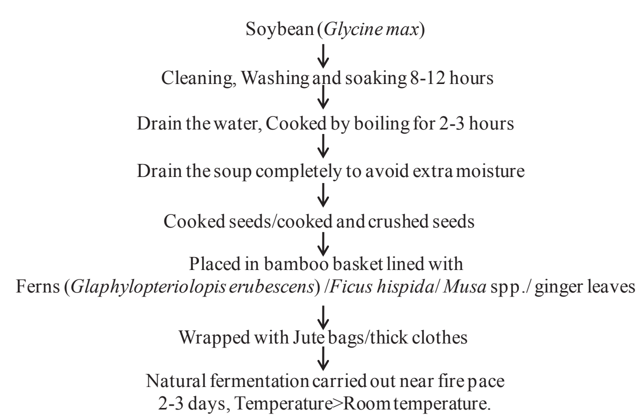

Fig. 1. Flow chart summarizing the process of production of fermented soybean products of North East India.