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Effect of soy protein subunit composition on tofu quality

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Abstract

Tofu was made, using two coagulants, from soybean lines which lacked specific glycinin and β-conglycinin protein subunits and the quality evaluated to determine the effects of specific protein subunits. The group IIb (A_3) glycinin subunit played the major role in contributing to tofu firmness, regardless of coagulant, while the group IIa (A_4) subunit had a negative effect on tofu quality in 2002. Soybeans with the group I (A_1A_2) subunit resulted in tofu with textural properties about one-third higher, expressed as a percent of Harovinton's values, than tofu prepared from soybeans without the group I subunit. The individual components of group I had contradictory effects on GDL tofu quality in 2002, with the A_1 subunit having a negative effect and A_2 having a major positive effect. Lack of the α′ subunit of β-conglycinin increased gel hardness relative to the complete 7S protein.

Keywords: Soybean; Tofu; Glycinin; β-Conglycinin; Protein subunits

1. Introduction

Soybeans have long been a staple of the human diet in Asia, especially as soymilk or tofu, which is prepared from soymilk (Liu, 1997; Tay & Perera, 2004; Watanabe, 1997). Soybeans are an inexpensive, high quality protein source. Consumption of soymilk, tofu, and other soy foods is increasing in North America due to an increase in Asian immigrants, greater acceptance by the general population, and increased recognition of the health benefits of soy foods, especially by those who wish to reduce their consumption of animal products (Murphy, Chen, Hauck, & Wilson, 1997).

In making tofu, soymilk is heated to cause protein dissociation and a coagulant is added to form a protein matrix, which gives the tofu its firmness and hardness (Liu, 1997). The quantity and quality of the protein in the seed is the major biochemical component influencing soybean quality for tofu and other soy food production (Poysa & Woodrow, 2002). A soybean genotype from which firmer tofu can be made at a given water:protein ratio, compared to a second soybean genotype, can be used for making a greater volume of tofu of a defined firmness, relative to the second genotype, making it more valuable.

The principal storage proteins in soybean are glycinin (11S) and β-conglycinin (7S), which account for about 70% of the total seed protein (Thanh & Shibasaki, 1976). β-conglycinin is a trimeric glycoprotein which consists of three sub-units, α, α′, and β (Thanh & Shibasaki, 1978). Glycinin is a hexamer composed of an acidic (A) polypeptide linked by a disulfide bond to a specific basic (B) polypeptide (Staswick, Hermodson, & Nielsen, 1984). Glycinin has five subunits, coded for by five genes, divided into group I ($A_{1a}B_2$; $A_{1b}B_{1b}$; A_2B_{1a}) [Gy1, Gy2, Gy3], group IIa ($A_5A_4B_3$) [Gy4] and group IIb (A_3B_4) [Gy5] (Beilinson et al., 2002; Nielsen et al., 1989; Yagasaki, Kaizuma, & Kitamura, 1996). Glycinin is the predominant soybean seed storage protein, accounting for over 50% of the seed protein in most

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varieties. Various spontaneous or induced mutations affect the accumulation of glycinin. Many commercial tofu-type cultivars lack group IIa; these are also known as A_4 nulls. A line lacking all 11S protein subunits was developed by combining a γ-irradiation induced mutation deficient for all group I subunits with a group IIa null cultivar and a group IIb null wild soybean accession (Yagasaki et al., 1996) The Japanese variety Keburi lacks the α′ subunit of β-conglycinin, while researchers in Japan developed soybean mutants with β-conglycinin lacking either or both of the α and α′ subunits (Takahashi, Banba, Kikuchi, Ito, & Nakamura, 1994, 1996).

The soy globulins differ in their functional properties, especially in gelation, with gels made from glycinin being harder than gels from β-conglycinin (Renkema, Knabben, & van Vliet, 2001; Rickert, Johnson, & Murphy, 2004; Saio, Kamiya, & Watanabe, 1969; Watanabe, 1997; Yagasaki, Kousaka, & Kitamura, 2000). The specific subunits within glycinin (Mujoo, Trinh, & Ng, 2003; Tezuka, Taira, Igarashi, Yagasaki, & Ono, 2000; Yagasaki et al., 2000) and β-conglycinin (Mohamad Ramlan et al., 2004; Mujoo et al., 2003) contribute differentially to protein gelling properties. Both Yagasaki et al. (2000) and Tezuka et al. (2000) reported the hardness of gels from glycinin decreased in the order group IIa, IIb, and I. The relative order of hardness for gels made from the β-conglycinin subunits is α, α′ and β (Mohamad Ramlan et al., 2004).

While the effects of isolated 11S and 7S proteins on tofu quality have been evaluated, there could be significant effects of the subunit composition on other seed components, particularly other proteins. Tezuka et al. (2000) evaluated the textural properties of tofu made from soy lines lacking different glycinin subunits, while Yagasaki et al. (2000) combined soymilk from low glycinin and low β-conglycinin soybeans for a series of 11S:7S ratios to evaluate the effects of different subunits on tofu gels. Further research is required to test the effects of different glycinin and β-conglycinin subunit combinations across genotypes, environments, and growing seasons. To test the effect of soy protein subunit composition on tofu quality, we have developed a series of lines differing in seed storage protein subunit composition. We studied the effects of soy protein subunit composition on tofu quality by making two types of tofu from soybean lines lacking some or all of the 11S glycinin subunits and the β-conglycinin subunit α′.

2. Materials and methods

2.1. Materials

The soybean lines used in this investigation included the tofu-type cultivar, Harovinton, with a complete complement of glycinin and β-conglycinin subunits, and 20 genotypes with different glycinin and β-conglycinin subunit compositions (Table 1). Some combinations of subunit nulls were duplicated in more than one line. These provided an additional check that the effects identified here were due to the subunit composition and not other non-evaluated factors in a specific genotype. These genotypes (subunit null lines) were developed by crossing and backcrossing Harovinton with selections from a population segregating for the lack of the glycinin subunits and the α′ subunit of β-conglycinin, which was kindly provided by Dr. N. Kaizuma, Iwate University, Morioka, Iwate, Japan. The line lacking all 11S protein subunits was developed by crossing a γ-irradiation induced mutation line deficient for all group I and IIa subunits with a group IIb null wild soybean accession (Yagasaki et al., 1996; Kaizuma, personal communication). BC_1-F_5 lines were grown in 2002 and BC_1-F_6 lines were grown in 2003. In the development of the lines used in this study, selection was for protein subunit composition, large seed size and high protein content, to resemble the adapted parent. Harovinton (Buzzell, Anderson, Hamill, & Welacky, 1991), a high-yielding, large-seeded, late maturity group I commercial cultivar, with 44% protein content on a dry matter (DM) basis, and yellow seed coat and hilum, suitable for tofu production, is the quality standard for Canadian tofu type soybeans.

Protein subunits of the soybean lines were identified after separation by SDS–PAGE using a PhastSystem™ (Amersham Biosciences, Piscataway, NJ, USA) apparatus and pre-cast gels. All chemicals were of molecular

Table 1
Seed protein, oil, and sugar content and seed mass of soybean lines deficient for specific glycinin and β-conglycinin subunits, along with Harovinton, grown in 2002 in Harrow

Line	Absent Subunits	Protein (%)	Oil (%)	Sugar (%)	Seed mass (mg seed^{-1})
1	A_2	41.7	20.6	10.9	200
2	A_3	43.0	20.7	10.1	160
3	$\alpha' A_2$	45.5	18.6	10.1	183
4	$\alpha' A_2$	44.8	19.7	10.0	192
5	$\alpha' A_3$	45.3	20.6	9.7	205
6	$\alpha' A_3$	42.9	21.5	10.5	167
7	$\alpha' A_4$	45.9	19.0	9.9	175
8	A_1A_2	44.4	19.3	10.4	172
9	A_2A_3	43.2	19.6	11.9	187
10	A_2A_4	41.9	20.9	10.6	189
11	$\alpha' A_1A_2$	42.8	20.3	10.5	214
12	$\alpha' A_1A_3$	42.4	20.3	10.4	180
13	$\alpha' A_2A_4$	44.2	19.8	10.3	209
14	$\alpha' A_3A_4$	41.0	20.5	10.6	193
15	$A_1A_2A_3$	41.6	19.5	11.0	153
16	$A_2A_3A_4$	42.8	19.9	10.3	191
17	$\alpha' A_1A_2A_3$	45.7	18.6	9.8	154
18	$A_1A_2A_3A_4$	41.2	19.9	11.5	216
19	$A_1A_2A_3A_4$	42.6	19.7	11.1	190
20	$A_1A_2A_3A_4$	42.9	19.8	10.9	199
21	Harovinton	44.6	20.3	9.7	213
	LSD	0.43	0.14	0.68	5.6