Genetic Improvement of Nutritional and Food Processing Quality in Soybean

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Abstract

Soybean 7S and 11S globulins are two major storage proteins, accounting for about 70% of the total seed protein. The 7S globulin is composed of α, α' and β subunits. The 11S globulin, on the other hand, is composed of group-I ($A_{1a}B_2$, $A_{1b}B_{1b}$, A_2B_{1a}), A_3B_4, and A_4, A_5, B^3 subunits. So far, several mutants which lack α, α', all the group-I, A_3B_4 and A_4, A_5, B_3 subunits, respectively and in which the levels of α and β subunits are reduced have been detected. The absence or reduction of the levels of the subunits has been shown to be controlled by respective single recessive alleles. Soybean seeds contain three lipoxygenase isozymes, L-1, L-2 and L-3, which are responsible for the beany flavor and bitter taste. Three mutant lines lacking L-1, L-2 or L-3 were detected. The absence of L-1, L-2 and L-3 is due to the operation of single recessive alleles, lx_1, lx_2 and lx_3. Double and triple recessive soybean lines which are physiologically normal have been obtained. Through breeding trials to manipulate the variant alleles, new soybean varieties and lines have been developed that should display improved quality and food processing characteristics.

Discipline: Plant breeding
Additional key words: Seed, storage protein, lipoxygenase

Introduction

About 50 years ago, soybeans were produced in a limited area of Asia and used in various traditional foods such as tofu, miso, natto and tempeh. By the 1950s, the crop had become one of the most important sources of edible oil and protein for the animal industry. Today, it is now generally recognized that soybeans are the most economical sources of food protein for various new soybean products such as protein concentrates, isolates, texturized protein products, and soy milk and related products as well as for the traditional foods. Recently, soybean production and use in human foods have become increasingly popular in developing countries in Africa, South Asia and Latin America.

Accordingly, it is essential to develop soybean varieties with new or modified seed components for various protein and oil uses as well as for whole soybean consumption.

In the last two decades, various mutant genes controlling the production of soybean seed proteins and enzymes closely related to nutritional and food processing quality have been identified in the world soybean germplasm. This paper describes a modification of the seed protein composition using mutant genes for various storage protein subunits and the elimination of the undesirable beany flavor by the use of lipoxygenase null mutants.

Genetic modification of seed storage proteins

Although soybeans have the highest protein content among seed crops, the protein quality is poor due to the low content of the sulfur-containing amino acids, cysteine and methionine. Soybean 7S globulin (β-conglycinin) and 11S globulin (glycinin) are the two major protein components, accounting for about 70% of the total seed protein. The content of sulfur-containing amino acids in the two globulins is very different: 11S globulin contains three to four times more methionine and cysteine per unit protein than 7S globulin[4]. Furthermore, the two globulins show considerable differences in key functional properties such as gel-making ability, thermal

stability, and emulsifying capacity[4,27]. It is anticipated that the increase of the content of 11S globulin and the decrease of the content of 7S globulin may result in enhanced protein quality, and that the manipulation of the 7S:11S ratios may improve the functionality of soybean proteins in various foods.

It has become possible to breed soybean varieties with a markedly modified protein composition ranging from extremely high to extremely low 7S:11S ratios using mutant genes for the subunits of the two globulins.

1) Manipulation of variant alleles for the 7S subunits

The 7S globulin is composed of three kinds of polypeptides, designated as α, α' and β subunits[3]. Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) has been used to identify two types of mutant varieties in the soybean germplasm collection. "Keburi" was characterized by the absence of the α' subunit, and "Mo-shi-dou (Gong 503)" was characterized by low levels of both the α and β subunits. The absence of the α' subunit is controlled by a single recessive allele, cgy_1[12] and the reduction of the levels of α and β subunits by their respective independent single alleles[24].

The 11S globulin, on the other hand, is composed of six non-identical intermediate subunits, each consisting of one acidic polypeptide and one basic polypeptide. The 11S globulins of various soybean varieties can be classified into two types, A_5 and A_4 (called A_4 and A_3, respectively according to the terminology of Nielsen[20]), depending on the presence or absence of the A_5 acidic and its paired basic polypeptides. The absence of the subunit is controlled by a single recessive allele, gy_4[7,12].

By gathering the mutant alleles in one genotype, we have obtained two types of 7S-low lines (the A and E lines in Plate 1). The content of 7S and 11S globulins in the 7S-low lines and ordinary varieties, grouped according to the type (A_4-type, with all the 7S subunits and an A_4-type 11S globulin, or A_5-type, with all the 7S subunits and A_5-type 11S globulin) was determined by single radial immuno-diffusion analysis using anti-7S and anti-11S sera[22].

The 7S globulin content of the 7S-low lines was only about half that of the ordinary varieties, whereas the 11S globulin content of the 7S-low lines was about 15% higher than that of the ordinary varieties. Consequently, the total protein content remained about the same in both mutant and ordinary varieties (Table 1). The presence of the A_5 intermediate subunit increased the content of 11S globulin in the seeds,
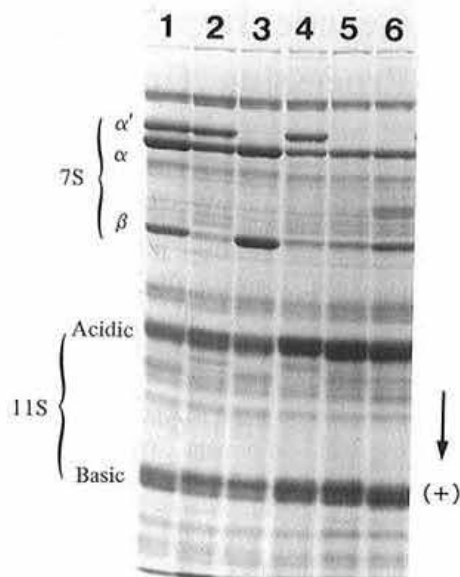

Plate 1. SDS-PAGE patterns of the total soybean seed protein
1 : Suzuyutaka (A_4-type),
2 : Williams 82 (A_5-type),
3 : Keburi (α' null),
4 : Mo-shi-dou Gong 503 ($\alpha \cdot \beta$ low),
5 : A line (α' null, $\alpha \cdot \beta$ low, A_5-type),
6 : E line (α' null, $\alpha \cdot \beta$ low, A_4-type).

which corresponded to the previous results[7]. A highly negative correlation was observed between the 7S and 11S globulin contents, suggesting that 11S globulin may be overproduced to compensate for the reduction in 7S globulin levels. The content of sulfur-containing amino acids in the 7S-low lines was 20% higher than that of ordinary varieties[22]. No deleterious effects have been observed despite the marked differences in the seed protein composition of the lines.

Recently, Takahashi et al.[23] have identified a mutant soybean line lacking both the α and α' subunits, and having a low level of β subunit in the progeny of gamma-ray-irradiated Karikei 434 which lacks the α' subunit and has a low level of α and β subunits (Plate 2). The mutant line has been observed over three generations and despite the marked reduction in the 7S globulin content, no reduction in the total protein content or deleterious effects on physiological processes such as seed development and germination have been observed. Absence of the α subunit has been shown to be controlled by an independent single recessive allele of cgy_1 which also controls the absence of the α' subunit.

By manipulating the variant alleles identified so

Table 1. Total and fractional protein contents of ordinary varieties and 7S-low lines

Group or variety	No. of lines tested	Total protein[1] (%)	% of total protein[2]	
			7S	11S
7S-low lines				
A lines (α' null, $\alpha \cdot \beta$ low, A_5-type)	5	43.4[a]	8.7	52.5
E lines (α' null, $\alpha \cdot \beta$ low, A_4-type)	5	44.4[a]	11.7	44.7
Ordinary varieties				
A_5-type	20	41.4[b]	17.3	38.3
A_4-type	20	41.1[b]	19.5	31.2

1): Figures designated with [a] differ from those designated with [b] at the 5% confidence level.
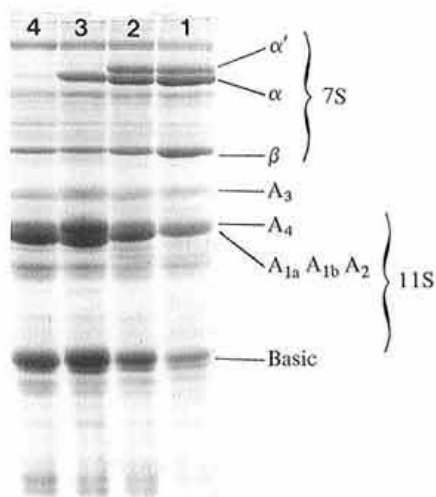2): All figures within each column differ from each other at the 5% confidence level.

Plate 2. SDS-PAGE patterns of the total soybean seed protein
1: Suzuyutaka (A_4-type),
2: Tachiyutaka (A_5-type),
3: Karikei 434 (α' null, $\alpha \cdot \beta$ low, A_5-type),
4: T line (an induced mutant line: $\alpha \cdot \alpha'$ null, β low, A_5-type).

far, it is possible to develop soybean varieties with extremely high 11S:7S ratios which would have improved nutritional value and food-processing properties.

2) Manipulation of variant alleles for the 11S subunits

As discussed above, there is a genetic polymorphism for 11S globulin independent of that of 7S globulin. All the U.S. varieties examined to date contain all five 11S subunits designated as $A_{1a}B_2$, $A_{1b}B_{1b}$, A_2B_{1a}, A_3B_4 and $A_4A_5B_3$ by Nielsen[20], while about 20% of the Japanese varieties lacked

the $A_4A_5B_3$ subunit (Kitamura et al., unpublished results). SDS-PAGE patterns of a typical U.S. variety Williams 82 and a Japanese variety Suzuyutaka lacking $A_4A_5B_3$ are shown in Plate 3.

Recently, Kaizuma et al.[9] have identified a mutant soybean line induced by gamma-ray irradiation. This line lacks all the group-I subunits consisting of $A_{1a}B_2$, $A_{1b}B_{1b}$ and A_2B_{1a} (Plate 3-3). Synthesis of the missing polypeptides has been shown to be controlled by a single recessive allele. Furthermore, recently, we have identified an additional novel mutant that lacks the A_3B_4 subunit (Plate 3-4) in one accession of wild soybean (*Glycine soja*)[17]. Since *G. soja* shows a complete cross-compatibility with the cultigen (*Glycine max*), this accession was crossed with the induced mutant soybean line lacking all the group-I subunits and $A_4A_5B_3$. A total of 560 F_2 seeds from the cross were examined for the 11S subunits by SDS-PAGE. The segregation of the F_2 seeds for the presence or absence of A_3B_4 fitted well to a 3:1 ratio, suggesting that the absence of A_3B_4 is controlled by a single recessive allele. As reported previously, the group-I subunits behaved like a single block, and no recombinant types of the group-I subunits were obtained in the F_2 seeds examined.

Eight phenotypes including one wild type with all the 11S subunits, three types of single mutants, three types of double mutants, and a triple mutant lacking all the subunits appeared in the F_2 seeds (Plate 3). The joint segregation for the presence or absence of the 11S subunits fitted well to a 27:9:9:9:3:3:3:1 ratio for independent inheritance among the three loci controlling the group-I, A_3B_4, and $A_4A_5B_3$ subunits, respectively (Table 2).

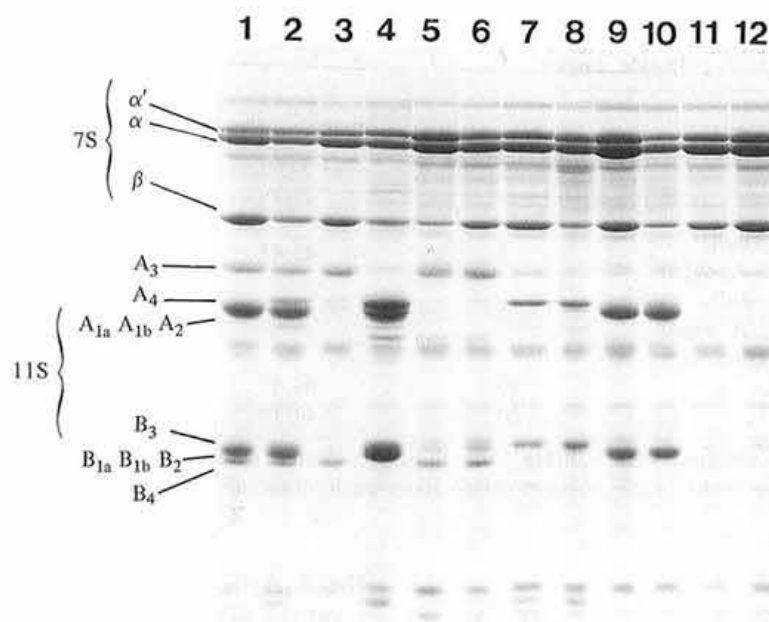The double and triple recessive alleles for the 11S subunits resulted in a marked decrease of the 11S

Plate 3. SDS-PAGE patterns of the total globulin fraction
  1: Suzuyutaka,
  2: Williams 82,
  3: a mutant *Glycine soja* line lacking the $A_3 B_4$ subunit,
  4: an induced mutant line lacking all the group-I and $A_4 A_5 B_3$ subunits,
  5-10: double recessive F_2 seeds lacking the group-I and $A_4 A_5 B_3$ subunits (5, 6), the group-I and $A_3 B_4$ subunits (7, 8), and the $A_3 B_4$ and $A_4 A_5 B_3$ subunits (9, 10),
  11-12: triple recessive F_2 seeds lacking all the 11S subunits.

Table 2. Observed and expected segregation of F_2 seeds from the cross between a line lacking group-I[a)] and $A_4 A_5 B_3$ subunits and a line lacking the $A_3 B_4$ subunit for the presence and absence of the 11S subunits

11S subunits			Seed number		Chi-square value $(27:9:9:9:3:3:3:1)$	Probability
Group-I	$A_3 B_4$	$A_4 A_5 B_3$	Observed	Expected		
+	+	+	223	(236.25)	0.743	
+	+	−	89	(78.75)	1.334	
+	−	+	80	(78.75)	0.020	
−	+	+	86	(78.75)	0.667	9.235	0.3>P>0.2
+	−	−	23	(26.25)	0.402	
−	+	−	31	(26.25)	0.860	
−	−	+	16	(26.25)	4.002	
−	−	−	12	(8.75)	1.207	

a): Group-I subunits include the $A_{1a} B_2$, $A_{1b} B_{1b}$ and $A_2 B_{1a}$ subunits.

globulin content in the seeds (Plate 3–5 to 12). It is interesting to note that no apparent increase of the 7S globulin content has been observed in the seeds, in contrast to the 7S-low lines, in which 11S globulin was overproduced to compensate for the reduction of the 7S globulin levels. Whether the marked reduction of the 11S globulin content adversely affects the total protein content or exerts a

beneficial effect on the oil content in the seeds remains to be studied. No deleterious effects on physiological processes such as seed development and germination were observed in the double and triple mutants.

Since 7S globulin is characterized by superior food functional properties, such as a greater emulsifying capacity and emulsion stability than 11S globulin,

soybean varieties with extremely high 7S:11S ratios could be effectively used by the soy food industry. Since the presence of the $A_4A_5B_3$ as well as A_3B_4 subunits in 11S globulin is closely related to the gelation rate and the gel properties[4,27], consequently soybeans with a modified subunit composition could be developed by manipulating the recessive alleles for the two subunits to modify the gel properties.

Genetic elimination of seed lipoxygenases

Lipoxygenase catalyses the hydroperoxidation of unsaturated fatty acids and polyunsaturated lipids. Soybean seeds contain three lipoxygenase isozymes, called L-1, L-2 and L-3, which are responsible for the generation of grassy-beany and bitter tastes, limiting the use of whole soybeans and soy proteins in certain food products[2,25,26].

Heat treatment has been used commercially to suppress the lipoxygenase activity in order to prevent the beany-flavor generation in soy protein products. However, heat treatment sufficient to inactivate the lipoxygenase action often results in some insolubilization of the soy proteins, and can generate an unpleasant "cooked" odor. Thus, the lipoxygenase problems have not been entirely solved yet.

1) Genetics of lipoxygenase null mutations and their application to breeding

In the early 1980s, three types of spontaneous mutant soybean varieties lacking L-1, L-2 or L-3 were detected (Plate 4). Genetic studies have demonstrated that the absence of L-1, L-2 and L-3 from the seeds is due to single recessive alleles, lx_1[8], lx_2[1,13] and lx_3[11] respectively, and that the lx_3 locus is independent of the lx_1 and lx_2 loci[1,13]. Two types of double mutant soybeans were obtained: one lacked both L-1 and L-3, and the other lacked both L-2 and L-3. No physiological problems were observed during the life cycles of the two double mutant lines.

Three to four backcrosses were made to the recurrent parent Suzuyutaka, a leading cultivar in Japan, and selection for the absence of the isozymes yielded isolines lacking L-1, L-2 or L-3. From crosses among the isolines, two new lines were obtained: one lacking both L-2 and L-3 was designated as Kanto 101, and the other lacking both L-1 and L-3 was designated as Kanto 102. There were no significant differences in the stage of germination, growth and seed development, disease resistance, protein and oil contents between the isolines and Suzuyutaka[14].
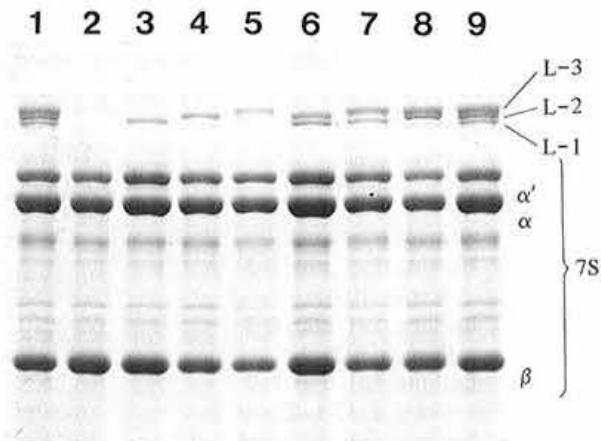

Plate 4. Resolution of the lipoxygenase isozymes in soybean seeds by SDS-PAGE
1, 9: Suzuyutaka (wild type),
2: a line lacking all three isozymes,
3: a line lacking L-2 and L-3 isozymes,
4: a line lacking L-1 and L-3 isozymes,
5: a line lacking L-1 and L-2 isozymes,
6: Wasenatsu (L-3 null),
7: PI 86023 (L-2 null),
8: PI 408251 (L-1 null).

Biochemical analysis using soybeans lacking lipoxygenase showed that the L-2 isozyme plays a major role in the formation of n-hexanal[19], the main constituent responsible for the grassy-beany flavor. Furthermore, careful sensory evaluation tests showed that the line lacking both L-2 and L-3 displayed much less off-flavor than the line lacking both L-1 and L-3. Therefore, we have attempted to breed new commercial cultivars lacking L-2 and L-3 that would be acceptable to the soybean food industry.

One of the lines exhibiting agricultural traits similar to those of Suzuyutaka was selected, and in 1992 it was registered as a new soybean cultivar named Yumeyutaka[16]. In performance trials, the average seed yield of Yumeyutaka during the period 1989–1991 was 95% of that of Suzuyutaka, while the protein and oil contents as well as resistance to soybean mosaic virus and cyst nematode were similar to those of Suzuyutaka.

Due to the close genetic linkage between the Lx_1–lx_1 and Lx_2–lx_2 loci, double mutants lacking both L-1 and L-2 or triple mutants lacking L-1, L-2 and L-3 have been obtained only recently. Two triple mutant lines lacking L-1, L-2 and L-3 were obtained[5,15]. One is Kyushu 111 produced from a progeny of γ-ray-irradiated F_2 seeds from a cross between Kanto 101 and Kanto 102. The other is a line which was obtained from M_2 seeds derived from the irradiated seeds of Kanto 101 with 40 kR

Table 3. Lipoxygenase activities of soybean seeds and leaves (± SD)

Plant type	Activity of seeds (ΔA_{234} nm/min, mg meal)		Activity of leaves (ΔA_{234} nm/min, mg protein)
	pH 6.5	pH 9.5	pH 7.0
Suzuyutaka	4.76 ± .13	7.76 ± .27	1.71 ± .14
Lacking L-2 and L-3 (Kanto 101)	0.70 ± .05	7.57 ± .91	1.62 ± .28
Lacking L-1 and L-3 (Kanto 102)	3.35 ± .04	0.15 ± .01	1.37 ± .17
Lacking L-1 and L-2	0.21 ± .04	0.04 ± .01	
Lacking L-1, L-2 and L-3 [1]	0.04 ± .01	0.14 ± .03	1.89 ± .26
Lacking L-1, L-2 and L-3 [2]	0.04 ± .02	0.13 ± .04	1.35 ± .30

Substrate: 2.5 mM linoleic acid.
1): Produced in Kyushu National Agricultural Experiment Station.
2): Produced in National Agriculture Research Center.

γ-rays. We have demonstrated that the double mutants lacking L-1 and L-2 obtained from crosses between normal soybean varieties and Kyushu 111 were able to develop normally[6]. Thus, the inability to identify mutants lacking L-1 and L-2 or L-1, L-2 and L-3 in previous studies must have been due to the close linkage between the L-1 and L-2 loci, and not to the lethality of the genotype[6].

Kyushu 111 has passed three generations from M_4 to M_6 in the field so far, and growth and seed production were similar to those of Suzuyutaka and Yumeyutaka. Lipoxygenase activities in the leaves of the triple-mutant lines have been shown to be almost the same as those of Suzuyutaka, while the lipoxygenase activities in the seeds of the mutants were considerably lower than those of the parent soybeans[15] (Table 3). Moreover, the new lipoxygenases, different from the L-1, L-2 and L-3 isozymes, which appear in the mutant soybean cotyledons after germination[10], may account for the fact that soybean varieties lacking L-1, L-2 and L-3 are physiologically normal. These facts have prompted us to breed commercial soybean cultivars lacking all three isozymes.

2) Effective use of soybeans lacking the seed lipoxygenases

Due to their low levels of beany flavor and bitter taste, Yumeyutaka which lacks both L-2 and L-3, and Kyushu 111, which lacks all the isozymes, could become economically valuable for the manufacture of soy products such as soy milk, soy yoghurt and ice cream, and light-plain tofu products that may be popular among the younger and non-Asian consumers.

Recently, we have analyzed the volatile compounds extracted by simultaneous distillation and extraction from soybean homogenate (soy milk) of the normal and the lipoxygenase-lacking soybean varieties by gas chromatography[18]. These products revealed that almost all the peaks of the volatile compounds obtained from soy milk of Yumeyutaka and Kyushu 111 were markedly lower than those of Suzuyutaka (Fig. 1). This fact implies that the concentration and composition of the flavor compounds of the lipoxygenase-lacking soybeans are quite different from those of the normal soybeans. The relationship between volatile compounds and the beany and bitter tastes of soy milk remains to be elucidated.

Obata et al.[21] demonstrated that the hydroperoxides formed by lipoxygenase action oxidize the sulfhydryl groups of proteins in soybean homogenates, resulting in a decrease of gel-forming ability. In fact, it was observed that the homogenate of Yumeyutaka contains a larger amount of -SH residues than that of Suzuyutaka, which may account for the fact that the tofu gel of Yumeyutaka is harder than that of Suzuyutaka[3,21].

Soybean cultivars lacking the lipoxygenases should become economically valuable due to their enhanced storage stability, since the lipoxygenase-induced oxidative deterioration of protein and oil in soy meals as well as whole soybeans is likely to be eliminated from these cultivars. It was observed in food processing studies for aburage (a deep-fried tofu) that aburages made from the old Yumeyutaka stored for several months at a cool room temperature expanded to a similar size to that of the products from the new crop, while aburages made from the old Suzuyutaka expanded poorly[3].
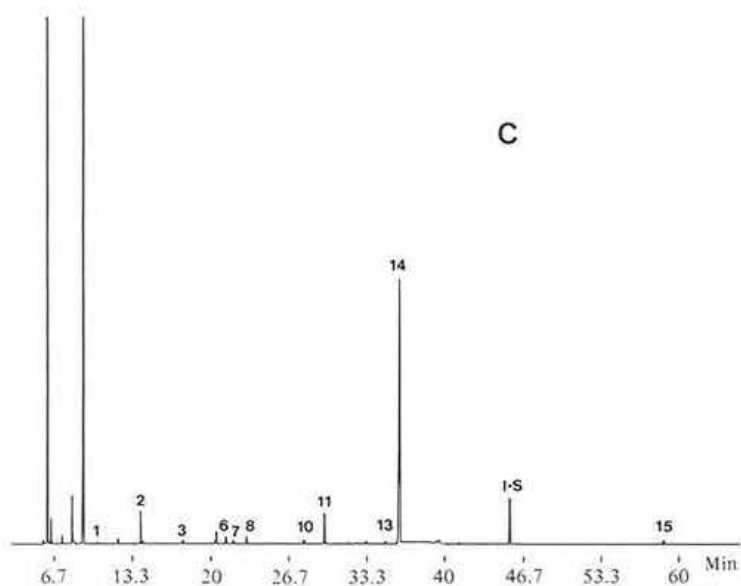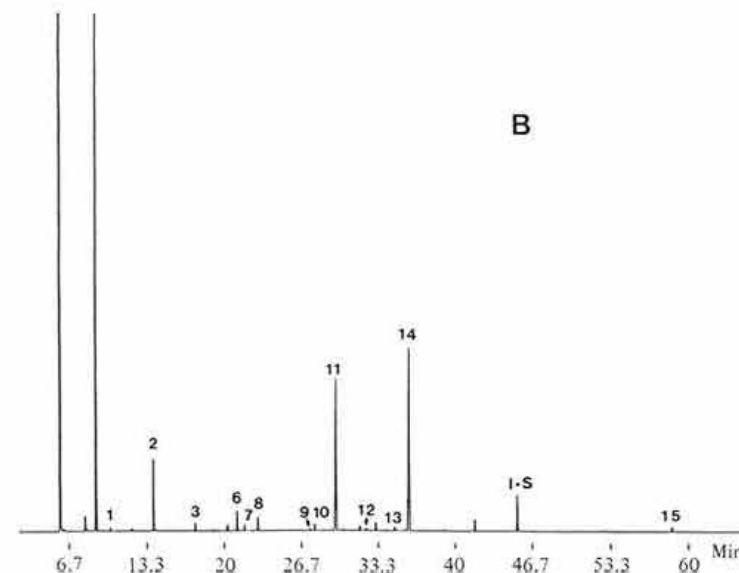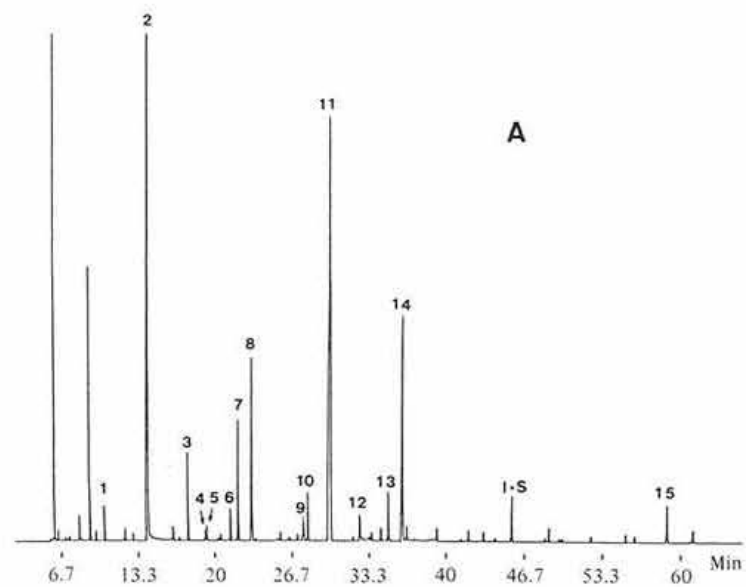
Fig. 1. Gas chromatogram of volatile substances extracted by simultaneous distillation and extraction from soybean homogenates (soy milk)

A: Suzuyutaka (wild type),
B: Yumeyutaka (L-2 and L-3 null),
C: Kyushu 111 (L-1, L-2 and L-3 null).
I•S: methyl decanoate.
Peak numbers correspond to compounds.
1: pentanal, 2: hexanal, 3: 1-penten-3-ol, 4: 2-heptanone,
5: heptanal, 6: trans-2-hexanal, 7: 2-pentyl furan,
8: 1-pentanol, 11: hexanol, 14: 1-octen-3-ol,
15: 2-hexyl furan.

A combination of appropriate processing technologies and the new cultivars may enable the production of various soybean-based foods suitable for people who do not like the traditional foods in future.

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