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(54) **SOY COMPOSITIONS HAVING IMPROVED ORGANOLEPTIC PROPERTIES AND METHODS OF GENERATION**

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(57) **ABSTRACT**

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(62) Division of application No. 11/177,943, filed on Jul. 8, 2005, now Pat. No. 7,704,540.

The present invention provides soy meat compositions with improved organoleptic properties and methods of identifying soybeans with improved organoleptic qualities. The invention also provides methods for producing soy compositions with improved organoleptic properties and for creating plants producing soybeans with such characteristics.

## SOY COMPOSITIONS HAVING IMPROVED ORGANOLEPTIC PROPERTIES AND METHODS OF GENERATION

[0001] This application claims the priority of U.S. Provisional Patent Appl. Ser. No. 60/521,846, filed Jul. 9, 2004, the entire disclosure of which is specifically incorporated herein by reference.

### BACKGROUND OF THE INVENTION

[0002] 1. Field of the Invention

[0003] The invention relates to the field of nutrition and food sciences. In particular, the invention relates to soy meat compositions with improved organoleptic properties such as decreased odor and methods for the use and production thereof.

[0004] 2. Description of Related Art

[0005] Soybeans provide high quality proteins that provide health benefits for humans (Hermansen et al., 2003; Bazzano et al., 2001; Food and Drug Administration, 1999). The demand for soybeans to make soy foods had not gone up as much as expected in last three decades (Wolfe and Cowan, 1975 and SoySource, The United Soybean Board 1999). This is in-part because of the undesirable odor associated with soy products (McLeod and Ames, 1988 and Freese, 1999). The undesirable soybean odor is commonly described as "beany." Components that impart beany characteristic to soybeans include many volatile fatty acids, aliphatic carbonyls, amines, alcohols, aldehydes, and furans derived from the action of enzymes on various compounds found in soybeans and their further oxidation that is caused by many mechanisms (Wolfe and Cowan, 1975; Sessa and Rackis, 1977).

[0006] Kobayashi et al. (1995) concluded the main contributors to the odor of uncooked soymilk were (trans, trans)-2,4-nonadienal, (trans, trans)-2,4-decadienal, hexanal, 2-pentyl furan, 1-octen-3-one, (trans)-2-nonenal, and (trans, cis)-2,4-nonadienal. The strongest odors extracted from heat-treated soymilk were identified as (trans, trans)-2,4 decadienal and n-hexanal (Feng, Cornell University Ph.D. Dissertation, 2000). The formation of (trans, trans)-2,4 decadienal take place at a slow rate at room temperature (Frankel, 1988), however this reaction is enhanced because of thermal degradation during soybean processing under hot conditions (Lin, 2003). Other contributors to odors were (trans)-4,5-epoxy-(E)-2-decenal (formed from 2,4 decadienal), (trans, cis)-2,6-nonadienal, (trans)-2-nonenal, (trans, trans)-2,4-nonadienal, 2,4 nonadienal, maltol, vanillin and  $\beta$ -damascenone. The most powerful odorants in soymilk determined by the minimum headspace volume required to detect by olfactometry, were hexanal, acetaldehyde, methanethiol, dimethyl trisulfide, and 2-pentyl furan (Boatright, 2002).

[0007] The strongest odorants in soy protein isolates were identified as dimethyl trisulfide, (trans, trans)-2,4-decadienal, 2-pentyl pyridine, (trans, trans)-2,4-nonadienal, hexanal, acetophenone, and 1-octen-3-one (Boatright and Lei, 1999). The mechanism of formation of methanethiol and dimethyl trisulfide involves free radicals formed by lipid oxidation (Lei and Boatright, 2003) and products of enzymes such as cysteine synthase (Boatright, 2003, poster 45C-26, LFT annual meeting, Chicago).

[0008] The formation of 2-pentyl pyridine occurs from a spontaneous reaction between 2,4 decadienal and ammonia at room temperature. Free amino acids arginine, lysine, aspar-

agine and glutamine increase 2-pentyl pyridine formation probably by providing ammonia during soy protein processing. (Zhou and Boatright, 2000; Kim et al., 1996). Free amino acids can also form other undesirable products. High temperature exposure of asparagine and glucose results in the formation of acrylamide (Jung et al., 2003). Arginine exposed at cooking temperatures can form mutagens (Knize et al., 1994). Free arginine was enriched in soybeans lacking both 3-conglycinins and glycinins (Takahashi et al., 2003).

[0009] Once formed, odors are difficult to remove from soy ingredients because they are associated with proteins (Franzen and Kinsella, 1974). The quality of natural flavors added to soy foods are also altered unfavorably because some of the odors bind to soy protein. Carbonyl compounds and 2-pentyl pyridine bound with greater affinity to glycinin fractions than  $\beta$ -conglycinin fractions (Zhou et al., 2002; O'Keefe et al., 1991). The extraction of oil-body-associated proteins and polar lipids significantly reduced the quantity of odors associated with soy protein isolate (Samoto et al., 1998).

[0010] Textures created by protein-protein interactions can have more effect on flavor intensity than the in-nose odor concentration (Weel et al., 2002). Soy proteins can contribute to the poor organoleptic quality of soy beverages by forming insoluble aggregates and chalky mouthfeel (Skarra and Miller, 2002). Among the main soy proteins, glycinins are more sensitive to pH and  $\text{Ca}^{+2}$ -induced insolubilization (Yuan, 2002) and soybeans containing a low ratio of glycinins to  $\beta$ -conglycinins are useful for creating soluble soy protein ingredients (U.S. Pat. No. 6,171,640). Lipid oxidation reactions also influence protein solubility. Antioxidants can be added during soy protein isolate manufacture to limit free radical induced oxidation of proteins and improve the yield of soluble protein (U.S. Pat. No. 5,777,080). Some peptides can react during processing with polysaccharides to form antioxidant compounds (Matsumura, 2003).

[0011] Color influences perceptions of freshness and taste (Joshi, 2000). Low amounts of reducing sugar and aldehydes formed from lipid oxidation react with amino groups of proteins on heating to form brown pigments by the Maillard's browning reaction (Kwok et al., 1999). Soymilk with a higher content of aldehydes will create a darker, less appealing color after heat processing. On the other hand lipid oxidation during soymilk processing decolorizes yellow pigments in soymilk (Obata and Matsuura, 1997).

[0012] Soybeans are refined to improve the flavor by extracting lipids and other components either by alcohol extraction, enzyme treatments, washing protein curds, ultrafiltration of protein and or use of flash vaporization. These processes add to the cost of the soy protein ingredients and typically lower the amounts of healthful components that are bioavailable (for example fiber, oligosaccharides, isoflavones, polyunsaturated fatty acids, tocopherols, phospholipids, bioactive peptides). Processing approaches used to improve the organoleptic properties of soy protein ingredients are limited in effectiveness by odors bound to soy proteins and by conditions that promote odor formation (pH 8-10). Soybeans that lack one to three of the lipoxygenases 1, 2, and 3 were created using mutation breeding to reduce the formation of beany odors (Hajika et al., 1991). Aroma analysis of soymilk and soy flour made from soybeans lacking the three lipoxygenases were found to contain lower amounts of several odors, but higher amounts of 1-octen-3-ol than the parent soybean line containing all three lipoxygenases (Hao et al., 2002). Similar levels of 2,4 decadienal were found in

defatted flour and soy protein isolate made from one soybean lacking three lipoxygenases and two other soybean lines (Boatright et al., 1998). Soy foods prepared from soybeans lacking lipoxygenases had improved flavor compared to foods made from control soybeans (Wilson, 1996). Soymilk prepared from soybeans lacking three lipoxygenases was perceived as more bitter than the control, especially after 15 months of seed storage, but this difference was expected to be eliminated by adding sugar (Torres-Penaranda and Reitmeier, 2001).

**[0013]** Transgenic modifications are proposed to improve the flavor of soybeans by reducing the levels of polyunsaturated fatty acids (U.S. Pat. No. 5,981,781), lipoxygenases (U.S. Patent Appl. 20030074693) and or hydroperoxide lyases (U.S. Pat. No. 6,444,874).

**[0014]** Soybeans containing less than 10% polyunsaturated fatty acids and greater than 75% oleic fatty acids yield frying oil that is less tasty compared to frying oil with higher polyunsaturated fatty acids (Warner et al., 2001).

**[0015]** Chemicals such as polyphosphates (U.S. Pat. No. 6,355,296) can be used to limit off-flavor production and improve protein solubility. Other additives such as gallic acid (PCT WO 01/06866) or aldehyde oxidase (Maheshwari et al., 1997) can be used to remove odors.

**[0016]** There is little published information on the effects of natural genetic variations on flavor and color attributes of soybeans. The thiobarbituric acid number for 16 soybean varieties was determined as a measure of lipid oxidation and no correlation was found with the vitamin E content of the soybeans (Dahuja and Madaan, 2004). The amounts of 2-pentyl pyridine and 2,4 decadienal in soy flour and soy protein isolate made from three soybean varieties were determined (Zhou and Boatright, 1999). The effects of drying conditions on the removal of the green pigment, chlorophyll from soybeans were studied (Salete et al., 2003; Sinnecker et al., 2002).

**[0017]** In past decades scientists showed that oils prepared from soybeans lacking lipoxygenases did not have improved oxidative stability. Soybean proteins produced from lipoxygenase null soybeans still contained significant levels of beany taste (Maheshwari et al., 1997).

**[0018]** The first step in making soymilk or soy protein ingredients is to dehull (or decoat) the soybeans to create soybean meats. Hypocotyls may also be separated from the cotyledons. Soybean meats are defined as dehulled soybeans and may or may not include cotyledons. A method for preparing meats is described, for example, in U.S. Pat. No. 5,727,689. One method for dehulling includes, but is not limited to, running seeds between counter-current rollers or a cracking mill and aspirating light weight hulls, leaving the meats. Meats may be soaked in water to produce soymilk or flaked and extracted using hexane as an initial step in making defatted soy flour, soy protein concentrates, soy protein isolates and purified protein fractions, as desired.

**[0019]** The present invention provides a new method to determine the ability of soybean meats to resist production of key odor compounds identified as 2,4 decadienal, hexanal, hexanol and 1-octen-3-ol. These compounds were selected as indicators of the extent of different types of oxidation reactions. Hexanal and hexanol result from the breakdown of hydroperoxide containing compounds (peroxides on 9 and 12 carbon positions of fatty acids) by hydroperoxide lyases and alcohol dehydrogenases. 2,4 decadienal is a breakdown product of the lipoxygenase pathway which is not known to

involve hydroperoxide lyases. 1-octen-3-ol is formed by the action of hydroperoxide lyases on hydroperoxides formed on the 10 carbon position of linoleic acid. These compounds can react further by additional processing to form more potent odors. For example, 2,4 decadienal is involved in the formation of 2-pentyl pyridine and 1-octen-3-ol is involved in the formation of 1-octen-3-one.

#### SUMMARY OF THE INVENTION

**[0020]** In one aspect, the invention provides a soybean meat composition produced from soybeans comprising lipoxygenases 1, 2 and 3, wherein the composition comprises greater than 10% linoleic acid as a percentage of total fatty acids and less than 20  $\mu\text{g}$  of total 2,4 decadienal plus hexanal plus hexanol per gram following oxidation under mild aqueous conditions. The composition may or may not comprise the lipoxygenases, or any combination thereof and may comprise deactivated lipoxygenase. In one embodiment, the composition comprises lipoxygenase-2. In certain embodiments, a composition provided by the invention may comprise less than about 15  $\mu\text{g}$  or less than about 18  $\mu\text{g}$  of total 2,4 decadienal plus hexanal plus hexanol. In further embodiments, the composition may comprise about 6  $\mu\text{g}$ -about 20  $\mu\text{g}$ , about 10  $\mu\text{g}$ -20  $\mu\text{g}$  or about 12-18  $\mu\text{g}$  total 2,4 decadienal plus hexanal plus hexanol. In other embodiments, the composition may comprise less than 4% linolenic acid as percent of total fatty acids, including less than about 3% and from about 1%-4% or about to about 2%-about 4%.

**[0021]** In another embodiment, a composition of the invention may comprise less than 2000  $\mu\text{g}$  per gram free arginine and/or less than 400  $\mu\text{g}$  free asparagine per gram, including less than about 1800  $\mu\text{g}$  per gram free arginine and/or less than about 350  $\mu\text{g}$  free asparagine per gram. Such a composition, in certain embodiments, may comprise about 300  $\mu\text{g}$ -2000  $\mu\text{g}$  per gram free arginine, including about 500-2000, about 1200-1800 and about 1000-2000  $\mu\text{g}$  per gram free arginine. A composition provided by the invention may further, in certain embodiments, comprise from about 50  $\mu\text{g}$  about 400  $\mu\text{g}$  free asparagine per gram, including about 100-400, 100-350, 200-400, 300-400 and 250-400  $\mu\text{g}$  free asparagine per gram.

**[0022]** In still another embodiment, the compositions provided by the invention may have a color measured as  $b^*$  value of less than 30 and an  $L^*$  value greater than 80, as monitored by the CIE- $L^*a^*b^*$  system where  $L^*$  indicates lightness and  $b^*$  indicates hue on a blue (-) to yellow (+) axis. In certain embodiments, a composition of the invention may comprise a color measured as  $b^*$  value of about 18-30, about 20-30, about 25-30 and less than about 25. In further embodiments, a composition provided by the invention may comprise an  $L^*$  value of, about 80-100, about 80-90 and greater than about 90. In certain embodiments, a composition provided by the invention may comprise less than 8  $\mu\text{g}$  of 1-octen-3-ol content per gram following oxidation under mild aqueous conditions, including less than about 6  $\mu\text{g}$ , less than about 5  $\mu\text{g}$ , from about 1.3-about 8  $\mu\text{g}$ , from about 2 about 8  $\mu\text{g}$ , and from about 4-about 8  $\mu\text{g}$ . A composition provided by the invention may also have greater than 30% of the protein as  $\beta$ -conglycinin and may have less than 25% of the protein as glycinin. Such a composition may be further defined as having greater than about 40% of the protein as  $\beta$ -conglycinin, and having a  $\beta$ -conglycinin content of from about 30%-about 60%, about 40%-60%, about 35%-55%, and about 30%-about 50% of the protein. Such a composition may be further defined as having

a glycinin content of less than about 20%, 15% and 10%, and may comprise from about 0%-25%, 5%-20%, 1%-25%, and about 10-25% of the protein as glycinin.

**[0023]** In another aspect of the invention, a soybean meat composition is provided having greater than 30% of the protein as  $\beta$ -conglycinin and less than 25% of the protein as glycinin, less than 5,000  $\mu\text{g}$  per gram of free arginine, and less than 900  $\mu\text{g}$  per gram free asparagine. Such a composition may comprise, in certain embodiments, from about 300-5,000  $\mu\text{g}$  per gram, from about 1,000-5,000  $\mu\text{g}$  per gram, from about 3,000-5,000  $\mu\text{g}$  per gram, from about 1,000-4,000  $\mu\text{g}$  per gram, and from about 500-2,000  $\mu\text{g}$  per gram of free arginine. Such a composition may comprise, in certain embodiments, less than 400  $\mu\text{g}$  per gram free asparagine, from about 50-400  $\mu\text{g}$  per gram, from about 100-400  $\mu\text{g}$  per gram, from about 100-700  $\mu\text{g}$  per gram, and from about 200-900  $\mu\text{g}$  per gram free asparagine. In one embodiment, the composition has less than 2,000  $\mu\text{g}$  per gram of free arginine and less than 400  $\mu\text{g}$  per gram free asparagine.

**[0024]** In another embodiment, the composition provided comprises less than 4  $\mu\text{g}$  per gram 1-octen-3-ol content following oxidation under mild aqueous conditions, including less than about 3  $\mu\text{g}$ , from about 1.3  $\mu\text{g}$ -3  $\mu\text{g}$ , from about 1.3  $\mu\text{g}$ -4  $\mu\text{g}$  and from about 2-4  $\mu\text{g}$  per gram. In still further embodiments, the composition has a linolenic acid concentration between 1% and 14% of the total fatty acids, including about 3-14%, about 5-14%, about 1.5%-12%, about 3-12% and about 7-14%. In yet another embodiment, the composition has a linoleic acid concentration between 10% and 60% of the total fatty acids, including between about 10% and 50%, between about 10% and 40%, between about 15% and 60%, between about 20% and 50%, and between about 20% and 60%.

**[0025]** In certain embodiments, a soybean meat composition provided by the invention may be defined as lacking one or more lipoxigenases. In one embodiment, a soybean meat composition provided by the invention may be defined as lacking lipoxigenase-2. In further embodiments, any combination of lipoxigenase-1, lipoxigenase-2 and/or lipoxigenase-3 are absent, including any two or all three of these lipoxigenases. A composition of the invention may also be defined as having a color characterized by  $b^*$  value less than 30 and an  $L^*$  value greater than 80 as monitored by the CIE- $L^*a^*b^*$  system where  $L^*$  indicates lightness and  $b^*$  indicates hue on a blue (-) to yellow (+) axis. In still other embodiments, a composition provided by the invention may comprise 67-69 mg lysine per gram of protein, may comprise 72-80 mg arginine per gram of protein and/or may comprise 28-30 mg histidine per gram of protein.

**[0026]** In yet another aspect, the invention provides a method of analyzing the odor producing properties of a soybean comprising determining the level of at least one compound selected from the group consisting of 2,4 decadienal, hexanol, hexanal, and 1-octen-3-ol. In one embodiment, the method may comprise determining the level of the compound comprises incubating a mixture of about 1 part of soybean seed flour and about 4 parts of water for a period in the range of about 1 to about 40 minutes and quantifying amounts of at least one compound selected from the group consisting of 2,4 decadienal, hexanol, hexanal, and 1-octen-3-ol and combinations thereof, using deuterated standards for hexanal, hexanol and decadienal. The soybean seed flour may be made from dehulled soybeans.

**[0027]** In still yet another aspect, the invention provides a method of obtaining a soybean variety producing soybeans and soybean meats with decreased odor producing properties comprising measuring the level of at least one compound selected from the group consisting of 2,4 decadienal, hexanol, hexanal, 1-octen-3-ol, and any combination thereof in one or more soybeans or soybean meats from first and second soybean varieties and selecting the variety producing seeds with lower levels of the compound. The method may further comprise crossing a plant of the selected variety with a different plant to produce progeny and measuring the level of at least one compound selected from the group consisting of 2,4 decadienal, hexanol, hexanal, 1-octen-3-ol, and any combination thereof in one or more soybeans or soybean meats from the progeny.

**[0028]** In still yet another aspect, the invention provides a method for selecting a soybean variety that resists fungal contamination comprising selecting a variety that comprises less than 5  $\mu\text{g}$  1-octen-3-ol per gram of seed as measured by incubating a mixture of about 1 part of soybean seed flour and about 4 parts of water for a period in the range of about 1 to about 40 minutes and measuring the 1-octen-3-ol.

**[0029]** In still yet another aspect, the invention provides a seed of the soybean plant designated 0119149, representative seed of which have been deposited under ATCC Accession No. PTA-6197. The invention further provides a soybean plant 0119149 or parts thereof produced by growing such seed. Such a plant of the invention may comprise a transgene. In still other embodiments, the invention provides a method of producing a soybean plant derived from the soybean plant 0119149, the method comprising the steps of: (a) preparing a progeny plant derived from soybean plant 0119149 by crossing a plant of the soybean plant 0119149 with a second soybean plant, wherein a sample of the seed of the soybean plant 0119149 was deposited under ATCC Accession No. PTA-6197; (b) crossing the progeny plant with itself or a second plant to produce a seed of a progeny plant of a subsequent generation; (c) growing a progeny plant of a subsequent generation from the seed and crossing the progeny plant of a subsequent generation with itself or a second plant; and (d) repeating steps (b) and (c) for an addition 3-10 generations to produce an inbred soybean plant derived from the soybean plant 0119149.

**[0030]** In still yet another aspect, the invention provides soybeans with improved organoleptic properties (i.e. soybeans with improved taste, color, odor and mouth feel properties), following oxidation under mild aqueous conditions. Also provided are soybeans with lighter color to improve the organoleptic properties of soybeans. Further provided are soybeans with low amounts of free arginine and asparagine to improve the organoleptic properties of soybeans. In another embodiment, soybeans with reduced levels of linoleic and linolenic acids are provided to improve organoleptic properties.

**[0031]** A soybean plant provided by the invention may comprise, in one embodiment, one or more transgenes. Examples include a gene conferring herbicide resistance that will produce plants with herbicide resistance and a gene conferring insect resistance.

**[0032]** In accordance with the invention, soybean seed are provided containing lipoxigenases 1, 2 and 3 and greater than about 10% linoleic acid as a percentage to total fatty acids that produces less than 20  $\mu\text{g}$  of total 2,4 decadienal plus hexanal

plus hexanol per gram of ground seeds following oxidation under mild aqueous conditions.

**[0033]** In accordance with another aspect of the invention, soybeans containing lipoxygenases are provided having less than about 4% linolenic fatty acid and greater than about 10% linoleic acid as percent of total fatty acid and producing less than 20  $\mu\text{g}$  of total 2,4-decadienal plus hexanal plus hexanol per gram of ground seeds following oxidation under mild aqueous conditions. The same soybeans may also produce less than 8  $\mu\text{g}$  of 1-octen-3-ol content per gram of ground seeds following oxidation under mild aqueous conditions.

**[0034]** In accordance with still yet another aspect of the invention, the invention also provides, soybeans having less than about 2000  $\mu\text{g}$  free arginine and less than about 400  $\mu\text{g}$  free asparagine per gram dry seed weight and producing less than about 20  $\mu\text{g}/\text{gm}$  of 2,4-decadienal, hexanal and hexanol per gram ground soybeans following oxidation under mild aqueous conditions. The same seeds may also produces less than 8  $\mu\text{g}$  of 1-octen-3-ol content per gram ground soy seeds following oxidation under mild aqueous conditions.

**[0035]** In accordance with still yet another aspect of the invention, soybeans are provided having yellow color measured as "b\* value" of less than 30 and producing less than 20  $\mu\text{g}/\text{gm}$  of 2,4-decadienal, hexanal and hexanol per gram ground soybeans following oxidation under mild aqueous conditions. The same seed may also produce less than 8  $\mu\text{g}$  of 1-octen-3-ol content per gram of ground seeds following oxidation under mild aqueous conditions.

**[0036]** In accordance with still yet another aspect of the present invention, soybeans are provided having greater than 30% of the protein as  $\beta$ -conglycinin and or less than 25% of the protein as glycinins that produce less than 20  $\mu\text{g}/\text{gm}$  of 2,4-decadienal, hexanal and hexanol per gram ground soybeans following oxidation under mild aqueous conditions. The same seed may also produce less than 8  $\mu\text{g}$  1-octen-3-ol per gram ground seeds following oxidation under mild aqueous conditions.

**[0037]** In accordance with the present invention, soybeans are provided comprising less than 5,000  $\mu\text{g}$  free arginine, less than 900  $\mu\text{g}$  free asparagine and greater than 30% of the protein as  $\beta$ -conglycinin and less than 25% of the protein as glycinins that produce less than 20  $\mu\text{g}/\text{gm}$  of 2,4-decadienal, hexanal and hexanol per gram ground soybeans following oxidation under mild aqueous conditions. The same seed may also produce less than 8  $\mu\text{g}$  1-octen-3-ol per gram of ground seeds following oxidation under mild aqueous conditions.

**[0038]** In accordance with still yet another aspect of the invention, soybeans are provided resulting from a cross of a first soybean seed having greater than 30% of the total protein as  $\beta$ -conglycinin and less than 25% of the protein as glycinins and a second soybean seed producing less than 20  $\mu\text{g}/\text{gm}$  of 2,4-decadienal, hexanal and hexanol per gram ground soybeans following oxidation under mild aqueous conditions.

**[0039]** In accordance with still yet another aspect of the invention, soybeans are provided resulting from a cross of a first soybean seed having less than 4% linolenic fatty acid and greater than 10% linoleic acid as percent of total fatty acids and a second soybean seed containing lipoxygenases 1, 2 and 3 and greater than 10% linoleic acid as a percentage of total fatty acids producing less than 20  $\mu\text{g}/\text{gm}$  of 2,4-decadienal, hexanal and hexanol per gram ground soybeans following oxidation under mild aqueous conditions.

**[0040]** In accordance with still yet another aspect of the invention, a method of analyzing the odor-producing property

of soybean seed varieties is provided comprising incubating a mixture of about 1 part of soybean seed flour and about 4 parts of water for a period in the range of about 1 to about 40 minutes and quantifying amounts of at least one compound selected from the group consisting of 2,4 decadienal, hexanal, hexanal, and 1-octen-3-ol and combinations of two, three or four thereof using deuterated standards for hexanal, hexanol and decadienal.

**[0041]** In accordance with still yet another aspect of the invention, a method of soybean breeding is provided comprising incubating a mixture of about 1 part of soybean flour or dehulled-soybean flour and about 4 parts of water for a period in the range of about 1 to about 40 minutes at room temperature and quantifying amounts of 2,4 decadienal, hexanol, hexanal, and 1-octen-3-ol using deuterated standards for hexanal, hexanol and decadienal and selecting seed from breeding populations based on the results.

**[0042]** In accordance with another aspect of the invention, soybeans are provided comprising a transgene, for example, a herbicide resistance gene imparting herbicide resistance or an insecticidal gene imparting insect resistance.

**[0043]** In accordance with another aspect of the invention, processed foods for human consumption are provided comprising soybeans having greater than 30% of the protein as  $\beta$ -conglycinin and less than 25% of the protein as glycinins that produce less than 20  $\mu\text{g}/\text{gm}$  of 2,4-decadienal, hexanal and hexanol per gram ground soybeans following oxidation under mild aqueous conditions.

#### DESCRIPTION OF ILLUSTRATIVE EMBODIMENTS

**[0044]** The invention provides soy compositions, soybeans, and soybean seed derivatives with improved organoleptic properties, and methods for producing the same. The soy compositions of the invention provide improved taste, color, odor and mouth feel properties. The invention also provides methods for producing such compositions and methods of determining the ability of a soybean variety to produce key odors identified as 2,4 decadienal, hexanal, hexanol and 1-octen-3-ol and the use of the results to select seeds from breeding populations.

**[0045]** Oxidation conditions may be produced in accordance with the invention when approximately 0.5 soy flour is mixed with 2 mL of water or 1 part of soybean flour is mixed with 4 parts of water to disperse the solid particles in water and allow oxidization reactions to occur for approximately 1-40 minutes at room temperature wherein room temperature could vary from 15° to 40° C. The concentrated suspension allows enzymes, substrates, free radicals, free radical scavenging compounds, enzyme inhibitors, and other factors impact the amounts of odors produced.

**[0046]** The invention provides lipoxygenase containing soybeans and compositions derived therefrom having less than 4% linolenic fatty acid and/or greater than 10% linoleic acid as percent of total fatty acid and producing less than 20  $\mu\text{g}$  2,4-decadienal ( $\text{CH}_3(\text{CH}_2)_4\text{CHCHCHCHCHO}$ , CAS No. 25152-84-5) plus hexanal ( $\text{CH}_3(\text{CH}_2)_4\text{-CHO}$ , CAS No. 66-25-1) plus hexanol ( $\text{CH}_3(\text{CH}_2)_5\text{OH}$ , CAS No. 111-27-3) per gram of ground seeds following oxidation under mild aqueous conditions. The same soybeans may also produce less than 8  $\mu\text{g}$  of 1-octen-3-ol ( $\text{CH}_3(\text{CH}_2)_4\text{-CHOHCHCH}_2$ , CAS No. 3391-86-4) per gram of ground seeds or dehulled soy flour following oxidation under mild aqueous conditions. The compounds 2,4 decadienal, hexanol, hexanal, and

1-octen-3-ol and their combinations were used to quantify odor-producing properties of soybeans. Odors are not restricted to these listed compounds. Other detectable aldehydes, ketones and alcohols can be used as measures of odor producing properties using the method of the invention. Examples of these compounds include but are not restricted to Propanal, Pentenal, Pentanal, Hexenal, Pentenol, Heptanal, Heptenal, Benzaldehyde, Hexadienal, Heptadienal, Heptanol, Octenol, Octenal, Nonanal, Octadienone, 2 pentyl Furan, Pentanal, 2,3-dimethyl, Nonenal, Maltol, Decenal, and 2-Undecenal. According to the invention the term "lipoxygenase" refers to an enzyme that catalyzes oxidation of unsaturated fatty acids with oxygen to yield peroxides. The term "lipoxygenase" (EC. 1.13.11.12) is also referred as lipoxidase and dioxygenase in the art. Odors of soymilk and soy protein ingredients from soybeans lacking one, two or three of the lipoxygenases 1, 2 and 3 were assessed by other researchers. High oleic soybeans exist with less than 4% linoleic acid. Some of the soybeans having those traits may produce less than 20  $\mu\text{g}$  of total 2,4 decadienal plus hexanal plus hexanol per gram of ground soybeans using the assay of this invention and some will not fall in that range. It was discovered in this invention that is possible to create soybeans that contain lipoxygenases 1, 2 and 3 that produce very low levels of odors and that lipoxygenase-null soybeans can produce high levels of off-flavors. In addition to high  $\beta$ -conglycinin compositions that were not previously screened for odor-producing properties, the invention provides in particular new soybean compositions having lipoxygenases 1, 2 and 3 and greater than 10% linoleic acid. Linoleic fatty acid (18:2 n-6) and linolenic fatty acid (18:3 n-6) are polyunsaturated fatty acids with two or three cis double bonds according to the invention. The methods of the invention for selecting low odor-producing lines from the progeny of lipoxygenase-null soybeans or high oleic soybeans are within the scope of the invention.

**[0047]** The odor 1-octen-3-ol is a cleavage product of fatty acids having hydroperoxides on the 10 carbon position of linoleic acid. It was discovered in this invention that the removal of the hull substantially reduces the 1-octen-3-ol forming property of soybean compositions. Fungal lipoxygenases and hydroperoxide lyases form 10-hydroperoxides and 1-octen-3-ol, respectively (Wurzenberger and Grosch, 1984; Husson et al., 1998). It was reasoned in this invention that soybeans that produce lower amounts of 1-octen-3-ol, resist contamination of the soybean hull by fungus such as *Phomopsis* (Minor et al., 1995) and/or contain components that inhibit the fungal lipoxygenases.

**[0048]** The organoleptic properties of soybean products depend on the contents of glycinins and  $\beta$ -conglycinins. The glycinins are more prone to retaining odors and to forming insoluble particles that adversely affect the sensory quality of soybean products. The present invention provides soybeans having greater than 30% of the protein as  $\beta$ -conglycinin and less than 25% of the proteins as glycinins and will produce less than 20  $\mu\text{g}$  of total 2,4-decadienal plus hexanal plus hexanol per gram of ground seeds following oxidation under mild aqueous conditions. The same seeds may also produce less than 8  $\mu\text{g}$  of 1-octen-3-ol per gram of ground seeds under similar conditions. According to the present invention  $\beta$ -conglycinin refers to a protein trimer with a molecular weight mass of 150-200 kDa. Three major subunits of  $\beta$ -conglycinin are the  $\alpha'$  (72 kDa),  $\alpha$  (68 kDa), and  $\beta$  (52 kDa). The alpha-prime and alpha subunits contain two covalently bound car-

bohydrate moieties and the beta-subunit contains one. A review of the structure and properties of  $\beta$ -conglycinin and the other major storage protein, glycinin is given by Utsumi et al., (1997). One can use public germplasm such as "Moshidou Gong 503" to change the ratio of  $\alpha$  to  $\alpha'$  subunits of  $\beta$ -conglycinin using traditional breeding methods. The term  $\beta$ -conglycinin in this application includes these subunit variations. The seeds of the present invention having greater than 30% of the protein as  $\beta$ -conglycinin and/or less than 25% of the protein as glycinins are provided that comprise less than 5,000  $\mu\text{g}/\text{g}$  free arginine and less than 900  $\mu\text{g}/\text{g}$  free asparagine. The term "free" refers to amino acids that are not bound with other molecules present in the soybeans or soybean seed flour and can be extracted and solubilized by 5% aqueous solution of trichloroacetic acid (TCA) at 4° C. overnight. The value of selecting soybeans comprising low levels of free amino acids for producing high quality soybean meats, soymilk, soy flour, soy protein concentrates and soy protein isolates had not previously been demonstrated.

**[0049]** The color of soy ingredients and foods made according to the invention may be improved by reducing the levels of aldehydes formed (e.g. hexanal and 2,4 decadienal) because aldehydes react with amines to form brown pigments. Reduced levels of lipid oxidation products can also limit the oxidative bleaching of yellow pigments causing a final product color to be less white. The potential low bleaching problem is solved in this invention by selecting soybeans containing low levels of yellow pigment. The invention provides soybean compositions having yellow color measured as "b\*" value" of less than 30 and producing less than 20  $\mu\text{g}$  of total 2,4-decadienal plus hexanal plus hexanol per gram of ground seeds following oxidation under mild aqueous conditions. The "b\*" value as used herein to describe the color of soybean seed represents the CIE-L\*a\*b\* Color Scale (CIE, Colorimetry, Publication 15.2, Second Edition, Vienna (1986) using Colorflex procedure) and relates to blueness (negative numbers) to yellowness (positive numbers) of soybeans or soybean flour, similarly "L\*" value refers to lightness of soybeans or soybean flour on CIE-L\*a\*b\* Color Scale. Soybeans or soybean flour in one embodiment of the invention will have L\* value greater than 80.

**[0050]** The desired wild-type soybean, commercial cultivar, or hybrid thereof may be crossed by conventional plant breeding methods with a soybean plant having seeds with the low odor producing phenotype of the invention to create seeds comprising the low odor trait plus other desired traits (e.g., yield, high  $\beta$ -conglycinin composition, herbicide resistance). Hybrid progeny exhibiting the low odor trait and others desired phenotypes are selected. Breeding methods used in accordance with the present invention include, for example, methods described in Knowles and Briggs (1967) or any like methods known in the art. Specific methods for the selection and development new soybean varieties, for example, are disclosed in U.S. Pat. No. 6,653,534.

**[0051]** The invention also provides processed food for human consumption made from soybean compositions of the invention. Example of processed foods for human consumption may be made, for example, from a dehulled soy flour composition of the invention. Examples of these derivatives include but are not limited to, bars, beverages, meat and meat alternatives, soy yoghurt, cheese alternatives, nutritional supplements.

**[0052]** The following examples are included to demonstrate preferred embodiments of the invention. It should be

appreciated by those of skill in the art that the techniques disclosed in the examples which follow represent techniques discovered by the inventors to function well in the practice of the invention, and thus can be considered to constitute preferred modes for its practice. However, those of skill in the art should, in light of the present disclosure, appreciate that many changes can be made in the specific embodiments which are disclosed and still obtain a like or similar result without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

#### EXAMPLE 1

##### Materials and Methods

**[0053]** This example describes an analytical method of the present invention. The purpose of this analytical method is to determine the odor-producing properties of different soybean lines.

**[0054]** The method determines selected odors by first initiating odor formation. Grinding the seed into fine flour and activating enzymes using water caused the odors to form. Studies on the rate of formation indicated that at room temperature the formation of the odors were mostly complete after about 20 minutes (Table 1). This time was important to successful quantitation of the odor compounds and assessment of the odor producing properties of different soybean lines. After 20 minutes deuterated surrogates of hexanal, hexanol and 2,4 decadienal were added to provide internal standards. The reaction was stopped by the addition of sodium sulfate which was immediately followed with the addition of 10% methanol:ether to extract the aldehydes, alcohols and ketones. The method was not restricted to the compounds listed. All other detectable aldehydes, ketones and alcohols can be quantified but not with the precision as the three using deuterated surrogates.

but can range from 0.2 g to 0.7 g. The range of an odor concentration on a wet weight bases was from 0.2 µg/g to 120 µg/g.

**[0056]** Soybean Meat Sample Preparation: Whole seed or soybean meats were collected as samples. Approximately 6 to 12 randomly selected seed or the equivalent weight as soybean meat pieces from a sample were ground in a ball mill at approximately 1200 revolution per minute (rpm) for 1 minute to produce a fine flour. Ball mill for grinding seeds is described in a U.S. Patent Pub. 2003/0146313 A1. Number of seeds or soybean meats was determined to obtain approximately 0.5 to 1.0 g of soybean flour at the end of grinding. Freshly ground soybean flour was used for further analysis.

**[0057]** Extraction of soybean flour: Freshly ground soybean flour was used for extraction of the key odors. Approximately 0.5 g (0.48 to 0.52 g) of soy flour was taken in a 20 milliliter (ml) vial (VWR TraceClean™ clear borosilicate Teflon lined closure vials) with a cap. De-ionized water (2 ml) was added to the flour in the vial before replacing the cap. Contents of the vial were mixed for approximately 30 seconds (sec) in a vortex mixer to ensure that all soybean flour was properly hydrated in the vial. Hydrated soybean flour was allowed to incubate under mild aqueous conditions, which are defined herein as incubation in water at room temperature (22° C.). for 20 minutes (min). After 20 min, 11-0.3 grams of anhydrous sodium sulfate was added in the vial followed by addition of 10 ml of 10% methanol:ether solution in the vial. 30 microliters of a standard surrogate (mixture of deuterated standards for Hexanal, hexanol and decadienal) spiking solution was further added in the vial before recapping and shaking it for 30-40 min on a reciprocating shaker at ~200 rpm. After 30-40 minutes 1 ml of methanol:ether extract was placed into a Autosampler vial (Autosampler vial for 7683 HP Autosampler. Vendor is VWR) for further analysis.

**[0058]** Analysis of Soybean Flour Extracts: Soybean flour extracts in methanol:ether extraction medium were further analyzed in a Gas Chromatograph Agilent 6890 (395 Agilent Technologies, Palo Alto Calif. 94306) equipped with Agilent 7683 series autosampler and Leco time of Flight mass spectrometer with LECO Chrom TOF software (LECO Corpora-

TABLE 1

Time (minutes)	Hexanal (µg/g)	2 x st dev.	1-Octen-3-ol (µg/g)		2,4 Decadienal (ug g)		pH (A-4)	pH (A-5)	pH (A-10)	pH (A-14)
			3-ol	2 x st dev.	Decadienal	2 x st dev.				
0	1.3	2.6	0.8	0.4	0.4	0.4	6.4	6.4	6.3	6.3
5	11.8	0.2	2.9	0.4	6.9	0.7				
10	11.4	1.4	3.7	0.7	6.5	0.6	6.3	6.3	6.3	6.3
20	14.0	0.8	5.8	0.6	7.8	0.9	6.3	6.3	6.3	6.3
40	15.7	1.9	7.8	0.5	8.6	2.3	6.3	6.3	6.3	6.3
240	17.0	4.2	8.9	1.8	9.9	1.7				

Effect of time after mixing soybean flour with water (0.5 g flour 2 mL water, i.e. 1:4 ratio) on formation of flavor components. The pH of the suspensions for four different soybean lines (A-4, A-5, A-10 & A-14) was about 6.3.

**[0055]** The analytical time to determine these components in 175 samples takes 24 hours. This includes extraction and determination using gas chromatography/mass spectrometry. The size of the sample for analysis was typically 0.5 grams (g)

tion, St. Joseph, Mich. 49085). Gas chromatograph was also equipped with 10-meter DB-WAX or DB1701 gas chromatography column with film thickness of 0.4 or higher and ID 0.18 mm (Agilent Technologies). Methanol (methanol is EM

Science methanol purge and trap grade) was procured from VWR (VWR International West Chester, Pa. 19380); Ethyl ether (ethyl ether anhydrous) was obtained from Mallinckrodt (Mallinckrodt, Hazelwood, Mo. 63042). 2,4 decadienal, 85% trans (15% cis), Hexanal 98%, Hexanol, 99%, 1 octen-3-ol 98%, 2 undecanone 99%, 2 nonenal 97%, and 2,4 nonadienal 99% were procured from Sigma-Aldrich Company (Saint Louis Mo. 63103). Deuterated D<sub>12</sub> Hexanal, D<sub>13</sub> Hexanol, and D<sub>2</sub> 2,4 decadienal were made in house as described by Lin et al. (1999). For analysis of samples 1 micro-liter (μL) of sample was injected through the injection port of the gas chromatography apparatus (gc). Parameter used for analyzing samples in chromatograph were as follows: Chromatograph Parameters Column: DB-Wax or DB 1701 capillary 10 m×0.18 mm, 0.4 mm Film Injection Volume: 1 ul Injection liner: Split/splitless liner.

**[0059]** Temperature Program: Initial 55° C. for 1 min 40° C. to 175° C. @ 40° C./min, hold 0 min 175° C. to 240° C. @ 35° C./min, hold 0 min Inlet temperature: 220° C. Injection mode: Pulsed splitless with 8 psi initial for 1.5 minutes Split ratio: 20:1 Carrier gas: Helium @ 1.8 mL/min constant flow.

**[0060]** LECO Time Of Flight Parameters Temperature interface: 250° C. Source Temperature: 200° C. Mass Spectrometer source temperature: 150° C. Scan parameters: 50 to 250 m/z at approx 50 scans per second.

**[0061]** Quality control of Analysis: For each batch of samples a method blank and a spike was run at the same time. The spike was made by splitting one of the samples to be analyzed into two portions. The sample should be as homogenous as possible. The second portion was spiked with a known amount of hexanal, 1 octen-3-ol and decadienal. This addition was made at the time of the addition of the deuterated compounds in extraction procedure as described above. The concentration of the spiked and unspiked compounds was determined as the % recovery by the following formula:

$$\% \text{ recovery} = 100 * \frac{(C_s)(w_t) - (C_0)(w_t)}{X_s}$$

Where:

C<sub>s</sub> = concentration of spiked sample in μg/(gm wet wt).

C<sub>0</sub> = concentration of the unspiked sample in μg/(gm wet wt).

W<sub>t</sub> = mass of sample spiked in gm.

X<sub>s</sub> = micrograms spiked into sample.

**[0062]** The method blank followed the extraction procedure without the addition of the soy flour.

**[0063]** Accuracy and Precision of Analysis: The accuracy and precision was determined by running a homogenous soy flour sample and spiking the sample with a known level of the three compounds hexanal, 1 octen-3-ol and 2,4 decadienal. Unspiked samples were also analyzed to determine the amounts of spiked compound recovery. Different spike levels can be used as a standard addition method to determine basis due to systematic errors. The average value along with the standard deviation was determined. The average value was compared to the known level of material added to give an estimate of the accuracy of the method. The standard deviation gives the precision of the measurement. Note it is important to understand that these approaches only measures the analytical variability since grinding over 40 seeds to make the homogenous soy flour averages out the seed variability. A

larger variation may occur for the actual determination because of seed to seed variation. For hexanal the % recovery was 83.8%. For 1 octen-3-ol the % recovery was 93.6%. For 2,4 decadienal the % recovery was 99.3%. The hexanal was the least accurate based on the spike approach. This is believed to be because of the volatility of the hexanal and the fact that the method has low recoveries for hexanal. The octen-3-ol and 2,4 decadienal have higher degrees of accuracy using the spike approach. The precision as indicated by the % relative standard deviation of the recoveries was 5% for the hexanal and the octen-3-ol. For the 2,4 decadienal the precision as measured by the % relative standard deviation of the recovery values was 1.1%. Sample size was not found to have an effect from 0.2 to 0.9 grams for this homogenous sample but it may be a problem between samples. This was not tested. Thus sample size should be about 0.5 grams until sample size is known not to be a problem or the method is adjusted.

**[0064]** The detection range for all compounds is from 0.5 micrograms to 25 micrograms. Adding more standards to the standard curve can extend the range.

#### EXAMPLE 2

##### Identification and Selection of Low Odor Producing Soybeans

**[0065]** Potent odors were quantified in soymilk made from a control soybean (Vinton 81), a soybean lacking lipoxigenase-2 (QT-1) and a soybean lacking lipoxigenases 1,2 and 3 (IA2025). Soymilk was made from each soybean variety by soaking desired clean seeds in water in 1:5 ratio (1 gm weight:5 ml water at 25° centigrade (C) for about eight hours. After discarding soaking water, soybeans weighing twice the original weight were drained and blended with fresh distilled water (2× dry weight of soybeans) for five minutes. Additional distilled water (7× dry weight of soybeans) was blended into the slurry for about 2 minutes (min.) at 20° C. The slurry was simmered at 95° to 98° C. for 20 min. in a water bath and filtered through coarse woven cheesecloth and squeezed by hand to express as much soymilk as possible. The soymilk was pasteurized by simmering in a water bath at 85° to 90° C. for 10 minutes to reduce microbial contamination and stored at 4° C. before the extraction of odors for further analysis.

**[0066]** Odors were extracted from the soymilk samples. Soymilk was extracted with a 0.67 portion of Freon™ 113 for at least 30 minutes. After removal of the Freon™ 113 extract, the aqueous phase was further extracted with 0.67 portion of ethyl acetate. After the collection of ethyl acetate extract, the aqueous phase was discarded. Both Freon™ and ethyl acetate were filtered thru magnesium sulfate to remove as much water as possible and concentrated to 1 ml using Buchi 0.1 rotary evaporator. Freon extracts were evaporated under 48 kilopascal (kPa) and ethyl acetate under 86 kPa.

**[0067]** Potent odors produced in soymilks were quantified using GC-Olfactometry (Acree T. E., Analytical Chem. 69:170 A-175A, 1997). GC-Olfactometry is gas chromatography with a sniffing port where potency of a chemical compound as an odorant is measured as a measurement of human response to odorants in air stream or puffs. Hewlett Packard 6890 gas chromatograph equipped with a 12 m×0.32 mm cross-linked methyl silicone fused silica capillary column (film thickness=0.33 μm) was used for CharmAnalysis™ (Acree, T. E.; Barnard, J.; Cunningham D. G.; Food Chem. 14,



273-286, 1984). The effluent consisted of helium (2 ml/min) as the carrier gas and nitrogen as the make up gas (ca. 30 ml/min). The effluent was mixed with sniffing air (20 L/min) which was 99% laboratory air humidified to between 50% and 75% and passed through the sniffer via a 10 mm diameter silylated pyrax tube. The GCMS oven was programmed to start increasing its temperature three mins from the initial temperature of 35° C. to 225° C. at 6° C./min. Further details of the method for CharmAnalysis™ in soybean milk can be found in Ph.D. Dissertation presented to the faculty of Graduate School of Cornell University by Yu-Wen Feng.

**[0068]** Both soybeans lacking lipoxigenase-2 (IA2025, QT-1) produced lower levels of hexanal than the control (Table 2). The soybean lacking all three lipoxigenases (IA2025) produced the highest levels of 2,4 decadienal and 1-octen-3-one, while the soybean lacking lipoxigenase 2 (variety QT-1) had the lowest levels of all five potent odors (Table 2). It was apparent from the results that unknown compositional factors other than lipoxigenase-2 were involved in controlling lipid oxidation in soybeans. The soybean variety QT-1, was identified as a useful variety for creating commercial low odor soybean varieties comprising or not comprising lipoxigenase-2. The method of example 1 was developed to identify progeny of QT-1 and other soybean lines that produce low amounts of 2,4 decadienal, hexanal, hexanol and 1-octen-3-ol.

TABLE 2

Charm value of odors present in soy milk prepared from three varieties of soybeans. The proprietary Monsanto line produced lower levels of off-flavors than a triple lipoxigenase-null (IA2025) and tofu soybean (Vinton 81).					
Soybean Lines	1-octen-3-one	2,4 decadienal	E,Z-2,6 nondienal	E-2 nonenal	Hexanal
Low odor line QT-1	3	25	11	67	69
IA2025	182	208	168	165	73
Vinton 81	65	71	186	165	224

**[0069]** Table 3 describes soybean crosses that were used to develop the progeny for describing the invention. Standard methods of plant breeding were used to generate these lines.

TABLE 3

Soybean crosses used to develop progeny used to describe the soybeans and methods of the invention.	
Crosses	Cross type
MonQT-1/A346E>	A
A22 47/6P24S	B
A2533/IA2027	C
IA2 032/A3469	D
CP3469/IA2025	E

## EXAMPLE 3

Demonstration of Year-to-year and Location-to-location Consistency of Low Odor-producing, Low Color and Low Free Amino Acid Characteristics in Soybean Lines Selected According to the Invention

**[0070]** For color evaluation whole seeds were collected as samples. Desired number of selected seed from a sample was

ground in a Mega-Grinder at an rpm of 1200 for 1 minute to produce a finely ground soybean flour. Mega-Grinder for grinding seeds is described in a U.S. Patent Pub. 2003/0146313 A1. Freshly ground soybean flour was used for further analysis.

**[0071]** ColorFlex Spectrocolorimeter Model 45/0 color measuring system made by Hunter labs (Hunter Associates Laboratory Inc, Reston Va., USA) was used for measuring color of soybean flour by standard operation procedure suggested by the manufacturer. The colors were measured on CIE-L\*a\*b\* color scale (CIE, Colorimetry, Publication 15.2, Second Edition, Vienna, 1986) using ColorFlex procedure. International Commission on Illumination—abbreviated as CIE from its French title Commission Internationale de l'Eclairage—is an organization devoted to international cooperation and exchange of information among its member countries on all matters relating to the science and art of lighting. The L\* value relates to the lightness of the soybean flour while the b\* value relates to the blue (negative numbers) to yellowness (positive numbers) of the soybean flour. Color values of Soybean Flour made from different lines are shown on Tables 4 and 5.

**[0072]** Free amino acids: Unground samples were stored in a temperature/humidity controlled, secured (APHIS approved) room. Samples were ground using the CAT Mega-Grinder to create soy flour and stored at 4° C. in seed storage room. Soy flour was extracted with 5% TCA at 4° C. overnight, centrifuged and extracts were stored at -80° C. Extracts were filtered, diluted if necessary and analyzed for free amino acids by the OPA method. The OPA method uses o-phthalaldehyde (OPA) to derivatize samples before injection onto a C18, reverse phase HPLC column. The derivatized, primary amino acids are efficiently separated by R-group and quantitatively detected by a sensitive fluorometer. The relative standard deviation for this method is ~3%.

**[0073]** Lipoxigenase activity: Samples were ground using a Mega-Grinder. Each freshly ground sample was weighed out in triplicate (5 mg±1) and placed into a specific well of a 2-ml 96-well extraction plate. Samples were extracted with 0.1M K<sub>2</sub>HPO<sub>4</sub> (pH 7.0 or pH 9.) for 1 hour at room temperature. After centrifugation, supernatant obtained was used to measure the consumption of linoleic acid (substrate) using a spectrophotometer followed by determination of total protein per sample using Bio Rad Protein Dye. Lipoxigenase unit was calculated by using the absorbance changes at 230 nm during a 1-minute reaction and using the extinction coefficient ( $\epsilon=23,000 \text{ M}^{-1} \text{ cm}^{-1}$ ). The concentration of substrate consumed during the reaction was calculated by substituting each value into the equation  $A=\epsilon bC$ . One unit of lipoxigenase was defined as the  $\mu\text{moles}$  of substrate consumed per min and mg total extracted protein. By using reagent solutions prepared at pH 7.0 or pH 9.0, this assay allows one to measure the levels (Units of enzyme) of lipoxigenase-2/-3 or lipoxigenase-1, respectively. Lipoxigenase unit results are given as LOX Units pH 7.0 for Lipoxigenase-2 and 3 activities and as a LOX Units pH 9.0 for Lipoxigenase-1 activity.

**[0074]** Results: Soybean odor characteristics persisted when grown in multiple locations (Table 4) and multiple years (Tables 4 and 5). Soybeans lines that produced low levels of hexanal, hexanol and 2,4 decadienal and 1-octen-3-ol in the odor assay (described in Example 1) were consistently different from lines that produced high levels (Table 4). The soybean lines in Table 4 were sorted in order of the levels of hexanal+hexanol+2,4 decadienal that the lines produced.

For example line A-1 at the top of the table produced 18.21+/-4.21 µg/g of the three odors in contrast with line A-18 at the bottom of the table that produced 65.74+/-21.97 µg/g of the three odors. The amounts of hexanal plus hexanol produced by the soybeans from the same cross (e.g., cross A) correlated with the amounts of 2,4 decadienal produced (Table 6, R<sup>2</sup>=0.85) suggesting similar mechanisms and control by genetic and compositional variations. The levels of 1-octen-3-ol produced were independent of hexanal, hexanol and 2,4 decadienal (Table 6, R<sup>2</sup>=0.01) suggesting a different mechanism of

formation and control. Soybeans lines grown in 2-3 locations that produce low levels of 1-octen-3-ol were consistently different from lines than produced high levels of 1-octen-3-ol. For example, line A-18 produced 4.70+/-0.88 µg/g 1-octen-3-ol and line A-12 produced 14.67+/-2.37 µg/g 1-octen-3-ol. One can select lines that have a combination of genetic compositions that produce lower levels of 1-octen-3-ol and lower levels of 2,4 decadienal plus hexanal plus hexanol (e.g., line A-6).

TABLE 4

A & B: Color and odors produced by ground soybeans made from the progeny of crosses A, B, C, D and E as shown on Table 3. Values are the mean and standard deviation (Stdev) for each line grown in two or three locations in 2002 (Ames, Iowa; Oxford, Indiana; Gladbrook, Iowa). Progeny of cross B listed had low linolenic acid content of 2.9 +/-0.4% of total fatty acids. All line shave yellow hilum. Ground soybeans had moisture content of 8%. Color values are L\* (lightness), a\* (green-red), and b\* (blue-yellow).

A.1:													
Cross Type	Hexanal (µg/g)	Hexanol (µg/g)	Hexanal + Hexanol (µg/g)	1-octen-3-ol (µg/g)	2,4 decadienal (µg/g)	2,4 Decadienal + Hexanal		Hexanol (µg/g)	Hexanol (Stdev)	Hexanal + Hexanol (µg/g)	1-octen-3-ol (µg/g)	2,4 Decadienal (µg/g)	2,4 Decadienal + Hexanal (µg/g)
						Hexanal (µg/g)	Hexanol (Stdev)						
C 1,L-null	9.78	1.06	10.84	10.79	6.23	17.07	3.36	0.93	4.29	0.34	0.64	4.93	
A-1	9.80	1.89	11.68	9.14	6.53	18.21	1.35	1.04	2.38	0.14	2.16	4.54	
B-1	9.88	0.34	10.22	10.03	8.52	18.75	3.93	0.48	4.42	3.39	0.06	4.48	
D-1,L-null	12.59	0.92	13.51	13.51	5.24	18.75	1.89	0.83	2.16	3.11	2.46	4.58	
A-2	11.86	1.25	13.11	8.59	6.55	19.66	2.15	1.09	3.23	2.90	0.63	2.64	
B-2	11.99	1.78	13.76	8.19	6.91	20.67	4.07	1.11	5.18	2.98	1.37	6.55	
A-3	12.60	1.53	14.12	10.87	7.29	21.41	3.23	0.23	3.45	3.48	2.09	5.34	
A-4	14.76	2.30	17.05	9.29	6.32	23.38	2.71	0.23	2.94	2.16	1.29	4.01	
A-5	14.95	1.38	16.33	9.08	7.65	23.98	4.94	0.17	5.08	1.02	4.55	9.55	
A-6	17.58	3.21	20.79	4.63	8.97	29.75	1.26	1.36	2.62	2.90	5.38	2.76	
A-7	19.38	3.35	22.73	7.71	8.39	31.12	3.03	1.76	3.49	1.54	2.69	3.73	
A-8	23.69	2.73	26.42	9.14	11.51	37.93	10.64	0.69	10.90	3.53	4.64	15.49	
A-9	25.45	3.41	28.86	10.12	10.53	39.39	6.71	0.52	6.25	0.93	1.70	7.60	
A-10	27.14	3.11	30.25	10.24	9.92	40.17	4.99	2.04	6.19	1.31	2.73	7.86	

  

A.2:													
Cross Type	Hexanal (µg/g)	Hexanol (µg/g)	Hexanal + Hexanol (µg/g)	1-octen-3-ol (µg/g)	2,4 decadienal (µg/g)	2,4 Decadienal + Hexanal		Hexanol (µg/g)	Hexanol (Stdev)	Hexanal + Hexanol (µg/g)	1-octen-3-ol (µg/g)	2,4 Decadienal (Mg'g)	Decadienal + Hexanal (µg/g)
						Hexanal (µg/g)	Hexanol (Stdev)						
A-11	31.27	4.05	35.32	8.71	11.62	46.94	11.65	1.21	11.65	2.18	5.95	17.16	
A-12	32.72	3.64	36.36	14.67	15.18	51.54	6.84	0.08	6.91	2.35	1.35	5.56	
A-13	35.16	4.30	39.46	10.03	12.33	51.78	8.20	0.99	9.20	3.74	2.74	11.94	
B-3	35.16	3.66	38.82	7.18	15.57	54.39	7.59	0.45	8.04	1.03	2.93	10.97	
E	34.26	4.41	38.67	5.35	16.27	54.94	10.47	0.24	10.23	0.88	5.00	15.22	
A-14	35.24	2.23	37.47	7.06	18.94	56.41	10.58	1.72	8.85	0.05	10.16	19.02	
A-15	40.01	4.12	44.14	8.25	14.89	59.02	0.81	1.13	1.86	1.91	2.39	4.20	
A-16	37.88	5.66	43.53	8.17	16.80	60.33	13.20	2.33	14.11	3.43	4.61	16.31	
D-3	49.52	2.26	52.38	11.40	10.69	63.07	15.72	3.94	18.82	0.86	7.14	25.31	
B-4	41.26	4.04	45.30	6.90	18.02	63.32	12.25	0.33	12.58	1.27	7.67	20.25	
B-5	37.99	7.22	45.21	9.04	18.51	63.72	18.14	1.04	19.17	0.01	7.55	26.72	
A-17	42.44	4.72	47.16	9.32	16.99	64.15	5.64	1.02	5.38	2.59	6.57	11.78	
D-2	43.54	10.71	54.26	7.62	10.08	64.34	14.71	1.41	13.30	2.41	4.44	17.74	
B-6	40.64	4.97	45.60	9.96	18.82	64.42	5.56	2.45	7.07	1.06	3.56	10.62	
A-18	42.56	5.46	48.01	4.70	17.72	65.74	11.59	2.08	13.50	0.88	8.51	21.97	

TABLE 4-continued

A & B: Color and odors produced by ground soybeans made from the progeny of crosses A, B, C, D and E as shown on Table 3. Values are the mean and standard deviation (Stdev) for each line grown in two or three locations in 2002 (Ames, Iowa; Oxford, Indiana; Gladbrook, Iowa). Progeny of cross B listed had low linolenic acid content of 2.9 +/-0.4% of total fatty acids. All line shave yellow hilum. Ground soybeans had moisture content of 8%. Color values are L\* (lightness), a\* (green-red), and b\* (blue-yellow).

B1:													
Cross Type	Free Arg (µg/g)	Free Asn (µg/g)	Free Arg Asn (µg/g)	Free Arg. (Stdev)	Free Asn (Stdev)	L*	a*	b*	L* (Stdev)	a* (Stdev) b*	(Stdev)	Lipoxygenase activity pH 7	Lipoxygenase activity pH 7 Stdev
C-1, L-null	845	185	1030	535	9	79.77	1.49	30.38	1.21	0.9	2.6	0.23	0.58
A-1	1824	331	2155	1241	39	84.35	0.03	25.35	0.9	0.15	1.25	0.14	0.94
B-1	968	95	1063	541	63	80.23	0.53	28.38	0.39	0.26	2.49		
D-1, L-null	1105	91	1196	89	2	82.95	0.54	25.78	3.02	0.47	4.47		
A-2	1621	321	1941	278	155	85.42	0.18	22.16	0.94	0.03	1.39		
B-2	979	78	1058	865	32	81.7	0.22	28.7	1.5	0.23	2.54		
A-3	3157	420	3578	278	119	84.84	0.27	23.28	0.16	0.21	1.44		
A-4	1666	179	1845	687	104	85.73	0.01	22.21	0.68	0.04	1.26	1.69	0.31
A-5	1860	184	2044	216	3	84.95	0.05	24.12	1.07	0.22	1.65	1.02	0.16
A-6	1144	183	1327	771	14	82.77	0.09	27.35	0.77	0.48	2.85		
A-7	2831	487	3318	496	137	85.06	-0.18	23.4	2.21	0.17	4.01		
A-8	1657	221	1878	181	46	84.75	0.18	24.59	1.78	0.22	2.78		
A-9	2458	541	2998	264	51	83.31	0.36	25.51	3.72	0.67	4.73		
A-10	2843	461	3304	612	170	83.82	0.3	24.54	3.65	0.59	4.14		

  

B2:													
Cross Type	Free Arg (µg/g)	Free Asn (µg/g)	Free Arg + Asn (µg/g)	Free Arg (Stdev)	Free Asn (Stdev)	L*	a*	b*	L* (Stdev)	a* (Stdev)	b* (Stdev)	Lipoxygenase activity pH 7	Lipoxygenase activity pH 7 Stdev
A-11	1921	334	2255	519	103	83.59	0.32	25.29	3.97	0.67	5.72		
A-12	956	139	1095	540	10	83.42	0.11	26.67	1.71	0.22	2.79		
A-13	654	76	729	460	11	83.52	-0.03	25.77	2.74	0.23	5.08		
B-3	1892	263	2154	1186	26	80.89	0.05	27.91	1.63	0.35	2.95		
E	911	85	996	678	4	83.47	0.64	27.86	0.69	0.38	2.07		
A-14	1554	311	1865	732	66	84.24	-0.12	25.17	0.93	0.09	1.74		
A-15	1847	292	2138	1045	217	83.68	0.28	25.01	3.41	0.63	4.15		
A-16	1860	305	2165	513	148	84.3	0.1	25.1	2.64	0.39	3.16	1.71	0.94
D-3	1280	188	1468	274	52	83.47	0.56	23.91	3.58	0.62	4.02		
B-4	992	127	1119	637	3	83.41	0.04	27.16	0.79	0.12	2.99		
B-5	976	106	1081	514	8	82.75	0.11	28.39	0.44	0.21	2.13		
A-17	1618	241	1859	760	107	85.61	-0.04	21.93	1.18	0.11	2.68		
D-2	1215	82	1298	758	6	82.76	0.57	28.69	0.59	0.32	0.19		
B-6	1430	157	1587	337	81	84.97	0.24	24.3	1.03	0.17	2.1		
A-18	2250	452	2702	281	128	85.25	0.12	23.48	1.15	0.18	0.75		

Abbreviations:

Stdev = standard deviation.

TABLE 5

A & B: Characteristics of soybean flour made from commercial commodity soybeans (controls) and the progeny of crosses A, B, C, D and E, grown in 2001. Ground soybeans had moisture content of 7%. Order of the lines is the same as in Table 4. Progeny of cross B listed had low linolenic acid trait (2.9 +/-0.4% of total fatty acids). Two progeny lacked one or more lipoxygenases, the others contained all three lipoxygenases designated as LI, L2, and L3. Lipoxygenase activities have units of micromoles of substrate consumed per mg of substrate. Color values are L\* (lightness), a\* (green-red), and b\* (blue-yellow).

A:													
Cross Type	2A Decadienal + Hexanal (µg/g)	2,4 decadienal (µg/g)	Hexanal + Hexanol (µg/g)	1-octen-3-ol (µg/g)	L*	a*	b*	Lipoxygenase activity pH 7	Lipoxygenase activity, pH 9	Free Arginine (µg/g)	Free Asparagine (µg/g)	Free Arg + Asn (µg/g)	
C-1, L-null	17.05	4.96	12.09	16.50	80.37	1.17	30.84	-0.092	0.297	314	39	353	

TABLE 5-continued

A &, B: Characteristics of soybean flour made from commercial commodity soybeans (controls) and the progeny of crosses A, B, C, D and E, grown in 2001. Ground soybeans had moisture content of 7%. Order of the lines is the same as in Table 4. Progeny of cross B listed had low linolenic acid trait (2.9 +/-0.4% of total fatty acids). Two progeny lacked one or more lipoxygenases, the others contained all three lipoxygenases designated as LI, L2, and L3. Lipoxygenase activities have units of micromoles of substrate consumed per mg of substrate. Color values are L\* (lightness), a\* (green-red), and b\* (blue-yellow).

A-1	17.96	3.04	14.92	14.20	83.36	0.05	28.05	4.015	6.687	866	69	935
B-1	24.80	5.16	19.64	9.90	81.71	0.84	28.71	3.462	7.072	1037	59	1096
D-1,	12.37	3.60	8.77	4.40	83.15	0.14	26.58	1.548	1.479	1124	82	1206
L-												
null												
A-2	17.44	4.00	13.44	8.70	83.42	0.17	26.40	2.860	15.800	2068	138	2206
B-2	27.10	5.38	21.72	9.90	81.52	0.21	28.59	3.591	4.592	558	47	605
A-3	20.58	4.42	16.16	9.30	84.25	-0.19	26.43	2.683	6.412	4662	240	4902
A-4	16.90	1.60	15.3	5.30	83.98	-0.18	26.09	4.400	4.752	3884	190	4074
A-5	15.73	2.30	13.43	4.90	82.53	0.11	29.00	4.681	5.624	2818	163	2981
A-6	19.62	3.85	15.77	8.60	83.09	0.17	28.98	2.664	5.949	687	58	745
A-7	21.96	4.20	17.76	4.80	83.53	-0.33	26.13	4.515	6.257	2722	269	2992
A-8	16.81	4.30	12.51	6.70	82.64	0.26	27.86	3.844	4.278	2126	108	2233
A-9	21.95	6.80	15.15	5.90	82.84	0.01	28.64	5.247	4.090	2594	275	2868
A-10	39.30	10.40	28.9	4.50	83.99	-0.33	27.35	5.520	4.901	4009	267	4276
A-11	27.46	6.30	21.16	4.30	81.88	0.54	29.37	4.748	5.681	1641	139	1780
A-12	42.10	7.46	34.64	17.30	83.60	-0.25	28.60	4.010	4.049	739	79	817
A-13	34.05	8.58	25.47	16.60	82.80	-0.11	29.21	4.983	5.377	547	42	589
B-3	31.06	7.43	23.63	6.30	82.99	0.18	28.84	4.304	4.132	1761	135	1896
E	33.98	8.33	25.65	9.80	83.83	0.29	29.71	4.444	4.608	1310	90	1400
A-14	41.75	9.81	31.94	11.20	82.98	-0.16	29.00	4.208	4.459	1025	86	1111

B

Cross Type	2,4			l-octen-3-ol (µg/g)	Lipoxygenase activity			Free Arginine (µg/g)	Free Asparagine (µg/g)	Free Arg + Asn (µg/g)		
	Decadienal + Hexanal (µg/g)	2,4 decadienal (µg/g)	Hexanal + Hexanol (µg/g)		pH 7	pH 9						
A-15	24.86	5.70	19.16	3.00	83.98	-0.16	26.22	4.236	4.925	2634	207	2841
A-16	31.99	9.70	22.29	5.50	82.58	0.40	29.04	5.659	6.237	1493	132	1625
D-3	40.16	5.80	34.36	5.20	84.08	0.20	27.30	7.354	6.773	1561	152	1713
B-4	30.70	5.79	24.91	7.90	82.94	0.24	29.64	4.724	4.675	669	61	736
B-5	38.59	10.05	28.54	13.80	82.76	-0.02	29.28	3.485	4.822	3060	239	3300
A-17	35.38	8.90	26.48	6.00	84.50	0.53	25.86	5.253	4.888	993	73	1066
D-2	43.55	5.89	37.66	8.60	82.75	-0.37	30.70	5.435	4.848	1724	114	1838
B-6	44.02	10.70	33.32	10.60	84.43	-0.31	26.47	5.334	5.708	4594	377	4971
A-18	27.88	6.70	21.18	4.10	84.71	0.06	27.35	4.604	4.263	3378	185	3564
Control 1	25.80	6.6	19.2	6.9	82.20	0.28	29.57	6.486	4.367	1078	88	1166
Control 2	28.10	6.7	21.4	12.3	79.88	0.64	29.16	3.901	6.000	619	156	774
Control 3	39.10	7.4	31.7	6	80.82	0.79	32.16	7.025	5.737	674	61	735
Control 4	23.70	7.4	16.3	5.4	81.53	0.39	30.89	3.220	3.575	1167	66	1233
Control 5	27.50	7.4	20.1	4.6	81.06	1.18	31.16	4.847	6.234	961	203	1164
Control 6	30.70	8.4	22.3	3.1	81.64	0.37	27.67	4.845	5.015	1002	81	1083
Control 7	29.40	8.5	20.9	6.7	80.35	0.22	28.51	3.748	4.885	1177	168	1345
Control 8	28.50	9	19.5	10.4	79.49	1.14	33.37	7.119	6.388	360	54	414
Control 9	27.90	9.6	18.3	8.4	82.45	0.62	27.88	3.181	5.730	1209	93	1302
Control 10	25.80	9.7	16.1	9.9	80.70	0.90	31.03	5.768	5.715	970	117	1087
Control 11	26.60	10.1	16.5	8.3	80.41	0.07	29.02	5.715	4.670	1214	200	1415
Control 12	34.00	11.6	22.4	6	82.71	0.22	30.63	4.142	4.585	1674	125	1798
Control 13	28.30	13.6	14.7	14.8	80.79	1.27	32.83	3.181	5.730	1358	303	1661

Abbreviations:

Stdev = standard deviation.

TABLE 6

Linear relations as determined by R-squared values, between odors produced from soybean progeny harvests m2001 & 2002.				
Cross Type A (2002)	H + H	D	DHH	O
H + H	1.00	0.85	0.99	0.01
D		1.00	0.91	0.02
DHH			1.00	0.01
O				1.00
All Crosses (01 & 02)	H + H (02)	D (02)	DHH (02)	O (02)
H + H (01)	0.56			
D (01)		0.59		
DHH (01)			0.62	
O (01)				0.07

Linear regressions were calculated from data in Tables 4 and 5. Abbreviations: H + H = hexanal + hexanol; D = 2,4 decadienal; O = 1-octen-3-ol; DHH = 2,4 decadienal + hexanal + hexanol.

[0075] It was possible to develop good yielding low odor-producing, low color varieties for the first time using the methods and soybeans of this invention. For example, lines A-1, A-4 and A-6 yielded 90, 90.5 and 104% of that of commercial checks.

[0076] There was little or no relationship between lipoxygenase activities at pH 7 and 9 and the formation of 2,4

decadienal+hexanal+hexanol from hydrated soybean flour (R-squared values<0.35, Table 7). Soybeans that produced significant lipoxygenase activities at pH 7 and 9 (line A-1) had similar odor properties as a line that lacked lipoxygenase activities (Line C-1) (Table 5). Commercially available soybean lacking lipoxygenases 1, 2 and 3 (IA2032 from 1999 harvest) was also tested for lipoxygenase activity and odor formation. No lipoxygenase activities were found at pH 7 and 9 for the triple lipoxygenase-null soybean flour, yet significant levels of hexanal (23.3 µg/g), hexanol (14.9 µg/g), and 2,4 decadienal (5.8 µg/g) were formed in the odor assay of the invention. Following experiments using lipoxygenase inhibitors, it was concluded that at least one other lipoxygenase was active in this soybean composition.

[0077] Protein ingredients that are light in color are valued by the food industry, especially for dairy type products. Soybeans were grown in 2-3 locations to determine if low color soybeans could be selected. It was discovered that low color soybeans could be selected as determined from the b\* value of ground soybeans (high b\* value indicates more yellow, less blue; Table 4). For example, soybean A-2 had a b\* value of 22.16+/-1.39 and soybean A-6 had a b\* value of 27.35+/-2.85 (Table 4). A correlation existed between the b\* value of soybean lines grown in 2001 and 2002 (R-squared=0.7; Table 7).

TABLE 7

	Linear relations as determined by R-squared values for odor, free amino acid, and color produced from soybean progeny harvested in 2001 and 2002. Linear regressions were calculated from data in Tables 4 and 5. Free amino acid correlations with 2002 data were made without three outliers (A-4, B-5 and B-6).							
	DHH (01)	Free Arg (01)	Free Asn (01)	Free Arg + Free Asn (01)	a* (02)	b + (02)	L* (02)	L* (01)
lox activity pH 7 (01)	0.34			0.01				
lox activity pH 9 (01)	0.00			0.10				
DHH (01)				0.01				
O (01)				0.15				
a* (01)					0.29			
b* (01)	0.00			0.28		0.65		0.48
L ± (01)							0.50	
b* (02)							0.79	
Free Arg (01)			0.81					
Free Arg (02)		0.77						
Free Arg (02)			0.66					
Free Arg + Asn (02)				0.75				

**[0078]** Environmental factors in addition to genetics affect compositions that influence the odor-producing properties of soybeans. Environmental effects were evident by comparing the odor-producing properties of soybean lines grown in two seasons, 2001 and 2002. The range of 2,4 decadienal+hexanal+hexanol for lines created were 12-44  $\mu\text{g/g}$  in 2001 and 17-65  $\mu\text{g/g}$  in 2002 (Tables 4, 5). The 1-octen-3-ol producing properties of soybeans appeared to be most sensitive to environmental factors as evidenced by the lack of correlation between 1-octen-3-ol produced by the same lines grown in 2001 and 2002 ( $R^2=0.07$ , Table 6). In contrast the levels of 2,4 decadienal+hexanal+hexanol produced by the soybean lines in 2001 and 2002 correlated ( $R^2=0.62$ , Table 6).

**[0079]** The amounts of free arginine (Arg) and asparagine (Asp) in soybean progeny were determined. Free arginine and asparagine amounts correlated ( $R^2=0.81$ , Table 7) and the total arginine plus asparagine ranged from 353 to 3300  $\mu\text{g/g}$  (2001)(Table 5). The free arginine+asparagine in the soybeans did not relate to the selected odor or color properties of the soybean ( $R^2<0.3$ , Table 7), so it is necessary to assay for free amino acids to select lines that have combinations of low 2,4 decadienal, low color and low free amino acids. The feasibility of selecting low free amino acid soybean lines was supported by the good correlation between free arginine+asparagine in lines harvested in two different years ( $R^2=0.8$ ; Table 7).

#### EXAMPLE 4

##### Combination of Low Odor Producing Property with High $\beta$ -conglycinin Composition and Low Free Arginine and Asparagine Compositions and Low Color Property

**[0080]** The source of the high  $\beta$ -conglycinin trait was a mutated soybean lacking glycinins and containing about 55% of the total protein as  $\beta$ -conglycinins (U.S. Pat. No. 6,171,640). A lipoxygenase assay was not useful for selecting low odor-producing lines as described above. Soybeans were created that had protein, fat and amino acid profiles that were within the normal ranges for commodity soybeans.

**[0081]** Quantitation of soybean protein subunits: About 8 seeds were ground using a Mega Grinder (U.S. Patent Pub. 2003/0146313 A1). For each sample, ~30 mg of the flour was extracted in 1.0 mL Laemmli SDS Buffer pH 6.8 with 0.1M DTT on a nutator or multiplate vortexer for 45-60 minutes. Tubes were centrifuged 3-5 min. A portion of the supernatant was transferred to microcentrifuge tubes and diluted with the above buffer to yield 1.2-1.5  $\mu\text{g}/\mu\text{L}$  total protein. The samples were boiled for 3 min, cooled and centrifuged. Pre-cast 10-20% gradient Tris-HCl Criterion gels were loaded with 15-20  $\mu\text{g}$  protein of each sample. The gels were electrophoresed at 180-200V, in 1 $\times$  Tris-Glycine-SDS Run Buffer, until the tracking dye reached the bottom of the gel, about 1.2 hours. The gels were fixed for 30-60 minutes in 40% methanol/10% acetic acid, and stained with Colloidal Coomassie Blue G-250; minimum overnight or up to 3 days. To remove the background, the gels were destained with deionized water. The gels were imaged using the GS 800 Calibrated Densitometer. Quantitation was performed using Bio-Rad Quantity One Software. The software is used to determine the relative quantity of each band in the sample lane. The % glycinin subunits and % beta conglycinin subunits were reported as the relative percent of the total protein in the lane.

**[0082]** Total amino acid analysis: The sample was assayed by three methods to obtain the full profile. Tryptophan required a base hydrolysis with sodium hydroxide. The sulfur containing amino acids required an oxidation with performic acid prior to hydrolysis with hydrochloric acid. Analysis of the samples for the remaining amino acids was accomplished through direct acid hydrolysis with hydrochloric acid. Once hydrolyzed, the individual amino acids were then quantitated using an automated amino acid analyzer (Official Methods of Analysis of AOAC INTERNATIONAL, 2000).

**[0083]** Ash: The sample was placed in an electric furnace at 550° C. and ignited to drive off all volatile organic matter. The nonvolatile matter remaining was quantitated gravimetrically and calculated to determine percent ash. Official Methods of Analysis of AOAC INTERNATIONAL, (2000).

**[0084]** Carbohydrates: The total carbohydrate level was calculated by difference using the fresh weight-derived data and the following equation: % carbohydrates=100%-(% protein+% fat+% moisture+% ash). United States Department of Agriculture (1973).

**[0085]** Fat by Soxhlet Extraction: The sample was weighed into a cellulose thimble containing sand or sodium sulfate and dried to remove excess moisture. Pentane was dripped through the sample to remove the fat. The extract was then evaporated, dried, and weighed. Official Methods of Analysis of AOAC INTERNATIONAL (2000).

**[0086]** Moisture: The sample was dried in a vacuum oven at approximately 100° C. to a constant weight. The moisture weight loss was determined and converted to percent moisture.

**[0087]** Protein: Nitrogenous compounds in the sample were reduced in the presence of boiling sulfuric acid and a mercury catalyst mixture to form ammonia. The acid digest was made alkaline. The ammonia was distilled and then titrated with a standard acid. The percent nitrogen was calculated and converted to protein using the factor 6.25. Official Methods of Analysis of AOAC INTERNATIONAL (2000). Bradstreet, (1965). Kalthoff and Sandell (1948).

**[0088]** Results: A population of high  $\beta$ -conglycinin soybean lines selected from a cross with a soybean containing the low odor producing trait exhibited a wide variation of odor-producing properties as measured by formation of hexanal, hexanol, 2,4 decadienal and 1-octen-3-ol (Table 8). Commodity soybeans contained about 22% of total protein as  $\beta$ -conglycinins and about 38% glycinins, compared to the soybeans in Table 8 that had greater than 30% of the protein as  $\beta$ -conglycinins and less than 25% of the proteins as glycinins. Soybeans were created having greater than 30% of the total protein as  $\beta$ -conglycinins that produced less than 20  $\mu\text{g/g}$  total of hexanal plus hexanol and 2,4 decadienal in the odor assay of example 1, and also comprise low levels of free asparagine and free arginine (Table 8). For example, 20 high  $\beta$ -conglycinin lines were created by the invention that produced less than 20  $\mu\text{g/g}$  of the total of 2,4 decadienal plus hexanal plus hexanol and a total of free arginine plus asparagine between 360 and 2,840  $\mu\text{g/g}$  of ground soybeans (Table 8). The free asparagine of these lines was between 35 and 1,000  $\mu\text{g/g}$  and the free arginine of these lines was between 500 and 2400  $\mu\text{g/g}$  of ground soybeans.

TABLE 8

A and B: Characteristics of high beta-conglycinin soybean progeny harvested in the United States. Soybeans having greater than 3 0% of the protein as beta-conglycinins and less than 2:5% of the protein as glycinins were created that also produce less than 20 fig/g 2,4 decadienal plus hex anal plus hexanol per gram of soybean flour in the odor assay of example 1. A lipoxygenase activity assay was not useful for identifying the low odor producing lines.

Sample ID	A					Hexanal + Hexanol + 2,4		Lipox. activity, pH 7	Lipox. activity, pH 9	Asn (μg/g)	Arg (μg/g)	Arg + Asn (μg/g)
	beta- conglycinin (% of total protein)	hexanal (μg/g)	hexanol (μg/g)	octen- 3-ol (μg/g)	decadienal (μg/g)	Hexanal + Hexanol (μg/g)	decadienal (μg/g)					
HiBCSoy52	38.4	5.4	4.3	1.5	3.2	9.7	12.9	7.734	5.731	2024	4421	6445
HiBCSoy70	39.5	6.1	5.3	6.1	2.8	11.4	14.2	6.483	11.347	771	2020	2791
HiBCSoy41	43.2	5.6	6.1	9.1	2.8	11.7	14.5	8.626	8.499	72	994	1066
HiBCSoy65	39.2	6.2	5.4	5.4	3.2	11.6	14.8	8.393	13.535	1939	5064	7003
HiBCSoy35	42.0	6.2	5.3	8.5	3.9	11.6	15.5	6.935	9.623	171	757	928
HiBCSoy67	40.4	6.7	5.5	2.6	3.4	12.2	15.6	7.019	8.377	1376	3323	4699
HiBCSoy34	40.6	5.2	7.8	7.4	3.0	13.0	16.0	7.279	10.633	311	1382	1693
HiBCSoy64	39.3	7.0	6.2	3.3	2.7	13.3	16.0	6.622	8.044	1723	3241	4964
HiBCSoy62	41.1	6.9	6.7	1.1	2.7	13.6	16.2	5.697	10.358	2882	3938	6820
HiBCSoy68	40.5	7.8	5.8	2.3	2.9	13.6	16.5	8.006	6.584	303	1655	1958
HiBCSoy61	30.3	9.6	4.7	4.1	2.3	14.4	16.6	6.709	6.110	372	1664	2036
HiBCSoy69	39.9	7.7	5.0	3.5	4.0	12.7	16.7	7.836	7.668	1393	4151	5544
HiBCSoy48	44.5	7.1	6.4	2.2	3.3	13.5	16.8	7.601	3.783	99	1496	1595
HiBCSoy40	45.0	5.7	6.7	6.9	4.4	12.4	16.9	7.893	8.897	956	1878	2834
HiBCSoy11	48.2	7.5	4.0	8.0	5.6	11.5	17.1	6.447	13.771	107	1680	1787
HiBCSoy42	38.6	7.4	6.0	6.0	3.7	13.4	17.1	6.825	11.446	77	289	366
HiBCSoy53	40.0	6.9	6.7	4.2	4.2	13.6	17.9	6.649	6.849	1154	2489	3643
HiBCSoy5	46.5	8.0	6.2	5.2	3.9	14.3	18.2	5.882	5.793	305	3109	3414
HiBCSoy36	40.6	8.0	5.0	10.4	5.2	13.1	18.3	9.259	9.062	38	536	574
HiBCSoy3	46.8	6.7	6.3	5.0	5.6	13.0	18.6	7.790	12.639	89	817	906
HiBCSoy4	44.9	7.4	6.7	8.6	4.6	14.1	18.6	6.929	8.660	723	3448	4171
HiBCSoy46	42.4	6.7	8.5	9.4	3.5	15.1	18.7	6.978	3.850	67	1114	1181
HiBCSoy26	44.7	7.8	7.6	14.4	3.4	15.3	18.8	6.148	6.667	225	863	1088
HiBCSoy18	44.8	9.3	4.6	7.6	5.0	13.9	18.9	9.482	7.439	121	504	625
HiBCSoy6	45.9	8.0	6.8	10.6	4.4	14.8	19.1	8.298	7.876	105	1149	1254
HiBCSoy17	43.7	7.7	5.7	12.6	5.8	13.3	19.1	9.689	6.337	298	2363	2661
HiBCSoy44	31.7	7.6	7.2	7.2	4.7	14.8	19.4	8.909	6.160	125	569	694
HiBCSoy63	33.5	10.3	6.7	1.9	2.7	16.9	19.6	6.177	10.239	677	1473	2150
HiBCSoy57	36.9	8.3	5.8	6.0	5.6	14.0	19.6	8.831	13.258	1329	3515	4844
HiBCSoy16	44.3	8.5	6.1	10.8	5.3	14.6	19.9	8.326	6.447	95	863	958
HiBCSoy24	43.7	6.5	9.3	6.2	4.2	15.8	20.0	7.293	9.330	1083	1909	2992
HiBCSoy58	36.4	7.7	7.3	3.2	5.0	15.1	20.1	8.078	14.134	1519	4252	5771
HiBCSoy39	41.7	7.2	6.5	4.5	6.3	13.7	20.1	9.084	5.718	95	400	495
HiBCSoy66	39.2	8.8	7.4	2.6	4.2	16.2	20.4	6.122	9.155	2573	4163	6736
HiBCSoy38	37.6	7.9	5.7	5.3	7.0	13.6	20.6	9.356	9.776	71	592	663
HiBCSoy59	37.2	9.6	5.2	5.0	5.8	14.8	20.6	10.126	8.492	271	1837	2108
HiBCSoy37	36.8	9.1	6.7	4.2	4.9	15.8	20.7	8.990	6.942	276	572	848
HiBCSoy2	45.9	11.5	5.2	3.1	5.0	16.7	21.8	8.604	8.144	103	1116	1219
HiBCSoy60	40.1	9.6	6.1	7.8	6.1	15.7	21.8	7.463	7.468	1671	4330	6001
HiBCSoy43	37.3	7.9	9.7	6.2	4.6	17.6	22.1	5.111	6.575	209	1083	1292
HiBCSoy25	44.2	10.0	8.5	8.3	3.9	18.4	22.3	6.642	7.495	686	1666	2352
HiBCSoy50	46.3	8.8	6.0	6.2	7.6	14.8	22.3	7.072	6.590	115	1339	1454
HiBCSoy13	45.4	11.4	5.3	2.8	5.9	16.7	22.6	7.421	8.778	508	2290	2798
HiBCSoy55	37.8	9.2	7.8	4.5	6.1	17.1	23.1	6.848	6.503	1705	4929	6634
HiBCSoy54	31.2	9.9	6.4	2.8	6.9	16.3	23.2	5.994	5.363	752	1569	2321
HiBCSoy19	44.3	12.5	5.7	9.3	5.1	18.2	23.3	9.434	11.218	108	1257	1365
HiBCSoy14	46.1	8.8	6.5	8.6	8.2	15.3	23.5	7.974	7.297	181	1334	1515
HiBCSoy20	41.4	10.2	8.9	10.4	4.6	19.1	23.7	6.392	10.686	1007	2329	3336
HiBCSoy45	45.7	9.1	9.3	3.8	5.5	18.4	23.9	7.637	15.149	169	744	913
HiBCSoy12	48.6	10.8	7.6	4.9	6.1	18.4	24.5	9.695	10.837	92	455	547
HiBCSoy31	42.0	12.5	6.9	11.0	5.4	19.4	24.8	12.072	6.823	66	1162	1228
HiBCSoy7	46.5	12.5	6.0	3.8	6.5	18.5	25.0	8.042	9.921	476	1641	2117
HiBCSoy1	45.1	12.3	6.7	6.2	6.5	19.0	25.6	8.351	7.376	187	1423	1610
HiBCSoy29	38.8	4.0	10.1	8.4	11.6	14.1	25.7	8.944	5.006	136	818	954
HiBCSoy8	48.7	11.6	8.6	8.2	5.6	20.2	25.8	6.888	8.407	321	2612	2933
HiBCSoy56	36.4	11.5	7.7	7.5	6.9	19.2	26.1	7.312	8.601	1155	2602	3757
HiBCSoy51	40.4	11.5	6.0	4.2	8.7	17.5	26.2	7.622	10.635	1695	3701	5396
HiBCSoy47	38.1	10.0	11.6	6.4	4.8	21.6	26.4	6.974	4.039	92	1013	1105
HiBCSoy30	36.6	9.9	11.6	3.0	5.1	21.4	26.6	11.349	4.735	158	812	970
HiBCSoy27	38.6	11.0	12.1	8.0	4.1	23.1	27.2	7.118	4.131	633	1082	1715
HiBCSoy28	38.8	12.7	9.6	6.9	6.3	22.2	28.6	4.762	5.822	2575	3204	5779
HiBCSoy15	45.8	15.5	6.6	2.2	6.6	22.1	28.6	9.056	9.945	129	620	749
HiBCSoy23	43.7	13.0	10.2	10.0	6.1	23.2	29.4	6.681	5.207	1136	2578	3714

TABLE 8-continued

A and B: Characteristics of high beta-conglycinin soybean progeny harvested in the United States. Soybeans having greater than 3 0% of the protein as beta-conglycinins and less than 2:5% of the protein as glycinins were created that also produce less than 20 fig/g 2,4 decadienal plus hex anal plus hexanol per gram of soybean flour in the odor assay of example 1.												
A lipoxygenase activity assay was not useful for identifying the low odor producing lines.												
Sample ID	Protein (% dry basis)	A1a, A1b, A2, A4 glycinins (% of total protein)	A3 glycinin	B1a, B1b, B2, B3, B4 glycinins	alpha beta-conglycinin	alpha-prime beta-conglycinin	beta beta-conglycinin	Total beta-conglycinins	Total Glycinins			
HiBCSoy22	29.7	9.3	11.0	8.9	9.5	20.4	29.9	6.645	8.996	192	844	1036
HiBCSoy33	41.3	16.7	7.6	6.0	7.8	24.2	32.0	8.162	9.203	576	2919	3495
HiBCSoy10	44.3	15.6	8.2	5.6	8.2	23.8	32.0	8.624	11.794	106	1108	1214
HiBCSoy21	35.7	14.5	12.0	10.8	6.1	26.5	32.6	8.634	11.921	99	581	680
HiBCSoy9	46.9	16.6	8.7	5.9	8.7	25.2	33.9	6.976	6.340	155	1430	1585
B												
Sample ID	Protein (% dry basis)	A1a, A1b, A2, A4 glycinins (% of total protein)	A3 glycinin	B1a, B1b, B2, B3, B4 glycinins	alpha beta-conglycinin	alpha-prime beta-conglycinin	beta beta-conglycinin	Total beta-conglycinins	Total Glycinins			
HiBCSoy52	42.76	4.872	4.784	9.097	20.849	12.381	5.123	38.353	18.753			
HiBCSoy70	41.78	4.179	3.563	7.039	22.621	11.596	5.262	39.479	14.781			
HiBCSoy41	40.49		5.784	1.555	23.558	14.185	5.502	43.245	7.339			
HiBCSoy65	43.82	4.759	5.007	7.737	22.044	12.875	4.263	39.182	17.503			
HiBCSoy35	40.56	2.312	4.538	4.913	22.903	12.727	6.415	42.045	11.763			
HiBCSoy67	42.46	4.167	3.608	8.694	23.331	11.983	5.038	40.352	16.469			
HiBCSoy34	41.31	3.352	4.615	4.318	22.04	12.555	5.961	40.556	12.285			
HiBCSoy64	43.10	4.949	5.047	7.773	21.646	12.79	4.858	39.294	17.769			
HiBCSoy62	44.64	4.891	5.045	6.098	22.73	12.045	6.309	41.084	16.034			
HiBCSoy68	42.56	3.849	3.209	7.343	22.829	11.403	6.297	40.529	14.401			
HiBCSoy61	38.94	4.274	4.172	10.014	17.347	9.747	3.206	30.3	18.46			
HiBCSoy69	42.64	4.468	3.81	8.614	22.386	11.791	5.713	39.89	16.892			
HiBCSoy48	39.72		6.168	2.907	24.062	15.07	5.414	44.546	9.075			
HiBCSoy40	41.18		6.068	1.264	23.629	14.878	6.525	45.032	7.332			
HiBCSoy11	44.68		6.886	3.09	24.802	13.863	9.552	48.217	9.976			
HiBCSoy42	38.13	1.822	4.93	3.401	20.961	11.577	6.06	38.598	10.153			
HiBCSoy53	43.66	4.782	4.783	8.585	22.103	12.521	5.373	39.997	18.15			
HiBCSoy5	45.03		5.869	2.944	24.794	13.653	8.089	46.536	8.813			
HiBCSoy36	38.50	2.463	4.221	3.665	22.435	11.65	6.532	40.617	10.349			
HiBCSoy3	42.55		6.308	3.171	25.442	14.402	6.927	46.771	9.479			
HiBCSoy4	43.56		5.449	2.765	23.395	14.297	7.2	44.892	8.214			
HiBCSoy46	39.89		6.149	1.453	23.323	14.892	4.155	42.37	7.602			
HiBCSoy26	41.21		5.742	2.31	23.643	14.391	6.636	44.67	8.052			
HiBCSoy18	40.14		5.385	1.875	23.836	14.999	5.934	44.769	7.26			
HiBCSoy6	42.40		5.598	2.769	24.62	13.114	8.123	45.857	8.367			
HiBCSoy17	39.74		5.788	2.281	22.884	13.984	6.816	43.684	8.069			
HiBCSoy44	39.01	4.766	4.241	8.322	17.716	9.373	4.63	31.719	17.329			
HiBCSoy63	41.95	3.372	3.558	11.179	17.431	10.613	5.467	33.511	18.109			
HiBCSoy57	43.94	5.167	5.105	8.459	21.038	11.288	4.558	36.884	18.731			
HiBCSoy16	40.83		5.284	2.94	23.351	14.266	6.694	44.311	8.224			
HiBCSoy24	43.66	2.799	4.931	5.949	23.351	13.832	6.508	43.691	13.679			
HiBCSoy58	43.60	5.132	4.262	8.898	21.14	11.418	3.834	36.392	18.292			
HiBCSoy39	37.61	2.666	5.814	5.48	21.436	13.648	6.63	41.714	13.96			
HiBCSoy66	44.77	5.063	4.755	9.443	21.266	12.448	5.5	39.214	19.261			
HiBCSoy38	38.42	3.923	3.871	5.194	20.716	11.702	5.154	37.572	12.988			
HiBCSoy59	40.54	4.696	4.26	7.023	21.554	12.26	3.384	37.198	15.979			
HiBCSoy37	38.99	4.581	4.392	6.76	19.84	10.994	5.954	36.788	15.733			
HiBCSoy2	41.47		5.872	2.553	25.298	13.934	6.684	45.916	8.425			
HiBCSoy60	43.40	5.019	4.688	7.532	22.035	12.045	5.976	40.056	17.239			
HiBCSoy43	43.12	4.856	4.538	7.757	20.84	12.396	4.097	37.333	17.151			
HiBCSoy25	42.23		5.976	3.074	23.404	14.578	6.257	44.239	9.05			
HiBCSoy50	41.44		6.019	1.903	25.113	16.165	4.972	46.25	7.922			
HiBCSoy13	45.51		5.732	2.562	24.536	13.744	7.135	45.415	8.294			
HiBCSoy55	43.70	5.036	4.671	8.171	20.641	12.032	5.142	37.815	17.878			
HiBCSoy54	42.39	3.604	3.3	12.991	16.118	10.006	5.063	31.187	19.895			
HiBCSoy19	41.60		6.109	2.67	22.8	14.199	7.284	44.283	8.779			
HiBCSoy14	42.22		5.431	2.557	24.628	14.439	7.002	46.069	7.988			
HiBCSoy20	41.04	1.577	4.913	4.422	21.592	13.422	6.411	41.425	10.912			
HiBCSoy45	40.13		5.347	2.737	24.384	14.056	7.308	45.748	8.084			
HiBCSoy12	39.59		5.842	3.582	26.33	15.218	7.082	48.63	9.424			
HiBCSoy31	40.25	1.7	4.33	2.41	22.579	13.279	6.133	41.991	8.44			
HiBCSoy7	42.12		5.905	2.788	25.149	13.985	7.401	46.535	8.693			
HiBCSoy1	40.11		5.451	2.366	23.373	13.84	7.865	45.078	7.817			
HiBCSoy29	40.01	3.919	3.571	6.9	20.207	13.234	5.345	38.786	14.39			
HiBCSoy8	46.86		6.055	3.495	25.584	14.699	8.464	48.747	9.55			
HiBCSoy56	42.14	4.915	4.981	8.294	21.474	12.173	2.736	36.383	18.19			
HiBCSoy51	42.24	4.303	4.695	7.503	22.456	13.402	4.535	40.393	16.501			



TABLE 8-continued

A and B: Characteristics of high beta-conglycinin soybean progeny harvested in the United States. Soybeans having greater than 3 0% of the protein as beta-conglycinins and less than 2:5% of the protein as glycinins were created that also produce less than 20 fig/g 2,4 decadienal plus hex anal plus hexanol per gram of soybean flour in the odor assay of example 1. A lipoxygenase activity assay was not useful for identifying the low odor producing lines.

HiBCSoy47	39.32		5.83	1.61	20.486	11.803	5.835	38.124	7.44
HiBCSoy30	43.28	4.796	4.07	8.302	19.29	11.788	5.555	36.633	17.168
HiBCSoy27	43.71	4.651	4.769	7.697	20.396	12.062	6.173	38.631	17.117
HiBCSoy28	44.14	5.017	4.986	8.742	20.351	12.578	5.915	38.844	18.745
HiBCSoy15	42.65		6.124	2.44	23.224	14.71	7.892	45.826	8.564
HiBCSoy23	41.51	2.38	5.072	5.094	23.148	13.627	6.932	43.707	12.546
HiBCSoy22	42.24	5.253	4.672	9.326	14.996	9.178	5.509	29.683	19.251
HiBCSoy33	41.71	2.126	5.273	4.404	22.994	13.025	5.292	41.311	11.803
HiBCSoy10	41.40		5.364	2.383	23.984	15.345	4.929	44.258	7.747
HiBCSoy21	39.50	3.867	3.566	6.852	18.347	11.398	5.944	35.689	14.285
HiBCSoy9	42.92		6.2	2.45	24.453	14.776	7.647	46.876	8.65

**[0089]** Lipoxygenase activities varied significantly for high  $\beta$ -conglycinin soybean samples. There was no relation between lipoxygenase activities at pH 7 and at pH 9 and the total amounts of 2,4 decadienal plus hexanal plus hexanol produced from the same soybean flour ( $R^2$  values<0.02).

**[0090]** An additional experiment was conducted to demonstrate that soybeans having greater than 30%  $\beta$ -conglycinin and less than 25% glycinins can be selected that have low color and an amino acid composition that is within the range of commodity soybeans. Four high  $\beta$ -conglycinin soybeans that also contained Roundup Ready® trait, were selected that yielded the same or better than the average of commercial checks in three locations. These soybeans had the low color trait as illustrated by an average  $b^*$  of about 22 and an average  $L^*$  of about 85 (Table 9). The amino acid compositions (Table 9) fell within the ranges for commodity soybeans (Table 10)

as published in the International Life Sciences Institute Crop Composition Database (Version 1.0, accessed Mar. 22, 2004). The average amino acid compositions of three high  $\beta$ -conglycinin soybean lines (Table 9) were compared to the average soybean composition in the ILSI database (Table 10). Four amino acids (arginine, lysine, histidine and serine) of the high  $\beta$ -conglycinin lines were between 10 and 15% different from the average composition of commodity soybeans but were still within the range of commodity soybean compositions.

**[0091]** It is also anticipated that the organoleptic properties of high  $\beta$ -conglycinin soybeans will be further improved by crossing with low linolenic, mid-oleic soybeans. The low linolenic, mid-oleic soybeans were created at Monsanto using traditional breeding and comprise a linolenic content of about 2%, the linoleic content of about 25% and the oleic acid content of about 59% of the total fatty acids.

TABLE 9

Composition, color and odor properties of soybeans grown in three locations in 2003. All had Roundup Ready ® trait and dark hilum.

	HBC-360		HBC-350		HBC-390				
	average	stdev	average	stdev	average	stdev			
Beta-conglycinins (% of total protein)	37.4	1.5	37.0	0.3	37.9	1.0			
Glycinins (% of total protein)	15.4	0.8	15.2	1.3	17.1	0.5			
Free Asn (ppm)	307	224	183	124	535	309			
Free Arg (ppm)	2825	1211	2050	829	2791	674			
<u>Color</u>									
$b^*$	22.0	1.6	23.3	1.9	22.7	0.6			
$L^*$	85.4	0.4	85.6	0.7	84.8	0.1			
<u>Odor</u>									
hexanal + hexanol + 2,4 decadienal ( $\mu\text{g/g}$ soybean)	25.1	1.2	53.7	17.0	29.4	1.0			
<u>Proximate (g/100 g soybean)</u>									
Moisture	5.91	0.06	5.855	0.09	5.97	0.11			
Protein	37.40	1.15	36.05	0.49	37.87	1.62			
Total Fat	17.53	0.25	18.2	0.42	16.27	0.15			
Ash	4.41	0.28	4.45	0.18	4.36	0.14			
Carbohydrates	34.77	0.80	35.45	0.21	35.57	1.59			
	mg/g soybean	mg/g protein	mg/g soybean	mg/g protein	mg/g soybean	mg/g protein			
Amino Acids									
Aspartic Acid	39.90	0.95	106.68	40.05	0.35	111.10	42.50	1.01	112.24
Threonine	12.70	0.60	33.96	12.3	1.70	34.12	12.37	0.21	32.66
Serine	19.70	0.72	52.67	20.25	1.20	56.17	21.40	0.46	56.51

TABLE 9-continued

Composition, color and odor properties of soybeans grown in three locations in 2003. All had Roundup Ready ® trait and dark hilum.									
Glutamic Acid	62.67	1.79	167.56	62.7	0.42	173.93	67.13	1.16	177.29
Proline	18.33	0.38	49.02	18.35	0.07	50.90	18.67	0.49	49.30
Glycine	15.27	0.32	40.82	15.2	0.00	42.16	15.80	0.26	41.73
Alanine	15.57	0.32	41.62	15.6	0.00	43.27	15.90	0.20	41.99
Cystine	5.31	0.34	14.21	5.915	0.06	16.41	5.81	0.33	15.33
Valine	17.13	0.72	45.81	17.1	0.14	47.43	17.63	0.25	46.57
Methionine	4.45	0.47	11.89	4.85	0.06	13.45	4.83	0.11	12.75
Isoleucine	16.33	0.55	43.67	16.4	0.14	45.49	16.67	0.25	44.01
Leucine	27.80	0.78	74.33	27.95	0.07	77.53	28.93	0.25	76.41
Tyrosine	12.33	0.15	32.98	12.55	0.07	34.81	12.90	0.53	34.07
Phenylalanine	18.37	0.61	49.11	18.4	0.00	51.04	18.83	0.31	49.74
Histidine	10.53	0.35	28.16	10.5	0.14	29.13	11.17	0.31	29.49
Lysine	25.07	0.93	67.02	24.85	0.07	68.93	26.13	0.80	69.01
Arginine	27.03	1.45	72.28	26.45	0.49	73.37	30.17	1.75	79.67
Tryptophan	3.96	0.11	10.58	3.945	0.02	10.94	4.20	0.17	11.08
Met + Cys			26.10			29.86			28.08

TABLE 10

Comparison of the average composition of three high beta-conglycinin soybeans with the average soybean composition in the International Life Sciences Institute Crop Composition Database (Version 1.0). Selection criteria used for obtaining the data: crop type, soybeans-*Glycine max.* tissue type: seed, crop year, all, country, all, state, all.

Analyte	Minimum	Maximum		n	Units	Average HBC soybeans (mg/g FW)	Difference from ILSI average (%)
<u>Avg.</u>							
Amino_Acids - Alanine	13.6	17.2	15.35	187	mg/g FW	15.7	2.2
Amino_Acids - Arginine	20.9	31.3	25.31	187	mg/g FW	27.9	10.2
Amino_Acids - Aspartic Acid	33.9	46.6	40.16	187	mg/g FW	40.8	1.6
Amino_Acids - Cystine/Cysteine	3.43	7.29	5.26	187	mg/g FW	5.7	8.0
Amino_Acids - Glutamic Acid	52.8	74	63.24	187	mg/g FW	64.2	1.5
Amino_Acids - Glycine	13.1	17.4	15.03	187	mg/g FW	15.4	2.6
Amino_Acids - Histidine	8.07	10.8	9.32	187	mg/g FW	10.7	15.2
Amino_Acids - Isoleucine	13.3	19	16.19	187	mg/g FW	16.5	1.7
Amino_Acids - Leucine	23.1	31.5	27.04	187	mg/g FW	28.2	4.4
Amino_Acids - Lysine	20	26.6	22.88	187	mg/g FW	25.4	10.8
Amino_Acids - Methionine	3.99	6.14	4.92	187	mg/g FW	4.7	-4.3
Amino_Acids - Phenylalanine	14.6	20.5	17.69	187	mg/g FW	18.5	4.8
Amino_Acids - Proline	15.1	21.2	18.05	187	mg/g FW	18.5	2.2
Amino_Acids - Serine	15	22.8	18.12	187	mg/g FW	20.5	12.9
Amino_Acids - Threonine	11.5	15.1	12.99	187	mg/g FW	12.5	-4.1
Amino_Acids - Tryptophan	3.26	4.7	3.903	187	mg/g FW	4.0	3.3
Amino_Acids - Tyrosine	8.7	14.3	11.78	187	mg/g FW	12.6	6.9
Amino_Acids - Valine	14.5	20.5	17.14	187	mg/g FW	17.3	0.9
<u>Average</u>							
Proximates - Ash	3.885	6.542	5.313	237	% DW		
Proximates - Carbohydrate By Calculation	29.6	50.2	38.1	237	% DW		
Proximates - Crude Protein	33.19	45.48	39.28	237	% DW		
Proximates - Total Fat	8.104	23.562	16.94	237	% DW		

Abbreviations:

FW = soybean flour weight.

DW = dry weight

#### EXAMPLE 5

Comparison of Odor and Color Properties of Commodity Soybeans with Soybeans Selected According to the Invention

[0092] The odor and color properties of commodity soybeans were determined to compare with the odor and color

properties of soybeans of the invention. Some soybeans produced less than 17.5 µg of hexanal plus hexanol and some produced less than 11 µg/g of 2,4 decadienal in the odor assay (Table 11). However, none of the commodity soybeans produced less than 20 µg of total 2,4 decadienal plus hexanal plus hexanol per gram of ground seeds following oxidation under mild aqueous conditions. Four lipoxigenase-containing lines

of this invention were created that produced less than 20 µg/g of hexanal+hexanol+2,4 decadienal in two seasons (Tables 4, 5). For example, line A-1 from 2001 and 2002 harvests produced 18.0 and 18.2 µg/g, respectively of the sum of hexanal+hexanol and 2,4 decadienal (Tables 4, 5). More than 20 high β-conglycinin lines were created by the invention that produced had less than 20 µg/g of the total of hexanal+hexanol

and 2,4 decadienal (Table 8). For example, one high β-conglycinin soybean produced 9.7 µg/g hexanal+hexanol and 3.2 µg/g of 2,4 decadienal (Table 8).

**[0093]** The color of commodity soybeans covered a broad range. For example the b\* value ranged from 27 to 34 (Table 11). The soybean lines of the invention were found to extend to values of b\* as low as 22 (Table 4, Table 9).

TABLE 11

A & B: Color and odor-producing properties of commodity soybeans harvested.						
A: Flavor						
Sample number	hexanal (pg/g)	Hexanal (pg/g)	Hexanol + (pg/g)	2,4 decadienal	1-octen-3-ol (MS'S)	2,4 decadienal + hexanal + hexanol (µg/g)
385	14.0	3.9	18.0	2.8	1.8	20.7
493	5.0	5.1	10.1	11.1	5.4	21.2
491-2	5.0	4.4	9.4	14.3	6.1	23.7
210	15.4	5.1	20.5	3.6	7.0	24.1
287	14.0	3.6	17.6	7.2	18.4	24.8
387	15.6	3.9	19.6	5.3	8.5	24.8
138	14.9	5.0	19.9	5.0	9.3	24.9
379	17.4	4.1	21.5	3.4	9.1	24.9
137	15.0	4.8	19.9	5.1	8.4	25.0
209	15.5	5.5	21.0	4.2	3.1	25.2
386	15.7	3.9	19.7	5.6	5.7	25.3
390	18.1	3.9	22.0	3.7	11.2	25.8
285	13.9	6.2	20.1	5.9	4.6	26.0
286	14.8	5.3	20.1	6.0	12.3	26.1
290	14.5	5.5	20.0	6.6	6.4	26.6
289	14.8	5.8	20.6	6.1	7.8	26.8
175	9.5	5.8	15.3	11.6	4.3	26.9
383	16.7	3.9	20.6	6.6	7.9	27.2
411	5.8	7.9	13.8	13.5	8.0	27.3
276	14.7	5.9	20.5	7.2	2.5	27.7
378	17.7	3.9	21.6	6.2	9.6	27.8
282	15.5	6.1	21.7	6.1	5.8	27.8
224	14.5	5.3	19.8	8.1	2.1	28.0
139	17.0	5.2	22.2	6.0	7.3	28.1
492	4.9	4.5	9.3	19.0	4.5	28.3
216	16.1	5.8	21.8	6.7	2.2	28.5
381	19.3	4.1	23.4	5.2	9.7	28.5
384	18.5	4.1	22.6	6.0	12.4	28.6
288	15.9	5.2	21.1	7.5	1.4	28.7
280	17.4	6.1	23.5	5.2	3.7	28.7
557	18.9	6.1	25.0	3.6	5.7	28.7
206-2	17.6	5.1	22.8	5.9	6.0	28.7
141	17.6	5.6	23.3	5.5	9.1	28.7
271	15.9	7.0	22.9	5.8	3.3	28.7
299	15.8	4.8	20.6	8.3	2.7	29.0
208	17.9	5.1	23.0	6.1	4.8	29.0
495	5.0	5.6	10.5	18.5	5.8	29.1
284	18.6	6.3	24.9	4.2	1.7	29.1
211	18.5	5.9	24.4	4.7	4.3	29.1
486	4.9	3.3	8.2	21.0	6.7	29.1
215	16.4	6.1	22.5	6.7	2.5	29.2
142	16.7	5.3	22.0	7.2	13.1	29.2
485	5.1	5.3	10.3	18.9	5.3	29.2
271-2	15.3	7.3	22.6	6.7	4.0	29.3
494	5.1	6.2	11.2	18.1	3.7	29.4
388	18.5	4.0	22.6	6.9	7.9	29.4
444	12.0	5.6	17.6	11.9	2.6	29.5
291	15.8	6.4	22.2	7.4	2.2	29.6
377-2	17.1	3.8	20.9	8.76	4.6	29.6
140	16.5	6.5	22.9	9	3.7	29.9
249	18.2	5.1	23.9	6.0	5.2	29.9
293	15.5	5.0	20.4	9.6	6.5	30.0
292	15.5	5.8	21.3	8.7	2.4	30.1
275	17.3	7.5	24.8	5.3	4.4	30.1
274	16.4	6.7	23.1	7.1	5.7	30.2
296	13.9	6.6	20.6	9.8	3.0	30.4
260	16.2	5.8	22.0	8.4	4.9	30.4
213	17.3	4.9	22.2	8.3	2.5	30.5

TABLE 11-continued

A & B: Color and odor-producing properties of commodity soybeans harvested.						
295	15.4	5.7	21.2	9.5	5.6	30.7
570	17.6	6.8	24.4	6.3	4.1	30.7
235	16.2	4.8	21.0	9.8	7.2	30.8
490	5.1	5.0	10.1	20.8	5.7	30.9
294	17.5	6.1	23.7	7.2	4.3	30.9
481	4.9	4.2	9.1	21.8	4.3	30.9
380	20.1	4.1	24.2	6.8	7.2	31.0
206	17.8	5.2	22.9	8.1	5.9	31.1
377	18.6	3.8	22.4	8.6	5.6	31.1
382	21.3	4.0	25.2	6.0	5.6	31.3
481-2	5.1	1.9	7.0	24.5	5.0	31.4
262	16.9	5.6	22.4	9.0	4.8	31.5
279	18.1	8.1	26.2	5.3	2.8	31.5
300	16.8	5.9	22.8	8.8	6.2	31.5
227	16.9	6.0	22.9	8.6	1.9	31.5
298	14.9	6.0	20.9	10.7	3.8	31.6
231	18.6	6.4	25.0	6.8	9.0	31.8
220	17.4	7.0	24.4	7.4	4.0	31.9
400	7.5	9.1	16.6	15.4	6.8	32.0
482	5.1	6.7	11.7	20.2	4.3	32.0
125	16.7	4.4	21.1	10.9	2.2	32.0
225	18.0	5.8	23.8	8.3	0.6	32.1
539	20.4	5.8	26.2	5.9	3.8	32.1
212	17.0	3.8	20.8	11.3	8.8	32.1
281	17.2	8.0	25.2	7.0	6.6	32.2
36	17.1	5.1	22.2	10.0	0.0	32.3
192	10.4	6.5	16.9	15.6	5.4	32.5
551	20.3	8.0	28.2	4.3	4.4	32.5
416	7.3	7.0	14.3	18.4	6.3	32.7
547	20.8	6.4	27.2	5.6	4.1	32.8
277	18.7	6.4	25.1	1.9	5.3	32.9
248	20.7	5.7	26.4	6.1	6.5	33.0
267	17.0	5.6	22.5	10.6	4.9	33.1
232	16.5	6.1	22.6	10.5	3.0	33.1
11	16.0	6.4	22.4	10.7	2.6	33.1
245	18.3	6.8	25.0	8.1	1.5	33.1
401	21.5	5.9	27.5	5.7	8.9	33.1
255	19.2	7.2	26.4	7.1	5.1	33.5
230	17.7	6.3	24.0	9.4	2.8	33.5
283	20.4	5.9	26.3	7.3	1.1	33.6
349	11.4	8.0	19.4	14.4	2.4	33.8
166	8.3	8.7	17.0	16.8	4.1	33.8
272	19.5	7.5	27.1	6.8	0.8	33.9
483	5.1	2.0	7.1	26.9	3.5	33.9
236	19.3	6.3	25.6	8.4	0.4	34.0
491	5.1	4.6	9.6	24.4	6.9	34.0
571	21.0	7.3	28.3	5.8	3.0	34.1
376-2	21.1	3.9	25.0	9.1	5.7	34.1
147	20.7	5.5	26.1	8.1	13.9	34.2
148	19.4	6.0	25.4	9.2	8.6	34.7
266	18.6	6.1	24.6	10.0	0.5	34.7
242	20.1	5.6	25.8	9.1	2.8	34.9
222-2	18.5	5.4	23.9	10.9	5.3	34.9
250	20.6	6.2	26.8	8.1	8.5	34.9
222	18.3	5.3	23.6	11.3	5.2	34.9
549	21.2	8.6	29.7	5.3	3.9	35.1
41	20.7	6.2	26.9	8.3	4.06	35.2
219	19.0	6.4	25.4	9.8	2	35.2
278	19.5	7.5	27.0	8.3	2.7	35.2
149	20.2	5.4	25.6	9.79	11.2	35.3
233	19.4	6.8	26.2	2	1.4	35.4
185	14.2	5.5	19.7	15.7	5.5	35.4
389	24.6	4.1	28.7	6.8	2.3	35.4
221	21.4	5.3	26.7	8.7	5.8	35.5
348	19.2	5.7	24.8	10.7	3.6	35.5
136-2	20.0	4.8	24.8	10.8	11.5	35.6
218	19.1	6.1	25.2	10.5	7.1	35.8
217	19.1	5.7	24.8	11.1	16.6	35.9
256	18.4	6.1	24.5	11.4	6.0	36.0
555	24.2	7.4	31.5	4.5	5.3	36.0
346	19.8	6.5	26.2	9.7	3.1	36.0
489	5.1	4.4	9.5	26.6	5.4	36.1
228	20.6	6.2	26.8	9.3	7.2	36.1
261	18.2	7.0	25.3	10.9	1.1	36.2

TABLE 11-continued

A & B: Color and odor-producing properties of commodity soybeans harvested.						
189	14.1	6.0	20.1	16.2	12.7	36.3
566	22.1	7.8	29.9	6.4	0.8	36.3
404	22.7	6.0	28.7	7.9	4.9	36.5
114	22.5	3.2	25.7	11.0	10.7	36.7
136	20.0	4.8	24.8	11.9	14.6	36.7
102	18.8	5.3	24.0	12.8	5.8	36.8
376	21.9	4.1	26.0	10.9	6.3	36.9
352	17.5	6.9	24.4	12.8	3.4	37.2
223	19.4	5.9	25.4	11.9	3.1	37.2
544	22.6	9.6	32.2	5.1	5.1	37.3
40	22.0	5.6	27.6	9.8	0.9	37.4
545	25.4	7.5	32.9	4.5	2.7	37.4
488	5.1	5.2	10.3	27.2	7.0	37.5
559	20.9	6.9	27.8	9.7	4.9	37.5
180	18.6	6.5	25.1	12.5	9.8	37.5
417	5.5	8.1	13.6	24.1	7.6	37.7
143	21.4	5.8	27.1	10.6	16.0	37.7
428	5.1	8.5	13.5	24.2	8.3	37.8
487	5.1	5.3	10.3	27.5	6.6	37.8
358	13.3	7.5	20.8	17.1	4.2	37.9
305	20.7	6.8	27.5	10.4	5.9	37.9
173	13.8	6.4	20.3	17.8	2.3	38.1
561	25.7	8.2	34.0	4.1	5.4	38.1
405	24.8	6.2	31.1	7.1	1.9	38.1
259	19.2	6.8	26.1	12.2	4.2	38.3
202	17.1	5.6	22.7	15.6	4.7	38.3
297	18.1	8.0	26.0	12.3	2.1	38.3
270	20.1	5.6	25.7	12.7	5.0	38.4
39	23.0	5.9	28.8	9.6	5.7	38.4
177	17.0	6.4	23.5	15.0	6.5	38.5
223-2	21.1	6.1	27.2	11.4	3.5	38.5
360	14.0	7.3	21.3	17.3	3.1	38.6
273	22.3	6.6	29.0	9.9	7.3	38.8
286-2	27.1	6.3	33.5	5.4	26.1	38.8
440	17.1	6.9	24.0	14.9	1.0	38.9
229	22.6	8.3	30.8	8.1	0.3	38.9
550	26.6	7.1	33.7	5.5	7.3	39.2
399	8.0	10.4	18.4	20.8	4.6	39.2
236-2	22.9	6.2	29.0	10.3	0.8	39.3
247	21.8	7.0	28.7	10.7	8.6	39.4
45	23.1	6.2	29.3	10.1	5.7	39.4
484	5.1	6.1	11.2	28.5	2.1	39.7
558	22.5	7.5	30.0	9.7	3.5	39.7
432	13.5	16.0	29.5	10.3	6.1	39.8
556-2	25.8	6.0	31.7	8.1	7.9	39.9
554	22.0	8.6	30.6	9.4	3.9	40.0
198	18.7	5.5	24.2	15.9	5.8	40.1
546	26.8	7.1	33.9	6.2	4.8	40.1
560	22.6	7.2	29.8	10.3	4.6	40.2
272-2	23.2	7.8	31.0	9.1	1.0	40.2
214	20.7	6.9	27.5	13.0	3.5	40.5
42	23.6	6.1	29.6	11.0	3.5	40.6
569	22.0	7.3	29.3	11.3	4.9	40.6
27	21.8	5.8	27.7	12.9	5.8	40.6
38	22.5	5.0	27.5	13.2	8.0	40.7
565	28.0	7.2	35.2	5.5	5.9	40.7
108	20.7	4.6	25.3	15.5	5.5	40.8
540	26.0	9.2	35.1	5.7	2.5	40.8
174	20.6	6.0	26.7	14.3	8.0	41.0
342-2	22.9	7.1	30.0	11.0	1.6	41.0
315	12.0	5.6	17.6	23.5	5.3	41.1
538	27.5	8.0	35.5	5.6	5.6	41.1
237-2	23.5	6.0	29.5	11.7	8.0	41.2
310	20.5	7.4	28.0	13.2	8.3	41.2
192-2	19.6	5.1	24.7	16.6	5.9	41.3
354	18.9	10.7	29.6	11.8	5.0	41.3
391	23.5	3.8	27.3	14.1	7.1	41.4
146	22.4	5.6	28.0	13.6	9.3	41.6
418	15.9	7.0	23.0	18.6	5.7	41.6
263	22.1	5.8	27.9	13.7	4.0	41.7
573	22.7	8.7	31.4	10.4	3.9	41.8
200	17.9	5.7	23.6	18.2	4.7	41.8
347	21.3	7.3	28.6	13.7	5.1	42.3
395	25.0	6.1	31.0	11.4	9.2	42.4

TABLE 11-continued

A & B: Color and odor-producing properties of commodity soybeans harvested.						
36-2	24.1	5.1	29.2	13.3	0.4	42.5
237	23.1	6.3	29.4	13.1	9.1	42.5
251	28.2	7.6	35.7	6.8	6.0	42.5
22	22.8	5.7	28.5	14.1	5.5	42.6
335	16.3	9.1	25.4	17.2	4.3	42.6
256-2	22.3	7.1	29.4	13.2	9.4	42.6
375	23.9	3.9	27.8	14.9	4.9	42.7
203	19.0	5.5	24.5	18.1	6.2	42.7
254	25.8	6.4	32.2	10.5	3.2	42.8
563	22.7	7.6	30.3	12.6	2.9	42.9
572	25.1	7.8	33.0	9.9	2.3	42.9
536	26.8	5.9	32.7	10.2	6.6	42.9
144	24.0	5.0	29.1	13.9	15.6	43.0
241	27.1	5.5	32.6	10.4	6.5	43.0
191	14.4	6.5	20.9	22.1	4.3	43.0
461	26.0	1.3	27.3	15.7	6.8	43.0
359	22.6	7.4	30.0	13.0	4.9	43.1
179	20.0	5.4	25.4	17.7	10.2	43.1
556	26.8	3.8	30.6	12.6	7.4	43.1
541-2	25.6	6.6	32.2	11.0	6.2	43.2
252	25.1	9.0	34.1	9.2	0.5	43.4
201	18.6	6.3	24.8	18.6	5.8	43.4
257	25.1	5.4	30.4	13.4	3.6	43.8
269	23.6	6.2	29.8	14.1	9.0	43.9
301	22.8	5.9	28.7	15.2	3.2	43.9
191-2	18.9	6.6	25.5	18.4	4.3	43.9
190	22.6	5.7	28.4	15.6	4.4	43.9
407	14.8	7.1	21.9	22.1	7.4	44.0
562	25.1	7.8	32.9	11.2	4.2	44.1
178	22.5	7.9	30.4	13.8	12.8	44.1
244	29.7	6.7	36.4	7.8	4.7	44.2
445	26.4	6.7	33.1	11.1	0.8	44.2
151	24.8	5.4	30.3	13.9	13.4	44.2
111-2	16.2	13.5	29.8	14.4	7.5	44.3
145	25.1	6.7	31.8	12.4	16.6	44.3
542	23.7	6.4	30.1	14.2	1.7	44.3
531	28.4	5.4	33.9	10.6	7.5	44.5
246	22.4	6.1	28.5	16.0	3.5	44.5
15	25.0	5.6	30.6	13.9	3.9	44.5
37	27.0	5.9	33.0	11.6	5.9	44.6
258	23.5	6.0	29.5	15.2	1.5	44.6
446	22.2	7.0	29.2	15.5	6.5	44.7
205	20.9	5.4	26.4	18.4	5.3	44.8
197	20.8	5.9	26.7	18.1	5.6	44.8
112	24.9	5.1	30.6	14.3	9.7	45.0
72	24.7	6.4	31.1	13.9	14.1	45.0
240	27.5	7.3	34.8	10.3	6.9	45.1
460	26.7	1.8	28.5	16.6	3.6	45.1
153	25.5	5.3	30.8	14.3	5.0	45.1
534	22.6	7.8	30.3	14.9	4.2	45.2
447	26.7	7.0	33.8	11.6	4.8	45.3
57	25.9	6.5	32.4	13.0	4.9	45.4
132	24.4	5.1	30.1	15.4	9.1	45.5
332	22.4	6.8	29.2	16.2	2.2	45.5
3	19.8	5.5	25.3	20.2	3.2	45.5
181	23.2	5.4	28.5	17.1	6.2	45.6
127	24.6	5.2	29.8	16.0	8.4	45.7
172	22.5	1.9	31.4	14.4	5.2	45.8
43	26.8	6.6	33.4	12.5	0.9	45.9
68	23.6	7.1	30.7	15.3	15.7	46.0
150	24.5	6.2	30.7	15.3	9.6	46.0
451	21.9	2.7	24.6	21.4	4.5	46.0
541	26.9	6.8	33.7	12.5	5.1	46.1
312	15.3	7.3	22.6	23.6	5.3	46.1
110	24.9	3.9	28.8	17.3	10.7	46.1
548	28.7	7.4	26.2	10.1	6.4	46.3
530	29.0	7.3	36.3	10.1	5.3	46.5
66	24.6	7.0	31.7	14.8	9.8	46.5
157	27.0	5.7	32.8	13.8	9.3	46.5
226	31.2	7.2	38.4	8.1	4.9	46.5
343	22.9	8.2	31.0	15.6	6.9	46.7
152	25.8	5.1	30.9	15.8	11.2	46.7
164	25.0	6.6	31.6	15.2	9.3	46.8
374	25.1	4.0	29.1	17.7	3.0	46.8

TABLE 11-continued

A & B: Color and odor-producing properties of commodity soybeans harvested.						
336	26.9	6.3	33.2	13.6	4.6	46.8
171	21.0	13.0	34.0	12.9	4.9	46.9
567	31.9	9.6	41.5	5.4	3.5	46.9
90	26.8	6.1	32.8	14.1	8.2	46.9
350	21.1	9.2	30.3	16.6	8.0	47.0
131	26.9	5.4	32.3	14.7	7.0	47.0
345	21.9	8.3	30.2	16.9	6.3	47.1
436	22.3	6.5	28.8	18.3	6.3	47.1
309	24.6	7.6	32.2	14.8	2.8	47.1
181-2	23.1	5.9	28.9	18.2	8.7	47.1
81	28.3	5.0	33.3	13.9	4.7	47.2
196	22.6	5.1	27.7	19.5	6.6	47.2
154	27.5	5.3	32.8	14.5	7.4	47.3
70	25.5	5.6	31.1	16.3	8.9	47.4
119	28.7	3.8	32.6	14.9	4.9	47.5
419	13.4	8.8	22.2	25.3	1.6	47.5
195	19.9	6.0	25.9	21.7	5.0	47.5
396	25.6	5.6	31.3	16.3	5.7	47.5
109	26.1	5.4	31.5	16.1	7.1	47.5
347-2	25.4	7.8	33.1	14.6	5.6	47.7
529	24.2	7.5	31.7	16.1	5.5	47.8
50	28.3	7.5	35.8	12.2	6.7	48.0
304	22.5	6.4	29.0	19.1	1.6	48.0
78	26.3	6.7	33.1	15.1	6.2	48.1
121-2	25.7	5.7	31.4	16.7	14.8	48.2
62-2	27.1	5.9	32.9	15.2	14.0	48.2
553	25.7	9.7	35.4	12.9	3.2	48.3
511	26.3	2.7	29.1	19.2	3.8	48.3
413	19.2	8.8	28.0	20.4	5.8	48.4
106	25.9	4.2	30.1	18.3	12.8	48.4
303	22.9	5.4	28.3	20.2	1.9	48.5
182	24.1	5.5	29.6	18.9	4.5	48.5
12	22.6	5.9	28.5	20.1	4.5	48.5
176	22.7	5.6	28.3	20.3	5.2	48.6
265	27.8	7.2	35.0	13.7	0.9	48.7
264	26.5	8.0	34.5	14.3	1.8	48.8
344	23.6	8.0	31.6	17.3	4.4	48.8
85	21.4	11.4	32.8	16.1	5.1	48.9
394	24.5	6.2	30.7	18.2	8.4	49.0
268	27.8	8.5	36.3	12.8	1.3	49.1
535	36.1	7.2	43.3	5.8	5.1	49.1
194	21.8	5.9	27.7	21.5	8.3	49.2
443	21.7	6.8	28.5	20.9	0.8	49.4
340	23.7	8.1	31.8	17.6	4.7	49.4
129	26.6	5.3	32.0	17.5	12.9	49.4
152-2	27.8	5.2	33.0	16.6	11.2	49.5
128	27.0	4.6	31.5	18.0	15.3	49.5
564	27.6	7.5	35.1	14.5	3.4	49.6
337	29.8	6.2	36.0	13.7	3.3	49.6
159	28.6	5.4	34.0	15.7	15.2	49.7
105	25.7	6.0	31.7	18.1	15.9	49.8
311	25.6	6.1	31.7	18.1	6.2	49.9
302	23.0	6.0	28.9	21.0	7.1	49.9
346-2	28.0	6.7	34.7	15.2	3.3	49.9
62	27.0	6.0	33.0	17.0	9.7	49.9
44	28.3	7.3	35.5	14.5	2.9	50.0
421	27.2	7.7	34.9	15.3	5.5	50.2
238	25.1	6.3	31.4	18.9	3.9	50.2
158	28.0	5.5	33.5	16.8	10.6	50.3
338	28.4	1.5	35.9	14.5	3.1	50.4
92	28.1	6.1	34.2	16.3	8.2	50.5
356	26.7	7.6	34.2	16.5	7.0	50.7
80	28.0	6.7	34.8	16.1	8.3	50.9
568	31.3	10.5	41.7	9.1	7.4	50.9
456	25.1	0.6	25.8	25.1	90.1	50.9
409	27.5	7.6	35.1	15.8	7.7	50.9
452	26.4	1.1	27.6	23.4	20.4	51.0
331	24.2	8.1	32.3	18.7	5.6	51.1
65	27.8	7.8	35.6	15.8	9.0	51.4
193	29.3	6.9	26.2	15.3	5.2	51.5
365	26.9	4.0	30.9	20.6	4.3	51.5
462	21.9	0.6	28.5	23.2	95.0	51.7
204	23.7	5.9	29.6	22.2	6.2	51.8
115	31.1	6.2	37.3	14.7	3.7	52.0

TABLE 11-continued

A & B: Color and odor-producing properties of commodity soybeans harvested.						
441	23.4	7.6	31.0	21.0	5.8	52.0
307	26.5	6.3	32.9	19.2	5.5	52.1
391-2	26.5	5.6	32.1	20.1	7.6	52.2
63	28.1	6.2	34.4	17.8	14.5	52.2
543	35.8	8.4	44.1	8.1	3.6	52.2
46	31.5	6.2	37.7	14.5	4.9	52.2
133	29.9	5.1	35.0	17.2	10.1	52.2
302-2	24.6	6.4	31.0	21.2	7.3	52.3
243	30.2	7.6	37.9	14.5	7.5	52.3
170	26.5	8.1	34.6	17.8	11.8	52.4
25	28.6	5.9	34.5	17.9	6.8	52.4
450	32.6	7.8	40.4	12.2	5.9	52.6
239	29.4	7.4	36.8	15.9	4.7	52.7
448	24.1	13.4	37.4	15.3	2.2	52.7
519	32.6	5.1	37.8	14.9	2.6	52.7
438	26.3	6.6	32.9	19.9	9.0	52.8
111	31.0	6.1	37.7	15.2	5.5	52.9
71	29.8	6.1	35.9	17.2	12.7	53.1
163	29.0	5.8	34.8	18.5	154	53.3
464	26.7	0.8	27.5	25.8	35.5	53.3
107	29.9	4.7	34.6	18.7	20.6	53.3
371	28.8	4.1	32.9	20.4	8.6	53.3
313	25.3	6.2	32.1	21.3	9.7	53.3
199	23.3	5.3	28.6	24.8	6.3	53.4
104	25.7	6.9	32.5	20.9	17.9	53.5
103	29.1	3.8	32.9	20.6	13.4	53.5
339	25.7	9.1	34.8	18.7	4.8	53.5
439	27.2	6.2	33.9	19.6	6.5	53.6
372	27.5	3.8	31.4	22.4	6.4	53.8
187	15.0	22.2	37.2	16.6	2.1	53.8
317	26.8	5.9	32.7	21.1	7.6	53.8
306	23.7	6.6	30.3	23.5	8.0	53.9
355	30.9	7.2	38.1	15.8	4.4	53.9
86	30.8	6.3	37.1	16.8	11.7	53.9
436-2	26.5	6.3	32..8	21.3	6.2	54.0
91	31.0	6.4	37.4	16.7	9.0	54.1
61-2	28.1	7.8	35.9	18.2	15.4	54.1
77	29.7	7.0	36.7	17.5	3.3	54.2
156	31.5	5.4	36.9	17.3	5.9	54.2
511-2	27.9	2.8	30.7	23.8	5.0	54.5
28	34.7	6.0	40.7	13.8	7.9	54.5
113	32.2	5.6	37..8	16.8	15.7	54.6
76	30.7	6.5	37.2	17.4	9.1	54.6
427	32.8	7.1	39.9	14.8	5.0	54.7
449	26.7	7.3	34.0	20.9	4.7	54.9
324	26.6	8.1	34.7	20.5	0.9	55.2
207	36.2	8.7	44.9	10.4	0.2	55.3
316	29.0	6.1	35.2	20.2	6.8	55.4
121	29.2	5.5	34.7	20.6	11.0	55.4
28	32.6	6.3	38.9	16.5		55.4
126	30.0	5.6	35.6	19.9	11.5	55.5
451-2	27.2	0.8	28.0	27.6	7.8	55.6
135	30.1	6.0	36.1	19.7	15.3	55.8
393	27.9	6.5	34.5	21.4	9.2	55.8
130	30.2	6.1	36.3	19.6	14.2	55.9
357	24.3	18.1	42.4	13.5	4.1	55.9
314	26.0	6.7	32.6	23.3	8.3	55.9
184	29.0	5.5	34.5	21.5	92	56.0
151-2	32.5	5.6	38.2	17.9	14.5	56.1
101	31.5	4.0	35.5	20.6	9.0	56.1
331-2	27.6	7.8	35.5	20.6	5.0	56.1
330	30.9	8.6	39.5	16.6	7.1	56.1
325	28.7	9.7	38.4	17.8	5.6	56.2
351	27.7	6.7	34.3	21.9	4.8	56.2
403	39.1	8.1	47.1	9.1	6.3	56.2
322	29.8	8.1	37..8	18.6	4.6	56.5
463	26.8	0.7	27.5	28.9	36.7	56.5
155	32.7	5.7	38.4	18.1	6.3	56.5
435	32.0	7.4	39.5	17.2	4.2	56.6
397	28.8	6.4	35.2	21.6	4.3	56.8
	29.2	7.0	36.3	20.6	10.4	56.9
465	26.5	1.0	27.5	29.5	44.8	56.9
183	29.4	8.1	37.5	19.5	12	57.0
321	35.0	9.4	44.4	12.9	4.0	57.3



TABLE 11-continued

A & B: Color and odor-producing properties of commodity soybeans harvested.						
457	31.2	0.7	31.8	25.5	15.4	57.4
21	32.9	6.3	39.2	18.2	2.4	57.4
316-2	28.5	6.1	34.6	22.9	7.9	57.5
34	33.6	7.1	40.7	16.9	9.4	57.6
69	30.5	6.9	37.4	20.2	11.4	57.6
94	31.9	8.3	40.2	17.4	8.3	57.6
317-2	28.3	6.0	34.3	23.5	8.8	57.8
422	25.9	10.0	35.9	22.0	6.2	57.9
366	35.1	4.3	39.4	18.5	6.5	51.9
188	28.9	9.4	38.3	19.7	9.2	58.0
87	32.2	7.3	39.5	18.5	7.3	58.0
473	27.8	4.6	32.4	25.6	10.7	58.0
527	31.5	6.7	38.2	19.9	4.1	58.1
341	28.6	7.9	36.5	21.7	3.7	58.2
117	33.6	6.6	40.2	18.0	8.7	58.2
342	28.6	1.9	36.5	21.8	4.3	58.2
4	28.9	1.1	36.6	21.7	11.7	58.3
67	31.7	7.0	38.7	19.7	12.0	58.4
47	34.1	6.9	41.0	17.4	3.2	58.4
165	33.2	6.2	39.4	19.0	12.9	58.5
369	30.5	4.1	34.6	24.0	12.3	58.6
370	38.8	4.1	42.9	15.9	4.2	58.8
421-2	29.1	7.8	37.0	21.9	5.3	58.9
533	31.1	7.4	38.5	20.7	5.7	59.2
35	34.1	6.3	40.4	18.8	6.4	59.2
434	22.0	10.2	32.2	27.0	8.6	59.2
364	30.8	3.9	34.7	24.5	4.6	59.2
326	27.0	8.7	35.7	23.6	2.9	59.3
123	32.0	5.9	37.8	21.5	11.4	59.4
120	33.2	4.0	37.2	22.2	20.2	59.4
116	34.5	6.2	40.8	18.7	8.7	59.4
361	33.1	4.1	37.2	22.3	2.3	59.5
84	34.8	6.3	41.1	18.8	1.5	59.9
24	31.1	5.7	36.8	23.1	3.4	59.9
353	30.5	8.8	39.3	20.6	5.9	60.0
122-2	32.7	5.7	38.4	21.6	17.5	60.1
528	32.7	6.6	39.3	20.9	5.3	60.2
186	35.3	8.2	43.5	16.7	6.2	60.2
21-2	32.6	6.2	38.8	21.4	3.2	60.2
415	26.9	7.0	33.9	26.4	5.8	60.3
328	26.5	8.1	34.6	25.8	5.9	60.4
118	36.0	3.9	39.9	20.5	7.1	60.4
402	32.8	6.5	39.4	21.2	4.6	60.5
466	31.8	1.0	32.8	27.9	38.5	60.7
13	31.8	6.6	38.4	22.4	8.2	60.8
442	27.1	12.1	39.2	21.8	2.6	60.9
61	32.9	8.1	41.0	20.3	15.7	61.3
455	28.4	0.8	29.2	32.1	30.6	61.3
423	29.2	6.1	35.3	26.1	3.8	61.4
169	36.0	8.5	44.5	17.0	7.8	61.4
458	27.8	2.2	30.0	31.6	5.4	61.5
362	35.8	3.9	39.7	22.1	5.2	61.8
124	33.6	5.9	39.5	22.4	11.9	61.9
33	35.7	6.4	42.1	19.9	7.2	62.0
22-2	32.7	6.0	38.6	23.3	6.2	62.0
23	36.2	6.7	42.9	19.2	2.3	62.1
32	34.0	6.5	40.5	21.8	9.7	62.3
253	36.1	11.2	47.3	15.0	1.7	62.3
75	36.9	6.1	43.0	19.5	8.2	62.5
318	28.4	9.2	37.6	25.0	4.7	62.6
420	26.8	11.3	38.1	24.5	7.8	62.6
161	34.0	6.1	40.1	22.6	8.2	62.7
74	34.3	7.4	41.7	21.1	12.2	62.8
48	39.0	6.4	45.4	17.5	2.5	62.9
26	33.8	6.7	40.5	22.6	5.7	63.1
406-2	32.4	9.1	41.5	21.7	7.4	63.2
552	42.2	7.8	50.0	13.9	4.1	63.9
51-2	37.5	6.5	44.1	19.8	4.0	63.9
424	31.4	1.6	39.1	24.9	7.6	63.9
88	37.9	6.1	44.6	19.4	8.3	63.9
122	34.9	6.2	41.2	23.0	13.0	64.2
160	36.7	6.1	42.9	21.5	11.8	64.4
398	36.1	8.0	44.1	20.4	15.7	64.4
89	36.6	8.5	45.0	19.9	11.4	64.9

TABLE 11-continued

A & B: Color and odor-producing properties of commodity soybeans harvested.						
323	34.5	7.3	41.8	23.2	0.9	65.0
512	32.0	3.1	35.1	29.9	11.8	65.0
429	30.6	7.3	37.9	27.3	113	65.2
91-2	37.1	6.7	43.7	21.6	8.6	65.3
64	38.9	11.8	50.7	14.7	9.6	65.4
433	32.4	8.5	40.9	24.5	2.3	65.4
453	31.1	0.8	31.9	33.6	41.5	65.5
367	36.2	4.0	40.3	25.4	4.7	65.7
10	35.6	9.3	44.9	21.7	2.1	66.5
437-2	33.9	8.2	42.1	24.5	3.4	66.6
521	35.1	3.6	38.7	27.9	7.8	66.6
472	33.4	2.7	36.1	30.6	6.6	66.8
56	40.7	9.8	50.6	16.3	15.2	66.8
5	36.1	6.0	42.1	24.8	3.2	66.9
31	41.3	6.3	47.5	19.7	7.5	67.3
532	48.9	8.9	57.8	10.1	5.6	67.9
329	34.9	9.1	44.1	24.0	5.1	68.1
134	38.9	5.3	44.2	24.0	14.9	68.2
2	34.0	11.2	45.2	23.0	7.9	68.3
526	45.9	7.1	53.0	15.7	2.7	68.8
408	36.3	7.8	44.2	24.6	8.2	68.8
523	35.9	5.4	41.3	27.7	7.4	69.1
373	42.4	4.0	46.4	22.7	6.0	69.1
407-2	37.7	6.8	44.5	24.8	7.6	69.3
79	37.9	7.9	45.8	23.7	7.7	69.4
524	44.3	3.9	48.2	21.3	7.6	69.5
478	34.1	7.0	41.1	28.4	3.9	69.5
515	36.4	4.7	41.2	28.5	8.0	69.6
476	33.1	3.3	36.4	33.3	6.2	69.7
8	37.5	9.1	46.6	23.1	6.9	69.8
516	35.7	3.6	39.2	30.6	8.3	69.9
520	38.5	5.5	44.0	26.1	8.7	70.2
503	39.1	3.4	42.5	27.8	6.8	70.3
406	38.7	6.8	45.5	24.9	9.7	70.5
363	36.4	3.9	40.3	30.3	6.9	70.6
49	47.0	8.5	55.5	15.1	3.0	70.7
98	39.4	7.5	46.8	23.9	11.9	70.8
414	32.0	12.2	44.2	26.7	3.9	70.9
308	30.9	6.9	37.8	33.3	3.8	71.1
514	36.9	3.9	40.8	30.4	4.8	71.2
509	39.7	5.0	44.7	26.8	7.3	71.4
234	39.1	10.2	49.3	22.4	3.8	71.7
466-2	32.5	1.9	40.4	31.5	36.6	71.9
101-2	38.7	10.5	49.2	22.9	8.1	72.1
162	40.2	7.7	47.9	24.6	12.0	72.5
361-2	40.3	3.8	44.1	28.4	3.4	72.5
334	39.8	10.4	50.2	22.8	3.9	73.0
327	35.5	12.8	48.3	24.8	2.8	73.0
525	38.4	8.3	46.8	26.6	7.9	73.4
52	48.8	6.6	55.4	18.5	4.0	73.9
54	43.7	10.8	54.5	19.4	16.6	73.9
459	35.8	3.0	38.8	35.6	4.1	74.4
497	39.2	4.8	44.1	30.8	6.6	74.9
83	44.9	6.4	51.2	23.8	8.7	75.1
81-2	54.4	6.3	60.7	14.3	4.1	75.1
467	42.8	0.9	43.7	31.4	21.8	75.1
526-2	46.9	6.9	53.7	21.8	4.3	75.5
469	36.0	5.5	41.5	34.1	6.5	75.6
500	33.2	5.9	39.1	37.3	7.2	76.3
73	44.6	7.3	51.9	24.6	9.8	76.6
100	43.1	9.3	52.4	24.2	6.4	76.6
518	40.0	4.3	44.3	32.4	5.4	76.7
437	28.3	15.4	43.7	33.1	4.9	76.8
58	42.6	15.2	57.8	19.2	16.9	77.1
19	44.7	7.3	52.0	25.1	12.7	77.1
467-2	45.3	1.8	47.1	30.1	3.8	77.2
508	48.0	4.9	52.9	24.3	8.7	77.2
454	37.7	1.2	38.9	38.4	8.3	77.2
499	41.0	5.0	46.0	31.7	7.0	77.7
333	44.0	8.7	52.7	25.1	4.5	77.8
431	40.1	7.0	47.1	30.8	8.3	77.9
167	54.4	6.9	61.2	16.8	9.5	78.0
480	41.3	5.5	46.8	31.3	5.9	78.1
6	46.4	9.1	55.5	22.7	11.9	78.2

TABLE 11-continued

A & B: Color and odor-producing properties of commodity soybeans harvested.						
501	48.8	7.3	56.1	22.3	8.4	78.4
410	48.2	9.0	57.2	21.6	3.7	78.7
471	38.8	3.9	42.7	36.1	7.2	78.8
468	38.7	5.6	44.3	34.9	7.4	79.1
475	36.7	6.3	43.0	36.1	6.5	79.1
470	36.7	7.4	44.1	35.2	6.5	79.3
502	39.4	3.9	43.3	36.1	12.5	79.3
477	43.2	2.9	46.1	33.6	5.9	79.7
479	41.0	6.5	47.5	32.9	6.2	80.4
368	46.8	4.0	50.8	29.6	8.6	80.4
60	49.0	4.0	53.0	27.7	13.7	80.6
166-2	49.7	8.1	57.8	23.2	3.8	81.0
412	47.3	8.4	55.8	25.3	6.9	81.1
93	52.5	4.2	56.8	26.5	6.4	83.2
510	42.4	6.0	48.4	35.3	7.5	83.7
474	41.4	5.8	47.2	37.2	10.8	84.4
53	55.2	6.5	61.7	24.3	7.6	86.0
99	51.5	8.1	59.6	26.5	10.1	86.0
537	68.9	8.9	77.8	8.4	4.0	86.2
522	45.2	6.1	51.3	35.6	7.9	86.9
11.2	53.2	15.8	69.0	18.1	3.5	87.1
55	56.0	9.8	65.8	21.5	11.4	87.3
504	43.5	5.2	48.7	38.8	8.2	87.5
320	51.0	12.2	63.2	24.6	5.8	87.7
319	51.6	10.6	62.2	26.5	4.8	88.7
506	55.0	5.1	60.1	28.9	6.0	88.9
95	54.3	7.5	61.8	27.9	7.9	89.7
30	57.4	6.6	64.0	25.9	8.2	89.9
517	59.1	5.4	64.5	25.7	10.5	90.3
96	53.5	7.9	61.4	28.9	9.6	90.3
29	61.2	7.1	68.3	26.1	3.8	94.4
496	60.6	5.2	65.9	29.6	4.5	95.5
512-2	53.4	3.5	56.9	38.6	13.7	95.5
59	55.8	9.5	65.3	30.5	14.1	95.9
7	56.6	11.3	67.9	28.7	8.5	96.6
16	60.4	11.6	72.1	29.3	11.8	101.4
20	67.2	8.1	75.3	26.8	9.9	102.1
513	64.3	4.1	68.4	37.2	10.8	105.6
498	60.3	4.8	65.1	41.4	10.3	106.5
9	67.4	8.4	75.8	32.1	9.1	107.9
14	65.8	7.4	73.2	35.8	7.8	109.0
496-2	79.1	6.0	85.2	28.4	4.5	113.5
507	78.1	6.2	84.3	29.5	6.5	113.8
97	69.2	10.8	80.0	34.9	11.2	114.9
430	76.1	14.0	90.1	25.1	3.7	115.2
12	74.1	11.5	85.6	30.6	2.9	116.2
505	83.3	6.5	89.8	29.9	7.8	119.6
18	77.1	6.7	83.8	36.0	7.6	119.8
17	81.7	8.2	89.8	36.6	10.1	126.5
392	92.1	14.7	106.8	20.5	15.0	127.4
425	90.2	12.7	102.9	28.8	8.2	131.7
60	86.9	8.3	95.2	41.3	25.8	136.5
426	119.6	15.4	135.0	24.1	4.19.0	159.1
82	119.9	50.5	170.4	13.9		184.4
Range	4.9-120	0.6-51	7.0-170	2.8-41	0-95	21-184
Average	28.5	6.6	35.0	17.4	7.3	52.4
Stdev	13.9	2.9	15.1	7.8	7.0	20.1

## B: Color

Sample Name	b*	L*
ID: 47	27.18	82.34
ID: 42	27.29	83.02
ID: 45	27.40	81.58
JA: 48	27.88	84.41
LB: 32	28.10	83.24
LB: 33	28.24	81.99
LB: 30	28.47	80.80
JA: 45	28.62	82.83
LB: 26	28.71	82.43
JA: 44	28.86	84.03
ID: 34	28.94	80.43
KA: 45	29.17	84.29

TABLE 11-continued

A & B: Color and odor-producing properties of commodity soybeans harvested.		
ID: 15	29.43	79.01
ID: 48	29.56	79.52
LB: 15	29.60	82.41
ID: 28	30.05	78.60
KA: 44	30.83	81.90
JB: 12	31.17	78.99
JB: 16	31.42	79.02
KA: 25	32.69	80.71
LB: 9	32.89	79.97
JA: 49	33.48	79.05
JB: 14	33.80	77.60
Range	27-34	77-84
Average	29.73	81:22

## EXAMPLE 6

Demonstration of the Ability to Combine the Low Odor-producing Trait with a Low Linolenic Acid Composition Trait

**[0094]** This example shows that it is possible to combine the low odor-producing trait with a low linolenic acid composition trait and further explores the effects of dehulling soybeans on the production of odors. The percentage of soybean fatty acids that are linolenic acid is normally about 8%. Using traditional breeding it was possible to create soybeans containing less than 6% linolenic acids and low odor producing property (Table 12).

**[0095]** The formation of 1-octen-3-ol is independent of the formation of other volatile compounds measured in the odor assay. One hypothesis is that fungal enzymes on the surface of the soybeans are the source of the rapid formation of 1-octen-

3-ol in the assay. It was reasoned that dehulling the seed before grinding to flour, should decrease the levels of fungal enzymes in the odor assay. Whole soybeans are normally ground to create flour that is used in the odor assay. A retest was performed using six low odor, low linolenic soybean lines that were carefully dehulled. The amount of 1-octen-3-ol formed from the dehulled seeds was about half that of the whole seeds, supporting the hypothesis that components such as fungus and fungal enzymes in the hull portion contribute to 1-octen-3-ol formation (Table 13). Lipoxigenase-null soybeans frequently produce higher levels of 1-octen-3-ol (Table 3). It was reasoned that lipoxigenases may play a role in inhibiting the growth of mold, so in the absence of lipoxigenases, mold infections may be greater allowing more fungal enzymes and formation of 1-octen-3-ol. Low odor soybeans containing lipoxigenases, that were identified by the screen of this invention tend to have a lower 1-octen-3-ol range than that of higher odor soybeans (e.g., Table 12).

TABLE 12

A1 to A4: Color and odor-producing properties of low linolenic soybean lines. Color values are L* (lightness), a* (green-red), and b* (blue-yellow).									
A1.									
Cross Type	Linolenic acid (%)	Hexanal (µg/g)	Hexanol (µg/g)	1-octen-3-ol (µg/g)	2,4 decadienal (µg/g)	12,4 decadienal, hexanal, hexanol (µg/g)	L*	a*	b*
J-2	4.0	5.0	0.5	4.4	4.0	9.6	79.9	1.5	27.5
J-1	3.7	7.3	0.6	5.1	4.8	12.7	82.6	0.4	27.0
J-3	3.5	7.6	0.7	6.3	8.6	16.8	81.9	1.0	26.8
J-4	4.6	5.8	5.1	4.3	9.4	20.3	81.9	0.9	27.5
J-5	4.2	6.5	5.1	4.7	8.9	20.5	81.8	0.7	26.3
F-2	2.7	14.8	0.4	3.9	7.6	22.7	82.6	0.6	26.2
E-11	2.7	19.9	5.1	4.4	7.0	32.0			
G-31	4.1	20.3	1.1	5.5	11.0	32.4			
I-7	3.1	16.0	5.1	3.5	12.2	33.3			
J-12	3.3	16.5	5.1	4.2	12.5	34.1			
I-4		15.5	5.5	3.6	13.7	34.3			
G-21	4.1	20.7	2.1	6.0	12.1	34.8			
F-2 0	4.9	24.6	1.4	3.8	9.3	35.3			
F-3	2.8	23.3	0.9	9.1	11.7	35.9			
E-13	3.0	23.4	5.1	4.1	7.4	35.9			
G-30	3.2	21.8	1.3	5.4	13.0	36.0			
F-6		23.0	1.7	5.9	11.6	36.3			
G-27		22.6	0.9	6.6	12.9	36.4			
G-16	4.2	19.5	3.5	4.6	13.6	36.6			

TABLE 12-continued

A1 to A4: Color and odor-producing properties of low linolenic soybean lines. Color values are L* (lightness), a* (green-red), and b* (blue-yellow).						
G-10	4.1	17.3	3.6	3.5	16.0	36.9
G-15	4.4	18.9	3.0	4.8	15.4	37.2
G-28	4.2	24.8	1.0	7.0	11.4	37.2
H-1	3.4	17.2	5.1	3.0	15.2	37.5
F-17	3.9	25.7	1.3	5.3	10.5	37.5
G-26	3.8	23.1	1.0	5.1	13.7	37.9
E-14	3.0	20.9	5.1	5.3	11.9	37.9
J-16	3.2	19.8	5.1	4.5	13.0	38.0
E-6	2.9	17.4	5.1	4.3	15.6	38.0
E-3	2.9	17.8	5.1	3.0	15.4	38.2
F-10	4.3	20.1	5.1	3.0	13.0	38.3
F-1	2.7	21.6	1.5	5.4	15.6	38.6

Cross Type	Linolenic acid (%)	Hexanal (µg/g)	Hexanal (µg/g)	l-octen-3-ol (µg/g)	2,4 decadienal (µg/g)	2,4 decadienal, hexanal, hexanol (µg/g)
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## A2.

E-7	3.0	20.1	5.1	4.0	13.5	38.7
E-5	2.7	20.2	5.1	4.2	13.7	38.9
G-8	4.2	27.1	3.5	4.1	8.3	38.9
E-16	3.1	22.2	2.3	3.8	14.6	39.1
F-19	3.6	26.2	5.1	3.3	7.9	39.1
G-19	4.3	20.0	4.2	4.6	15.2	39.4
F-25	3.9	23.4	0.7	9.5	15.3	39.4
F-21	2.8	22.9	1.1	7.2	15.7	39.6
G-11	4.2	22.1	4.0	4.6	13.6	39.7
J-17	3.0	22.8	5.1	4.9	12.4	40.2
E-15	3.0	19.5	5.1	5.0	15.8	40.4
E-4	2.8	21.1	5.1	3.2	14.5	40.6
J-14	3.2	20.6	5.1	4.3	15.2	40.9
G-24	4.0	22.9	1.9	7.0	16.1	40.9
J-13	3.2	20.9	5.1	4.1	15.0	41.0
E-10	2.8	22.9	5.1	4.0	13.1	41.1
F-31	3.5	26.8	0.0	9.6	14.3	41.2
J-34	3.2	26.2	5.1	3.5	10.1	41.3
G-25	3.5	23.6	2.5	7.2	15.3	41.4
F-5	2.7	25.7	1.3	9.6	14.5	41.5
J-30	3.2	26.5	5.1	4.8	10.0	41.5
H-7	3.2	22.2	5.1	4.2	14.6	41.9
G-22	4.0	22.6	1.5	7.5	17.9	42.0
E-11	2.9	27.6	5.1	4.1	9.4	42.1
J-19	3.0	22.2	5.1	3.8	14.9	42.2
F-13	4.0	30.4	1.2	4.3	10.8	42.3
G-23	3.2	23.6	2.2	6.2	16.6	42.4
E-9	2.9	25.2	5.1	3.4	12.3	42.6
F-22	3.4	25.3	1.0	8.7	16.4	42.7
E-8	2.9	25.2	5.1	4.1	12.7	42.9
E-1	2.8	21.9	5.1	2.6	16.6	43.6
F-8	3.7	26.0	5.1	3.1	12.6	43.6
F-32	2.8	25.6	0.9	9.1	17.4	43.8
F-23	3.6	28.6	0.9	6.5	14.8	44.3

## A3.

J-6	2.7	23.8	5.1	4.2	15.7	44.6
G-29	4.0	23.7	1.5	6.8	19.3	44.6
F-7	4.1	25.4	5.1	3.8	14.1	44.6
I-12	2.9	22.0	5.1	3.9	17.7	44.8
G-17	4.1	20.8	4.2	4.1	20.1	45.1
F-4	2.8	24.0	5.1	3.6	16.2	45.3
H-6	3.1	24.7	5.1	3.5	15.7	45.4
G-9	4.5	23.9	3.8	3.8	17.8	45.5
I-11	3.1	21.2	5.1	3.6	19.2	45.5
G-14	4.1	22.8	3.3	4.9	19.5	45.7
I-13	2.9	21.1	5.1	4.8	19.6	45.8
J-15	3.1	24.4	5.1	5.6	16.8	46.3
F-33	3.0	29.0	0.9	10.1	17.6	47.5
E-2	2.8	25.3	5.1	3.4	17.4	47.8
J-32	3.0	31.5	5.1	4.6	11.2	47.8

TABLE 12-continued

A1 to A4:						
Color and odor-producing properties of low linolenic soybean lines.						
Color values are L* (lightness), a* (green-red), and b* (blue-yellow).						
E-18	3.0	28.8	2.9	3.2	16.2	48.0
H-5	3.0	30.0	5.1	3.3	13.0	48.0
E-17	3.2	29.0	3.2	2.5	16.3	48.6
I-6	2.9	30.8	5.1	4.1	12.8	48.7
J-33	2.9	28.6	5.1	2.6	15.2	48.9
G-2	2.9	26.8	1.5	6.5	20.7	49.0
G-12	4.1	23.1	4.9	4.5	21.4	49.4
I-8	2.8	26.5	5.1	5.7	18.0	49.5
G-5	2.9	31.1	3.4	8.1	15.2	49.7
G-6	3.2	31.7	1.6	8.9	16.7	50.1
J-23	2.9	30.0	5.1	6.9	15.0	50.1
F-35	2.9	28.8	1.2	6.5	20.4	50.4
I-5	2.8	30.4	5.1	3.1	15.0	50.6
G-20	4.0	23.8	5.0	5.3	22.0	50.8
J-29	3.0	31.3	5.1	4.5	14.6	51.0
G-13	4.4	35.8	3.8	4.8	11.5	51.0
I-0	3.0	32.7	5.1	4.1	13.3	51.1
H-2	2.8	34.0	5.1	3.2	12.6	51.7
G-18	3.9	27.6	4.4	4.5	19.9	51.9
<u>A4.</u>						
H-3	3.0	28.6	5.1	3.3	18.5	52.2
F-18	2.9	33.3	5.1	4.5	14.0	52.4
F-26	4.6	31.4	1.6	4.5	19.3	52.4
G-4	3.1	28.7	2.4	7.6	21.4	52.5
J-9	3.5	29.9	5.1	5.4	17.7	52.7
J-31	3.0	33.6	5.1	4.7	14.1	52.8
J-27	3.1	34.5	5.1	5.9	13.9	53.5
G-7	2.6	32.2	5.1	8.8	16.3	53.6
J-24	3.1	31.1	5.1	6.2	17.7	53.9
I-1	2.9	32.1	5.1	4.5	17.0	54.2
I-2	3.0	32.1	5.1	4.8	17.2	54.3
I-3	2.9	34.0	5.1	5.2	16.2	55.3
J-11	3.3	34.8	5.1	6.2	16.1	55.9
G-1	2.7	29.8	4.2	5.4	22.2	56.1
H-4	2.9	36.0	5.1	3.8	16.1	57.2
I-9	3.1	30.4	5.1	4.7	22.0	57.4
F-14	4.3	41.7	2.0	4.4	13.8	57.5
J-10	3.1	35.8	5.1	4.8	17.3	58.2
J-25	3.1	37.6	5.1	7.0	16.7	59.3
J-28	3.1	38.3	5.1	4.8	16.1	59.5
J-18	3.3	39.1	2.4	7.8	18.2	59.6
J-21	3.1	37.7	5.1	5.3	17.1	59.9
J-22	3.2	38.0	5.1	7.2	16.9	60.0
F-30	3.1	35.7	0.8	13.2	23.8	60.2
J-26	3.1	37.1	5.1	5.2	19.0	61.2
J-8	3.3	37.6	5.1	5.5	18.9	61.6
J-7	3.2	38.6	5.1	5.5	18.3	62.0
F-12	4.8	36.5	5.1	3.9	21.0	62.5
G-3	2.9	32.6	2.5	9.8	27.7	62.7
F-28	2.9	43.5	2.4	6.2	16.9	62.8
F-24	4.4	42.0	3.5	6.4	18.3	63.7
F-34	3.0	35.3	1.5	7.8	27.0	63.8
E-19	3.0	40.9	4.6	1.7	19.3	64.8
F-29	3.6	42.0	3.5	4.6	20.8	66.2
E-12	2.8	39.9	5.1	4.4	21.8	66.7
J-20	2.9	41.5	5.1	5.3	21.5	68.1
F-27	2.9	42.1	0.9	8.1	26.5	69.6
F-11	3.6	41.3	5.1	3.5	23.7	70.1
F-9	4.6	46.7	1.4	5.2	21.9	70.1
F-16	4.1	42.8	5.1	4.5	22.5	70.4
F-15	3.7	54.5	2.2	6.7	25.8	82.5

TABLE 13

Formation of 1-octen-3-ol in odor assay was reduced when soybeans were dehulled prior to making soy flour.			
Soybean 14	1-octen-3-ol( $\mu\text{g}/\mu\text{g}$ )		
	Whole seed	Dehulled	Dehulled Stdev
J-2	4.39	2.17	0.13
J-1	5.08	2.25	0
J-3	6.26	2.36	
J-4	4.27	1.35	0
HIBC	10.36	3.19	0.09

## EXAMPLE 7

## Effects of pH on Odor Formation

**[0096]** A standard odor assay provided by the invention involved the addition of soy flour to water, resulting in odor formation at about pH 6.3. The purpose of the following experiment was to determine if varieties that produce low levels of odors under this condition also produce low levels of odors under other pH conditions. The odor producing properties of a commercial control soybean were compared with a low odor producing line (A-4). Line A-4 produced lower levels of decadienal and hexanal at pH 3.0 and pH 5.5, and pH 7 and pH 9.2 (Table 14). These data support the use of the assay without buffer as a method for selecting soybeans that will produce low levels of odors over a wide range of pH conditions. The highest concentrations of hexanal and 2,4 decadienal were produced at pH 9, the pH where the least amount of 1-octen-3-ol was produced (Table 14).

TABLE 14

Effects of pH on formation of odors from commodity control and low odor producing soybean (A-4 of FIGS. 4 and 5 harvested in 2003). Odor assay use for this measure was the same as in example 1 except soy flour was added to 0.1M $\text{K}_2\text{HPO}_4$ at the respective pH (3.02, 5.45, 7.01, and 9.16).												
pH	Decadienal ( $\mu\text{g}/\text{g}$ )				Hexanal ( $\mu\text{g}/\text{g}$ )				1-octen-3-ol ( $\mu\text{g}/\text{g}$ )			
	Control	Stdev	A-4	Stdev	Control	Stdev	A-4	Stdev	Control	Stdev	A-4	stdev
3.02	3.60	0.98	1.79	0.96	18.47	4.40	7.35	0.76	13.37	7.86	11.71	2.79
5.45	8.03	1.97	3.65	2.27	36.49	2.66	11.30	1.78	12.41	6.56	8.06	3.62
7.01	8.10	1.90	3.51	0.64	30.75	9.50	10.68	0.74	10.57	4.17	6.03	3.87
9.20	37.72	33.17	9.12	3.66	60.38	30.20	27.93	6.11	2.10	1.71	2.98	1.67

## EXAMPLE 8

## Effects of Soybean Protein Composition on Soy milk Sediment

**[0097]** This example describes the lower levels of sediment formed in soy milk made from soybeans having the modified protein composition of the invention. Protein-containing sediment has a negative impact on the organoleptic quality of soy milk as it is not desired to feel particles in beverages.

**[0098]** Soy milk preparation. A control low odor soybean (A-4) and a soybean having about 39%  $\beta$ -conglycinin and about 13% glycinin were ground using a Mega Grinder to make soy flour. Each flour sample was added to 36.75 grams of water (4° C.) in a 50 mL disposable polypropylene centri-

fuge tube so the final concentration of protein in the mixtures were 3.3% weight basis and sonicated for 15 sec at output power control setting of 8. The sonicated samples were centrifuged for 10 minutes at 8,000 rpm at 4° C. using the Eppendorf Centrifuge 5804 R. The supernatants (soy milk) were decanted into 50 ml disposable polypropylene centrifuge tubes. A portion of each soy milk (27.25 grams) was transferred to another 50 ml disposal centrifuge tube. The above samples were prepared in duplicate to accommodate the following variation in sucrose addition. Sucrose (0.6987 grams) was added to the 27.25 grams of soy milk either before or after heat-treatment.

**[0099]** Soy milk samples were heat-treated by placing the 50 ml disposal polypropylene centrifuge tube into the 95° C. silicon oil bath for 5 minutes, then the samples were transferred to an ice bath to cool, followed by refrigerated storage for 30 days. Sediment formed in the soy milk samples over time. The amount of sediment formed was quantified as follows. The sediment in the bottom of the centrifuge tubes were dispersed by tipping the tubes back and forth. The soy milk samples were transferred into a weighed centrifuge tube and the final weight was recorded. The tubes were placed into the Eppendorf Centrifuge 5804 R and centrifuged for 2 minutes at 8,000 rpm at 25° C. The soy milk supernatant was decanted and the weight of the sediment remaining was calculated. The amount of sediment was recorded as percentage of soy milk weight (% Sediment=100×Wt of Sediment/Wt of Soy milk).

**[0100]** Results. The control soy milks had at least twice as much sediment as the high  $\beta$ -conglycinin soy milks (Table 15).

TABLE 15

Effects of soybean protein composition on soy milk sediment.		
Soybean Type	Sucrose addition	% Sediment
A-4	Before heating	1.8
A-4	After heating	2.0
HBC	Before heating	0.7
HBC	After heating	1.0

A-4 is control soybean. HBC is a high  $\beta$ -conglycinin, low glycinin soybean

**[0101]** The above examples show that unique compositions were created that can produce low levels of odors even when soybeans contained lipoxigenases 1, 2 and 3. Also demon-

strated was the ability to select soybeans that have improved organoleptic properties where soybeans were selected based on the amounts of glycinin proteins, free arginine and asparagine, yellow pigments and polyunsaturated fatty acids in the soybeans and the amounts of the odors 2,4 decadienal, hexanal, hexanol, and 1-octen-3-ol produced by the aqueous ground soybean suspensions. A method was disclosed for estimating the potential for soybeans to produce 2,4 decadienal, hexanal, hexanol and 1-octen-3-ol and other even more potent odors in soybean ingredients and foods. In this method of estimation of soybean odors, the value of incubated the soy flour in water at a 1 to 4 ratio resulted in original observations that 2,4 decadienal forms rapidly at room temperature in the suspensions and that it is possible to breed soybeans containing lipoygenases 1, 2 and 3 that produce very low levels of odors.

**[0102]** It was also shown in the examples that it is possible to create endogenous soy compositions having greater than 30%  $\beta$ -conglycinins and less than 25% glycinins that have normal or low free arginine and free asparagine. It was further demonstrated that low odor and color properties can be combined with high  $\beta$ -conglycinin compositions and further combinations were conceived with low linolenic and mid-oleic soybeans. Glycinins are a source of insoluble proteins in soy ingredients and foods, creating sediment in beverages and undesirable mouthfeel. Free arginine and asparagine can form ammonia during processing which further react with odor compounds such as 2,4 decadienal to form potent odors such as 2 pentyl pyridine. Linoleic and linolenic acids are polyunsaturated fatty acids that are substrates for odor formation; lowering their content in soybeans will help lower formation of odors. Pigments of soybeans contribute to the off-color of soybean products, further impacting the organoleptic response. All together, the high  $\beta$ -conglycinin, low free arginine and asparagine, low odor and low color and low polyunsaturated fatty acid soybean composition is the most valued composition of the invention for creating soy protein ingredients and foods that are organoleptically pleasing. It is also recognized that these compositions will not lack the health properties associated with soy protein ingredients as  $\beta$ -conglycinins are associated with the cholesterol and triglyceride lowering properties of soy protein (Duranti et al., 2004) and in the inhibition of arteriosclerosis (Adams et al., 2004).

#### EXAMPLE 9

##### Additional Sedimentation Analysis of Low Odor Soy Compositions

**[0103]** The study in Example 8 was repeated with the following changes. A commodity soybean control was included (AG3302). The soybeans were dehulled before making flour and the flour was sifted prior to addition to water. The supernatants (soymilk) were transferred to preweighed weighed centrifuge tubes. Sucrose was added before heat-treatment to make the final sucrose concentration 2.5% (w/w basis). The samples were stored in a refrigerator for 21 days. The heights of soymilk sediments in the centrifuge tubes were measured, then samples were centrifuged for 5 minutes at 8,000 rpm. The supernatants were decanted and weighed, the pH of the supernatants were determined (all samples had pH=6.7), and the tubes containing pellet were weighed. The weight percent of the wet soy sediments were calculated for each sample

(weight % of wet soy sediment=100×weight of wet soy sediment/(weight of wet soy sediment+weight of soy supernatant)).

**[0104]** The beneficial effects of the HBC and low odor soybeans in reducing protein sediment formation were found using dehulled soybeans (Table 16). The HBC soymilk had a 2.2 fold reduction of sediment compared to the low odor soybean line and a 7 fold reduction of sediment compared to control soybean. A surprising 3.1 fold reduction was found in sediment in the low odor soybean compared to the control. It is possible that the low odor trait reduced sediment formation by limiting free radical formation and protein oxidation. This therefore indicated the further beneficial properties of the optimized composition combining high  $\beta$ -conglycinin and low odor traits.

TABLE 16

Effects of high beta-conglycinin and low odor traits on soymilk sediment height and sediment-pellet weight compared to a control soybean compositions			
	Sediment Height (mm)	Pellet weight (%)	Pellet wt. Stdev
HBC	0	2.1	0.14
A-4	8	4.7	0.42
Control	14	14.8	2.8

#### EXAMPLE 9

##### Preparation of Dehulled Soybean Flour and Isolated Soy Protein Ingredient

**[0105]** A control soybean (AG3302) and soybeans comprising greater than 30% of the total proteins as beta-conglycinins and less than 25% of the total proteins as glycinins and less than 2500 micrograms/g of free arginine plus asparagine (DJB2104GOR, EXP319AP) were dehulled to make dehull-soybean flour and then further processed to make isolated soy protein ingredient according the steps below.

TABLE 17

Composition of beta-conglycinins and glycinins in different soybean lines.				
Soybean line	Beta-conglycinins (% of total protein)	Glycinins (% of total protein)	Free Arg (ug/g)	Free Asn (ug/g)
AG3302	24.1	33.6	1455	155
DJB1804BOR	33.9	12.9	1230	93
EXP319AP	40.1	12.5	2197	224

**[0106]** 1. Adjust soybeans to about 10% moisture and temper at room temperature.

**[0107]** 2. Crack soybeans by using a cracking mill.

**[0108]** 3. Dehull the cracked soybeans by using an Aspirator.

**[0109]** 4. Condition the cracked and dehulled soybeans at 50-60 degrees C. by using a cooker.

**[0110]** 5. Flake the conditioned soybeans using a Flaking Mill.

**[0111]** 6. Extract soybean flakes with hexane.

**[0112]** 7. Desolventize the defatted soybean meal, resulting in flakes.

**[0113]** 8. Flakes were ground to make flour.



**[0114]** 9. Water was added to a 300 liter jacked tank and adjusted to 50 degrees C. and pH 9.0 using 40% NaOH. Defatted soybean flour was added and mixed. The water to soy flour ratio was 12/1 (w/w). Extraction time was 45 minutes.

**[0115]** 10. The solubilized soy protein was recovered from the extraction slurry by using a desludging disk centrifuge (back pressure, 58-60 psi).

**[0116]** 11. The clarified protein solution was adjusted to pH 4.5 by adding hydrochloric acid (18%) and allowed to react for 30 minutes at 45 degrees C.

**[0117]** 12. The precipitated protein was recovered using a desludging disk centrifuge.

**[0118]** 13. The protein curd was washed two times using acidified water (pH 4.5+/-0.1, 30-35 degrees C.). Ratio of washing water to packed wet solids was 6:1 (w/w). The protein curd was recovered using a desludging disk centrifuge after each washing (back pressure, 58-60 psi).

**[0119]** 14. The washed curd was mixed with sodium hydroxide (30%) to adjust pH to 6.8 using 30% NaOH and then heat treated at 116 degrees C. for 7.5 seconds. Then the pH was adjusted to pH 6.8.

**[0120]** 15. The protein solution was adjusted to 45-55 degrees C. and was spray dried using an inlet air temperature of 204-215 degrees C., outlet air temperature of 82-88 degrees C.

**[0121]** 16. The Nitrogen solubility index of the isolate soy protein ingredients were determined. A portion of sample was suspended in water with stirring at 30° C. for two hours. It was then diluted to a known volume with water. A portion of sample extract was centrifuged and an aliquot analyzed for protein. A separate portion of sample was analyzed for total protein by the same method. Water-soluble protein was calculated as a percent of total protein, which was proportional to water-soluble nitrogen as a percent of total nitrogen.

**[0122]** Results:

**[0123]** The nitrogen solubility index of the of soy protein powders (Table 18) were directly proportional to the amounts of beta-conglycinin in the soybeans used to make the soy protein ingredients:  $NSI=1.4716(\% \text{ beta-conglycinin})+7.4502$ ;  $R\text{-squared}=0.9975$ . A reduction in the levels of insoluble protein improves the organoleptic quality (e.g. smoother more refreshing mouthfeel) of food products which are formulated with the soy protein ingredients.

TABLE 18

<u>Nitrogen Solubility Index</u>		
Soybean line used to make Isolated Soy Protein.	NSI (%)	Standard Deviation
AG3302	42.7	2.5
DJB1804BOR	58.0	2.5
EXP319AP	66.0	2.3

**[0124]** The amino acid composition of the isolated soy protein ingredients made from soybeans had similar amino acid composition with the exception that the lysine content of the high beta-conglycinin (HBC) ingredients were about 6% higher than the control.

TABLE 19

<u>Total amino acid composition of isolated soy protein ingredients.</u>			
	AG3302	DJB1804BOR	EXP319AP
Aspartic	114.5	111.2	114.7
Threonine	37.6	37.3	36.8
Serine	50.8	52.0	52.7
Glutamic	203.3	192.0	202.2
Proline	55.5	53.8	55.2
Glycine	41.6	40.0	39.8
Alanine	42.9	42.2	41.9
Valine	49.1	49.6	49.7
Methionine	13.3	13.1	12.3
Isoleucine	44.3	44.5	44.9
Leucine	81.1	81.3	82.6
Tyrosine	38.4	39.1	39.3
Phenylalanine	50.9	51.8	53.7
Histidine	23.7	25.9	26.3
Lysine	62.3	65.4	66.5
Arginine	73.8	71.9	75.8
Cysteine (after ox)	13.1	14.6	13.6
Methionine (after ox)	14.7	14.5	13.3
Tryptophan	12.1	12.0	11.4

## EXAMPLE 10

## Preparation of Cultured Soy Product from Dehulled Soybean Flour

**[0125]** Dehulled soybean flour was prepared from a commodity soybean (AG3302), a high beta-conglycinin soybean (DJB2104GOR) and a low odor producing soybean line (03JBK8-25), all harvested in the U.S. in 2004.

**[0126]** Methods use to prepare and test cultured product made from soybeans:

**[0127]** 1. Soybeans were cracked using a cracking mill.

**[0128]** 2. Cracked soybean seeds were dehulled using an aspirator.

**[0129]** 3. The soybean meats (dehulled soybeans) were milled for 1 pass through a hammer mill and 5 passes through a pin mill.

**[0130]** 4. Dehulled soybean flour was packaged plastic bags within fiber drums.

**[0131]** 5. Protein content of the flour was determined.

**[0132]** 6. Dehulled soybean flour (3 degrees C.) was added to water (3 degrees C.) so the protein content would be 3.5% (weight basis) and mixed using a hand held homogenizer for about 1 to 2 minutes.

**[0133]** 7. Soy flour suspension was warmed in a plate heat exchanger and then injected with steam to treat the suspension at 141 degrees C. for 3.5 seconds and deaerated before cooling to about 4 degrees C.

**[0134]** 8. Heat-treated suspensions were filtered to remove fiber, resulting in soymilk.

**[0135]** 9. Dairy flavor ingredients, sugar (3%), and salt (0.2%) were added to the soymilk and mixed using a hand held homogenizer.

**[0136]** 10. Flavored soymilk was warmed in a plate heat exchanger and then injected with steam to treat the suspension at 141 degrees C. for 3.5 seconds and deaerated before cooling to about 4 degrees C. and packaged in sterile containers.

**[0137]** 11. Soymilk samples, each containing 2.2% protein, were weighed into sterilized, quart jars and warmed in the microwave from 45 to 60 seconds (about 24 degrees C.).

[0138] 12. Sugar (3.1%), vanilla extract (0.4%) and cultured soy yoghurt (*L. Bulgaricus*, *S. Thermophilus*, *L. Acidophilus*, *B. Bifidum*, *L. Casei*, *L. Rhamnosus*) (8%) were added to the soymilk and the samples were mixed.

[0139] 13. Soymilk containing culture was placed in an incubator at 43 degrees C. and incubated to 4 hours, removed and refrigerated overnight (4 degrees C.).

[0140] 14. The pH and viscosity measurements were taken on the refrigerated samples and a sensory evaluation was conducted by a 3 person panel who were blind to sample formulations. Viscosity was measured using a Brookfield viscometer with Spindle #3 at 20 rpms

[0141] Results: The flavor profile of the cultured product made from DJB2104GOR was pleasant and would lend itself to a fruit flavored smoothie. The profile of the product made from 03JBK8-25 was pleasant and would lend itself well a sour cream or dip product. It was reasoned that a combination of high beta-conglycinin and low odor producing traits would also create pleasant cultured soymilk products. The lower viscosity of the high beta-conglycinin protein material may help in creating higher protein products at the same thickness level.

Grinder (SOP Ascii-01-0002). Ground samples were stored at 4° C. For analysis, ~30 mg of flour from each was weighed into one well of a 96 well 2 ml microtiter plate. Protein was extracted for 1 hour with shaking in 1.0 ml 1xLaemmli SDS buffer pH 6.8 containing 0.1M dithiothreitol (DTT) as a reluctant. Following centrifugation, a portion of each extract was further diluted in SDS buffer to yield 0.2-0.5 µg/µL total protein, heated to 90-100° C. for 10 min, and cooled. For each sample, 1-2 µg total protein was loaded using a 12 channel pipet onto a 26 lane 15% T gradient Tris/HCl Criterion gel. Molecular weight standards and a parental control were included in two of the lanes in each gel. The gels were electrophoresed until the tracking dye reached the bottom of the gel ~1.2 hrs, then stained overnight in Colloidal Coomassie Blue G-250, destained in DI water, and imaged using the GS800 Calibrated Densitometer. Quantitation was performed using Bio-Rad Quantity One™ Software. The software was used to determine the relative quantity of each band in the sample lane. The percent glycinin and percent β-conglycinin protein sub-unit bands are reported as the relative percent of the total protein in the lane. The α5-glycinin subunit was not

TABLE 20

Soybean composition and odor property.										
Soybean	Beta-conglycinins (% of total protein)	Glycinins (% of total protein)	Oil (Dry Matter Basis)	Protein (Dry Matter Basis)	Free Arg (ug/g)	Free Asn (ug/g)	Hexanal (ug/g)	Hexanol (ug/g)	1-octen-3-ol (ug/g)	2,4-decadienal (ug/g)
DJB2104GOR	37.7	14.7	18.9	39.5	2197	142	8.8	3.17	5.9	11.7
AG3302	24.1	33.6	22.4	38.1	1455	155	12.5	4.47	3.0	13.7
03JBK8-25	25.7	34.3	19.1	38.2	1711	116	2.6	1.39	3.3	5.6

TABLE 21

Flavor profile of the cultured product					
Sample Identification	Initial pH (4° C.)	Final pH (4° C.)	pH Change	Viscosity (Centipoise)	Sensory Comments
AG3302	6.828	4.629	2.199	2025	Has a smoothie consistency, some coagulated bits, earthy, fermented flavor, tart, vanilla, cooked, not as sweet as others.
DJB2104GOR	6.922	4.581	2.341	1095	Thinner than other samples, sweet, "bright", balanced, vanilla comes through, tart.
03JBK8-25	6.835	4.662	2.173	1948	Thickest sample, rice pudding like texture, flavor like a sour cream, seems to be a different type of sour profile versus the others, fairly bland, some mouth drying.

## EXAMPLE 11

## Demonstration of Combination of Low Odor and High β-Conglycinin

[0142] This example describes the combination of low odor producing properties with high β-conglycinin compositions. It demonstrates the ability to produce low odor soybean compositions comprising decreased glycinin and increased β-conglycinin content. Cross types such as A and E (Table 3) were combined with high beta-conglycinin germplasm having a pedigree of A3233/B2G2/A1923.

[0143] Protein analysis was carried out as follows: Eight soybean seeds were pooled and ground using the CAT Mega-

quantitated and was not included in total acidic glycinin value. The sample identities and weights are tracked using Master LIMS™.

[0144] Low odor producing soybeans compositions with high β-conglycinin trait are illustrated along with some lines that did not have the low odor trait (Table 22). Duplicate odor analyses are shown for the first line in the table. Several lines are shown that lack glycinins and produced compositions with less than 20 µg/g of total 2,4 decadienal plus hexanal plus hexanal per gram of ground seeds following oxidation under mild aqueous conditions. The compositions produced will be further evaluated for other characteristics (e.g., yield, free amino acids, color and fatty acid composition).

TABLE 22

Sample ID	Odor producing and protein subunit makeup of high beta-conglycinin soybean compositions.								
	hexanal (ug/g)	Hexanol (ug/g)	Hexanal + Hexanol (ug/g)	1-octen- 3-ol (ug/g)	2,4 decadienal (ug/g)	Alpha' BC (%)	Alpha BC (%)	Beta BC (%)	AcGly (%)
JB0305620.0102.0001	ND	2.5	2.5	6.9	3.8	16.0	24.4	8.1	0.0
JB0305620.0102.0001	4.4	2.5	7.0	8.2	4.0	16.0	24.4	8.1	0.0
JB0305616.0349.0016	5.7	2.3	8.0	8.9	3.0	14.7	18.3	6.3	12.1
JB0305597.0181.0008	7.1	2.2	9.4	5.4	3.0	14.0	15.2	4.4	5.0
JB0305617.0178.0015	5.3	2.0	7.3	1.5	5.2	15.4	17.4	3.5	0.0
JB0305618.0035.0025	7.2	1.7	8.9	8.9	3.8	16.4	19.2	6.6	0.0
JB0305616.0349.0011	7.1	2.2	9.3	10.9	3.8	13.6	18.8	8.7	2.6
JB0305611.0213.0020	5.9	2.7	8.6	9.9	4.5	15.3	18.5	5.7	1.2
JB0305620.0102.0018	7.8	2.6	10.4	5.5	3.0	16.8	23.9	10.1	0.0
JB0305620.0102.0004	7.9	2.3	10.2	9.7	3.3	16.0	23.0	9.0	0.0
JB0305617.0094.0031	7.3	2.3	9.5	1.6	4.6	16.8	21.4	6.6	3.2
JB0305617.0178.0010	8.0	1.9	9.9	12.1	4.4	14.8	16.3	3.4	0.0
JB0305611.0098.0009	7.7	2.3	10.0	8.8	4.4	14.9	18.2	10.3	6.7
JB0305611.0213.0004	7.3	2.2	9.5	12.7	5.0	14.0	16.1	4.1	0.9
JB0305611.0380.0010	7.5	2.2	9.7	6.7	4.9	16.3	19.5	6.5	3.8
JB0305611.0213.0022	7.0	2.4	9.4	11.6	5.3	13.6	15.4	6.0	1.6
JB0305619.0275.0007	16.8	4.5	21.3	14.0	14.0	14.5	13.4	3.9	0.0
JB0305620.0078.0012	19.4	9.2	28.6	4.5	7.5	17.0	18.7	7.7	0.0
JB0305618.0035.0021	21.1	5.1	26.2	7.0	10.2	11.6	13.5	3.4	0.0
JB0305618.0047.0021	20.5	4.2	24.7	16.5	12.2	12.5	13.5	2.3	0.0
JB0305620.0282.0008	24.7	3.9	28.6	7.4	8.4	12.3	13.9	3.3	0.0

Gly = acidic glycinins.  
ND = not detectable.

#### Deposit Information

**[0145]** A deposit of Monsanto Technology LLC, soybean 0119149 seed disclosed above and recited in the claims, has been made under the Budapest Treaty with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, Va. 20110. The deposited line 0119149 also has the designations "A-4" and "03JBK8-25", which are used herein in the working examples and tables. The ATCC accession number for the deposit is PTA-6197 and the date of deposit was Sep. 10, 2004. The deposit will be maintained in the depository for a period of 30 years, or 5 years after the last request, or for the effective life of the patent, whichever is longer, and will be replaced as necessary during that period.

**[0146]** All of the compositions and methods disclosed and claimed herein can be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of this invention have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be applied to the compositions and methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the invention. More specifically, it will be apparent that certain agents which are both chemically and physiologically related may be substituted for the agents described herein while the same or similar results would be achieved. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the invention as defined by the appended claims.

#### REFERENCES

**[0147]** The following references, to the extent that they provide exemplary procedural or other details supplementary to those set forth herein, are specifically incorporated herein by reference.

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**[0149]** U.S. Pat. No. 5,981,781  
**[0150]** U.S. Pat. No. 6,171,640  
**[0151]** U.S. Pat. No. 6,171,640  
**[0152]** U.S. Pat. No. 6,171,640.  
**[0153]** U.S. Pat. No. 6,355,296  
**[0154]** U.S. Pat. No. 6,444,874  
**[0155]** U.S. Pat. No. 6,653,534.  
**[0156]** U.S. Patent Appln. 20030074693  
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- 1-26. (canceled)
27. A method of analyzing the odor producing properties of a soybean comprising determining the level of at least one compound selected from the group consisting of 2,4 decadienal, hexanol, hexanal, and 1-octen-3-ol.
28. The method of claim 27, wherein determining the level of the compound comprises incubating a mixture of about 1 part of soybean seed flour and about 4 parts of water for a period in the range of about 1 to about 40 minutes and quantifying amounts of at least one compound selected from the group consisting of 2,4 decadienal, hexanol, hexanal, and 1-octen-3-ol and combinations thereof, using deuterated standards for hexanal, hexanol and decadienal.
29. The method of claim 28, wherein soybean seed flour is made from dehulled soybeans.
30. A method of obtaining a soybean variety producing soybeans with decreased odor producing properties comprising measuring the level of at least one compound selected from the group consisting of 2,4 decadienal, hexanol, hexanal, 1-octen-3-ol, and any combination thereof in one or more soybeans from first and second soybean varieties and selecting the variety producing seeds with lower levels of said compound.
31. The method of claim 30, further comprising crossing a plant of the selected variety with a different plant to produce progeny and measuring the level of at least one compound selected from the group consisting of 2,4 decadienal, hexanol, hexanal, 1-octen-3-ol, and any combination thereof in one or more soybeans from said progeny.
32. A method for selecting a soybean variety that resists fungal contamination comprising selecting a variety that comprises less than 5 µg 1-octen-3-ol per gram of seed as measured by incubating a mixture of about 1 part of soybean seed flour and about 4 parts of water for a period in the range of about 1 to about 40 minutes and measuring the 1-octen-3-ol.
- 33-36. (canceled)
37. The method of claim 27, wherein said determining the level of at least one compound comprises determining the level of 2,4, decadienal.
38. The method of claim 27, wherein said determining the level of at least one compound comprises determining the level of 2,4, decadienal and at least one compound selected from the group consisting of hexanol, hexanal, and 1-octen-3-ol.
39. The method of claim 30, wherein said measuring the level of at least one compound comprises measuring the level of 2,4, decadienal.
40. The method of claim 30, wherein said measuring the level of at least one compound comprises measuring the level of 2,4, decadienal and at least one compound selected from the group consisting of hexanol, hexanal, and 1-octen-3-ol.
41. The method of claim 31, wherein said measuring the level of at least one compound comprises measuring the level of 2,4, decadienal.
42. The method of claim 31, wherein said measuring the level of at least one compound comprises measuring the level of 2,4, decadienal and at least one compound selected from the group consisting of hexanol, hexanal, and 1-octen-3-ol.
43. The method of claim 32, comprising dehulling a sample of seed and measuring the 1-octen-3-ol level from the sample.
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