HIGH BETA-CONGLYCININ PRODUCTS AND THEIR USE

CROSS REFERENCE

This application is a continuation-in-part of International application number PCT/US98/06579, designating the U. S., which was filed with the U.S. Receiving Office on Apr. 3, 1998 and claims priority from U. S. Provisional Application Serial No. 60/042,643 filed Apr. 4, 1997.

BACKGROUND OF THE INVENTION

The present invention relates to a high beta-conglycinin composition, meat analog, cheese analog, beverage and animal feed and to methods of producing a high beta-conglycinin composition, cheese analog, beverage, meat analog and animal feed.

The publications and other materials used herein to illuminate the background of the invention or provide additional details respecting the practice, are incorporated by reference.

Glycinin and beta-conglycinin (BC) account for approximately 70% of the proteins in soybeans. It has been postulated that the functional properties of soy protein ingredients in food systems can be improved by modifying the ratio of these proteins. Previous attempts have been to increase the ratio of glycinin to beta-conglycinin to improve the yield and quality of tofu-type soybean gels and to improve the content of sulfur amino acids for nutritional purposes (Kitamura, K., *Trends Food Science & Technology* 4:64–67, (1993), Murphy, P., et al., *Food Technology* 51:86–88, 110 (1997)).

Dietary proteins are needed to replace metabolic losses of tissue and organ proteins, to form and deposit protein in new tissues and to replenish tissue loss as a consequence of pathological conditions. These needs are met by indispensable (essential) amino acids and dispensable amino acids that comprise dietary proteins. It is largely in this context that the nutritional value of dietary proteins is defined as the ability to meet daily requirements for essential amino acids (Steinke, F. et al. New Protein Foods in Human Health: Nutrition Prevention and Therapy, CRC Press, 1992). High quality proteins contain all the essential amino acids at levels greater than reference levels and are highly digestible so that the amino acids are available. In this context, egg white and milk proteins are the standards to which other proteins are evaluated and plant proteins are considered to have inferior nutritional value. The essential amino acids whose concentrations in a protein are below the levels of a reference protein are termed limiting amino acids, e.g., the sum of cysteine and methionine are limiting in soybeans.

Glycinin contains 3 to 4 times more cysteine and methionine per unit protein than beta-conglycinin (Fukushima D., *Food Rev. Int.* 7:323–351, 1991). Thus it is expected that an increase in the content of glycinin and a decrease in the content of beta-conglycinin results in enhanced protein quality (Kitamura, K. *Trends Food Science & Technology* 4:64–67, 1993; Kitamura, K., *JARQ* 29:1–8, 1995). This is consistent with the finding that the mean value of the sulfur-containing amino acid contents in the seeds of four representative lines which were low in beta-conglycinin was about 20% higher than that of four ordinary varieties (Ogawa, T. *Japan. J. Breed.* 39:137–147, 1989). A positive correlation was also reported between the glycinin:beta-conglycinin ratio (1.7–4.9) and the methionine or cysteine concentration of total protein, among wild soybeans (Kwanyuen et al., *JAOCS* 74:983–987, 1997). There are no reports of the amino acid composition of high beta-conglycinin soybeans (glycinin:beta-conglycinin ratio less than 0.25).

In addition to the ability of proteins to meet the body's daily needs for essential amino acids, dietary proteins can also contribute bioactive peptides and amino acid patterns which can reduce the risk factors for cardiovascular diseases, cancer and osteoporosis. These compositional factors should also be considered in assessing protein quality, especially in countries such as the United States where people on the average consume a large excess of dietary protein. Researchers (Sugano, et al. PCT No. W089/01495; Sugano, M. *J. Nutr* 120:977–985,1990; Sugano. M. & Kobak, K. Annu. *NY Acad. Sci.* 676:215–222, 1993; Wang, M. *J Nutr. Sci. Vitaminol.* 41:187–195, 1995) have identified a pepsin-resistant fraction of soybean protein (5,000–10,000 molecular weight) that represents about 15% of the protein in isolated soy protein. Humans fed a diet with the pepsin-resistant fraction at 24 g or 48 g per day had lower LDL-Cholesterol and more fecal neutral and acidic steroid excretion than those fed diets with isolated soy protein or casein. The soy proteins which contribute to this pepsin-resistant fraction were not identified. Purified beta-conglycinin is more pepsin-resistant than purified glycinin (Astwood, J. & Fuchs, R. In Monographs in Allergy, Sixth International Symposium on Immunological and Clinical Problems of Food Allergy, Ortolani, C. and Wuthrich, B. editors, Basel, Karger, 1996), so it follows logically that beta-conglycinin may be a primary contributor to the bioactive fraction. This possibility has not been demonstrated yet in a feeding study, or with protein made from soybeans having altered protein compositions.

The alpha and alpha-prime subunits of beta-conglycinin specifically interacted with membrane components of human and animal liver cells in tissue culture experiments (Lovati, M. R., et al., *J. Nutr.* 126:2831–2842). The beta-conglycinin subunits were incorporated by the liver cells, degraded and caused an increase in the maximal binding of LDL to high-affinity receptors. It is proposed that such a mechanism could be responsible for the cholesterol lowering properties of soy protein isolates. However, it is not clear if significant amounts of dietary soy proteins can get to the liver in vivo. Lavarti et al. (*J. Nutr.* 122:1971–1978, 1992) reported a study in which hypercholesterolemic rats were fed either glycinin or beta-conglycinin for two weeks. Both groups showed a ⅓ reduction in total serum cholesterol. There are no studies which determine the effects of soy protein isolates from soybeans with modified soy protein compositions on the cholesterol lowering properties of soy protein isolate in animal models or humans.

It is reasoned from Rhesus monkey studies using alcohol extracted (which removes isoflavones) and non-alcohol extracted soy protein isolate, that soybean isoflavones are the primary components of soy protein isolates responsible for the cholesterol lowering effects (Anthony, M. S., *J. Nutr.* 126:43–50, 1996). However, subjecting soy protein to ethanol extraction did not have any effect on its lipid-lowering effects in other studies using hamsters (Balmir et al., *J. Nutr.* 126:3046–3053, 1996) or rats (Topping et al., *Nutr. Res.* 22:513–520, 1980). Alcohol extraction processes can extract some proteins and can denature and aggregate the unique structures of soy proteins, likely affecting how they act in the GI tract. For example, Sugano et al., (*J. Nutr.* 120:977–985, 1990) observed that methanol extraction completely eliminated the ability of high molecular weight soy protein peptides to bind and excrete steroids. Feeding isolated soy isoflavones (genistein and daidzein) had no flavorable effect on serum lipids or lipoproteins in humans (Colquhoun, et al., *Atherosclerosis,* 109:75, 1994; Nestel, P. J., *Arterioscler. Thromb. Vasc. Biol.* 17:3392–3398, 1997).