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ENZYMATIC IMPROVEMENT OF SOYBEAN FLAVOR AND STABILITY

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1 Claim

ABSTRACT OF THE DISCLOSURE

Incubation of soybean curd or defatted soybean flour with certain highly specific proteolytic enzymes liberates extraction-resistant beany and astringent flavor constituents and reversion-sensitive lipid materials from their apparent extremely close association with the proteinaceous constituents. The removal of the enzymatically liberated objectionable taste and oxidation susceptible constituents by washing with aqueous ethanol then provides stabilized soybean materials having little if any astringent and beany flavor and a distinctly diminished tendency to flavor reversion.

A nonexclusive, irrevocable, royalty-free license in the invention herein described, throughout the world for all purposes of the United States Government, with the power to grant sublicenses for such purposes, is hereby granted to the Government of the United States of America.

FIELD OF THE INVENTION

This invention pertains to a process for improving the flavor and taste of soybean curd or defatted soybean flour. More particularly this invention pertains to an enzymatic process for substantially eliminating the highly objectionable astringent and beany flavors that are sensed upon the ingestion of raw soybeans, and which, though markedly lessened by cooking or toasting, still are still strong enough to limit consumption of the soybean material unless masked by extensive admixture with bland additives that then limit the protein value of the foodstuff. Still more particularly this invention relates to a process whereby soybean curd or defatted soy bean flour is subjected in aqueous suspension to selective action of one or more specific proteolytic enzymes that liberate or degrade much of the poorly defined objectionable taste constituents and also liberate tightly bound lipid components that cause flavor reversion, and then washing from the still essentially intact soybean protein the thusly liberated or degraded grassy or beany as well as the reversion-sensitive components by extracting with 50 percent aqueous ethanol.

DESCRIPTION OF THE PRIOR ART

The presently vast cultivation of soybeans in the United States is due more to its content of edible oil than to its protein, which in the raw state has an astringently beany taste that largely prevents its food use for humans unless converted by fermentation to such Japanese foods as miso, shoyu (soy sauce), or tofu, or unless the above tastes or flavors are substantially attenuated by toasting or cooking the hexane-extracted soybean meal, in either case of which the still rather unpalatable soy protein has become completely denatured and insoluble and cannot be satisfactorily used as a substitute for sodium caseinate, particularly in vanilla ice cream or in the synthetic milks where an utterly bland flavor would be absolutely essential.

Many distinct proteolytic enzymes are commercially

available. Papain and bromelin, both of plant origin, are widely used for tenderizing meat. Pepsin and trypsin are powerful, nonspecific proteolytic enzymes of animal origin that are used clinically for replacement therapy and in fundamental protein research. Lastly, there are a number of microbiologically produced proteases that are used for the preparation of protein hydrolysates and amino acids and for sophisticated research in the fields of enzymology, medicine, protein chemistry, microbiology, and foods. However, to our knowledge, none of these proteases including "Molsin," the proprietary name of Seishin Seiyaku Company (Japan) for its brand of aspergillopeptidase-A from cultures of *Aspergillus saitoi*, and "C-Pase," Sigma Chemical Company's proprietary name for the carboxypeptidase-A obtained from bovine pancreas, or "Takadiastase-SS," from culture of *Aspergillus oryzae* have heretofore been employed on soybean protein either for analytical purposes or for flavor improvement.

The instant invention wherein we subject hexane-defatted soy flour or soy curd to 2-6 hours of selective digestion by a microbial protease selected from the group consisting of the acidic aspergillopeptidase-A produced by *Aspergillus saitoi*, the neutral protease produced by *Aspergillus oryzae*, and the alkaline protease produced by *Bacillus subtilis*, or the carboxypeptidase-A isolated from bovine pancreas, and removing the thereby liberated or degraded astringent and beany principles and the also liberated hexanol, hexanal, saponin, and reversion-susceptible bound lipids by then extracting with highly aqueous ethanol, is the outgrowth of our slightly preceding discoveries that the free amino acids and particularly the peptides liberated from soy protein by extensive hydrolysis with pepsin comprise significant amounts of leucine, isoleucine, phenylalanine, and valine, which specific amino acids per se are known to have a bitter flavor, which bitterness we also found to be even more intense in the therewith concurrently formed or liberated diffusible peptides, analysis of which showed the C- or N-terminals thereof to be mostly composed of leucine, the specific amino acid sequences of the seven arbitrarily designated constituent peptides being set forth in Table I. These findings, incidentally, are not inconsistent with the several literature reports that bitter peptides have been isolated following tryptic or bacterial enzyme hydrolysis of different dairy products including cheese, casein, and defatted milk solids.

However, the bitter amino acids and the still more intensely bitter peptides characterized in Table I are not per se germane to the present invention other than for indicating the background research and for showing the unobviousness of the operative enzymes of our invention in view of the fact that we have found that most proteolytic enzymes actually produce a distinctly bitter taste, apparently by promoting the formation of the bitter peptides.

TABLE I

Peptide:	Amino acid sequence
A-1	H·Gly·Leu·OH
A-2	H·Leu·Phe·OH
B-1	H·Leu·Lys·OH
C-1	H·Ser·Lys·Gly·Leu·OH
D-1	H·Phe·(Ile, Leu ₂)·Gln·Gly·Val·OH
D-2	H·Arg·Leu·Leu·OH
D-3	H·Arg·Leu·OH

SUMMARY

In view of our elucidation of the bitter and beany (astringent) factors increasingly liberated from soybean protein during up to 24 hours of peptic hydrolysis, the primary object of the instant invention is the provision