

SUPPLEMENTARY NOURISHMENT

This invention relates to supplementary nourishment (i.e. a food supplement). More particularly, this invention relates to supplementary nourishment containing in abundance nutritive ingredients such as saccharides and vitamins as well as sulfides of amino acids and unsaturated fatty acids which can provide excellent immunity.

Of late, a number of supplementary nutriments were introduced and marketed with an aim of providing the retention and/or promotion of health. Many of these supplementary nutriments contain various nutritive elements such as amino acids, fatty acids, vitamins and minerals, correspondingly providing those who take them with effects such as blood pressure control provided by fatty acids like linoleic acid and vasodilatation provided by amino acids.

The inventor has found, through a strenuous study of fermentation of substrates such as rice bran, wheat and soybeans containing abundant vegetable protein and vegetable lipid, that sulfides of the amino acids and unsaturated fatty acids in such a substrate can provide excellent immunity, both of which are, therefore, very useful to human body.

The present invention provides a method of preparing a food supplement comprising the steps of

- a) preparing an aqueous substrate containing vegetable protein and vegetable lipid by heat treatment in an autoclave;
- b) adding lipase and proteinase to the aqueous substrate in a total amount of 0.01 to 0.1 wt.% to form a substrate mixture; and
- c) fermenting the substrate mixture at 30°C to 55°C for 8 to 15 hrs.

A further embodiment of the present invention is characterized in that the food supplement contains a fermented product obtained by fermenting at 30°C to 55°C for 8 to 15 hours an aqueous substrate containing vegetable protein and vegetable lipid and 0.01 to 0.1 wt.% in combination of lipase, proteinase and amylase.

A yet further aspect of the present invention is characterized in that the food supplement contains a fermented product obtained by fermenting at 40°C to 50°C for 12 to 15 hours an aqueous substrate mixture prepared by heat treatment in an autoclave containing vegetable protein and vegetable lipid and 1 to 3 wt.% of koi (a kind of yeast).

Substrates to be used for the present invention may be anything that contains vegetable protein and vegetable lipid, for example, rice bran, sake (rice wine) lees, wheat embryo buds, wheat bran, whole buckwheat, whole barnyard millet, soybeans, bean-curd refuse, soybean milk, adzuki

beans, soybean flour, coffee grounds, yams, taros, tea grounds, honey, sesame, peanuts, lotus seeds, ashitaba leaves, beefsteak plants. Wakame seaweed and sea tangles. Among them, rice bran, sake lees, wheat bran, coffee grounds, wheat embryo buds, sesame, bean-curd refuse, soybeans and adzuki beans are especially preferred to be substrates for the present invention since these materials contain well-balanced vegetable protein and vegetable lipid.

A substrate as such is fermented with use of enzymes which are capable of decomposing both the vegetable protein and vegetable lipid in the substrate. This may optionally be achieved with use of a yeast. Such enzymes may comprise lipase and proteinase, or lipase, proteinase and amylase. Such yeast may be koji or malted rice which is capable of producing lipase, proteinase, amylase and/or other various kinds of enzymes found in organisms.

The most preferable proteinase to be used is an alkali proteinase (pH 9 to 13), which can be obtained from, for example, *Bacillus*, *Bacillus subtilis*, *Bacillus licheniformis*, *Bacillus alcalophilus*, *Bacillus cereus* and *Bacillus mycoides*. A preferred buffer solution is urea, preferably of 0.1 to 1.0 mol/l.

When the alkali proteinase is to be neutralized or acidified (by papain, for example), it may be mixed with proteinase obtained from *Aspergillus*. A group of weak alkaline/neutral proteinases which function best between pH

6 to 8, but which can be reasonably stable at pH 9, (for example, neutral proteinase from *Streptomyces griseus*) may be used together with the alkali proteinase. Such alkali proteinases can also be mixed with animal proteinase. Pancreatic proteinase and proteinase which functions best at pH 8 to 9 or under, (for example, a proteinase composition from *Streptomyces griseus* and enzyme from *Bacillus natto*, *Bacillus cereus* or *Bacillus mycodies*, enzyme from *Asp. orizae*, *Rhiz. Chinensis* or *Mucor. pusillus* and vegetable proteinase, for example, papain, ficin and bromelain) can be used. It is necessary to adjust pH values of said proteinase for obtaining the best functionability of the enzyme used. Urea or a buffer solution (for example, sodium hydrogencarbonate or potassium dihydrogenphosphate) may be added thereto for the adjustment of the pH value.

Said lipase may be obtained from various kinds of mold, yeast, bacilli, body fluid or from internal organs (for example, pancreatic lipase, liver lipase, tubercle bacteria lipase, FIB lipase and hima lipase).

Polysaccharides contained in substrates employed, especially disaccharides and trisaccharides possess excellent immunity power, and amylase can be utilised to decompose the saccharides.

It is also necessary to adjust pH values with regards to the lipase and amylase in order to attain good decomposition. A buffer solution of sodium

hydrogencarbonate-sodium carbonate, borax-sodium hydroxide, borax-sodium carbonate, sodium dihydrogenphosphate-sodium hydroxide, etc. can be used. It is possible to use a plurality of such buffer solutions.

The amount of enzyme to be used depends on the kinds of enzyme and the activities. The amount of enzymes added is in the range of 0.01 to 1.0 wt%. The amount of lipase or amylase is preferably half the amount of proteinase. The fermentation temperature is not essential in the present invention. It depends on the property of enzyme used and as such is the most suitable temperature within the range of enzyme stability. The temperature is between 30°C and 55°C. The fermentation time is 8 to 15 hrs. Before each fermentation the substrate is heat treated in an autoclave.

Koji such as malted rice or malt which can produce said enzymes such as lipase, proteinase and amylase in organisms, can be used also for fermentation. A condition for fermentation when koji is utilised, is 1 to 3 wt.%, 40°C to 50°C and 12 to 15 hrs. When koji is used for decomposition of a substrate, it is not necessary to use a specially prepared buffer solution.

A unit induced from the Anthon's method is utilized to measure activities of enzyme. The unit is called the proteinase unit (hemoglobin) U. The unit U corresponds to the amount of enzyme producing 1 micro mol/min. (at 40°C: 280 nm) of tyrosine from hemoglobin. The method is widely

utilised in determining activities of proteinase. Trichloroacetic acid is added to an enzyme decomposition to precipitate undigested protein and then the peptides (containing tyrosine) left in solution are quantitatively analysed.

Fermented products are obtained by adding water to a substrate and then adding enzymes (lipase, proteinase and optionally amylase), or koji (malted rice and/or malt) to the aqueous substrate and fermenting the liquid.

The filtrate of the fermented product prepared by adding the enzymes or koii to the aqueous substrate containing vegetable protein and vegetable lipid was analyzed by HPLC. The analysis was made by using a detector (LC-9A, SHIMADZU SEISAKUSHO CO., LTD., Japanese), a column system (3 meters long) and an acetonitrile solvent as an elution layer under the condition that the column temperature was 30 °C and the liquid flow speed was 10 ml/min. The following result was obtained.

It was confirmed that said fermented filtrate contained fatty acids such as myristic acid, palmitic acid, stearic acid, arachic acid, behenic acid, lignoceroic acid, dodecenic acid, tetradecenic acid, tetradecadienic acid, pentadecenic acid, hexadecenic acid, oleic acid, linoleic acid, linolenic acid, eicosaenic acid, eicosadienic acid, arachidonic acid, eicosatrienic acid, docoenoic acid and docosahehexanic acid. Said filtrate was found also containing amino acids such as leucine, isoleucine, lysine, methionine, cysteine, phenylalanine, tyrosine, threonine, tryptophan, valine, histidine, arginine, aspartic acid, alanine, glutamic acid, glycine, proline and serine. Saccharides, ash, calcium, phosphorus, carotin, vitamin B₁, vitamin B₂, vitamin B₆, pantothenic acid and nucleic acids were also found to be contained in the filtrate.

As is clear from the analysis, the fermented product according to the present invention contains many kinds of

amino acids and fatty acids. The sulfides of amino acids and the unsaturated fatty acids are considered to be vital to retention and promotion of immunity. As regards a fermented product prepared from an aqueous substrate where proteinase, lipase and amylase, or koii such as malted rice or malt is added, the saccharides in the substrate are decomposed and polysaccharides such as disaccharides and trisaccharides are produced, which are believed to also promote immunity.

A method for producing Supplementary nourishment according to the present invention is described hereunder.

An aqueous substrate where vegetable protein and vegetable lipid are added is prepared. Enzymes are then added thereto. For the decomposition of the substrate, proteinase, lipase and optionally amylase, koii which can produce a variety of enzyme in organisms are used. Said enzymes may be added separately or together. Other types of enzyme may additionally be used. The fermentation temperature when enzyme is used is between 30°C and 55°C. The fermentation time under agitation of the substrate is between 8 hrs. and 15 hrs. The pH values should be determined according to the types of the enzyme used. When koii is used, the fermentation temperature is between 40 °C and 50 °C. The fermentation time then is between 12 hrs. and 15 hrs. under agitation. The fermented products prepared as such are filtrated.

The food supplement prepared by filtration of the fermented products may be added to juice, candy, soy sauce, etc.

The preferred features and advantages of the present invention will be better understood with reference to the following non-limiting examples.

EXAMPLE 1

Rice bran was heat treated for 30 min. by hot steam (150 °C to 180 °C) in an autoclave. The heat treated rice bran was then press treated by a press till the moisture content was 40 wt.%. *Aspergillus orizae* and *Hanzenula anorama* had separately been cultured on MY media. *Bucillus subtilis* had also been cultured on bouillon. The press treated rice bran (10 kg) was sterilized and said three cultured substances were mixed therein. The mixture was agitated at 30 °C. After about 8 hours the temperature was raised to 45 °C and agitated again for 5 hours. The then fermented product was filtrated and 300 g of filtrate was obtained. The filtrate was analyzed. The following is the result of the analysis.

Components:	wt. %
water	3.5
amino acids	55.8
fatty acids	20.1
saccharides	2.5
ash	8.3

calcium	2.3
phosphorus	2.2
carotin	0.09
vitamin B ₁	0.15
vitamin B ₂	0.0005
vitamin B ₆	0.0025
panthothenic acid	0.0058
nucleic acids	0.1160

EXAMPLE 2:

Water was added to wheat embryo buds (40 wt.%) and the aqueous substrate was heat treated in an autoclave for 25 min. *Aspergillus soya* had been cultured on MY media. *Saccharomyces subtilis* had also been cultured on bouillon media. Papain, malted rice, kidney lipase and sodium hydrocarbonate were added to the mixture of said two cultured substances and the resultant mixture was mixed into the aqueous substrate (10 kg). The whole mixture was agitated for 10 hrs. at 45 °C. The fermented product was filtered. a filtrate (310 g) was obtained. The filtrate was analyzed and the following result was obtained.

Components:	wt. %
saccharides	2.0
amino acids	65.5
fatty acids	25.5
ash	12.3
carotin	0.009

vitamin B ₁	0.110
vitamin B ₂	0.0005
vitamin B ₆	0.0015
panthothenic acid	0.0038
nucleic acids	0.150

EXAMPLE 3

Immunity Test:

The filtrates obtained in Embodiments 1 and 2 were tested on 20 mice, ten of which were healthy and the other ten were suffering from cold. They were grouped in two, 10 each, five of which were healthy. Two each of the healthy mice and the sick mice in each group were given a physiological saline solution (2 ml. twice a day) only. The other three each of the healthy mice and the sick mice in each group were given said filtrates of Embodiments 1 and 2 (2 ml. twice a day) respectively. The following is the result after 30 days' observation.

Healthy Mice:

No abnormality was seen in the four mice in the two groups which were given only physiological saline;

No abnormality was seen in the six mice in the two groups which were given either filtrate;

Sick Mice:

All the four mice in the two groups which were given

only physiological saline died within 5 days:

All the six mice in the two groups which were given either filtrate survived the 30-day-long test.

An immunogenic effect of the filtrates was thus confirmed.

Examples showing practical usages of such a filtrate of the fermented products according to the present invention are given below.

EXAMPLE 4

Fully ripened tomatoes (1 kg) were crushed with a mixer. The crushed paste was sent into a Liebig cooler where 90 °C hot water was running and the oxidizing enzyme in the paste was destroyed. The paste was then put in a grinder. Salt (7 g) and the filtrate obtained from Embodiment 1 (20 g) were added thereto. The mixture was ground for 5 sec. The ground paste was vacuum treated and sterilized at 120 °C for 2 sec. Then, it was cooled and became tomato juice.

EXAMPLE 5

Powder sugar (1 kg) and thick malt syrup (500 g) were mixed with the filtrate of the fermented product of Embodiment 1 (10 g). The mixture was heated to 120 °C in an aluminum pan. The thickened syrup was little by little dropped into alcohol which was below 0 °C and became drops.

EXAMPLE 6

Natural gum base (20 g) was heat melted and the filtrate of Embodiment 1 (2 g), calcium carbonate (2 g), powder sugar (40 g), grape sugar (20 g), malted syrup (15 g) and aromatic (1 g) were added thereto. They were well kneaded and pressed flat. Chewing gum was thus made.

EXAMPLE-7

Rice powder (100 g) and sugar (100 g) were put in a container. Water (80 ml) was little by little added in the container. They were mixed well and the filtrate of Embodiment 1 (10 g) was added thereto. The whole mixture was kneaded well. The kneaded mixture was steamed in a steamer for 25 min. The steamed mixture was then placed in a bowl and a little salt was mixed therein. The mixture was divided into smaller pieces, each weighing roughly 5 g and became dumplings.

The above examples show only a few of many possible ways to utilize the fermented products prepared according to the present invention. By consuming such foods, health will be better retained and/or promoted.



CLAIMS

1. A method of preparing a food supplement comprising the steps of
 - a) preparing an aqueous substrate containing vegetable protein and vegetable lipid by heat treatment in an autoclave;
 - b) adding lipase and proteinase to the aqueous substrate in a total amount of 0.01 to 0.1 wt.% to form a substrate mixture; and
 - c) fermenting the substrate mixture at 30°C to 55°C for 8 to 15 hrs.
2. A method according to claim 1 wherein step b) comprises;
 - b) adding lipase, proteinase and amylase in a total amount of 0.01 to 0.1 wt.%.
3. A method of preparing a food supplement comprising the steps of
 - a) preparing an aqueous substrate containing vegetable protein and vegetable lipid by heat treatment in an autoclave;
 - b) adding koii in an amount of 1 to 3 wt.% to the aqueous substrate to form a substrate mixture; and
 - c) fermenting the substrate mixture at 40°C to 50°C for 12 to 15 hrs.
4. A food supplement prepared by the method of any preceding claim.
5. A food supplement or method of preparation thereof substantially as described in any of Examples 1 to 6.