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(54) Title: METHOD FOR PRODUCING A FAT MIXTURE

(57) Abstract

The present invention relates to a method for producing a fat mixture of β-sitosterol lowering the serum total cholesterol and LDL-cholesterol levels. β-sitosterol or a starting material containing β-sitosterol is dissolved in oil or fat, or in mixtures of oils and fats, using heat and mechanical energy, and water is added to the mixture during cooling.

Method for producing a fat mixture

The present invention relates to a method for producing a fat mixture of ß-sitosterol, which mixture is health beneficial, homogeneous and stable, lowers the serum total cholesterol and LDL-cholesterol levels, and contains the ß-sitosterol in a partly dissolved and/or microcrystalline form.

A high serum total cholesterol level, hypertension and smoking are the main risk factors associated with a heart disease (1). There are several sterols of plant origin that are distinguished from cholesterol only by side chain substituents and by the degree of saturation. Most of the higher plants produce 24α-substituted sterols (24-methyl- and 24-ethylsterols). Sitosterols are mixtures of ß-sitosterol (stigmasta-5-en-3ß-ole) and certain saturated sterols, such as ß-sitostanol, containing sterols not less than 95%, and unsaturated sterols not less than 85%. Sitosterols are broadly present in plants, such as in wheat and rye germ oils, corn oil, and commonly in seed oils. Sitosterols are antihypercholesterolemic agents that inhibit absorption of cholesterol in the intestine, and through the inner walls of blood vessels (2). Sitosterols play a role in the treatment of atherosclerosis when administered in doses of 2—3 grams, three times a day. In the western diet, the daily intake of ß-sitosterol, stigmasterol and campesterol from food is about 200—400 mg (3), which is of about the same order as our daily cholesterol intake from food.

At the beginning of 1950's, it was recognized that as a result of the addition of ß-sitosterol to the feed of cholesterol fed chicken and rabbits, the cholesterol levels were lowered in both test animals, and moreover, this addition of ß-sitosterol prevented atherogenesis in rabbits (4). The use of sitosterol and soy bean sterols for lowering cholesterol levels was studied intensely in 1950's and 1960's (5), and indeed, preparations thereof lowered cholesterol levels by about 10% (6). It was then dis- covered that the activity of ß-sitosterol was based on the inhibition of absorption

of cholesterol, and that sterols of plant origin were themselves poorly absorbed (7). The mechanism which inhibits the cholesterol absorption was considered to be based on crystallization and coprecipitation of cholesterol and ß-sitosterol. **Mattson** et al. (8) showed that 1 gram of ß-sitosterol reduces by 42% the absorption of cholesterol from food containing 500 mg of cholesterol. The reduction of plasma cholesterol may be due to the increased activity of LDL-receptors.

ß-Sitosterol is a lipophilic compound. In contact with the lipid membranes of intestinal walls, ß-sitosterol will not be absorbed due to its poor water solubility, or only a minor proportion of it will be absorbed; when administered orally, only less than 5% of it will be absorbed (9). The activity of ß-sitosterol is based on competitive inhibition of cholesterol absorption in the intestine (10). B-sitosterol interferes with cholesterol resorption and reresorption in the small intestine (11). It is considered that this results from the similarity between the chemical structures of cholesterol and ß-sitosterol (12). Several studies carried out under various conditions have shown that phytosterols lower LDL-cholesterol levels. It is further recognized that serum phytosterols correlate with HDL-levels. ß-sitosterol reduces synthesis of cholesterol in the liver by affecting the gene expression of HMG-CoA reductase (13). **Richter W** et al. (14) have shown that ß-sitosterol lowers by 10—15% the total serum cholesterol level, and by 19% the LDL-cholesterol level, by inhibiting the absorption of cholesterol in the intestine. In a study, nine adult patients were administered for 5 days with 500 mg of cholesterol, as well as with 1 gram of ß-sitosterol, or 2 grams of ß-sitosteryl oleate. The absorption of cholesterol was decreased by 42% when administering ß-sitosterol, and by 33% when administering ß-sitoteryl oleate (15). **Uchita** et al. (16) have recognized that in female rats, sitosterol inhibits absorption of cholesterol, and lowers cholesterol balance in plasma and liver. **Vahouny** et al. (17) have discovered that sitosterol inhibits absorption of cholesterol in rats by 54%.

Finnish patent application No. 964951 discloses an agent for lowering the cholesterol level in serum, and the use thereof. This application relates to the use of an ester of ß-sitostanol with a fatty acid or a mixture of esters of ß-sitostanol with a fat acid as

a fat component or as a fat substitute in food products, to the use thereof as such, complementing the diet, and to the compound itself.

Finnish patent publication No. 98 730 discloses a method for producing a substance to lower high serum cholesterol levels. In the method ß-sitostanol, obtained from ß-sitosterol by hydrogenating in an organic solvent in the presence of palladium on carbon as a catalyst, and a plant oil are used to produce an ester of ß-sitostanol with a fatty acid, or a mixture of such esters, employing the transesterification technique in the presence of a sodium ethylate catalyst.

Both of these patent publications mentioned above disclose a method for modifying ß-sitosterol to obtain a derivative thereof soluble in fats, wherein soluble ß-sitostanol fatty acid esters are produced therefrom, as well as the use of the compounds obtained as agents to lower serum cholesterol levels.

Naturally occurring ß-sitosterol is a crystalline compound. As is known, free sterols like ß-sitosterol are dissolved only scarcely in oil and fat, and therefore, derivatives of ß-sitosterol, for instance esters that are significantly more soluble in fats are produced for practical reasons, even though, according to some studies (15), these derivatives do not inhibit the absorption of cholesterol as effectively as the free ß-sitosterol. Such derivatives soluble in fats may be mixed much more easily into nutrition products to form a homogeneous mixture than a solid, insoluble, coarse ß-sitosterol powder. However, such processing to obtain a ß-sitosterol derivative entails additional costs for the product. Further, the hydrogenation of ß-sitosterol to ß-sitostanol is necessarily carried out using an organic solvent so that traces of it, as well as traces of the metal catalysts used, may be present in the esterified end product. In addition, the esterified product is no longer a naturally occurring substance but a man made artificial chemical compound.

An object of the invention is to provide a method for producing a fat mixture of ß-sitosterol, which is health beneficial, homogeneous and stable, lowers the total serum cholesterol and LDL-cholesterol levels, and contains the ß-sitosterol in a

partly dissolved and/or in a microcrystalline form. Another object of the invention is to use such a homogeneous stable fat mixture of ß-sitosterol containing it in a partly dissolved and/or in a microcrystalline form in fat preparations or food products as an agent that lowers the cholesterol level in serum, as well as to use this mixture as such to complement the diet.

The main characteristic features of the method and use according to the present invention are disclosed in the appended claims.

We have discovered that ß-sitosterol may be made partly soluble and/or microcrystalline with the following procedure. The problems and disadvantages associated with the state of the art may be avoided with the solution of the present invention. According to the method of the invention, ß-sitosterol and food grade oil are mixed, and this mixture is heated until all solids are dissolved in oil. After cooling, water is added into the mixture at the temperature thereof, thereby dispersing it. The result will be a homogeneous, stable, fat-like, almost white mass with a consistency closely resembling that of butter, or an oily mixture, depending on the amounts of the components. This homogeneous and stable paste is particularly suitable for being mixed into food products, for instance.

The starting material in this method is ß-sitosterol that may comprise 80—100% of ß-sitosterol and ß-sitostanol, and as impurities 0—20% of other sterols and stanols. This starting material containing ß-sitosterol, or ß-sitosterol may be mixed with the food grade oil in an amount of 0.5—80%, preferably 10—30%, the resulting pasty product having an appearance and a viscosity closely resembling those of butter, and being easy to handle. The higher the percentage of ß-sitosterol in the mixture, the harder the mass will be. On the other hand, if the amount of ß-sitosterol present in the mixture is less than 10% calculated from the amount of the oil, the viscosity of the mass will decrease, and the consistency thereof is clearly more like that of an oil. As a food grade oil, any cooking oil or any food grade oil or fat, or oil or an oily compound of animal origin, and suitable for human consumption may be used, for instance cod-liver oil, or any edible oily substance of plant or animal origin, or

mixtures thereof. Preferred oils are rapeseed oil, turnip seed oil, sunflower oil, soy bean oil, corn oil, and olive oil. The amount of oil is 5—90%, preferably 60—85%, by weight of the mass of the mixture. Water used may be any food grade water, the percentage thereof being 5—30%, preferably 10—20%, by weight of the mass of the mixture.

In this method, a mixture of the starting material containing ß-sitosterol and oil is heated at the temperature of 80—140 °C, preferably at 100—120 °C, until the solid starting material containing ß-sitosterol is dissolved in oil. After cooling the mixture in a known manner to the temperature of 40-80°C, preferably to 50—70 °C, water is added thereto essentially at the temperature of the mixture. Texture stabilizing surface active agents, such as polysorbate (Tween 80, Polysorbat 80), egg lecithin, or soy bean lecithin, known as emulsifying agents, may optionally be added to the mixture in an amount of 0.05—8.0%, by weight. If necessary, stabilizing agents, antioxidants, or other suitable food additives well known in the art, such as sodium chloride, mineral salt, preserving and flavouring agents, and/or various vitamins, for instance the vitamins A and E, food colours and phytophenols may be added. The mixture thus prepared is homogeneous and stable under conventional conditions for storing food products. In the mixture, ß-sitosterol is in a partly dissolved and/or microcrystalline form. If required, ß-sitosterol may also be dissolved in an oil as described above, and this ß-sitosterol/oil mixture may be used as such in food production.

The method of the invention makes it possible to produce, in a simple and economic way, a fat mixture of ß-sitosterol which is health beneficial, homogeneous and stable, reduces absorption of cholesterol in the intestine, thus lowering the serum total cholesterol and LDL-cholesterol levels, and contains the ß-sitosterol in a partly dissolved and/or microcrystalline form. This method uses a naturally occurring ß-sitosterol, and a food grade oil or fat, without any organic solvents or complicated process steps. Even high doses of the resulting homogeneous stable fat mixture containing a naturally occurring ß-sitosterol may be consumed safely in food products daily, and used in food production and cooking to replace fat partly or

totally. ß-sitosterol cannot be detected from food products by senses. By means of the thus prepared food products, absorption of cholesterol in the intestine may be inhibited, and total serum cholesterol and LDL-cholesterol levels may be lowered significantly. In addition, since ß-sitosterol replacing fat is not substantially absorbed, the proportion of absorbed fat is reduced, and thus the energy intake lowered.

Fat mixture containing ß-sitosterol may be added into food products that comprise fats of animal or plant origin, or mixtures thereof. Suitable food products are various processed meat products such as sausages and cold cuts, processed fish products, food products containing natural fatty acids, dairy products such as cheese, and several other food products containing edible fats or mixtures thereof, for instance sauces and dressings, mayonnaise, spices and spice mixtures, cereal, noodle and pasta products, ice cream, candies, chocolate, cakes, pastries, and the like, as well as edible fats for cooking and baking, and mixtures thereof.

The invention will now be illustrated with some preferred embodiments thereof described in the following examples, however, without intending to limit the invention solely thereto.

Examples

Method for producing a mixture of ß-sitosterol and fat, i.e. a so-called basic mixture

In the examples, the starting material was a mixture containing ß-sitosterol and ß-sitostanol in a total amount of 89.2%, α-sitosterol in an amount of 0.1%, campesterol and campestanol in a total amount of 8.9%, and arthenols in a total amount of 0.9%. The starting material had a solid matter content of 98.8%, melting range of 137—138 °C, and density of 0.49 kg/dm^3.

For convenience, this starting material is referred to in the following as the **starting material containing ß-sitosterol** according to the main component thereof.

Example 1

Basic mixture containing ß-sitosterol

A mixture was prepared containing 20% by weight of ß-sitosterol and 80% by weight of rapeseed oil. The mixture was heated while stirring in a glass jar until the starting material containing ß-sitosterol was dissolved in oil. At that point, the temperature was about 110 °C, and the test was carried out at normal atmospheric pressure.

After cooling the mixture to about 60 °C, tap water having the same temperature as the mixture (60 °C) was added thereto in an amount of about 15%, by weight of the amount of the mixture, triturating in a mortar.

The mixture was initially transparent and oily yellow when examined visually. Suddenly, the addition of water being almost complete, the mixture became opaque and off-white. The mixture was allowed to cool to room temperature (22 °C) while mixing. The final composition of the mixture is shown in Table 1.

Table 1. Composition of the basic mixture containing ß-sitosterol

Ingredient	Amount (%, by weight)
Starting material containing ß-sitosterol	17
Rapeseed oil	68
Water	15
Total	100

Based on sensory examination, the mixture of Table 1 was a white fat mass, having a consistency closely resembling that of butter, and containing ß-sitosterol in a partly dissolved and/or microcrystalline form. The mixture was practically tasteless.

When stored in a refrigerator, the basic mixture of Table 1 has remained unchanged, based on sensory examination. Up to now, the mixture has been stored for about 6 months.

Example 2

Variation of concentrations of the starting material containing ß-sitosterol in the mixture containing oil and water

With the method of Example 1, mixtures were prepared wherein the proportion of the starting material containing ß-sitosterol was 2.5—60% of rapeseed oil. It was observed that the consistency of the basic mixture was most preferable when the concentration of the starting material containing ß-sitosterol was between 10% and 20%. The mixture has then an appearance and a viscosity comparable to those of butter, and it is easy to handle.

The higher the proportion of the starting material containing ß-sitosterol in the mixture, the harder the consistency thereof will be. Despite the hardness of the mixture, even high proportions of the starting material containing ß-sitosterol may be used, as described in Example 11 (Addition of the starting material containing ß-sitosterol to a pasta product).

Example 3

Preparation of the mixture using various food oils

With the method of Example 1 mixtures were prepared replacing rapeseed oil with sunflower oil, corn oil and olive oil. Mixtures were prepared with each of these oils using three different percentages of ß-sitosterol: 5%, 10% and 20%.

Based on a sensory and microscopic examination, the mixtures were similar to those prepared with rapeseed oil, except that the mixture containing olive oil had a greenish colour. This suggests that all food grade oils are very suitable for use in the method of the invention.

Example 4

Addition of a surface active agent to the basic mixture of example 1

It is generally known that surface active agents are necessary for the preparation and stabilization of disperse systems, particularly emulsions. It is often important to use surface active agents able to stabilize the consistency of dispersions, especially for long term storage, for instance to prevent any separation of the emulsion components, or crystallization thereof.

Essentially, the mixtures of example 1 were prepared, containing 2% by weight, calculated from the proportion of the water phase, of emulsifiers generally known as surface active agents such as polysorbate (Tween 80, Polysorbat 80), egg lecithin or soy bean lecithin. The resulting compositions were essentially as shown in Table 1, but each containing about 0.3%, by weight, of a surface active agent.

Addition of a fat mixture containing ß-sitosterol to a food product

Example 5

Addition of the mixture described in example 1 to traditional commercially available butter

50% by weight of the basic mixture according to example 1 and 50% by weight of butter (Meijerivoi of Valio, low salt content) were mixed in an ordinary steel mortar at room temperature (about 22 °C). The mixing could be readily carried out without any difficulties.

Based on a sensory evaluation, the result was a uniform, light yellow mass of the colour of butter that felt like ordinary butter in every respect. The taste of the mixture was good and could not be distinguished from that of real butter, except perhaps for a lower salt content.

Example 6

Addition of the mixture described in example 1 to conventional commercially available rapeseed margarine

50 % by weight of the basic mixture according to example 1 and 50% by weight of rapeseed margarine (Kultarypsi margariini 60, Van der Bergh, Sweden) were mixed in an ordinary steel mortar at room temperature (about 22 °C). The mixture was slightly softer, but having a consistency otherwise similar to that in Example 3 above. The mixing could be readily carried out without any difficulties.

Based on a sensory evaluation, the result was a uniform, light yellow mass of the colour of the original rapeseed margarine that felt like ordinary rapeseed margarine in every respect. The taste of the resulting mixture was good and could not be distinguished from that of the initial margarine, except perhaps for a lower salt content.

Example 7

Addition of the mixture described in example 1 to ordinary commercially available light spread

50% by weight of the basic mixture according to example 1 and 50% by weight of light spread (Kevyt Voilevi 40% of Valio having a low salt content) were mixed in an ordinary steel mortar. The mixing could be carried out easily without any problems.

Based on a sensory evaluation, the result was a uniform mass having a light yellow colour, and a feeling throughout similar to those of the initial light spread used. The

taste of the resulting mixture was good, and practically, could not be distinguished from that of the initial light spread, except perhaps for a lower salt content.

Example 8

Addition of salt (sodium chloride) to mixtures prepared as described in examples 1 and 2

Mixtures were prepared as described in examples 1 and 2, adding thereto 0.9% of sodium chloride of the final weight of the mass, using a generally known method. Based on a sensory evaluation, the addition of salt did not impair in any way the properties of these basic mixtures.

Example 9

Evaluation of the frying properties of the masses described above

The behaviour of the mixtures containing butter or vegetable margarine described in examples 5 and 6 was studied under simulated frying conditions by frying them in beakers. For comparison, frying of pure butter and margarine was also studied. The mixture containing light spread was not fried because the light spread used as a starting material is not intended for frying.

9.1 Butter as such

Initially, when pure butter was heated, it formed a bright yellow oil with little bubbles. When the heating was continued, brown precipitated layers appeared. This is how butter normally turns brown.

9.2 Mixture containing butter and ß-sitosterol

When frying the mixture of example 5, it melted slightly slower and sizzled more than butter heated similarly. Like butter, it formed a bright yellow oil. When the heating was continued, brown spots appeared having the same colour as the spots in

butter, but of smaller size. This suggests that said mixture of example 5 is as suitable for frying as commercially available butters.

9.3 Margarine as such

Also pure margarine formed a bright yellow oil when melted. It sizzled more than butter when heated. On heating, brown spots appear on it, as in the mixture of example 5 containing butter and the basic mixture of example 1.

9.4 Mixture containing margarine and ß-sitosterol

When frying the mixture of example 6, it was observed that it behaved in the frying test in the same way as pure margarine and substantially in the same way as the mixture of example 5. This suggests that the mixture is as suitable for heating as commercially available margarines.

9.5 Conclusions of the tests in example 9

In this frying test, differences in fat browning seemed to be due to the type of fat used (butter or rapeseed margarine), not to the presence of the basic mixture containing ß-sitosterol.

Example 10

Addition of the mixture described in example 1 to dairy products

50% by weight of the basic mixture according to example 1 and 50 % by weight of mayonnaise (Heinz Mayonnaise, H.J. Heinz B.V., Holland) were mixed in an ordinary steel mortar at room temperature (about 22 °C). The mixture was mixed in the same manner with a sour cream product (Smetana from Valio) and with a cream cheese product (Hovi cream cheese from Valio). Mixing with these food products could be carried out easily without any difficulties.

Example 11

Addition of the starting material containing ß-sitosterol to a pasta product

ß-sitosterol was heated with the amount of oil needed for pasta preparation until either ß-sitosterol dissolved in oil, or an opalescent uniform flowing liquid was formed, depending on the ratio of ß-sitosterol to oil. This liquid was allowed to cool while triturating it. To the resulting cooled mixture was added either water, or an egg mixture and water, or an egg mixture while triturating at the same time to form an emulsion. A suitable amount of durum wheat flour and salt were then added by kneading the dough. Water was added as required during kneading. The result was uniform pasta wherein ß-sitosterol could not be detected visually, nor tasted. The amount of ß-sitosterol in the pasta was as much as 2 g/100 g of fresh pasta. Amounts exceeding this are not necessary in view of the weight of a pasta portion (125 g of fresh pasta/portion) and considering the suitable concentration of ß-sitosterol for the activity thereof. Table 2 shows examples of pasta dough compositions.

Pasta sheets were prepared in a usual way from this pasta dough. The pasta may be served either as fresh pasta or it may be dried for a longer term storage. Both fresh and dried pasta products were cooked in ample water for as long as 10 minutes. ß-sitosterol was not released from the pasta either into the cooking water or into the rinsing water of the cooked pasta.

The properties of the pasta completely corresponded to those of ordinary pasta prepared without ß-sitosterol.

Table 2. Examples of the compositions of pasta doughs

Ingredient	Pasta without egg amount (g) / 100 grams of pasta dough	Pasta with egg amount (g) / 100 grams of pasta dough
ß-sitosterol	1.7	1.7
Rapeseed oil	1.3	1.3
Durum wheat flour	62.6	61.8
Egg	-	29.0
Water	about 34 [1)]	about 5 [1)]
Salt	0.8	0.8

[1)] Suitable water amount may vary depending for instance on the type of durum wheat flour.

Example 12

Addition of the basic mixture containing ß-sitosterol to pizza

Table 3. Pizza recipe

	Ingredient	Pizza	Pizza with basic mixture containing ß-sitosterol
Dough	water	200.0 g	200 g
	yeast	25.0 g	25.0 g
	salt	3.0 g	3.0 g
	wheat flour	298.8 g	298.8 g
Garnish	ground meat (pork + beef)	250.0 g	250.0 g
	onion	130.0 g	130.0 g
	crushed tomatoes	400.0 g	400.0 g
	oregano	1.2 g	1.2 g
	basil	1.0 g	1.0 g
	crushed garlic	3.0 g	3.0 g
	salt	6.0 g	6.0 g
	black pepper powder	0.5 g	0.5 g
	paprika powder	0.5 g	0.5 g
	basic mixture containing ß-sitosterol*	**0.00 g**	59.0 g
Topping	grated cheese	150.0 g	150.0 g
	total	1471.0 g	1530.0 g

* The composition of the basic mixture used is shown in Table 1.

The pizzas were baked at 225 °C for about 25—30 minutes.

The properties of the pizza containing ß-sitosterol completely corresponded to those of the pizza prepared without ß-sitosterol.

Example 13

Addition of the basic blend containing ß-sitosterol to meatballs

Table 4. Meatball recipe

Ingredient	%	g
Prefabricated meatball mix**	10.5	52.5
Water	36.5	182.5
Ground meat (pork + beef)	53	265.0
	100	500.0

Table 5. Meatball recipe added with the basic blend containing ß-sitosterol

Ingredient	%	g
Prefabricated meatball mix**	10.1	52.5
Water	35	182.5
Ground meat (pork + beef)	50.8	
Basic blend containing ß-sitosterol*	**4.1**	**21.25**
	100	521.25

* Table 3 shows the composition of the basic blend used.

** Meatball Mix is an industrially produced mixture of dry ingredients for meatballs of Northern type containing seasonings, starch, soyaflour and bread crumbs.

Meatballs were baked at 225 °C for 20 mins.

The meat mixture did not stick to hands, and the properties of the meatballs containing ß-sitosterol were comparable to those of ordinary meatballs without any ß-sitosterol.

Example 14

Addition of the basic mixture containing ß-sitosterol to bread rolls

Table 6. Recipe for bread rolls

Ingredient	%	g
Water	44	461.0
Yeast	4.5	47.5
Salt	1.1	11.7
Syrup	0.3	3.6
Bread flour mixture	46.3	525.3
Oat flakes	3.8	40.0
	100	1089.1

Table 7. Recipe for bread rolls with a basic mixture containing ß-sitosterol added thereto

Ingredient	%	g
Water	3.7	461.0
Yeast	3.8	47.5
Salt	0.9	11.7
Syrup	0.2	3.6
Bread flour mixture	42.2	525.3
Oat flakes	3.2	40.0
Basic mixture containing ß-sitosterol*	**12.7**	**158.0**
	100	1247.1

* Composition of the basic mixture containing ß-sitosterol is shown in Table 1.

The basic mixture containing ß-sitosterol could be mixed into the dough very well and the dough was easy to handle and knead since it was not sticky. The properties

of the bread rolls containing ß-sitosterol totally corresponded to those of the rolls prepared without ß-sitosterol.

Example 15

Addition of the basic mixture containing ß-sitosterol to a sauce made with milk

Table 8. Recipe for a sauce with milk

Ingredient	%	g
Milk	93.7	390.0
Salt	1	4
Bread flour mixture	5.4	22.3
	100	416.3

Table 9. Recipe for a sauce with milk and with a basic mixture containing ß-sitosterol added thereto

Ingredient	%	g
Milk	85.9	390
Salt	0.9	4
Bread flour mixture	4.9	22.3
Basic mixture containing ß-sitosterol*	**8.3**	**37.5**
	100	453.8

* Composition of the basic mixture containing ß-sitosterol is shown in Table 1.

Fat was melted in a saucepan, and then flour was added thereto. The mixture was allowed to boil, and cold milk was added in two portions. The fat was well and evenly mixed in the sauce. The properties of a milky sauce wherein fat was replaced with the basic mixture containing ß-sitosterol completely corresponded to those of the sauce prepared using ordinary fat.

References:

1) Jousilahti P, Vartiainen E, Tuomilehto J, Puska P: The Lancet 348/9027), pp. 567—572. 1996

2) Claus EP, Tyler VE & Brady LR: *Pharmacognosy* 6th edition, Lea & Febiger, London, 1970, pp. 165—157

3) Jones PJH *et al.:* Canadian Journal of Physiology & Pharmacology 75(3): 217—227, 1997

4) Pollak OJ, Kritchecsky D: Monogr Atheroscler. 10: 1—219, 1981

5) Vahoyny GV, Kritchavsky D.: Plant and marine sterols and cholesterol metabolism. In Spiller GA, ed. Nutritional Pharmacology. New York, NY: Alan R Liss Inc; 1981 pp. 31—72

6) Vahoyny GV, Kritchevsky D.: Plant and marine sterols and cholesterol metabolism. In Spiller GA, ed. Nutritional Pharmacology. New York NY: Alan R Liss Inc; 1981 pp. 31—72

7) Tilvis RS, Miettinen TA: Am J Clin Nutr.: 43; 92—97, 1986

8) Mattson FH; Grundy SM, Crouse JR: Am J Clin Nutr. 35, 697—700, 1982

9) Steinegger E & Hänsel R: Pharmakognosie, 5. Aufl., Springer-Lehrbuch, Berlin-Haidelberg-New York, 1992, p. 195

10) Hänsel R & Haas H.: Therapie mit Phutopharmaka, Springer-Verlag, Berlin-Heidelberg-New York-Tokyo, 1983 pp. 187—188

11) Hänsel R: Phutopharmaka, Grundlagen und Praxis, 2. Auflage, Springer-Verlag, Berlin-Heidelberg-New York, 1991 pp. 192—193

12) Jones PJH *et al.:* Canadian Journal of Physiology & Pharmacology 75(3): 217—227, 1997

13) Field, FJ *et al.:* Journal of Lipid Research. 38(2): 348—360, 1997

14) Richter W *et al.:* Current Research. 57(7): 497—505, 1996

15) Mattson FH *et al.:* American Journal of Clinical Nutrition. 35(4): 697—700, 1982

16) Uchita E *et al.:* Japanese Journal of Pharmacology. 33(1): 103—12, 1983

17) Vahouny GB *et al.:* American Journal of Clinical Nutrition. 37(5): 805—9, 1983

Claims

1. A method for producing a fat mixture of ß-sitosterol lowering the serum total cholesterol and LDL-cholesterol levels, **characterized** in that ß-sitosterol or a starting material containing ß-sitosterol is dissolved in oil or fat, or in mixtures of oils and fats, using heat and mechanical energy, and water is added to the mixture during cooling.

2. A method according to claim 1, **characterized** in that the mixture comprises 0.5—80% of ß-sitosterol or the starting material containing ß-sitosterol, 5—90% of oil or fat, and 5—30% of water, all percentages being by weight and relative to the total mass of the mixture.

3. A method according to claim 1 or 2, **characterized** in that ß-sitosterol is present in the fat mixture in a partly dissolved and/or in a microcrystalline form.

4. A method according to any one of claims 1—3, **characterized** in that the oil or fat is any food grade oily substance of plant or animal origin, or a mixture thereof.

5. A method according to any one of claims 1—4, **characterized** in that the oil comprises sunflower oil, rapeseed oil, turnipseed oil, soy bean oil, olive oil, or corn oil.

6. A method according to any one of claims 1—5, **characterized** in that, in the method, a surface active agent or an emulsifying agent, polysorbate, lecithin of plant or animal origin, or a mixture thereof is/are added to the mixture.

7. A method according to any one of claims 1—6, **characterized** in that a food additive, sodium chloride, mineral salt, preserving and flavouring agents and vitamins, such as the vitamins A and E, or mixtures thereof is/are added to the mixture.

8. A method according to any one of claims 1—7, **characterized** in that the mixture of ß-sitosterol, or the starting material containing ß-sitosterol, and oil is heated at the temperature of 80—140 °C until the solid starting material is dissolved, the mixture is cooled to the temperature of 40—80 °C, and then water is added thereto essentially at the temperature thereof.

9. A fat composition containing ß-sitosterol that lowers the serum total cholesterol and LDL-cholesterol levels, **characterized** in that the composition comprises ß-sitosterol, oil or fat, or mixtures of oils and fats, and water.

10. A composition according to claim 9, **characterized** in that the composition comprises 0.5—80% of ß-sitosterol or the starting material containing ß-sitosterol, 5—90% of oil or fat, and 5—30% of water, all percentages being by weight and relative to the total weight of the mixture.

11. A composition according to claim 9 or 10, **characterized** in that ß-sitosterol is present in the composition in a partly dissolved and/or in a microcrystalline form.

12. A composition according to any one of claims 9—11, **characterized** in that the oil or fat is any food grade oily substance of plant or animal origin, or a mixture thereof.

13. A composition according to any one of claims 9—12, **characterized** in that the oil comprises sunflower oil, rapeseed oil, turnipseed oil, soy bean oil, olive oil, or corn oil.

14. A composition according to any one of claims 9—13, **characterized** in that the composition comprises a surface active agent or an emulsifying agent, polysorbate, lecithin of plant or animal orgin, or a mixture thereof.

15. A composition according to any one of claims 9—14, **characterized** in that the composition comprises a food additive, sodium chloride, mineral salt, preserving and flavouring agents and vitamins, such as the vitamins A and E, or mixtures thereof.

16. A composition according to any one of claims 9—15, **characterized** in that the composition is stable and homogeneous at room and refrigerator temperatures, based on physical and sensory examinations.

17. The use of a composition according to any one of claims 9—16 in food products, food grade oils and fats, such as butter, fat mixtures containing butter, light fat spreads, vegetable margarine, and various mixtures thereof, as well as fats for cooking and baking.

18. The use of a composition according to any one of claims 9—16 in food products, in food products comprising fats of animal or plant origin, or mixtures thereof, such as processed meat and fish products, food products containing natural fatty acids, dairy products, food products containing edible fats or mixtures thereof, for instance sauces and dressings, mayonnaise, spices and spice mixtures, cereal products, ice cream, candies, chocolate, cakes, pastries.

19. A method for adding ß-sitosterol into food products for lowering serum total cholesterol and LDL-cholesterol levels, **characterized** in that ß-sitosterol or a starting material containing ß-sitosterol is dissolved in oil, fat, or mixtures thereof using heat and mechanical energy, and the resulting mixture is mixed into food ingredients during a food production process.

AMENDED CLAIMS

[received by the International Bureau on 12 July 1999 (12.07.99);
original claims 1-19 replaced by amended claims 1-16 (3 pages)]

1. A method for producing a fatty blend of plant sterol lowering the serum total cholesterol and LDL-cholesterol levels, **characterized** in that 0.5—80 wt% of a starting material containing ß-sitosterol is dissolved in 5—90 wt% of oil, fat, or of mixtures of oils and fats, by heating at a temperature of 80—140 °C until the starting material containing ß-sitosterol is dissolved, then the mixture is cooled to a temperature of 40—80 °C, and during cooling 5—30 wt% of water with a temperature essentially similar to the one of the mixture, is added to the mixture, and the mixture is agitated whereby a homogeneous and stable mixture is obtained wherein the starting material containing β-sitosterol is in a partly dissolved and/or microcrystalline form.

2. The method according to claim 1, **characterized** in that the starting material containing β-sitosterol comprises β-sitosterol and/or β-sitostanol.

3. The method according to claim 1 or 2, **characterized** in that the oil or fat is any food grade oily substance of plant or animal origin, or a mixture thereof.

4. The method according to any one of claims 1—3, **characterized** in that the oil comprises sunflower oil, rapeseed oil, turnipseed oil, soyabean oil, olive oil, or corn oil.

5. The method according to any one of claims 1—4, **characterized** in that in the method, a surface active agent, an emulsifying agent, polysorbate, lecithin of plant or animal origin, or a mixture thereof is/are added to the mixture.

6. The method according to any one of claims 1—5, **characterized** in that a food additive, sodium chloride, mineral salt, preserving or flavouring agents or vitamins, such as vitamins A and E, or mixtures thereof is/are added to the mixture.

7. A fat composition containing plant sterol that lowers the serum total chloesterol and LDL-cholesterol levels, **characterized** in that the composition comprises 0.5—80 wt% of a starting material containing ß-sitosterol, oil or fat or mixtures thereof, and 5—30 wt% of water, and the starting material containing ß-sitosterol is in a partly dissolved and/or microcrystalline form, and the composition is stable and homogeneous at room and refrigerator temperature, based on physical and sensory evaluations.

8. The composition according to claim 7, **characterized** in that the starting material containing β-sitosterol comprises β-sitosterol and/or β-sitostanol.

9. The composition according to claim 7 or 8, **characterized** in that the oil or fat is any food grade oily substance of plant or animal origin, or a mixture thereof.

10. The composition according to any of claims 7—9, **characterized** in that the oil comprises sunflower oil, rapeseed oil, turnipseed oil, soybean oil, olive oil, or corn oil.

11. The composition according to any of claims 7—10, **characterized** in that the composition comprises a surface active agent, an emulsifying agent, polysorbate, lecithin of plant or animal orgin, or a mixture thereof.

12. The composition according to any of claims 7—11, **characterized** in that the composition comprises any food additive, sodium chloride, mineral salt, preserving or flavouring agents or various vitamins, such as the vitamins A and E, or mixtures thereof.

13. Use of a composition according to any one of claims 7—12 in food products, in food grade oils and fats, such as butter, in fat mixtures containing butter, in light fatty spreads, in vegetable margarine, and in various mixtures thereof, as well as in fats for cooking and baking.

14. Use of a composition according to any of claims 7—12 in food products, in food products comprising fats of animal or plant origin or mixtures thereof, such as processed meat and fish products, in food products containing natural fatty acids, in dairy products, in food products containing edible fats or mixtures thereof, for instance sauces and dressings, mayonnaise, spices and spice mixtures, cereal products, ice cream, candies, chocolate, cakes and pastries.

15. A method for adding plant sterol for lowering serum total cholesterol and LDL-cholesterol levels into food products, **characterized** in that 0.5—80 wt% of a starting material containing ß-sitosterol is dissolved in 5—90 wt% of oil, fat or mixtures thereof by heating at a temperature of 80—140 °C until the starting material containing ß-sitosterol is dissolved, then the mixture is cooled to a temperature of 40—80 °C, and during cooling 5—30 wt% of water with a temperature essentially similar to the one of the mixture, is added to the mixture, and the mixture is agitated whereby a homogeneous and stable mixture is obtained wherein the starting material containing ß-sitosterol is in a partly dissolved and/or microcrystalline form, and the resulting mixture is mixed into food ingredients during a food production process.

16. The method according to claim 15, **characterized** in that the starting material containing ß-sitosterol comprises ß-sitosterol and/or ß-sitostanol.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/FI 99/00121

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: A23L 1/30, A23L 1/29
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CAPLUS, WPI

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 3085939 A (MILTON WRUBLE ET AL), 16 April 1963 (16.04.63) --	1-19
X	WO 9742830 A1 (UNILEVER N.V.), 20 November 1997 (20.11.97) --	1-19
X	STN International, File WPIDS, WPIDS accession no. 1987-224314, Document no. C87-094358, RIKEN VITAMIN CO: "Sterol-contg. composite for food industry, etc. - also contains specified emulsifier, and dispersant, e.g. liq. paraffin"; & JP,A,62148424, 870702, --	1-19

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
10 June 1999	15 -06- 1999
Name and mailing address of the ISA: Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Eva Johansson/Els Telephone No. +46 8 782 25 00

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 3751569 A (BILLY ARTHUR ERICKSON), 7 August 1973 (07.08.73) --	1-19
A	US 3865939 A (RONALD JAMES JANDACEK), 11 February 1975 (11.02.75) --	1-19
A	STN International, File WPIDS, WPIDS accession no. 1983-10783K, Document no. C83-010564, AJINOMOTO KK: "Edible oil having cholesterol suppressing effect - contains vitamin-E and vegetable sterol"; & JP,A,57206336, 821217 --	1-19
A	STN International, File WPIDS, WPIDS accession no. 1975-18814W, LENINGRAD FOOD RES INST: "Dietetic pastries contg. beta-sitosterol - for use by patients with lipid exchange disorder"; & SU,A,414989, 740918 --	1-19
A	STN International, File WPIDS, WPIDS accession no. 1979-66146B, LITH FOOD IND DES: "Canned dietetic food mfr. - includes addn. of oil contg. beta-sitosterol before heating, useful in lipid metabolism disorders"; & SU,A,635951, 781215 --	1-19

INTERNATIONAL SEARCH REPORT

Information on patent family members

03/05/99

International application No.
PCT/FI 99/00121

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 3085939 A	16/04/63	NONE	
WO 9742830 A1	20/11/97	AU 3028297 A	05/12/97
US 3751569 A	07/08/73	NONE	
US 3865939 A	11/02/75	BE 811452 A	22/08/74
		CA 1024814 A	24/01/78
		DE 2408067 A,C	05/09/74
		FR 2218838 A,B	20/09/74
		GB 1413102 A	05/11/75
		JP 1135221 C	14/02/83
		JP 50040605 A	14/04/75
		JP 57026732 B	07/06/82
		NL 185384 C	02/04/90
		NL 7402426 A	27/08/74

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代理人 李 瑛

权利要求书2页 说明书16页 附图页数0页

[54]发明名称 脂肪混合物的生产方法

[57]摘要

本发明涉及一种生产β-谷甾醇脂肪混合物的方法,所说的混合物降低血清总胆固醇和LDL-胆固醇含量。用热能和机械能将β-谷甾醇或含β-谷甾醇的原材料溶解于油或脂肪、或油和脂肪的混合物中,并且在冷却过程中向混合物添加水。

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权 利 要 求 书

1、一种生产降低血清总胆固醇和LDL-胆固醇含量的β -谷甾醇脂肪混合物的方法，其特征在于利用热能和机械能将β-谷甾醇或含β-谷甾醇的原材料溶于油或脂肪，或溶于油与脂肪的混合物中，并且在冷却过程中向混合物加入水。

2、权利要求 1 的方法，其特征在于混合物含有 0.5-80%的β-谷甾醇或含β-谷甾醇的原材料、5-90%的油或脂肪、和 5-30%的水，所有百分数均以重量计并相对于混合物的总量。

3、权利要求 1 或 2 的方法，其特征在于β-谷甾醇以部分溶解和/或微晶形式存在于脂肪混合物中。

4、权利要求 1-3 中任一项的方法，其特征在于油或脂肪是来源于植物或动物的任何食品级油性物质，或其混合物。

5、权利要求 1-4 中任一项的方法，其特征在于油包括向日葵油、菜子油、芜菁籽油、大豆油、橄榄油或玉米油。

6、权利要求 1-5 中任一项的方法，其特征在于，在该方法中，向混合物中加入表面活性剂或乳化剂、聚山梨醇酯、起源于植物或动物的卵磷脂、或其混合物。

7、权利要求 1-6 中任一项的方法，其特征在于，向混合物中加入食品添加剂、氯化钠、矿盐、防腐剂和调味剂以及维生素，如维生素 A 和 E、或其混合物。

8、权利要求 1-7 中任一项的方法，其特征在于，将β-谷甾醇或含β-谷甾醇的原材料与油的混合物在 80-140℃加热，直至固态原材料溶解，将混合物冷却至 40-80℃的温度，然后向其中加入基本上为该温度的水。

9、一种降低血清总胆固醇和 LDL-胆固醇含量的含有β-谷甾醇的脂肪组合物，其特征在于组合物含有β-谷甾醇、油或脂肪、或油和脂肪的混合物、以及水。

10、权利要求 9 的组合物，其特征在于，组合物含有 0.5-80%的β-

谷甾醇或含β-谷甾醇的原材料、5-90%的油或脂肪、和 5-30%的水，所有百分数均以重量计并相对于混合物的总重。

11、权利要求 9 或 10 的组合物，其特征在于β-谷甾醇以部分溶解和/或微晶形式存在于组合物中。

12、权利要求 9-11 中任一项的组合物，其特征在于油或脂肪是来源于植物或动物的任何食品级油性物质，或其混合物。

13、权利要求 9-12 中任一项的组合物，其特征在于油包括向日葵油、菜子油、芜菁籽油、大豆油、橄榄油或玉米油。

14、权利要求 9-13 中任一项的组合物，其特征在于组合物含有表面活性剂或乳化剂、聚山梨醇酯、起源于植物或动物的卵磷脂、或其混合物。

15、权利要求 9-14 中任一项的组合物，其特征在于，组合物含有食品添加剂、氯化钠、矿盐、防腐剂和调味剂以及维生素，如维生素 A 和 E、或其混合物。

16、权利要求 9-15 中任一项的组合物，其特征在于，根据物理和感官检查，组合物在室温和冷藏或冷冻温度下是稳定且均匀的。

17、权利要求 9-16 中任一项的组合物在食品、食品级油和脂肪如黄油、含黄油的脂肪混合物、轻质脂肪涂抹料、植物人造奶油及其各种混合物、以及用于烹调和焙烤的脂肪中的应用。

18、权利要求 9-16 中任一项的组合物在食品、包含来源于动物或植物的脂肪或其混合物的食品，如加工肉和鱼制品、含天然脂肪酸的食品、乳制品、含食用脂肪或其混合物的食品，如调味汁和调味料、蛋黄酱、香辛料和香辛料混合物、谷物产品、冰淇淋、糖果、巧克力、蛋糕、焙烤食品中的应用。

19、一种向食品中添加β-谷甾醇以降低血清总胆固醇和 LDL-胆固醇含量的方法，其特征在于，利用热能和机械能将β-谷甾醇或含β-谷甾醇的原材料溶解于油、脂肪或其混合物中，并且在食品生产过程中将所得的混合物混入食品配料中。

说　明　书

脂肪混合物的生产方法

本发明涉及β-谷甾醇脂肪混合物的生产方法，该混合物有益于健康、均匀、稳定，它降低血清总胆固醇和LDL-胆固醇含量，并且含有部分溶解和/或微晶形式的β-谷甾醇。

高血清总胆固醇含量、高血压和吸烟是与心脏病有关的主要危险因素(1)。一些甾醇起源于植物，它们与胆固醇的区别仅在于侧链取代基及饱和程度。大部分高级植物产生24α-取代的甾醇(24-甲基－和24-乙基甾醇)。谷甾醇是β-谷甾醇(豆甾-5-烯-3β-醚)与某些饱和甾醇如β-豆甾醇的混合物，其中甾醇含量不少于95%，不饱和甾醇含量不少于85%。谷甾醇广泛存在于植物中，例如在小麦和黑麦的胚芽油、玉米油中，并常存在于种子油中。谷甾醇是抗高胆固醇剂，抑制胆固醇在肠中以及通过血管内壁的吸收(2)。谷甾醇当以2-3克/每天三次的剂量给药时在治疗动脉粥样硬化中起作用。在西方饮食中，每天从食物中摄取的β-谷甾醇、豆甾醇和菜油甾醇为约200-400mg(3)，这大约与我们每天从食物中摄取的胆固醇为同一数量级。

在20世纪50年代初期，认识到向喂养鸡和兔的胆固醇饲料中添加β-谷甾醇降低了这两种测试动物中的胆固醇水平，此外，β-谷甾醇的这种添加防止了兔中动脉粥样硬化的形成(4)。使用谷甾醇和大豆甾醇来降低胆固醇含量在20世纪50年代及20世纪60年代得到了深入地研究(5)，并且事实上其制剂降低了约10%的胆固醇含量(6)。进而发现，β-谷甾醇的活性是基于对胆固醇吸收的抑制，而且来源于植物的甾醇本身吸收性差(7)。抑制胆固醇吸收的机理被认为是基于胆固醇和β-谷甾醇的结晶和共沉淀。Mattson 等(8)指出，1g β-谷甾醇将从含500mg胆固醇的食物中的胆固醇吸收降低了42%。血浆胆固醇的降低可能是由于LDL-受体活性的增加。

β-谷甾醇是一种亲脂性化合物。在与肠壁的脂质膜接触中，β-谷甾

醇由于其水溶性差而不被吸收，或者仅仅吸收一小部分；当口服给予时，仅仅吸收少于 5%的β-谷甾醇 (9)。β-谷甾醇的活性是基于肠内胆固醇吸收的竞争性抑制(10)。B-谷甾醇妨碍小肠内胆固醇的吸收和再吸收(11)。认为，这是由于胆固醇和β-谷甾醇化学结构的相似性(12)。在各种条件下进行的一些研究表明植物甾醇降低 LDL-胆固醇含量。进一步认识到，血清植物甾醇与 HDL-含量相关。β-谷甾醇通过影响 HMG-CoA 还原酶的基因表达而降低胆固醇在肝中的合成(13)。Richter W 等(14)指出β-谷甾醇通过抑制胆固醇在肠内的吸收，使总血清胆固醇含量降低了 10-15%，使 LDL-胆固醇含量降低了 19%。研究中，给 9 名成年患者 5 天施用 500mg 胆固醇、以及 1g β-谷甾醇或 2g 油酸β-谷甾醇酯。当施用β-谷甾醇时，胆固醇的吸收降低了 42%，当施用油酸β-谷甾醇酯时，胆固醇的吸收降低了 33%(15)。Uchita 等(16)认为在雌性大鼠中，谷甾醇抑制胆固醇的吸收，并且降低血浆和肝中的胆固醇平衡。Vahouny 等(17)发现在大鼠中谷甾醇抑制了 54%的胆固醇吸收。

芬兰专利申请 964951 公开了一种用于降低血清中胆固醇含量的试剂及其用途。该申请涉及使用β-豆甾醇与脂肪酸的酯，或β-豆甾醇与脂肪酸的酯的混合物作为食品中的脂肪组分或作为脂肪替代物，该申请涉及其作为这样的补充饮食的用途，以及化合物本身。

芬兰专利公开 98 730 公开了一种用于降低高血清胆固醇含量的物质的生产方法。在该方法中，使用将β-谷甾醇在负载于碳上的钯催化剂存在下在有机溶剂中氢化而获得的β-豆甾醇以及植物油，利用酯交换技术，在乙醇钠催化剂的存在下生产β-豆甾醇与脂肪酸的酯或这样的酯的混合物。

上述这两篇专利公开都公开了将β-谷甾醇改性来获得其可溶于脂肪的衍生物的方法，从中生产可溶性β-豆甾醇脂肪酸酯，以及使用得到的化合物作为降低血清胆固醇含量的试剂。

天然存在的β-谷甾醇是晶体化合物。众所周知，类似β-谷甾醇的游离甾醇几乎不溶于油和脂肪，因此生产β-谷甾醇衍生物，如明显更溶于脂肪的酯类用于实际需要，尽管根据一些研究(15)，这些衍生物并不象

游离β-谷甾醇一样有效地抑制胆固醇的吸收。这样的可溶于脂肪的衍生物比固体不溶性粗β-谷甾醇粉末可以更容易混合到营养品中形成均匀的混合物。然而，为获得β-谷甾醇衍生物的这种加工给产品带来额外的成本。另外，将β-谷甾醇氢化成β-豆甾醇必然使用有机溶剂，这样，在酯化的最终产品中可能存在微量有机溶剂以及微量所用金属催化剂。此外，酯化的产品不再是天然存在的物质而是人造仿真化合物。

本发明的一个目的是提供一种β-谷甾醇脂肪混合物的生产方法，该混合物有益于健康、均匀、稳定，它降低总血清胆固醇和 LDL-胆固醇含量，并且含有部分溶解和/或微晶形式的β-谷甾醇。本发明的另一个目的是在脂肪制品或食品中使用这样的含有部分溶解和/或微晶形式的β-谷甾醇的均匀稳定的β-谷甾醇脂肪混合物，作为降低血清中胆固醇含量的试剂，以及使用该混合物作为这样的物质以补充饮食。

在附加的权利要求中公开了根据本发明的方法和用途的主要特征。

我们发现，β-谷甾醇可以通过以下步骤制成部分可溶的和/或微晶状。用本发明的溶液可以避免与本领域现有技术有关的问题和缺点。根据本发明的方法，将β-谷甾醇与食品级油混合，加热该混合物直至所有固体溶于油中。冷却后，向混合物中加入在此温度下的水，由此使其分散。得到均匀、稳定、类似脂肪的几乎白色的物质，其稠度与黄油或油状混合物非常近似，取决于组分的量。该均匀稳定的糊状物特别适合混到例如食品中。

本方法中的原材料是可以含有 80-100%的β-谷甾醇和β-豆甾醇以及作为杂质的 0-20%的其它甾醇和 stanol 的 β-谷甾醇。可以将该含有β-谷甾醇的原材料或β-谷甾醇与用量为 0.5-80%、优选 10-30%的食品级油混合，得到的糊状产品具有非常近似黄油的外观和粘度，并且容易处理。混合物中β-谷甾醇的百分含量越高，物质越硬。另一方面，如果存在于混合物中的β-谷甾醇的量，以油的量计算，小于 10%，物质的粘度将降低，其稠度明显更类似于油。作为食品级油，可以使用任何烹饪用油或任何食品级油或脂肪，或者来源于动物并适合人食用

的油或油状化合物，例如鳕鱼肝油，或者来源于植物或动物的任何可食用的油性物质，或其混合物。优选的油是菜子油、芜菁籽油、向日葵油、大豆油、玉米油和橄榄油。油的量，以混合物块的重量计，为5-90%，优选60-85%。所用的水可以是任何食品级水，其百分含量以混合物块的重量计，为5-30%，优选10-20%。

在本方法中，将含β-谷甾醇的原材料与油的混合物在80-140℃的温度，优选在100-120℃加热，直至含β-谷甾醇的固体原材料溶于油中。以已知方式将混合物冷却至40-80℃、优选至50-70℃之后，向其中加入基本上在混合物温度下的水。可以任选地向混合物中加入量为0.05-8.0重量%的结构稳定化表面活性剂，例如聚山梨醇酯（polysorbate）(Tween 80，Polysorbat 80)、卵磷脂或大豆卵磷脂（已知的乳化剂）。如果需要，可以加入稳定剂、抗氧化剂或其它合适的本领域公知的食品添加剂，如氯化钠、矿盐、防腐剂和调味剂、和/或各种维生素如维生素A和E、食用色素和植物酚类（phytophenols）。由此制备的混合物在常规储存食品的条件下是均匀且稳定的。混合物中，β-谷甾醇是部分溶解的和/或微晶形式。如果需要，也可以如上所述将β-谷甾醇溶于油中，可以将该β-谷甾醇/油混合物用作食品生产中这样的物质。

本发明的方法使得以简单和经济的方式生产β-谷甾醇脂肪混合物成为可能，该混合物有益于健康、均匀稳定，降低肠内胆固醇的吸收，从而降低血清总胆固醇和LDL-胆固醇含量，并且含有部分溶解和/或微晶形式的β-谷甾醇。本方法使用天然存在的β-谷甾醇和食品级油或脂肪，不需任何有机溶剂或复杂的工艺步骤。即使高剂量的所得的含天然存在的β-谷甾醇的均匀稳定的脂肪混合物，也可以在食品中每天安全食用，并且可以用于食品生产和烹调以部分或全部取代脂肪。β-谷甾醇不能依靠感官从食品中检测出来。通过由此制备的食品，可以抑制胆固醇在肠内的吸收，可以显著降低总血清胆固醇和LDL-胆固醇含量。此外，由于取代脂肪的β-谷甾醇基本上不被吸收，减少了所吸收的脂肪比例，从而降低了能量摄入。

含β-谷甾醇的脂肪混合物可以加入到含有源于动物或植物的脂肪或其混合物的食品中。适宜的食品是各种加工肉制品，如香肠和绞肉制熟食品；加工鱼制品；含天然脂肪酸的食品；乳制品，如干酪；以及其它一些含食用脂肪或其混合物的食品，例如调味汁和调味料（dressings）、蛋黄酱、香辛料和香辛料混合物、谷类食物、面条和面食制品、冰淇淋、糖果、巧克力、蛋糕、焙烤食品等；以及用于烹调和焙烤的食用脂肪；及其混合物。

现在将通过下面实施例中所述的本发明的一些优选的实施方案来说明本发明，但是这些实施例仅仅是为了说明而非限制本发明。

实施例

β-谷甾醇与脂肪的混合物即所谓的基础混合物的生产方法

在实施例中，原材料是混合物，其中含有总量为 89.2%的β-谷甾醇和β-豆甾醇、量为 0.1%的α-谷甾醇、总量为 8.9%的菜油甾醇和 campestanol 以及总量为 0.9%的 arthenols。原材料的固体物质含量为 98.8%，熔程为 137-138℃，密度为 0.49kg/dm^3。

为方便起见，以下将该原材料根据其主要组分称为含β-谷甾醇的原材料。

实施例 1

含β-谷甾醇的基础混合物

制备含 20 重量%β-谷甾醇和 80 重量%菜子油的混合物。将混合物在玻璃瓶中边搅拌边加热，直至含β-谷甾醇的原材料溶于油中。此时，温度为约 110℃，在标准大气压下进行试验。

将混合物冷却至约 60℃之后，向其中加入量为约 15%（以混合物量的重量计）的与混合物温度(60℃)相同的自来水，在研钵中研磨。

混合物一开始当用肉眼观察时是透明的并且呈黄色油状。突然，几乎加完水，混合物变得不透明并呈灰白色。使混合物冷却至室温(22℃)，同时混合。混合物的最终组成见表 1。

表 1、含β-谷甾醇的基础混合物的组成

成分	含量(重量%)
含β-谷甾醇的原材料	17
菜子油	68
水	15
总计	100

根据感官检验，表 1 的混合物为白色脂肪物质，其稠度非常类似黄油，并且含有部分溶解和/或微晶形式的β-谷甾醇。该混合物实际上是无味的。

当在冰箱中贮存时，根据感官检验，表 1 的基础混合物保持不变。迄今为止，混合物已贮存了约 6 个月。

实施例 2

含β-谷甾醇的原材料在含油及水的混合物中的浓度变化

按照实施例 1 的方法制备混合物，其中含β-谷甾醇的原材料的比例为菜子油的 2.5-60%。经观察，当含β-谷甾醇的原材料的浓度为 10-20%时，基础混合物的稠度是最优选的。于是，该混合物具有类似于黄油的外观和粘度，并且容易处理。

混合物中含β-谷甾醇的原材料的比例越高，其稠度越硬。如实施例 11 所述，尽管混合物是硬的，即使高比例的含β-谷甾醇的原材料也可以使用 (向面食制品中添加含β-谷甾醇的原材料)。

实施例 3

使用各种食品级油制备混合物

按照实施例 1 的方法制备混合物，用向日葵油、玉米油和橄榄油代替菜子油。用这些油中的每一种，使用三种不同的β-谷甾醇百分含量制备混合物：5%、10%和 20%。

根据感官和显微镜检验，除了含橄榄油的混合物带绿色外，这些混合物与用菜子油制备的混合物类似。这表明所有食品级油都非常适合用在本发明的方法中。

实施例 4

向实施例 1 的基础混合物中加入表面活性剂

通常已知，表面活性剂对分散体系、特别是乳液的制备和稳定是必需的。使用能够稳定分散体稠度的表面活性剂常常是重要的，特别是为了长期贮存，例如为了防止乳液组分的任何分离或结晶。

基本上制备了实施例 1 的混合物，其中含有 2 重量%（以水相比例计）的乳化剂，该乳化剂作为表面活性剂通常是已知的，如聚山梨醇酯 (Tween 80，Polysorbat 80)、卵磷脂或大豆卵磷脂。所得组成基本上如表 1 所示，除了每种含有约 0.3 重量%的表面活性剂。

将含β-谷甾醇的脂肪混合物加到食品中

实施例 5

将实施例 1 所述的混合物加到传统的市售黄油中

室温下(约 22℃)在普通钢研钵中将根据实施例 1 的 50 重量%的基础混合物与 50 重量%黄油(Valio 的 Meijerivoi，低盐含量)混合。混合容易进行而没有任何困难。

根据感官评价，得到黄油颜色的均匀浅黄色物质，该物质每个方面都感觉类似普通的黄油。混合物的味道不错，可能除了盐含量较低外，与真正的黄油无法区分。

实施例 6

将实施例 1 所述的混合物加到常规的市售菜子人造奶油中

室温下(约 22℃)在普通钢研钵中将根据实施例 1 的 50 重量%的基础混合物与 50 重量%的菜子人造奶油(Kultarypsi margariini 60，Van

der Bergh，瑞典)混合。混合物略微较软，但稠度在其它方面类似于上述实施例 3。混合容易进行而没有任何困难。

根据感官评价，得到原始菜子人造奶油颜色的均匀浅黄色物质，该物质每个方面都感觉类似普通的菜子人造奶油。所得混合物的味道不错，可能除了盐含量较低外，与开始的人造奶油无法区分。

实施例 7

将实施例 1 所述的混合物加到普通的市售轻质涂抹料（light spread）中

在普通钢研钵中将根据实施例 1 的 50 重量%的基础混合物与 50 重量%的轻质涂抹料(Valio 的 Kevyt Voilevi 40%，低盐含量)混合。混合容易进行而没有任何问题。

根据感官评价，得到浅黄色均匀物质，自始至终的感觉类似于开始使用的轻质涂抹料。所得混合物的味道不错，并且实际上可能除了盐含量较低外，与开始的轻质涂抹料无法区分。

实施例 8

向按实施例 1 和 2 所述制备的混合物中加入盐(氯化钠)

按实施例 1 和 2 所述制备了混合物，使用通常已知的方法，向其中加入 0.9%（以物质的最终重量计）的氯化钠。根据感官评价，加入盐在任何方面均未损害这些基础混合物的性质。

实施例 9

上述物质油炸特性的评价

通过将实施例 5 和 6 所述的含黄油或植物人造奶油的混合物在烧杯中油炸，研究了它们在模拟的油炸条件下的行为。为了比较，也研究了纯黄油和人造奶油的油炸。含轻质涂抹料的混合物未进行油炸，因为用作原材料的轻质涂抹料不是用来油炸的。

9.1 黄油本身

开始，当将纯黄油加热时，它形成带有小气泡的亮黄色油。当继

续加热时，出现褐色沉淀层。这就是通常黄油怎样变褐。

9.2 含黄油和β-谷甾醇的混合物

当将实施例 5 的混合物油炸时，它比同样加热的黄油熔化得略慢且发出更多嗤嗤声。与黄油类似，它形成了亮黄色油。当继续加热时，出现与黄油中的点颜色相同但尺寸更小的褐色点。这表明所述实施例 5 的混合物与市售黄油一样适合油炸。

9.3 人造奶油本身

纯的人造奶油当熔化时也形成了亮黄色油。加热时，它比黄油发出更多嗤嗤声。一加热，如同含有黄油和实施例 1 的基础混合物的实施例 5 的混合物一样，在其上出现褐色点。

9.4 含人造奶油和β-谷甾醇的混合物

当油炸实施例 6 的混合物时，观察到它在油炸试验中的行为方式与纯的人造奶油相同，并且基本上与实施例 5 的混合物相同。这表明该混合物与市售的人造奶油一样适合加热。

9.5 实施例 9 中试验的结论

在该油炸测试中，脂肪褐变的区别似乎是由于所用脂肪的类型(黄油或菜子人造奶油)，而不是由于存在含β-谷甾醇的基础混合物。

实施例 10

将实施例 1 所述的混合物加到乳制品中

室温下(约 22℃)在普通钢研钵中将根据实施例 1 的 50 重量%的基础混合物与 50 重量%的蛋黄酱(Heinz Mayonnaise，H.J. Heinz B.V.，荷兰)混合。将混合物与酸性乳制品(Valio 的 Smetana)和乳酪制品(Valio 的 Hovi 乳酪) 以相同方式混合。与这些食品的混合容易进行而没有任何困难。

实施例 11

将含β-谷甾醇的原材料加到面食制品中

将β-谷甾醇与制备面食制品所需量的油加热，直至β-谷甾醇溶解

于油中，或者形成乳色的均匀流动的液体，取决于β-谷甾醇与油之比。使该液体冷却同时研磨。向所得的冷却的混合物中加入水，或蛋混合物与水，或蛋混合物，同时研磨以形成乳液。然后通过揉和面团加入适宜量的硬质小麦粉和盐。在揉和过程中根据需要加入水。得到均匀的面食制品，其中β-谷甾醇不能用肉眼检测出来，也不能品尝出来。面食制品中的β-谷甾醇量多达2g/100g新鲜面食制品。鉴于面食制品部分的重量(125g新鲜面食/部分)并考虑用于其活性的β-谷甾醇的合适浓度，不必超过这个量。表2给出了面食制品面团组成的实例。

用通常的方式从该面食制品面团制备了面食片。该面食制品可以作为新鲜面食食用，也可以干燥以较长期储存。新鲜的和干燥的面食制品，都在足够的水中煮长达10分钟。β-谷甾醇不从面食中释放出来，既不进入蒸煮水中也不进入煮熟面食的漂洗水中。

该面食的特性与不用β-谷甾醇制备的普通面食完全一致。

表2、面食制品面团组成实例

成分	不含蛋的面食量(g)/100g面食面团	含蛋的面食量(g)/100g面食面团
β-谷甾醇	1.7	1.7
菜子油	1.3	1.3
硬质小麦粉	62.6	61.8
蛋	-	29.0
水	约34 [1)]	约5 [1)]
盐	0.8	0.8

[1)]适宜的水量可以根据例如硬质小麦粉的类型而变化。

实施例12

将含β-谷甾醇的基础混合物加到比萨饼中

表 3、比萨饼配方

	配料	比萨饼	有含β-谷甾醇的基础混合物的比萨饼
面团	水	200.0g	200g
	酵母	25.0g	25.0g
	盐	3.0g	3.0g
	小麦粉	298.8g	298.8g
饰菜	肉馅(猪肉 + 牛肉)	250.0g	250.0g
	洋葱	130.0g	130.0g
	碎番茄	400.0g	400.0g
	牛至（oregano）	1.2g	1.2g
	罗勒	1.0g	1.0g
	碎蒜	3.0g	3.0g
	盐	6.0g	6.0g
	黑胡椒粉	0.5g	0.5g
	辣椒粉	0.5g	0.5g
	含β-谷甾醇的基础混合物*	0.00g	59.0g
顶饰料（topping）	碎干酪	150.0g	150.0g
	总计	1471.0g	1530.0g

*所用基础混合物的组成见表 1.

将比萨饼在 225℃焙烤约 25-30 分钟。

含β-谷甾醇的比萨饼的特性与不用β-谷甾醇制备的比萨饼完全一致。

实施例 13

将含β-谷甾醇的基础混合物加到肉丸子中

表 4、肉丸子配方

配料	%	g
预制肉丸子混合料**	10.5	52.5
水	36.5	182.5
肉馅(猪肉 + 牛肉)	53	265.0
	100	500.0

表 5、加有含β-谷甾醇的基础混合物的肉丸子配方

配料	%	g
预制肉丸子混合料**	10.1	52.5
水	35	182.5
肉馅(猪肉 + 牛肉)	50.8	
含β-谷甾醇的基础混合物*	4.1	21.25
	100	521.25

*表 3 给出了所用基础混合物的组成

**肉丸子混合料是工业生产的用于含有佐料、淀粉、大豆粉和面包屑的 Northern 型肉丸子的干配料混合物。

肉丸子在 225℃焙烤 20 分钟。

肉混合物不粘手，并且含β-谷甾醇的肉丸子的性质与不含任何β-谷甾醇的普通肉丸子类似。

实施例 14

将含β-谷甾醇的基础混合物加到面包圈中

表 6、面包圈配方

配料	%	g
水	44	461.0
酵母	4.5	47.5
盐	1.1	11.7
糖浆	0.3	3.6
面包粉混合物	46.3	525.3
燕麦片	3.8	40.0
	100	1089.1

表 7、添加有含β-谷甾醇的基础混合物的面包圈配方

配料	%	g
水	3.7	461.0
酵母	3.8	47.5
盐	0.9	11.7
糖浆	0.2	3.6
面包粉混合物	42.2	525.3
燕麦片	3.2	40.0
含β-谷甾醇的基础混合物*	12.7	158.0
	100	1247.1

*含β-谷甾醇的基础混合物的组成见表 1。

含β-谷甾醇的基础混合物可以很好地混入面团中，并且因为面团不发粘，所以容易处理及揉和。含β-谷甾醇的面团圈的特性与不用β-谷甾醇制备的面团圈完全一致。

实施例 15

将含β-谷甾醇的基础混合物加到乳制调味汁中

表 8、乳制调味汁配方

配料	%	g
乳	93.7	390.0
盐	1	4
面包粉混合物	5.4	22.3
	100	416.3

表 9、添加有含β-谷甾醇的基础混合物的乳制调味汁配方

配料	%	g
乳	85.9	390
盐	0.9	4
面包粉混合物	4.9	22.3
含β-谷甾醇的基础混合物*	8.3	37.5
	100	453.8

*含β-谷甾醇的基础混合物的组成见表 1。

在平底锅中将脂肪熔化，然后向其中加入面粉。将混合物煮沸，并且分两批加入冷乳。脂肪彻底并且均匀地混在调味汁中。其中用含β-谷甾醇的基础混合物替代脂肪的乳状调味汁的特性与用普通脂肪制备的调味汁完全一致。

参考文献:

1) Jousilahti P, Vartiainen E, Tuomilehto J, Puska P: The Lancet 348/9027）, pp.567-572, 1996

2) Claus EP, Tyler VE & Brady LR: Pharmacognosy, 第 6 版, Lea & Febiger, 伦敦, 1970, pp. 165-157

3) Jones PJH 等:《加拿大生理学和药理学杂志》(Canadian Journal of Physiology & Pharmacology), 75(3): 217-227, 1997

4) Pollak OJ, Kritchecsky D: Monogr Atheroscler. 10:1-219, 1981

5) Vahoyny GV, Kritchavsky D.:《植物和海水甾醇和胆固醇代谢》, Spiller GA 编《营养药理学》(Nutritional Pharmacology), 纽约, NY: Alan R Liss Inc; 1981 pp.31-72

6) Vahoyny GV, Kritchevsky D:《植物和海水甾醇和胆固醇代谢》, Spiller GA 编《营养药理学》(Nutritional Pharmacology), 纽约 NY: Alan R Liss Inc; 1981 pp31-72

7) Tilvis RS, Miettinen TA: Am J Clin Nutr.: 43;92-97, 1986

8) Mattson FH; Grundy SM, Crouse JR: Am J Clin Nutr. 35,697-700, 1982

9) Steinegger E & Hänsel R: Pharmakognosie, 5. Aufl., Springer-Lehrbuch, 柏林-海得堡-纽约, 1992, p.195

10) Hänsel R & Haas H.: Therapie mit Phutopharmaka, Springer-Verlag, 柏林-海得堡-纽约-东京, 1983 pp.187-188

11) Hänsel R : Phutopharmaka, Grundlagen und Praxis,2. Auflage, Springer-Verlag, 柏林-海得堡-纽约, 1991 pp.192-193

12) Jones PJH 等:《加拿大生理学和药理学杂志》, 75(3): 217-227, 1997

13) Field FJ 等:《脂质研究杂志》(Journal of Lipid Research). 38(2):348-360, 1997

14) Richter W 等: Current Research. 57(7):497-505, 1996

15) Mattson FH 等:《美国临床营养杂志》(American Journal of

Clinical Nutrition). 35(4): 697-700, 1982
16) Uchita E 等《日本药理学杂志》.33(1): 103-12, 1983
17) Vahouny GB 等:《美国临床营养杂志》.37(5): 805-9, 1983