

Milk Product Comprising Unesterified Sterol

The present invention relates to a composition comprising an unesterified sterol and a crystal modifier and a method for production of the composition. The composition can be used for reducing LDL cholesterol concentration. In addition the invention relates to a method for production of the composition, use of the composition in reducing LDL cholesterol concentration, use of the composition in the manufacture of a functional food product or medicament for the treatment or prevention of high LDL cholesterol concentration and a method of treatment or prevention of high LDL cholesterol concentration which comprises administration or consumption of the composition.

Within the context of this specification the word "comprises" is taken to mean "includes, among other things". It is not intended to be construed as "consists of only".

It is well known that a high concentration of LDL cholesterol is associated with the problems of serious health risks including heart disease, atherosclerosis, high blood pressure, and cardiovascular conditions. A typical western diet exacerbates this problem because it is itself high in cholesterol and dietary lipids that lead to increased circulating LDL cholesterol in humans. In view of the risk, a diet low in cholesterol is

generally recommended which typically includes fruits, vegetables, and grains, and a minimum of red meat, eggs, and fried food.

Furthermore, it has been observed that consumers generally prefer foods high in saturated fats, because of their sensory attributes including mouth feel, texture, and flavours associated with these foods. In contrast, diets rich in fruits, vegetables, and grains appear to be less desirable. In the light of this, there is a need for new food products which taste desirable, but which do not have negative health characteristics associated with foods having large amounts of fat. In addition, there is a need for new foods which counter the negative effects of a diet which provides large amounts of cholesterol.

Sterols, which include phytosterols, stanols, esters of stanols, esters of phytosterols, oligosidic derivatives of the above and combinations thereof are known to lower cholesterol levels in humans when eaten on a regular basis and in a sufficient amount. It is known that sterols lower cholesterol by binding to sites in the gastrointestinal tract and competing with cholesterol for binding sites, thus inhibiting the absorption of cholesterol. Plant sterols have structures that are similar to cholesterol, but differ by having ethyl, methyl groups or unsaturated groups in their side chain. Because of their structural similarities to cholesterol, plant sterols are capable of inhibiting cholesterol absorption. However, despite the fact that sterols are present in high concentrations in plants such as corn, rice, and soybean, the sterols are largely removed during

processing and are not consumed. Therefore, typical foods consumed in typical quantities, of a human diet generally do not contain sufficient amounts of sterols to contribute effectively to reducing cholesterol absorption.

In order to obtain an adequate amount of sterol required to lower cholesterol levels, the diet must be supplemented. Therefore, there is a demand for new foods which deliver sufficient amounts of sterols to humans to lower their cholesterol concentrations.

Thus, a key issue regarding the use of sterols for their cholesterol-lowering properties concerns their bioavailability, which is linked to their molecular state within food matrices – sterols are able to decrease cholesterol absorption only when solubilized in molecular (uncrystallised) form. Thus, simply increasing the amount of sterols in a diet does not ensure that cholesterol levels are lowered because even if sterols are included in a person's diet the benefits of consuming sterols may not be obtained. In particular, sterols in the form of crystals, are not readily absorbed by the gastrointestinal (GI) tract. In contrast, sterols must be in a solubilized form in order that they can be absorbed by the GI tract.

Therefore, overcoming the problem of solubilization of sterols in food matrices represents a key challenge in the formulation of anti-cholesterol foods. Research in this area has been focussed on increasing solubility by production of esterified and

hydrogenated sterols or esterified and non-hydrogenated sterols and known products are on the market, such as spreads, salad dressings and yogurts which include this form of sterol. All sterols available on the market today suffer from the problem that they are very poorly soluble in unesterified form.

A composition comprising one or more sterols is described in US patent no. 6190720. The composition has one or more sterols, one or more fats and one or more emulsifiers and it is suggested that the disclosed composition can be used as an agent for lowering cholesterol levels. However, it has been found that the known compositions comprising sterols are not stable and therefore they do not have a long shelf-life. Within the context of this specification not stable is taken to include 1) separation of fat and water phases, and 2) recrystallisation of sterols.

The present invention addresses the problems set out above.

Remarkably, it has now been found that a composition comprising a crystal modifier selected from the group consisting of sorbitan tristearate (STS) provides a stable composition wherein sterols are kept in molecular form for a shelf-life of at least several months. Surprisingly, comparative test data shows that known compositions comprising emulsifiers have not been effective in this regard.

Consequently, in a first aspect the present invention provides a composition which comprises an unesterified sterol and a crystal modifier comprising sorbitan tristearate (STS).

Preferably, an embodiment of a composition according to the present invention comprises a crystal modifier which includes sorbitan tristearate (STS) and optionally additionally comprises an additional compound selected from the group consisting of sorbitan monostearate (SMS), one or more monoglycerides, one or more diglycerides, propyleneglycolmonostearate (PGMS) or a combination of two or more thereof. This composition provides the advantage that sterols remain in a stable molecular form and unlike previously known products the composition therefore has a long shelf life.

Remarkably, the composition provides a product having a long shelf life at room temperature (even under tropical conditions).

Preferably the monoglycerides are provided in the form of saturated distilled monoglycerides, eg Dimodan PV (TM).

Preferably an embodiment comprises at least one monoglyceride and at least one diglyceride.

Preferably, an embodiment of a composition according to the present invention comprises STS in an amount of from about 50% to about 200% by weight the amount of sterol in the composition. More preferably it is about the same amount of sterol by weight.

Preferably, an embodiment of a composition according to the present invention comprising a mixture of STS and SMS comprises STS and SMS in an amount of from about 50% to about 200% by weight the amount of sterol in the composition. More preferably it is about the same amount of sterol by weight. Preferably the amount of SMS in the mixture is from about 15% to about 35% by weight the total amount of the mixture of STS and SMS. More preferably, the amount of SMS in the mixture is about 25% by weight the total amount of the mixture of STS and SMS.

Preferably, an embodiment of a composition according to the present invention comprising a mixture of STS and PGMS comprises STS and PGMS in an amount of from about 50% to about 200% by weight the amount of sterol in the composition. More preferably it is about the same amount of sterol by weight. Preferably the amount of PGMS in the mixture is from about 15% to about 35% by weight the total amount of the mixture of STS and PGMS. More preferably, the amount of PGMS in the mixture is about 25% by weight the total amount of the mixture of STS and PGMS.

Preferably, an embodiment of a composition according to the present invention comprising a mixture of STS and saturated distilled monoglyceride comprises STS and saturated distilled monoglyceride in an amount of from about 50% to about 200% by weight the amount of sterol in the composition. More preferably it is about the same amount of sterol by weight. Preferably the amount of saturated distilled monoglyceride in the mixture is from about 15% to about 35% by weight the total amount of the mixture of STS and saturated distilled monoglyceride. More preferably, the amount of saturated distilled monoglyceride in the mixture is about 25% by weight the total amount of the mixture of STS and saturated distilled monoglyceride.

Preferably, an embodiment of a composition according to the present invention comprises a sterol selected from the group consisting of unesterified sterols. More preferably the sterol is selected from the group consisting of unhydrogenated sterols. Even more preferably, the sterol is predominantly from soy origin and non-hydrogenated. Most preferably it has a purity of about 90% or more in a powder form, its colour is white and not-discoloured, its particle size is fine and there is no off-taste.

This provides the advantage that the sterol is not subject to the risk of contamination with heavy metals including lead. These metals have been present as contaminants in the solvents used for dissolving sterols in known products.

Preferably, an embodiment of a composition according to the present invention comprises sterol in an amount of about 400mg to about 1200mg per serving, more preferably about 600mg to about 800mg per serving wherein one serving comprises about 200ml to about 250ml of composition. These amounts refer to the amount of pure sterol in the composition.

Preferably, an embodiment of a composition according to the present invention comprises milk. Preferably the milk comprises about 0.8% to about 2.2% by weight fat, more preferably 1.58% by weight fat (including emulsifier). Preferably an embodiment of a composition according to the present invention comprises from about 90% to about 99% by weight milk, more preferably about 95% to about 99% by weight milk, most preferably about 98.16% by weight milk.

Preferably the milk is cows milk or milk from another source including soy milk. Alternatively the milk can be a fat free or low fat aqueous extract including for example whey.

Preferably, an embodiment of a composition according to the present invention comprises oil. Preferably the oil is a vegetable oil having applicable amounts of mono-unsaturated and polyunsaturated fatty acids. Preferably the oil comprises rapeseed oil and/or corn oil. Preferably an embodiment of a composition according to the present invention comprises from about 0.4% to about 0.8% by weight rapeseed oil, more

preferably about 0.5% to about 0.7% by weight rapeseed oil, most preferably about 0.645% by weight rapeseed oil. Preferably an embodiment of a composition according to the present invention comprises from about 0.2% to about 0.5% by weight corn oil, more preferably about 0.3% to about 0.4% by weight corn oil, most preferably about 0.39% by weight corn oil.

Preferably, an embodiment of a composition according to the present invention comprises water. Preferably an embodiment of a composition according to the present invention comprises from about 1.5% to about 3.0% by weight water, more preferably about 2.0% to about 2.5% by weight water, most preferably about 2.1% by weight water. Alternatively, no water is added to the composition and the percentage by weight of milk is increased by a corresponding amount.

In preferred embodiments the composition is optionally enriched with vitamins and or minerals including calcium. This provides the advantage of a balanced nutritional composition which is, for example, beneficial to bone formation and/ or inhibits reduction of bone mass.

In preferred embodiments the composition is optionally flavoured. This provides the advantage that an embodiment of the composition has a good taste which increases desirability of the composition.

In preferred embodiments, the composition includes a sweetener. Preferably the sweetener is sugar although other known sweeteners can be included.

In a preferred embodiment, one or more oligosaccharides and/or polysaccharides are included. The polysaccharide(s) provide the advantage that they are capable of suspending insoluble minerals which may also be included, for example calcium carbonate (CaCO_3). The oligosacchrides provide the advantages associated with prebiotics including promotion of gut health.

In a second aspect the invention provides a method for production of the composition which comprises the steps of:

- i) integrating a sterol in an oily matrix comprising at least one crystal modifier;
- ii) transferring the oily matrix to an aqueous phase in such a manner that the oily matrix remains intact and a proper emulsion is obtained; and
- iii) processing the oil in water emulsion to make it shelf-stable in a way that the emulsion remains unaffected and stable over several months without excessive re-crystallisation of the sterols over time.

Preferably, an embodiment of a method according to the invention comprises the steps of heating and keeping the oily matrix at about 80°C preferably until a clear solution is obtained. Preferably sterol is added to the oily matrix consisting of or comprising STS and the oily matrix is maintained at 80°C.

Preferably, a water phase comprising milk is prewarmed to about 80°C and the oily matrix is added to the prewarmed water phase.

Preferably the oil in water emulsion is kept at 80°C and is not cooled below 80°C until further processing is carried out.

Preferably the further processing comprises UHT treatment, preferably followed by homogenisation.

Preferably the composition is cooled and filled following the further processing.

In a third aspect the invention provides use of a composition according to an embodiment of the invention in reducing LDL cholesterol concentration.

In a fourth embodiment the invention provides use of the composition in the manufacture of a functional food product or medicament for the treatment or prevention of high LDL cholesterol concentration

In a fifth embodiment the invention provides a method of treatment or prevention of high LDL cholesterol concentration which comprises administration or provision of the composition for consumption.

Additional features and advantages of the present invention are described in, and will be apparent from, the description of the presently preferred embodiments which are set out below with reference to the drawings in which:

Figure 1 shows micro-photographs illustrating the emulsion obtained according to the present invention;

Figure 2 shows micro-photographs illustrating an emulsion obtained according to the prior art for illustration only;

Figure 3 shows a schematic showing an embodiment of a method of the invention;

Figure 4 shows electronically generated images of microscope slides showing embodiments of a milk product comprising sterols which have very few crystalline plant sterols. These embodiments are considered to be biologically active at inhibiting the absorption of cholesterol.

For the purposes of clarity and a concise description features are described herein as part of the same or separate embodiments, however it will be appreciated that the scope of the invention may include embodiments having combinations of all or some of the features described.

As seen in Figures 1 and 2 an embodiment of a composition according to the invention is smooth and provides sterols which are integrated in the composition in a molecular form. In contrast a similar composition according to the prior art has sterols which have crystallised to form needle like structures.

As seen in figure 3, an embodiment of a process according to the invention comprises the steps of forming a first solution by emulsifying oils, emulsifier and sterols at a temperature of at least 85°C; and separately forming a second solution by prewarming skimmed milk at a temperature of at least 80°C. The two solution are mixed to form a mixture and stored at a temperature of at least 80°C. The mixture is subjected to further processing including UHT and cooling followed by homogenisation. The mixture is then cooled and subjected to aseptic filling.

Study 1

The following comparative study shows that negligible plant sterol crystals are present in an embodiment of the invention comprising a filled milk containing free sterol solubilised with STS.

Principle of Assay:

Non-esterified plant sterols are hydrophobic and remain in crystalline form in low-fat liquid milk and low-fat vegetable oil partly filled liquid milks. Specially designed emulsifiers, and mixing, heating, homogenization, and UHT treatment steps are

necessary in order for the emulsifier to stay bound to the plant sterol and keep it in a soluble form in the milk for long periods of time (months) at room temperature.

Under these conditions, only a small percentage of plant sterols will be in the biologically less efficous crystalline form. Conversely, if the plant sterols are not properly solubilised, a greater relative percentage will be in the crystalline form. A procedure has now been developed to quantify the amount of crystalline plant sterols in liquid milks in the presence of various emulsifiers (results with the emulsifier STS are described below), using radiolabeled plant sterols. Special caution was taken to add radiolabeled sitostanol to unlabeled plant sterols in such a way to produce a homogenous mixture without changing the structure of the plant sterols. The hydrogenated sitostanol tracer behaves similarly to the unlabeled plant sterols from emulsion and re-crystallization perspectives and is thus considered a valid marker. In the procedure described below, the pelleted material following centrifugation was representative of crystalline sterol. However, the act of centrifugation itself can result in plant sterols that were once solubilised re-crystallizing and thus becoming pelleted. The amounts of pelletable material is nevertheless proportional to the amount of originally crystalline plant sterols in a given food preparation.

Method:

³H Sitostanol/Sterol Powder Preparation. Radioactive sitostanol was added to the non-hydrogenated, non-esterified plant sterols predominately of soybean origin in such a manner to achieve a homogenous mixture of 5 μ Ci ³H per gram of sterols.

Milk Preparation. All milks used in this study contained 1.58% vegetable (soybean) oil (O), 0.4% sterols (S), 2% water (W), plus or minus 0.4% STS, and non-fat liquid milk (M) varied to attain a final volume of 100%. STS was heated to 110°C, powdered, unheated S was added to the heated STS solution, and the S/STS mixture was heated to 120°C. O was preheated to 45°C, added to the S/STS heated solution, and the S/STS/O mixture was brought to a temperature of 85°C. MW was pre-heated to 85°C and added to a Polytron mixer. The S/STS/O mixture at 85°C was then immediately added to MW in the Polytron. The final mixture described above was removed from the Polytron and cooled to room temperature. Milks prepared without O or STS were otherwise prepared identically.

Quantification of Crystalline Plant Sterols in the Milk Preparation. One mL of the above milk preparation was added to test tubes. After centrifugation at 20,000*g for 3 minutes, the mixture consisted of a combined liquid layer (supernatant) and a solid precipitate on the bottom of the tube (pellet). The supernatant was placed in a scintillation vial following aspiration with a pasteur pipette. The pellet was rinsed repeatedly with scintillation fluid and the washings placed in a scintillation fluid. Ten mL of scintillation fluid was added to each scintillation vial and the radioactivity in the supernatant and pellet counted by autoradiography.

Results:

Sample	³ H sitostanol (cpm pellet/cpm total in the test tube ± Std Deviation)
	Centrifugation 20000*g 3 min

SWM	0.86 ± 0.007
SWM+O	0.80 ± 0.026 (no change)
SWM+O+STS	0.35 ± 0.019 (-)
SWM+O+STS	0.25 ± 0.062 (-)

The values in the table above represent the mean of 4 replicates. A higher fraction number is representative of more crystalline sterols (proportionately more pellet sterols relative to the total amount of sterols added to the test tube). The minus sign refers to the change in this ratio relative to the amount in SWM. A difference of less than 10% is considered "no change". The last two rows of the table show independent experiments with the same mixture.

The results from the table above show that the sterols placed into WMO without emulsifiers are 80% pelletable (SWM+O above). Adding STS dramatically decreased the amount of pelletable material to 25-35% (SWM+O+STS above). These results show that the addition of STS keeps the sterols in a more soluble (less-pelletable) state. Plant sterols in the soluble state are known to effectively inhibit the absorption of dietary cholesterol in the gut.

Study 2

The following comparative study shows that negligible plant sterol crystals are present in filled milks containing free sterols solubilised with STS, in the presence of bile acid.

Principle of Assay:

In Study 1, we showed that following centrifugation of milks, the pelleted material is proportional to the amount of non-solubilised crystalline plant sterols. Radiolabeled plant sterols were used as a marker to quantify the amount of material in the pellet. This quantification scheme was employed in Study 2. For a food preparation containing plant sterols to be biologically efficacious at inhibiting the absorption of dietary and biliary cholesterol, two conditions must be met: 1. the plant sterols must be solubilised and mostly non-crystalline in the original food matrix (Study 1 above); and 2. during the intestinal digestive process in the presence of naturally occurring bile acid, lysophospholipids, and fatty acids, the plant sterols must remain solubilised and not re-crystallized. A method has now been developed to quantify the amount of non-solubilised plant sterols in the presence of bile acid, lysophospholipids, and fatty acids to simulate the intestinal digestive process. It is considered that large, non-solubilised crystalline sterols cannot be appreciably re-solubilised in the gut, and cannot effectively inhibit the absorption of dietary and biliary cholesterol; whereas small plant sterol crystals have a better chance of being re-solubilised in the gut matrix.

Method:

³H Sitostanol/Sterol Powder Preparation, Milk Preparation, and Quantification of Crystalline Plant Sterols were as described in Study 1, except for quantification, test tubes were spun in a centrifuge at 20,000*g for 3 minutes and 50,000*g for 3 minutes to see if a faster spin speed would result in more radiolabeled pelleted material than slower spin speeds.

Bile Acid Preparation. A bile acid solution contained 150 mM sodium chloride and 15 mM sodium phosphate, brought to pH 7.4 with 10 M sodium hydroxide. Then, sodium taurocholate and egg lecithin were added to achieve final concentrations of 8 and 5 mM, respectively. The solution was gently stirred overnight at room temperature with a magnetic bar and filtered through 5 μ m filter paper. The above mixture is referred to as bile acid solution (BA)

Plant Sterol Solubilization in the Presence of Bile Acid. One mL of the milk preparation was added to test tubes along with 0.5 mL of bile acid. Thus, the final concentrations of bile salts were 1.5 times less than the starting concentration in BA. The milk/BA solution was incubated at 37°C and agitated at 30 rpm for 1 hour.

Results:

Sample	³ H sitostanol (cpm pellet/cpm total in the test tube) $\pm$ Std Deviation	
	Centrifugation 20000*g 3 min	Centrifugation 50000*g 3 min
SWM+O	0.80 $\pm$ 0.026	0.86 $\pm$ 0.0183
SWM+O+BA	0.52 $\pm$ 0.054(-)	0.77 $\pm$ 0.037(-)
SWM+O+STS+BA	0.19 $\pm$ 0.082(-)	0.19 $\pm$ 0.011(-)

The values in the table above represent the mean of 4 replicates. A higher fraction number is representative of more crystalline sterols (proportionately more pellet sterols relative to the total amount of sterols added to the test tube). The minus signs refer to the change in this ratio relative to the amount in SWM+O.

Similarly to results of study 1, the sterols placed into WMO without emulsifiers are 80% pelletable (SWM+O; 20,000 g condition). In the presence of bile acid, without emulsifiers, about 65% of the sterols are no longer pelletable (SWM+O vs. SWM+O+BA above; 20,000 g condition) indicating the BA solution helps to either prevent original crystallization from occurring since the milks were prepared only one hour before addition of BA; and/or that the BA helps to re-solubilised previously crystalline the plant sterols. Importantly, as compared to the condition without STS, in the presence of STS, a lesser proportion of plant sterols were pelletable (SWM+O+BA vs. SWM+O+STS+BA above; 20,000 g or 50,000 g condition), indicating that in the presence of BA, the plant sterol/STS complex will be in a mostly soluble, efficous form. It is thus considered that in an actual gut matrix, the plant sterol/STS complex inhibits the absorption of dietary and bilary cholesterol, and subsequently reduces LDL cholesterol concentrations.

Study 3

This study shows the effects of phytosterols on absorption of cholesterol by caco-2 intestinal cells.

Principle of assay:

Intestinal cells in culture behave similarly to intestinal cells in the living organism.

Radioactive cholesterol is incubated with intestinal cells, and added plant sterols inhibit the uptake of the radiolabeled plant sterol into the intestinal cells, similarly to

what happens in a living organism. As compared to incubations without added plant sterols, with plant sterols added there is proportionately more radiolabeled cholesterol in the media surrounding the intestinal cells, and less radiolabeled cholesterol taken up by the cells.

Method:

Milk preparation. All milks contained 2.7% omega vegetable oil mix, plus or minus 0.4% plant sterols, plus or minus 0.4% emulsifier, and non-fat liquid milk was varied to bring the total volume to 100%. Emulsifier and plant sterols were first heated at 140°C, then the omega vegetable oil mix oil was added and mixed with a Polytron with non-fat liquid milk at 85°C. The emulsion was homogenized in two stages at 300bars then 150 bars. The emulsion was pasteurised for 1 hour at 85°C, then stored at 4°C.

Cell incubation preparation. Caco-2 cells were incubated in 24 well plates in medium containing DMEM enriched with and antibiotics (78.2% DMEM with 4.5 g/L glucose plus 19.5% FCS, 0.8% penicillin-streptomycin, 0.5% gentamycin, and 1.0% 100x DMEM) for 21 days at 37 °C in a 10% CO₂ environment. Medium was changed every 2 days. 0.6 mM lysophosphatidylcholine, 0.2 mM oleic acid, 50 µM cholesterol and 0.2 µCi/mL (414C)-cholesterol were solubilised in chloroform/methanol (2:1 v/v), evaporated under nitrogen, and to the dried mixture, bile salts in DMEM plus 0.3% BSA, were added to achieve a final concentration of 1 mM taurocholate, 0.5 mM taurochenodeoxycholate, 0.5 mM taurodeoxycholate, and 2 mM glycocholate

(micellar solution). Radioactivity was counted in an aliquot of these micelles (5 μ L counted in 2 mL scintillation fluid), and the remainder of the micellar solutions were distributed into glass tubes. In quadruplicate, non-hydrogenated, non-esterified plant sterols (S) predominately of soybean origin (plus or minus emulsifier) were added in low fat milk containing an Omega vegetable oil mix (OM for Omega Milk) to achieve a final concentration of 40 mM sterols. Tubes were incubated for 2 hours at room temperature, then an aliquot of each tube was counted. After washing cells 2x with HBSS, 0.5 mL of the plant sterol/bile salt/ lysophosphatidylcholine/ fatty acid/ radiolabeled cholesterol mixture was added to the cells for 2 hours at 37 °C in a 10% CO₂ environment, then washed 3x with HBSS. Following aspiration of the media, the cells were lysed with 1 N NaOH, agitated 30 min at ambient temperature, and following removal of 25 μ L for protein determination, the remaining material was counted by autoradiography in 10 ml of liquid scintillation fluid.

Results of three separate experiments

Sample	414C-cholesterol (cpm/mL in Caco-2 cells)		
	Exp. 1	Exp. 2	Exp.3
Cells+micellar solution	42'600		34'900
+ OM	51'700(+)	24'000	24'900(-)
+OM + 0.4% S	44'600(-)	33'000(+)	30'200(+)
+OM + 0.4% S + 0.4% emulsifier	41'700(-)	13'100(-)	19'500(-)
+OM + 0.4% emulsifier	39'400(-)	35'700(+)	34'400(+)

Each value in the table above represents the mean of 3-4 replicates. The plus and minus signs refer to the change relative to the condition immediately above.

The addition of low fat milk containing an Omega vegetable oil mix (OM) either increased or decreased the uptake of cholesterol into Caco-2 cells, and thus had an inconsistent effect (+OM vs Cells+micellar solution).

Adding sterols in a crystalline form to OM, increased the uptake of cholesterol into Caco-2 cells in two experiments and decreased the absorption in Exp. 1, thus having an overall inconsistent effect (OM + 0.4% S vs. +OM).

Adding sterols in a solubilised form to OM in the presence of emulsifier decreased dramatically the uptake of cholesterol into Caco-2 cells in two experiments and produced a smaller decrease in uptake in Exp. 1 (+OM + 0.4% S + 0.4% emulsifier vs. +OM + 0.4% S). Thus, the results show that plant sterols solubilised in the presence of emulsifier in OM more effectively inhibit the uptake of radiolabeled cholesterol into Caco-2 cells than plant sterols in OM without this emulsifier.

Adding sterols in a solubilised form to OM in the presence of emulsifier was also more effective at inhibiting the uptake of cholesterol into Caco-2 cells than OM in the presence of emulsifier in two of three experiments (+OM + 0.4% S + 0.4% emulsifier

vs. +OM + 0.4% S vs. +OM + 0.4% emulsifier) , showing that the active compound at inhibiting uptake of cholesterol is in fact solubilised plant sterols.

Human Clinical Trial Results

Subjects: Consisted of 16 mildly hypercholesterolemic men, age 35 - 65 years, having starting total cholesterol 5.6-8.4 mmol/L, and total triacylglycerol <3.5 mmol/L.

Vehicle: Subjects consumed 2 x 250 mL per day of a low fat milk containing an Omega vegetable oil mix (OM). Milks were consumed with meals at breakfast and lunch. All meals were provided. This milk contained 0.63% butter oil, 0.56% rapeseed oil and 0.39% corn oil and thus 1.58% total oil. Control milks contained no emulsifiers or plant sterols, whereas sterol-containing milks contained 0.4% non-hydrogenated, non-esterified, predominately soy plant sterols (90% pure sterols) plus 0.4% sorbitan tristearate emulsifier (STS). Skim milk liquid was added to equal to 100%. The sterol-containing milk is referred to as OMS. The sterol-containing milk contained very few crystalline plant sterols and was thus expected to be biologically active at inhibiting the absorption of cholesterol (Fig. 4).

Parameter measured:

Cholesterol absorption was assessed following daily oral consumption of 15 mg of pure D6-cholesterol in 1 mL of normal oil taken spread on bread for 3 days, combined with administration of 30 mg of pure ¹³C5-cholesterol in 10% Intralipid, for 3 days.

*Study Results:***Table 1:**

Cholesterol absorption (%)

ID	Control	Sterol
D	87.2	46.8
E	66.1	55.9
G	66.2	84.3
H	86.7	54.1
J	73.5	41.6
K	46.1	36.5
L	99.4	48.3
M	59.1	34.0
N	89.0	35.6
O	43.9	37.7
P	57.2	37.6
R	69.9	37.3
S	47.5	18.5
T	77.2	45.6
U	64.1	23.4
W	87.8	19.7
mean	70.1	41.1
sd	16.9	15.9

Table 1 shows the cholesterol absorption in 16 subjects who consumed both control and 1.8 g of plant sterols in low fat milks with OM.

Conclusion:

Relative to control, cholesterol absorption was reduced from 75.4% to 41.9%, a relative reduction of 41.1% (1.7 fold less cholesterol absorbed), demonstrating that the STS solubilised plant sterols in milks containing OM were highly efficacious at inhibiting the absorption of dietary and biliary cholesterol. Such large reductions in cholesterol absorption inhibition are expected to be correlated with reductions in LDL cholesterol.

Example 1

An example of an embodiment of a composition according to the invention comprises a 250ml serving having the following ingredients in the following amounts by weight:

milk (0.655% fat):	96.15%
rapeseed oil:	0.56%
corn oil:	0.39%
STS:	0.40%
Sterol composition:	0.40%
Water:	2.10%.

Given the purity of the sterol composition used (about 91% purity of sterols) the quantity of pure/ free sterols in the composition according to this embodiment of the invention in a 250ml sample is about 910mg.

In view of the fact that the milk has a fat content of 0.655% by weight the quantity of milk fat in the composition is 0.63% by weight.

Example 2

In an alternative embodiment, water is replaced by an corresponding increased percentage by weight of milk.

In contrast to the known compositions, this embodiment provides a stable emulsion having a long shelf life. The known compositions have been found to suffer from not

being able to form a stable emulsion during mixing; formation of an emulsion which quickly deteriorates with time with or without the appearance of sterol crystal formation (typically eg saturated with monoglycerides); or a stable emulsion is formed having a reasonably long shelf life, but clearly visible crystallisation fo sterols over time occurs (typically, eg unsaturated monoglycerides).

It should be understood that various changes and modifications to the presently preferred embodiments described herein will be apparent to those skilled in the art. Such changes and modifications can be made without departing from the spirit and scope of the present invention and without diminishing its attendant advantages. It is therefore intended that such changes and modifications are covered by the appended claims.

Claims

1. A composition which comprises an unesterified sterol and a crystal modifier comprising sorbitan tristearate (STS).
2. A composition according to claim 1 which comprises a crystal modifier which includes sorbitan tristearate (STS) and optionally additionally comprises an additional compound selected from the group consisting of sorbitan monostearate (SMS), one or more monoglycerides, one or more diglycerides, propyleneglycolmonosterate (PGMS) or a combination of two or more thereof.
3. A composition according to claim 1 or 2 wherein the monoglycerides are provided in the form of saturated distilled monoglycerides.
4. A composition according to claim 1 or 2 wherein the crystal modifier is in an amount of from about 50% to about 200% by weight the amount of sterol in the composition.
5. A composition according to any preceding claim comprising a mixture of STS and SMS wherein the amount of SMS in the mixture is from about 15% to about 35% by weight the total amount of the mixture of STS and SMS.

6. A composition according to any one of claims 1 to 4 comprising a mixture of STS and PGMS wherein the amount of PGMS in the mixture is from about 15% to about 35% by weight the total amount of the mixture of STS and PGMS.
7. A composition according to any one of claims 1 to 4 comprising a mixture of STS and monoglyceride wherein the amount of monoglyceride in the mixture is from about 15% to about 35% by weight the total amount of the mixture of STS and monoglyceride.
8. A composition according to any preceding claim wherein the sterol is selected from the group consisting of unesterified sterols.
9. A composition according to any preceding claim wherein the sterol is selected from the group consisting of unhydrogenated sterols.
10. A composition according to any preceding claim wherein the sterol is predominantly from soy origin.
11. A composition according to any preceding claim wherein the sterol has a purity of about 90% or more in a powder form, its colour is white and not-discoloured, its particle size is fine and there is no off-taste.

12. A composition according to any preceding claim wherein the sterol is in an amount of about 400mg to about 1200mg per serving and one serving comprises about 200ml to about 250ml of composition.
13. A composition according to any preceding claim which comprises milk.
14. A composition according to any preceding claim which comprises a vegetable oil having mono-unsaturated and polyunsaturated fatty acids.
15. A composition according to claim 11 wherein the oil comprises rapeseed oil and/or corn oil.
16. A method for production of a composition according to any preceding claim which comprises the steps of:
 - i) integrating a sterol in an oily matrix comprising at least one crystal modifier;
 - ii) transferring the oily matrix to an aqueous phase in such a manner that the oily matrix remains intact and a proper emulsion is obtained; and
 - iii) processing the oil in water emulsion to make it shelf-stable in a way that the emulsion remains unaffected and stable over several months without excessive re-crystallisation of the sterols over time.

17. A method according to claim 16 which comprises the steps of heating and keeping the oily matrix at about 80°C preferably until a clear solution is obtained; adding sterol to the oily matrix consisting of or comprising STS and maintaining the oily matrix at 80°C.
18. A method according to claim 16 or 17 wherein a water phase comprising milk is prewarmed to about 80°C and the oily matrix is added to the prewarmed water phase to create an oil in water emulsion.
19. A method according to claim 18 wherein the oil in water emulsion is kept at 80°C and is not cooled below 80°C until further processing is carried out.
20. A method according to any one of claims 16 to 19 further comprising the steps of UHT treatment, optionally followed by homogenisation.
21. Use of a composition according to any one of claims 1 to 15 in reducing LDL cholesterol concentration.
22. Use of a composition according to any one of claims 1 to 15 in the manufacture of a functional food product or medicament for the treatment or prevention of high LDL cholesterol concentration

23. A method of treatment or prevention of high LDL cholesterol concentration which comprises administration or provision of a composition according to any one of claims 1 to 15 for consumption.
24. A composition as described herein with reference to the accompanying drawings.