Research progress on the function and synthesis of phytosterol esters of fatty acids

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Abstract

Phvtosterol esters are kinds of novel serum cholesterol lowering functional food additives. A detailed introduction to progresses in the function and the synthesis of phvtosteryl esters, especially the enzymatic synthesis,are discussed in this paper in order offering consults for the application research of phytosterol ester.

Key words: phytosterol esters; phytosterols; function; synthesis; lipase

Introduction

Plant sterols (phytosterols) are sterols derived from plant sources, such as vegetable oils and cereals a. In plants, more than 40 sterols have been identified with β-sitosterol being the most abundant. Phytosterols are important, due to their recent recognition and application in the food and nutraceuticals industries as cholesterol lowering agents, which are known to have a hypocholesterolemic effect by lowering plasma total and low density lipoprotein (LDL) cholesterol levels without affecting plasma high density lipoprotein (HDL) cholesterol concentration(Pollak, 1953; Beveridge, 1964; Lees, 1977). The safety of phytosterols has been affirmed by government agencies such as the US Food and Drug Administration and the European Union Scientific Committee. Phytosterol is one of 10 function ingredients in the future, 2000 year FDA authorized foods added phytosterol or ester could use "healthy" label (Jones, 2000). 2004 year European Union committee passed the foods added phytosterol or esters of ARCHER DANIELS MIDLAND Company (ADM) and Unilever Company.

1 Function

1.1 Absorption of phytosterols and Toxicity studies

An early summary of phytosterol absorption by various animal species showed absorption ranging from 0% (rabbit) to 4% (rat), and 6% (human being) (Pollak, 1981). Salen et al (Salen, 1949) fed human sitosterol and estimated absorption at 1.5% to 5%. Ostlund et al (Ostlund,2002) studied the absorption of different phytosterols in human subjects and found out that sitosterol and campesterol were absorbed at levels of 0.512% ±0.038% and 1.89% ±0.27%, respectively. Salo et al. (Salo, 2002) thought the minimum amount of sterols and stanols required to produce a significant LDL cholesterol lowering effect is about 0.8~1.0 g/day. European Union recommended the quantity was 3g/d.

From Shipley et al (Shipley,1958) to Hepburn et al (Hepburn, 1999) the authors found no detectable effects on growth, serum proteins, blood urea nitrogen, or gross or microscopic appearance of any organ or tissue. No treatment-related changes were observed (Baker, 1999; Weststrate, 1999; Ayesh,1999; Sanders,2000; Wolfreys,2002; Lea,2004;) No indications of estrogenic effects were observed, nor were there effects on levels of reproductive hormones in female volunteers. Phytostanol esters are not genotoxic in in vitro gene mutation assays using mammalian or bacterial cells (Whittaker, 1999; Turnbull, 1999). To date, no evidence of toxicity or genotoxicity was found.

1.2 Cholesterol-lowering effects

Phytosterols have been widely studied for their hypocholesterolemic, anticarcinogenic, and other health effects. For more than 50 years now, phytosterols are known to have a hypocholesterolemic effect allowing the reduction of LDL (low-density lipoprotein) cholesterol in plasma whereas high-density lipoprotein (HDL) cholesterol concentration is not affected by their consumption. The cholesterol lowering mechanism of plant sterols is attributed to an inhibitory effect on cholesterol absorption due to the chemical structure similarities of phytosterols with cholesterol. Thousands of people were enrolled in clinical trials which showed the efficacy and safety of the preparation based on phytosterols. Exhaustive results compiled from recent studies are summarized in the following table 1:

2 Synthesis methods

Phytosterols have generated interest in the functional food industry as they have been shown to reduce the levels of "bad" serum low-density-lipoprotein (LDL) cholesterol in human. However, free sterols have limited interest owing to their low solubility and high melting point. Apparently, fat-soluble plant steryl esters are able to lower plasma cholesterol levels more efficiently than the corresponding homogenized crystalline plant sterol preparations. Therefore, fatty esters of sterols are generally preferred in food formulation. These fatty esters sterols can be produced by chemical esterification and lipase-catalyzed esterification.

2.1 chemical esterification

The reaction by chemical esterification is faster than by lipase-catalyzed method. Phytosterol esters of FAs are presently synthesized by chemical esterification and transesterification. According to the reagents, the chemical method includes acid-direct esterification, acid-anhydride esterification method, acyl-chloride esterification method, transesterification method and so on.

US patent 5502045 Tatu Miettinen etc introduced a method using beta-sitostarol reacted with rapeseed oil methyl ester under vaccum at temperature 90~120°C using Na ethylate and the conversion of 98%. US Patent 6147236 provide a synthrtic route of conversion 95% that was amenable to large scale production of the esters in high yields. Oleic and stearic acid reacted with phytosterol was studied, the best parameters were 7~8 hour, 135°C, oleic or stearic acid/phytosterol mol/mol 1:1.2~1:1.3 (Chen Maobing,Huang Qin,2005).

These fatty esters sterols can be produced by chemical esterifications generally requiring higher temperature than enzymatic technologies and favoring consequently the formation of side products (e.g., dehydrated or oxysterols) and staining

2.2 Lipase-catalyzed esterification

Lipase-catalyzed esterification reactions are an important area of research in lipid chemistry, and enzyme technologies offer now a good alternative for their production allowing mild and environmental friendly reactions conditions. In the last decade, a little research has been performed on the production of phytosterol ester by lipase-catalyzed reaction. Lipase-catalyzed esterification can be acted in supercritical carbon dioxide.

2.2.1 Lipase-catalyzed esterification

Yuji shimidae etc attempted to synthesize steryl esters of PUFA by enzymatic methods (Yuji shimidae, 1999). Among lipases used, *Pseudomonas* lipase was the most effective for the synthesis of cholesteryl docosahexaenoate. When a mixture of cholesterol/docosahexaenoic acid(3:1,mol/mol), 30%water, and 3000units/g of lipase was stirred at 40°C for 24h, the esterification extent attained 89.5%. Sterols contained in three different steam distillates by physical refining by Weber etc have been converted to a high degree of long-chain acyl esters via esterification and/or transesterification with fatty acids and/or triacylglycerols using lipase from *Candida rugosa* as biocatalyst in vacuo (20-40mbar) at 40°C, 30~40°C. The lipase active could be stable used twice in vacuum but after the third time the lipase activity drop seriously (Weber N., 2001; Weber N.,2002).

Vu etc produced the sitosteryl esters from CLA and MCFAs using various lipases as a biocatalyst, Among lipases, AYS (from *C. rugosa*) was the most effective for the synthesis of sitosteryl esters in the presence of either water (maximum 26.8%) or hexane (maximum 28.3%) during a 48 h reaction, and the effect in non-polar insolvent was better than in polar condition. The optimized parameters were 55°C, magnetic whisking rate 175rpm, mol/mol of CLA with phytosterol 3:1, 48h (Vu, 2004).P.Villeneuve etc established the feasibility of lipase-catalyzed esterification of canola phytosterols with oleic acid. Among the tested lipases, namely plant lipases from *Candida rugosa* to be the most. When optimal enzyme load (5%) or temperature (35°C) were determined to allow a final production of steryl esters close to 85% after 72 h.

Condensation condition was studied for lipase-catalyzed synthesis of β- sitosterol ester with conjugated linoleic acid (CLA) in organic solvent (Li Ru, 2006).N-hexane as the reaction solvent, 4A molecular sieve as desiccant. The fittest parameters were the molar ratio 1:1,50 °Cfor 72 hours, the amount of molecular sieve 60 mg/ mL. The conversion of β-sitosterol ester of CLA was 72. 63 %.

As the difference of the reagents, lipase kinds and origin, there are differente yield of esterification from 25%~96%. While the lipase from *C. rugosa* effects better, and as the amount of lipase is enhanced or the mol/mol of fatty acid between phytosterol enlarged or in a polar condition, the rate of esterification will be increscent.

2.2.2 Lipase-catalyzed esterification in supercritical carbon dioxide

Jerry W. King et al evaluated several enzymes to determine the best catalyst and optimal conditions for the reaction between various fatty acids and cholesterol or sitostanol in SC-CO_2. The lipase derived from *Burkholderia cepacia*, Chirazyme L-1 was determined to be the most selective for facilitating the desired reactions. Fatty acids C_8–C_{18}, pressures between 20.7 MPa and 31 MPa, a temperature range of 40–60 °C, along with variable flow rates, and initial static hold times were used to evaluated the feasibility of the above reaction. The yield of the cholesterol esters, as measured by supercritical fluid chromatography (SFC), ranged from 90% for caprylic acid to 99% for palmitic acid, while the corresponding reaction between sitostanol and the same fatty acids produced yields of 92% for caprylic acid and 99% for palmitic acid, respectively.

3 Conclusion

Blood cholesterol levels in human beings in the present era still reflects efficacy and safety [36]. The safety of phytosterols has been affirmed by government agencies such as the US Food and Drug Administration and the European Union Scientific Committee. Scholarly reviews [36,37,108-110] have all confirmed the health benefits and safety of phytosterols. The use of phytosterol esters are inhibitted by solution.while the solution could be increased by esterification found, phytosterols are widely used.

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Table 1 Studies on Cholesterol-lowering effects of phytosterol

Phytosterol or Food type	Number of subject duration	Diary dose		Reference
Soybean sterols	chicks		First showedsterol could inhibit the elevations of plasma and liver cholesterol and reduce severity of therosclerotic lesions.	Peterson et al 1951
	52 human being for 2 weeks	an average of 8.1 g of plant sterols daily (5.7-10.0 g)	an average of 17 subjects fed 13±1 g of phytosterol daily for 3 to 5 weeks showed a 20% decrease in blood cholesterol levels	Pollak et al 1953
soy sterol–supplemented butter		5.7 g of phytosterols per day	11% reduction in plasma cholesterol	Peterson et al 1956
free or sterifed sitosterol	rats		hypolipidemic	Duncan and Best et al 1956
sitosterol	human being	from 3 to 53 g/d	successfully for lowering plasma cholesterol levels and shown to be safe for half a century.	Pollak et al 1981
			Interesterify sitostanol with rapeseed oil and incorporate the formed sitostanol esters into a spread which is lower serum cholesterol in subjects with hypercholesterolemia	Miettinen et al 1995
Phytosterol in corn oil or in olive oil			-compared effects on cholesterol metabolism of phytosterol-rich corn oil and phytosterol-poor olive oil. -Phytosterol of Corn oil was more hypocholesterolemic, olive oil increased cholesterol fractional synthesis rates.	Howell et al 1998
Spreads containing stanol esters			stanol esters shown to exert equivalent cholesterol-lowering effects	Plat J, et al allikainen MA et al 2000
phytosterol-free corn oil		150or300mg added per meal	reduced cholesterol absorption by 12% or 28% respectively	Ostlund et al 2002

Phytosterol or Food type	Number of subject duration	Diary dose		Reference
spreads enriched with plant sterols	42 healthy subjects 8 weeks	Sterol content of the enriched spread 8.3%. Intake of 25 g/day	Serum total and LDL-cholesterol concentrations lowered by 7% and 10%, respectively, with the plant sterol-enriched compared to the control spread. Serum HDL-cholesterol concentration did not significantly differ between the two spreads. Apolipoprotein B concentrations lowered by 8% with the plant sterolenriched spread.	Temme et al.,2002
various enriched sitosterol food products	human being		found that the optimal daily dosage of sterols or stanols is 2 g/d,which can result in a 10% reduction in LDL-C, whereas higher doses provide only a small additional effect.	Katan et al 2003
500mL Sterol enriched Milk blend	71Healthy subjects 4 weeks for each product	- Place 0,1.2,1.6 g/day;	*Double blind, cross over placebo controlled study.* Substantial reduction of LDL cholesterol and the two treated group: no significative difference between the two administered doses.	Thomsen et al., 2004
300ml/d sterol enriched milk	39 Healthy subjects 12 weeks	2.0g/day terol ester alone or combined with 25 g/day of placebo or spread.	*single blind crossover design with 4 phases of 3-week interventions* Sterol enriched milk and sterol enriched spread were equally efficacious in lowering total and LDL-cholesterol as compared to placebo by 6-8% and 8-10%, respectively.	Noakes et al 2004
low-fat milk-based beverage	26 Healthy subjects 1 week for each product	Placebo, 2.2 g plant sterol equivalents or 2.2 plant sterol ester equivalent	*Double-blind, randomized, crossover study.*Both milks containing plant sterols and plant sterol ester reduced beta-carotene and alpha-tocopherol bioavailability and cholesterol absorption in normocholesterolemic men.	Richelle et al., 2004
4 phytosterol ester enriched low-fat foods	58 Healthy subjects 3 weeks each product	1.6 g/day of phytosterols as sterol esters.	Serum LDL cholesterol levels fell significantly by 6.5% with bread and 5.4% with cereal. Lipid-adjusted beta-carotene was lowered by 5-10% by sterols in bread and milk, respectively. Plant sterols in low-fat milk were almost three times more effective than in bread and cereal.	Clifton et al., 2004
Orange juice	72 mildly hypercholesterol emic subjects 8 weeks	placebo orange juice -or plant sterol fortified orange juice (2g/d)	Sterol supplemented orange juice significantly decreased total (7.2%), LDL(12.4%), and non- HDL cholesterol (7.8%) compared with baseline and compared with placebo. Apolipoprotein B levels were significantly decreased (9.5%) with sterol orange juice. There were no significant changes in HDL cholesterol or triglycerides with the sterol orange juice.	Devaraj et al., 2004
Margarine containing sterol	42 healthy subjects	30 g/day in 2 servings	The subjects consuming margarine with sterols showed a significant (11%) decrease in LDL-C (P<.001). After the consumption of margarine with sterols, the adhesion and aggregation time of blood platelets was significantly prolonged after collagen-epinephrine activation.	Kozlowska-Wojciechowska et al., 2003
Phytosterol-enriched margarines	85 subjects with type 2 diabetes ;12 weeks	2 x10g/day of spread with or without 8% esters.	After 4 weeks, total and LDL cholesterol were significantly reduced in the phytosterol group by 5.2 % and 6.8 %, HDL cholesterol was significantly increased in the phytosterol group	Lee et al., 2003
polyunsaturated spread	50 Healthy subjects total of 11 weeks	25 g of PUFA spread with or without 2g of sterols for 4 weeks, crossing over in the last 4 weeks to the alternate spread.	Replacing butter with a standard polyunsaturated fat spread reduced mean plasma total cholesterol concentrations by 4.6% and low-density lipoprotein cholesterol by 5.5%. Replacing butter with a polyunsaturated spread containing plant sterols reduced plasma total cholesterol by 8.9% and low density lipoprotein cholesterol by 12.3%. Plasma high density lipoprotein cholesterol concentration was the same on the three diets.	Cleghorn et al., 2003

Note :Figue comes of < Request for scientific evaluation of "Substantial equivalence" for Lipofoods' -phytosterol product, intended to be used in specified foods and under regulation EC 258/97 of the European Parliament>and< Phytosterols—health benefits and potential concerns>.