Study on extraction, isolation and bioactivities of phytosterol from rapeseed

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Abstract

In this paper, the research results that were carried out in our laboratory have been reported about the composition, extraction, isolation and structure of phytosterol from deodorizer distillates of rapeseed in our country. The preparation of phytosterol ester that is the derivant of phytosterol has also been covered in this paper. Furthermore, the results about the bioactivities of phytosterol and phytosterol ester, such as antioxidant, serum lipid-lowering, inhibiting tumor and immune enhancement, have also been reported in this paper. These research results provided reliable and theoretical basis for the preparation process of phytosterol and phytosterol ester from deodorizer distillates of rapeseed, and also for the comprehensive utilizations of products with high additional value.

Key words: Rapeseed, Deodorizer distillates, Phytosterol, Phytosterol ester, Bioactivities

1. Introduction

Phytosterol is a kind of natural substance which structure is similar to cholosterol. It is mainly composed of ß-sitosterol, stigmasterol, brassicasterol and campesterol. The study on phytosterol has attracted many research interests in the word because phytosterol bears several functions, such as high safety, inhibiting the absorption of cholesterol and preventing from coronary atherosclerosis. The main method to obtain phytosterol is extracting from deodorizer distillates of vegetable oil when oil was refined. And phytosterol product can be widely used in medicine, foods, cosmetic and feeds. In our laboratory, we have studied the composition, extraction, isolation and structure of phytosterol from deodorizer distillates of rapeseed, studied the preparation of phytosterol ester that is the derivant of phytosterol. The bioactivities of phytosterol and phytosterol ester, such as antioxidant, serum lipid-lowering, inhibiting tumor and immune enhancement were also reported through our research. The application of plytosterols ester was also discussed in this paper.

2. The analytical method of phytosterol ester and its composition

Gas-chromatography(GC) method for the quantitative determination of phytosterols was developed. Gas-chromatography separate condition were selected as follows: HP-5 capillary gas-chromatography column; flame ionization detector(FID); injector temperature 300ºC; oven temperature 285ºC; squalane as internal standard. The method has the advantages of exact and quick.

The determination method of phytosterols ester was established. Methanol sodium as catalyzer, phytosterols ester sample were interesterificated with methanol. The compose and relatively content of fatty acid methyl ester were determinated by GC, accordingly, species and content of fatty acid could be determined. Phytosterols ester sample were saponificated in KOH-alcohol solution, reaction temperature 80ºC, reaction time 2h. Phytosterols ester was translated into free phytosterols. Then, through determining the content of free phytosterols by GC, make a conversion to the content of corresponding phytosterols ester respectively.

UV spectrum, IR spectrum and GC-MS were used to determine the structure of the phytosterols and phytosterols ester. GC-MS crack law of phytosterols and phytosterols ester were summarized.

3. Study on preparation of plytosterols ester

Using pyridine as catalyst, phytosterols acetate (PSA) was synthesized by direct esterification of phytosterols and acetic anhydride. The effect of factors on the yield was studied. The optimal synthetic conditions were as follows: molar ratio of phytosterols to acetic anhydride to pyridine was above 1:9:5; Reaction temperature was 85ºC and reaction time was 2h. The recrystallization method phytosterols acetate were researched. Results of GC analysis of the product show that the purity of product was above 98%.

Phytosterols oletate(PSO) were synthesized by direct esterification of phytosterols and oleic acid. The optimal synthetic conditions obtained were as follows: using sodium bisulfate as catalyst(2.0% molar of phytosterols); molar ratio of oleicacid to phytosterols was 1.3, reaction temperature was 135ºC and reaction time was 8h. The yield of esterify reached 84.3%, the purity of product was above 90%.

Phytosterols stearic (PSS) were synthesized by direct esterification of phytosterols and stearic acid. Through factorial design, the optimal synthetic conditions were obtained as follows: molar ratio of phytosterols to stearic acid was above 1:1.2; reaction temperature was 135ºC and reaction time was 7h. The yield of esterification was 87.8%. Recrystallization method of

phytosterols stearic were researched, the purity of product was above 95%.

4.The antioxidant experiment of phytosterol in lard

The experiments adopted different dosage of phytosterol and different synergism to study the antioxidantive of phytosterol in lard. The results of experiment showed that phytosterol had strong antioxidantive activity in lard, which was concentration dependent. EDTA was the best synergism, next was vitamine E, vitamine C and citric acid.

5.Study on serum lipid-lowering bioactivities of plytosterols ester

Study on the preventive and therapeutic effects of three phytosterol esters (PSA, PSO, PSS) on diet-induced hyperlipidemia in mice and their structure-activity relationships. On preventive experiments, forty healthy, male Kunming mice were randomly divided into five groups on body weight, eight mice per group. The control groups were fed with normal weight, while hyperlipidemic group were fed with the high-cholesterol diet. The rest three groups were given the high-cholesterol diets. At the same time, they were respectively given with three phytosterol esters at the level of 100mg/kg body weight. The effect of phytosterol ester on lipid metabolism in mice was studied. The experiment lasted twenty-eight days. Results showed that compared with the hyperlipidemic group, phytosterol ester could significantly lower the cholesterol levels of mice, including the serum TC, LDL-C and arteriosclerosis index (AI). They also could lower the liver weight, TC and TG. PSA and PSO had ideal preventive effects on diet-induced hyperlipidemia in mice and better than PSS.

6.Study on effect of inhibiting tumor and function of immune enhancement of plytosterols ester

Investigated the anti-tumor effect of Phytosterol Acetate(PSA) and Phytosterols Oletate(PSO). PSA (10,50mg/kg.d) and PSO(10, 50mg/kg.d) were given respectively to mice bearing transplanted tumor S_{180} by intraperitoneal injection for 9 days. The inhibiting rate of tumor growth, thymus index, spleen index, activity of catalase were detected. Results showed that both PSA group could inhibit the growth of S_{180} cell efficiently and increase the activity of catalase in erythrocyte of S_{180} cell in mice, exhibiting dosage-depended. Effect of inhibiting tumor of PSO was better than PSA.

Evaluated the effect of phytosterol oletate (PSO) on the immune function of S_{180}-bearing mice. Fifty mice bearing transplanted tumor S_{180} were used as animal model. The effects of PSO on the immune function were observed by intraperitioneal injecting different doses of PSO-1 (10mg/kg.d), PSO-2 (50mg/kg.d) and cyclophosphamide (20mg/kg.d) to mice respectively. Compared with the S_{180}-bearing mice model group, PSO could enhance significantly the phagocytosing ratio and the phagocytosing index ($\rho<0.05$), the effects of S_{180} were better than cyclophosphamide; PSO also could improve evidently delayed-type hypersensitivity ($\rho<0.05$), but worse than cyclophosphamide; PSO could increased formation of antibody in splenic cells of S_{180}-bearing mice, but there is not remarkable different ($\rho>0.05$). The results showed that PSO could improved immunologic function of mice.

7. Study on application of plytosterols ester

We have studied the soluble capacity of phytosterols ester and phytosterols. There are significant different in the soluble capacity among three phytosterols esters. Solubility(g/100mL, 20ºC) of phytosterols in soy salad oil was 1.55, PSO was 31.8, PSA was 8.07, PSS was 1.82; The soluble capacity of solubility was: PSO>PSA>PSS>Phytosterols.

The functional mayonnaise could be make up by adding PSO(2.5%) and natural tocopherol(0.5%) in the ordinary mayonnaise food. The optimum ingredients of the functional mayonnaise product were as follows: special soy salad oil(include PSO 3.5%, V_E 0.7%) 74 shares, yolk 14 shares and vinegar 9 shares.

References

A.E.Brynes, R. Mandeno. (2002). Acomparison of the LDL-lowering efficacy of plant sterol and stanol esters. Atherosclerosis Supplements. 3(2):176-178.

A.M.Wolfreys and P.A.(2002). Safety evaluation of phytosterol esters. Food and Chemistry Toxicology. 40(4): 461-470.

Award A B, et al.(2000) Dietary phytosterel inhibits the growth and metastasis of MDA-MB-231 human breast cancer cells grown in SCID mice. Anticancer.20:821-822

Arjan J.H.Louter, Christina G. Bauer-Plank and Guus S.M.J.E.(2002). Analysis of plant sterol esters as functional food ingredients. Lipid Technology. 14(4): 87-89.

Chonglun Xie, Laura A.,Woollett, Stephen D.(2002). Fatty acids differentially regulate hepatic cholesteryl ester formation and incorporation into lipoproteins in the liver of the mouse. Journal of Lipid Research. 43:1508-1519.

Dutta, Paresh C, Normen, Lena.(1998). Capillary column gas-liquid chromatographic separation of Δ5-unsaturated and saturated phytosterols. Journal of Chromatography A. 816:177-184.

J.C.kim, B.H. Kang.(2002). Subchronic toxicity of plant sterol esters administered by gavage to Sprague-Dawley rats. Food and Chemical Toxicology. 40:1569-1580.

Lena Normen, Paresh Dutta.(2000). Soy sterol esters and β-sitostanol ester as inhibitors of cholesterol absorption in human small bowel. American J of Clinical Nutrition. 71: 908-913.

Malcolm R Law.(2000). Plant sterol and stanol margarines and health. 173:43-47.

Yuji shimada. Enzymatic Synthesis of Steryl Esters of Polyunsaturated Fatty Acid. J.Am.Oil Chem.Soc. 76:713-717