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Extraction of lipids from the raw materials for beer production and development of methods for phytosterols' determination by high performance liquid chromatography (HPLC)

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Abstract
The study of phytosterols' content in the raw materials for beer production is one of the new research problems that arose with a significant increase in beer consumption in the recent years. There is a theory that the content of phytosterols in beer is so significant that could have a negative impact on the human body. Hops and malt were selected to study the phytosterols' content in the raw materials for beer production. Studies using the high performance liquid chromatography (HPLC) with mass detection showed presence of following phytosterols in malt and hops: stigmasterol, beta-sitosterol, campesterol, and brassicasterol.

Keywords: Phytosterols; cholesterol; sex hormones; the raw materials for beer production; lipids; high performance liquid chromatography (HPLC); beta-sitosterol; campesterol; stigmasterol.

Introduction

Lipids are a group of natural molecules, including fats, waxes, sterols, fat-soluble vitamins (A, D, E, and K), monoglycerides, diglycerides, triglycerides, phospholipids, and others.

The main biological functions of lipids: to act as structural components of cell membranes and to store energy. Phytosterols are plant sterols isolated from the unsaponifiable lipids of the plant. Phytosterols are structurally similar to animal sterols. However, unlike animal sterols (e.g., cholesterol), a side chain of molecules of phytosterols unsaturated and contains 8 and 9 or 10 carbon atoms. Cholesterol is one of the most important fats in human, since it is involved in lots of useful and harmful processes. Cholesterol is the main material for construction of the most important hormones – cortisol, progesterone, testosterone, and estrogen. It serves as a structural component of cell membranes and is essential for the production of bone restorative vitamin D. Phytosterols are biological very active and play an important role in normalization of lipid and cholesterol metabolism. As food components or special food additive phytosterols have the property of cholesterol reduction. The mechanism by which phytosterols reduced cholesterol is as follows: the penetration of a cholesterol micelle in digestive system is inhibited, resulting a total volume of intake cholesterol is reduced. This property of phytosterols helps to control cholesterol in the human body.

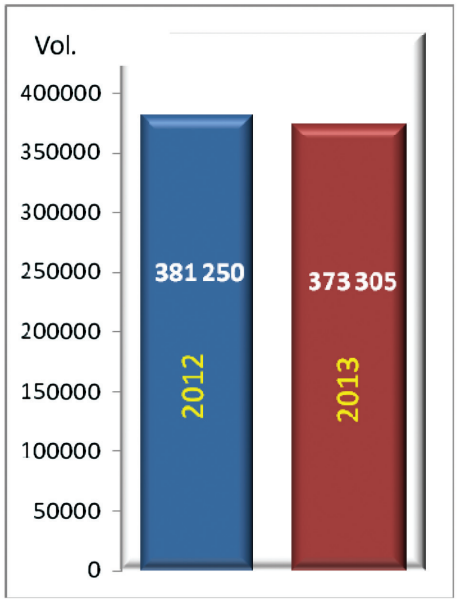
Hypercholesterolemia (high content of cholesterol in blood) is a hormonal disease associated with impaired receptor mechanism of input cholesterol inside the cell structures [1-3].

There are more than 200 of phytosterols in nature. The most common phytosterols are beta-sitosterol, stigmasterol, and brassicasterol. Melting points of beta-sitosterol: 140°C; stigmasterol: 170°C; brassikasterin: 148°C [4].

Phytosterols get into the human body with plant food. There is a theory that the content of phytosterols in beer is so significant that could have a negative impact on the human body: presence in the body, supposedly can have a significant impact on human hormones (the totality of hormones in the body), and the ratio of the number of which varies under the

influence of internal and the external environment. Sex hormones regulate the formation and functioning of the reproductive organs. The difference between men and women is primarily determined by the fact that male hormone (testosterone) is produced in men's body and female (estrogen) in women's. The action of these substances determines the external difference between men and women. If a person begins to take uncharacteristic hormone, his appearance, his voice, character begin to change. The biosynthesis of steroid hormones in male testis (mainly testosterone) and female ovaries occurs due to cholesterol presence in the free form and in sulfate derivative form. Plant sterols operate the structural components of the cell membrane, they may reduce the concentration of cholesterol in the human body, but with substitution of existing cholesterol by plant sterols there is a risk that their hormone properties will appear. The study of of phytosterols' content in the raw materials for bee production is one of the new research problems that arose with a significant increase in beer consumption in the recent years [5]. Sales of the top five beer producers (LLC "Baltika Breweries" company Heineken Russia, the Company Efes Rus, Kompaniya "SUN InBev, Moscow Brewing Company") in Russian Federation for 2012-2013 years are shown in Figure 1.

Figure 1: Sales of the five largest beer producers in the Russian market for the 2012-2013 years, in hectoliters.



Technologically, it is possible to affect beer parameters, by changing models of the processes while preserving flavour and quality of the final product. Considering different approaches to the definition of phytosterols in lipid feedstock for the beer production, it is important to take into account that the precise definition of phytosterols in the plant matrix is very complex due to phytosterols' presence in plants, in "free", and the "conjugate" form, same as in esters or glycosides forms [6,7].

The content of phytosterols in a number of foods, such as soybeans, carrots, figs, coriander, and other food sources are regulated by *the Uniform sanitary, epidemiological and hygienic requirements for goods subject to sanitary and epidemiological supervision (control) of the Customs Union.* According to *the Uniform sanitary, epidemiological and hygienic requirements for goods subject to sanitary and epidemiological supervision (control) of the Customs Union* tolerable upper intake level of phytosterols ranges from 450 mg to 600 mg per day [8].

Materials and Methods

Currently in the Russian Federation, there is no approved method for the phytosterols determination in foods. Abroad phytosterol content in foods is performed by gas chromatography and high performance liquid chromatography (HPLC) [9]. High sensitivity is the advantage of these methods. Taking into account international experience HPLC was chosen to determine the phytosterols in the raw materials for beer production. The aim of the work was to develop a method for the separation of total lipids from the raw materials for beer production and with further phytosterols determination by HPLC.

Chromatographic determination was performed on liquid chromatography system such as Agilent Technologies 1200 Series with the light scattering detector: Alltech 3300 ELSD, column type: Agilent Hypersil ODS 2.0 × 125 mm. Also, in the system of liquid chromatography type Agilent 1100 Series LC/MS with the mass detector, detector type: LC/MSD Trap SL. Centrifugation of the samples was carried out in a centrifuge: Rotina 38 (FRG), shaking is on a shaker for shaking the tubes Biosan OS-10 (USA); Biosan OS-10 (CIIIA). When preparing samples the following reagents were used: distilled water, methane, acetonitrile HPLC grade

dichloromethane. Hops and malt have been selected for the experiment. These products were purchased from the online store Novopermskiy Brewery Plant, http://pivoperm.ru/magazin.html.

Preparation of the standard solution

1 mg of cholesterol sample was placed in a volumetric flask of 100 cm^3, 1 mg sample of beta-sitosterol was added, dissolved in a mixture of 50 ml of ethanol and 15 ml of dichloromethane, 35 ml of distilled water was added, stirred until complete dissolution.

Sample preparation

50 g of malt sample was subjected to grinding in a laboratory mill. 5 mg of crushed malt sample was placed in a volumetric flask of 100 cm^3, 10 ml of distilled water was added, together with 5 ml of dichloromethane, stirred until complete dissolution. Volumetric pipet transferred 1 ml of this solution in a polymer tube of 15 cm^3 with an airtight lid.

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The resulting solutions were centrifuged for 5 min, the supernatant liquid was analysed.

HPLC method with light scattering detector have been chosen for phytosterols detection during the first part of the experiment. Detection conditions: mobile phase: distilled water, acetonitrile. Detector type: Agilent Technologies 1200 Series; the light scattering detector: Alltech 3300 ELSD. Chromatographic conditions: column type: Agilent Hypersil ODS 2.0 × 125 mm, thermostat temperature 20°C.

Results and Discussion

The experiment showed that the phytosterols determination was not possible via detection with light scattering. Large number of peaks of various substances was observed in the chromatograms. The resulting chromatograms are shown in Figures 2 and 3. It was decided to use the HPLC method with mass detection [10]. Detection conditions were selected as follows: mobile phase: distilled water, acetonitrile. Detector type: Agilent 1100 Series LC/MS; ion source: APCI; velocity of nebulizer gas (nitrogen): 10 l/min; nebulizer pressure: 50 psi; temperature of drying gas (nitrogen): 350°C; polarity: positive; type detection: LC/MSD Trap SL; fracture energy (amplitude): 1,2 Chromatographic.

Studies have shown the possibility of qualitative determination of phytosterols in the raw materials for beer production by HPLC with mass detection. Qualitative and quantitative research of the phytosterols composition in different beer types is planned for future experiments. It is also planned to develop and improve methods of sample preparation, depending on the object of study.

During determination with MS, beta-sitosterol, stigmasterol, and campesterol were detected in the hop sample. The resulting chromatograms are shown in Figure 4.

Figure 2: Chromatogram of the hops sample.

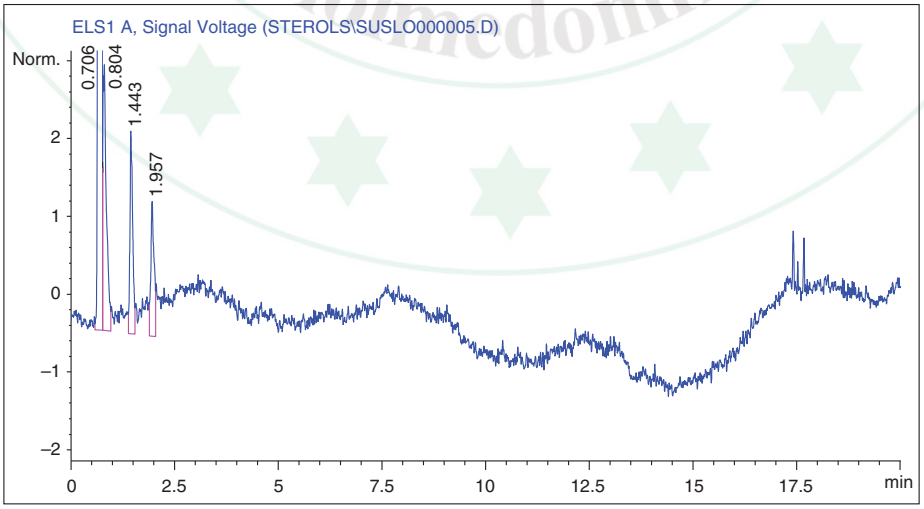


Figure 3: Chromatogram of the malt sample.

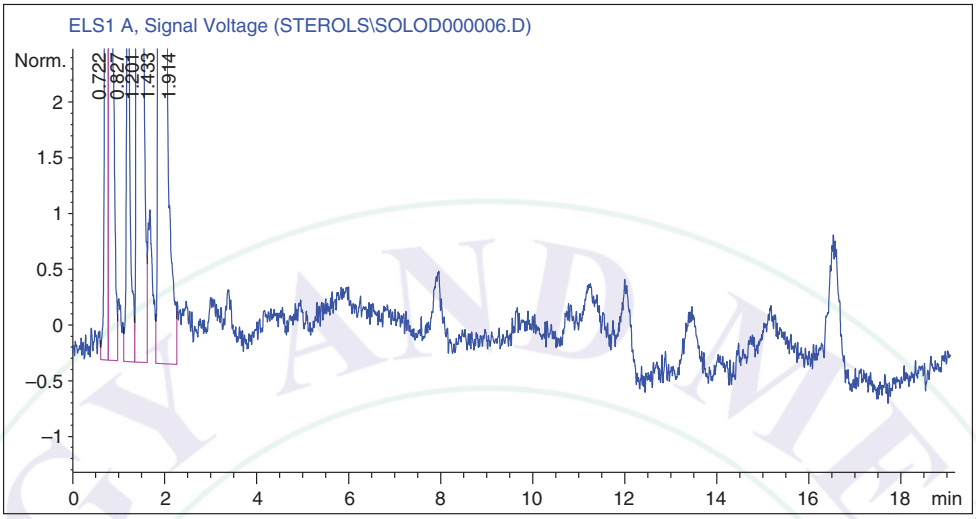
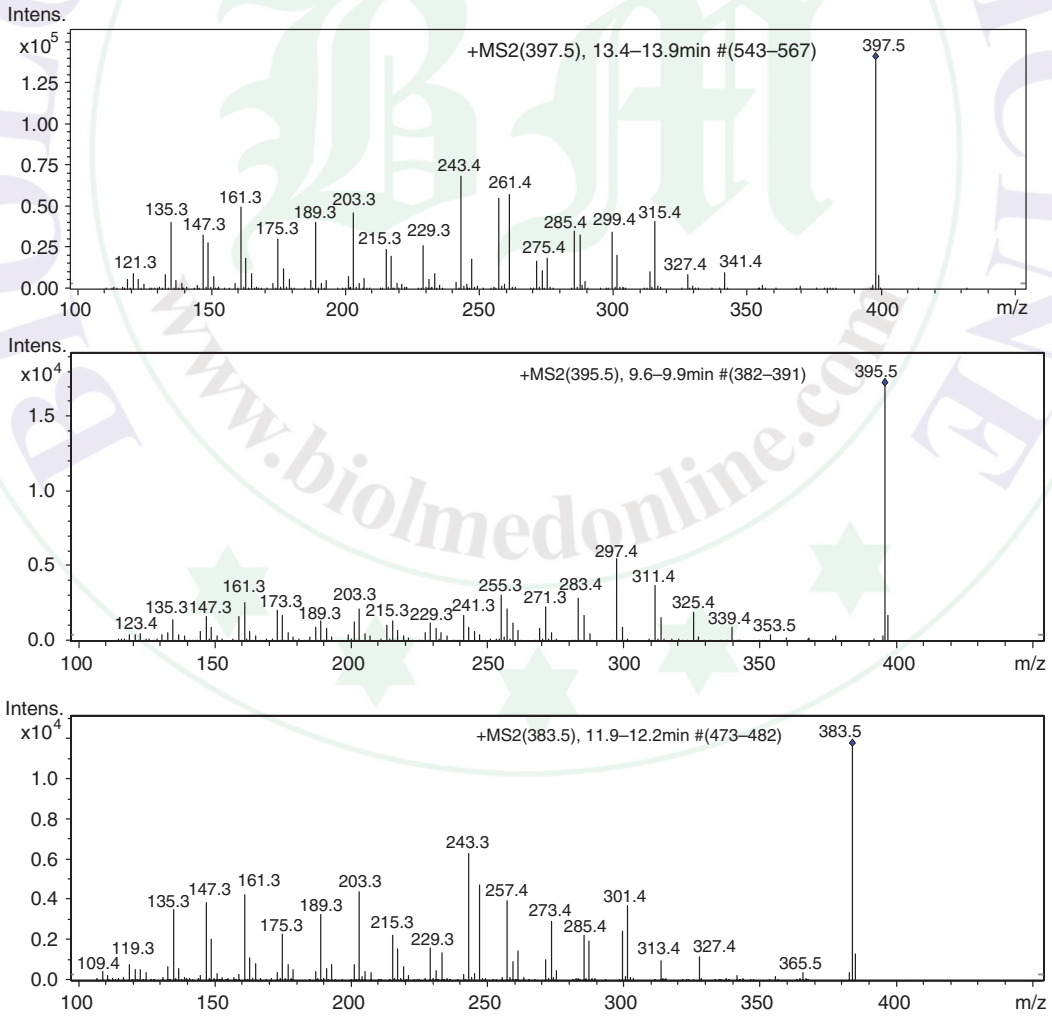


Figure 4: Chromatogram of the hops sample with destruction of the major ions.



Studies using HPLC with mass detection allowed us to conclude that following phytosterols are present in malt and hops based on their molecular weights: stigmasterol, beta-sitosterol, campesterol, and brassicasterol.

Conclusion

Studies have shown the possibility of qualitative determination of phytosterols in the raw material for the beer production by HPLC with mass detection. In future, qualitative and quantitative research of the phytosterols composition in different beers is planned. It is also planned to develop and improve methods of sample preparation, depending on the object of study.

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