Glycomics & Lipidomics

Extended Abstract

# Steryl glycosides: The emerging bioactive glycolipids of olive oil

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## Abstract

Plant sterols have been used as cholesterol-lowering agents in humans for a very long time. Within this group of compounds, steryl glycosides (SG) considered as non-nutrients plant-secondary metabolites are separately categorized as glycolipids thanks to both the sugar molecule(s) bound to the 3-C atom of the sterol backbone and to their favorable effect in human health. Different sources of SG have been identified, being olive oil (OO) the most recent one. To this respect some considerations should be taking into account. First of all, from the analytical point of view one must realize that the European Commission official method for sterol analysis in OO overlooks the presence of SG, causing underestimations when reporting on total phytosterol concentration. Furthermore, it is important to provide data also on the SG profile, since it has been pointed out that different species may have dissimilar biological effects. Under this perspective the development of a proper analytical approach prevailed and new procedures to determine these sterol derivatives in OO are have been developed. Secondly, OO production and consumption is no longer restricted to the Mediterranean basin, which have resulted on an increasing competitiveness, lack of a centralized databank for validated methods of analysis, absence of harmonization, etc., driving to a significant weakness in the OO production and supply chain, which is nowadays being exploited by counterfeiters. Our purpose here is to present the information available on the field of SG, giving an overview of what these molecules are and of what has been done in different fields of research. Furthermore, we will comment on one of our specific objectives in the European OLEUM project: The search for novel markers focused on the development and validation of innovative analytical solutions to solve part of the OO fraud problems presently under the microscope of the international community.

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Glycosylated plant sterols (steryl glycosides, SG and acylated steryl glycosides, ASG) are minor glycolipids found in a variety of plants at different levels and in different compositions. They have until now been far less studied than other sterol conjugates, though they possess at least the same potential health benefits as free or esterified plant sterols. Additionally, due to the more hydrophilic conjugated part, they may possess further beneficial properties in forming water-soluble structures. We studied the content and sterol profiles of glycosylated sterols in various plant foods to be able to better understand their levels in human diets, as well as to identify sources with high levels and/or unique sterol profile for possible extraction for ingredients. Glycosylated sterols contributed up to almost 60% of total sterols in e.g. potatoes, indicating that they indeed are an important group of sterols in plants. The ratio of ASG and SG varied from 0.4 to 3.6, indicating significant differences in the proportions of these two conjugates in various plants. Furthermore, extremely large differences were observed in the sterol profiles not only between but also within different plants, which indicates that the sterol profile of the glycosylated sterols does not always reflect the total sterol composition in the same plant tissue

Avoiding any kind of oil sample pretreatment, a fraction containing the steryl glucosides (SG) was directly isolated by

SPE. This fraction was derivatised and analysed by GC in order to quantify the SG content. The limit of detection of the method was 0.37 mg/kg and the recovery 90%. Additionally, the identity of the SG was confirmed by MS. We applied the procedure to oil samples of different categories and origins indicating that the only SG that could be quantified in olive oil was β-sitosteryl glucoside, which was present at concentrations not higher than 3.00 mg/kg. Practical applications: Olive oil is of upmost importance from both the nutritional point of view and from the economic repercussion that fraud may have.

Among the many parameters required by the EU in order to determine olive oil characteristics, SG are completely overlooked and no specific methodology has been elaborated so far. We have developed a straightforward protocol for the determination of SG in olive oil. This method can be used to provide an additional identity parameter and a new blend indicator. This method is fast, cheap and easy to perform. Besides, it has a great potential for automation which would allow its routine application. The possibility of applying the procedure to study vegetable oils used in the biodiesel industry is also pointed out.

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