Different Strategies for the Production of Phytosterol Esters

Guzmán Torrelo, Carlos Torres, F.J. Señorans, Guillermo Reglero
Sección Departamental de Ciencias de la Alimentación, Facultad de Ciencias,
Universidad Autónoma de Madrid, 28049 Cantoblanco, Madrid, Spain.
E-mail: guzman.torrelo@uam.es

Enzymatic esterification and supercritical fluid extraction was combined to produce phytosterol esters from soybean oil deodorizer distillates. Firstly, the original SODD was
mixed with oleic acid to reduce its melting point from 65–70 to 30–35 °C and also to produce a reaction mixture with a ratio of free fatty acids (FFA) to sterols close to 2 to improve the progress of sterols esterification. Two enzymatic steps were used in order to separate sterols esterification and ethyl esterification in time and space. The first enzymatic step (*Candida rugosa* lipase) allowed to efficiently transform more than 90% of the original sterols in a short period of time (5 h). The second enzymatic step (Novozym 435) converted more than 95% of the FFA in less than 3 h. In addition, the stability of both biocatalysts has been evaluated and both bioprocesses have been scaled-up reutilizing the same batch of lipase up to 8 and 3 times for the first and the second enzymatic step, respectively. The final product obtained was used as starting material for the purification of sterol esters, tocopherols, and fatty acid ethyl esters via supercritical fluid extraction. At 250 bar, 55°C and solvent-to-feed ratio of 35 phytosterol esters were concentrated in the raffinate product up to 82.4 % w/w with satisfactory yield (72 %).

In addition, we conducted a near quantitative esterification of phytosterols from soybean oil deodorizer distillate with conjugated linoleic acid in a solvent free medium. We used a 1:1 molar ratio of sterols to conjugated linoleic acid. For that matter, stepwise addition of sterols was investigated. Using this methodology, purities of up to 80% steryl esters were obtained that consumed more than 90% of the total conjugated linoleic acid. In addition, the effects of temperature, amount, and stability of lipase were also evaluated. We also performed an enzymatic esterification of phytosterols with fatty acids from butterfat in equimolecular conditions to produce phytosteryl esters in solvent free medium. Commercial and immobilized *Candida rugosa* lipases were used as biocatalysts for the reaction. By this methodology, under simple and mild reaction conditions (without solvents, 50ºC and short reaction times), 94% and 99% (w/w) of phystosteroyl esters were obtained in 48 hours and 9 hours with the commercial and the immobilized lipase respectively. The effect of temperature, fatty acid specificity, enzyme amount and residual activity of each lipase were also evaluated.