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Synthèse et caractérisation de biomolécules antioxydantes

Synthesis and characterization of antioxidant biomolecules

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Summary and Conclusions

In this study, an enzymatic process was developed and optimized, using the lipase B from *C. Antarctica*, for the synthesis of bimodular derivatives combining highly oxidizable fatty acids and a phenolic antioxidant. A model derivative was firstly synthesized through an alcoholysis reaction between DHA ethyl ester and vanillyl alcohol.

In that case the synthesis was carried out in organic solvents or in solvent-free medium. Initially, the enzymatic synthesis was carried out in organic solvents such as 2-methyl-2-butanol or acetonitrile, in the presence of a slight excess of DHA (molar ratio vanillyl alcohol: PUFA equal to 1:2). Kinetics obtained showed that these conditions required 8h of reaction to reach the equilibrium and a maximum conversion of vanillyl alcohol of 60%ester. The product was purified; its structure was determined showing that acylation took place on vanillyl alcohol primary hydroxyl group. Although traditional, processes performed in organic solvents are increasingly criticized today because of their cost and the use of media of high environmental impact. Due to growing safety and environmental concerns and with the objective to increase the conversion yield of vanillyl alcohol together with the ester production, a solvent-free process appeared as an attractive alternative. Under these conditions, the acyl donor substrate (DHA ethyl ester) was introduced in large excess comparing to the acyl acceptor substrate (vanillyl alcohol). The main advantages of this approach are the absence of organic solvents (the large excess of one substrate allowed the solubilisation of the other substrate) and the possibility to shift the equilibrium of the reaction in favour of the ester synthesis. This effect can be reinforced by working under reduced pressure, that allows the continuous

elimination of the by-product of the reaction, ethanol. Such conditions were shown to improve the conversion of vanillyl alcohol, and then the production of the ester. The concentration of vanillyl alcohol and the feeding of the reactor with the phenolic substrate were optimized. Finally, a fed-batch process was proposed, leading to high concentration of ester (440 g/L) and avoiding the oxidative degradation of the reaction medium. Alternatively, a high amount of vanillyl alcohol can be introduced at one time (saturation conditions).

Given all these results, different elements must be taken into account to choose the most appropriate process for the production of phenolic fatty acid esters as the efficiency of the process, the complexity and the cost of synthesis and purification steps, the stability of the reaction medium towards oxidation.

The biological activities of the ester were investigated, leading to promising conclusions. In fact, the ester exhibits interesting potential for food industry and nutrition: (i) improved organoleptic qualities of DHA-VE-supplemented diet; (ii) elevated antioxidant activity that should stabilize DHA as well as various food matrices such as oils, fats and emulsions; (iii) increased bioavailability of DHA leading to higher DHA levels in erythrocytes and neurons; (iv) combined beneficial effects of phenols and ω3 PUFAs; (v) increased neuroprotection against amyloid stress; (vi) no visible toxicity.

Moreover, its oxidative stability was monitored by using different spectroscopic methods. Conjugated dienes (CD) determination is a widespread and inexpensive technique providing information about the first stage of oxidation that leads to primary oxidation products. In the present work, CD determination was particularly effective to study the thermal and temporal stability of DHA-based compounds. Furthermore, FTIR

is now well-known for its high efficiency to follow structural changes in complex evolving systems. The different regions of FTIR spectra provide useful information about functional groups and their chemical environment. On a practical point of view, this rapid and non-destructive method does not require any sample or chemical preparation, and then allows significant time- and cost-savings in comparison with classical analyses. In this work, FTIR was shown to be an efficient tool to follow lipid oxidation thanks to significant changes in the frequency and the intensity of characteristic bands. More specifically, the intensity of the band related to =C-H bond stretching vibration of cis-double bonds at 3013 cm-1 depended on the degree of unsaturation of the samples, and then was used as a marker for DHA oxidation. Another sensitive indicator was the ratio between the absorbance at 3013 cm-1 and the absorbance at 2853 cm-1 that corresponds to the vibration of saturated C-H bonds.

Unsurprisingly, the oxidative stability of the compounds was negatively affected by increasing temperature and storage time. All results indicated a higher stability of DHA-VE in comparison with DHA-EE, showing the interest of combining this highly oxidizable lipid with vanillyl alcohol in a single structure. According to FTIR data, oxidation was delayed till 8 weeks in the case of pure DHA-VE stored at 20°C against 2 weeks in the case of pure DHA-EE. A midway stability was determined for the crude reaction medium made of 45% DHA-VE. Main advantages of such a medium are, firstly, a high stability despite a significant content in DHA, secondly, easy preparation and use that do not require any purification step. Phenolic esters of DHA undoubtedly appear as promising derivatives that could make easier the use of polyunsaturated lipids.

Lipase-catalyzed alcoholysis of salmon oil with vanillyl alcohol was performed through an efficient solvent-free bioprocess, leading to a mixture of SFA, MUFA and PUFA vanillyl esters (FA-VE). Structural analyses allowed identifying the major products. Vanillyl alcohol was almost totally converted after 24 h of reaction, starting from an initial concentration of 50g/L. The crude reaction medium recovered from salmon oil alcoholysis exhibited a high stability during storage compared to salmon oil.

The overall experimental results obtained through the present study could lay the ground for the use of vanillyl fatty acid esters as nutraceuticals and/or functional ingredients for food products. Future work should focus on bioavailability studies in order to get a better understanding of the transport and the absorption of these molecules in the human body.

To develop and complete this work, some ideas seem promising:

- Biological studies must be undertaken in order to get information about the metabolism the esters produced.
- The organoleptic properties of the synthesized products must be evaluated through sensory analyses.
- The process could be even more improved by studying the effect of specific parameters like water activity, enzyme concentration, reaction temperature and agitation speed.
- Biological studies about the neuroprotective effect of the esters must be pursued and intensified. The mechanism of action of the esters appears as a key issue of this project.

- The alcoholysis process could be applied to other types of oils aiming to extend it to other application areas like cosmetic ingredients.