SYNTHESIS OF L-TYROSINE GLYCERYL ESTER CATALYZED BY
α-CHYMOTRYPSIN IN WATER-MISCIBLE ORGANIC SOLVENTS:
A POSSIBLE SUN-TAN ACCELERATOR PRODUCT

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SUMMARY
The synthesis of L-tyrosine glycercyl ester, from glycerol and L-tyrosine methyl ester, was carried out by a transesterification reaction catalyzed by α-chymotrypsin. Values of 60 % (v/v) for glycerol and 200 mM for L-tyrosine methyl ester were optimal for the transesterification reaction. Additionally to glycerol, several other water miscible cosolvents (acetonitrile, N,N'-dimethyl formamide and tetrahydrofurane) were tested in the reaction media, but their presence did not give an enhancement on the transesterification activity with respect to the glycerol/water medium. However, increasing the hydrophobicity of the cosolvent resulted in a reduction of the enzyme activity, the water:glycerol mixture being the best reaction media.

INTRODUCTION
Peptides and amino acid derivatives have important applications in food and pharmacological areas. In the cosmetic industry, hydrophilic L-tyrosine derivatives have a great interest because of their properties as sun-tan accelerators. The very poor solubility of L-tyrosine in water limits its direct use for cosmetic formulations. Thus, recently, the use of hydrophilic L-tyrosine derivatives synthesized by enzymic ways as sun-tan accelerators in novel cosmetic formulations has been described (Monsan and Paul, 1990). In fact, L-tyrosine is the first substrate to produce the skin pigmentation, which is firstly hydroxylated to L-DOPA, and then oxidized to L-dopaquinone (the key intermediate in the melanin pigmentation) by the consecutive action of both the hydroxylase and oxidase activities of tyrosinase.

The use of proteases to catalyze peptide bonds or amino acid esters synthesis in hydrophilic organic solvents has been extensively reported (Ingalls et al., 1975; Mori et al., 1987; Kise et al, 1990, Cardillo-Theobaldo et al., 1991; Lozano et al., 1992). There are many advantages in employing one-phase liquid cosolvent systems for enzymic synthesis, such as high reactants

The aim of this paper was to study the synthesis of an hydrophilic L-tyrosine derivative, as the L-tyrosine glyceryl ester, by a transesterification reaction catalyzed by α-chymotrypsin. The influence of the substrate concentration, the glycerol content, as well as the presence of other water-miscible cosolvents, was analyzed.

MATERIALS AND METHODS
Materials
α-Chymotrypsin (EC 3.4.21.1., type II from porcine pancreas) and L-tyrosine methyl ester were obtained from Sigma Chem. Co., and used without previous purification. Glycerol, N,N'-dimethyl formamide, tetrahydrofurane and acetonitrile were Merck, analytical grade.

Hydrolysis/transesterification reactions
Into an Eppendorf tube of 1-ml total volume, 200 μl of 1 M L-tyrosine methyl ester dissolved in 0.2 M phosphate buffer pH 7.0, was placed. The reaction volume was adjusted to 980 μl by addition of the corresponding volume of water, glycerol or organic cosolvent, and then, 20 μl of a 2.5 mg/ml α-chymotrypsin solution in water added. The reaction mixture was incubated without stirring at 40 °C. Aliquots of 50 μl were extracted at several time intervals from the reaction mixture, previously homogenized by mechanical shaking, and mixed with 950 μl of 10 % (w/v) trichloroacetic acid (TCA) to stop the reaction. TCA-treated samples were immediately centrifuged (10 min at 2,800 g) at 6 °C to separate the precipitated protein and stored at -20 °C until analysis.

HPLC analysis
Substrates and products concentrations were determined by HPLC. An AminoQuant (model 1090, Hewlett-Packard) chromatograph, equipped with a Nucleosyl C-18 column (Touzart and Matignon, 25 cm length and 3.9 mm internal diameter, 5 μm particle size, and 10 nm pore size), was used. Samples were eluted isocratically with water:acetonitrile:acetic acid (89:10:1, v/v/v) at 1 ml/min flow rate and elution profiles detected at 280 nm. One unit of activity was defined as the amount of enzyme which produced 1 μmol of the hydrolysis (L-tyrosine) or transesterification (L-tyrosine glyceryl ester) product per minute.

RESULTS AND DISCUSSION
Synthesis of L-tyrosine glyceryl ester.
As a kinetically-controlled process, the degree of hydrolysis of an amino acid ester by a serine protease is highly dependent on the water (nucleophile acceptor of the aminoacyl-enzyme complex) content of the reaction medium. Thus, the presence of water-miscible organic solvents, able to reduce the water content/activity, should affect negatively the hydrolytic action of the enzyme, and favour the transfer of the acyl moiety of the acyl-enzyme intermediate complex to another nucleophile in the reaction medium. Thus, the influence of