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TERPENE ESTER SYNTHESIS BY LIPASE-CATALYZED TRANSESTERIFICATION

Lisa N. Yee[1], Casimir C. Akoh[1]* and Robert S. Phillips[2]

[1]Department of Food Science and Technology, The University of Georgia, Athens, Georgia 30602-7610, USA

[2]Departments of Chemistry and Biochemistry, The University of Georgia, Athens, GA

SUMMARY

Five lipases were screened for their ability to synthesize terpene esters by transesterification. The nature of terpene alcohol and enzyme, as well as the chain length of the acyl donor used affected the product yields. Lipase AY from *Candida rugosa* gave the best overall yield (96.2%). Geraniol and tributyrin were also found to be the best reactants.

INTRODUCTION

Flavors represent a small but significant segment of the food industry. It is the most important reason for repeated sales (Risch and Reineccius, 1988). In the United States, consumer market is one of the key forces in the shift towards high quality foods. In recent years, consumers are demanding products that are perceived as natural, nutritional and healthy.

Consumers are becoming more involved and interested in the source and compositions of flavorings in foods (Stofberg, 1986). The safety of food as related to health is often associated with the term "natural", therefore there is an increasing demand for flavor and fragrance chemicals which are considered to be natural. Traditionally, these flavors have been isolated from natural sources or obtained through chemical synthesis. Disadvantages such as expense of isolation, restriction of supply for natural materials, cost of natural material, as well as the negative impact associated lately with the word "synthetic or "artificial" have led to the search for other alternatives. One approach is the application of biotechnology to produce flavors. Using enzymatic techniques it is possible to carry out relatively large scale preparative syntheses of natural flavor-active esters using natural raw materials as substrates (Gatfield, 1988).

Terpenes, due to their variety of organoleptic properties, are among the most important flavor compounds. Enzymatic reactions in organic solvents offer opportunities for the production of esters of some of these flavor compounds, especially those of geraniol and citronellol. Geranyl and citronellyl esters are essential oils widely used in the food, cosmetic and pharmaceutical industries.

*To whom correspondence should be addressed.

Lipase-catalyzed synthesis of these esters using short chain acyl donors has been studied (Claon and Akoh, 1993, 1994). This study focuses on the ability of five lipase enzymes to synthesize terpene esters by transesterification using tributyrin as the acyl donor. Other triacylglycerols were also used to test the effect of chain length on terpene ester synthesis.

MATERIALS AND METHODS

Materials: Non-specific lipase AK (22,000 U/g) from *Pseudomonas sp.*, AY (33,600 U/g) from *Candida rugosa*, sn-1,3 specific lipase L (11,000 U/g) from *Candida lipolytica*, N (88,000 U/g) from *Rhizopus niveus*, and G (50,000 U/g) from *Penicillium cyclopium* were kindly provided by Amano International Enzyme Co. (Troy, VA). All five lipase preparations were in crude powder form. Terpene alcohols geraniol (99% pure), DL citronellol (95% pure), as well as triacylglycerols triacetin, tributyrin, tricaproin and tricaprylin (all 99% pure) were purchased from Sigma Chemical Co. (St. Louis, MO). n-Hexane (HPLC grade) was obtained from Fisher Scientific (Norcross, GA).

Transesterification method: Ester synthesis took place in screw capped test tubes. 0.1 mole terpene alcohol (geraniol or citronellol, respectively) and 0.03 mole of triacylglycerol (tributyrin for the first part; triacetin, tributyrin, tricaproin and tricaprylin for the second part) were added to 2 ml hexane, followed by 200 units of the enzyme in their respective tubes. The samples were incubated in an orbital shaking water bath at 30°C and 200 rpm for 24 hr, along with their controls (samples with no enzyme). Experiments were carried out in duplicates for the first part, and in triplicates for the second part.

Extraction and analysis: After the 24 hr incubation period, samples were removed, cooled in ice and passed through anhydrous sodium sulfate (Na_2SO_4) column to remove the enzyme as well as any residual water. Two hundred micrograms of internal standard (linalool), was added to each sample. Analysis was done by injecting a one microliter aliquot in a splitless mode into a GLC (Hewlett Packard HP 5890 Series II Hewlett Packard, Avondale, PA) equipped with a flame ionization detector (FID). A DB-5 fused-silica capillary column (30 m x 0.25 mm i.d.; J & W Scientific, Folsom, CA) was used. Injector and detector temperatures were set at 250°C and 260°C, respectively. Oven temperatures ranged from 150°C to 200°C depending on the chain length of the acyl group. The carrier gas was helium, used at a total flow rate of 24 ml/min. Synthesis of terpene esters was calculated from the amount of terpene alcohol that reacted, and further quantified by an on-line computer.

RESULTS AND DISCUSSION

Screening of enzymes: All five lipases were capable of synthesizing terpene esters but to very different degrees. For geranyl butyrate, the highest yield was obtained with lipase AY (96.2%), followed by L, G, N and AK (Table 1). For citronellyl butyrate, the highest yield (31.6%) was obtained with lipase G, followed by AY, AK, N, and L (Table 1). Geranyl butyrate had higher yields than citronellyl butyrate samples with all enzymes, except for lipase AK. Lipase AY showed the highest yield for geranyl butyrate and also showed the second to the highest values for citronellyl butyrate. Lipase G worked fairly well for both terpene esters, while lipase N and L were fairly good for the geraniol ester but not for the citronellol ester. Lipase AK performed its

lowest for geraniol but higher values were obtained with citronellol. This results suggest that lipase AK has a higher affinity for citronellyl butyrate than for geranyl butyrate. Values for lipase AK for both esters were very similar to those reported by Claon and Akoh (1994) under the same conditions studied in this experiment. Previously, Claon and Akoh (1994) reported a higher yield for citronellyl butyrate than geranyl butyrate compared to their acetates, caproates, and caprylates with lipase AK. Overall geraniol seems to be a better substrate than citronellol for the five enzymes studied.

Table 1. Effect of terpene alcohol and lipase on the reaction yield[1]

Lipase	Geraniol	Citronellol
AK	15.2	27.5
AY	96.2	28.2
G	36.1	31.6
L	37.8	0.3
N	26.2	0.5

[1]Reactions were performed in duplicate in 2 ml hexane with 0.1 mole geraniol or citronellol and 0.03 mole tributyrin, and 200 units of lipase. Yields are expressed in percent yield after 24 hr incubation.

In general, all lipases performed well with geraniol as the acyl group acceptor. These results suggest that lipase AY exhibited the best yields for both terpene esters. The high yields from lipase AY can possibly be attributed to the fact that the microorganism that this enzyme originates from, *Candida rugosa*, is selective for butyric acid (Langrand et al., 1990). In this study tributyrin was chosen as the acyl donor for convenience purposes. Previous studies have shown that triacylglycerols are the best substrates when used under conditions similar to the ones in this experiment (Macrae, 1983; Claon and Akoh, 1994). Nevertheless, they have not been used extensively in terpene ester synthesis (Gray et al., 1990), hence the reason for the great interest in these acyl donors.

Effect of chain length: Since lipase AY performed the best with geraniol and tributyrin as substrates, we continued to study the effect of acyl donor chain length on terpene ester synthesis. Yields were obtained for all four acyl donors but results definitely stood out for tributyrin compared to the rest (Table 2). Tributyrin showed the highest yields, while triacetin, tricaproin and tricaprylin had closer but lower values. Claon and Akoh (1994) also observed highest yields with tributyrin as the acyl donor in a reaction catalyzed by SP 435, an immobilized *Candida antarctica* lipase.

Table 2. Effect of acyl donor chain length on geranyl ester yield[1]

Acyl Donor	Percent Yield
Triacetin	22.1
Tributyrin	96.2
Tricaproin	17.6
Tricaprylin	29.9

[1] Reactions were performed in triplicates in 2 ml hexane with 0.1 mole geraniol and 0.03 mole triacetin, tributyrin, tricaproin and tricaprylin, respectively, and 200 units of lipase AY. Yields are expressed in percent yield after 24 hr incubation.

Thus, findings indicate that overall a higher affinity was shown for geraniol than for citronellol and that the nature of the lipases used and chain length of acyl donor have an effect on the yield of terpene esters. Lipase AY showed the best overall yields, followed by lipase G. These results suggest that both enzymes, especially AY, has potential for and could be used to catalyze terpene ester synthesis via transesterification. Immobilizing the enzyme could also improve yields as well as reduce costs. Geraniol and tributyrin were the best substrates under the conditions of this study. Therefore, reaction conditions and parameters need to be studied more extensively to develop optimum product yields.

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