Inhibition of Pancreatic Lipase by Phenolic Acids – Examination *in vitro*

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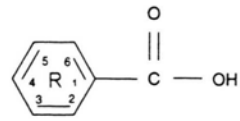
The influence of addition 2, 4, 6, 8, and 10 μM of benzoic and cinnamic acids and selected phenolic acids (salicylic, *p*-hydroxybenzoic, gentisic, protocatechuic, vanillic, syringic, *o*-coumaric, *p*-coumaric, caffeic, ferulic, sinapic) on the activity of pancreatic lipase was examined *in vitro*. The strongest inhibition activities were observed with caffeic, ferulic and benzoic acid, while sinapic and gentisic acids produced the lowest inhibition.

Introduction

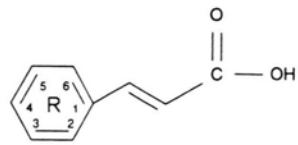
Phenolic acids, derivatives of hydroxybenzoic and hydroxycinnamic acids, are the main phenolic compounds of rapeseed, canola and mustard (Wanasundara *et al.*, 1994; Shahidi *et al.*. 1994; Amarowicz and Fornal , 1995). This group of phenolic compounds has antioxidative and bacteriostatic properties (Nowak *et al.*, 1992; Amarowicz *et al.*, 1995; Amarowicz *et al.*, 1996) . Moreover, they show abilities to bind with proteins of rapeseed (Amarowicz *et al.*, 1993). Phenolic acids could exert an inhibitory effect of mutagenesis induced by aflatoxin B_1 (Chan , 1992) , benzo(a)pyrene-7,8-diol-9,10-epoxide-2 (Wood *et al.*, 1982). Inhibition of soya lipoxygenase and mould lipase from *Aspergillus oryzae* by extracts of phenolic acids occurred in model examinations (Zadernowski and Nowak-Polakowska, 1992). The results obtained determined us to examine an influence of pure phenolic acids on the activity of pancreatic lipase.

Materials and Methods

Pancreatic lipase (2000 IU / g) of Schuchard was applied. Moreover, the following acids were used: benzoic, *p*-hydroxybenzoic, gentisic, vanillic, syringic, cinnamic, *o*-coumaric, *p*-coumaric, caffeic, ferulic, sinapic (all from Sigma). Chemical structures of the compounds mentioned above are described in Fig. 1.



Acids:	
Benzoic	R: 4-H
Salicylic	R: 2-OH
p-Hydroxybenzoic	R: 4-OH
Gentisic	R: 2,5-di-OH
Protocatechuic	R: 3,4-di-OH
Vanillic	R: 3-OCH_3, 4-OH
Syringic	R: 3,5-di-OCH_3, 4-OH



Acids:	
Cinnamic	R: 4-OH
o-Coumaric	R: 2-OH
p-Coumaric	R: 4-OH
Caffeic	R: 3,4-di-OH
Ferulic	R: 3-OCH_3, 4-OH
Sinapic	R: 3,5-di-OCH_3, 4-OH

Fig. 1. Chemical structure of applied acids.

Lipase activity was measured by a colorimetric method according to Bier (1955). An enzymatic hydrolysis of *p*-nitrophenol acetate to *p*-nitrophenol was applied in this method; *p*-nitrophenol acetate was synthesized according to Huggins and Lapides (1947).

0.1 ml of methanol including 2, 4, 6, 8, and 10 μM of acids were added to the tubes with 2 mg of lipase dissolved in 2 ml of phosphate buffer (1/15 M, pH 7) and 2.4 ml of distilled water. After heating up to 37 °C, 1 ml of a substrate solution containing 0.33 μM of *p*-nitrophenol acetate was added. Following incubation for 20 min at a temperature of 37 °C the absorbance at 400 nm was measured. Simultaneously the sample, free from lipase, with

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addition of a substrate and phenolic acid was incubated.

Inhibition of lipase was calculated as follows:

$$\% \text{ Inhibition} = \frac{A_1 - (A_2 + A_3)}{A_1 - A_3} \times 100\%.$$

A_1, sample absorbance with lipase and substrate;
A_2, sample absorbance with lipase and substrate and phenolic acid;
A_3, sample absorbance with substrate and phenolic acids.
All measured were conducted in triplicate.

K_m and type of inhibition for ferulic acid was evaluated using Lineweaver-Burk plot. An addition of 10 μM of ferulic acid to the sample was used in investigations.

Results and Discussion

The strongest inhibitors among derivatives of hydroxybenzoic acid were salicylic and *p*-hydroxybenzoic acids. Inhibition exceeded the value of 25% at an addition of 10 μM of salicylic acid. In the case of the addition of gentisic, protocatechuic and syringic acids at the same level an inhibition index was in the range from 10% to 15% (Fig. 2). The reaction rate for the control was 0.017 μM of *p*-nitrophenol / min.

Derivatives of hydroxycinnamic acid inhibited more powerfully the activity of pancreas lipase (Fig. 3). The strongest inhibitors were cinnamic and caffeic acids. At an addition level of 10 μM of phenolic acids an inhibition index was slihtly more than 30%. The weakest inhibitors of pancreas lipase were sinapic and *p*-coumaric acids.

The Lineweaver-Burk plot (Fig. 4) suggests noncompetitive inhibition of panreatic lipase by ferulic acid. The K_m calculated from the plot is about 0.152 mM / l.

Comparing the results obtained with the chemical structure of phenolic acids, it appears that compounds with a methoxy group (syringic and sinapic) are weak inhibitors of lipase. Comparatively small inhibition of lipase by a compound without a hydroxy group (benzoic acid) shows that a carboxy group takes part in the activity between phenolic acids and the active centre of lipase. The activity intensity depends on molecular size. In the case of cinnamic acid it is distinctively lower than that of benzoic acid. Position of the -OH group in the ring also affected lipase inhibition. Inhibitory effect at *ortho-* position was stronger than at *para-* position and salicylic acid was a stronger inhibitor than *p*-hydroxybenzoic; *o*-coumaric had stronger inhibitory properties than *p*-coumaric.

The inhibitory effect of pancreas lipase activity by phenolic acids in this paper, was clearly smaller

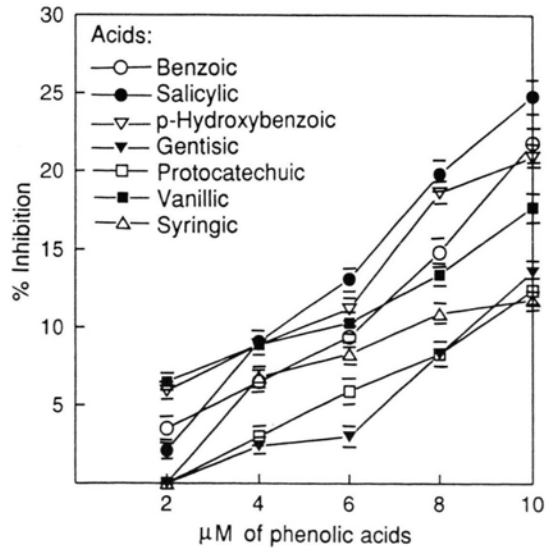

Fig. 2. Inhibition of pancreatic lipase by benzoic acid and hydroxybenzoic acid derivatives.

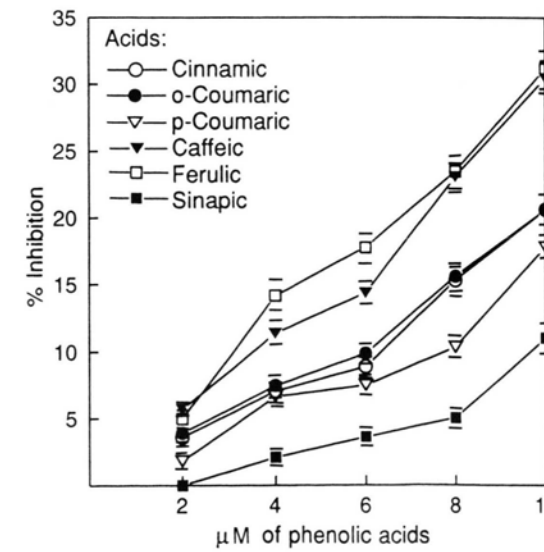

Fig. 3. Inhibition of pancreatic lipase by cinnamic acid and hydroxycinnamic acid derivatives.

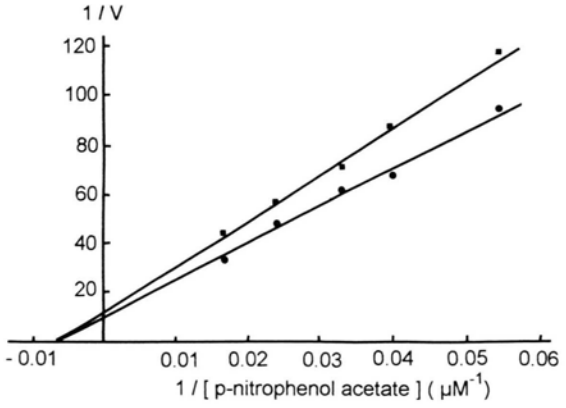

Fig. 4. Lineweaver-Burk plot for inhibition of pancreatic lipase by ferulic acid. *V* is epressed as μM of *p*-nitrophenol / min. ● samples without phenolic acid; ■ samples with addition of 10 μM of ferulic acid.

than inhibition of the same enzyme by chlorophenoxyacetic acid derivatives (Amarowicz *et al.*, 1988). In the case of these compounds, an inhibitory effect occurred at 10 times smaller contents of inhibitors in the same sample.

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