LIPOLYTIC ACTIVITY OF POLISH RAPESEED VARIETIES

J. GRABSKA, L. JĘDRYCHOWSKI

Centre for Agrotechnology & Veterinary Sciences PAN, 10 Tuwima street, 10718 Olsztyn, Pl.

ABSTRACT

The dynamic of changes in lipolytic activity during germination of double improved BOLKO and high erucic SKRZESZOWICKI rapeseeds was studied. The most effective method ensuring about 55-fold purification of rapeseeds lipase was the methods of molecular filtration on Sephadex G-100 gel preceeded by ultracentrifugation 100 000g x 90 mn.

INTRODUCTION

Due to its low specificity against polyunsaturated fatty acids (PUFA) containing double bonds cis-6 or cis-4 rapeseed lipase (E.C. 3.1.1.3) is more and more often applied for the processes of "enriching" plant oils with PUFA . The aim of the study was to isolate and purify native lipase from Polish varieties of rapeseeds for its further application for enzymatic modification of plant oils (Hills M.J. et al., 1990).

EXPERIMENTAL

Plant material

Double-improved (Brassica napus cv. BOLKO) and high erucic (Brassica napus cv. Skrzeszowicki) rapeseeds were used in ouer investigations.

Lipase isolation and purification

Lipase was obtained from enzymatic extracts prepared after 5 days of germination acc. to Lin Y-H et Huang H.C. (Lin Y-H. et Huang H.C., 1983). Lipase purification was conducted in two stages in two ways:

1 - ultracentrifugation of enzymatic extracts at acceleration 100 000 g for 90 min followed by molecular filtration on gel Sephadex G-100;

2 - ultracentrifugation of enzymatic extracts at acceleration 23 000 g for 30 min and molecular filtration on gel Sephadex G-100 or ionexchange chromatography on gel DEAE Sephadex A-50 in NaCl gradient (0 - 500 mM).

Methods

Lipolytic activity was measured by diffusion method acc. to Lawrence (Lawrence R.C., Fryer T.F., Reiter B., 1967) taking change in tributyriloglycerol hydrolysis zone by 1 mm caused by 5 μl of enzymatic extract after 16 h incubation at 30°C as activity unit A.U. or A.U./mg protein.

Protein content was determined by measuring absorbance at 280 nm and calculated based on the standard curve of rapeseed albumin.

RESULTS AND CONCLUSIONS

The greatest increase in lipolytic activity of BOLKO rapeseeds was observed between the 2nd and 3rd day of germination and for SKRZESZOWICKI rapeseeds also between the 4th and 5th day. For both varieties maximum activity was observed after the 5th day of germination .

As a result of centrifugation (100 000 g) enzymatic extracts from the seeds of both varieties were separated into 3 fractions: *lipid bodies, supernatant and pellet*. About 90 to 96% of lipolytic activity remained in *the supernatant.* Specific activity of this fraction was 76 and 71 A.U./mg protein and was 3 to 4 times greater than the activity of enzymatic extracts prior to centrifugation. Following separation of the *supernatant* of both rapeseed varieties with molecular filtration on gel Sephadex G-100 7 fractions were obtained of which only the first revealed lipolytic activity (Fig.1). Its specific activity was about 14 times higher the specific activity of *the supernatant* prior to separation and about 56 times higher than that of enzymatic activity prior to centrifugation. As a result of molecular filtration on gel Sephadex G-100 of *the pellet* from BOLKO and SKRZESZOWICKI varieties chromatograms of similar profile were obtained (Fig.2). In both cases lipolytic activity was found for the fractions with the highest molecular weight at 12- to 17-fold purification index/coefficient.

Acceleration 23 000 g allowed to obtain *the supernatant* with specific lipolytic activity 71.8 A.U./ mg protein i.e. 3 times higher than that of the initial extract. Separation of this fraction on gel Sephadex G-100 ensured 5- to 10-fold purification of enzymatic proteins, wich made the first peak (Fig. 3). Their specific lipolytic activity was 333.1, 394.8, and 896.7 A.U./ mg protein.

Ionexchange chromatography on gel DEAE-Sephadex A-50 permitted a more advantageous separation of protein compounds from phenolic compounds (Fig. 3) than the method based on molecular filtration on gel Sephadex G-100.

Lipolytic activity was found for the fractions with elution volume from 112 ml to 123 ml within the range of the first peak.

Among the isolation and purification methods applied the best results, i.e. the highest purification degree was obtained applying ultracentrifugation of enzymatic extracts at acceleration 100 000 g x 90 min followed by separation of *the supernatant* on gel Sephadex G-100 or centrifugation at 23 000 g x 30 min and purification of *the supernatant* by ionexchange chromatography.

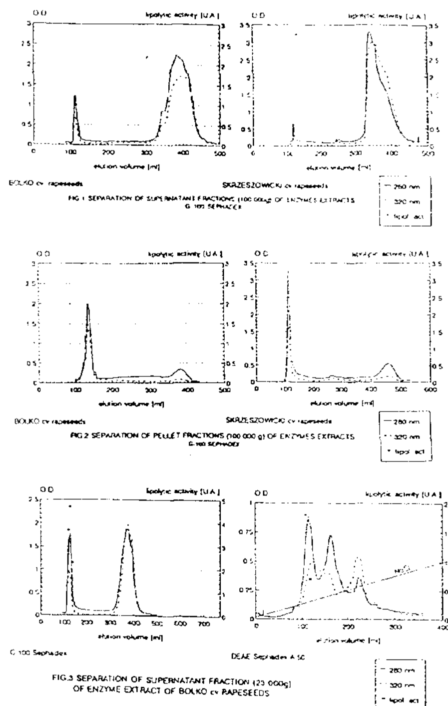

FIG 1 SEPARATION OF SUPERNATANT FRACTIONS (100 000g) OF ENZYMES EXTRACTS

FIG 2 SEPARATION OF PELLET FRACTIONS (100 000 g) OF ENZYMES EXTRACTS

FIG.3 SEPARATION OF SUPERNATANT FRACTION (23 000g) OF ENZYME EXTRACT OF BOLKO cv RAPESEEDS

REFERENCES

Hills, M.J., Kiewitt,I., Mukherjee,K.D., (1990). Enzymatic fractionation of fatty acids: enrichment of γ-linolenic acid and docopsahexanic acid by selective esterification catalyzed by lipases. Journal of the American Oil Chemist's Society, 67, 561-564.

Lawrence, R.C., Fryer, T.F., Reiter, B. (1967). Rapid method for the quantitative estimation of microbial lipases. Nature, 25, 1264-1265.

Lin, Y-H., Huang, H.C. (1983). Lipase in lipid bodies of cotyledons of rape and mustard seedling. Archives of Biochemistry and Biophysics, 225, 360-369.