ZESZYTY PROBLEMOWE POSTĘPÓW NAUK ROLNICZYCH 1995 z. 427, 121-126

INFLUENCE OF SELECTED AGRICULTURAL OPERATIONS ON LIPOLYTIC ENZYMES ACTIVITY OF RAPESEED*

L. Jędrychowski, J. Grabska, E. Kubicka, D. Mierzejewska

Centre for Agrotechnology and Veterinary Sciences, Polish Academy of Sciences, Tuwima 10, 10-718 Olsztyn, Poland

A b s t r a c t. The activity of triacylohydrolases of rapeseed varieties and phylums available in Poland: Bolko, Ceres, Liporta, Mar-3 was estimated. The variable factors were the moisture of seeds, the level of mechanical damage (micro and macro) resulting from the method of harvest and the type of dessicants used in the final stage of seed ripening. A clear (statistically highly significant) diversification of the activity of lipolytic enzymes was found. It related to the varieties and experimental groups of the different methods of harvest (one- and two-stage harvest) and the moisture of seeds. The maturity degree, the kind and amount of the ripening regulator applied in the final stage of vegetation, differentiated the activity of lipolytic enzymes in a highly significant way.

K e y w o r d s: lipolytic enzymes, rapeseed, one-stage harvest, two-stage harvest

INTRODUCTION

The physiological properties of seeds correspond clearly with the occurrence of particular storage substances (protein, fat, carbohydrate substances). Their content depends on the presence of specific and characteristic for particular varieties of seeds enzymatic systems. The physicochemical and biochemical conditions affect the physiological conditions of seeds. Activity of enzymes that influence forming and abatement of a state of dormancy, germination, etc. Apart from climate conditions also measures relating to the technique of field-crop production, carried out during seed ripening, and mechanical damage caused by, e.g., the way of harvest, influence the physiological condition of seeds. Lipolytic enzymes (hydrolases of triacyloglyceroles E.C. 3.1.1.3) occurring in raw food, mainly in cereals, seeds of oil plants and food products, can cause physicochemical and organoleptic changes of a negative character [1]. The activity of lipases (native and microbiological originating from microbiological infections) occurs especially at high seed moisture and at higher temperature of their storage [3].

The aim of the study was to estimate the influence of moisture and the degree of rapeseed mechanical damages occurring during harvest on lipolytic enzyme activity of rapeseeds.

Moreover, the effect of applying ripening regulators on the level of lipolytic enzyme activity of these seeds was determined.

MATERIALS AND METHODS

The experimental material was rapeseed coming from field experiments of the Institute of Agrophysics of the Polish Academy of Sciences in Lublin. The level of seed damage was varied and resulted from different conditions of harvest:

*This work was supported by the State Committee for Scientific Research, Poland, under grant No. 5 S307 029 01

- parameters of the threshing drum: width of the working slot: accordingly 16 and 18 mm, rotation speed: 600 and 800 r.p.m.,
- moisture of seeds during harvest: I block - 12-20 %, II block - approximately 7 %,
- method of harvest: one-stage and two-stage,
- different degrees of seed maturity (Bolko, Ceres, Liporta, Mar-3) with respect to the time of harvest (very early, early, optimal and retarded harvest) [6].

The level of micro- and macrodamage was estimated at the Department of Physical Properties of Food in the Institute of Animal Reproduction and Food Research of the Polish Academy of Sciences according to the methods described elsewhere [2].

In the final stage of vegetation the plants were subjected to the activity of different ripening regulators: SPODNAM, HARVADE, REGLONE, and a mixture of: SPODNAM + REGLONE.

In the case of the seeds of Bolko variety, each desiccant was applied in three different concentrations:

- SPODNAM - 0.6; 1.2; 1.8;
- HARVADE - 1.0; 2.0; 3.0;
- REGLONE - 1.5, 2.0; 3.0;
- SPODNAM+HARVADE - 0.6+2; 1.2+2; 1.8+2;
- SPODNAM+REGLONE - 0.6+2; 1.2+2; 1.8+2;

Enzymatic extracts were obtained from rapeseed according to the method of Lin and Huang [5]. Protein content was calculated basing on a standard curve formed by a spectrophotometric method at the wavelength of 280 nm and using rapeseed albumin as a standard. The activity of lipolytic enzymes was measured by a diffusion method according to Lawrence in relation triburylglicerol TC4:0 [4]. Incubation was conducted at 30 oC for 16 h. Lipolytic activity measure was the size of a basis brightening sphere estimated in mm and measured by an optical microscope. It was assumed that a lipolytic activity unit was an increase of the brightening sphere by 1 mm, caused by 5 µl of enzymatic extract after incubation in conditions examined. The results of all research were evaluated statistically applying a variance analysis and a Duncan test.

RESULTS

The degree of maturity and the physiological condition of seeds obtained during one-stage harvest are differentiated. These differences may decrease by changing the method of harvest and using two-stage harvest that enables to match the maturity degree of seeds from upper and lower parts of plants.

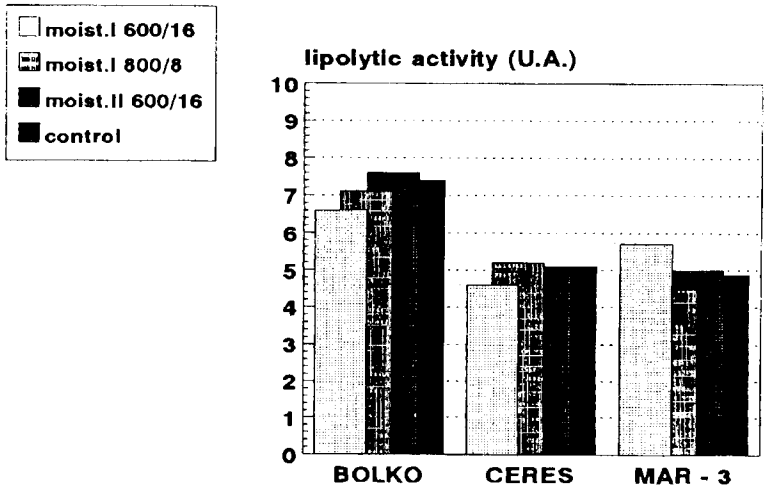
A distinct differentiation of lipolytic enzymes activity among experimental groups, which seeds were harvested in different ways (one- and two-stage harvest), was found in the whole experiment. During one-stage harvest the highest ability for TC:4 hydrolysis among the varieties examined had enzymatic extracts of the Bolko variety seeds, while in two-stage harvest - extracts of Mar-3 seeds (Fig. 1).

Differences of lipolytic activity, estimated in J.A. units, among the varieties were more significant for samples from one-stage harvest than from two-stage one.

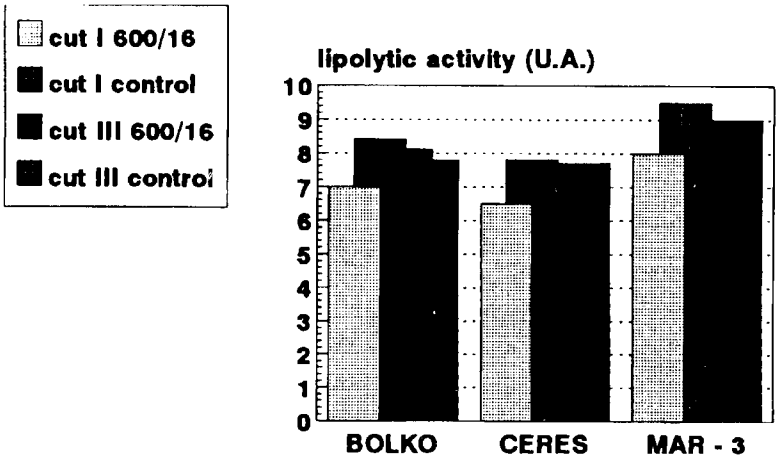
Different parameters of threshing drum work applied during one-stage harvest did not comply with differentiated level of seed damage. Such level (sum of micro- and macrodamage) was in both cases low and fell in the range from 2.4 % to 6.8 %. Rapeseed obtained at higher rotations of threshing drum and a smaller working slot had a damage level only approx. 15 % higher than in seeds obtained during harvest at lower rotations and a bigger working slot. The results of statitical analysis confirmed the lack of a clear influence of this parameter on the activity of lipolytic enzymes (estimated in J.A.). In the case of seeds harvested at the same conditions, the activity of the hydrolases examined was significantly dependent on the moisture of seeds and their variety.

Sum of micro- and macrodamage of seeds harvested during two-stage harvest using a harvester was ten times higher than the sum of seed damage in seeds harvested by hand at the same way and was approx. from 11 % to 18 %.

Statistical analysis showed a significant influence of seed damage degree (dependent on the method of harvest: mechanical or manual) on the activity of lipolytic enzymes in seeds. The activity of these hydrolases was



ONE - STAGE HARVEST



TWO-STAGE HARVEST

Fig. 1. Lipolytic activity of three varieties of rapeseed harvested in one- and two-stage harvest.

also significantly dependent on the variety. However, the time of harvest using a harvester, accordingly in an early and retarded stage of seed maturity (I mowing and III mowing), did not affect the activity of the lipases examined.

DEGREE OF SEED MATURITY

The maturity degree of seeds indicates both the technological value of seed for processing and its reproductive value. Native enzymatic systems and storage substances, occurring in seeds, which are substrates in enzymatic changes, play an important role in both ways of seed use utilization.

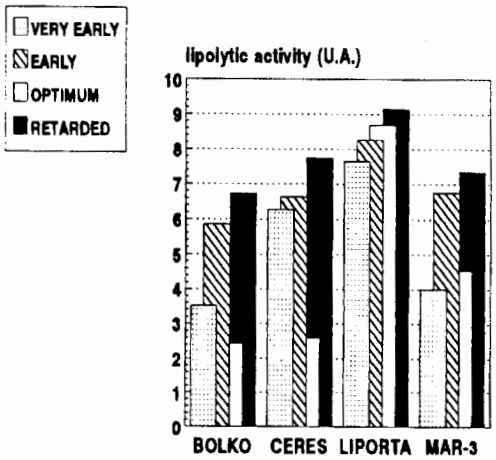
The results of a two-factor analysis of variance and a Duncan test show that the degree of seed maturity of the varieties examined was a factor that clearly differentiated the activity of lipolytic enzymes (Table 1). Based on group means of lipolytic activity (J.A./mg of protein) for all the varieties examined, it can be found that seeds from early and retarded harvest had high activity of lipases which was significantly different from the activity of these

Table 1. Analysis of variance and Duncan test of changes of lipolytic enzymes activity (U.A.) of four varieties of rapeseed characterized by different degree of maturity

Maturity degree	Ceres	Liporta	Mar-3	Bolko	x_{av}	F_{cal}
V. early	A 6.27 A	A 7.65 B	*b* 4.00 C	A 3.14 D	5.26 A	431.83**
Early	A 6.33 A	B 8.27 B	*b* 6.75 A	*B* 6.67 A	7.08 B	22.71**
Optimal	B 2.60 A	C 8.70 B	*a* 4.53 C	*A* 3.53 D	4.84 C	283.29**
Retarded	C 7.67 A	D 9.15 B	*a* 7.35 A	*B* 6.73	7.72 D	50.19**
			a	***b***		
x_{av}	5.79 A	8.44 B	5.67 A	5.02 C		
F_{cal}	167.27**	29.13**	108.19**	236.34**		

Factor 1 - maturity degree of seeds (very early, early, optimal, retarded); Factor 2 - variety of rapeseed (Ceres, Liporta, Mar-3, Bolko);

Significance of the factor influence indicated: ** - differences highly significant $F_{cal.} > F_{0.001}$; In Duncan test - one factor analysis of values of a difference on the level of 0.01 - A,B,C, etc., values of a difference on the level of 0.5 - a,b,c etc. The same type of letters, in line or column, indicate the same analytical group.



TWO-STAGE HARVEST

Fig. 2. Lipolytic activity of four varieties of rapeseed harvested in different maturity degrees.

enzymes in seeds gathered in the very early and optimum stage of maturity (Fig. 2).

A lower technological value of seeds harvested before complete maturity, expressed by lowered fat content, higher acid and peroxide numbers, was found during examinations in the Institute of Agrophysics of the Polish Academy of Sciences in Lublin [6].

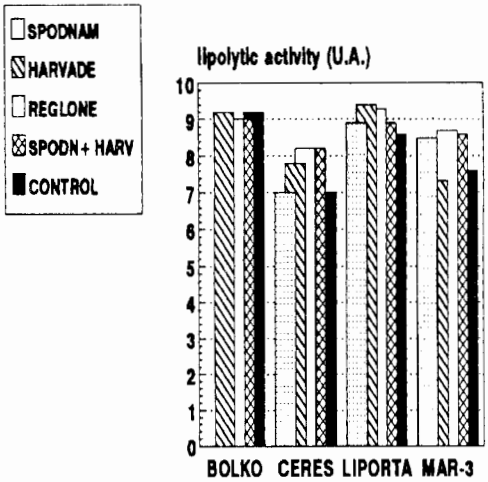
ADDITION OF DESICCATION AGENTS

Desiccants (regulators of seed ripening), apart from changing the water content in seeds, affect the chlorophyl content in tissues and accelerate seed ripening.

The examinations carried out in the Institute showed different influence of the desiccants SPODNAM, HARVADE and REGLONE on the technological value or rapeseed. Similarly, the authors own examinations proved that the specific activity of lipolytic enzymes of the four rapeseed varieties was significantly related to the desiccants applied and the variety.

In the case of seed of the Bolko variety such dependence was statistically significant, whereas for the rest of the varieties examined significantly dependent on this factor.

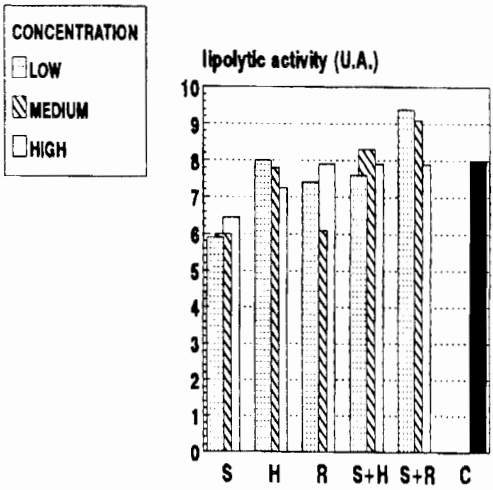
The highest average specific activity (J.A. /mg of protein) both in samples with an addition of desiccants and in the control sample, was that rapeseed of the Ceres variety. The highest activity of lipolytic enzymes estimated



ONE-STAGE HARVEST

pH 8.0

Fig. 3. Dependence of lipolytic activity of different desiccation agents for four varieties.



S=Spodnam ; H=Harvade ; R=Reglone ; C=Control ;

pH 8.0

Fig. 4. Influence of different concentrations of desiccation agents for lipolytic activity of Bolko cv rapeseeds.

in J.A. was observed for seeds of the Liporta variety. Enzymatic extracts of seeds of all the varieties treated with REGLONE preparations and mixtures of SPODNAM and HARVADE showed similar lipolytic activity expressed in J.A. (Fig. 3). This dependence was additionally confirmed when the activity of the enzymes examined was expressed in J.A./mg of protein (except the Ceres variety).

The quantity and kind of the desiccant added showed a significant influence on the lipolytic activity of rapeseed of the Bolko variety. The lowest specific lipolytic activity for a particular desiccant (except REGLONE) was that of the enzymatic extracts from seeds with an addition of the lowest concentrations of the desiccants examined. Enzymatic extracts of almost all samples examined revealed specific lipolytic activity lower than the activity in the control sample. Only the activity of lipolytic enzymes from seeds with addition of 1.2 l/ha of preparations SPODNAM, 1.8+2 l/ha of mixture SPODNAM +HARVADE, also 1.2+2 l/ha of mixture SPODNAM+REGLONE was higher than the activity in the control sample. Similarly in the case of the activity of lipolytic enzymes expressed in J.A., when only samples of seeds with the addition of mixtures of desiccants: SPODNAM + HARVADE (1.2+2 l/ha) and SPODNAM+ REGLONE (0.6+2 l/ha) showed a higher activity of examined enzymes than the activity in the control sample (Fig. 4).

CONCLUSIONS

As a result of the research conducted, a distinct (statistically significant) differentiation of lipolytic enzymes activity was found both between the varieties examined and in the experimental groups of different types of harvest (one- and two-stage harvest), as well as of different moisture contents of seeds:

- the kind of desiccant applied in the final stage of vegetation differentiated significantly the activity of lipolytic enzymes of examined varieties of seeds;
- the activity of lipolytic enzymes of seeds obtained during very early and retarded harvest was distinctly higher than in seeds from the other stages of harvest. The highest lipolytic activity was revealed by the enzymatic extracts of seeds of the varieties Ceres and Liporta.

REFERENCES

1. **Cyperowicz A.S.**: Enzymes (in Polish). Wydawnictwa Naukowo-Techniczne, Warszawa, 1974.
2. **Fornal J., Sadowska J., Jaroch R., Szot B.**: Effect of rape seed damages on protein and fat quality. I. Effect of rape seed damages and time of storage of rapeseeds on fat quality (in Polish). Zesz. Probl. IHAR, 19(1), 123-134, 1992.
3. **Jędrychowski L., Grabska J.**: Effect of rape seed damage on protein and fat quality. III. Effect of rapeseeds damages and moisture on lipolytic enzymes activity (in Polish). Zesz. Probl. IHAR, 19(1), 141-150, 1992.
4. **Lawrence R.C., Fryer T.F., Reiter B.**: Rapid method for the quantitative estimation of microbial lipases. Nature, 25, 1264-1265, 1967.
5. **Lin Y.H., Huang A.H.**: Lipases in lipid bodies of cotyledons of rape and mustard seedlings. Archiv. Biochem. Biophysics, 225(1), 360-369, 1983.
6. **Tys J.**: Effect of desiccation agents on rapeseed quality (in Polish). Conf. Mat., Kazimierz Dolny, 1995.

WPŁYW WYBRANYCH OPERACJI I ZABIEGÓW AGROTECHNICZNYCH NA AKTYWNOŚĆ ENZYMÓW LIPOLITYCZNYCH NASION RZEPAKU

Oceniono aktywność triacylohydrolaz nasion rzepaku dostępnych w kraju odmian i rodów: Bolko, Ceres, Liporta i Mar-3. Czynnikami zmiennymi była wilgotność nasion, poziom mechanicznych uszkodzeń (mikro- i makro-), wynikający ze sposobu zbioru oraz zastosowanie środków desykacyjnych w końcowym okresie dojrzewania nasion. Stwierdzono zdecydowane (statystycznie wysokoistotne) zróżnicowanie aktywności enzymów lipolitycznych zarówno między odmianami, jak również w grupach doświadczalnych różniących się sposobem zbioru (zbiór jedno- i dwuetapowy) i wilgotnością nasion. Również stopień dojrzałości, rodzaj i ilość stosowanego w końcowym okresie wegetacji regulatora dojrzewania w sposób wysokoistotny różnicował aktywność enzymów lipolitycznych.

S l o w a k l u c z o w e: enzymy lipolityczne, nasiona rzepaku, zbiór jednoetapowy, zbiór dwuetapowy.