Determination of the Lipoxygenase Activity of Rapeseed

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Oilseeds generally contain lipoxygenases (linoleate oxido-reductases) which catalyze the oxidation of linoleic and linolenic acids. The oxidation products present in seeds deteriorate the sensory quality of refined rapeseed oil produced by the seed processing. The deterioration is manifested by lower sensory quality and oxidative stability of refined oil (1).

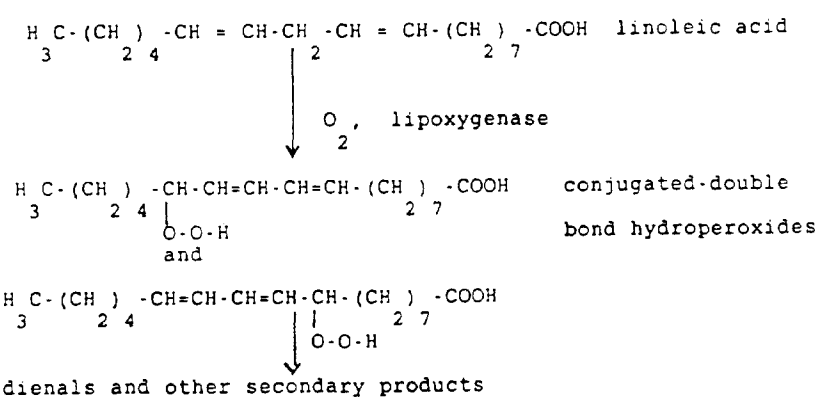
In spite of the importance of rapeseed lipoxygenases, very little is known about their properties and activities. They are deactivated by erucic acid (2), but their activity is not negligible in zero-erucic rapeseed.

Lipoxygenases are fully active only after crushing the seed and in the presence of water, but some low activity may be assumed even at the natural water content in whole seeds, especially in case of damaged seed or seed cropped before the degree of full ripeness, and when seeds are stored for several months before the processing.

During the industrial processing in oilseed plants, lipoxygenases present in zero-erucic rapeseed may be deactivated by rapid heating or steaming, particularly during the conditioning of crushed seed.

By catalytic action of lipoxygenases, linoleic acid is oxidized into a hydroperoxide possessing a system of conjugated double bonds (Fig.1). The composition of the resulting mixture of isomers depends on the ratio of isoenzymes present, and on the reaction conditions. Linolenic acid oxidation is catalyzed in similar way.

Fig. 1: Mechanism of lipoxygenase-catalyzed oxidation of polyenoic fatty acids in rapeseed.



The activity of lipoxygenases may be determined by measuring the amount of oxygen consumed, and the amount of hydroperoxides or of conjugated double bond systems formed. We have obtained (3) good results determining the content of total oxidation products by reverse-phase HPLC.

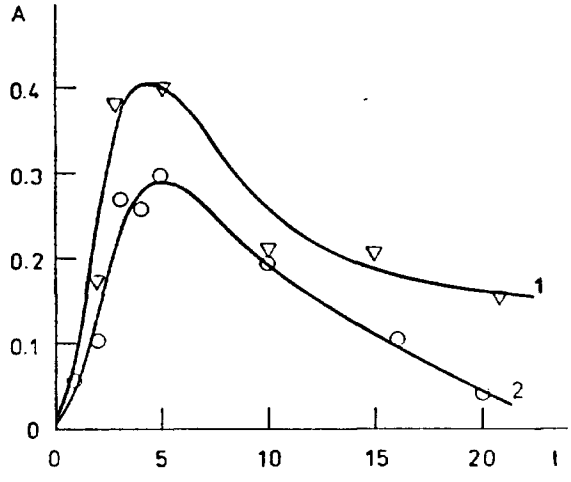
Hydroperoxides are not stable, but are decomposed by the enzyme hydroperoxide lyase into unsaturated aldehydes and other low-molecular weight products.They may be decomposed by non-enzymic reactions as well.

There are several lipoxygenases known, possessing their optimum activities at different pH-values.

The lipoxygenase activity of rapeseed was determined under conditions close to those in seeds on storage. Seeds were crushed, mixed with the respective buffer, and the solution of either sodium linoleate, or of methyl linoleate and Tween 20 was added. The mixture was incubated at 20°C, and the reaction was interrupted at defined reaction times by addition of ethanol, and the absorbance of conjugated hydroperoxides produced was measured at 234 nm.

Two activity maxima were observed, namely at the pH = 9,0 and at the pH = 6,8 respectively. The maximum at the pH = 9,0 was by 30 - 40% higher than the maximum at the pH-value = 6,8 (Fig.2), but the latter maximum is more important as the pH-value of 6,8 is closer to the natural pH-value of rapeseeds.

Fig. 2: The course of lipoxygenase-catalyzed oxidation in rapeseed at ifferent pH-values.
A = absorbance at 234 nm; t = reaction time (min); 1 = at the pH = 9,0; 2 = at the pH = 6,8.



Crude lipoxygenase preparation effectively catalyzed the oxidation of linoleic acid at the pH = 6,8. The absorbance of conjugated double bonds rapidly increased during 5 - 10 min., and remained nearly constant afterwards.

The activity of pure lipoxygenase or of crude lipoxygenase preparations may not correspond to the catalytic effect in ground rapeseeds where many other substances are present, which may activate or deactivate the lipoxygenases, or may affect the reaction course in another way.

In mixtures of ground rapeseed with the buffer solution, the amount of oxidation products was measured by the determination of hydroperoxides, absorbance at 234 nm, and by reverse-phase HPLC. The results obtained with the use of the three analytical methods were very similar, e.g. the relation between the two latter methods are given in Fig.3.

The content of oxidation products rapidly increased to attain the maximum after 3 - 6 minutes. During the further incubation, the content rapidly decreased again (Fig.4). The reaction course was thus substantially different from the course obtained with crude lipoxygenase extracts where no decrease took place.

Fig. 3: Relation between the absorbance at 234 nm and the content of total oxidized fatty acids in lipoxygenase-catalyzed oxidation in rapeseed.
A = absorbance at 234 nm; X = content of oxidized fatty acids (% of total fatty acids).

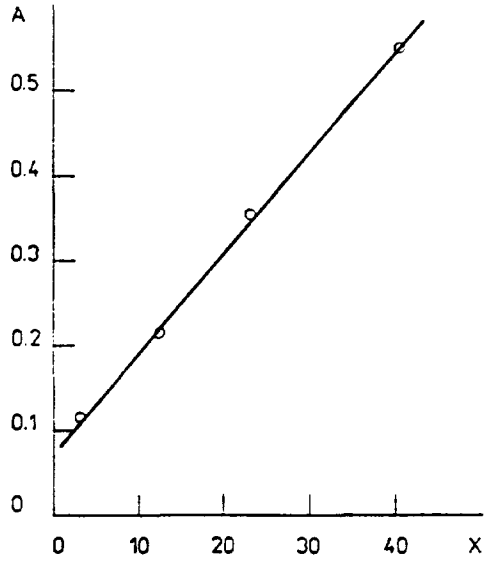
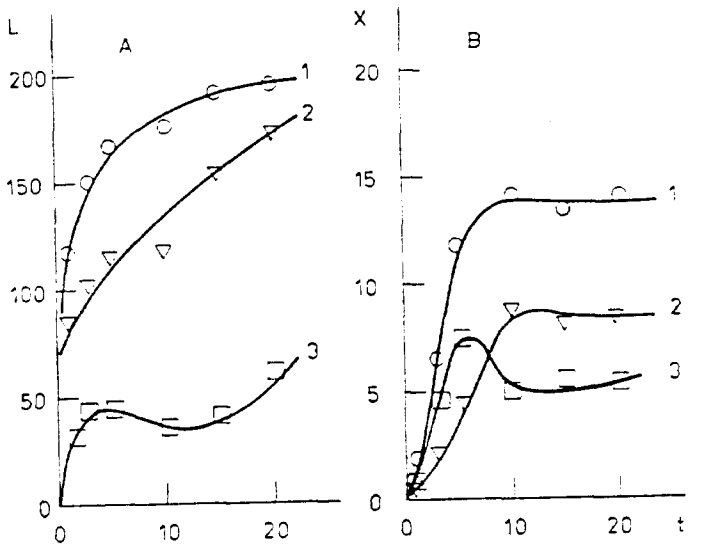


Fig. 4: Changes of lipids during the lipoxygenase-catalyzed oxidation in crushed rapeseed. L = total lipids extracted (mg) ; X = oxidized lipids (mg); t = reaction time (min); seeds extracted with acetone, then linoleate added; 3 = fraction extractable under the conditions of the methods (free lipids); 2 = fraction extractable with chloroform - methanol (bound lipids); 1 = total lipids (sum of A and B).



The decrease of the content of oxidized fatty acids needed explanation. It is known (4) that oxidized fatty acids react with proteins forming relatively stable lipoprotein complexes where the lipidic and protein moieties are bound together by hydrogen bonds, and partially, even by covalent bonds. The hydrogen-bonded lipids are not extracted by common non-polar solvents, but may be extracted by mixtures of chloroform and methanol after Folch.

As shown in Fig.4., the amount of hydrogen-bonded lipids is negligible in the beginning of the reaction but rapidly increases during the incubation, and continues to increase even at the stage of rapid decrease of free lipids.

The decrease of absorbance and of oxidized lipids observed by direct measurement was thus caused by the reaction of oxidized lipids with non-lipidic components, particularly proteins, with formation of hydrogen-bonded non-extractable fraction. The true lipoxygenase activity is found only if both free and hydrogen bonded oxidized lipids are determined.

If proteins are added to crude lipoxygenase extracts, the same decrease of oxidized lipids after the maximum was observed, as in the case of ground rapeseed.

CONCLUSIONS

1 - The determination of oxidation products of lipoxygenase-catalyzed oxidation by iodometric, spectrophotometric and chromatographic methods give similar results.

2 - Products of the lipoxygenase-catalyzed oxidation are partially bound to seed proteins, which should be taken into account in the determination of enzyme activity.

3 - Linolenic acid is oxidized at the same rate as linoleic acid.

4 - There is great variability in lipoxygenase activities among samples of rapeseed so that some samples are very prone to oxidative deterioration during their storage and processing. Such samples should be processed soon after the harvest, and the processing must be rapid in order to deactivate the lipoxygenase rapidly. The samples with low lipoxygenase activities can be stored for a longer time and processed later.

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