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STUDIES ON RANCIDIFICATION OF GROUND POPPY-SEED

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To estimate the quality deterioration caused by lipid peroxidation in stored ground poppy-seed samples, the kinetic behaviour of different biochemical parameters characteristic of lipid peroxidation was studied and compared with flavour deterioration. It was found that the probability of rapid deterioration of samples is low when the change in AC values (the ratio of the values at the time of measurement and at the beginning of investigation) for peroxide values is a linear function of storage time and when lipoxygenase activity is low. When the kinetics is immediately non-linear and high activity of lipoxygenases is detected, the possibility of rapid quality deterioration can be anticipated.

Keywords: lipid peroxidation, lipoxygenases, poppy-seed storage, rancidification

It is known that lipid peroxidation leads to rancidity in food and food raw materials stored in the presence of oxygen. Lipid peroxidation is a process mediated by the formation of free radicals at the α-methylene groups in the unsaturated long-chain fatty acid components of fats and oils (NIKI, 1993). The formation of hydroperoxides, an important step in lipid peroxidation, is often a simple autoxidation, but an alternative enzymatic reaction catalyzed by lipoxygenases is also known (BERGSTRÖM & HOLMAN, 1948). Decomposition of hydroperoxides by a radical mechanism is a complex process that leads to the formation of different short-chain ketones, aldehydes and carboxylic acids with an unpleasant odour or flavour and to dialdehydes, especially malondialdehyde (CECONI, 1993). Besides the important role that lipid peroxidation plays in flavour deterioration and rancidity in food and food raw materials (GUTTERIDGE & HALLIWELL, 1990), there is also considerable interest in its role and the role of other free radical reactions in human diseases (e.g. arteriosclerosis, myocardial infarction and cancer) (HALLIWELL & GUTTERIDGE, 1990).

Poppy-seed is used in pastries as a filling and flavouring agent in several countries, especially in Europe (MESHEHDANI et al., 1990a). The oil content of poppy-seed is relatively high (38–57%) and the oil is rich in linoleic acid (about 72%). MESHEHDANI and co-workers (1990b) found no correlation between the lipoxygenase activity and the degree of oxidation of lipids in poppy-seed.

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The applicability of biochemical parameters characteristic of lipid peroxidation, to characterize the quality of unground and ground poppy-seed was studied by KOSÁRY and CSALÁRI (1998). During these preliminary studies a special ratio of these parameters called activation coefficient (AC), was introduced. AC is the ratio of the value of the given parameter at the time of the measurement and at the beginning of the investigation. Therefore, the higher the AC value of a particular parameter, the higher is the degree of lipid peroxidation. It was found that AC values could illustrate changes in the concentration of hydroperoxides and malondialdehyde in stored poppy-seed samples better than the measured data.

In this paper we report the results of a study carried out to determine the change in the concentration of hydroperoxides and malondialdehyde, lipoxygenase activity and flavour deterioration in ground poppy-seed stored at different temperatures to estimate the quality deterioration caused by lipid peroxidation.

1. Materials and methods

1.1. Materials

Chemicals were Sigma products. Selected samples of commercial poppy-seed produced in Hungary (peroxide value: 0.018 and thiobarbituric acid assay: 0.51) were used. Selection was made on the basis of kinetic behaviour of the biochemical parameters. Before grinding the selected unground samples were stored in a deep freezer at –18 °C. Freshly ground poppy-seed samples were examined without any pre-treatment (e.g. blanching). Their water content was less than 10% during the entire storage period. We applied storage conditions that are most frequently used in the food industry (4, 20 °C) and 40 °C.

1.2. Methods

1.2.1. Measurement of peroxide values. Stages of lipid peroxidation in samples were characterized by the peroxide value (concentration of hydroperoxides, measured iodometrically) and the thiobarbituric acid test, according to literature (DAHLE et al., 1962). Ground poppy-seed samples (0.10 g) were extracted with an acetic acid–chloroform mixture (3:2) (2 ml). The mixture was centrifuged (2000 g) and a saturated aqueous potassium iodide solution (0.10 ml) was added to the supernatant. After 15 min the mixture was diluted with water (7.5 ml) and titrated with sodium thiosulfate (0.005 M) in the presence of a starch indicator (1%). One unit of peroxide value is defined as the amount (g) of liberated iodine from potassium iodide by ground poppy seed (100 g).

1.2.2. Measurement of thiobarbituric acid assays. The concentration of dialdehydes, especially malondialdehyde was measured by the thiobarbituric acid assay (PLACER et al., 1966). Ground poppy-seed samples (0.10 g) were extracted with trichloroethylene (3 ml). The mixture was centrifuged (2000 g) and thiobarbituric acid

(10 mg) in 50% aqueous acetic acid (3.0 ml) was added to the supernatant (1.0 ml). Each tube was placed in a boiling water bath for 20 min, then cooled and centrifuged to give a clear aqueous phase. The absorbance was measured at 535 nm in a DMS 100S-UV-Visible Spectrophotometer (Varian Techtron Pty. Ltd., Mulgrave, Victoria, Australia). One unit of thiobarbituric acid assay is defined as the amount (mg) of malondialdehyde in ground poppy seed (100 g).

1.2.3. Measurement of lipoxygenase activity. The lipoxygenase (EC 1.13.11.12) activity of poppy-seed samples extracted with 0.05 M TRIS acetate buffer (pH 8.2) [containing sucrose (0.38 M) and calcium chloride (0.02 M)] (100 mg ml^{-1}) was determined using linoleic acid as substrate and by measuring concentration changes of conjugated dienes at 234 nm in potassium phosphate (0.025 M), sodium borate (0.10 M) or TRIS hydrochloride (0.050 M) buffers of different pH containing 0.02% citric acid as stabilizing agent following a modified method described by MESHEHDANI and co-workers (1990b). Reaction mixtures contained the poppy-seed extract (0.030 ml), linoleic acid substrate solution (0.025 ml) and buffer (1.345 ml). One unit of lipoxygenase activity was defined as the amount of enzyme that caused one unit change in absorbance of the reaction mixture (1.0 ml) in 1 min at 234 nm. For the linoleic acid substrate solution, the mixture of 0.10 M borate buffer (pH 9.0) (5.5 ml), TWEEN 20 (0.025 ml), linoleic acid (0.025 ml) and 1 M NaOH (0.10 ml) was diluted to 10 ml with water.

1.2.4. Measurement of flavour deterioration. Flavour deterioration was determined *via* sensory evaluation by detecting the odour threshold of unpleasant flavour agents formed during storage in poppy-seed samples (AMERINE et al., 1965). Before testing the vessels of the samples were closed for 30 min at room temperature. Poppy-seed samples were tested once a week over 10 weeks by a team of five semi-trained university students.

All data are mean values obtained from three replicate experiments.

2. Results and discussion

2.1. Kinetics of peroxide values and thiobarbituric acid assays

Lipid peroxidation is one of the most serious causes of quality deterioration of oil-seeds. The quality of poppy-seed depends on both harvesting and storage conditions. In some cases when quality deterioration is in an early phase, changes in the characteristic parameters are not yet detectable, even though rancidification may occur later. To avoid marketing of such seeds, an early diagnosis of these undesirable changes is necessary.

The results of the AC values of the peroxide values and thiobarbituric acid assays of ground poppy-seed samples stored at 4, 20 and 40 °C are presented in Figs 1 and 2. Regression analysis was used to determine significant differences in AC values, and coefficients of determination were about 0.8. The kinetic behaviour of AC values of both peroxide values and thiobarbituric acid assays of ground poppy-seed samples were compared to the signs of rancidity (flavour deterioration).

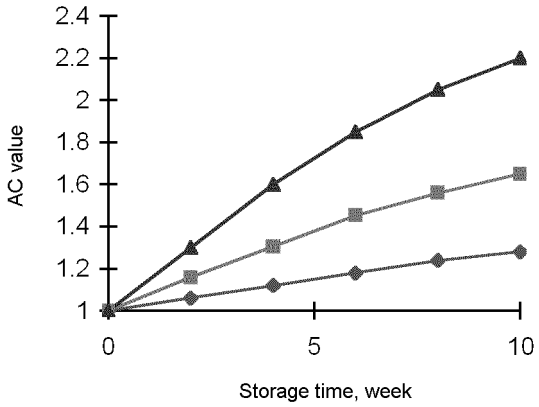

Fig. 1. Change in AC values of peroxide values (PV) with respect to storage time at 4 °C (◆), 20 °C (■) and 40 °C (▲) in ground poppy-seed samples

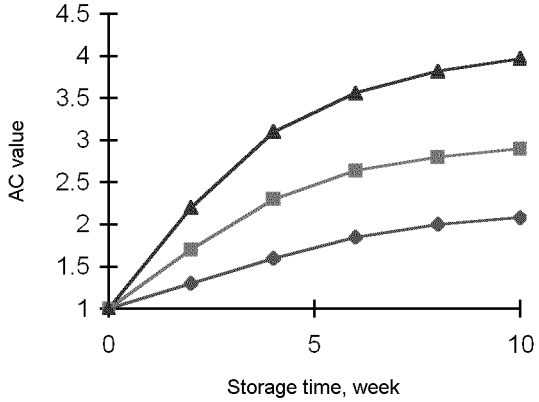

Fig. 2. Change in AC values of thiobarbituric acid assays (TBA) with respect to storage time at 4 °C (◆), 20 °C (■) and 40 °C (▲) in ground poppy-seed samples

Even after 10 weeks at –18 °C no lipid peroxidation was detected. At higher temperatures in the first few weeks (at 4 °C – 8 weeks, at 20 °C – 6 weeks, at 40 °C – 4 weeks) the change in AC values of peroxide values as a function of storage time was linear and no symptoms of rancidity were found. During this time the formation of hydroperoxides was faster than their conversion, but their concentration was rather low. In these first weeks the formation of different volatile oxidation products was slow and their concentration was below the odour threshold.

After longer storage and/or at higher temperatures the increase in AC values for hydroperoxides was not linear and the signs of rancidity could be observed only in this

non-linear period. It is supposed that in this period the decomposition of hydroperoxides becomes a complex process that leads to the intensive formation of various short-chain ketones, aldehydes and carboxylic acids having an unpleasant odour or flavour associated with rancidity. The increase in malondialdehyde concentration as a function of storage time was not linear. This increase was more rapid in the first weeks than later.

We suggest that linear kinetics of peroxide values in the initial storage period is characteristic of a good quality ground poppy-seed sample, while a ground poppy-seed sample with non-linear kinetics of peroxide values deteriorates rapidly during storage. This means that the degree of tendency for rancidification in poppy-seed samples can be predicted considering the kinetic behaviour of AC values for hydroperoxide content as a function of storage time. For example we measured non-linear kinetics in the initial storage period of ground poppy-seed samples stored in a paper bag for several months at room temperature before grinding. It is supposed that different commercial samples of poppy-seed with unknown histories can be tested by this kinetic characterisation. Among commercial poppy-seed samples, some samples were found with a tendency for rapid deterioration. We suggest that ground poppy-seeds can be stored for a prolonged time without quality deterioration only in a deep freezer (–18 °C).

The odour thresholds of ground poppy-seed samples with a low tendency for rancidification were detected after a storage period of 10 weeks (stored at 4 °C), 8 weeks (stored at 20 °C) and 6 weeks (stored at 40 °C).

The study was conducted for ten weeks, but in an attempt to shorten it an accelerated test was done by storing the samples at 50 °C for a few weeks. We found that in this case AC values of peroxide values were higher than expected on the basis of earlier results, however the linear part of the change in AC values of peroxide values with respect to storage time was practically as long as at 40 °C. On the basis of our preliminary data we hope that testing at 50 °C can also be used for a preliminary estimation of the tendency for rancidification of ground poppy-seed samples. We presume that this tendency is low when the correlation between AC values of peroxide values and storage time of the sample at 50 °C is linear for a period of at least two or three weeks.

This slightly altered kinetic behaviour of the accelerated test is thought to be due to the fact that the process of lipid peroxidation becomes more complex at 50 °C than at lower temperatures. It is supposed that the share of autoxidation and enzymatic oxidation in lipid peroxidation is slightly different at lower and higher storage temperatures. This suggested that the activity of lipoxygenases of ground poppy-seed samples should be studied.

2.2. The pH optima of lipoxygenases

It is known that poppy-seeds contain several lipoxygenase isoenzymes with different pH optima, e.g. in poppy-seeds grown in Czechoslovakia in 1985–88 pH optima were 6.5, 9.5 and 10.5 as measured in phosphate (pH<8.5) and borate (pH>8.5) buffers (MESHEHDANI et al., 1990b).

The pH optima of lipoxygenase isoenzymes of ground poppy-seed samples grown in Hungary in 1996 were determined in different buffers. It is known that for different types of lipoxygenases different substrates are the best (e.g. linoleic acid for lipoxygenase-1, arachidonic acid and methyl linoleate for lipoxygenase-2, methyl linoleate for lipoxygenase-3). Linoleic acid was found to be a satisfactory substrate for the different types of lipoxygenases, therefore for preliminary tests only this substrate was used. In these preliminary tests we found that all the three pH optima (pH 5.0, 7.3 and 8.5) of lipoxygenase isoenzymes could be established not only in potassium phosphate and sodium borate buffers, as described in the literature, but also in TRIS hydrochloride buffer.

The maximum value measured at pH 7.3 (LOX 7.3) (13 U min^{-1} g^{-1} poppy-seed) was taken as 100% and the other pH optima were at pH 5.0 (LOX 5.0) (78%) and 8.5 (LOX 8.5) (50%). The effect of storage time at 50 °C on the measured activity of different lipoxygenase isoenzymes at their pH optima is shown in Fig. 4. The measured activity of different lipoxygenase isoenzymes rose dramatically during storage. This change can be attributed to various factors e.g. the activating effect of oxygen on lipoxygenases in damaged (ground) poppy-seeds or inactivation of the antioxidant content of the seeds and it may be caused by a change in solubility or other properties of different lipid components in the samples stored at 50 °C for weeks or the combination of these factors.

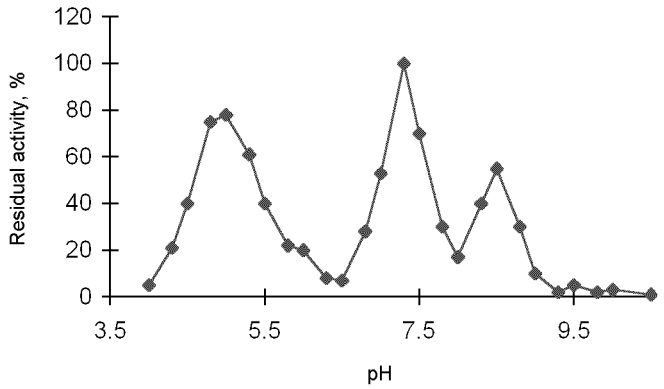

Fig. 3. Change in lipoxygenase activity of ground poppy-seed with respect to pH. The maximum activity of LOX 7.3 was taken as 100%

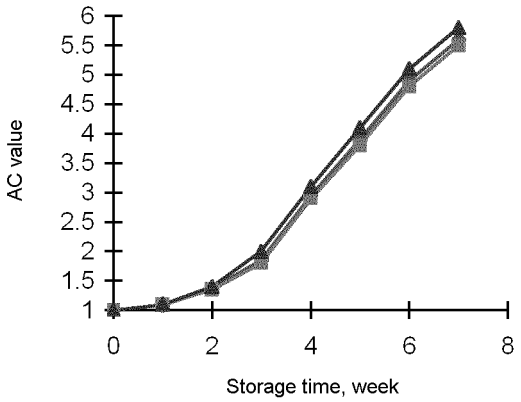

Fig. 4. Change in AC values of lipoxygenase isoenzyme LOX 5.0 (◆), LOX 7.3 (■) and LOX 8.5 (s) activities with respect to storage time at 50 °C in ground poppy-seed samples

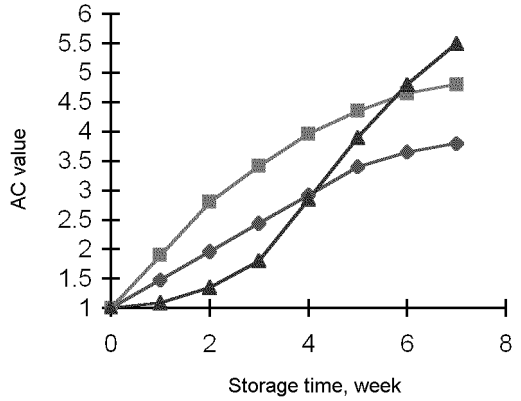

Fig. 5. Change in AC values of peroxide values (◆), thiobarbituric acid assays (■) and LOX 7.3 (s) activities with respect to storage time at 50 °C in ground poppy-seed samples

The change in measured activity of different lipoxygenase isoenzymes and the change in AC values of both peroxide values and thiobarbituric acid assays of ground poppy-seed samples stored at 50 °C are compiled in Fig. 5. The results are presented only for LOX 7.3 but the behaviour of LOX 5.0 and LOX 8.5 was almost the same.

Conclusions

On the basis of the dramatic increase in the measured activity of lipoxygenases as compared to the change in AC values of both peroxide values and thiobarbituric acid assays we suppose that the slightly altered kinetic behaviour of both peroxide values and

thiobarbituric acid assays of ground poppy-seed samples stored at 50 °C might be connected to a change in the contribution of different mechanisms to total lipid peroxidation, however a more detailed study is necessary to elucidate the behaviour of lipoxygenases and other components of the lipid peroxidation system in stored ground poppy-seed samples.

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