Aroma Compounds Derived from Oxidized Lipids. Some Biochemical and Analytical Aspects

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The formation of aliphatic aldehydes and alcohols, primarily from unsaturated fatty acids with the participation of lipoxygenase, hemoproteins, alcohol dehydrogenase, and esterase, is briefly reviewed with special regard to plant foods. The controlling or avoiding of aldehyde formation in food systems by the use of alcohol dehydrogenase and of certain sugar-amino acid reaction products was studied by use of a gas chromatographic technique.

The formation of a number of aroma compounds can be traced to lipid degradation at the various stages of food processing. This is true particularly for the formation of aldehydes belonging to the groups *n*-alkanals, *n*-alk-2-enals, and *n*-alka-2,4-dienals. These aldehydes occur in a variety of foods both of high and low fat content such as milk, meat, poultry, fish, edible oils, vegetables, tubers, fruits, and berries. Especially in the foods of plant origin the corresponding alcohols also occur either free or esterified.

In our laboratory, investigations have been carried out for a couple of years on some important biocatalysts which are involved in the formation of aldehydes and alcohols particularly from unsaturated fatty acids. Emphasis was placed on the basal conditions for lipoxygenase, hemoproteins, and alcohol dehydrogenase to act as catalysts in fresh, heat treated, and frozen vegetables.

The physiological function of lipoxygenase is unknown; its strict specificity to produce hydroperoxides by oxygenation of the *cis*-1,*cis*-4-pentadiene structures together with the fact that particular enzymes exist for the further metabolizing of hydroperoxides, suggest, however, that linoleic and linolenic acids are primary substrates of lipoxygenase. The similar nonenzymatic catalytic activity by hematin compounds most probably has no physiological function, their activity being, however, of great importance in food technology.

The catalytic properties of lipoxygenase and hematin compounds in relation to food have been comprehensively reviewed (Watts, 1954; Tappel, 1961, 1962; Love and Pearson, 1971; Grosch, 1972). Hematin compounds like hemoglobin, myoglobin, and cytochrome *c* are important lipid oxidation catalysts in animal tissues where they occur in high concentration. Corresponding knowledge for plant tissues is lacking, probably due to the fact that lipoxygenase is far superior to hemoproteins as a lipid oxidation catalyst in enzymically active tissue. In enzymically inactive material, however, the role of hemoproteins may be increased. Lipoxygenase is much more substrate specific than hemoproteins due to the enzyme nature of the former and the metal catalyst nature of the latter. Lipoxygenase is active primarily with free unsaturated fatty acids and for that reason more intimately linked to hydrolytic reactions than hemoprotein catalysts. The catalytic activity is much higher for native lipoxygenase than for native hemoproteins. The activation energy for both of these catalytic reactions was found to be in the order of 12–20 kJ mol^{-1}. Chain scission occurs to some degree during lipid oxidation resulting in a variety of volatile compounds, the major ones being aliphatic aldehydes with some hydrocarbons and alcohols also produced. The exact nature of these reactions is not yet known. Enzymatic isomerization and reduction of hydroperoxides to produce ketohydroxy and hydroxy acids have recently been reviewed (Grosch, 1972). It should be noticed that the routes leading to the formation of volatile compounds are much more important in terms of odor effect than in the actual amount of fatty acids oxidized.

The assumed participation of alcohol dehydrogenase in the conversion of these kinds of aldehydes into alcohols and *vice versa* was based on the broad substrate specificity found for both the liver and the yeast enzyme and that alcohol dehydrogenase is of widespread occurrence in the plant kingdom.

LIPOXYGENASE

Cotyledons of green peas were found to contain a significantly higher specific activity of lipoxygenase than the skins, while peroxidase (the most abundant hematin compound in green peas), free linoleic, and linolenic acid were found to be evenly distributed over the entire pea seed. The latter fact is also indicative of an even distribution of lipid hydrolases in the seeds (Eriksson, 1967).

In order to study the production of hydroperoxides and volatile compounds from oxidized linoleic acid as well as the thermal inactivation of pea lipoxygenase and the activation energy of the reaction catalyzed by it, this enzyme was purified, by ammonium sulfate precipitation, gel chromatography, and ion exchange chromatography. One important purity criterion was the complete absence of hemoproteins like peroxidase and catalase because of their possible interference with the activity measurements (Eriksson and Svensson, 1970).

The pea enzyme, like that of hemoproteins, was found to produce equal amounts of the 9- and 13-hydroperoxyoctadecadienoic acid at pH 6.8 at 0° both when the supply of oxygen was free and when restricted (Leu, 1974). This was measured by a gas chromatographic technique after conversion of the hydroperoxides into trimethylsilyl ethers of methyl hydroxystearate (Eriksson and Leu, 1971).

The volatile compounds produced in linoleic acid oxidation catalyzed by purified pea lipoxygenase were analyzed by gas chromatography–mass spectrometry. The principal compounds identified were: *n*-propan-1-ol, *n*-pentan-1-ol, *n*-hexanal, *n*-hept-*trans*-2-enal, *n*-oct-*trans*-2-enal, *n*-non-*trans*-2-enal, *n*-nona-*trans*-2,*trans*-4-dienal, *n*-deca-*trans*-2,*cis*-4-dienal, *n*-deca-*trans*-2,*trans*-4-dienal, and 2-*n*-pentylfuran.

Thermal inactivation of purified pea lipoxygenase was carried out under carefully controlled conditions in a continuous flow apparatus. By this technique, basic data on the time–temperature–pH influence on the thermal inactivation of pea lipoxygenase were presented, showing first-order inactivation kinetics and maximal thermal stability of the enzyme around pH 6. No reactivation of thermally inactivated lipoxygenase was observed (Svensson and Eriksson, 1972a). Exposure of pea lipoxygenase to increasing amounts of linoleic acid hydroperoxides (the products of lipoxygenase-catalyzed reactions) during the heat treat-

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ment resulted in a drastic lowering of the stability of the enzyme. The maximal effect was obtained when the molar ratio of linoleic acid hydroperoxide to enzyme reached unity, indicating an interaction at a single site of the enzyme molcule (Svensson and Eriksson, 1972b).

HEMOPROTEINS

The investigations of hemoproteins as lipid oxidation catalysts were performed with purified bovine liver catalase and horseradish peroxidase after a preliminary study of pea peroxidase. Using a spinning ampoule technique for heat treatment the nonenzymatic lipid oxidation activity of heat-treated peroxidase and catalase was found to increase considerably and simultaneously with the decrease in their enzymatic activities (Figure 1). This indicated that protein denaturation was involved in the increased nonenzymatic activity which is supported by the fact that denaturation with acid and 8 *M* urea gave almost identical results (Eriksson *et al.*, 1971).

The thermal increase of the nonenzymatic lipid oxidation activity of peroxidase was found to occur mainly in the pH region 5.5–6.5. Heat treatment below pH 5.5 gave no activity increase, while treatment at gradually higher pH values resulted in a steep increase of the thermally induced activity. Electron microscopy of treated peroxidase samples indicated that the catalytically inactive material obtained by heat treatment at pH 4.94 consisted of irregularly shaped aggregates, *e.g.*, rods 3000–4000 Å in length, while identical treatment at pH 7.24 gave a catalytically active material consisting of spheres 100–200 Å in diameter. Both types of aggregates were much larger than native peroxidase identically prepared for electron microscopy. Small angle X-ray scattering measurements revealed an average gyration radius of 70 Å for a large molecular size fraction from a peroxidase sample heat treated at pH 7.24. By gel chromatography, using tandem columns containing Sephadex G-150 and Sepharose 6B respectively, peroxidase exposed to varied time–temperature combinations at pH 6.5 could be separated according to aggregate dimensions into three major fractions. The most interesting observation was the almost complete association of lipid oxidation activity with the aggregate fractions, whose heme content per peroxidase unit had been doubled due to heme migration (Eriksson and Vallentin, 1973).

ALCOHOL DEHYDROGENASE

The C_2–C_{10} saturated and C_6–C_{10} 2-unsaturated aliphatic *n*-aldehydes were all reduced to alcohols in the presence of NADH and pea alcohol dehydrogenase. Except for ethanal, there was a marked dependence of the reaction rate on the chain length of the saturated aldehydes, a maximum appearing at C_6, *i.e.* *n*-hexanal. For the 2-unsaturated aldehydes, which are poorer substrates than the saturated ones, the reaction rate increased only slightly with chain elongation. The equilibrium constant, *K*, of alcohol-aldehyde systems in the presence of cofactors was found to vary with carbon chain length and unsaturation of the substrates. From the standpoint of flavor chemistry, these results were most interesting because of the inherent possibility of variation within the alcohol–aldehyde pool of plants. This may best be illustrated by: *n*-hexan-1-ol–*n*-hexanal and *n*-hex-*trans*-2-en-1-ol–*n*-hex-*trans*-2-enal, where the presence of the 2-double bond increased the *K* value 100-fold from 1.4×10^{-11} to 1.4×10^{-9} *M* at pH 8 (Eriksson, 1968). Recent studies on the substrate specificity of dehydrogenase from oranges (Bruemmer and Roe, 1971) and potato (Davies *et al.*, 1973) basically arrived at the same result.

ESTER HYDROLYSIS

Hydrolytic activity toward volatile aliphatic esters was found to be located mainly in the skins of green peas. A purified pea esterase was found to be active, in increasing order on ethyl, propyl, butyl, pentyl, and hexyl acetates when the assays were made below the saturation concentration of hexyl acetate, the least soluble substrate (I. Yamashita and C. E. Eriksson, unpublished data). Thus, esters may also be sources of some of the alcohols found in green peas.

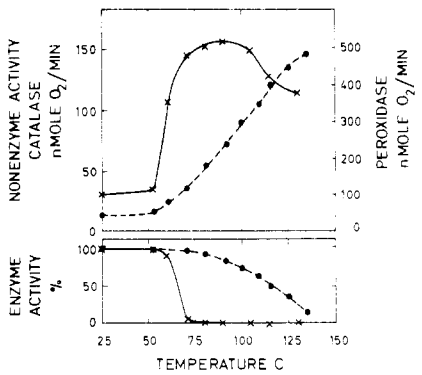

Figure 1. Nonenzymatic linoleic acid oxidation activity (upper part) and enzyme activity (lower part) of bovine liver catalase (×–×) and horseradish peroxidase (●- - -●) after heat treatment at pH 6.5 for 2 min at the temperatures indicated by the abscissa (Eriksson *et al.*, 1971).

ACTIVATION ENERGY AT FREEZING CONDITIONS

The major supercooling agents in plant tissues are sugars, notably sucrose which accounts for approximately 5% of the fresh weight of green peas. The various enzymes in frozen vegetables may thus operate in concentrated sugar solutions, a fact that could influence quality of fruits and vegetables during freezing (Joslyn, 1966).

While *in vitro* activation energy of aldehyde reduction catalyzed by alcohol dehydrogenase in concentrated sucrose or glycerol solutions was found two to three times as high as in buffers containing no supercooling agent, the activation energy of linoleic acid oxidation by lipoxygenase, thermally denatured peroxidase, and hematin was decreased in the presence of such agents (Table I).

The different activation energies of aldehyde production on one side (lipoxygenase, hematin compounds) and that of aldehyde reduction on the other (alcohol dehydrogenase) could be used to demonstrate the theoretical effect of lowering the temperature. By setting the reaction rate to 100% at 20° for linoleic acid oxidation catalyzed by hematin, denatured peroxidase, and lipoxygenase and for *n*-hexanal reduction by alcohol dehydrogenase, the relative rates at −20° in supercooled media will be 40, 30, 20, and 3%, respectively, thus a considerably larger effect on the reduction of aldehydes (Eriksson and Svensson, 1974).

ALDEHYDE CONTROL

The above results may give a biochemical explanation to the formation of aliphatic alcohols in fresh vegetables and the accumulation of aliphatic aldehydes in frozen blanched and dried peas, the latter phenomenon being known to cause flavor deterioration not only in stored vegetables, but also in most other stored foods.

Efforts to avoid or control the appearance of the aldehydes due to lipid oxidation in various foods may be made by conventional methods such as removal of oxygen and addition of synthetic antioxidants or by as yet undeveloped methods, such as conversion of the aldehydes into nonrancid compounds, particularly alcohols, or induction of the formation of lipid oxidation protectives during fermentation or thermal processes, *e.g.*, from combinations of sugars and amino acids.

In our laboratory we have studied the unconventional methods in more detail. The importance of the aldehyde–alcohol conversions in foods was stressed by experiments

Table I. Activation Energy of Linoleic Acid Oxidation and Aldehyde Reduction in Buffer and Supercooled Media (from Eriksson and Svensson, 1974)

Substrate	Catalyst	Activation energy, kJ·mol^{-1} (temp range, °C) Buffer	Supercooled media
Linoleic acid	Pea lipoxygenase	23 (10–20)	
		39 (0–10)	27 (– 3 to – 20)
	Denatured horseradish peroxidase	44 (0–20)	19 (0–20)
	Hematin	49 (0–10)	15 (– 3 to – 20)
n-Hexanal	Pea alcohol dehydrogenase	18 (1–20)	56 (– 10 to 25)
n-Hex-*trans*-2-enal	Pea alcohol dehydrogenase	19 (1–20)	44 (– 10 to 25)
n-Hept-*trans*-2-enal	Pea alcohol dehydrogenase	19 (1–20)	36 (– 10 to 25)

Table II. Formation of *n*-Hexanal during Linoleic Acid Oxidation Catalyzed by Denatured Horseradish Peroxidase in the Presence of Varying Amounts of *n*-Propyl Gallate

Mol of *n*-propyl gallate/mol of linoleic acid	*n*-Hexanal, ppm, in medium at time, hr 0	1	2.5	4.5	24
0	0.017	0.088	0.183	0.312	1.550
0.0016	0.005	0.021	0.043	0.081	0.921
0.008	0.022	0.048	0.078	0.119	0.142
0.040	0.017	0.016	0.019	0.020	0.021

where the odor detection concentrations in water of the aldehydes originating from lipid degradation were found to be 20–250 times lower than those of the corresponding alcohols (C. E. Eriksson *et al.*, unpublished data). In other words, the conversion of even a small amount of the aldehyde above the detection concentration into the alcohol will cause considerable odor reduction and *vice versa*. Some experiments along these lines were made in our laboratory with milk and rehydrated instant potato where additions of alcohol dehydrogenase and NADH considerably reduced the concentration of *n*-hexanal, *n*-hex-*trans*-2-enal, *n*-hept-*trans*-2-enal, and *n*-oct-*trans*-2-enal in the headspace and simultaneously the smell of these materials (C. E. Eriksson, unpublished data). Attention has also been paid to the possibility of influencing the alcohol-aldehyde flavor contribution by controlling the NAD/NADH ratio, *e.g.* in citrus fruits (Bruemmer, 1969). Techniques like these ones may be applicable to certain foods of low fat content which are still disposed to rancidity.

Another approach concerns the possibility of reducing or preventing lipid oxidation and thus aldehyde formation by sugar–amino acid reaction products. In order to obtain a more concrete connection of the results with sensory data, a gas chromatographic technique has been developed. This allows direct recording of the influence of various additions on the formation of aldehydes during lipid oxidation rather than using conventional lipid oxidation measurements like peroxide value, thiobarbituric acid reactivity, etc., which usually correlate poorly with sensory responses. In addition, the gas chromatographic procedure gives much more information about the oxidizing system.

The procedure mentioned is based on a precolumn concentration technique; the collected volatile compounds are separated on a capillary column. For convenience, a few easily recognizable compounds have been selected for studying the kinetics of their formation from linoleic acid oxidized in the presence of air and denatured horseradish peroxidase as the catalyst. *n*-Hexanal is formed at a constant rate for 24 hr, while *n*-hept-*trans*-2-enal is formed at a decreasing rate and *n*-oct-*trans*-2-enal at an increasing rate in the same linoleic acid oxidation. The effect of antioxidant addition, *e.g.*, *n*-propyl gallate, on the formation of *n*-hexanal can be recorded directly from the gas chromatograms (Table II) (Eriksson *et al.*, 1974).

By this technique we studied the retardation of lipid oxidation with different sugar–amino acid reaction products (produced by refluxing water solutions of the components for 5 hr) whereby results similar to those where *n*-propyl gallate had been used were obtained. The effect of such products varied greatly with the sugar and amino acid involved, xylose–arginine and xylose–lysine combinations being the most efficient ones. These results might be useful for application studies with foods which are formulated from protein, fat, and carbohydrates. Intense work along these lines is presently being carried out in our laboratory.

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