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Studies on the stability and antioxidant properties of butylated hydroxyanisoles

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STUDIES ON THE STABILITY AND ANTIOXIDANT PROPERTIES OF BUTYLATED HYDROXYANISOLES

by

RONALD KIRKBY

A Master's Thesis
submitted in partial fulfilment of the requirements
for the award of Master of Philosophy of the
Loughborough University of Technology, March 1988

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ABSTRACT

Butylated hydroxyanisole (BHA) is one of the most widely used synthetic antioxidants added to foodstuffs. BHA consists of a mixture of isomers, 3-tert-butyl-4-hydroxyanisole (3-BHA) and 2-tert-butyl-4-hydroxyanisole (2-BHA). According to the EEC Regulations, BHA must not be less than 98.5% $C_{11}H_{16}O_2$ and not less than 85% of the 3-BHA. Since the 3-BHA is considered to be the active form of BHA, the permitted level of the 2-BHA, up to 15%, is very high when considered in relation to the strict regulations applied to the purity of other food additives.

Samples of 3-BHA and 2-BHA were prepared and their stability to heat and light together with their antioxidant properties were investigated. Samples of the separate isomers and a 9:1 mixture of 3-BHA to 2-BHA (typical proportions in food grade BHA) were heated (115°C) in water at varying pHs under either air or nitrogen in sealed tubes. The aqueous mixtures were extracted with chloroform and the amount of decomposition determined by g.l.c. using diphenylamine as an internal standard. Similar mixtures were prepared and left in daylight for several weeks, before extraction and examination by g.l.c. The antioxidant properties of the separate isomers and a 9:1 mixture of 3-BHA to 2-BHA were examined using a Rancimat and various samples of oils.

The results obtained showed that both isomers were in general more stable under acidic or neutral conditions than alkaline, and more stable under nitrogen than air. The light treatment caused more decomposition than the heat treatment. The 2-BHA was in general found to be more stable than the 3-BHA; the stability of the 3-BHA did not differ appreciably from that of the mixture. The results obtained with the Rancimat indicated little difference between the 3-BHA and the 9:1 mixture. The 2-BHA was less effective, but did show some antioxidant activity.

The results indicate that using pure 3-BHA as a food antioxidant, rather than a mixture of 3-BHA and 2-BHA, would be feasible with regard to stability and antioxidant properties, and would involve relatively little extra cost.

DEDICATION

This work is dedicated to my wife, Lyn, whose love and encouragement has helped me to complete this and my previous studies with the Open University. I also wish to include my daughter, Anne, and son, Paul, for the support they have given me.

ACKNOWLEDGMENTS

I should like to acknowledge the assistance provided by my Supervisor, Dr. S.W. Hanson, and my Director of Studies, Professor John Mann. I also wish to thank Dr. W.R.B. Arthur, Head of School of Food Studies, Humberside College for his help with administrative matters.

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1. INTRODUCTION

Foods being composed of complex and reactive compounds such as lipids, proteins, carbohydrates and vitamins are susceptible to quality changes when subjected to manufacturing processes and storage. Lipid material undergoes various deterioration processes, including both enzymic processes and chemical processes such as hydrolysis and the action of atmospheric oxygen, i.e. autoxidation.

Numerous edible lipid foodstuffs, such as lard, cooking oil and margarine, and foods containing oils and fats, such as baked products and potato crisps, frequently become rancid on storage due to autoxidation. The rate of lipid autoxidation depends on numerous factors including light and heat, traces of certain transition metals and the composition of the lipid itself.

Some naturally occurring compounds such as ascorbic acid (vitamin C) and α-tocopherol (vitamin E) exhibit antioxidant properties, but are normally present in insufficient amounts in food products to completely prevent autoxidation. It is therefore often found to be necessary to add synthetic antioxidants. Two of the most common synthetic antioxidants in use are butylated hydroxyanisole and butylated hydroxytoluene.

Butylated hydroxyanisole (BHA) consists of a mixture of 3-tert-butyl-4-hydroxyanisole and 2-tert-butyl-4-hydroxyanisole. According to the EEC regulations BHA (EEC code : E320) must not be less than 98.5% $C_{11}H_{16}O_2$ and not less than 85% of the 3-isomer. Since the 3-isomer is considered to be the active form of BHA, the permitted level of the 2-isomer, up to 15%, is very high

relative to the strict limits applied to the purity of other food additives. BHA is now probably the most commonly used synthetic antioxidant in the U.K. partly due to its good carry over properties in baked food-stuffs.

The work described in this dissertation investigated the stability and antioxidant properties of the two BHA isomers and a typical commercial mixture of the isomers.

2. LITERATURE SURVEY

2.1 LIPID AUTOXIDATION

2.1.1 Lipid autoxidation in relation to other types of rancidity

The development of rancidity, i.e. off-flavour in foodstuffs resulting from reactions of lipid, may be hydrolytic or oxidative and may involve enzymic or non-enzymic processes.

Hydrolytic rancidity occurs as a result of hydrolysis of lipids to glycerol and free fatty acids. The effect of hydrolysis is dependent upon the fatty acid composition of the triglycerides. Triglycerides containing low molecular weight fatty acid groups, i.e. 4-12 carbon atoms, yield characteristically unpleasant odours. For example, hydrolysis of butter yields the rancid smelling butyric acid. Lipids containing fatty acid groups with 14 or more carbon atoms are not liable to hydrolytic rancidity as the free acids are involatile and hence odourless. Hydrolysis can be either enzymic, catalysed by lipase or phospholipase enzymes, or non-enzymic (Dugan, 1976).

Lipid rancidity developing as a result of oxidation, i.e. oxidative rancidity, is a major cause of food deterioration. Oxidative rancidity is caused by the reaction of unsaturated lipids with oxygen and can be both enzymic and non-enzymic. A good example of an enzyme catalysed oxidation is the off-flavour occurring in soya flour. Investigation showed that this off-flavour was caused by oxidative breakdown of unsaturated fatty acids to small molecular weight compounds, in particular pent-1-en-3-one (Mattick and Hand, 1969; Wilkins, Mattick and Hand, 1967). Oxidative

reactions that are catalysed by enzymes such as lipoxygenase cannot be stopped by antioxidants and generally the enzyme must be inactivated. Although there are similarities with the lipid autoxidation process discussed below, one major difference is that the fatty acid chains must be polyunsaturated for the enzymic process to occur (Heimann, 1980).

The major non-enzymic lipid oxidation process, which involves oxidation of lipids by atmospheric oxygen, is generally referred to as autoxidation since the rate of oxidation increases as the reaction proceeds. The process occurs with both monounsaturated and polyunsaturated fatty acid groups and is catalysed by certain transition metals.

A less important non-enzymic lipid oxidation process is the so-called photosensitised oxidation process which is thought to involve singlet oxygen reacting directly with carbon-carbon double bonds (Frankel, 1984). Although under conditions of high light input and in the presence of sensitizers such as chlorophyll, this process may become significant, it is generally considered to be of only minor importance in food storage compared with the lipid autoxidation process.

2.1.2 Importance of lipid autoxidation in foodstuffs

Rancidity of edible oils and fatty foods due to lipid autoxidation is a serious problem in many sectors of the food industry and is the limiting factor in the storage life of a wide range of "fatty" food products or foods with added fats, e.g. margarine, lard, biscuits, crisps and other snack foods. Factors which have contributed to this problem in recent years are the increased emphasis on polyunsaturated dietary lipids and the fortification of certain foods with iron.

Problems also occur due to rancidity in "non-fatty" food products. Many living tissues, contain very low amounts of lipid and in these triglycerides may represent a minor proportion of the lipids. Nevertheless, rancidity caused by lipid oxidation may be a major problem in such materials. This is true of many plant products, for example dehydrated potato preparations may undergo very rapid lipid oxidation to give unacceptable products although the total lipid content of potatoes is only 0.1% of the fresh weight. The cause of rancidity in such low fat products lies mainly in the polar lipids (phospholipids and glycolipids) which are components of membrane structures in all living cells. These membrane lipids commonly contain high proportions of polyunsaturated fatty acids. In the case of potato lipids, linoleic and linolenic acids together represent 75% of the total fatty acids (Galliard, 1973).

2.1.3 Mechanism of lipid autoxidation

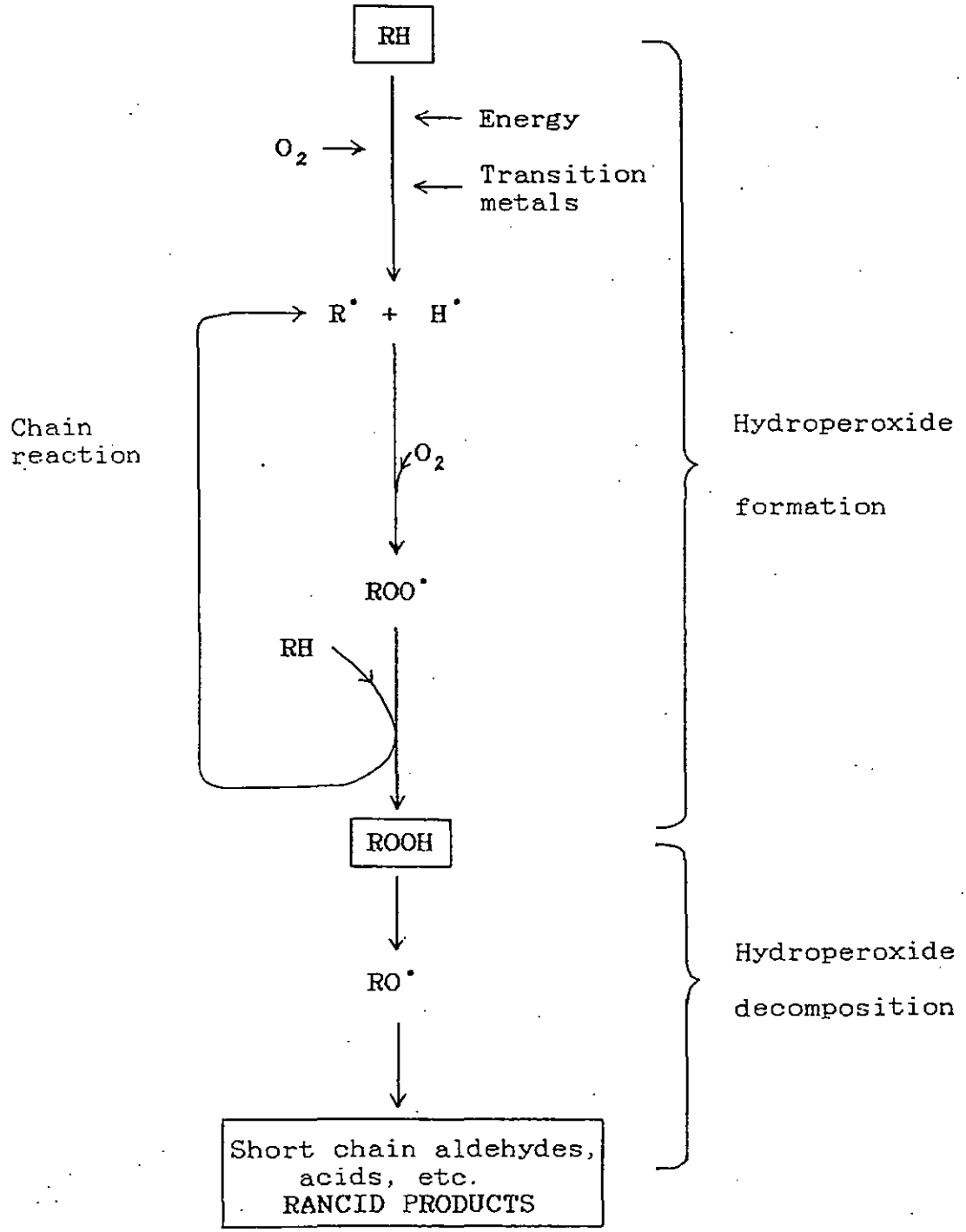
The autoxidation of unsaturated fatty acyl groups occurs by a free radical mechanism. The overall scheme is summarised in Fig. 1. As with all free radical reactions the process can be divided into three phases:

Initiation : oxygen, energy (light, heat), traces of transition metals and peroxides are involved in decomposition of the unsaturated lipid RH to give highly reactive free radicals ($R^{\bullet}$)

$$RH \xrightarrow{\text{initiators}} R^{\bullet} + H^{\bullet}$$

In practice initiation is a complex and poorly understood process possibly involving oxygen in a singlet state (Dugan, 1976). Normally oxygen exists in a

Fig. 1: Scheme for the conversion of RH (an unsaturated fatty acyl compound) to rancid products



triplet state but it has been shown that olefinic molecules react directly with electrophilic singlet oxygen. Singlet oxygen could react with RH with change of spin and with conservation of energy since RH is in a singlet state. The presence of chlorophyll or haem pigments, i.e. myoglobin, haemoglobin and their derivatives, could convert oxygen to the singlet state by photosensitisation. Thus trace amounts of pigments or other sensitisers may be responsible for the initial formation of hydroperoxides in some systems.

The actual hydroperoxides formed from a particular fatty acyl compound, and hence also the rancid products, are determined by the positions of the double bonds within the fatty acid chain. The hydrogens α to the double bonds are most labile because of the electron distribution at the double bond and because of stabilisation of the allylic radicals that are formed. These hydrogens are therefore readily abstracted by a peroxy radical in the propagation stage.

Propagation : Free radicals react with oxygen to produce peroxide radicals (ROO$^\bullet$). Peroxide radicals have the ability to attack unsaturated fatty acyl compounds RH. The result is a hydroperoxide and a free radical (R$^\bullet$). Hence a chain reaction occurs:

$$R^\bullet + O_2 \longrightarrow ROO^\bullet$$

$$ROO^\bullet + RH \longrightarrow ROOH + R^\bullet$$

Hydroperoxides are unstable compounds which decompose to give radicals, e.g. RO$^\bullet$ and $^\bullet$OH, which can initiate further breakdown of RH. The RO$^\bullet$ radicals react further to give the rancid products, short-chain aldehydes, acids and ketones as discussed below.

Termination : The quantity of highly reactive radicals rises constantly until they begin to interact. Then the concentration of radicals and peroxide falls and stable deterioration products are formed, thus limiting the rate of the hydroperoxide formation process.

$$R^{\bullet} + R^{\bullet} \longrightarrow RR$$
$$ROO^{\bullet} + R^{\bullet} \longrightarrow ROOR$$

Hydroperoxides are readily decomposed by heat, light and metal catalysis with the means depending on the system in which they exist. Decomposition of hydroperoxides produces additional radicals which add to the chain process thus causing acceleration of oxidation without requiring new initiation events.

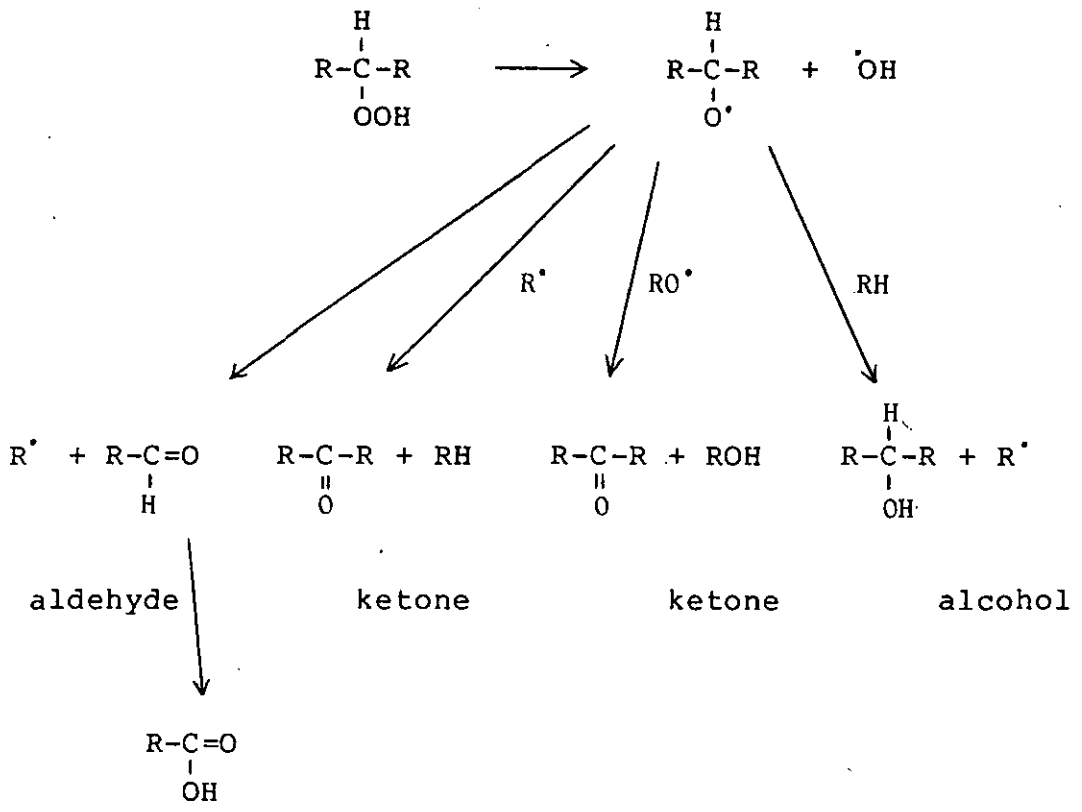
A monomolecular decomposition can occur as $ROOH \longrightarrow RO^{\bullet} + {}^{\bullet}OH$ when hydroperoxide concentration is low. High hydroperoxide concentration can give rise to a bimolecular decomposition, as $2ROOH \rightarrow RO^{\bullet} + ROO^{\bullet} + H_2O$ (Dugan, 1976). The more energetic $ROO^{\bullet}$ radical predominates in the chain propagation stage whilst the $RO^{\bullet}$ radicals participates to a lesser extent.

Metal pro-oxidants, such as copper or iron, act as hydroperoxide decomposers contributing to the formation of additional radicals. A metal capable of existing in two valence states functions typically as :

$$M^{+} + ROOH \longrightarrow RO^{\bullet} + {}^{-}OH + M^{2+}$$
$$M^{2+} + ROOH \longrightarrow ROO^{\bullet} + H^{+} + M^{+}$$
$$2ROOH \longrightarrow RO^{\bullet} + ROO^{\bullet} + H_2O \qquad \text{net reaction}$$

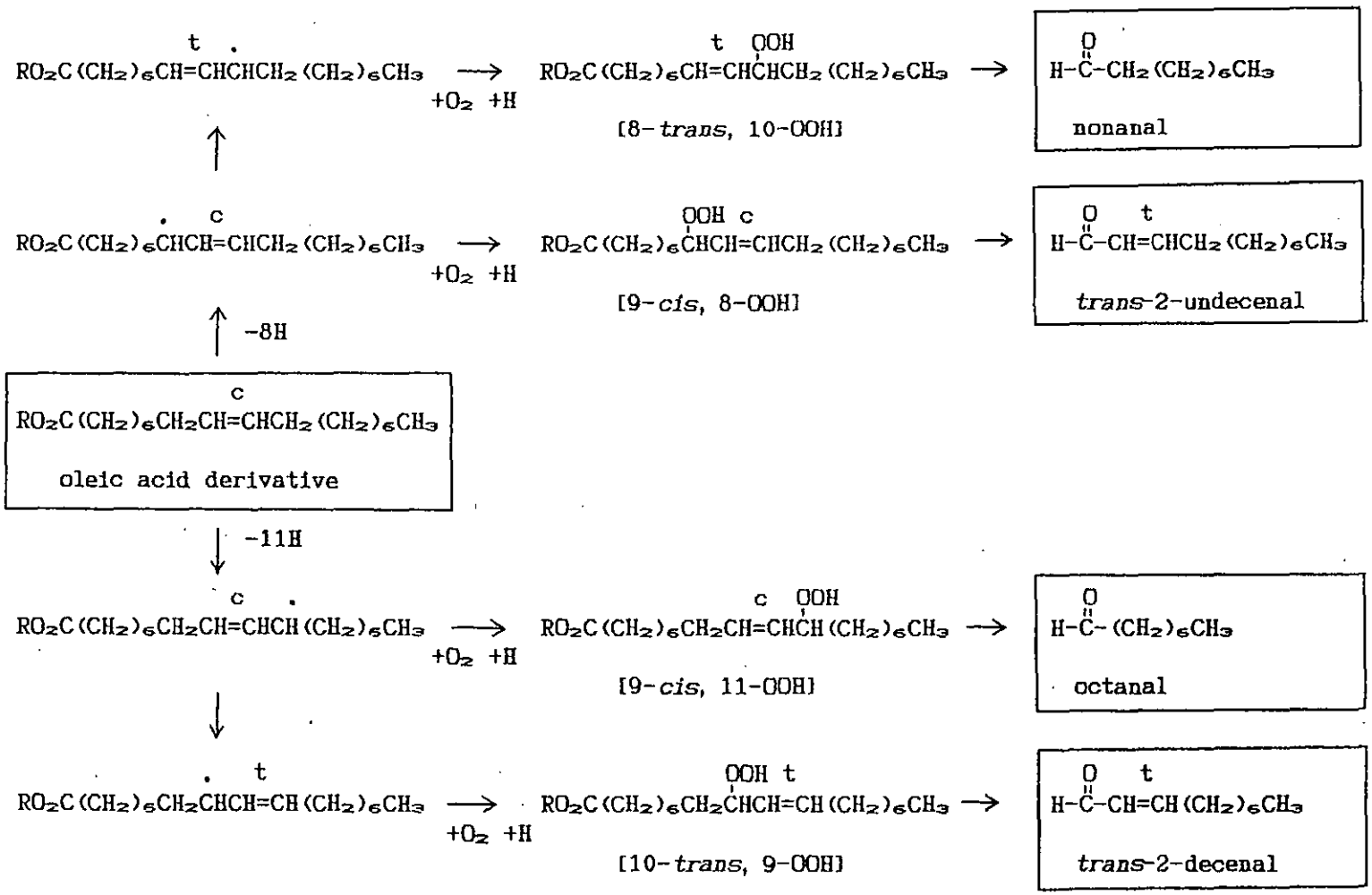
When a hydroperoxide decomposes to form $RO^{\bullet}$ radicals, these are capable of a series of reactions leading to several products which can be isolated from

oxidizing lipid systems. The following reactions appear to be involved (Dugan, 1976).



Some of these products are radicals and are capable of continuing in the chain progagation process. Others, such as the aldehydes and acids are commonly found in oxidizing lipid systems. The aldehydes, many of which are short chain, and the short-chain acids derived by further oxidation of these aldehydes, are largely responsible for the off-odours characteristic of rancid foods. The derivation of short-chain aldehydes in oleate autoxidation is shown in Fig. 2. Nonanal and trans-2-decenal are normally the major products and trans-2-undecenal and octanal minor products.

Fig. 2: Autoxidation of an oleic acid derivative



2.1.4 Factors affecting the rate of autoxidation

Some of the major factors which affect the rate of lipid autoxidation are discussed in this section, with the exception of antioxidants which are dealt with separately in Section 2.2.

2.1.4.1 Lipid structure

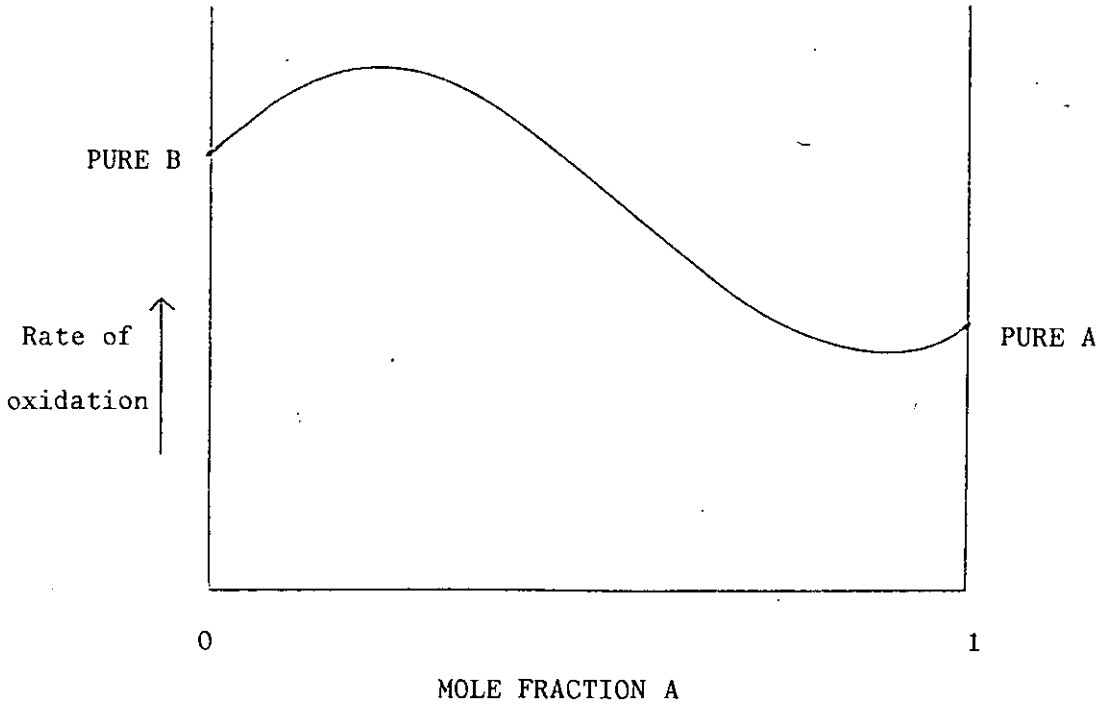
The rate of autoxidation is not a direct function of the number of unsaturated groups but increases drastically as the number increases. For example in the series stearic (C18:0; 18 carbon atoms, 0 double bonds), oleic (C18:1 Δ9; 18 carbon atoms, 1 double bond at the 9 position), linoleic (C18:2 Δ9,12) and linolenic (C18:3 Δ 9,12,15) acids, stearic is not oxidised, linoleic oxidises 10 times faster than oleic and linolenic 20 to 30 times faster than oleic.

The reason for the increased rate with more highly unsaturated fatty acids is due to the stability of the conjugated systems which is formed on abstraction of a hydrogen from the CH_2 group between the two double bonds.

2.1.4.2 Competitive oxidation

Work by Russel (1955), showed that in pure systems the expected dilution effect did not occur when mixing a fast oxidizing substrate with a low one. In fact both pro-oxidant and anti-oxidant phenomena were observed as seen diagrammatically in Fig. 3. Thus the addition of a small amount of highly reactive substrate slows the oxidation rate and vice-versa, due to cross termination steps.

Fig. 3: Rate of autoxidation of a binary mixture



2.1.4.3 Energy input

Increase in temperature results in increased rates of reaction, due in part to the usual activation energy considerations. However, it has also been proposed that increased temperature can be responsible for direct attack of oxygen. Perhaps at higher temperatures the singlet state oxygen formation increases. Ultra violet light would be a good initiator under this hypothesis if the proper sensitizers were present. Chahine and DeMan (1971) found very rapid oxidation of corn oil in u.v. light. A calculation of the activation energy from their results showed a value of about 4 kcal/mole, greatly reduced from the 35-65 kcal/mole predicted for RH/O_2 interaction. Again, this supports singlet oxygen initiation possibly through the photosensitization with the traces of chlorophyll in the corn oil or the free metals. Under extreme conditions, the light induced, non-radical, photosensitized oxidation can predominate resulting in the rapid production of a different product mixture to lipid autoxidation (Frankel, 1984).

2.1.4.4 Availability of oxygen

Since oxygen is involved stoichiometrically in the lipid autoxidation process, access to oxygen is necessary for the reaction to take place.

Access can be reduced by vacuum packaging, nitrogen packaging, glazing of fish and canning. Access can be increased in comminuted products and powders.

2.1.4.5 Moisture content

The properties of water most relevant to lipid oxidation are :

1. Water acts as a solvent.
2. Water mobilizes reactants.
3. Water can interact either chemically or by hydrogen bonding with other species.

It has been found that there is an optimum moisture level for dehydrated foods which prevents or reduces the rate at which rancidity develops. The basic protective function that water exhibits when the moisture content increases from the absolute dry state can be accounted for by two factors:

1. Metal catalysts become less effective through changes in their co-ordination sphere due to interaction with water.

2. Hydrogen bonding of water with hydroperoxides ties them up so that they are no longer available for decomposition through initiation reactions.

The rate of initiation is slowed by these two factors and other work showed that water causes a rapid loss in free radicals in dehydrated systems possibly through recombination reactions.

Salwin (1959) proposed an important theory that water attached to sites on the food surface which excluded oxygen from the lipid and this slowed lipid oxidation. Therefore one would expect that if lipid oxidation was the only possible reaction, the higher the water content or water activity, the slower the reaction. However, Labuza (1971) pointed out that above a certain water activity there seemed to be an increase in the rate of

oxidation. It was found that in the intermediate moisture range i.e. a water activity range of 0.55 to 0.85, the oxidation rate increased to a maximum. At these higher water activities it has been found that the solvent and mobilization properties of water became more important. Easier mobilization of catalysts present together with possible swelling of the solid matrix exposing new catalytic sites increases oxidation rates even higher than in the dry state. Thus the presence of water significantly affects the rate of lipid oxidation.

2.1.4.6 Transition metals

The induction period is decreased and the rate of oxidation increased by transition metals which possess two or more valency states with a suitable oxidation-reduction potential between them. These metals include: copper, iron, cobalt, manganese and nickel, as well as others of less importance (Labuza, 1971). These metals are normally present in the necessary trace amounts in foods to catalyse lipid autoxidation.

A metal hydroperoxide is most likely formed by addition into the co-ordination sphere of the metal, before decomposing to free radicals The most active metals usually have a valence state of change from +3 to +2, with reduction of the hydroperoxide decomposition activation energy being due more to the oxidized +3 state. Competition for the metal by other co-ordination species may either reduce or raise the oxidation rate by changing the electronic structure of the outer shell of the complex.

Certain chelating complexes may reduce the catalytic effectiveness of a metal by steric hindrance even though all co-ordination positions are not filled.

2.1.5 Measurement of autoxidative rancidity development

Since the consequences of lipid autoxidation are extremely important in the food industry, various methods have been devised to measure the onset of rancidity. The methods developed can give a reasonably accurate indication of the product shelf-life in a relatively short time period. Since the rate of a reaction increases exponentially with the absolute temperature, this parameter is usually singled out to speed up oxidation giving rise to accelerated shelf life tests (A.S.L.T.).

The most commonly used methods for assessing stability or rancidity of lipids are the Schaal Oven Test, Active Oxygen Method (A.O.M.) Oxygen Absorption Method (O.A.M.), and a new method, the Rancimat.

2.1.5.1 The Schaal Oven Test

The test is normally carried out in an oven with forced draft ventilation at 63°C. The sample is stored in glass beakers covered with watch glasses and smelled daily until rancidity is detected. Usually, as the end of the induction period approaches, the sample darkens in colour at which stage organoleptic rancidity soon becomes evident. The oven test is reported as the number of days required for rancidity to be detected.

The principal limitation of this method is personal evaluation of the point at which organoleptic rancidity occurs. Lipids with shorter induction periods are easier to judge because they become rancid so quickly at the end-point that there is a distinct change in odour between daily inspection. Those with longer induction periods are more difficult since the day to day change is less.

2.1.5.2 The Active Oxygen Method

The Active Oxygen Method (A.O.M.) is based on the principle that lipid autoxidation is greatly accelerated by aeration at a constant elevated temperature. The aeration tubes, containing the sample are first brought to a temperature of 98°C by immersion in hot water, and then placed in the bath and connected to the air-distributing system. The rate of air flow is adjusted to 2.33 ml per second by means of a flowmeter. At regular intervals, the samples are examined, and as soon as any off-odours are detected small samples (ca. 0.2 g) are removed for peroxide value determination. The tests are continued until the samples are definitely rancid, during which time peroxide values for at least three time intervals are obtained. The stability is then estimated from the peroxide-time curve and reported to the nearest half-hour.

The peroxide value achieved by A.O.M. at which fat will be rancid by organoleptic evaluation varies with the nature of the fat. General practice is to use a PV of 20 mEq/kg for lard and 100 mEq/kg for vegetable oils and hydrogenated fats.

2.1.5.3 Oxygen Absorption Method

The principle behind the Oxygen Absorption Method (O.A.M.) is determination of the time for a fixed weight of lipid to absorb a fixed volume of oxygen when held under controlled conditions in air without illumination.

Many versions of the O.A.M. are available. The most commonly used methods are those of Sylvester-Martin and Eckey (Ragnarsson and Labuza, 1976). Samples of lipid are kept in flasks connected to mercury manometers which are connected to a pressure recorder. The sample is kept at atmospheric pressure in oxygen at 100°C. The end point is

taken when a marked drop in pressure occurs. If the sample absorbs oxygen gradually, the end point is the organoleptic rancid point.

Two disadvantages of this method are the high temperature, which can affect even simple lipids and the relatively low oxygen pressure since the rate can be dependent on oxygen dissolution. The dependence on oxygen will be expected to increase with the degree of unsaturation of the lipid.

2.1.5.4 Rancimat method

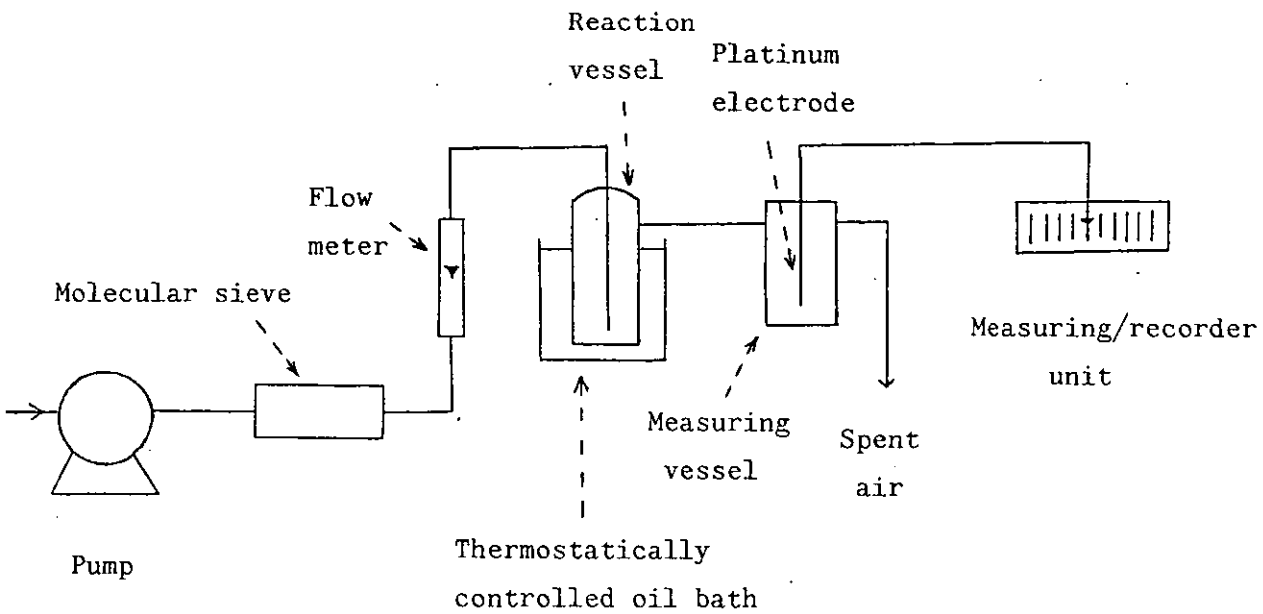
The Rancimat equipment, which is produced by Metrohm Herisan (Switzerland), consists of a pump to pump air through the sample which is placed in one of six reaction vessels placed in a thermostatically controlled oil bath (see Fig. 4). The air is passed through a molecular sieve to absorb any oxidizing gases and moisture before passing through the sample at a set rate determined by the flow meter, approximately 18 to 20 litres/hour.

The reaction vessels are connected to measuring cylinders, containing 50 ml distilled water and equipped with a platinum electrode. The electrode is connected to a six pen measuring recorder unit.

As oxidative rancidity develops the organic acids characteristic of oxidative degeneration pass from the reactor to the measuring vessel. This increases the conductivity of the water and this is picked up by the measuring recorder unit.

The duration of the analysis depends on the proneness to rancidity of the samples: it may be short, 1 to 5 hours, or much longer, 14 to 20 hours or even more.

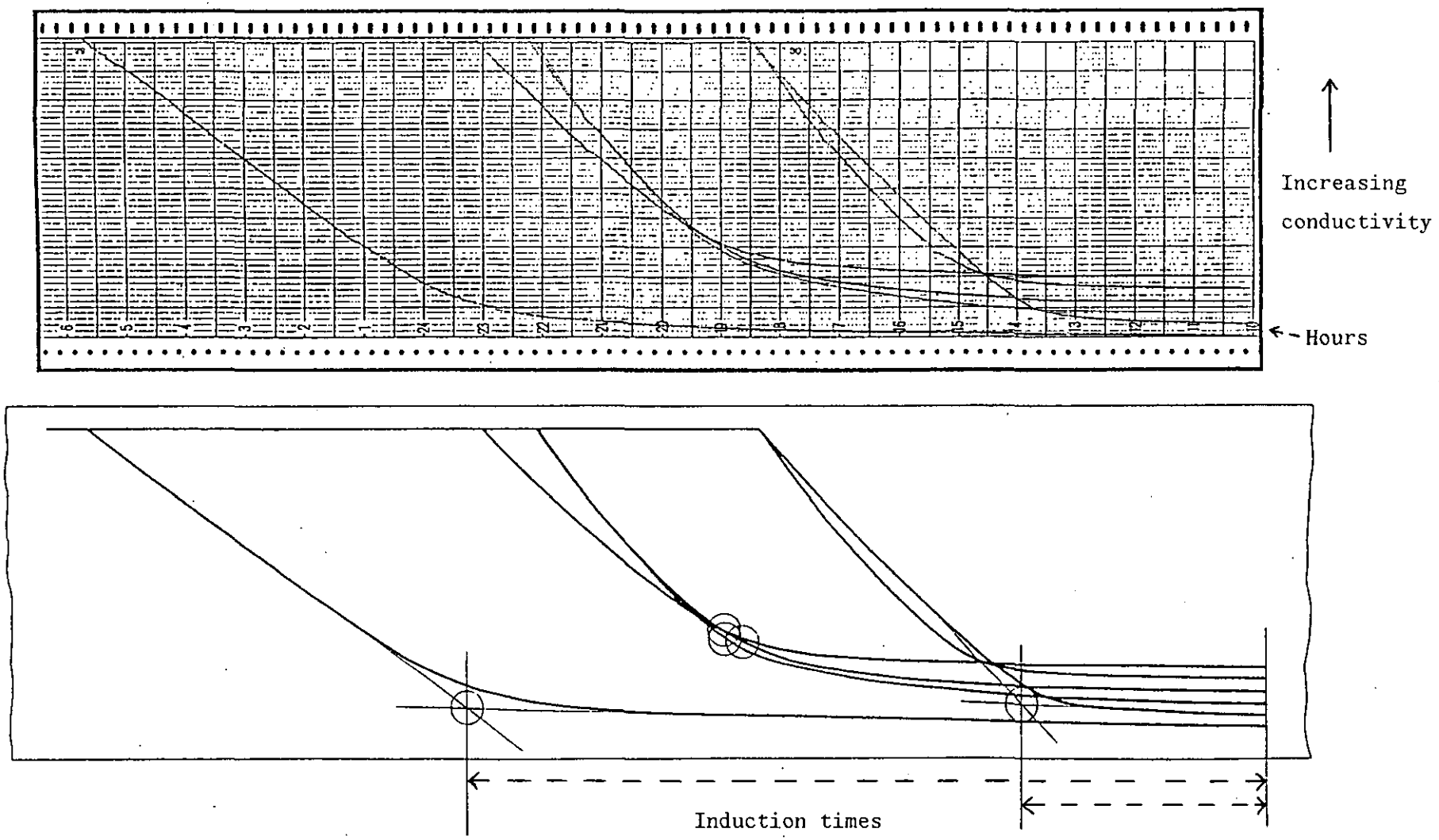
Fig. 4: Schematic diagram of the Rancimat



A typical trace from the recording unit is shown in Fig. 5. To evaluate the results, the sharpest inflection on each of the traces is found, either by visual examination or by means of a simple tangent construction. The times at which these points of sharpest inflection occurred are then read off (accuracy = to within 1/4 of an hour). These times are known as induction times and represent the characteristic parameter for the oxidation stability of the samples under investigation under the temperature and air-flow conditions used.

The various parameters influencing the analysis make it not possible to determine the induction time with an uncertainty of less than 1/4 hour and unless the equipment is absolutely clean, less precision is obtainable since traces of impurities can initiate oxidative rancidity.

Fig. 5: Typical Rancimat trace and illustration of the method for determining induction times



2.2 RANCIDITY INHIBITORS

There are three main classes of additives which can inhibit lipid autoxidation. These are:

(a) antioxidants or chain terminators,
(b) metal chelators,
(c) oxygen scavengers.

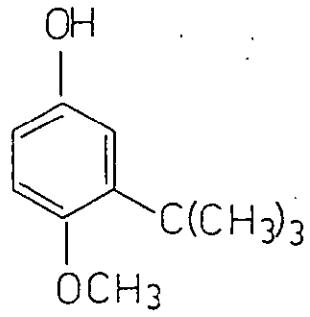
The three classes of additives frequently complement each other in their inhibitory response. Some compounds may also act in a dual capacity.

2.2.1 Antioxidants

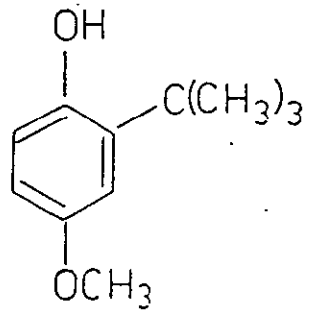
It has been shown by Scott (1965) by both kinetic and chemical evidence that phenolic and amino compounds interfere with the process of autoxidation, primarily by transferring either a hydrogen atom or an electron to the most abundant chain-propagating species and are, therefore, classed as chain breakers or chain terminators. The phenylenediamines are extremely potent antioxidants for inhibiting autoxidation of most triglycerides as well as other organic compounds subject to autoxidation. Most amino-type compounds however are toxic and not satisfactory as food-grade antioxidants. Naturally occurring antioxidants as well as synthetic antioxidants used in direct food applications are phenolic, ethoxyquin (6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline) being an exception. Fig. 6 gives the chemical structures of the main food-approved antioxidants. These compounds are structurally similar in that they have an unsaturated aromatic ring with either a hydroxyl or amine group to provide an electron or hydrogen atom for free radical abstraction. The tocopherols are the most important class of naturally occurring antioxidants.

Fig. 6: Structures of some food approved antioxidants

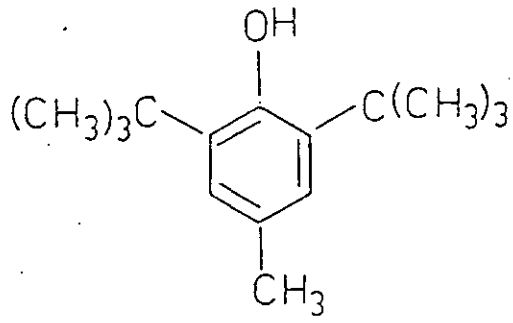
Butylated hydroxyanisole (BHA), a mixture of 2-tert-butyl-4-hydroxyanisole (2-BHA) and 3-tert-butyl-4-hydroxyanisole (3-BHA)



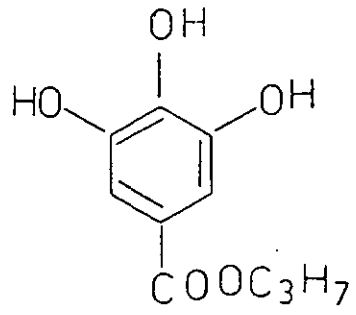
2-BHA



3-BHA

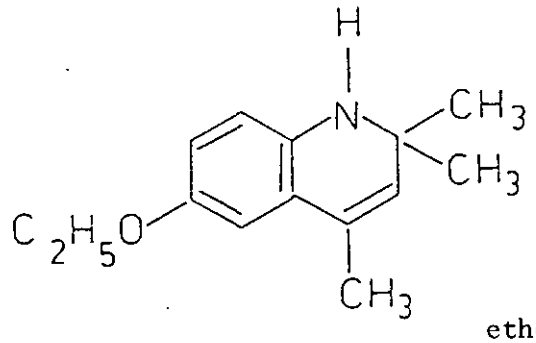


butylated hydroxytoluene (BHT)

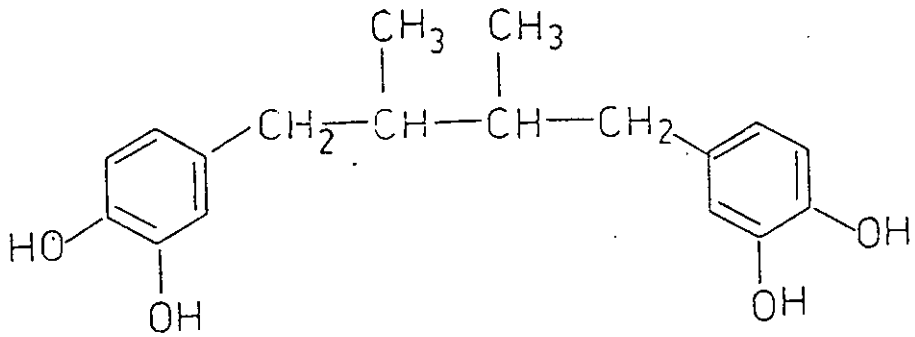


propyl gallate (PG)

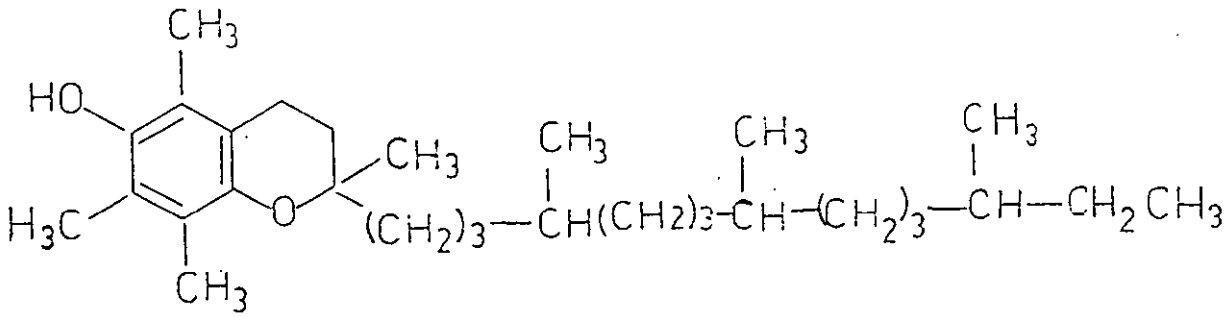
(Fig. 6 continued)



ethoxyquin



nordihydroguaiaretic acid (NDGA)



α-tocopherol

A review of a number of studies on the inhibition of lipid oxidation indicated that more than one antioxidant mechanism may occur, depending upon the conditions of the reaction and the type of system being studied (Shelton, 1959). Some possible mechanisms were described by which an inhibitor may function as a chain terminator for the free radical chain mechanism of lipid oxidation:

a. hydrogen donation by the antioxidant,
b. electron donation by the antioxidant,
c. addition of the lipid to the aromatic ring of the antioxidant,
d. formation of a complex between the lipid and the aromatic ring of the antioxidant.

Later studies showed that when the labile hydrogen atom on a typical antioxidant was replaced with deuterium, the antioxidant was not effective; this indicated that the inhibitor donated hydrogen rather than an electron.

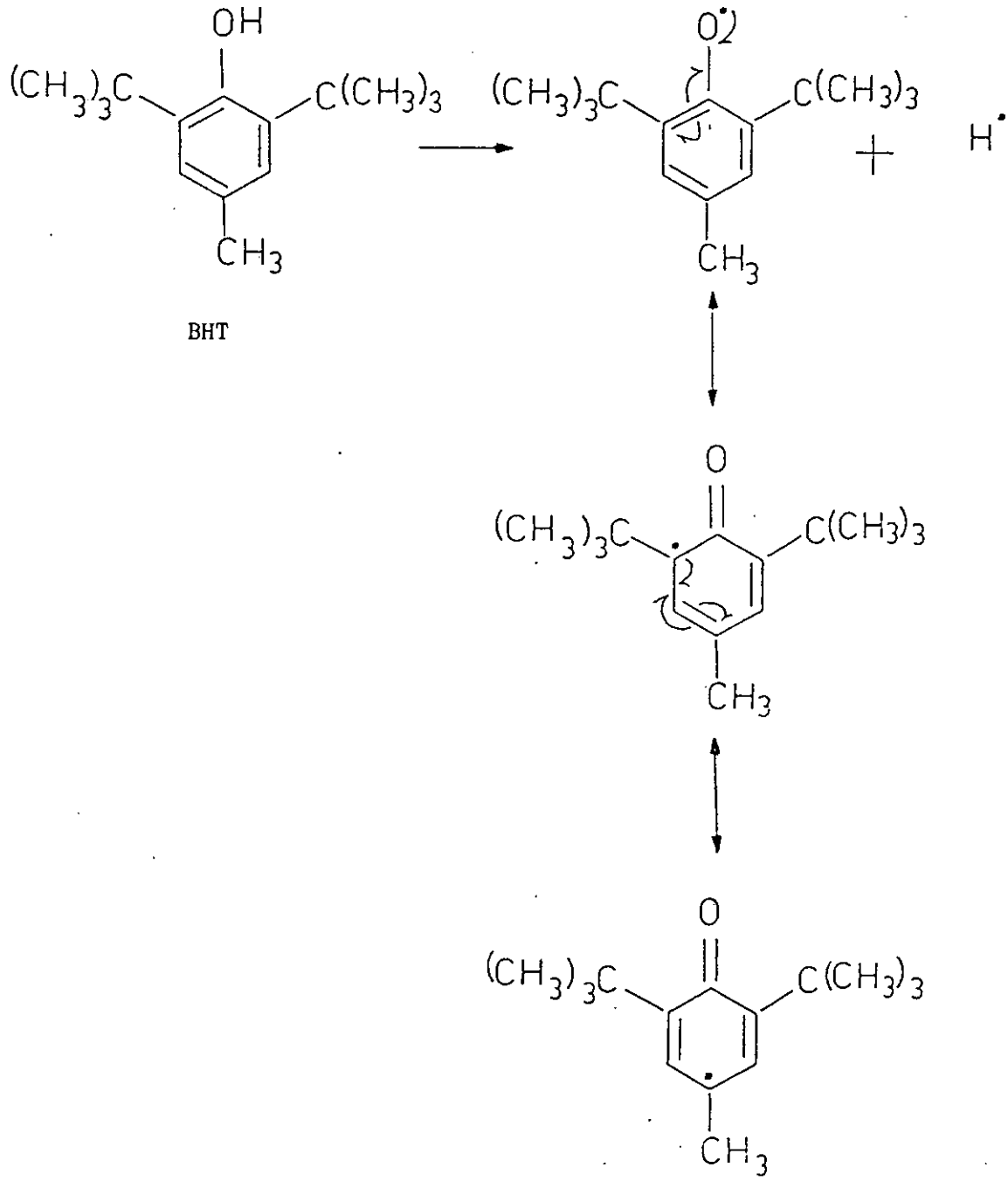
A scheme proposed to explain the chain terminating effect of organic compounds which possess a labile hydrogen atom is as follows:

$$R^{\bullet} + AH \text{ (antioxidant)} \longrightarrow RH + A^{\bullet}$$

$$ROO^{\bullet} + AH \longrightarrow ROOH + A^{\bullet}$$

The antioxidant radical $A^{\bullet}$ has a much lower reactivity than the substrate radical $R^{\bullet}$ and does not react with the substrate to form further substrate radicals. The low reactivity of the antioxidant free radical, $A^{\bullet}$, arises from the stability as a result of delocalisation of the electron density around the benzene ring, as for example for BHT in Fig. 7. Steric hindrence due to the bulky tert-butyl group also tends to lower the reactivity of the radical. The antioxidant radicals are removed by the following reactions:

Fig. 7: Resonance structure for the radical formed by hydrogen abstraction from BHT



$$A^{\cdot} + ROO^{\cdot} \longrightarrow ROOA$$

$$A^{\cdot} + R^{\cdot} \longrightarrow RA$$

$$A^{\cdot} + A^{\cdot} \longrightarrow A\text{-}A$$

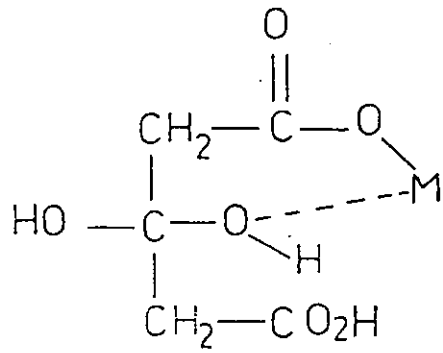
2.2.2 Sequestering agents

Heavy metals are important initiators even in concentrations as low as 0.1 ppm. Sequestering agents or chelators are compounds which, when added to a food system, co-ordinate the metal ions, making them less effective in promoting free radical formation thus reducing the initation rate (Section 2.1.3). It has been stated by Porter (1980) that for effective chelation: (a) sequestering agents must have a suitable steric and electronic configuration in relation to the metal being complexed; and (b) the pH, ionic strength and solubility proportion must be suitable for complex formation. Citric acid, EDTA, and phosphoric acid are common sequestering agents. It has been stated that the chelate stability increases with an increase in the number of chelate rings formed (Hole, 1980). Citric acid forms 1 to 3 rings whereas EDTA forms 5 rings, which produces a very stable configuration preventing the metal ion from promoting oxidation (see Fig. 8).

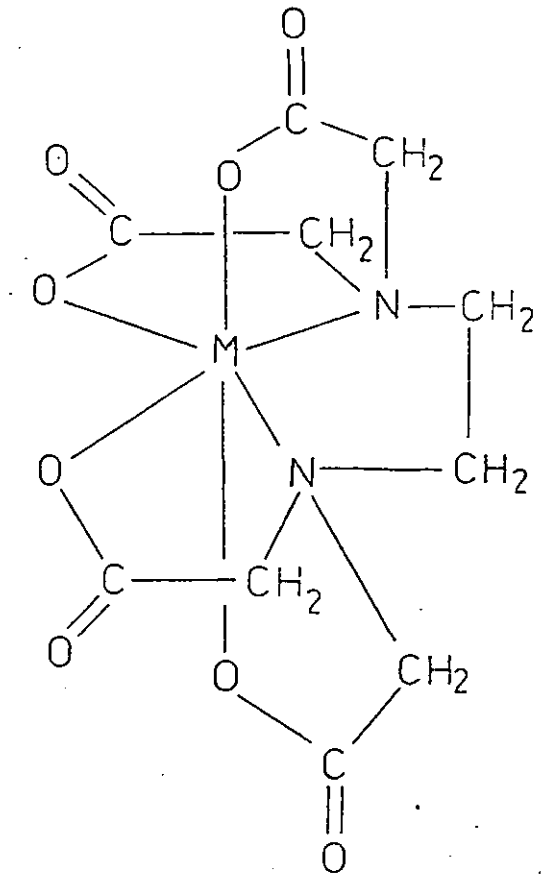
2.2.3 Oxygen scavengers

Oxygen can be removed by oxygen scavengers which are themselves converted to oxidized forms. Examples include ascorbic acid, its fatty acid esters such as ascorbyl palmitate and ascorbyl stearate, and also sodium erythorbate (see Fig. 9). Ascorbic acid combines with oxygen to give dehydroascorbic acid.

Fig. 8: Structure of citric acid-metal chelate and EDTA-metal chelate

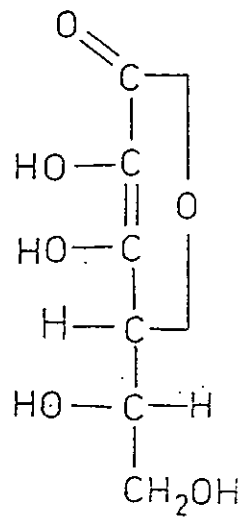


citric acid - metal chelate

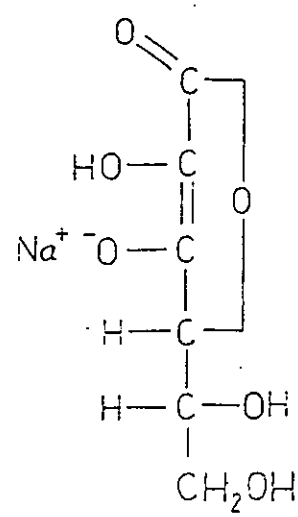


EDTA - metal chelate

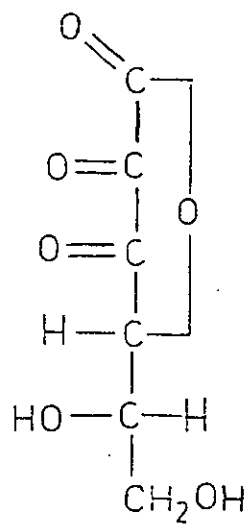
Fig. 9: Ascorbic acid and related compounds



ascorbic acid
(vitamin C)



sodium erythorbate



dehydroascorbic acid

2.3 BUTYLATED HYDROXANISOLE

Butylated hydroxyanisole (BHA) is one of the most widely used sythetic antioxidants added to foodstuffs. BHA consists of a mixture of isomers, 3-tert-butyl-4-hydroxy-anisole (3-BHA) and 2-tert-butyl-4-hydroxyanisole (2-BHA) (See Fig. 6). According to the EEC Regulations, BHA must not be less than 98.5% $C_{11}H_{16}O_2$ and not less than 85% of the 3-BHA.

2.3.1 Utilisation

Most countries have laws designed to protect the quality of food and to ensure that only safe additives are used in food manufacture.

In the U.K., antioxidants are controlled by "The Antioxidant in Food Regulations, 1978" which succeeds the previous regulations of 1958 and 1966. This statute implements EEC Directive No. 70/357/EEC on food antioxidants. The permitted antioxidants are listed below.

Name	
L-ascorbic acid	E300
Sodium L-ascorbate	E301
Calcium L-ascorbate	E302
6-0-palmitoyl-L-ascorbic acid	E304
Extracts of natural origin rich in tocopherols	E306
Synthetic alpha-tocopherol	E307
Synthetic gamma-tocopherol	E308
Synthetic delta-tocopherol	E309
Propyl gallate	--
Octyl gallate	E311
Dodecyl gallate	E312
Butylated hydroxyanisole	E320
Butylated hydroxytoluene	E321
Ethoxyquin	--

In Table 1 some food products available in the U.K. that contain antioxidants are listed. The permitted maximum concentration in specific foodstuffs are shown in Table 2 (Pearson, 1970).

In many fats and oils it has been found that a mixture of two antioxidants delays the onset of rancidity to a much greater extent than does either antioxidant on its own. This phenomenon is known as synergism and is an important aspect of BHA. The effect for BHA with BHT (butylated hydroxytoluene) in lard is shown diagrammatically in Figure 10.

Synergism is also observed between BHA and the esters of gallic acid, however, BHT does not show a synergistic effect with the esters of gallic acid (Dugan, 1976).

2.3.2 Toxicology

The toxicology of BHA and BHT was reviewed by Branan (1975). The review reported estimates that man consumes ca 0.1 mg/kg body weight daily of these antioxidants. At levels 500 times this level (50 mg/kg/day) both butylated hydroxyanisole and butylated hydroxytoluene appear to be free of any obviously injurious effects. However at larger doses (500 mg/kg/day), both butylated hydroxyanisole and butylated hydroxytoluene lead to certain pathological, enzyme and lipid alterations in both rodents and monkeys, and butylated hydroxytoluene, in some cases, has been reported to have certain teratogenic and carcinogenic effects upon rodents. These alterations appear to differ markedly between rodents and monkeys, apparently as a result of differences which exist in the metabolism and excretion of butylated hydroxyanisole and butylated hydroxytoluene by these two species. However, in both animal species, the

Table 1: Some foodstuffs that contain BHA and other antioxidants

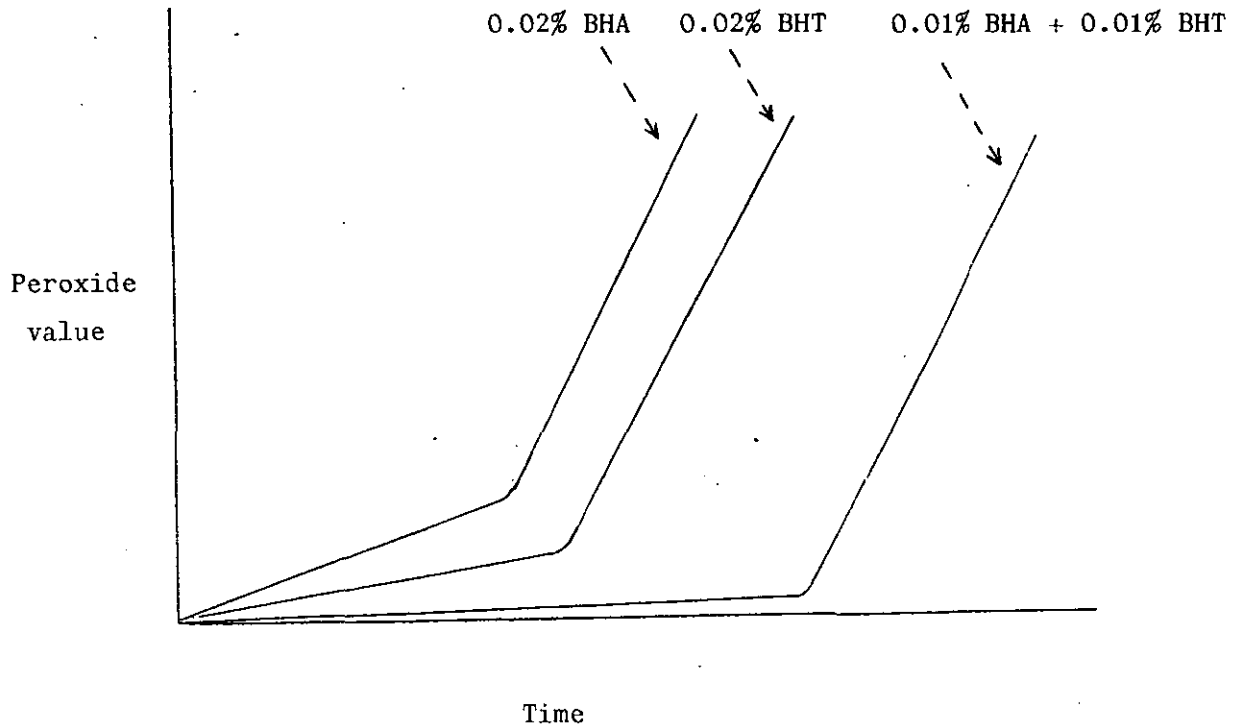
Product	Additives
Batchelors Savoury Rice	Preservative and antioxidant
Cadbury's Pint Size	Less than 1% of emulsifiers, antioxidants
Greens Cake Mixes	Fat with emulsifiers and antioxidants
Huntley & Palmers Butter & Raisin Biscuits	BHA
Jacobs Club Biscuits	BHA
Jacobs Mallows	BHA
Knorr Soups	Edible fat (with antioxidant)
KP Crisps, Skips, Hula Hoops	Antioxidant
Krona Margarine	BHA
Morrell Mushrooms in Sauce	Antioxidant
Rowntree Mackintosh Cheddar Sauce	Antioxidant
Smiths Crisps, Monster Munch	Vegetable oil with antioxidant
Vesta Meals	Antioxidant
Yeoman Mashed Potato	Antioxidant, vitamin C.

Table 2: Permitted maximum concentration of chain terminating antioxidants in foodstuffs (in mg/kg)

Antioxidant	1 BHA	2 BHT	3 BHA + BHT	4 gallates*	5 mixtures†
Fats & oils, vitamin oils, partial glycerol esters & concentrates	200	200	200	100	300
Essential oils, isolates & concentrates	1000	1000	1000	1000	1000
Butter for manufacturing purposes	160	160	160	80	240
Vitamin oils, preparations containing > 100,000 i.u. vitamin A per g	10‡	10‡	10‡	0	0
Dehydrated potato	25	25	25	0	0

* n-Propyl, n-octyl or n-dodecyl gallates, or any mixture of these three.
† Any mixture of 4 with 1 or 2 or 3 to this total, provided limits on 1, 2, 3 and 4 are not exceeded.
‡ In mg/kg for each 1000 i.u. per g.

Fig. 10: Synergistic effect of BHA and BHT in lard



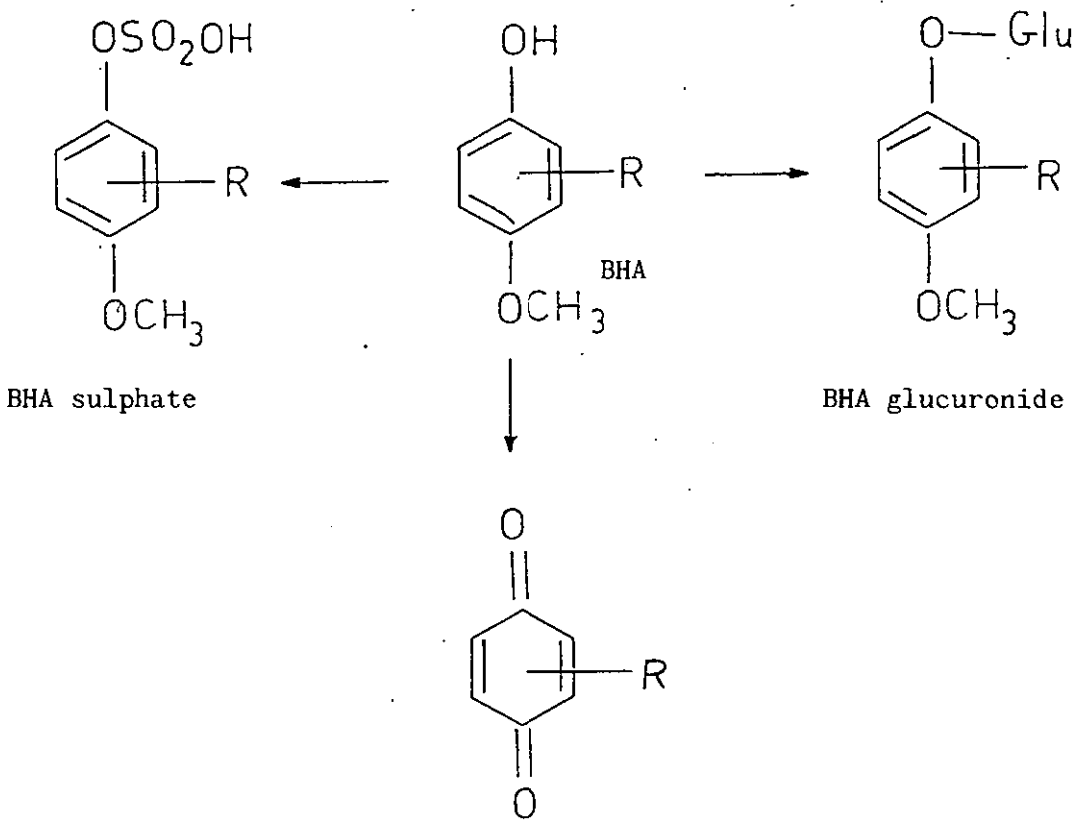
alterations appear to be physiological responses which are reversible upon removal of BHA and BHT from the diet.

Major differences have been reported to exist between rodents and primates in the pattern of metabolism, the rate of excretion, and causes of delayed excretion of BHA and BHT.

BHA was metabolized rapidly by all animal species studied. Within 24 hours, high quantities of BHA and its metabolites were excreted in the urine of rats, dogs, rabbits, monkeys and man. Differences did exist, however,in the pattern of BHA metabolism of different animal species (Fig. 11). Rabbits excreted BHA primarily as glucuronide conjugates, although small amounts were excreted as ether sulphates and as the free phenols. In the rat, the predominant 3-tert-butyl isomer of BHA was converted primarily to the glucuronide ester whereas the 2-tert-butyl isomer was converted mostly to ether sulphate. Dogs excreted BHA in the urine primarily as ether sulphates, although tert-butyl-hydroquinone also was formed. Man excreted 27 - 77% of a low dose of BHA as the glucuronide conjugate. No demethylated or hydroxylated products were found. It was concluded that small doses of BHA in man and large doses in rats and rabbits have similar metabolic patterns.

The Hyperactive Children's Support Group recommend that BHA should be avoided and in the UK, BHA is not permitted in foods intended for babies or young children (Hanssen, 1984).

Fig. 11: BHA metabolites



R represents a tert-butyl group

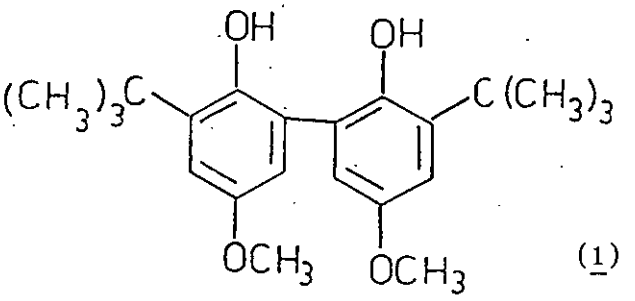
2.3.3 Chemical changes during processing and storage

Stability of butylhydroxyanisole in water under stresses of sterilization was investigated by Monte and Maga (1973). 20 ml of distilled demineralised water and 0.6 g BHA were placed in 20 ml ampoules as reaction vessels. At the reaction temperatures only approximately 0.75% of the BHA will go into solution producing a 0.02% aqueous solution of BHA i.e. about 200 ppm. The additional BHA was introduced to raise 2-BHA levels high enough for quantitation. The tops of the ampoules were hermetically sealed with the minimum amount of air remaining and the ampoules were autoclaved at 15 psi steam for 15 minutes.

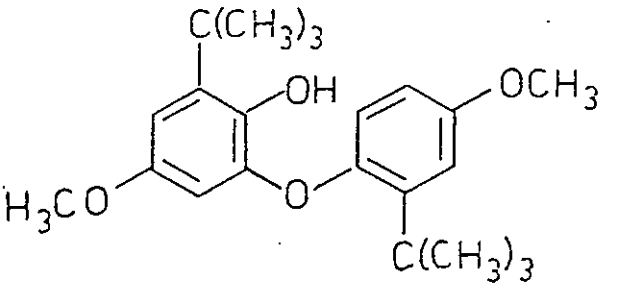
Extraction of the reacted mixtures was carried out with two 20 ml portions of redistilled chloroform. The chloroform layers were combined and evaporated under reduced pressure at room temperature to a volume of 1 ml. The solution was analysed by gas-liquid chromatography and mass spectometry. Twenty separate peaks were visually identified by GLC. Two of these peaks represented the isomeric forms of BHA (Peak 1: 3-tert-butyl-4-hydroxyanisole and Peak 2: 2-tert-butyl-4-hydroxyanisole) as verified by use of commercial standards. A third peak was postulated to represent the oxidised form of both isomers.

The most significant finding was the identification of two of the compounds as dimer structures identical to those found by Kurechi (1967) resulting from the photodegradation of BHA, namely: 2,2'-dihydroxy-5,5'-dimethoxy-3,3'-di-tert-butylbiphenyl (1) and 2',3-di-tert-butyl-2-hydroxy-4',5-dimethoxybiphenyl ether (3), as shown in Fig. 12. In addition they identified two other dimer structures, namely: 2,2'-dihydroxy-5,5'-dimethoxy-3,4'-di-tert-butylbiphenyl (2) and 2',4-di-tert-butyl-2-hydroxy-4', 5-dimethoxybiphenyl ether (4).

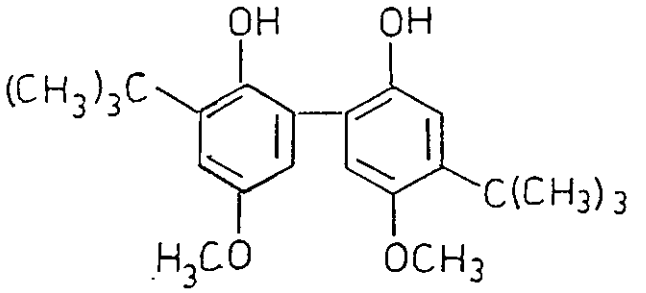
Fig. 12: Dimers obtained from BHA



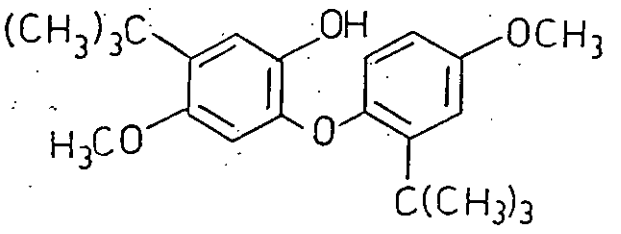
(1)



(2)



(3)



(4)

The value of this study is reduced by the absence of any information on the origin, purity and isomeric ratio of the BHA used.

The influence of various storage and processing variables on BHA dimer production was investigated by Maga and Monte (1977), using "commercial BHA". They concluded that sunlight, water, pH, temperature, time and air decreased the level of 3-BHA (which they thought was the minor isomer) while increasing the levels of dimeric forms of BHA, whereas nitrogen had a preserving effect. Sunlight had a dramatic effect on BHA stability, as had also been reported by Kurechi (1967). The results (see Tables 3 and 4) showed that in a water system, after exposure to sunlight for 1 day, approximately half of the 3-BHA was lost, and after 5 days, 3-BHA was not detected. When autoclaved, higher losses of BHA occurred with higher temperatures, but losses were in general lower than when BHA was exposed to sunlight. Acidity affected the stability of BHA with more decomposition occurring under alkaline conditions.

The value of these results is however limited by the confusion of the authors with regard to the BHA mixture used. It was stated in this paper that most commercial BHA in the USA is an isomeric mixture of 4-methoxy-3-tert-butylphenol and 4-methoxy-2-tert-butylphenol normally found in an approximate ratio of 96:4. This ratio is incorrect and the reverse is true, i.e. the 4-methoxy-2-tert-butylphenol (i.e. 3-tert-butyl-4-hydroxyanisole, 3-BHA) is the major isomer.

2.3.4 Analysis

2.3.4.1 Total BHA

Analytical methods were developed initially for total BHA; more sophisticated methods can now give the amounts of each isomer, as discussed in the next Section.

Table 3: Influence of storage time, media and sunlight on the amounts of isomeric and dimeric BHA detected (Maga & Monte, 1977)

Storage Time (Days)	Medium	Sun light	Total Antioxidant Recovered (ppm)	% Isomeric BHA		Percent Dimeric BHA				
				BHA-3	BHA-2	Dimer 1	Dimer 2	Dimer 3	Dimer 4	% Total Dimer
0	–	–	199 (1.2)	95.72	4.23	0.05	–	–	–	0.05
1	water	+	193 (2.9)	94.17	2.37	2.39	0.49	0.53	0.05	3.46
1	water	–	195 (0.8)	95.72	4.23	0.05	–	–	–	0.05
1	oil	+	198 (2.7)	94.48	2.61	1.83	0.25	0.83	0.10	3.01
1	oil	–	195 (1.5)	95.71	4.24	0.05	–	–	–	0.05
2	water	+	196 (3.6)	93.65	0.62	3.87	0.71	1.04	0.11	5.73
2	water	–	193 (2.9)	95.72	4.23	0.05	–	–	–	0.05
2	oil	+	193 (2.0)	94.15	0.76	3.41	0.57	0.87	0.24	5.09
2	oil	–	194 (2.0)	95.73	4.22	0.05	–	–	–	0.05
3	water	+	198 (1.3)	93.08	0.01	4.36	0.81	1.61	0.13	6.91
3	water	–	201 (1.2)	95.71	4.24	0.05	–	–	–	0.05
3	oil	+	197 (2.1)	93.72	0.02	4.07	0.72	1.13	0.34	6.26
3	oil	–	199 (1.9)	95.71	4.24	0.05	–	–	–	0.05
5	water	+	194 (2.0)	92.79	–	4.97	0.79	1.21	0.24	7.21
5	water	–	196 (2.1)	95.70	4.25	0.05	–	–	–	0.05
5	oil	+	195 (1.5)	93.14	0.01	4.61	0.62	1.11	0.51	6.85
5	oil	–	194 (2.8)	95.71	4.24	0.05	–	–	–	0.05
20	water	+	202 (1.2)	92.82	–	4.72	0.73	1.25	0.48	7.18
20	water	–	198 (2.7)	95.73	4.20	0.07	–	–	–	0.07
20	oil	+	196 (1.9)	92.80	–	4.87	0.75	1.31	0.27	7.20
20	oil	–	198 (1.8)	95.74	4.20	0.06	–	–	–	0.06
40	water	–	195 (3.1)	95.75	4.16	0.09	–	–	–	0.09
40	oil	–	190 (3.1)	95.74	4.18	0.08	–	–	–	0.08
80	water	–	198 (0.7)	95.75	4.14	0.10	–	0.01	–	0.11
80	oil	–	196 (0.6)	95.74	4.16	0.09	–	0.01	–	0.10
130	water	–	195 (0.9)	95.80	4.03	0.15	–	0.02	–	0.17
130	oil	–	198 (2.7)	95.75	4.10	0.14	–	0.01	–	0.15
180	water	–	199 (0.7)	95.93	3.84	0.19	–	0.04	–	0.23
180	oil	–	194 (2.6)	95.91	3.89	0.17	–	0.03	–	0.20

All data represent the composite means and (standard deviation) for 3 analyses
Percent of Dimers 1–4 relative to total antioxidant recovered

Table 4: Influence of autoclaving at various temperatures and pH's on the amounts of isomeric and dimeric BHA detected (Maga & Monte, 1977)

Temp. (°C)	pH	Total Recovered Antioxidant (ppm)	% Isomeric BHA		Percent Dimeric BHA				Percent Total Dimer
			BHA-3	BHA-2	Dimer 1	Dimer 2	Dimer 3	Dimer 4	
118	3	196 (2.3)	96.00	2.07	1.41	0.10	0.38	0.04	1.93
118	7	192 (2.1)	95.67	1.98	1.64	0.12	0.56	0.03	2.35
118	11	185 (1.2)	95.38	1.91	1.89	0.17	0.60	0.05	2.71
124	3	187 (2.1)	95.87	1.86	1.58	0.20	0.41	0.08	2.27
124	7	181 (2.7)	95.80	1.79	1.67	0.18	0.50	0.06	2.41
124	11	174 (2.1)	94.64	1.74	2.43	0.25	0.82	0.12	3.62

All data represent the composite means and (Standard Deviation) for 3 analyses
Percent of Dimers 1–4 relative to total antioxidant recovered

(a) Colorimetric Methods

Several colorimetric methods have been developed for determining BHA. In the presence of a borax buffer, BHA will produce a blue colour with 2,6-dichloroquinone-chlorimide (Gibb's reagent). A quantitative determination can be obtained by measuring the colour intensity at 610 nm (Anglin et al., 1956). Under basic conditions BHA will form a purple red colour with diazotised sulphanilic acid and by measuring the optical density at 535 nm, a quantitative determination of BHA can be obtained. BHA can also be determined by measuring the intensity of its coloured reaction product with ferric chloride and 2,2'-dipyridyl (Kahan, 1954). Unfortunately, all these methods suffer from interference by other antioxidants, in particular BHT and the gallates.

Komaitis and Kapel (1985) developed a colorimetric method based on the reaction of BHA with N'N-dimethyl-p-phenylenediamine in the presence of a mild oxidising agent in alkaline solution. Other antioxidants do not interfere with this method, however some phenols do interfere and have to be removed by distillation.

(b) Thin layer and paper chromatography

Although thin layer chromatography (TLC) and paper chromatography methods are suitable for semi-quantitative quality control work, it is difficult to get precise quantitative results.

Using TLC with a mixture of 25 parts silica gel and 5 parts kieselguhr on the plate and hexane as the mobile phase, BHA may be separated from other antioxidants. However, a double development is necessary to give adequate separation. Development with a phosphomolybdic acid spray gives blue/green coloured spots on a yellow background. On treatment with ammonia vapour, the background becomes white and the spots are more easily recognised (Meyer, 1961).

As an alternative, silver nitrate may be used to make the spots visible. (Gander, 1955; Roy et al., 1960). Using a silver silica gel plate and chloroform as the mobile phase satisfactory separation can be obtained with one development. Kurechi and Yamaguchi (1980) determined BHA and degradation products by a TLC method using chloroform/methanol/acetic acid (90:2:2 v/v) as eluting solvent.

A similar method has also been reported using paper chromatography, with a variety of mobile phases (Association of Public Analysts, 1963).

(c) Gas-liquid chromatography

Several gas-liquid chromatography (GLC) methods have been developed for determining total BHA in the presence of other antioxidants. Columns suggested include, 1 per cent "Tween" 80 and 2 per cent Silicone Gum SE-30 on 70-80 mesh "Chromosorb" W-type ABS (Schwecke and Nelson, 1964), 20 per cent Silicone Gum SE-20 on 60-80 mesh Firebrick or "Chromosorb" W, (Choy et al., 1963), and 10 per cent DC 200 or 10 per cent "Carbowax" 20M on 80-100 mesh "Gas Chrom" Q, (Hartman and Rose, 1970). Internal standards used include 3,5-di-tertbutyl-4-hydroxyanisole and methyl undecanoate.

(d) Titrimetric methods

As little as 30 ppm of an antioxidant in a fat can be detected by titration with ceric sulphate solution with the end point being determined potentiometrically or by means of a redox indicator (Wenger, 1954). Complexometric titrations have also been used for the determination of BHA (Sedlacek, 1959; Sedlacek, 1960). However, both methods lack specificity.

(e) Ultra-violet absorption methods

Although BHA has a characteristic UV absorption, other antioxidants and naturally occurring materials can interfere with the measurement. In practice only BHT can be effectively determined by this method since it can be easily separated from intefering materials by column chromatography (Phillips and Hintel, 1957; Roos, 1959).

2.3.4.2 Determination of 2-BHA and 3-BHA

(a) Colorimetric methods

A method for the estimation of the isomers in commercial BHA was developed by Mahon and Chapman (1952). It was found that 3-BHA on reaction with a 2,6-dichloroquinonechlorimide borax reagent produces 5.2 times as much absorbance per unit weight at 620 nm as does 2-BHA. However, 2-BHA on reaction with a ferric chloride 1,1'— bipyridine reagent produces 17 times as much absorbance per unit weight at 515 nm as does 3-BHA. Consequently the ratio of absorbances measured at 620 and 515 nm using the appropriate reagents can be used for estimating the proportions of these isomers. The method suffers from serious interference by gallates.

(b) Infra-red method

An infra-red method has been developed based on absorption bands in the 830-900 cm^{-1} region of the spectrum. Although this method is accepted by the Food Chemicals Codex it is not clear whether other antioxidants interfere with the procedure.

(c) Gas-liquid chromatography

A GLC method for determination of the isomers in BHA was developed by Mann et al. (1970). The developed procedure involves dilution of the sample with acetone and

injection into a gas chromatogram with a XE-60 column using a hydrogen flame ionization detector and diphenylamine as an internal standard. The method is quantitative and the chromatogram is completed in approximately 20 minutes.

An alternative GLC method was developed by Monte and Maga (1973, 1977). A Beckman GC-45 equipped with a 1.2 m x 16 mm OD stainless steel column packed with a 1% OV-7 on 60-80 mesh HMDS treated Chromosorb-W was used, with helium as the carrier gas, the first 10 minutes of each run was held at 100°C, then the temperature was programmed to reach 300°C in the next 22 minutes.

2.4 AIMS OF THE PRESENT WORK

The literature survey revealed a large amount of information regarding the use of BHA as an antioxidant and reservations about its safety as a food additive. It is suprising that up to 15% of the 2-BHA is permitted in commercial BHA in view of the reservations regarding its safety. The majority of the references dealt with the improved stability of various lipid materials due to the presence of BHA.

The stability of BHA has been investigated with respect to autoxidation, photodegradation, degradation by sunlight, U.V. light and X-ray radiation. Very few papers have been published concerned with the stability of BHA under processing conditions, and the authors of one of the papers concerned with this aspect confused the isomeric mixture in the commercial BHA.

Based on these considerations the present work had the following general aims:

1. To determine the stability of the two BHA isomers and a typical commercial mixture under processing and storage conditions involving the following factors: heat, light, pH, nitrogen atmosphere.
2. To compare the antioxidant properties of the two BHA isomers and a commercial mixture and to determine whether pure 3-BHA could be used in place of the commercial mixture.

3. EXPERIMENTAL

3.1 MATERIALS

Commercial grade butylated hydroxyanisole (BHA) was obtained from BDH Limited (containing about 99% 3-BHA to 1% 2-BHA) and from May and Baker (containing 90% 3-BHA to 10% 2-BHA).

The following oils which contained no antioxidant were obtained from Sigma Chemicals Ltd: peanut oil, safflower oil, soybean oil and olive oil. Glass ampoules (20 ml) were obtained from Pyrex Limited. Analar chloroform was used throughout the experimental work, other reagents were laboratory grade unless stated otherwise.

3.2 EQUIPMENT

A Shimadzu GLC-RIA gas chromatograph with flame ionisation detector and Shimadzu data processor GC-1 was used for most of the chromatographic analysis.

A Perkin-Elmer F11 gas chromatograph with flame ionisation detector was used for some of the gas chromatographic analysis.

A Status autoclave (Northern Media Supplies) was used to heat treat the samples.

A Pye-Unicam SP800A spectrophotometer was used in the colorimetric analysis.

A Rancimat (Metrohm, Switzerland) was used to assess antioxidant activity of samples.

3.3 METHODS

3.3.1 Preparation of 3-BHA

BHA (4 g) commercial grade material from BDH Limited was recrystallised twice from cyclohexane (5 ml) to yield 2.3 g of 3-BHA (3-t-butyl-4-hydroxyanisole), m.p 61-62°C.

3.3.2 Synthesis of 2-BHA

2-BHA (2-t-butyl-4-hydroxyanisole) was prepared following the method described by Lam and Farhat (1978).

Trimethylacetylchloride (20 g) was added to a solution of t-butylhydroquinone (30 g) in pyridine (187 ml) at 5-10°C. The reaction mixture was stored for 4 days at 5-10°C and then tested for the presence of t-butylhydroquinone by thin layer chromatography. A further 2 g trimethylacetylchloride was added and the mixture was tested again after 48 hours. A further 1 g of trimethylacetylchloride was added and the mixture retested after 24 hours. t-Butylhydroquinone was still present, but after leaving for a further 7 days at 5-10°C the reaction was complete. The pyridine hydrochloride was filtered off and the remaining pyridine removed by distillation under vacuum. The crude product was dissolved in ether (80 ml) and the solution washed with 6 M hydrochloric acid (40 ml), water, (50 ml) and finally with 10% sodium carbonate (2 x 50 ml). The extract was dried over anhydrous magnesium sulphate and after filtration the ether was distilled off under vacuum. The residue was dissolved in methanol (25 ml) at reflux then cooled to crystallise and finally cooled in ice/water. The product was filtered off, washed with methanol (10 ml) and dried at 60°C under vacuum to yield 19 g of 3-t-butyl-4-hydroxyphenol trimethyl acetate, m.p. 153.6-154.8°C (lit. m.p. 157-159°C, Lam and Farhat, 1978).

3-t-Butyl-4-hydroxyphenol trimethylacetate (18 g), potassium carbonate (20 g) and dry acetone (216 ml) were added to a 250 ml flask. The mixture was stirred and heated to reflux, dimethyl sulphate (11 ml) was added over 1 hour and the mixture refluxed for a further 24 hours. The reaction mixture was cooled to 20°C and the potassium carbonate filtered off. The filter cake was washed with acetone (25 ml) and the filtrates were combined and the acetone was removed by distillation under vacuum. The residue was dissolved in methanol (20 ml) at reflux then cooled to crystallise. The product was filtered off, washed with methanol (10 ml) and then allowed to air dry to yield 15.5 g of 3-t-butyl-4-methoxyphenol trimethylacetate mp 66.8-67.4°C (lit. mp 67-68°C, Lam and Farhat, 1978).

3-t-Butyl-4-methoxyphenol trimethylacetate (14 g), potassium hydroxide (37.4 g) and 50% aqueous ethanol (146 ml) was added to a 250 ml flask. The mixture was stirred for 64 hours at reflux under an atmosphere of nitrogen. The reaction mixture was cooled and acidified with concentrated hydrochloric acid. The yellowish organic layer was separated off and the aqueous layer was extracted with ether (3x50 ml). The organic layer and ether extracts were combined and washed with water (50 ml) and with saturated sodium bicarbonate solution (2x50 ml). After drying over anhydrous magnesium sulphate, the mixture was filtered and the ether removed by distillation under vacuum. Cyclohexane was added and warmed to dissolve the residue. The solution was crystallised by cooling in ice and the product was filtered off and washed with cold cyclohexane (10 ml). The product was allowed to air dry to yield 5 g of 2-BHA, m.p. 61.6-62.4°C.

2-BHA (4 g) was recrystallised from cyclohexane (5 ml), filtered off, washed with cold cyclohexane (3 ml) and air dried to yield 3.6g of 2-BHA, m.p 62-62.4°C (lit. mp

63.5-64°C, Lam and Farhat, 1978; 62.5°C, Akhrem, Kamernitskii and Prodhoda, 1967).

3.3.3 Colorimetric determination of total BHA

The method used was that described by Mahon and Chapman (1952).

The test solution of BHA in methanol (2 ml) was added to a 20 ml graduated flask. 95% Aqueous methanol (2 ml), 0.5% borax solution (8 ml) and Gibbs reagent (2 ml, 0.01% w/v solution of 2,6-dichloroquinonechlorimide in 95% aqueous methanol) were added. After 15 minutes, the flask was made up to the mark with n-butanol. The absorbance was measured at 608 nm against 95% aqueous methanol. A calibration curve was obtained using BHA solutions in 95% aqueous methanol (0, 1, 2, 3, 4, 6 and 8 mg/ml).

3.3.4 TLC method for total BHA

An ethanolic solution of BHA was spotted on to an Eastern Chromatogram Sheet 6060 which had been previously activated at 100°C for 15 to 30 minutes. The spotting solvent was allowed to evaporate then the sheet was developed using chloroform as the eluent. Visualisation was achieved by either exposing the dried sheet to iodine vapours or by spraying with a 3% solution of phosphomolybdic acid in ethyl alcohol. After spraying with phosphomolybdic acid, the sheet was heated at 100°C for several minutes to develop the deep blue colours. Colours were fixed and the background made white by exposing to ammonia.

3.3.5 GLC method for total BHA

The method was as described by Monte and Maga (1973).

The test solution of BHA was added to a 25 ml volumetric flask containing 5 ml of diphenylamine (DPA) solution (80 mg DPA: 100 ml chloroform) as internal standard, and the volume made up to 25 ml with chloroform. The solution (1.5 µl) was analysed by GLC (Perkin-Elmer F11) using a stainless steel 16 mm diameter, 1m long column packed with 1% Silicone OV-7 Chromosorb W EMDS 60-80 mesh, with an oven temperature of 125°C and a nitrogen gas flow rate of 4.5 ml/minute.

Initially the BHA and standard DPA peaks were cut out and weighed. BHA standards containing DPA, were also run and the ratios of the peak weight were compared to determine the amount of BHA in the test solutions. However, the ratios of the peak heights were found to be equal to the ratios of the peak weights, therefore, peak heights were subsequently used for determining the amounts of BHA in the test solutions rather than the less convenient peak areas (peak weights).

3.3.6 GLC method for 3-BHA and 2-BHA

The method was as described by Mahn et al. (1970).

The test solution of BHA was added to a 25 ml volumetric flask containing 5 ml of diphenylamine (DPA) solution (80 mg DPA: 100 ml chloroform) as internal standard, and volume made up to 25 ml.

The solution was analysed by a Shimadzu gas chromatrograph using a Pyrex coiled column, 1.5 m x 3 mm outside diameter, packed with 10% XE-60 on 100-120 mesh Aeropack 30. The oven temperature was 125°C, injection temperature 180°C and nitrogen carrier gas flow rate 42 ml/minute.

3.3.7 Heat and light treatment

Aliquots of 5 ml of standard BHA solutions (containing 160 mg BHA in 200 ml chloroform) were pipetted into 20 ml glass ampoules. After evaporating off the chloroform under vacuum, water (20 ml at different pH values) was added. The ends of the ampoules were sealed in a gas flame either in the presence of air or in an atmosphere of nitrogen.

Some of the ampoules were heated in an autoclave at 115°C, other ampoules were placed on a south facing window ledge and left for six weeks.

3.3.8 Work-up after heat or light treatment

The contents of the ampoules were transferred to a separating funnel and extracted with chloroform (3x5 ml). The extracts were combined and dried over anhydrous sodium sulphate. The sodium sulphate was filtered off and washed with chloroform (5 ml). The chloroform solution was transferred to a 25 ml volumetric flask containing diphenylamine solution (5 ml) and the volume made up to 25 ml with chloroform. The solution was analysed by GLC as described in Sections 3.3.5 and 3.3.6.

3.3.9 Use of the Rancimat

The Rancimat was used to determine the antioxidant activity of 2-BHA, 3-BHA and a 9:1 mixture of 3-BHA:2-BHA using the following oils which did not contain any antioxidant: safflower, soybean, peanut and olive oil.

4. RESULTS AND DISCUSSION

4.1 SCHEME OF WORK

In order to fulfil the aims of the project it was necessary:

1. To obtain pure samples of 3-BHA and 2-BHA.

2. To develop analytical procedures suitable for determining losses of total BHA and the separate isomers.

3. To develop a procedure for subjecting sample mixtures to heat or light at different pH levels, followed by estimation of the residual BHA.

4. To assess the breakdown of 3-BHA, 2-BHA isomers under the influence of heat or light.

5. To assess the antioxidant activity of the separate BHA isomers and a typical commercial mixture of the isomers.

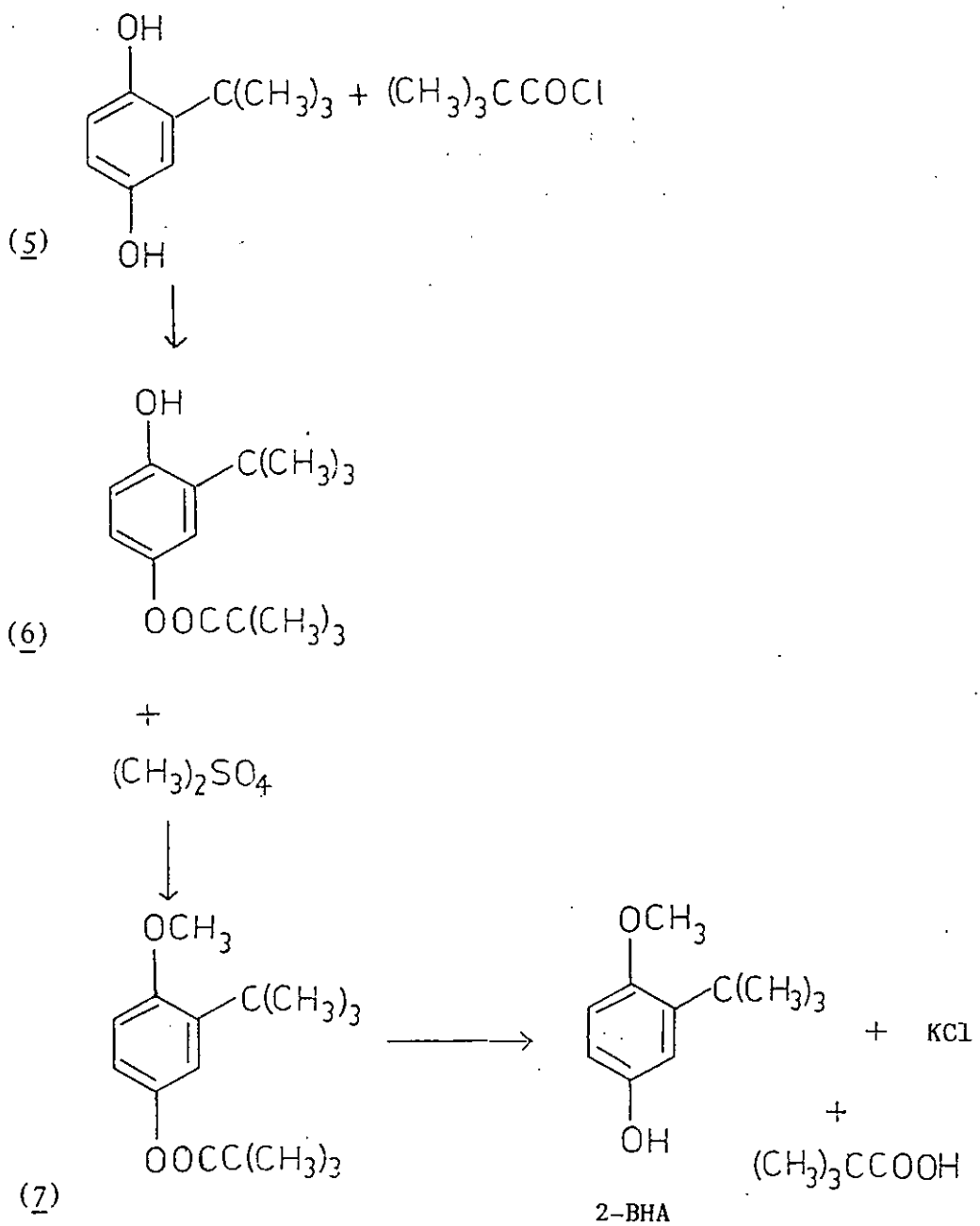
6. To assess the economics of producing pure 3-BHA rather than a mixture.

4.2 PREPARATION OF 3-BHA AND 2-BHA

Commercially available BHA contains two isomers in approximately 85:15 ratio of 3-BHA to 2-BHA. Reported sythesis of BHA, either by t-butylation of hydroxyanisole (Akhrem et al., 1967) or by methylation of t-butylhydroquinone (Daniewski et al., 1964) resulted in a mixture of 3-BHA and 2-BHA in approximately the same proportions. 3-BHA can be obtained in 99.5% purity from fractional crystallisation of the commercial mixture but attempts to isolate 2-BHA are complicated by its rapid autoxidation in solution. 3-BHA was prepared by recrystallising commercial grade BHA (as supplied by BDH Limited) twice from cyclohexane.

Pivaloyl chloride has been used successfully in the synthesis of peptides as a carboxyl protecting group (Leplawy et al., 1960; Schwyzer and Sieber, 1963). Lam and Farhat (1978) used pivaloyl chloride as a phenol protecting group in the synthesis of 2-BHA without contamination by the 3-BHA isomer. The synthetic scheme is outlined in Fig. 13. t-Butyl hydroquinone (5) was esterified with pivaloylchloride in pyridine at 10°C to give the monoester (6). The pivaloyl chloride optimised the steric interactions between the t-butyl groups resulting in the esterification of the less hindered hydroxyl group of (5). Methylation of (6) with dimethyl sulphate in acetone in the presence of potassium carbonate gave (7). 2-BHA was obtained by saponification of (7) with potassium hydroxide.

Fig. 13: Scheme for 2-BHA synthesis



4.3 DEVELOPMENT OF ANALYTICAL PROCEDURES FOR TOTAL BHA AND SEPARATE ISOMERS

4.3.1 Total BHA

Mahon and Chapman (1952) developed a colorimetric method for the detection and determination of BHA. This method depends upon the property of BHA to produce a blue colour with 2,6-dichloroquinone-chlorimide (Gibbs reagents) in the presence of a borax buffer which absorbs at 608 nm. This method was investigated in some detail and was found to suffer from the problem that the calibration curves varied greatly from run to run, even when reagent concentrations, temperature, reaction times, etc, were carefully controlled. The method therefore appeared unsuitable for determing the small changes in BHA concentrations that might be expected under processing and storage conditions.

The use of thin layer chromatography for the detection of BHA was investigated. Eastman chromatogram sheet no. 6060 (silica gel) was used together with either chloroform, ethyl alcohol or hexane/toluene 80:20 as eluents. The vizualization reagent used was a 3% solution of phosphomolybdic acid in ethyl alcohol or iodine crystals. BHA was easily detected but difficult to determine quantitatively.

A GLC method for determining total BHA was developed based on the method described by Monte & Maga (1973). The samples of BHA after treatment were extracted with chloroform and added to a 25 ml volumetric flask containing 5 ml of DPA solution (80 mg DPA:100 ml chloroform). The test solution was analysed by GLC (Perkin Elmer F11) using the following conditions:-

Column: stainless steel diameter 16 mm, length 1m
1% silicone OV-7 Chromosorb W HMDS 60-80 mesh.
Injection temperature: setting 2
Oven tempemperature: 125°C
Sensitivity: 50
Flow rate: 4.5ml/min
Sample volume: 1.5 µl

The BHA peak and the standard DPA peak were cut out and weighed. Standards of non-reacted BHA and DPA were also run and the ratios of the peak weights were compared to determine the percentage loss of BHA.

Standard solutions of 2-BHA, 3-BHA and DPA in chloroform were prepared at varying concentrations of BHA, and examined by GLC using the conditions described above. The ratio of BHA to DPA was determined by measuring the peak heights. A graph of this ratio against concentration of BHA was plotted and found to be linear for 2-BHA and 3-BHA, therefore the peak heights for the BHA are directly proportional to the peak areas and peak heights were subsequently used for determining the amounts of BHA remaining after reaction, rather than the less convenient peak areas (peak weights). A Shimadzu gas chromatograph equipped with a data station became available and was used to give the results reported. The oven temperature was 125°C, injection temperature 180°C and nitrogen carrier gas flow rate 42 ml/min. The ratio of the percentage concentration of BHA to DPA was calculated and by comparison with standard samples the loss of BHA after treatment was determined.

4.3.2 Separate isomers

A GLC method for determining the separate isomers of BHA was developed based on the method described by Mahn et al. (1970). The test solution was analysed using a Shimadzu

data station using the following conditions:-

Column: Pyrex coiled column, 1.5 m x 3 mm outside diameter, packed with 10% XE-60 on 100-120 mesh Aeropak 30
Injection temperature: 180°C
Oven temperature: 125°C
Flow rate: 42 ml/min
Sample volume: 1.5 µl

Under these conditions, 2-BHA had a retention time of about 14.7 minutes, 3-BHA about 12.4 minutes and DPA about 18.9 minutes. The ratio of the percentage concentration of BHA isomers to DPA was calculated and by comparison with standard samples the loss of BHA isomers after treatment was determined.

4.4 PROCEDURE FOR SUBJECTING SAMPLE MIXTURES TO HEAT OR LIGHT

Initially the following procedure was devised to study the stability of the two isomers of BHA when subjected to either heat or light at various pH values.

Approximately 4 mg of BHA was weighed into a 25 ml glass ampoule. 25 ml boiled distilled water, adjusted to varying pH values, was added and the ampoule was sealed in a gas flame under either air or nitrogen. The ampoule was either heated at 115^{o}C for 15 minutes in an autoclave or left standing in the light in a window for several weeks. After treatment, the contents of the ampoule were extracted with chloroform. The extracts were dried and transferred to a 25 ml volumetric flask containing 5 ml of diphenylamine solution (80 mg DPA:100 ml chloroform) as internal standard, and the volume adjusted to 25 ml. The solution was examined by GLC (Perkin Elmer F11) as described in Section 4.3.1. Standards of non-reacted BHA and DPA were also run and the ratios of the peak heights were compared to determine the percentage loss of BHA in the various samples. The experiments showed that breakdown was occurring, but the results were very variable. This was attributed to difficulties encountered in weighing BHA sufficiently accurately.

A more accurate method was devised whereby 160 mg BHA was dissolved in 200 ml chloroform in a volumetric flask. Aliquots of 5 ml of this solution were pipetted into 25 ml ampoule and the chloroform removed in vacuo. Boiled distilled water at varying pH was added and the ampoule sealed in a gas flame under air or nitrogen. The sample was then treated and worked up as previously described. The final solution was examined by GLC. (Shimadzu gas chromatograph) using either the OV-7 Chromosorb column or the Pyrex XE-60 column. This method was used to give the results reported below.

Experiments were carried out to assess the accuracy of the method with respect to physical losses. A 5 ml aliquot of the standard BHA solution was pipetted into a 25 ml ampoule and the chloroform removed in vacuo. Boiled distilled water adjusted to pH 3.0, pH 7.0 and pH 11.0 was added and the ampoule shaken. The mixture was extracted and examined by GLC against standard solutions as previously described. Each experiment was carried out in duplicate.

$$\frac{\text{The ratio of BHA/DPA experimental}}{\text{The ratio of BHA/DPA standard}} \times 100 = \% \text{ loss}$$

The average loss was found to be 5%. This factor was used to correct subsequent results.

4.5 DECOMPOSITION OF 3-BHA AND 2-BHA UNDER THE INFLUENCE OF HEAT AND LIGHT

4.5.1 The effect of heat

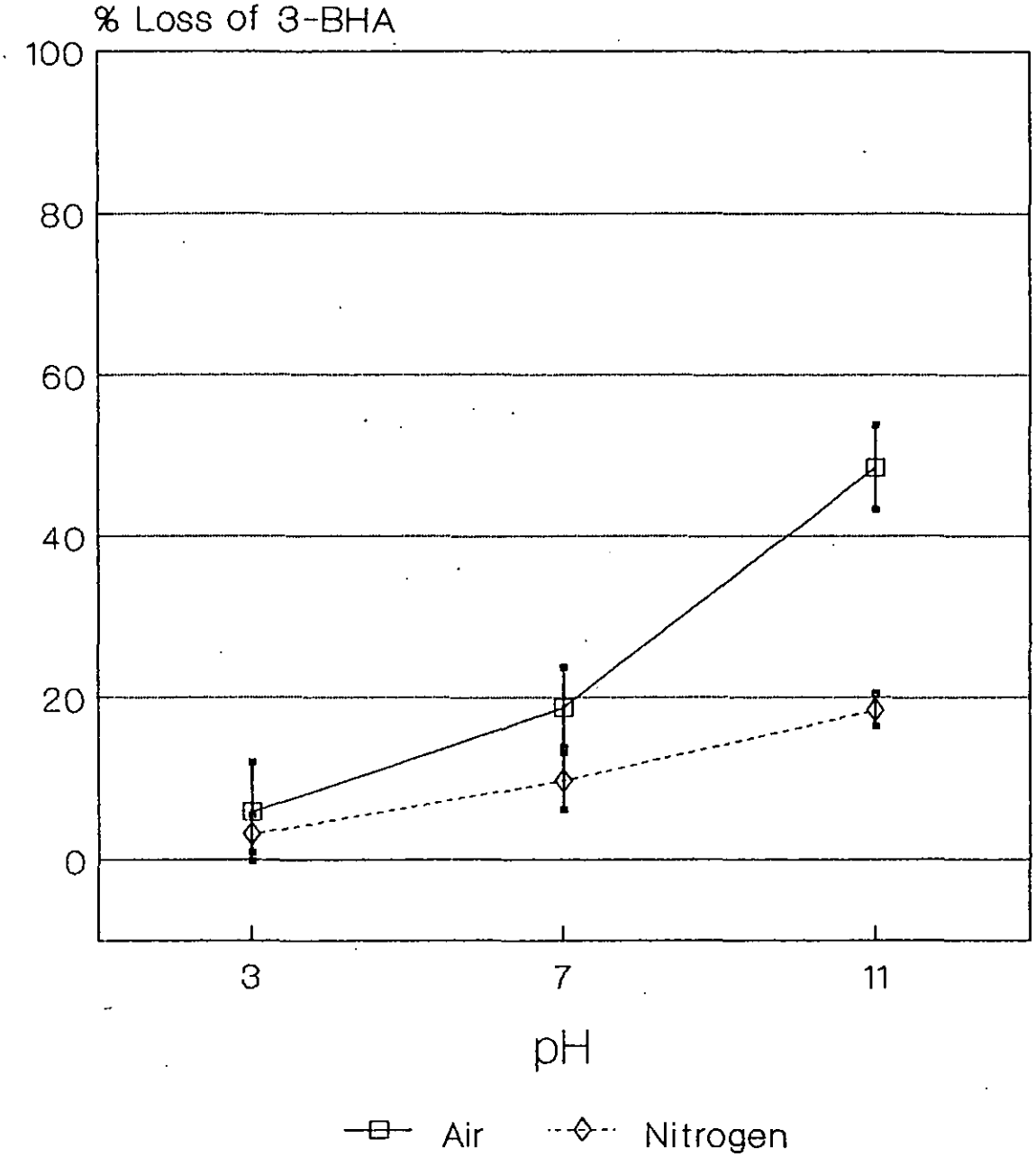
Ampoules containing 2-BHA and 3-BHA were prepared, in triplicate, as previously described, Section 4.4. The samples were heated for 3 hours at 115^{o}C at pH 3.0, pH 7.0 and pH 11.0, extracted into chloroform then examined by GLC (Shimadzu) using the OV-7 column. The results are plotted in Figs 14 & 15.

From Figs 14 & 15 it can be seen that in air significantly greater losses occurred at pH 11.0 than 7.0 and 7.0 than 3.0. The losses under nitrogen were much less than under air but the same effect of pH was observed. The 2-BHA appears to be slightly more stable than the 3-BHA. The experiment was repeated using a 9:1 mixture of 3-BHA to 2-BHA. The results for the separate isomers, using the XE-60 column, are given in Figs 16 & 17, and the total loss, using the OV-7 column, is given in Fig. 18. The total losses of BHA were calculated from the results obtained using the XE-60 column and are plotted in Fig. 19.

From Figs 16 & 17 it can be seen that the greatest loss occurred at pH 11.0 but at pH 7.0 the results are not as clear. The results obtained using the OV-7 column for total BHA, Fig. 18, clearly shows stability decreasing with increasing pH and less stability in air than in nitrogen.

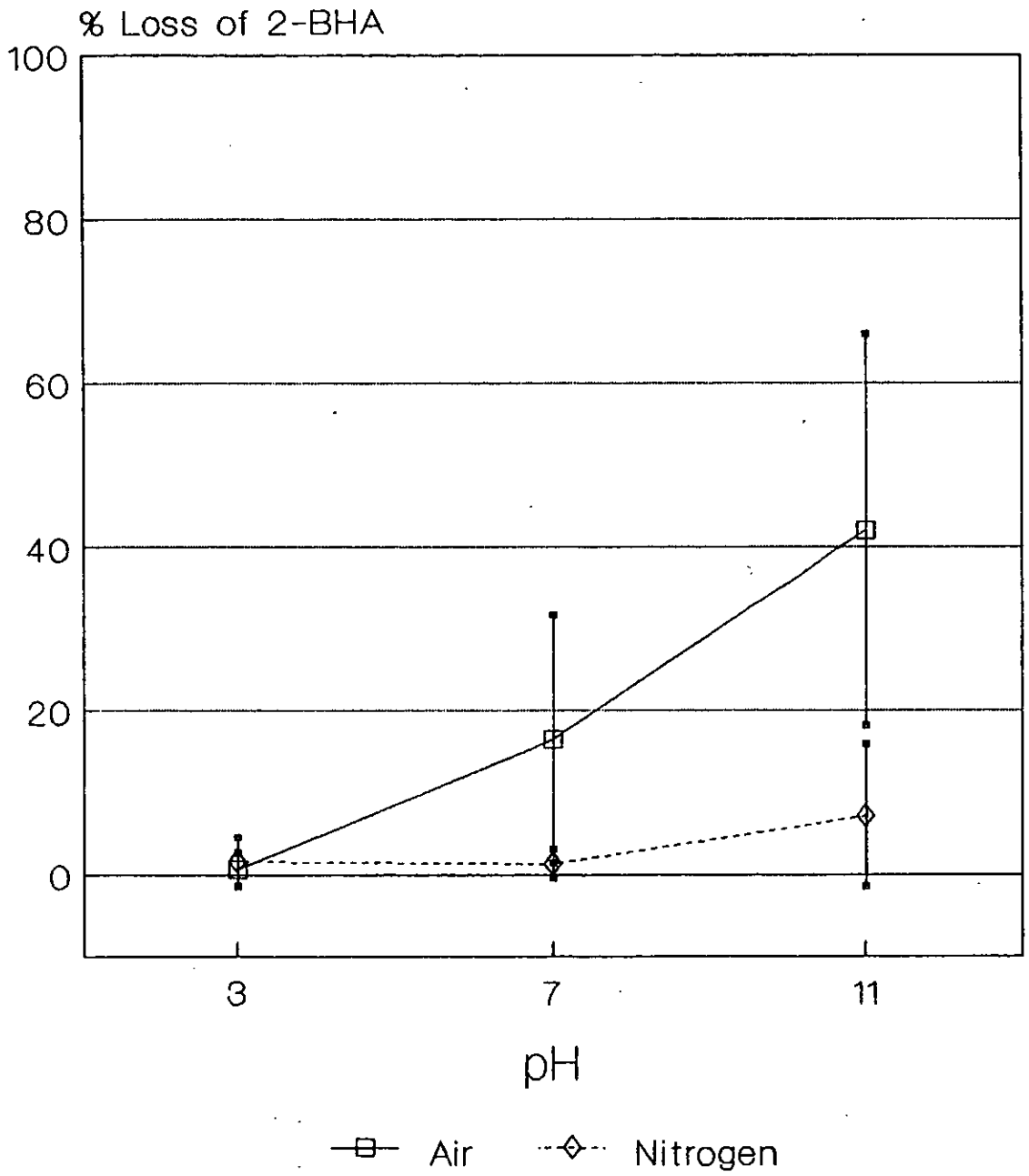
The calculated total loss, Fig. 19 compared with Fig. 18, shows similar results at pH 3.0 and 11.0, but the results at pH 7.0 are less clear. None of the results indicate any greater or lesser losses when the isomers are mixed on a 9:1 ratio rather than one heated separately.

Fig. 14: 3-BHA heated 3 hours at 115°C
(GC column OV-7)



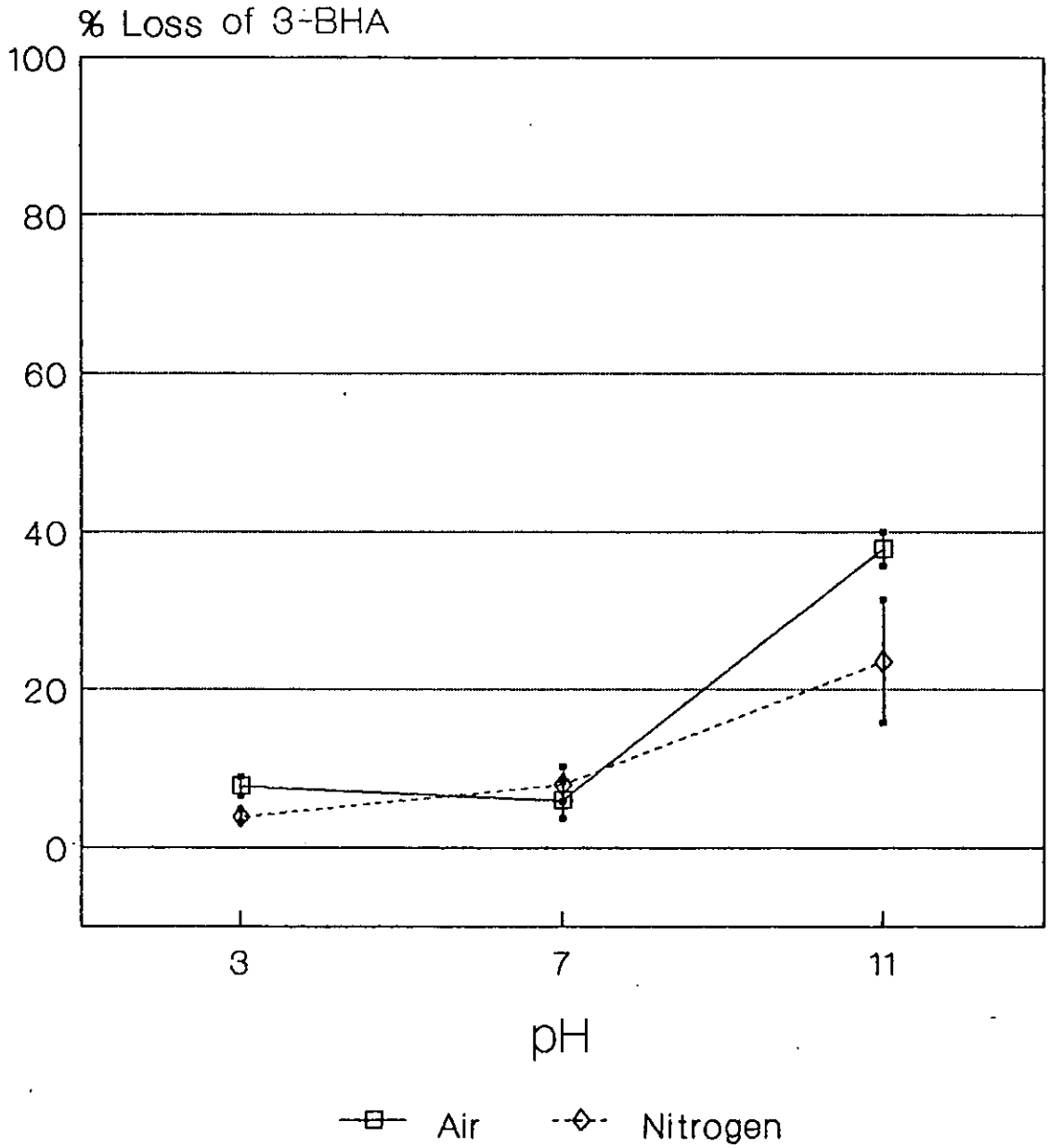
Points are means of 3 determinations.
Vertical lines represent 1 standard
deviation to each side of the points.

Fig. 15: 2-BHA heated 3 hours at 115°C
(GC column OV-7)



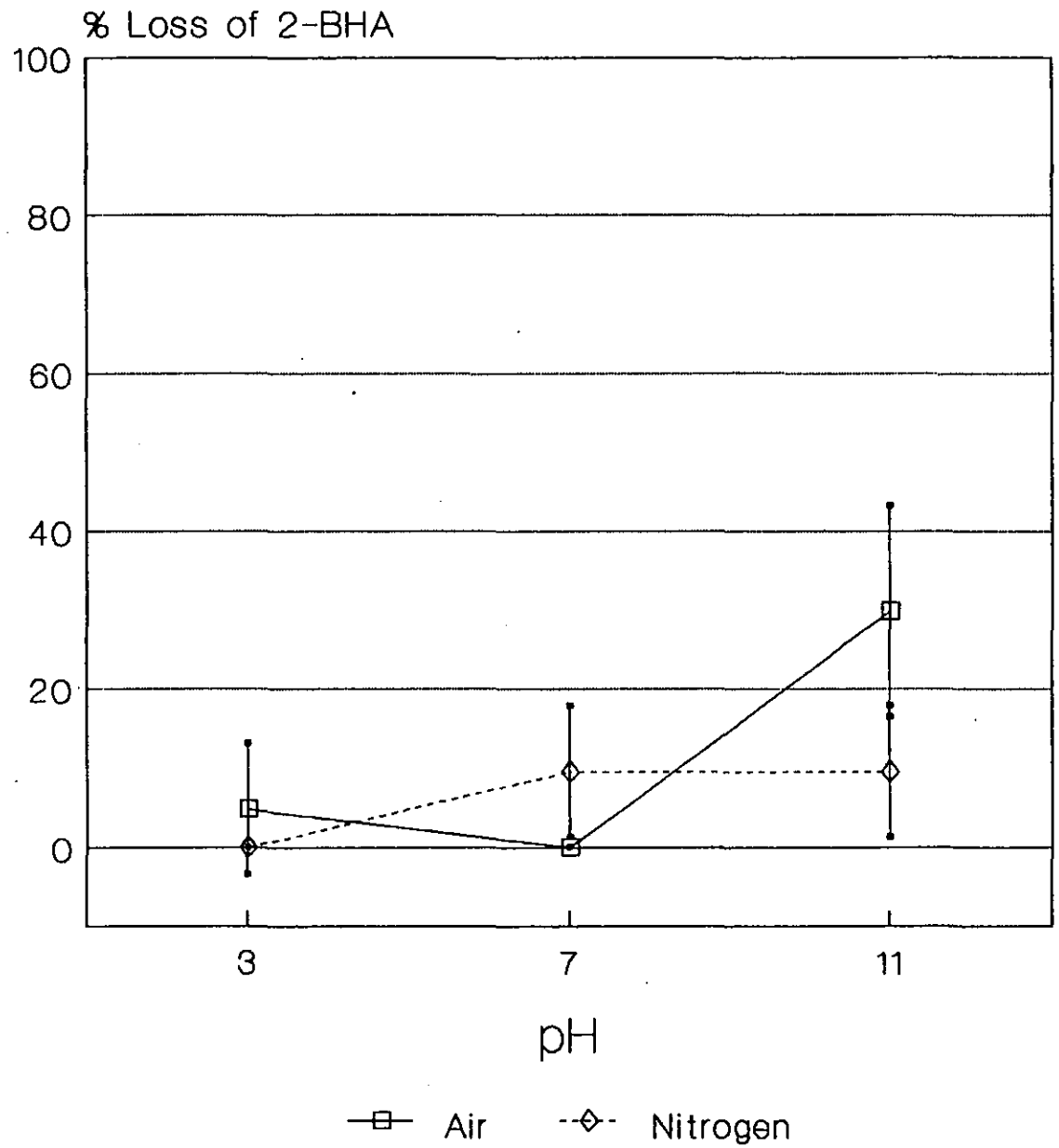
Points are means of 3 determinations.
Vertical lines represent 1 standard
deviation to each side of the points.

Fig. 16: 3-BHA heated 3 hours at 115°C in a 9:1 mixture of 3-BHA:2-BHA
(GC column XE-60)



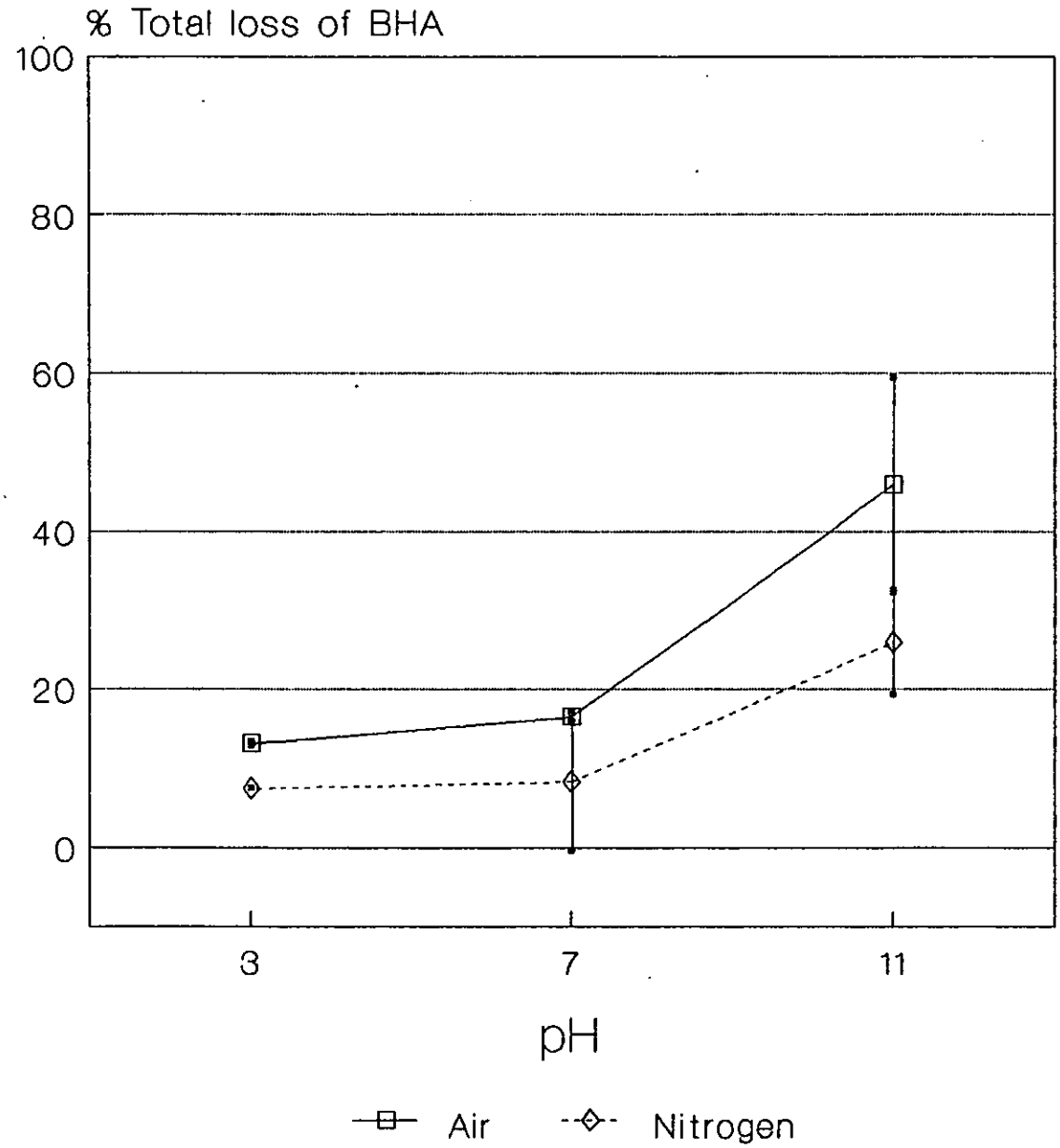
Points are means of 3 determinations.
Vertical lines represent 1 standard deviation to each side of the points.

Fig. 17: 2-BHA heated 3 hours at 115°C in a 9:1 mixture of 3-BHA:2-BHA
(GC column XE-60)



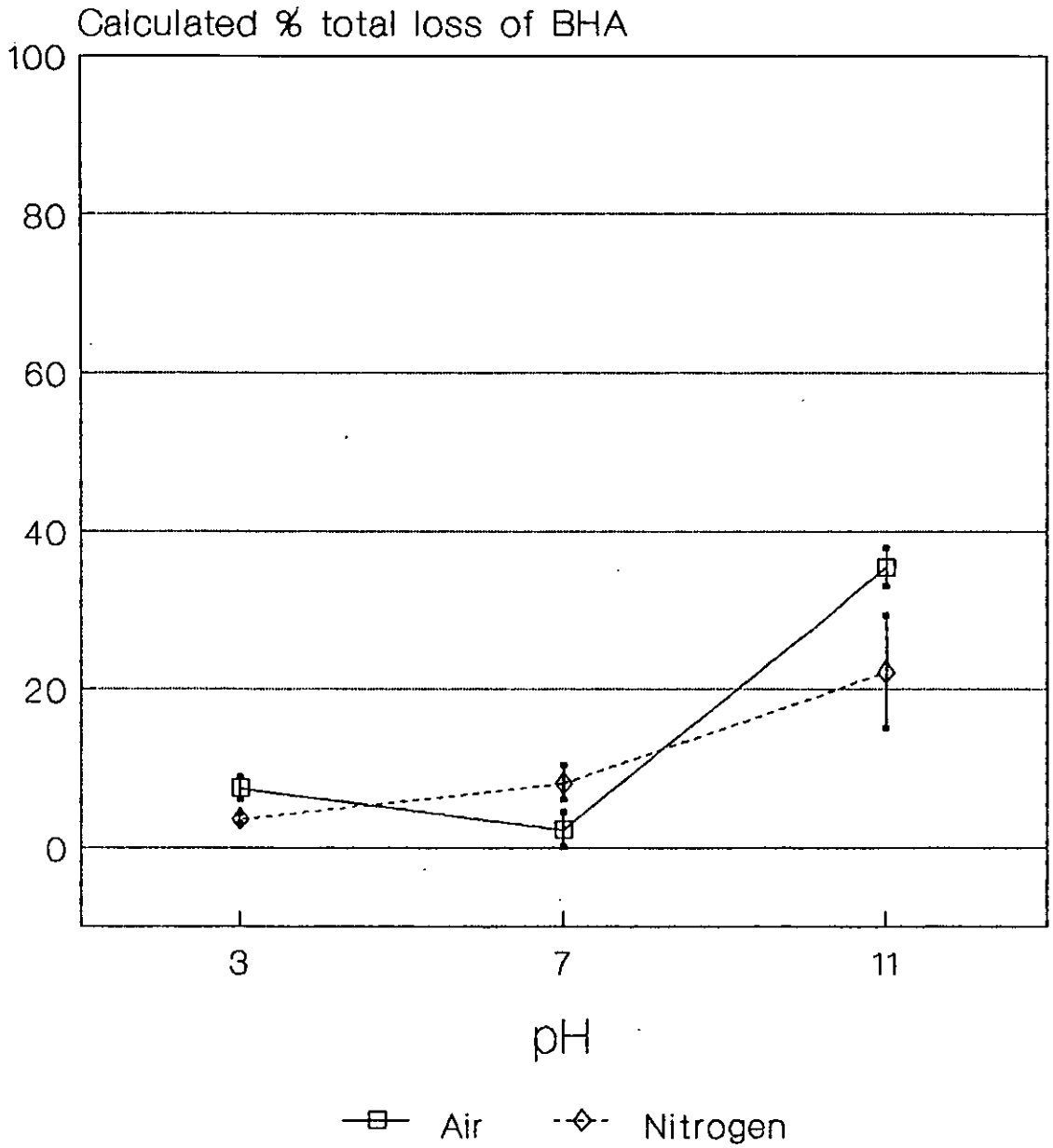
Points are means of 3 determinations.
Vertical lines represent 1 standard deviation to each side of the points.

Fig. 18: 9:1 mixture of 3-BHA:2-BHA heated for 3 hours at 115°C
(GC column OV-7)



Points are means of 3 determinations.
Vertical lines represent 1 standard deviation to each side of the points.

Fig. 19: 9:1 mixture of 3-BHA:2-BHA heated for 3 hours at 115°C
(GC column XE-60)



% Total loss of BHA and standard deviations are calculated from the data plotted in Figs 16 and 17.

Maga and Monte (1977) heated samples of commercially available BHA in air at pH 3.0, 7.0 and 11.0 for 15 minutes at 118°C and 124°C. The original composition of the BHA was 95.7% 3-BHA and 4.23% 2-BHA. Losses calculated from their results are shown below, Table 5. It is assumed that the 3-BHA and 2-BHA are as normally designated and not as given in the paper (see Section 2.3.3).

Table 5: Results of Maga and Monte for BHA isomers heated

Temp °C	pH	% loss 3-BHA	% loss 2-BHA
118	3.0	1.7	52
118	7.0	4.0	55.1
118	11.0	7.8	58.2
124	3.0	6.3	58.9
124	7.0	9.4	61.7
124	11.0	14.0	64.2

The results indicate that the stability of BHA decreases with increasing pH, in agreement with the results of this study, but that the 2-BHA is much less stable than the 3-BHA. In this study 3-BHA gave greater percentage decomposition than 2-BHA even when in a 9:1 mixture. Even though Maga and Monte were working with a different mixture of 3-BHA to 2-BHA, and at different temperatures, this cannot explain the difference in results. The validity of their results is questionable, although maybe explained by their confusion with isomers, as discussed above (Section 2.3.3).

4.5.2 The effect of light

Ampoules containing 2-BHA, 3-BHA and a 9:1 mixture of 3-BHA to 2-BHA were prepared in water at pH 3.0, 7.0, and 11.0. The samples were exposed to sunlight for six weeks then extracted into chloroform and examined by GLC using a OV-7 column for total BHA and an XE60 column for the separate isomers.

The results obtained for the separate isomers are shown in Figs 20 & 21. It can be seen that at pH 11.0 in air all the 3-BHA was lost and virtually all of the 2-BHA. Significantly less loss was observed at pH 7.0 and 3.0, with pH 7.0 being the most stable. In nitrogen the losses were much less but did increase with increasing pH values.

The results for the separate isomers from the 9:1 mixture are shown in Figs 22 and 23. In air the 3-BHA is less stable than the 2-BHA but changing from pH 7.0 to 11.0 did not affect the stability of the 3-BHA. However, the 2-BHA appears to be completely stable at pH 7.0 but with more decomposition at pH 11.0 than at pH 3.0.

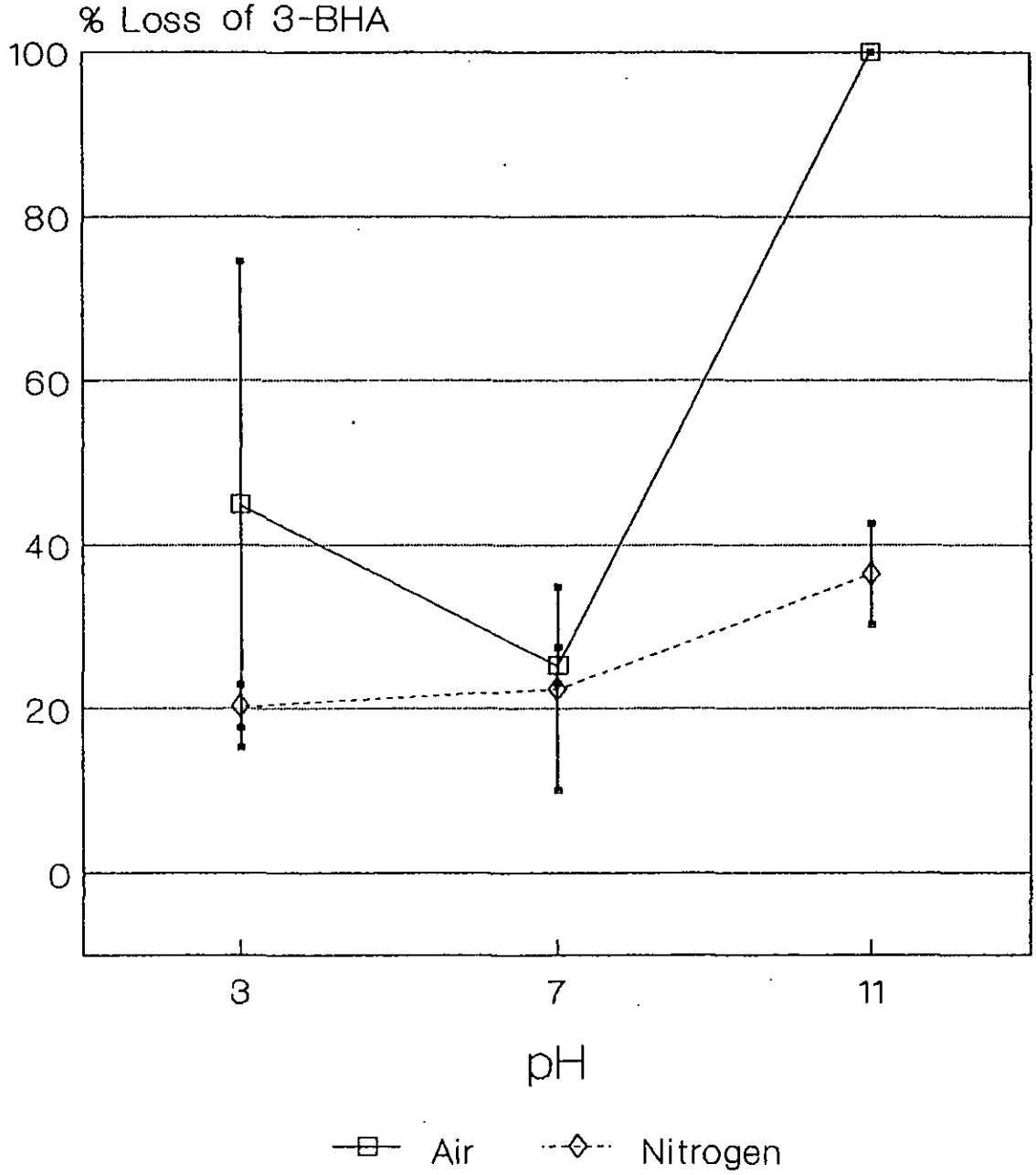
Under nitrogen the 2-BHA was significantly more stable than the 3-BHA. The stability of the 3-BHA was hardly affected by pH but the stability of 2-BHA decreased at higher pH values.

The results obtained for the loss of total BHA are shown in Fig. 24. The effect of no apparent loss of 2-BHA is reflected in the result obtained at pH 7.0. At pH 3.0 and 11.0 the stability of BHA is much increased under nitrogen compared with air.

The calculated total losses of BHA obtained from the results of Figs. 22 and 23 are shown in Fig. 25. This shows decreasing stability of BHA with increasing pH and a significant increase in stability under nitrogen compared with air.

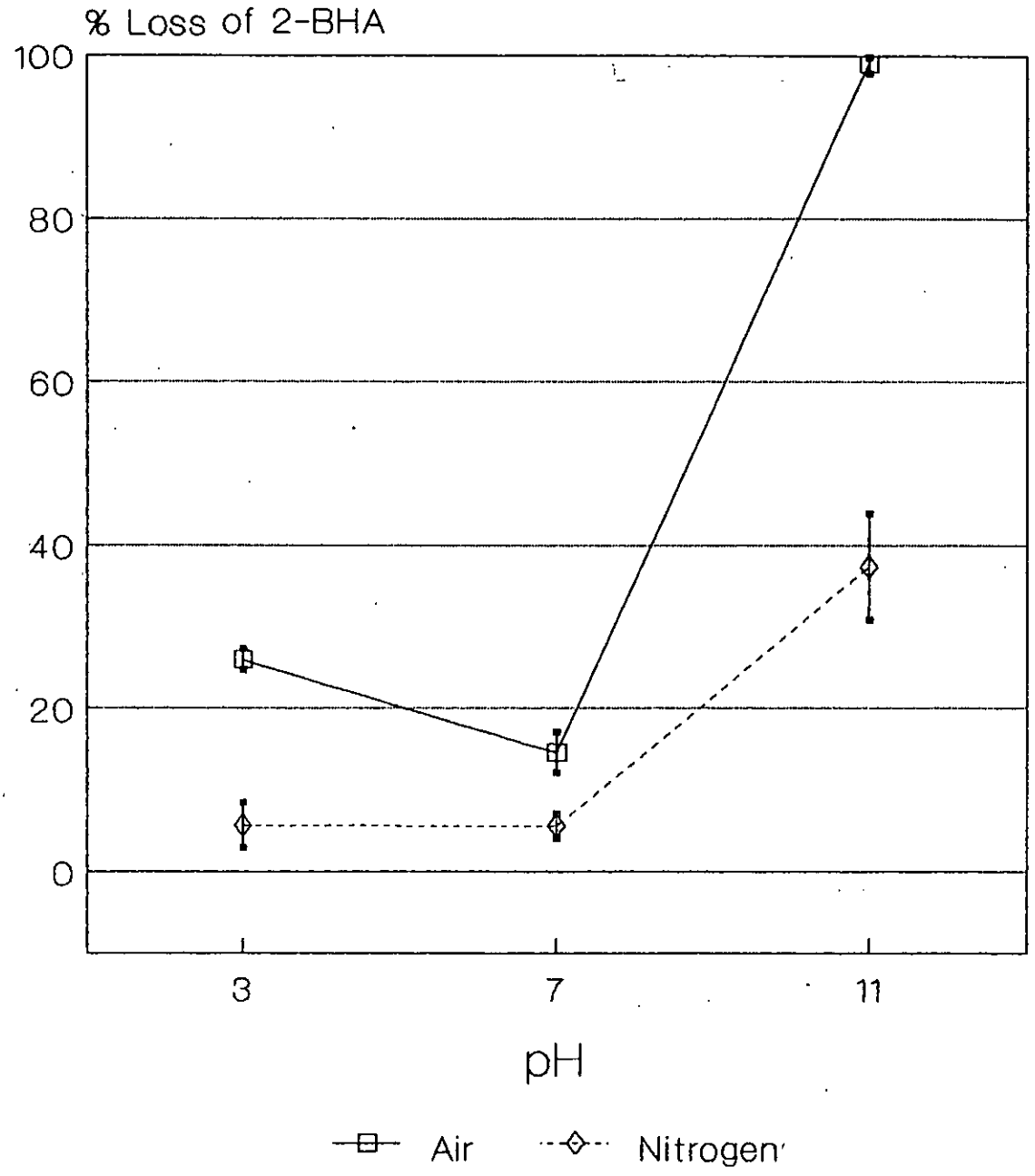
Maga and Monte (1977) subjected ampoules containing 5.00 g of 0.02% suspension of commercial BHA (95.7% 3-BHA, 4.3% 2-BHA) in water to sunlight. Losses calculated from their results are shown in Table 6. As with the heating experiments, their results show the 2-BHA to be much less stable than the 3-BHA. In this study, when the two isomers are treated separately as well as in a mixture, the two isomers are seen to have similar stabilities in sunlight.

Fig. 20: 3-BHA after storage in light
(GC column OV-7)



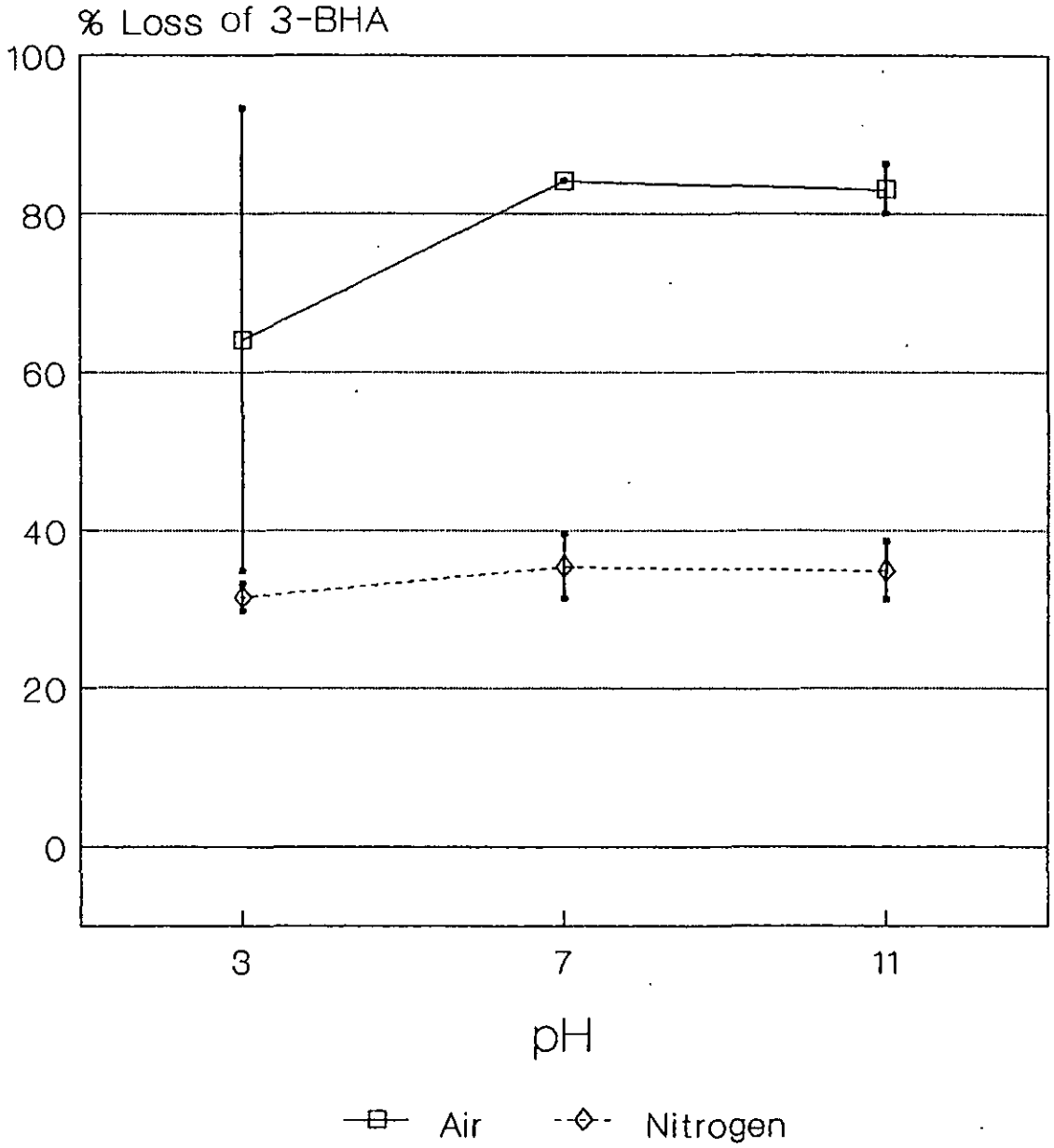
Points are means of 3 determinations.
Vertical lines represent 1 standard
deviation to each side of the points.

Fig. 21: 2-BHA after storage in light
(GC column OV-7)



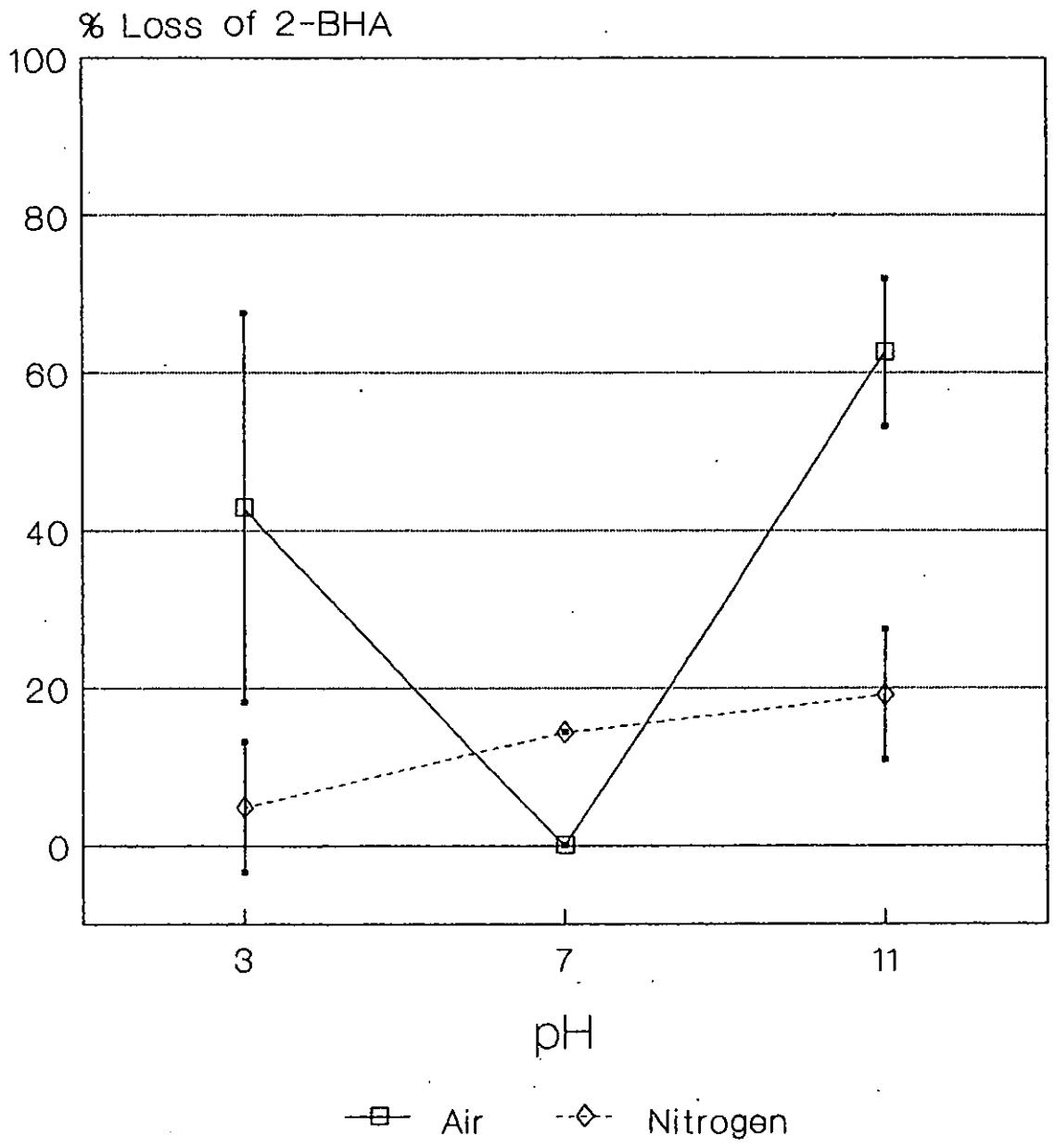
Points are means of 3 determinations.
Vertical lines represent 1 standard deviation to each side of the points.

Fig. 22: 3-BHA after storage in light in a 9:1 mixture of 3-BHA:2-BHA
(GC column XE-60)



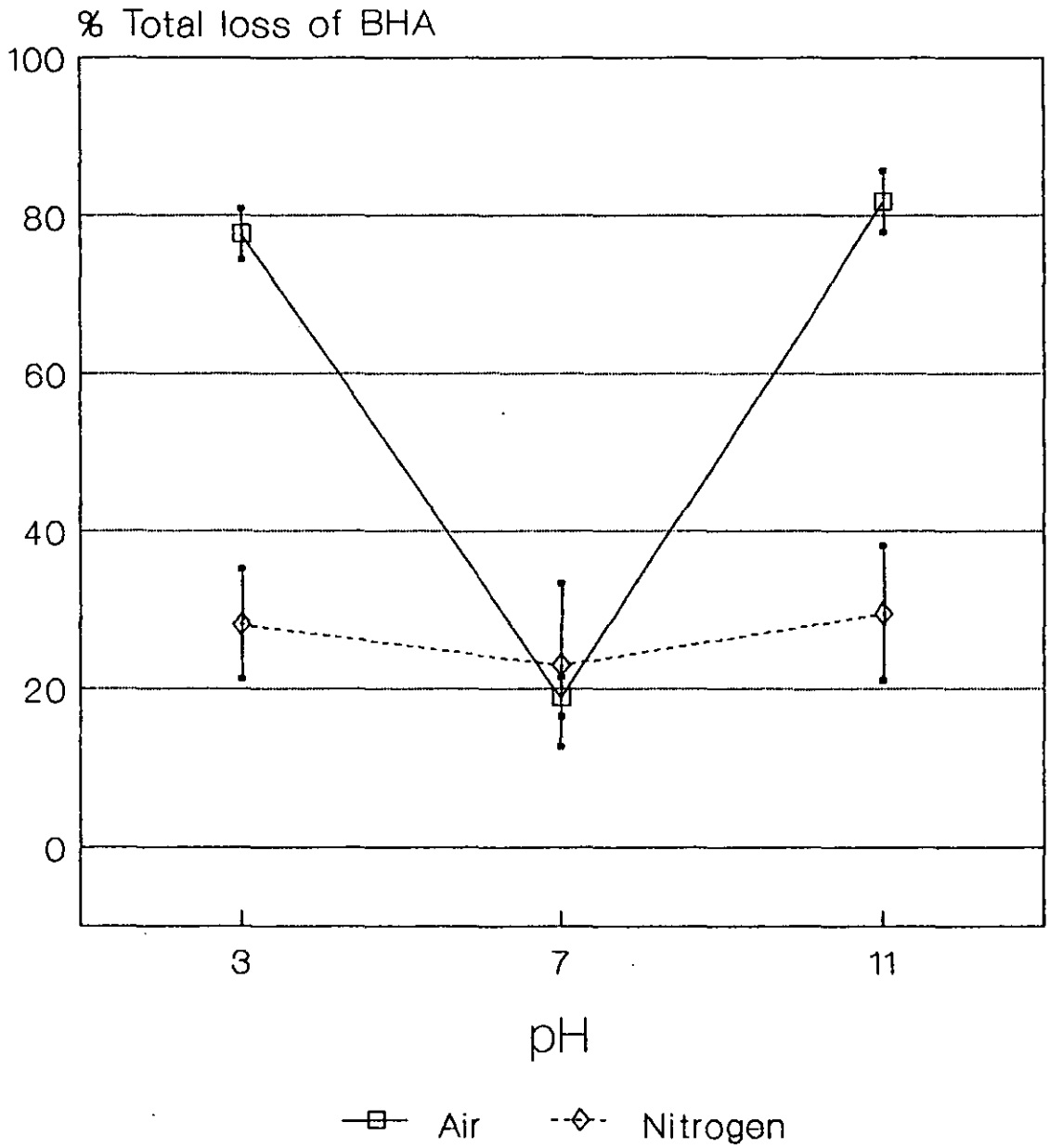
Points are means of 3 determinations.
Vertical lines represent 1 standard deviation to each side of the points.

Fig. 23: 2-BHA after storage in light in a 9:1 mixture of 3-BHA:2-BHA
(GC column XE-60)



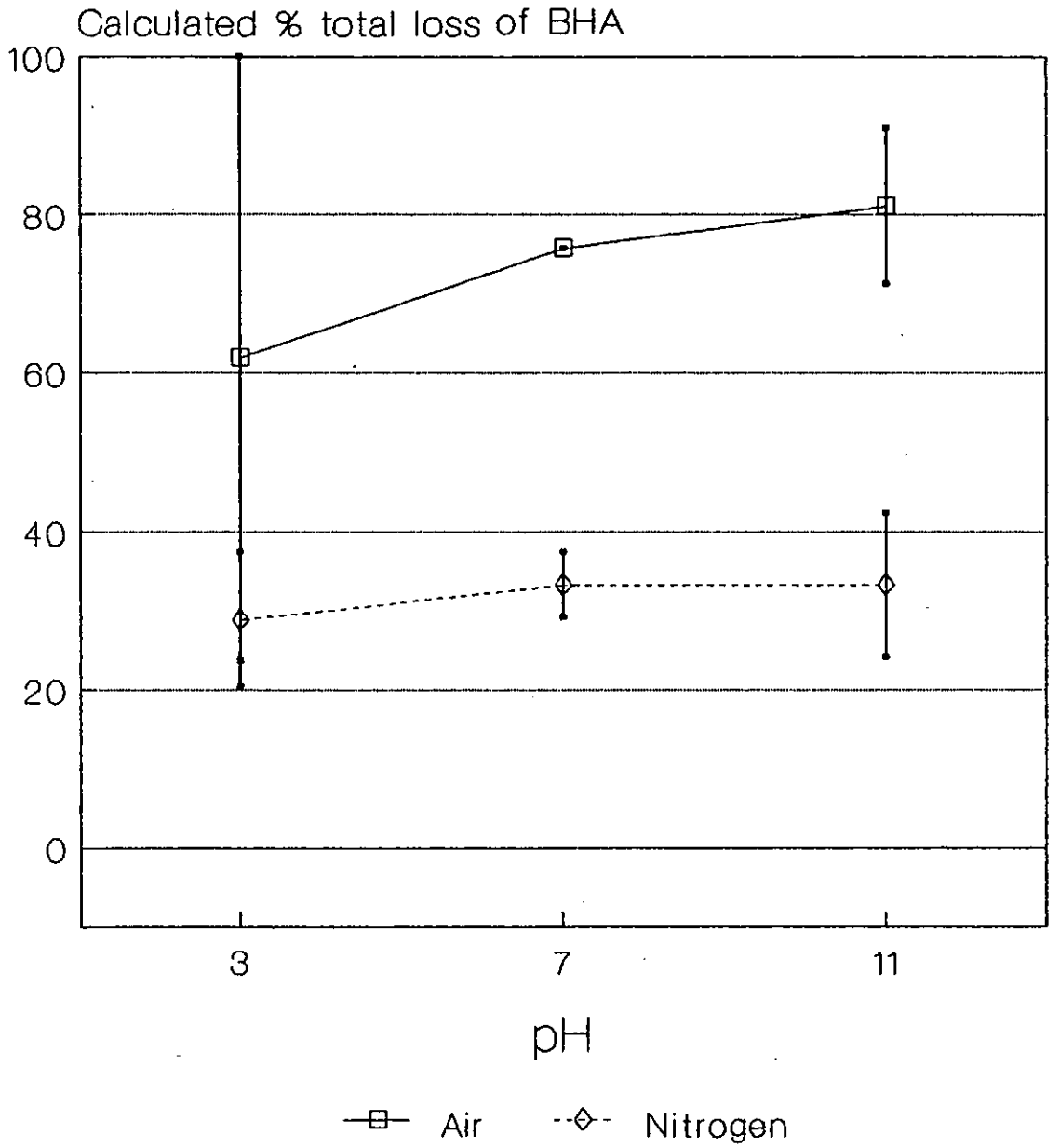
Points are means of 3 determinations.
Vertical lines represent 1 standard deviation to each side of the points.

Fig. 24: 9:1 mixture of 3-BHA:2-BHA after storage in light
(GC column OV-7)



Points are means of 3 determinations.
Vertical lines represent 1 standard deviation to each side of the points.

Fig. 25: 9:1 mixture of 3-BHA:2-BHA after storage in light
(GC column XE-60)



% Total loss of BHA and standard deviations are calculated from the data plotted in Figs 22 and 23.

Table 6: Results of Maga and Monte (1977) for BHA isomers in sunlight

No. of days	% Loss of 3-BHA	% Loss of 2-BHA
1	5.0	45.9
2	4.1	85.6
3	3.7	99.8
5	5.9	100.0
20	2.0	100.0

4.5.3 General discussion of the decomposition results

In this study it was found that: the BHA isomers are in general more stable under nitrogen than in air, the BHA isomers are more stable at acid or neutral than alkaline pH, that light has a greater effect than heat, that the 2-BHA is marginally more stable than the 3-BHA and a mixture of the isomers does not markedly effect their individual stabilities.

Since the products of decomposition are dimers formed by oxidation processes (See Section 2.3.3), it is not suprising that nitrogen increases the stability of the BHA isomers. The effect of pH may be that the phenolate anion is more reactive than the unionised forms of BHA. The greater effect of light than heat can be explained in terms of initiation of free radical reactions which are probably predominant in the light induced decompositions. The relative stabilities of the individual BHA isomers cannot be explained without further study.

Although these results show that 2-BHA is not greatly more unstable than 3-BHA, as suggested by Monte and Maga, nevertheless, the fact that 2-BHA does decompose on heat or light treatment, and may interact with 3-BHA indicates that pure 3-BHA would be preferable as a food additive than the 9:1 mixture presently used.

4.6 ANTIOXIDANT ACTIVITY OF BHA ISOMERS AND A COMMERCIAL MIXTURE

The antioxidant activity of 2-BHA, 3-BHA and a 9:1 mixture of 3-BHA to 2-BHA was examined using the Rancimat. The results are given in Table 7.

For the safflower oil a relatively small antioxidant effect was observed with the 3-BHA not being significantly more effective than the mixture, but more effective than the 2-BHA. For the peanut oil a small antioxidant effect was observed but there was no significant difference between the 3-BHA, 2-BHA and the mixture. For the soy bean oil and the olive oil a relatively small antioxidant effect was observed for the 3-BHA and the mixture with no significant difference between the two.

Overall it is seen that the antioxidant activity of the 3-BHA is either greater or not significantly different to the 9:1 mixture of 3-BHA and 2-BHA. In general both the 3-BHA and the mixture were more effective antioxidants than 2-BHA.

Table 7: Effect of BHA isomers and a commercial 9:1 mixture of 3-BHA: 2-BHA on induction times for rancidity development for various oils using the rancimat*

Oil	Induction time in hours			
	No antioxidant	200 ppm 2-BHA	200 ppm 3-BHA	200 ppm 9:1 3-BHA:2-BHA
Safflower	4.8^{a}(0.3)	5.4^{ab}(0)	6.3 (0.1)	5.7^{b}(0.2)
Peanut	16.2^{a}(0.5)	17.1^{ab}(0.4)	17.9^{b}(0.5)	17.9^{b}(0.4)
Soybean	8.5^{a}(0.4)	8.7^{a}(0.4)	10.3^{b}(0.2)	10.1^{b}(0.3)
Olive	3.7 (0.9)	11.4^{a}(1.3)	15.5^{a}(2.4)	14.6^{a}(1.2)

* The induction times are means of between 2 to 8 replicates; standard deviations are given in parentheses. For each oil, values with the same superscript are not significantly different at the 5% probability level by the Student t-test.

4.7 ECONOMIC ASPECTS OF USING 3-BHA COMPARED WITH A TYPICAL 9:1 MIXTURE OF 3-BHA TO 2-BHA

3-BHA can be obtained by the fractional crystallisation of a commercial mixture from cyclohexane. The following estimation is based on the author's many years of experience in the chemical industry. It is assumed that two crystallisations would produce sufficiently pure 3-BHA (>99.5% purity) with a yield of 58% (see Section 3.3.1), the overall loss of cyclohexane is expected to be in the order of 15%.

Scale of manufacture

1000 litres charging 100 kg BHA and 500 kg cyclohexane.
Yield of 3-BHA = 58 kg
Loss of cyclohexane = 75 kg.

Costs

Time for 2 crystallisations = 36 hours
Labour input 4.5 hours per crystallisation at £11 per hour = £99
Cost of cyclohexane = £0.55 per kg
= 75 x 0.55 = £41
Utilities cost = £10
Depreciation/maintenance = £0.45 per kg of product
= £26
Let cost of BHA = £y per kg

Increase in costs.

Labour	£99
Cyclohexane usage	£41
Utilities	£10
Dep/maintenance	£26
BHA	£100y
	£139 + 100y

58 kg costs £139 + 100y

$$\text{Cost per kg} = \frac{£139 + 100y}{58}$$

$$\text{Increased cost per kg} = \frac{£139 + 100y}{58} - y$$

$$\% \text{ increase} = \left[\frac{139 + 100y}{58}\right] - y \times \frac{100}{y}$$

Increased costs of purification

If y = £1 increase = 312%

If y = £5 increase = 120%

If y = £10 (May and Baker price 1980) increase = 96%

Although the cost of BHA is increased, the increase in foodstuff cost would be relatively small since the maximum BHA allowed is only 200 ppm. Also, since the 3-BHA is a more effective antioxidant than the mixture less would be required.

Increased cost of a food commodity

Example of the increase in cost of using 3-BHA instead of a commercial mixture for a product containing the maximum permitted amount of BHA.

Krona margarine costing 70p for 500 g.

200 ppm of BHA is equal to 0.1g of BHA per 500 g of margarine.

Assume cost of commercial BHA to be £10 per kilo. Then 0.1g costs 0.1p. Increase in cost on using pure 3-BHA is 0.096p per 500g pack; which is 0.14% of the total product price.

For other food products which contain less than the 200 ppm maximum permitted amount of BHA, the increased costs would be proportionately less.

5. CONCLUSIONS

1. 3-BHA is marginally less stable than 2-BHA under heating but both isomers were generally more stable under acid conditions than alkaline. Both isomers were more stable under nitrogen rather than air.

2. Light produced more decomposition than heating. Again, both isomers were more stable under acidic conditions than alkaline and were also more stable under nitrogen. The 2-BHA appeared to be marginally more stable than 3-BHA.

3. The antioxidant activity of the 3-BHA was either greater or not significantly different to that of the 9:1 mixture of 3-BHA to 2-BHA. In general both the 3-BHA and the mixture were more effective antioxidants than the 2-BHA.

4. The 3-BHA is at least as effective an antioxidant as the 9:1 mixture and would also produce less decomposition products than the mixture under heating or light conditions.

5. Economically there appears to be no real justification for not recrystallising the commercial 9:1 mixture to produce pure 3-BHA since the extra cost would be relatively small in typical foodstuffs.

6. SUGGESTIONS FOR FURTHER WORK

1. To examine the effect of heat and light at more pH values between pH 3.0 and pH 11.0 using the separate isomers and a 9:1 mixture.

2. To repeat the work of Monte and Maga using the separate isomers and a 9:1 mixture to try to clarify the discrepancies in their results.

3. To check the effect of other additives on the decomposition of BHA isomers, e.g. potassium sobate, sodium nitrite.

4. To isolate the decomposition products to check for possible toxic compounds.

5. To carry out further assessment of the relative antioxidant activity of 3-BHA compared to the 9:1 mixture with a view to recommending the use of pure 3-BHA as a food additive rather than a mixture of 3-BHA and 2-BHA.

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