Oxidation of Lipids in Fish Meal

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ABSTRACT

Oxidation of lipids in fish meal has been studied. The course of the reaction has been followed by means of oxygen absorption, peroxide values and chromatographic analyses. The latter has been used quantitatively, because the method of methylation of the unreacted fatty acids obtained by hydrolysis of the glyceryl esters has proved to be quantitative. The results indicate: (a) there are other oxygen-consuming reactions which account for up to 10 times the oxygen consumed by simple peroxidation of the polyunsaturated lipids; (b) increasing moisture content has a marked prooxidant effect in all the oxidative reactions; (c) the overall rate of reaction is apparently independent of the peroxide concentration in the lipid, possibly due to the fact that there is some resistance to the diffusion of oxygen which produces deviations from the normal autocatalytic path of the reaction occurring when oxygen is fully available.

INTRODUCTION

One of the primary problems in the storage of food is the oxidation of its lipids. Normally, this oxidation proceeds rather slowly, due to the low degree of unsaturation of the fatty esters usually present. However, lipids in pilchard and anchovy meal have fatty acids with five or six degrees of unsaturation. This fact, together with the conditions of temperature and oxygen availability which occur during the production of the meal, produces a rate of oxidation high enough to cause spontaneous heating of the meal during its shipping and transportation, thus creating serious economical problems. These problems seem even more serious when the interaction between proteins and oxidized lipids is taken into account (1-2), because this phenomenon might cause a decrease in the nutritional value of the meal.

It has been shown (3-4) that after the initiation period, and with enough oxygen, the mechanism of autoxidation of unsaturated lipids corresponds to a free radical process in which the resulting peroxy radicals produce an autocatalytic reaction, that can be represented by:

$$\frac{d(O_2)}{dt} = -\frac{d(RH)}{dt} = k(RH)\ (ROOH)$$

Furthermore, it has been proved that the rate of this process is strongly influenced by the presence of water, inorganic and organic substances (4-7). In particular, it has been found that below the monolayer value, water has a protective effect against oxidation. Above this value (5), however, this effect would not be so clear.

The purpose of the present work was to provide information about the parameters affecting the rate of oxidation of lipids in fish meal, and to gather some information on the analytical techniques necessary to follow the oxidation quantitatively.

EXPERIMENTAL PROCEDURES

Material

Whole pilchard or anchovy meal was obtained directly from factories in Arica, Chile. Immediately after production, the meal was put into sealed cans and sent to our laboratories, where it was kept at -30 C until it was used in the experiments. Except for the meal used in the tests related to the influence of grinding in the velocity of oxidation, all the material was screened between 50 and 100 Tyler mesh.

Oxygen Absorption

The velocity of oxygen uptake was measured using the standard Warburg manometric technique.

Lipid Extraction

The method of lipid extraction was the one described by Bligh and Dyer (8), with a slight change consisting in the acidulation of the water-methanol phase immediately after extraction, to obtain a better phase separation. This change improved the efficiency of the procedure by nearly 10%. The amount of lipid extraction remained approximately constant during the course of the oxidation, and the emission of volatiles has proved to be comparatively low.

Peroxide Value

The peroxide value was determined by the AOCS method (Cd 8:53) with minor alterations, consisting in the addition of water and titration not only 1 min after the addition of KI, but also after 10 and 20 min. The curves obtained are shown in Figure 1. These curves suggest that some secondary oxidation of the sample occurs during titration and, consequently, the peroxide value was taken as

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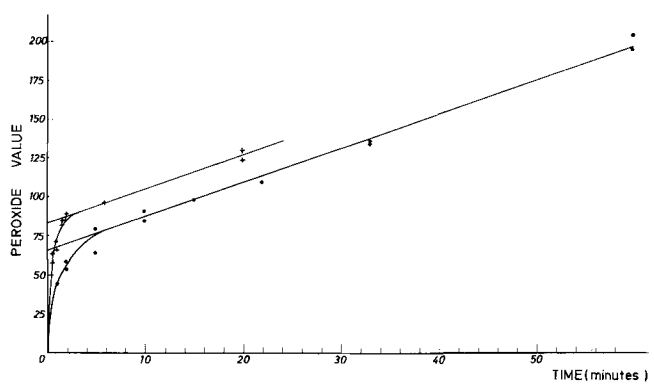

FIG. 1. Peroxide value as a function of the time elapsed between the addition of KI and the addition of water. + Run 1, • Run 2.

TABLE I

Fatty Acid Distribution in Fish Meal Lipids

Fatty acid	wt %
14:0	8.95
16:0+16:1+17:0+17:1	39.47
18:0+18:1	24.07
20:1+18:4	1.13
Unknown	1.94
20:5	14.72
22:5+22:6	8.08

Less than 1%: 15:x, 18:2, 18:3, 20:0, 20:4, 22:1, 24:0

TABLE II

Quantitative Validity of the Method of Esterification

Run 1, 45 C, 45% R.H.				Run 2, 45 C, 65% R.H.			
Time, hr	A[a]	B[b]	C[c]	Time, hr	A[a]	B[b]	C[c]
0	18.9	23.3	42.2	0	17.6	22.9	40.5
4.2	16.5	26.0	42.5	19.6	13.2	26.5	39.7
23.7	14.2	27.5	41.7	91.4	8.5	30.4	38.9
29.0	13.7	28.8	42.5	189.8	7.0	32.9	39.9
47.6	13.0	29.8	42.8	262.3	5.3	34.5	39.8
52.9	12.4	30.5	42.9				
72.0	11.2	32.1	43.3				

[a]A, per cent of polyunsaturated lipid in total fat.
[b]B, per cent of polymers, related fatty substances and oxidized lipids.
[c]C, per cent of polyunsaturated lipids, polymers, related fatty substances and oxidized lipids = A+B.

the extrapolation of the straight line to its intersection with the axis corresponding to time 0.

Concentration of Polyunsaturated Lipids

The method used to analyze the mixture of fatty compounds that exist in the fish meal has two parts: (a) the hydrolysis of the lipids, followed by an esterification with methanol of the fatty acids thus obtained, and (b) the chromatographic analysis of the resulting methyl esters. Part (a) was performed using the procedure of rapid preparation of fatty acid esters developed by Metcalfe et al. (9). Part (b) was carried out in a Perkin Elmer 800 flame ionization gas chromatograph. Conditions were: column, 1/8 x 100 in., 10% BDS on Chromosorb 60-80; temperature, 145 to 205 C, with a programmed increase of 0.8 C/min; carrier gas, N_2, 1 cc/sec.

A typical distribution of methyl esters is seen in Table I. All the runs performed have shown that acids 20:5 and 22:6 account for about 90% of the fatty esters being oxidized. The remaining 10% is made up of minor amounts of acids 18:3, 18:4, 20:4 and 22:5. The knowledge of the molecular weights has permitted the concentration of reactive lipids to be expressed as μmol of polyunsaturated lipid per gram of fat.

Lipids in fish meal consist mainly of fatty triglycerides,

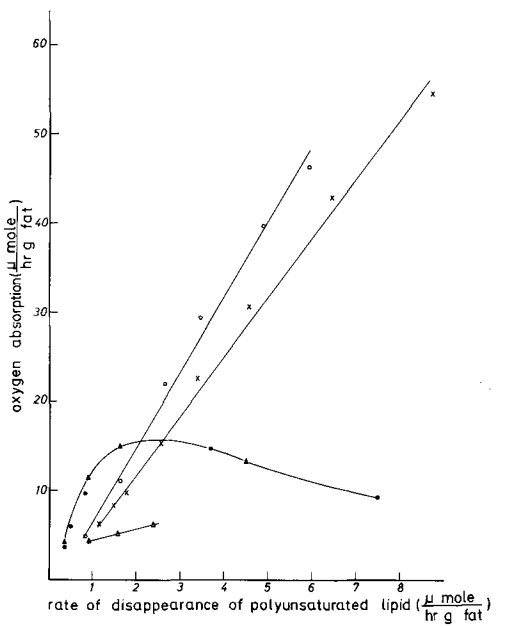

FIG. 2. Rate of oxygen absorption as a function of the rate of disappearance of polyunsaturated lipid. • 45 C, 20% R.H.; ▲ 45 C, 20% R.H.; △ 30 C, 45% R.H.; ○ 45 C, 45% R.H.; x 45 C, 45% R.H.

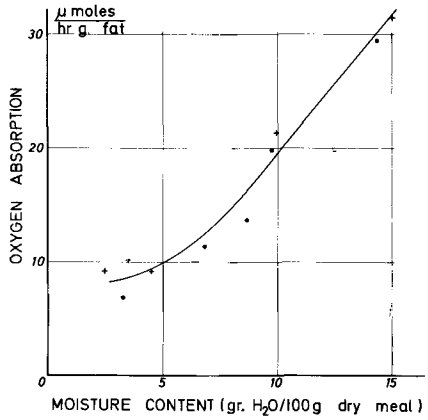

FIG. 3. Rate of oxygen absorption as a function of the moisture content of the fish meal. • Run 1, + Run 2.

plus a certain quantity of polymers, related fatty compounds and products of previous oxidation. When the hydrolysis and further esterification, which rapidly produce a phase of methyl esters dissolved in petroleum ether, are conducted, two possibilities of behavior can be expected: (a) The polymers, related fatty compounds and products of oxidation are easily esterified. In this situation it should be expected that the yield of the esterification, expressed as grams of methyl ester per gram of fat, should remain approximately constant during the oxidative process. (b) On the other hand, if these polymers and products of oxidation are not easily esterified, or if even esterified they are not obtained in the petroleum ether phase due to polar effects, a decrease in the yield of esterification should be expected during the process of oxidation. This decrease should therefore be related to the decrease in polyunsaturated lipids detected in the chromatograph.

Table II suggests that in our experiments the second possibility occurred quite accurately. In fact, it can be seen that the percentage of polymers and oxidized substances, considered as 100 minus the yield of the esterification, increases during oxidation to the same extent as the percentage of polyunsaturated lipids decreases, since the sum of both quantities remains constant.

This result indicated that, due to the rapidity of the Metcalfe method, it is possible to measure easily the amount of polymers and oxidized substances in lipids, making it also useful as an industrial test. This test, combined with only one chromatographic measurement, provides direct information about the evolution of the concentration of reactive lipids in the meal. From another point of view, this procedure should provide an easy way of isolating the products of oxidation, because they are obtained as a residue below the petroleum ether phase. Unfortunately, it has not been possible to determine the exact composition of these residues and, therefore, it is not

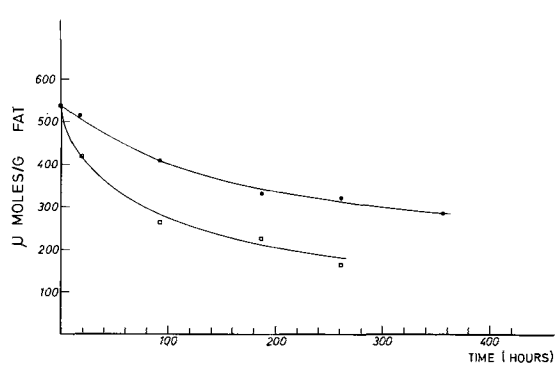

FIG. 4. Evolution of the concentration of polyunsaturated lipid for meal under 65% R.H. (□) and under 20% R.H. (•).

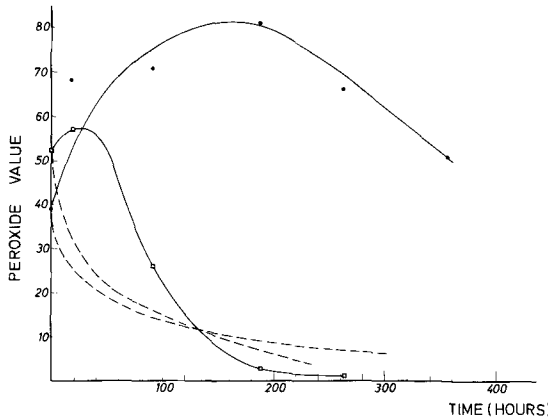

FIG. 5. Evolution of the concentration of hydroperoxides for meal under 65% R.H. (□), and under 20% R.H. (●); - - - is the value of d(RH)/(RH)dt for each condition.

known whether these substances have been esterified or not.

The chromatographic technique has permitted the identification and measurement of the reactive lipids in the meal in a very accurate and reproducible way. This technique, when compared with the iodine index, has shown considerable advantages, due to the fact that different meals revealed different correlations between their iodine index and chromatographic values, probably due to the tendency to react with iodine shown by polymers and products of oxidation.

Moisture Sorption Isotherm

The sorption isotherm was prepared by means of the desiccator method (18) at 30 C. A B.E.T. plot showed that the monolayer value occurs at a moisture content of approximately 3-4%, on a dry basis.

Oxygen Diffusivity

In order to estimate the oxygen diffusivity in fish meal, a sample of material was allowed to oxidize until its rate of oxygen absorption was negligible. Then it was kept in vacuum for several hours in order to produce the desorption of the oxygen physically solved in the meal. Once the vacuum was broken the velocity of oxygen absorption was measured during 3 hr. Since the average diameter of these particles was known, it was possible to deduce the value of the diffusion coefficient from the time needed to return to equilibrium (18).

Oxidation of Samples

The oxidation of the samples was performed in several small fixed bed reactors submerged in a thermostatic bath. The flow of air had controlled relative humidity and

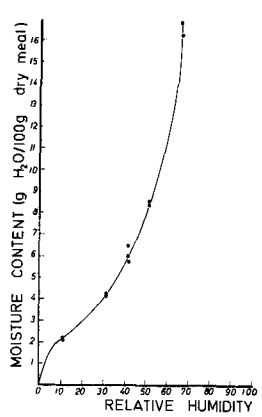

FIG. 6. Moisture adsorbed as a function of equilibrium water activity (per cent R.H. 100) for fish meal at 30 C.

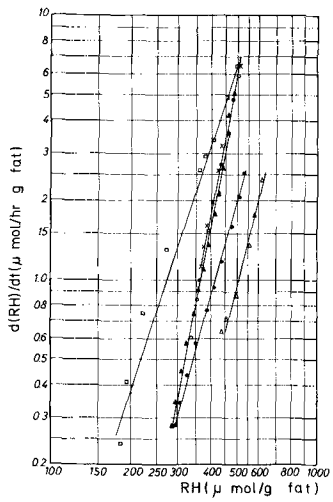

FIG. 7. Rate of disappearance of polyunsaturated lipid as a function of the concentration of polyunsaturated lipid. • 45 C, 20% R.H.; ▲ 45 C, 20% R.H.; △ 30 C, 45% R.H.; ○ 45 C, 45% R.H.; x 45 C, 45% R.H.; □ 45 C, 65% R.H.

temperature, and it was high enough to insure that the oxygen concentration did not have any noticeable variations throughout the reactor.

RESULTS AND DISCUSSION

Oxygen Consumption

Meal was oxidized under controlled temperature and relative humidity. The concentration of polyunsaturated lipid was followed by the above method, and, simultaneously, the velocity of oxygen uptake was measured. The curves thus obtained were computer-fitted to evaluate accurately the velocity of disappearance of polyunsaturated lipids.

The relationship between the velocity of oxygen absorption and the velocity of consumption of reactive lipids, both expressed as μmole/hr/g fat, is shown in Figure 2. It can easily be seen that there are other oxygen-consuming reactions which account for up to 10 times the oxygen consumed by the simple peroxidation of polyunsaturated lipids. The relationship between oxygen absorption and lipid consumption is variable, depending on the temperature, moisture content and time of reaction.

There are three possible explanations for this phenomenon: (a) the existence of simple protein oxidation; (b) the existence of protein oxidation catalyzed by the presence of lipid peroxides, according to the mechanism suggested by Zirlin and Karel (2); and (c) the existence of further lipid peroxidation, due to its high degree of polyunsaturation. In

TABLE III

Influence of Grinding on the Rate of Oxygen Absorption

Run	Sample	Oxygen absorption, μmol/hr/g meal
1	Normal fish meal	2.68
	Same meal, thoroughly milled	5.57
2	Normal fish meal	2.32
	Same meal, mildly milled	2.90
3	Meal screened between 18 and 50 mesh	4.08
	Same meal, milled and screened between 50 and 100 mesh	7.29
	Id., screened between 100 and 170 mesh	13.71

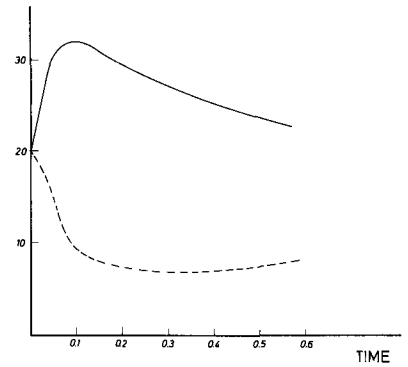

FIG. 8. Values of $(ROOH)_{average}$ ——, and $d(RH)/(RH \cdot dt)_{average}$ - - -, as a function of time (F) (dimensionless units). $kL^2 (RHo)^2/D = 200$; $k_1L^2 (RHo)/D = 12$; $kL^2(RHo) (O2 ext)/D = 100$; $(ROOHo)/(RHo) = 0.2$; $f(x) = 0.05 (x/L)^2$ (O_2 ext).

1955 Tappel (10) analyzed the copolymerization of oxidized fats with protein, reporting an absorption of 4 moles of oxygen per linoleic acid mole incorporated in the copolymer. Other workers reported that dimers and trimers formed during the autoxidation of ethyl linoleate contained 2 moles of oxygen per mole of ethyl linoleate.

The first explanation is easily discarded. In fact, solvent-extracted meal does not show any tendency to absorb oxygen. Similar findings have been reported by Martinez in freeze dried salmon (7). The second and third explanations seem equally acceptable, though recent research carried out by Heiss (personal communication) in our laboratories strongly suggests that the extra consumption of oxygen is primarily due to post oxidation of previously peroxidated lipids. In other words, having five or six degrees of unsaturation makes the lipid so reactive that it is able to undergo several oxidative reactions. It should be pointed out that in some cases the oxygen absorbed is more than five or six times the quantity of disappeared lipids, thus leaving the possibility that some protein oxidation exists.

Influence of Moisture Content

To investigate the influence of moisture content on the rate of oxidation of the meal, parts of the same sample were humidified to different levels, and then their velocity of oxygen absorption was measured. The result of this test is shown in Figure 3, where it can be concluded that an increasing moisture content increases the rate of oxidation. As this increment could have been associated either with the reactions of direct peroxidation of polyunsaturated lipids, or with the secondary reactions mentioned above, two samples of the same meal were humidified to different moisture contents, and were allowed to oxidize at 45 C. Figure 4 shows the changes of the concentration of polyunsaturated lipid in both samples, and Figure 5 shows the changes in their peroxide value (the dotted lines in Figure 5 will be discussed later). It can be inferred that both the consumption of polyunsaturated lipid and the reactions involving peroxide disappearance are enhanced by high moisture contents.

In principle, these results seem to contradict the findings of other workers (4,7) who have reported a decrease in the rate of oxidation with increasing moisture content. However, this contradiction is clarified by the observation of the water adsorption isotherm of fish meal (Fig. 6) where it can be seen that the whole range of moisture content studied falls within the capillary range, because in fish meal the monolayer value occurs at a low moisture content.

According to the mechanism suggested by Labuza et al. (5), it is possible that in this region of capillary condensation the rate of oxidation increases due to an enhancement of the mobility of the reactants. Nachenius (11) has reported that the shape of the adsorption isotherm in fish

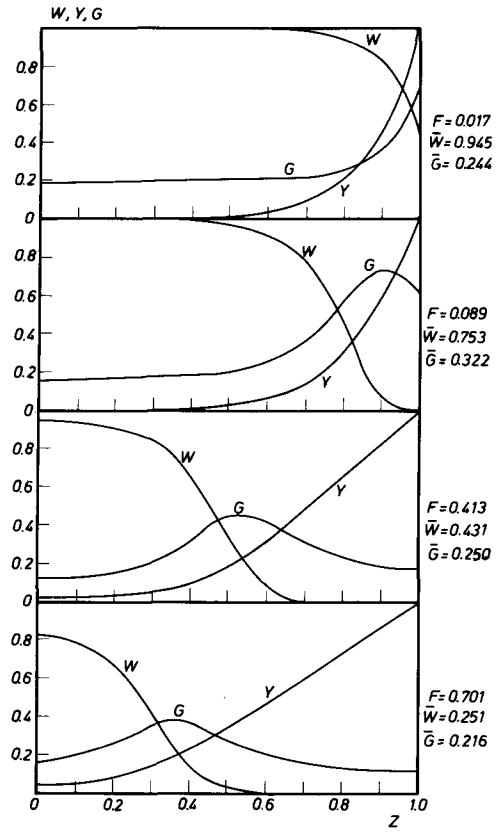

FIG. 9. Evolution of the profiles of polyunsaturated lipid (W), peroxide (G), and oxygen concentration (Y) inside a particle, (dimensionless units). $F = tD/L^2$; $G = ROOH/RHo$; $Z = x/L$; $\bar{G} = G_{average}$; $W = RH/RHo$; $Y = (O_2)/(O_2 \text{ ext})$; $\bar{W} = W_{average}$.

meal is strongly influenced by the quantity of soluble salts present. This fact should confirm that water in fish meal exists mainly in the state of capillary consensation (12).

Kinetics

Figure 5 shows the changes of peroxide value for two runs conducted at different relative humidities. The dotted lines, which have an initial value corresponding to the initial peroxide value, are the values of the function $d(RH)/(RH \cdot dt)$, obtained by means of chromatographic analysis and multiplied by a constant factor to have them coincide with the initial values of the peroxide curves.

If there should be a kinetic relationship of the type

$$d(RH)/dt = -k(RH)(ROOH)$$

it is clear that the dotted and full lines should coincide exactly. The big difference that can be observed between these lines clearly indicates that the overall kinetic behavior is quite different from the normal autocatalytic behavior of peroxidizing lipids. In fact, Figure 7 shows that the overall rate of reaction can be empirically expressed as simply as $d(RH)/dt = -k(RH)^n$, in which n is an exponent not above 5.0 and not below 4.0, and in which the peroxide value has no significant importance.

There is one fact, closely related to these experimental findings, which deserves further discussion. In 1957, Dreosti and Rowan (13) measured the heating rates of milled and unmilled meals in adiabatic calorimeters, and concluded that at all stages of storage milled fish meal oxidizes faster than unmilled meal. On the other hand, Table III shows three short tests in which the rate of oxygen absorption of milled and unmilled meal are compared. These results suggest that oxygen diffusion might play an important role in the phenomenon. Based on this assumption, it is possible to develop a mathematical model of the peroxidation of lipids included in a solid support

which resists the penetration of oxygen. The main assumptions of this model are: (a) there is no external resistance to the diffusion of oxygen at the gas-particle interphase; (b) the phenomenon is isothermal; (c) the rate of oxygen consumption varies linearly with partial oxygen pressure and with lipid and peroxide concentration inside the particle; (d) there is a slab-type geometry; and (e) there is a bimolecular rate of peroxide decomposition (16).

With these assumptions three simultaneous equations are readily obtained, each describing the balance of polyunsaturated lipid, peroxide and oxygen, respectively:

$$d(RH)/dt = -k(RH)\ (ROOH)\ (O_2)$$
$$d\ (ROOH)/dt = k(RH)\ (ROOH)\ (O_2) - k_1(ROOH)^2$$
$$D\,\delta^2(O_2)/\delta x^2 = \delta(O_2)/\delta t + k(RH)(ROOH)(O_2)$$

The boundary conditions are:

$(RH)=RHo$	$O<x<L$	$t=O$
$(ROOH)=ROOHo$	$O<x<L$	$t=O$
$(O_2) = f(x)$	$O<x<L$	$t=O$
$\delta(O_2)/\delta x=o$	$x=O$	$t>O$
(O_2) =constant	$x=L$	$t>O$

where k and k_1 are kinetic constants, L is the dimension of the particle, D is the oxygen diffusivity, t is the time of reaction, RHo and ROOHo are the initial concentrations of reactive lipid and peroxide, and f(x) indicates that initially there is a small amount of oxygen inside the particle.

Since this system is nonlinear, it has been put in a dimensionless form and it has been computer-solved with a finite difference method (16) for several values of the dimensionless parameters thus created. The tendency of these results was always the one seen in Figure 8, which describes the change of $(ROOH)_{average}$ and $d(RH)/(RH \cdot dt)_{average}$ as a function of time. These values were obtained by computing the average value of the concentrations of reactive lipid and peroxide inside the particle, because the experimental measure is based on the total extraction and measurement of the fat in the particles being studied. The comparison between Figures 5 and 8 is remarkable, and it readily suggests that oxygen diffusion in fish meal produces a deviation from the normal autocatalytic path of reaction, since the average concentration of one reactant has less importance than the concentration in those parts of the particle in which the rate of reaction is maximum. Figure 9 shows the evolution of the oxygen, reactive lipid and peroxide profiles inside a particle.

It must remain clearly stated that an accurate description of the peroxidation of lipids in fish meal should take into account other important facts, such as: (a) the existence of other oxygen consuming reactions; (b) the rate of peroxide decomposition is not necessarily bimolecular; and (c) further experimental work is needed to determine more accurately the value of the different parameters that define the problem, especially the ratio between diffusional and kinetic constants and the relationship between the microscopical structure of the meal and its diffusional constants. In any case, this mathematical model proves, in general, that diffusional resistances not only produce a decrease in the rate of peroxidation, but also changes in the overall kinetic behavior.

Finally, one point which must be discussed is that the rate of oxygen absorption in fish meal varies strongly with temperature (the activation energy was found to be 19.6 Kcal/mole), and in diffusionally controlled processes a small temperature dependance is usually expected. However, the estimation of the oxygen diffusivity gave a value as low as 10^{-7} cm^2/sec, which is similar to the values of oxygen diffusivity in polymers such as nylon and polyolefins, and in this kind of material diffusion is an activated process, with activation energies as high as the one mentioned above.

Furthermore, it is important to take into account that in polymeric materials the presence of water vapor often accelerates the diffusion of oxygen (14). This fact shows that high moisture contents could possibly enhance lipid peroxidation in fish meal, at least partially, through the mechanism of raising the oxigen diffusivity.

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