Antioxidant Activity of Some Plant Extracts of the Family Labiatae

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The antioxidant activities of methanol extracts of oregano, dittany, thyme, marjoram, spearmint, lavender and basil were tested in lard stored at 75°C. The concentration of extracts in lard varied from 0.01 to 0.20%. Oregano extract was found to be the most effective in stabilizing lard, followed by thyme, dittany, marjoram and lavender extracts, in a decreasing order. The induction period of lard increased with antioxidant concentration. After the induction period, peroxide formation proceeded rapidly, following pseudo-zero order reaction kinetics. The rate of the reaction decreased slightly with increasing plant extract concentration. Combined addition of plant extracts in lard showed a low synergistic action between thyme extract and spearmint extract.

KEY WORDS: Antioxidant activity, natural antioxidants, oxidative deterioration rate, plant extracts, synergistic action.

Natural antioxidants of plant origin have been used in oils, or lipid-containing foods to prevent their oxidative deterioration. The most effective ones seem to be rosemary *(Rosmarinus officinalis)* and sage *(Salvia officinalis)*, two plants belonging to the Labiatae family (1-5). Some other plants of the same family have also been tested (5), but they showed sometimes contradictory antioxidant activity when used under different conditions.

Ground oregano, thyme, marjoram and spearmint at concentration 2.5% in a freeze-dried model system of corn oil and carboxymethyl cellulose, stored at 65°C, showed protection factors (i.e., the ratio of the induction period of the sample containing the additives to the induction period of the control sample) of 4, 2, 3 and 2.5, respectively (6). Thyme was more effective in stabilizing pork fat subjected to an accelerated oxidation test. The protection factor obtained was 4.60, equal to those of rosemary and sage, while marjoram under the same conditions showed a moderate antioxidant activity and a protection factor of 1.7 (7). On the contrary, ground thyme or oregano added into sardine oil stored at 40°C and to minced sardine muscle stored at 0°C did not show any remarkable antioxidant activity according to the peroxide and p-anisidine values. Basil was also tested under the same conditions and showed a slight activity in sardine muscle (8). Marjoram, on the other hand, exhibited a prooxidant effect in precooked minced meat products stored under refrigerated and frozen conditions and negated the antioxidant effects of rosemary and sage when used in combination with these two spices (9). Rosemary and sage were tested as antioxidants in all the studies mentioned above and proved to be effective.

In other lipid-containing foods, like mayonnaise and French salad dressing, ground oregano proved the most effective of all the spices tested, while thyme showed low activity (10).

In addition to ground spices, extracts of some plants of the Labiatae family also have been tested as antioxidants. In most cases the extracts seemed to be more effective than the dry spices. Rosemary and sage extracts showed the best antioxidant activity while thyme and marjoram extracts displayed moderate activity (11-14). Methanol is a widely used and effective extraction solvent (3). Thus, extraction of basil with methanol followed by a subsequent extraction with n-hexane gave oleoresins that increased the induction period of lard. Methanol-extracted oleoresins were much more effective than those from n-hexane extraction (15). Oregano leaves were also successively extracted with dichloromethane and methanol. The methanol extract was found to contain two compounds that showed antioxidant activity comparable to BHA (butylated hydroxyanisole) when added to a mixture of linoleic acid, ethanol and water (16,17).

It is evident from the reports mentioned so far that the plants tested showed different antioxidant activities. Their effectiveness seems to depend on the substrate, the preparation procedure and the oxidation test.

The present study was undertaken to determine and compare the antioxidant activity of the most common Greek plants of the Labiatae family under the same conditions, i.e., addition of methanol extracts into lard and storage at 75°C. These plants are oregano *(Origanum vulgare)*, thyme *(Thymus vulgaris)*, marjoram *(Origanum majorana)*, spearmint *(Menta viridis)* and basil *(Ocymum basilicum)*, which have been tested already as antioxidants with the results mentioned above, as well as dittany *(Origanum dictamnus)* and lavender *(Levandula vera)*, for which there is no literature on their antioxidant activity presently available. Methanol extract of rosemary is used for comparison reasons only, as the antioxidant properties of this plant, as well as of sage, are well known. Results of combinations of these extracts in lard are also reported.

EXPERIMENTAL PROCEDURES

Preparation of plant extracts. All plants were collected in Greece, dried at room temperature and ground to pass a 0.5 mm sieve. The ground materials were extracted with methanol for 2 hr in a stirred vessel, at a liquid-to-solid ratio of 8:1 and a temperature of 60°C. The mixture was filtered and the residue was extracted again under the same conditions. The combined filtrate was partially concentrated in a rotary evaporator and kept in sealed bottles, in the dark, until used. Rosemary extract was also prepared under the same conditions to be used for comparison.

Sample preparation. Lard was melted at 85°C in an oil bath and filtered before use. A calculated quantity of the extract was added into lard and the mixture was stirred for 30 min at 50°C. The samples were placed in open beakers. A control sample was prepared each time, under the same conditions, without adding any antioxidant.

Analytical methods. Oxidative deterioration was studied by using the oven test at 75°C. The peroxide value was determined by the method Cd 8-53 of the American Oil Chemists' Society (18).

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RESULTS AND DISCUSSION

The data of lard autooxidation, measured as peroxide values, at 75°C after the addition of extracts of oregano, dittany, marjoram, thyme, spearmint, lavender and basil are plotted in Figures 1-7, respectively. The concentration of the extracts in lard, calculated on dry basis, varied from 0 to 0.20% w/w.

The reproducibility of peroxide values was determined by using three samples of the same batch of lard prepared simultaneously. Oregano extract was added in all the samples at a concentration of 0.02%. Peroxide values of all three samples were determined at equal storage times and their differences varied from 0 to 7. It should be mentioned that in experiments with the same plant extract at various concentrations the same batch of lard was used.

It is evident from Figures 1-7 that all extracts showed some effect in stabilizing lard, which increased with their concentration in lard. Oregano, thyme, dittany and marjoram extracts prolonged the induction period and protected lard appreciably, whereas spearmint, lavender and especially basil seemed much less effective.

The relative efficiency of oregano, dittany, marjoram, thyme, spearmint and lavender is given in Figure 8. Figure 8 presents the induction period of lard after the addition of each plant extract as a function of the concentration of the plant extract in lard. The induction period was considered as the number of days needed for the peroxide value of the sample to become 20. This is in agreement with a general consideration that lard becomes rancid at peroxide values larger than 20 (19,20), and with our experimental data, which showed that the rate of autoxidation of most samples increased sharply after that peroxide value.

Significance of variance of induction period was tested by using ten batches of lard in which oregano was added at a concentration of 0.02%. Control samples were also prepared each time. Calculations were made with the Student t-test for a significance level of 0.05. Standard deviation was s = 0.45 and confidence interval was ($\overline{IP}$ − 0.3, $\overline{IP}$ + 0.3), where $\overline{IP}$ represents the mean induction period.

Figure 8 shows that the oregano extract is much more effective than the others in stabilizing lard and that it is followed by thyme, dittany, marjoram, spearmint and lavender extracts in a decreasing order. Differences in

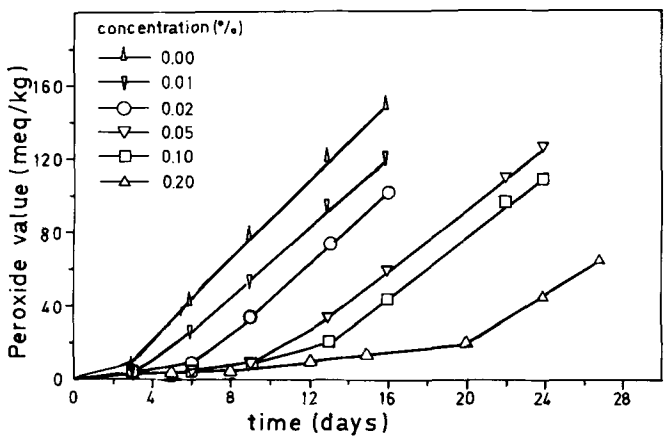

FIG. 1. Autooxidation of lard containing oregano extract, during storage at 75°C.

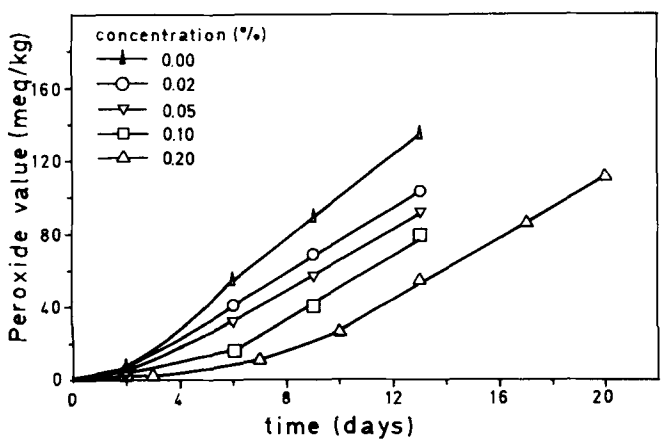

FIG. 3. Autooxidation of lard containing marjoram extract, during storage at 75°C.

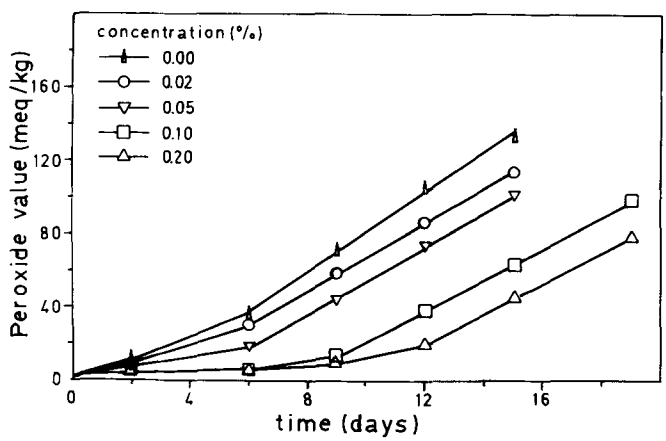

FIG. 2. Autooxidation of lard containing dittany extract, during storage at 75°C.

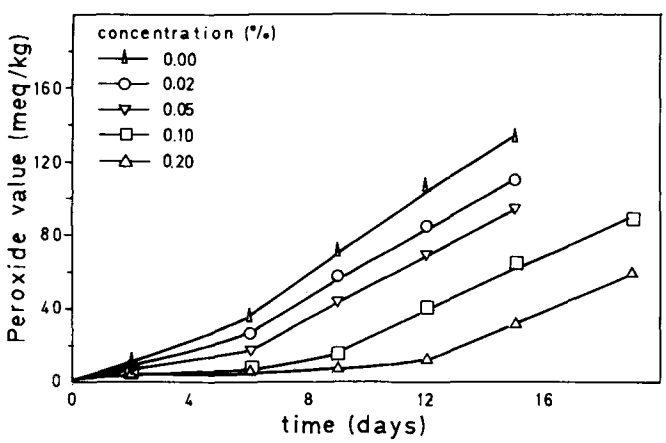

FIG. 4. Autooxidation of lard containing thyme extract, during storage at 75°C.

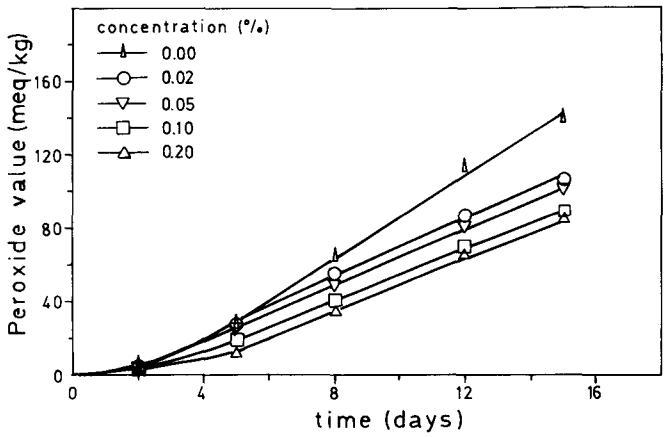

FIG. 5. Autooxidation of lard containing spearmint extract during storage at 75°C.

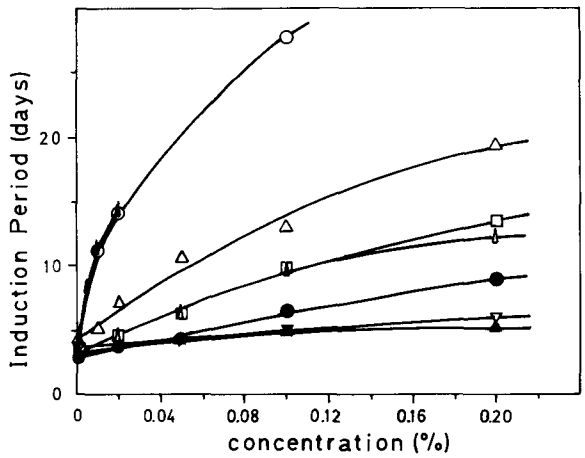

FIG. 8. Induction period of lard after the addition of oregano (△), thyme (□), dittany (Δ), marjoram (●), spearmint(▽), lavender (▲), rosemary (○), and BHA (▲) at concentrations varying from 0.0 to 0.20%.

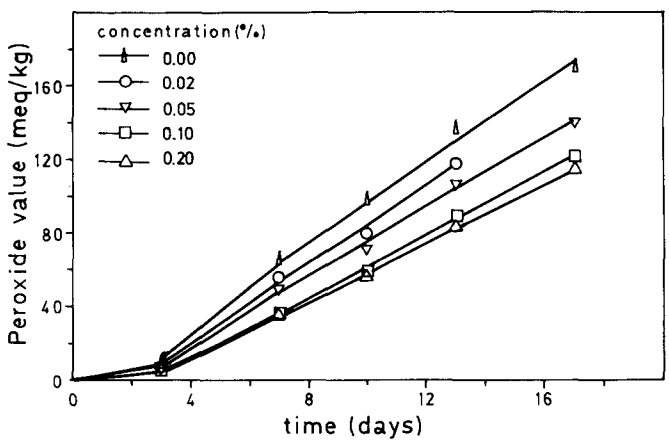

FIG. 6. Autooxidation of lard containing lavender extract during storage at 75°C.

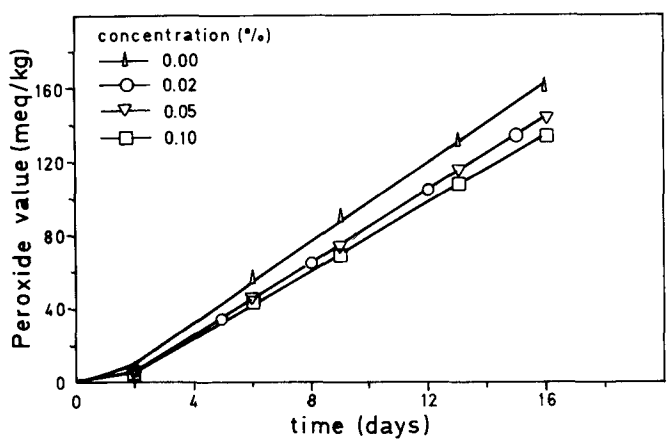

FIG. 7. Autooxidation of lard containing basil extract during storage at 75°C.

induction periods between thyme and dittany, as well as between spearmint and lavender, however, are not statistically significant. Results for basil are not presented in Figure 8 as there is no noticeable increase of the induction period with increasing concentration of basil extract (Fig. 7).

The antioxidant activity of basil extract is slightly weaker than the activity measured by Huang *et al.* in their experiments with methanol extract of basil in lard (15). However, it is in agreement with the result obtained when ground basil was added into minced sardine muscle (8).

Results for rosemary extract prepared and tested under the same conditions, as well as for BHA, are given in the same Figure (Fig. 8) for comparison. BHA is used at concentration not greater than 0.02%, because that is usually the highest level of its use in oils, fats and lipid-containing foods (21). Rosemary extract prolongs the induction period of lard equally to BHA and significantly more than the other plant extracts used. This is in disagreement with results reported in the literature, where ground oregano was found to be a better antioxidant than ground rosemary in some lipid substrates (6,10), and ground thyme proved to be equally as good in stabilizing lard under more accelerated oxidation conditions (7). By means of a heamoglobin peroxidation test, Cort found that the activity of ground thyme was quite similar to that of ground rosemary, while the ethanol extract of thyme had much lower activity than the ethanol extract of rosemary (11). This means that ground whole spices and their extracts may act quite differently.

Marjoram extract was much less effective as antioxidant than rosemary extract, a result generally in agreement with those reported in the literature (12–14), but in no case was it a prooxidant. Spearmint extract showed a very slight antioxidant activity, much less than that given for the dry whole spice (6). Lavender extract, one of the new antioxidants tested in lard, also showed a minor effect in increasing the induction period. Dittany extract, on the other hand, proved to be almost as good as thyme in stabilizing lard.

TABLE 1

Rate Constants of the Autooxidation Curves of Lard Containing the Plant Extracts at Concentration 0.0–0.20% (w/w)

Concentration (%)	Additive													
	Oregano		Thyme		Dittany		Marjoram		Spearmint		Lavender		Basil	
	k	R^2	k	R^2	k	R^2	k	R^2	k	R^2	k	R^2	k	R^2
0.00	10.56	0.998	10.87	0.996	10.87	0.996	11.43	1.000	11.40	1.000	10.53	0.992	10.37	0.999
0.01	9.53	1.000												
0.02	10.03	0.999	9.20	0.999	9.35	1.000	8.92	1.000	7.74	0.996	10.22	0.985		
0.05	8.42	1.000	8.53	1.000	9.35	0.999	8.51	1.000	7.57	0.999	9.23	0.994	9.34	0.999
0.10	8.16	0.999	7.42	0.995	8.73	1.000	9.11	0.998	7.15	0.999	8.72	0.998	9.23	0.999
0.20	6.85	1.000	6.73	0.999	8.42	1.000	8.51	0.999	7.17	0.997	7.96	0.998		

According to these results, oregano, dittany and thyme extracts are promising antioxidants, and investigation is carried on in our laboratory for the purification of these extracts and the isolation of antioxidant components.

Figures 1–7 show that after the induction period the rate of peroxide formation does not depend on the concentration of the reactants. The slope (k) and the coefficient of determination (R^2) of all the autoxidation curves presented in these figures were calculated from the linear part of the curve by assuming the peroxide formation to be a pseudo-zero order reaction. The results are given in Table 1. It is evident that the coefficients of determination are high in all cases. The rate of peroxide formation decreases after addition of the plant extract, and the decrease becomes slightly greater as the concentration of the extract in lard increases. The intercept of these curves is not tabulated as it depends on the induction period and it follows the increase of the induction period as expected.

These results show that the antioxidants act mainly by increasing the induction period, but also by slightly decreasing the rate of peroxide formation, which follows pseudo-zero order kinetics, after that period.

To examine the stability of antioxidant activity upon storage of the plant extracts some experiments were repeated after three and six months of storage. The antioxidant activity was found to be constant in all cases. Oregano was also tested for stability of the raw material. The same raw material was extracted just after being collected and after one year of storage. The antioxidant activities of both extracts were identical.

A major problem in the investigation of the oxidative deterioration of lard is that the rate of deterioration depends on the condition of the lard used. An effective antioxidant such as oregano extract can prolong the induction period and affect the rate of peroxide formation equally, independent (within certain margins) of the oxidative deterioration rate of the raw material. Results are presented in Figure 9. The same result was obtained with the rosemary extract (unpublished data).

The plant extracts were also added to lard in combinations of two to detect any synergistic effects of the antioxidant components they contain. A synergistic effect could be expressed as:

$$IP_m - IP_c > (IP_1 - IP_c) + (IP_2 - IP_c) \qquad [1]$$

where IP_m, IP_1, IP_2 and IP_c are the induction periods of the sample with the mixture of additives, the sample with additive 1, the sample with additive 2 and the control sample, respectively.

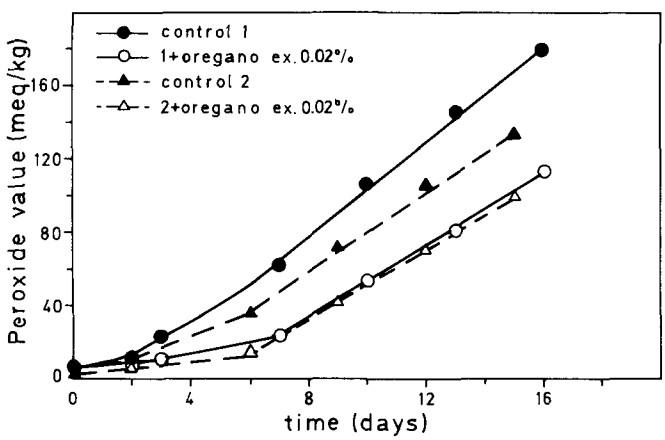

FIG. 9. Autooxidation of two samples of lard showing different deterioration rates.

Equation [1] can be expressed as:

$$(IP_m - IP_c) - (IP_1 - IP_c) - (IP_2 - IP_c) > 0$$

or

$$\frac{IP_m}{IP_c} - \frac{IP_1}{IP_c} - \frac{IP_2}{IP_c} + 1 > 0$$

which also can be written as

$$PF_m - PF_1 - PF_2 + 1 > 0 \qquad [2]$$

where PF is the protection factor.

Results for the protection factors of mixtures of the extracts added into lard, as well as the protection factor of each component, are given in Tables 2 and 3. Synergism between components is also given in these tables, calculated according to equation [2] and expressed as positive, negative or zero. It can be seen from Table 2 that when thyme, marjoram, spearmint, lavender or basil were combined with oregano, which is a more effective antioxidant (each one at concentration 0.02%), the protection factor is more or less equal to that obtained when oregano extract was added alone into lard at the same concentration, with the exception of basil. The differences are not

TABLE 2

Antioxidant Activity of Plant Extracts Added Together with Oregano Extract (Concentration = 0,02%, PF[a] = 2,0) Into Lard

Combined additive			PF of the mixture	Synergism
Name	C(%)[b]	PF		
Thyme	0.02	1.2	2.0	0
Thyme	0.05	1.8	3.0	0
Marjoram	0.02	1.1	2.1	0
Spearmint	0.02	1.0	2.1	0
Lavender	0.02	1.1	2.1	0
Basil	0.02	1.0	1.7	—

[a]PF, protection factor.

[b]C%, concentration of the additive, on dry basis, % w/w on lard.

TABLE 3

Antioxidant Activity of Plant Extracts Added in Combination into Lard

Combined additives						PF of the mixture	Synergism
A			B				
Name	C%[a]	PF[b]	Name	C%	PF		
Thyme	0.02	1.2	Marjoram	0.02	1.1	1.6	+
Thyme	0.05	1.8	Marjoram	0.05	1.3	2.0	0
Marjoram	0.02	1.2	Spearmint	0.02	1.0	1.3	0
Thyme	0.02	1.2	Spearmint	0.02	1.0	1.7	+
Thyme	0.02	1.2	Lavender	0.02	1.1	1.5	0
Lavender	0.02	1.1	Spearmint	0.02	1.0	1.2	0

[a]C%, concentration of the additive, on dry basis % w/w on lard.

[b]PF, protection factor.

statistically significant because confidence interval, calculated as mentioned before for induction period, was ($\overline{PF} - 0.16$, $\overline{PF} + 0.16$). The rate of peroxide formation was also almost identical to that of oregano.

Table 3 presents the combinations of the other plant extracts, except oregano, which had shown a moderate antioxidant effect at concentration 0.02% in lard. It can be concluded from the results that thyme showed some noticeable synergistic action with spearmint, while the synergistic action observed with most of the other combinations is not statistically significant. Thyme extract showed a low synergistic action with marjoram extract too, when both antioxidants were added at concentration 0.02%, but the same result was not obtained at higher concentration (0.05%). This may be attributed to a parallel combined action of some constituents of the plant extracts that promote oxidation. Such a reaction must depend on the concentration of these constituents, its order being higher than the order of the synergistic action. Dependence of the synergistic effect on concentration has been reported in the literature (6,22).

REFERENCES

1. Ostric-Matijasevic, B., *Rev. Fran. Corps Gras 8*:443 (1963).
2. Russo, J.R., *Food Eng. 48*:19 (1976).
3. Chang, S.S., B. Ostric-Matijasevic, O.A.L. Hsieh and C.-L. Huang, *J. Food Sci. 42*:1102 (1977).
4. Bracco, U., J. Löliger and J.L. Viret, *J. Am. Oil Chem. Soc. 58*:686 (1981).
5. Dewdney, P.A., B.A. Meara and M.L. Meara, in *Natural Fat Soluble Antioxidants*, The British Food Manufacturing Industries Research Association, 1977, pp. 8–18.
6. Bishov, S.J., Y. Masuoka and J.G. Kapsalis, *J. Food Processing and Preservation 1*:153 (1977).
7. Gerhardt, U., and P. Blat, *Fleischwirtschaft 64*:484 (1984).
8. Pizzocaro, F., F. Caffa, A. Gasparoli and E. Fedeli, *Riv. Ital. Sostanze Grasse 62*:351 (1985).
9. Korozak, J., E. Flaczyk and Z. Pazola, *Fleischwirtschaft 68*:64 (1988).
10. Chipault, J.R., G.R. Mizuno and W.O. Lundberg, *Food Technol. 10*:209 (1956).
11. Cort, W.M., *Ibid. 28(10)*:60 (1974).
12. Palitzsch, A., H. Schulze, F. Metzl and H. Baas, *Fleischwirtschaft 49*:1349 (1969).
13. Palitzsch, A., H. Schulze, G. Lotter and A. Steichele, *Ibid. 54*:63 (1974).
14. Burgheimer, Y.F., Ph.D. thesis, University of Illinois (1968).
15. Huang, J.K., C.S. Wang and W.H. Chang, *J. Chinese Agric. Chem. Soc. 19*:200 (1981).
16. Nakatani, N., and H. Kikuzaki, *Agric. Biol. Chem. 51*:2727 (1987).
17. Kikuzaki, H., and N. Nakatani, *Ibid. 53*:519 (1989).
18. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 3rd edn., edited by R.O. Walker, American Oil Chemists' Society, Champaign, IL, 1986.
19. Sherwin, E.R., *J. Am. Oil Chem. Soc. 55*:809 (1978).
20. Swern, D., in *Bailey's Industrial Oil and Fat Products*, 4th edn., Wiley Interscience Publication, N.Y., 1979, p. 145.
21. Dziezak, J.D., *Food Technol. 40(9)*:94 (1986).
22. Dziedzic, S.Z., and B.J.F. Hudson, *J. Am. Oil Chem. Soc. 61*:1042 (1984).

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