The Effect of Primary Antioxidants and Synergists on the Activity of Plant Extracts in Lard

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The antioxidant activities of combinations of plant extracts and primary antioxidants or citric acid were studied in lard stored at 75°C. Methanol extracts of oregano, thyme, marjoram, dittany, rosemary and sage were used. When combined with butylated hydroxyanisole, butylated hydroxytoluene or ascorbyl palmitate the extracts showed an additive antioxidant effect, while combinations with propyl gallate did not increase the induction period obtained by the phenolic antioxidant. Negative synergism was observed when the plant extracts were mixed with DL-α-tocopherol. Citric acid showed a high synergistic efficiency with marjoram extract and a minor one with thyme extract, but it had a negative effect with all the others. Ternary mixtures of the plant extracts with citric acid and ascorbyl palmitate had lower activity than the corresponding dual combinations with ascorbyl palmitate, except for the mixture of marjoram extract.

KEY WORDS: Antioxidants, plant extracts, synergistic effect.

Some plant extracts of the family Labiatae, namely oregano, thyme, marjoram and dittany, have shown noticeable antioxidant activity in lard (1). Extracts of rosemary and sage, which belong to the same family, also have been found effective in preventing autoxidation of lard (2,3). The present work was undertaken to examine the effect of primary antioxidants and synergists on the activity of these plant extracts in lard. The primary antioxidants selected were phenolic, such as butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT) and propyl gallate (PG), as well as ascorbyl palmitate (AP), which functions as an oxygen scavenger and has no restrictions on usage levels, and DL-α-tocopherol (α-Toc), which is a natural antioxidant. The objectives for the phenolic antioxidants were to replace part of them by the plant extracts and for AP and α-Toc to obtain mixtures of improved antioxidant activity. Citric acid (CA), which is a well-known chelating agent, was tested as a possible synergist of the plant extracts.

EXPERIMENTAL PROCEDURES

The plant extracts were prepared according to Economou *et al.* (1). BHA, BHT, PG and CA were purchased from Fluka Chemika (Bucks, Switzerland). The AP and α-Toc were kindly donated by Roche Products (Switzerland). For preparation of the samples, lard was melted at 85°C in an oil bath and filtered. The calculated quantities of each antioxidant were added, and the mixtures were stirred for 20 min at 40°C. The AP and CA were dissolved in 95% ethanol before the addition to lard. A control sample was prepared each time under the same conditions, without adding any antioxidant.

The samples (25 g each) were placed in open beakers, and oxidative deterioration was studied by using the oven test at 75°C as described by Economou *et al.* (1). The experiments were repeated and data are the average of the duplicates. The peroxide value was determined by method Cd 8-53 of the American Oil Chemists' Society (4).

Copper and iron ions were determined in the plant extracts by the following procedure. The extracts (5 mL) were digested with concentrated sulfuric acid and hydrogen peroxide (50%) in a Digesdahl digestion apparatus (Hach Company, Loveland, CO) to free the bound metal ions (5). Aliquots were then taken for analysis by atomic absorption spectrophotometry. A Perkin Elmer spectrophotometer Model 305; Perkin Elmer Corp., Norwalk, CT was used. Wavelengths used for copper and iron were 324.7 and 248.3 nm, respectively.

RESULTS AND DISCUSSION

The results obtained for dual combinations of the plant extracts with the primary antioxidants are given in Tables 1 and 2. The concentrations of all the additives are referred to on a dry basis, w/w % in lard. The induction periods (*i.e.*, the time needed for peroxide value to become 20) were determined by plotting the peroxide values of the samples *vs.* time. To test the significance of variance, six experiments were run with the same batch of lard under the same conditions. In each experiment, oregano (0.10%), BHA (0.01%) and their mixtures were tested. Calculations were made with the Student's *t*-test at a significance level of 0.05 (6). The standard deviation was s = 0.23 and the confidence interval was (IP−0.2, IP + 0.2), where IP represents the mean induction period. The BHA, BHT and AP showed an additive effect with the plant extracts in stabilizing lard, and the induction periods of the samples containing the mixtures were increased compared to the induction periods of the samples containing the corresponding individual components. Percent synergism, calculated according to Bishov *et al.* (7), approximated zero in all cases with statistically insignificant differences. The confidence interval, calculated as mentioned for induction period, was (% Syn − 20, % Syn + 20), where % Syn represents mean percent synergism. When percent synergism was calculated in a manner similar to that used for time needed to reach higher peroxide values, the same results were shown. It can be concluded that mixtures of the plant extracts with BHA, BHT or AP could be used in lard without any loss of antioxidant activities of the individual components.

The PG at concentration 0.01% proved to be effective in preventing autoxidation of lard, and its inhibitory action was virtually unaffected by the addition of the mild plant antioxidants. The α-Toc showed a strong negative effect with the plant extracts. In all cases the induction period of the mixture was lower than the induction period of the corresponding plant extract. This negative effect might be responsible for the inability of plant extracts to protect vegetable oils with high tocopherol content (ref. 8, and Oreopoulou, V., unpublished data).

Rosemary and sage extracts, which proved to be the most effective of the plant antioxidants tested in the present research, also were added to lard at a much lower con-

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TABLE 1

Antioxidant Activity in Lard of Plant Extracts (0.10%) and of Their Mixtures with BHA (0.01%), BHT (0.005%), PG (0.01%), AP (0.01%) or α-Toc (0.02%)

Additive	Peroxide value[a] (meq/Kg) after days															
	2	3	4	5	6	7	8	9	10	11	12	13	14	16	19	21
None	20	40	65	97	131											
Oregano		5	8	10	13	16	26	38	60	75			142			
Thyme		11	16	28	50	70	100		125							
Marjoram		15	25	44	65	88	123									
Dittany		8	14	26	34	61	81		116							
Rosemary		3	4		8			12	14	16	20	25	45	57	73	
Sage		3			7			9				13		17		
Sage[b]			5	5			16		28		53		64			
BHA			6		8		16		21				50	72		
Oregano + BHA		3			4				8		11			21	60	
Thyme + BHA					7				13		18		31		84	
Dittany + BHA					7				12		20		34		87	
Rosemary + BHA					3				7					12	22	
Sage[b] + BHA							6		9				14	33	65	
BHT		6		21	40		80		122							
Oregano + BHT		3			7				14		24		54			
Thyme + BHT		4			15		26		70							
Marjoram + BHT		4			16		28		72							
Dittany + BHT				10			34				75					
Rosemary + BHT					6				12		15	17	25			
Sage[b] BHT				3			9		12		28			80		
PG	3				6				9				13		21	36
Oregano + PG					5				7						17	30
Thyme + PG		3			6				8						20	36
Marjoram + PG		3			6				8						21	36
AP		8	21	38		73										
Oregano + AP		3		5		9		16		52				92		
Thyme + AP		3		9		35			63							
Marjoram + AP		3		16		37			69							
Dittany + AP		3		7		30										
Rosemary + AP		3						9				14		28		
Sage + AP		3						5				8		14		
α-Toc	16	24	31		72		126									
Oregano + α-Toc		12	18		41		68		98							
Thyme + α-Toc		21	27		63		103									
Marjoram + α-Toc		21	33		77		134									
Rosemary + α-Toc		9			22				44		63					

[a]Data are the average of at least two separate experiments. The initial peroxide value of the samples was 2.

[b]At concentration 0.05%.

TABLE 2

Antioxidant Effects in Lard of Combinations of Plant Extracts and Primary Antioxidants

Plant extract (concentr. 0.10%)	Primary antioxidant											
	None		BHA 0.01%		BHT 0.005%		PG 0.01%		AP 0.01%		α-Toc 0.02%	
	IP[a]	Syn.[b] %	IP	Syn. %	IP	Syn. %	IP	Syn. %	IP	Syn. %	IP	Syn. %
None	2.0[c]	—	10.0	—	5.0	—	19.0	—	4.0	—	2.8	—
Oregano	7.4	—	15.9	5	11.5	10	20.0	−25	9.4	0	4.1	−200
Thyme	4.4	—	12.4	0	7.4	0	19.0	−13	6.0	−10	3.0	−220
Marjoram	3.5	—	—	—	7.0	10	19.0	−8	5.3	−5	3.0	−130
Dittany	4.4	—	12.0	−4	7.4	0	—	—	6.2	5	—	—
Rosemary	12.0	—	18.8	−7	13.4	−14	—	—	14.2	2	5.9	−180
Sage	15.5	—	—	—	—	—	—	—	16.5	−7	—	—
Sage[d]	9.0	—	14.8	−17	11.4	−6	—	—	—	—	—	—

[a]IP, induction period, days needed for peroxide value to become 20.

[b]Syn % = 100 $[(IP_m - IP_c) - (IP_p - IP_c) - (IP_a - IP_c)]/(IP_m - IP_c)$, where IP_m, IP_c, IP_p and IP_a are the induction periods of the sample containing the mixture of the additives, the control sample, the sample containing the plant extract and the sample containing the primary antioxidant, respectively.

[c]Data are the average of at least two separate experiments.

[d]At concentration 0.05%.

TABLE 3

Antioxidant Activity of Combinations of BHA with Rosemary or Sage Extract

Antioxidant	Concentration w/w % in lard	IP[a]	Syn[a] %
None	—	1.8[b]	—
BHA	0.01	9.0	—
Rosemary extract	0.01	5.5	—
Sage extract	0.01	6.0	—
BHA, rosemary extract	0.01 each	12.6	0
BHA, sage extract	0.01 each	12.8	−3
BHA	0.02	11.7	—

[a]Symbols as in Table 2.

[b]Data are the average of duplicate experiments.

centration (0.01% instead of 0.10%) together with BHA 0.01%. The results are given in Table 3. The induction period of the mixture of either extract with BHA was higher than the induction period obtained by doubling the phenolic antioxidant concentration. Thus, a partial replacement of the phenolic antioxidant by any of these plant extracts gave better results in stabilizing lard than did doubling of the phenol.

The results of dual combinations of CA and plant extracts are presented in Tables 4 and 5. It is evident that a high synergistic efficiency was obtained when CA was added together with marjoram extract into lard, and a minor one when it was combined with thyme extract

TABLE 4

Antioxidant Activity in Lard of Combinations of Plant Extracts with Citric Acid (CA)

	Peroxide value[a] (meq/Kg) after days													
Additive	0	1	2	3	4	5	6	7	8	9	10	11	12	14
None (A)[b]	1		13	24	57	91								
CA 0.01	1		11	24	55	88								
CA 0.02	1		11	23		88								
CA 0.04	1		10	23		86								
Marjoram 0.05	1		6		20	45	73	97		154				
Marj. 0.05 + CA 0.01	1		2		4	8	14	28		86			170	
Marj. 0.05 + CA 0.02	1					7	12	23		74			164	
Marj. 0.05 + CA 0.04	1					6	10	21		65			156	
Marjoram 0.10	1				12	20	41	64		114				
Marj. 0.10 + CA 0.01	1				3	6		14	30	47			111	
Marj. 0.10 + CA 0.02	1					5		11	16	26			81	
Marj. 0.10 + CA 0.04	1					5		10		19			69	
Marjoram 0.20	1					10	19	35		87				
Marj. 0.20 + CA 0.01	1					4		10		22			68	
Marj. 0.20 + CA 0.02	1					3		8		14	17		47	
Marj. 0.20 + CA 0.04	1					2		7		10	13		22	
Dittany 0.05	1				12	19	37		73					
Ditt. 0.05 + CA 0.01	1				17	28	46		86					
Ditt. 0.05 + CA 0.04	1				20	31	51		95					
Dittany 0.10	1				9		19		44		77			
Ditt. 0.10 + CA 0.01	1				11		24		50		84			
None (B)[b]	2	10	25	48	69	98								
Oregano 0.02	2	5		15	28	48		93						
Oreg. 0.02 + CA 0.01	2	6		23	41	60		112						
Oregano 0.05	2	5		12	15		28		53				137	
Oreg. 0.05 + CA 0.01	2	8		13	24		43		68				157	
Oregano 0.10	2	2			9		16	26	35				99	
Oreg. 0.10 + CA 0.01	2	4			14	22	29		54				121	
Oreg. 0.10 + CA 0.04	2	5			16	26	33		61				130	
Thyme 0.10	2			14	23	38		76						
Thyme 0.10 + CA 0.01	2			11	20	32		70						
Thyme 0.10 + CA 0.04	2			9	17	29		69						
Thyme 0.20	2				10	19	33		67					
Thyme 0.20 + CA 0.01	2				8	17	30		63					
Rosemary 0.02	2				7		15	24	39					
Rose. 0.02 + CA 0.01	2				12		24		58					
Rosemary 0.05	2				4		9		14		39			
Rose. 0.05 + CA 0.01	2				7		12	16	24		57			
Rose. 0.05 + CA 0.04	2				7		13	18	27		60			
Rosemary 0.10	2				4		7		11		17		36	
Rose. 0.10 + CA 0.01	2				5		9		13		25		54	
None (C)[b]	1		15	25	59			157						
Sage 0.02	1							7	12	24	34		88	
Sage 0.02 + CA 0.01	1							12	26		62		120	
Sage 0.05	1							3			11		25	53
Sage 0.05 + CA 0.01	1							4			14	22	30	63
Sage 0.05 + CA 0.04	1							4			17	25	33	75

[a]Data are the average of duplicate experiments.

[b]Three batches of lard were used (A) in the experiments with marjoram and dittany; (B) in the experiments with oregano, thyme and rosemary; and (C) in the experiments with sage. CA added alone to (B) and (C) gave the same results as to (A).

TABLE 5

Synergistic Efficiency in Lard of Citric Acid and Plant Extracts

Plant extract			Citric acid at concentration (w/w %)					
			0.01		0.02		0.04	
Name	C%[a]	IP[b]	IP	Syn[b] %	IP	Syn %	IP	Syn %
Oregano	0.00	1.7[c]	1.7	—			1.7	—
Oregano	0.02	3.4	2.7	−71				
Oregano	0.05	5.0	3.7	−65				
Oregano	0.10	6.4	4.9	−45			4.4	−73
Thyme	0.00	1.7	1.7	—			1.7	—
Thyme	0.10	3.7	4.0	14			4.3	22
Thyme	0.20	5.0	5.3	8				
Marjoram	0.00	2.7	2.7	—	2.7	—	2.7	—
Marjoram	0.05	4.0	6.4	64	6.7	67	6.9	68
Marjoram	0.10	5.0	7.3	50	8.4	59	9.0	63
Marjoram	0.20	6.0	8.9	38	10.3	50	11.8	58
Dittany	0.00	2.7	2.7	—			2.7	—
Dittany	0.05	5.0	4.2	−53			4.0	−80
Dittany	0.10	6.1	5.5	−20				
Rosemary	0.00	1.7	1.7	—			1.7	—
Rosemary	0.02	6.6	5.5	−28				
Rosemary	0.05	8.6	7.5	−20			7.2	−30
Rosemary	0.10	10.2	9.5	−9				
Sage	0.00	2.5	2.5	—			2.5	—
Sage	0.02	8.6	7.7	−16				
Sage	0.05	11.4	10.9	−6			10.4	−12

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

[b]Symbols as in Table 2.

[c]Data are the average of duplicate experiments.

TABLE 6

Antioxidant Activity in Lard of Ternary Mixtures of the Plant Extracts (0.10%) with Citric Acid (CA) and Ascorbyl Palmitate (SP) at Concentration 0.01% Each

Additive	Peroxide value[a] (meq/Kg) after days														
	0	2	3	4	5	6	7	8	10	11	12	13	15	16	18
None	2	17	29	60	92	123									
CA	2	17	28	58	91	120									
AP	2		11	16	30	56	80								
CA + AP	2		16	35	67	86									
Oregano	2			5		12	14	19		64					
Oregano + CA + AP	2			5		13	20	28		83					
Thyme	2			14	25	50		90							
Thyme + CA + AP	2			15	26	53		96							
Marjoram	2		16	25		62		110		162					
Marjoram + CA + AP	2		8	11	17	26		63		123					
Dittany	2			14	23	35		71							
Dittany + CA + AP	2			14	24	39		78							
Rosemary	2			4		7		10	14		21		55		
Rosemary + CA + AP	2			4		6		9	13		18		38		
Sage	2					7						12		20	45
Sage + CA + AP	2					7						14		24	59

[a]Data are the average of duplicate experiments.

(Table 5). With all the other plant extracts a negative effect was observed. In general, the positive or negative effect was increased with increasing CA concentration and decreased with increasing plant extract concentration, although some differences were found in the range of the confidence interval. CA alone showed no antioxidant activity in lard, as was expected. The concentration of the prooxidative metal ions, namely copper and iron, was determined by duplicate experiments and was less than 2 ppm in all the plant extracts, as well as in lard; therefore it is doubtful that CA acted as a metal binder. The effect of CA on the activity of the plant extracts and possible interactions with the components of these extracts should be examined further. Research on the composition of these plant extracts and their mode of action in stabilizing lipids is being studied in our laboratory.

A series of experiments also was run for ternary mixtures of each plant extract with CA and AP at concentrations 0.10, 0.01 and 0.01%, respectively, in lard. The results are presented in Tables 6 and 7. CA, when added

TABLE 7

Antioxidant Effect in Lard of Ternary Mixtures of the Plant Extracts with Citric Acid (CA) and Ascorbyl Palmitate (AP)

Plant extract (0.10%)		Mixture with CA + AP (0.01% each)	
Name	IP[a]	IP	Syn[a] %
None	2.2	3.3[b]	−100[c]
Oregano	8.0	7.0	−50
Thyme	4.6	4.6	−45
Marjoram	3.7	5.4	18
Dittany	4.8	4.6	−50
Rosemary	12.0	12.2	−10
Sage	16.0	15.8	−12

[a]Symbols as in Table 2.

[b]Data are the average of duplicate experiments.

[c]The IP of lard with no additives (control) was 2.2, with CA 2.2, and with AP 4.4 days.

alone to lard, showed an induction period of 2.2 days (equal to that of the control), and AP gave an induction period of 4.4 days. The induction period of the sample containing the mixture of CA and AP was 3.3 days, showing a negative synergism of −100% of these additives in lard. The induction periods of the ternary mixtures of all the plant extracts were lower than the induction periods of the corresponding dual mixtures with AP, presented in Table 2, with the exception of marjoram extract. However, the high synergistic efficiency of marjoram extract with CA (Table 5) was decreased by the addition of AP, and synergism of the ternary mixture was 18%. The results showed that CA could be added with marjoram extract into lard to provide extra stability, while all the other combinations of the plant extracts with CA or CA and AP had no positive effect.

REFERENCES

1. Economou, K.D., V. Oreopoulou and C.D. Thomopoulos, *J. Am. Oil Chem. Soc. 68*:109 (1991).
2. Chang, S.S., B. Ostric-Matijasevic, O.A.L. Hsieh and C.-L. Huang, *J. Food Sci. 42*:1102 (1977).
3. Dewdney, P.A., B.A. Meara and M.L. Meara, in *Natural Fat Soluble Antioxidants*, The British Food Manufacturing Industries Research Association, 1977, pp. 8–13.
4. *Official Methods and Recommended Practices of the American Oil Chemists' Society*, 3rd edn., edited by R.C. Walker, American Oil Chemists' Society, Champaign, 1986.
5 Hach, C.C., B.K. Bowden, A.B. Kopelove and S.V. Brayton, *J. Assoc. Off. Anal. Chem. 70*:783 (1987).
6. Cramer, H., *The Elements of Probability Theory*, John Wiley & Sons Inc., NY, 1955, pp. 171 and 198.
7. Bishov, S.J., Y. Masuoka and J.G. Kapsalis, *J. Food Processing and Preservation 1*:153 (1977).
8. Manganari, F., and V. Oreopoulou, *Riv. Ital. Sostanze Grasse 68*:305 (1991).

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