TABLE II

Lowest Detectable Quantity (in ng) on TLC plate; Recovery (%); and Minimum Detectable Concentration (in μg/kg) for Aflatoxin B_1 and Ochratoxin A in Olive Oil

	Sensitivity on TLC plate (ng)		Recovery (%)		
	Solution of standard	Standard mycotoxin added to 6 μL extract spot	Quantity added to olive oil (μg/kg)	Recovery (%)	Lowest detectable concentration (μg/kg)
Aflatoxin B_1	0.4-0.5	0.6	20	90	4
Ochratoxin A	12	20	150	90-100	40

at low level, leads us to suppose that ochratoxin producing molds can grow on olives and secrete this mycotoxin. However, we have to verify whether or not olives are a good medium for ochratoxin production by toxicogenic *A. ochraceus* strains.

The recovery of ochratoxin in pressed oil and fatty cakes when processing contaminated olives should also be measured.

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The Antioxidant Activity of Amino Acids in Two Vegetable Oils

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ABSTRACT

Of 27 amino acids studied, most had some antioxidant activity when added in aqueous solution to either safflower oil or a mixture of sunflower and cottonseed oil (active oxygen and storage methods). Cysteine-HCl, glutamic acid-HCl (in the mixture), and glutamic acid-HCl (in safflower oil) behaved as prooxidants. When added as a solid, most amino acids were ineffective. The protection factors of these amino acids were less than 1.3 in safflower oil with methionine, proline, lysine and cysteine providing the highest activity. In the oil mixture (which had a higher metal content) lysine, arginine, glutamic acid, methionine, and hydroxyproline were antioxidant with protection factors of up to 1.85. Chelation of metals by amino acids was presumably responsible for the antioxidant activity. The increase in cysteine concentration up to 1% has more than doubled the protection factor in Bint oil (compared with the 0.01% level), whereas with some other amino acids the increase was either small or slight.

INTRODUCTION

Many contradictory results are found in the literature when comparing the relative effectiveness of amino acids as antioxidants in different oils and fats. For example, methionine was reported to have a good antioxidant effect in some fatty materials, rapeseed, olive and soy oils (1-4). It was also described as more effective than some other amino acids, including cystine or cysteine in some vegetable oils and lard (1, 3). In contrast, some reports found cystine as an effective antioxidant in soy, rice and corn oils as well as in lard and tallow (5, 6) and it was ranked as better than methionine in olive oil (7). However, other reports described cystine, proline and some other amino acids as ineffective in lards (8), with cysteine behaving as prooxidant in soy oil (5). Still, proline was found as an effective antioxidant in dry butter (9), in fresh sardine oil (10), and as proline nitroxide in menhaden oil (11), although it was not an effective metal deactivator in lard (12). Other amino acids were also investigated by many workers with different order of antioxidant effectiveness frequently reported (4, 6, 8, 12-15).

In view of this, it would not be possible to extend any findings in the literature in this respect to other oils. Therefore, it was the interest of the present work to investigate the antioxidant effects of 27 amino acids (including some derivatives) in two Iraqi vegetable oils.

MATERIALS AND METHODS

Materials

Oils. Safflower seeds (*Carthamus tinctorius* L.) of the "Gila" variety were supplied by the cotton and oilseed

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entire filtrate to a separating funnel and extract with three 20 mL portions of $CHCL_3$. Combine chloroform fractions, dry over anhydrous sodium sulfate, filter and evaporate chloroform on rotary evaporator. Transfer to a small conic-shaped graduated glass tube and continue evaporation to near dryness. Adjust final volume of extract to 500 μL with benzene.

Thin layer chromatography (TLC). 20 × 20 cm glass plates, coated with 0.25 mm Silica Gel HR 60 (Merck). Follow directions of manufacturer. Activated plates by heating for 90 min at 105 C. Allow to cool to room temperature in dessicator.

Samples extracts and mycotoxins standards are spotted on TLC plate as shown in Table I.

The plate is first developed with benzene/hexane (3+1). In this system, lipids move with the solvent front (11), and some pigments are also eluted; while mycotoxins remain at the origin. After development, the plate is dried in a 60 C force-draft oven for 5 min and allowed to cool a few min in a dessicator.

Next, a second development in toluene/ethyl acetate/formic acid (6+3+1) separates mycotoxins.

After drying, examine plates under longwave UV light (366 nm). Aflatoxin B_1 standard should fluoresce blue and ochratoxin A standard should appear as a greenish-blue spot. Determine whether similar spots appear at same R_f in developed sample extracts. Expose plates to NH_3 vapor. Ochratoxin A should now fluoresce bright blue under longwave UV light.

Confirmation procedures. Aflatoxin B_1: using plates prepared as mentioned above, place two 6 μL spots of each suspect extract, and on one of them, superimpose one 5 μL spot of aflatoxin B_1 standard solution (1 μg/mL). Then, on the two spots, add 2 μL trifluoroacetic acid and let react 5 min (7). Blow warm air over plate to remove all unreacted trifluoroacetic acid. Four different samples can be run on each plate.

Develop first with benzene/hexane (3+1) to eliminate lipids and some pigments, next with ethyl acetate/formic acid (99+1). This double elution leads to cleaner chromatograms.

After drying, observe plate under longwave UV light. Determine whether blue fluorescent derivative of aflatoxin B_1 is present in reacted sample extract line, by comparison with superimposed standard line. This will confirm identity of aflatoxin B_1 in sample extract.

Ochratoxin A: using plates prepared as mentioned above, place two 6 μL spots of each suspect extract, and on one of them, superimpose one 5 μL spot of ochratoxin A standard solution (10 μg/mL). Four different samples can be run on each plate.

Develop first with benzene/hexane (3+1); next with either chloroform/methanol (92+8) or benzene/methanol/acetic acid (18+1+1). After drying, examine plates as described above.

This method does not need ochratoxin esterification (7), which is time-consuming, when running many samples.

Semiquantitative analysis. Using plates prepared as mentioned above, place 6 spots of respective composition: 6, 5, 4, 3, 2, 1 μL of contaminated sample extract; and 0, 1, 2, 3, 4, 5 μL of mycotoxin-free sample extract.

Add a seventh spot of 6 μL contaminated sample and superimpose with 5 μL concerned mycotoxin standard. Develop first with benzene/hexane (3+1), next with toluene/ethyl acetate/formic acid (6+3+1); and examine as described above. Determine its lowest spotted volume of contaminated sample extract at which mycotoxin can still be found. From the sensitivity on TLC plate, calculate the approximate quantity of mycotoxin. If necessary, dilute the contaminated sample extract with mycotoxin-free sample extract and rechromatograph.

RESULTS AND DISCUSSION

Recover (%) and Detection Levels of the Method

The lowest detectable mycotoxin levels on TLC plates were determined by adding standard solution to 6 μL mycotoxin-free sample extract spot.

Crude olive oil was spiked simultaneously with aflatoxin B_1 (20 μg/kg) and ochratoxin A (150 μg/kg), and subjected to the complete procedure in order to determine the recovery percentages.

Minimum detectable concentration was verified experimentally. See Table II for data.

These results are similar to those obtained by authors using the TLC technique for aflatoxin B_1 and ochratoxin A determination in other commodities (10, 12-14).

Commercial Samples Screening Test

We analyzed 60 different samples of crude farm olive oil collected in the area of Beni-Mellal. None of these samples showed any contamination with aflatoxin B_1, but ochratoxin A was detected in 3 samples at level of 40 μg/kg similar to the lowest detectable concentration of the method. The presence of ochratoxin A in olive oil, even

TABLE I

Volumes (μL) of Mycotoxin Standards Solutions and Sample Extract Spotted on Each Plate

Spot no.	1	2	3	4	5	6	7	8
Unspiked sample extract	6	6						
Aflatoxin B_1: 1 μg/mL	5							
Ochratoxin A: 10 μg/mL	5							
Unknown samples extracts no.								
1			6					
2				6				
3					6			
4						6		
5							6	
6								6

Six different samples can be run on the same plate.

which cystine was more effective than methionine, and another report (4) indicating the effectiveness of asparagine as antioxidant in butter.

Bint oil had higher metal contents (16) and relatively higher effectiveness for most amino acids. This effectiveness is also apparent when comparing the rate of peroxide formation in the presence of seven selected (and generally more effective) amino acids in Bint oil (Fig. 1) and safflower oil (Fig. 2). Therefore, chelation of metals by amino acids would partly account for their higher antioxidant activity in Bint oil. This observation is in general accordance with some published work (12, 15, 18).

Storage stability tests (days at 45 C) were further determined for seven amino acids and were compared with the AOM values (Table II). The increase in storage stability resulting from amino acids was not always of the same magnitude for the corresponding increase in the AOM values. This added another source of variation when comparing results from different laboratories. In Bint oil, the protection factors ranged from 1.05 for tryptophan to 1.2 for methionine. In safflower oil, the range was from 1.01 for cysteine to 1.17 for methionine.

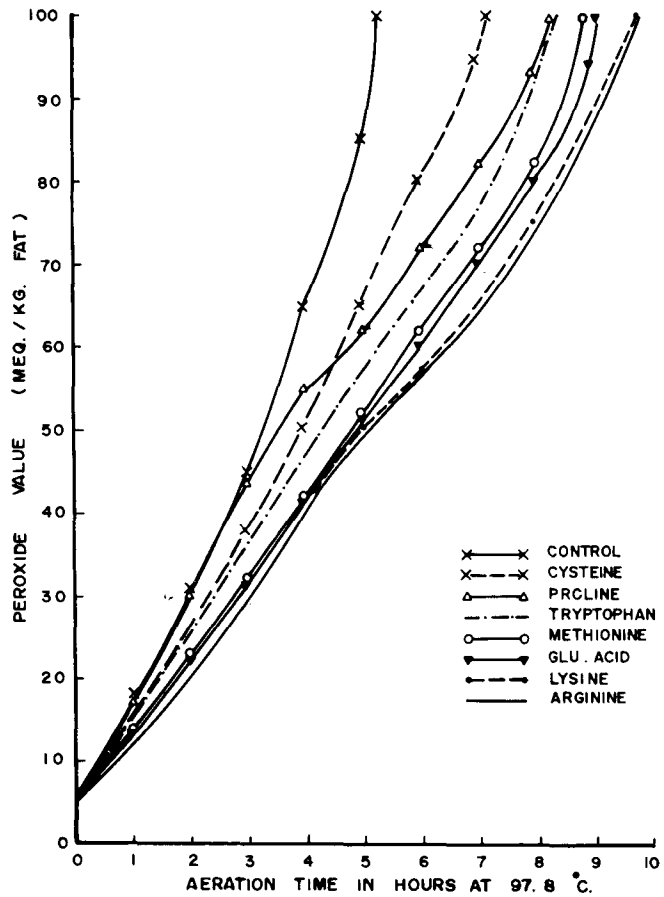

FIG. 1. Influence of amino acids on peroxide formation during oxidation process of the commercial brand vegetable oil (Bint oil).

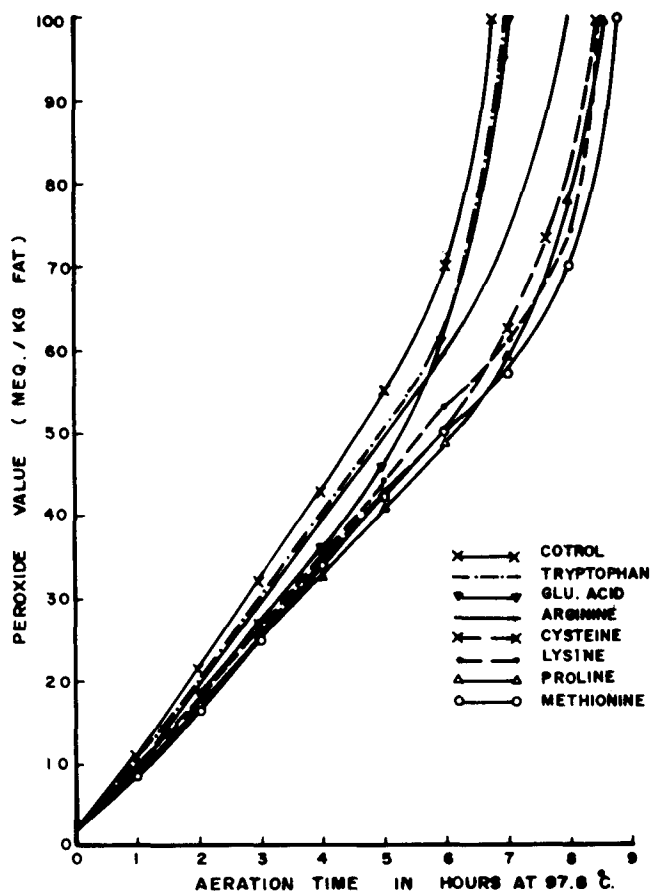

FIG. 2. Influence of amino acids on peroxide formation during oxidation process of crude safflower oil.

TABLE II

Antioxidant Effect of Amino Acids on the Oxidation of Safflower and Bint Oils under the Conditions of the Active Oxygen Method (AOM, hr at 97.8 C) and Storage Stability Method (days at 45 C) for Both Peroxides to Reach 100 meq/kg fat

Amino acids (0.02%)	Bint oil[a]		Safflower oil[b]	
	AOM	Storage method	AOM	Storage method
Methionine	1.68	1.20	1.29	1.17
Proline	1.57	1.15	1.26	1.04
Lysine	1.85	1.19	1.25	1.14
Cysteine	1.36	1.13	1.25	1.01
Arginine	1.85	1.28	1.18	1.06
Tryptophan	1.58	1.05	1.04	1.04
Glutamic acid	1.72	1.19	1.04	1.03

[a]Protection factors: AOM control 5.3 hr, storage method control 23.3 days.
[b]Protection factors: AOM control 6.8 hr, storage method control 31.5 days.

TABLE III

The Effect of Increasing the Concentration of Seven Amino Acids (from 0 to 1%) on the Protection Factors (AOM) of the Two Vegetable Oils

Amino acid concentration (%)	Protection factors[a]													
	Lysine		Proline		Arginine		Methionine		Cysteine		Glutamic acid		Tryptophan	
	Saf.[b]	Bint[c]	Saf.	Bint	Saf.	Bint	Saf.	Bint	Saf.	Bint	Saf.	Bint	Saf.	Bint
0.01	1.23	1.81	1.23	1.55	1.19	1.79	1.19	1.47	1.19	1.30	1.04	1.68	1.02	1.53
0.04	1.29	1.87	1.28	1.58	1.32	1.87	1.31	1.70	1.25	1.36	1.07	1.77	1.07	1.58
0.07	1.28	1.90	1.29	1.64	1.35	1.87	1.31	1.75	1.26	1.49	1.07	1.89	1.10	1.66
0.10	1.31	2.02	1.37	1.70	1.37	1.89	1.35	1.77	1.29	1.60	1.09	1.89	1.12	1.72
0.40	1.48	2.07	1.66	1.87	1.40	1.89	1.43	1.87	1.72	2.09	1.10	2.04	1.15	1.98
0.70	1.54	2.07	1.93	2.13	1.47	1.92	1.48	1.92	1.87	2.43	1.15	2.41	1.19	2.13
1.00	1.84	2.11	1.94	2.19	1.57	2.00	1.51	2.00	1.93	2.79	1.18	2.40	1.53	2.30

[a]Control stability values (AOM) were 6.8 and 5.3 hr (at 97.8 C) for safflower and Bint oils, respectively.
[b]Safflower oil.
[c]Bint oil.

The Effect of Amino Acid Concentration

The seven amino acids displayed a general pattern of slowly increasing stability with concentration (from 0 to 1%) in both oils (Table III). In the case of cysteine, the increase was th largest in Bint oil where the protection factor (PF) at the 1% level was more than double its value at the 0.01%, compared with an increase of ca. 60% (of the 0.01% level) in safflower oil. In other cases, the increase in PF was much smaller and it ranged between 11.7 (arginine) and 50.3% (tryptophan) in Bint oil, whereas in safflower oil the increase ranged between 13.5 (glutamic acid) and 57.7% (proline).

The present data with regard to the effect of amino acid concentration are in general agreement with one report (19). However, it was observed (20) that the increase in methionine and proline concentration had little effect on the stability of lard by the AOM.

Assuming that antioxidants would be of: very low, low, medium and high antioxidant activity when their PF values are 1.5, 1.5-2, 2-2.5 and 2.5-3, respectively, all of the amino acids activities observed here are either very low or low at the 0.01-0.07% levels with some having medium activity at the 1% level, and cysteine alone being of high antioxidant activity in Bint oil. However, it remains to be seen how such activities would compare with those of conventional antioxidants in the same oils.

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