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Chemical Abstracts
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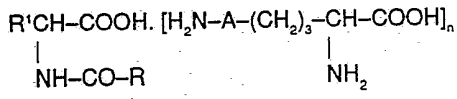
(58) Field of search
C2C

(54) Antioxygen agents

(57) Antioxygen agents, useful in the protection of oils and fats against oxidative degradation, have the formula



R = hydrocarbon chain of 4 to 32 C atoms,
A = $-CH_2-$ or $-C(=NH).NH-$,
R^1 is a residue such that $R_1-CH(NH_2)-COOH$ is a naturally occuring essential aminoacid or protein,
n is 0 or 1 if $R^1=CH_3SCH_2CH_2-$ or 1 otherwise.

Oil and fat compositions, protected against oxidative degradation by such antioxygen agents, may be included in cosmetic or pharmaceutical substances or in nutriments.

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TITLE

Antioxygen Agents

DESCRIPTION

The present invention relates to new anti-oxygen substances and compositions consisting of selected amino-acids or of associations of the same with basic amino-acids such as lysine and arginine. Numerous works have shown the important biological role of lipoperoxides and of the free radicals deriving from the same.

Many publications indicate that the peroxides, like free radicals, are involved in the settlement of various pathological conditions, as well as in the ageing process.

It is known that the peroxidation of lipids leads to the formation of malonadialdehyde which, in turn, may react with a variety of biological structures, such as phosphoaminolipids and amino-acids, for instance, and synthetizes a fluorescent pigment called lipofuscine and which is found in both animal and vegetable aged tissues.

It has been also shown that anti-oxygen agents play an important role in human as well as in animal longevity, and that the dismutase superoxide could be considered as the most important defense agent in the organism, against the toxic effects of oxygen metabolism. (KUNIO YAGI - Lipid peroxides in biology and medicine - Academic Press 1982), (FRIDOVICH I - Adv. in enzymology 1986), (MELBORN R - COLE G - Adv. Free Radical Biology and Medicine 1985), (Cutler R - antioxidants and longevity of mammalian species - Life Sci. 1985).

A large number of publications deals with the formation of the peroxides and the means of acting against them by means of anti-oxygen agents. That is why the use of anti-oxygen agents in human and animal food is highly desirable both from a physiological and from an economic point of view and, in this regard, numerous substances such as tocopherol, the gallates, ascorbates and mainly the butylhydroxypanisols, or BHA, and butylhydroxytoluenes, or BHT, are presently legally used.

In our previous British patent application No. 2181647 it was shown that associations of fatty acids and basic amino-acids presented anti-oxygen properties. The present invention relates to new anti-oxygen substances, characterised by the fact that they contain certain lipoaminoacids or associations of the same with basic amino-acids such as lysine and arginine, which may be advantageously used for protection against oxidative degradation either of emulsions containing lipids,or of vegetable or animal oils and fats.

Depending on the nature of the fatty substances or of the compositions which contain the same and which must be protected against auto-oxidation, the present invention provides a number of strictly biological compositions, (anti-oxygen compounds of this kind are usually found in living organisms) which are deprived of any toxic action whatsoever and that can, moreover, be considered as nutriments. This kind of anti-oxygen agent is characterised by highly anti-oxygen properties which are sometimes superior to those of the most active synthetic anti-oxygen agents used in legal doses for the protection of fats i.e. the above mentioned BHT and BHA, for instance.

The invention provides an antioxygen agent having the formula

$$R^1\underset{\underset{NH\text{-}CO\text{-}R}{|}}{CH}\text{-}COOH.\ [H_2N\text{-}A\text{-}(CH_2)_3\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}COOH]_n$$

wherein R represents a saturated or unsaturated hydrocarbon chain having from 4 to 32 carbon atoms, A represents a methylene group or a group of the formula $-\overset{\overset{NH}{\|}}{C}\text{-}NH-$, R^1 is a residue such that $R^1\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}COOH$ represents a naturally occuring essential animoacid or protein, and n is 0 or 1 if R^1 represents a 2-methylthio-ethyl group or 1 otherwise.

In order to determine the anti-oxygen activity of the various biological substances or compositions subject of the present invention, various fatty compositions (oils, fats or emulsions) are submitted to U.V. radiation (252 nm), over a period of fifteen hours, at temperature of 50 to 60°C, depending on the case.

For each tested fatty composition 10 g were used, placed in small Petri boxes of the same size; the irradiation in this temperature range accelerated the peroxidation process.

After irradiation, 1g of fatty composition was taken and placed in a 100 ml flask fitted with a stopper and a glass tube of approximately 50 cm; 1 g of potassium iodide and 25 ml of a mixture of 3/2 acetic acid/chloroform were added. The flask, fitted with its tube, was placed in a dark vessel so as to be protected from light and brought to the boiling point for exactly three minutes. The solution was cooled and then about fifty ml of water and a few drops of starch paste were added. It was titrated with a N/200 solution sodium hyposulfite until the

solution became colourless. The number of ml used to neutralise the freed iodine corresponds to the peroxide index of the non protected fatty composition indicated in the following tables by the letter A and retained as control. By comparsion with A it was easy to calulate the protection rate indicated by the letter B for the fatty compositions protected by various substances.

The results are reported in the following tables.

From this experimentation it may be concluded that:

1-) The lipoaminoacidic structures are generally weakly anti-oxygen for oils as well as for fats;

2-) The combination of lipoaminoacids + basic amino acids such as lysine and arginine leads to very active anti-oxygen agents but they cannot be used in fats because of their insolubility;

3-) The lipoaminoacids resulting from the acylation of methionine by saturated or unsaturated fatty chains have highly anti-oxygen properties; such structures have a particularly interesting advantage: their components are fundamental elements of living tissues. Finally, due to its biological nature, this group of anti-oxygen agents presents also nutritional properties.

Tables Nos.1 and 2 indicate the protective action of associations of lipoaminoacids with 2.5% of lysine and arginine on different vegetable oils, compared with lipoaminoacids which are not associated with basic amino-acids. The BHT (E 321) used as a reference, at 0.2%, i.e. at a higher concentration than legally admitted (0.01% or 150 ppm according to the case).

Table No. 1

Tested substances	Sunflower Oil		Sesame Seed Oil		Almond Oil	
	A	B	A	B	A	B
Control oils	6.6		5.8		9.2	
B H T - 2 %	5.5	17 %	2.5	57 %	2.7	71 %
Palmitoylkeratinic acid	4.9	26 %	3.0	48 %	3.6	61 %
Lysine palmitoyl-keratinate	0.9	86 %	0.1	98 %	0.1	99 %
Palmitoylcaseinic acid	3.8	42 %	2.4	55 %	4.2	54 %
Lysine palmitoylcaseinate	0.8	88 %	0.1	99 %	0.1	99 %
Palmitoyl-collagenic acid	5.7	14 %	3.3	43 %	3.8	59 %
Lysine palmitoyl-collagenate	0.6	91 %	0.6	90 %	0.1	99 %
Palmitoylcystinic acid (+)	1.8	73 %	1.0	83 %	0.9	90 %
Lysine palmitoylcystinate	0.1	99 %	0.1	99 %	0.1	99 %
Butyryl-lysinic acid	5.5	17 %	4.3	26 %	8.2	11 %
Lysine butyryllysinate	0.7	89 %	0.5	90 %	1.5	84 %
Lysine butyrylcystinate	0.8	84 %	0.7	88 %	1.8	80 %

Table No. 2

Tested substances	Sunflower Oil		Sesame Seed Oil		Almond Oil	
	A	B	A	B	A	B
Control oils	7.5		5.2		9.2	
BHT - 2 %	5.0	33 %	3.6	31 %	2.7	71 %
Palmitoylkeratinic acid	4.5	40 %	1.0	80 %	3.6	61 %
Arginine palmitoylkeratinate	0.1	99 %	0.1	99 %	0.7	93 %
Palmitoylcaseinic acid	5.4	28 %	1.3	75 %	4.2	54 %
Arginine palmitoylcaseinate	0.1	99 %	0.1	99 %	0.1	99 %
Palmitoyl-collagenic acid	6.2	17 %	2.2	58 %	3.8	59 %
Arginine palmitoylcollagenate	0.3	94 %	0.1	99 %	0.6	93 %
Palmitoylcystinic acid (+)	2.4	68 %	0.8	85 %	0.9	80 %
Arginine palmitoylcystinate	1.1	86 %	0.3	94 %	0.6	94 %

(+) Poorly soluble in oils unless heated at a temperature that may alter the same.

The following table No. 3 shows the protection provided to four vegetable oils by different lipomethionic acids, after a period of 15 hours of irradiation at 60°C.

Table No. 3

Tested substances	Sunflower Oil		Hazelnut Oil		Sesame Seed Oil		Almond Oil	
	A	B	A	B	A	B	A	B
Control oils	13.0		20.2		6.9		15.0	
B H T - 2 %	7.7	41%	11.0	46%	5.9	15%	7.0	53%
Palmitoyl-methionic acid - 2 %	1.9	86%	1.0	95%	0.9	87%	1.0	94%
Caprylyl-methionic acid - 2 %	1.9	86%	1.0	95%	0.6	91%	1.2	92%
Lauroyl-methionic acid - 2 %	1.9	86%	1.0	95%	0.8	89%	1.2	92%
Butyryl-methionic acid - 2 %	2.0	85%	1.2	95%	0.8	89%	1.8	88%
Oleoyl-methionic acid - 2 %	0.8	94%	0.8	96%	0.4	94%	0.7	95%

The following Table No. 4 shows the protection provided to three different fats by lipomethionic acids having contents of 3 %, 2 % and 1 %, in comparison with the 0.2 % BHT and an acyleted derivative of sarcosine which is not biochemically a lipoaminoacid.

Table No. 4

Tested substances	Lard		Raw tallow		Refined tallow	
	A	B	A	B	A	B
Control fats	31.5		3.5		14.0	
B H T - 0,2 %	2.3	93 %	1.5	58 %	1.5	89 %
Palmitoylmethionic acid						
- 3 %	3.2	90 %	1.0	72 %	1.6	89 %
- 2 %	3.2	90 %	1.2	66 %	1.7	88 %
- 1 %	9.7	69 %	2.1	40 %	4.5	68 %
Caprylylmethionic acid						
- 3 %	3.1	90 %	1.2	66 %	1.9	87 %
- 2 %	3.1	90 %	1.2	66 %	2.0	86 %
- 1 %	5.4	83 %	1.6	55 %	4.9	65 %
Lauroylmethionic acid						
- 3 %	3.2	90 %	1.0	72 %	1.4	90 %
- 2 %	3.2	90 %	1.1	69 %	2.0	86 %
- 1 %	6.8	78 %	2.2	37 %	5.0	65 %
Butyrylmethionic acid						
- 3 %	5.2	84 %	1.3	63 %	4.0	72 %
- 2 %	5.6	83 %	1.3	63 %	4.9	64 %
- 1 %	12.2	62 %	2.3	35 %	6.0	57 %
Oleoylmethionic acid						
- 3 %	0.1	99 %	0.0	100 %	0.9	94 %
- 2 %	0.3	98 %	0.0	100 %	0.9	94 %
Lauroylsarcosine - 3 %	26.7	15 %	4.7	-	13.7	-

The following table reveals the weak antioxygen protection of some examples of lipoaminoacids, with regard to refined tallow, in comparison with table No. 4.

Table No. 5

Tested substances	Refined tallow	
	A	B
Control	11.0	
B H T - 0.2 %	2.0	82 %
Palmitoylcollagenic acid - 2 %	7.2	35 %
Palmitoylcaseinic acid - 2 %	5.0	55
Palmitoylkeratinic acid - 2 %	4.7	58 %
Palmitoylhydroxyprolinic acid - 2 %	10.5	0.5 %
Palmitoylcystinic acid - 2 %	9.7	1.4 %
Palmitoylprolinic acid - 2 %	10.5	0.5 %
Palmitoylphenylalanic acid	9.8	1.2 %

The following table shows the evolution of peroxydation eight days after the irradiation of refined tallow (initial figures given in table No. 4).

Table No. 6

Tested substances	Immediately after UV irradiation		8 days later	
	A	B	A	B
Control	14.0		24.5	
B H T - 0.2 %	1.5	89 %	1.9	93 %
Palmitoylmethionic acid				
- 3 %	1.6	89 %	1.9	93 %
- 2 %	1.7	88 %	2.1	91 %
Caprylylmethionic acid				
- 3 %	1.9	87 %	2.5	90 %
- 2 %	2.0	86 %	2.0	92 %
Lauroylmethionic acid				
- 3 %	1.4	90 %	2.8	89 %
- 2 %	2.0	86 %	2.2	91 %
Oleoylmethionic acid				
- 3 %	0.8	94 %	1.1	95 %
- 2 %	0.8	94 %	1.1	95 %

As can be seen from these tables Nos. 4 and 6, it is surprising that an unsaturated fat chain, such as oleic acid, which is easily auto-oxydisable and methionine acylated, provides such remarkable antioxygen properties. It is also surprising that only the lipomethionic acids ensure good protection for vegetable as well as animal oils ; this is not the case for one of the most efficient antioxygen agents, such as the BHT.

Table No. 7 gives the results of the comparison of the protective activity of some lipoaminoacids and their arginine and lysine derivatives on three emulsified oils. Oil : 10 %, polyoxyethylenated fatty alcohol 10 % , lipoaminoacid or derivative : 5 %, water : sufficient amount to 100 % ; 15 hours of irradiation at 50°C. (As water evaporates, its content is completed to 5 %).

Table No. 7

Tested substances	Sunflower		Hazelnut		Sesame	
	A	B	A	B	A	B
Controls	18.0		9.0		4.4	
B H T - 0.2 %	6.8	62 %	2.8	69 %	2.1	52 %
Palmitoylcollagenic acid	8.5	53 %	4.5	50 %	3.6	18 %
Lysine palmitoyl-collagenate	4.5	75 %	2.5	72 %	2.0	55 %
Arginine palmitoyl-collagenate	4.2	77 %	3.8	58 %	2.2	50 %
Palmitoylkeratinic acid	8.2	54 %	3.7	57 %	3.7	16 %
Lysine palmitoyl-keratinate	3.2	82 %	3.2	65 %	2.2	50 %
Arginine palmitoyl-keratinate	3.1	83 %	3.2	65 %	2.4	45 %
Palmitoylcaseinic acid	7.8	57 %	3.4	62 %	3.4	33 %
Lysine palmitoyl-caseinate	3.5	80 %	2.9	68 %	2.0	55 %
Arginine palmitoyl-caseinate	3.2	82 %	3.1	66 %	2.3	75 %
Palmitoylcystinic acid	7.0	61 %	3.2	65 %	2.8	47 %
Lysine palmitoyl-cystinate	4.5	75 %	2.2	76 %	2.6	61 %
Palmitoylhydroxypro-linic acid	10.3	43 %	6.0	33 %	3.8	14 %
Lysine palmitoyl-hydroxyprolinate	4.5	75 %	4.2	53 %	2.8	47 %

Table No. 8 shows the protective activity on emulsions containing 10 % oil, by five lipomethionic acids with contents from 2 to 0.2 %.

Table No. 8

Tested substances	Sunflower		Hazelnut	
	A	B	A	B
Controls	23.0		6.5	
B H T - 0.2 %	1.9	92 %	2.9	53 %
Palmitoylmethionic acid - 2 %	1.5	95 %	0.0	100 %
Caprylylmethionic acid - 2 %	0.3	99 %	0.0	100 %
Lauroylmethionic acid - 2 %	0.3	99 %	0.0	100 %
Butyrylmethionic acid - 2 %	4.5	80 %	0.0	100 %
Oleoylmethionic acid - 2 %	0.2	99 %	0.0	100 %
Controls	20.5		7.8	
B H T - 0.02 %	15.0	27 %	6.7	24 %
Palmitoylmethionic acid - 0.2 %	15.2	26 %	5.5	30 %
Caprylylmethionic acid - 0.2 %	14.0	32 %	5.8	26 %
Lauroylmethionic acid - 0.2 %	15.3	27 %	5.9	25 %
Butyrylmethionic acid - 0.2 %	14.8	28 %	5.9	25 %
Oleoylmethionic acid - 0.2 %	14.2	30 %	5.5	30 %

The invention applies to all cosmetic, pharmaceutical, nutritional or industrial compositions comprising fats or oils of animal origin (land or sea), or of vegetable origin, or even mineral origin, which would be protected from oxydative deterioration by the incorporation of lipoaminoacidic lysine or arginine salts, or by a lipoaminoacid derived from the acylation of a fatty chain of methionine, in conformity with the structures hereinabove described.

The invention is more specifically interesting for it affords a protection of fats, oils and the like against oxydative alteration by substances of strictly biological nature, the structure of which is very close to that one of the living tissues ; due to this similarity of structure, these substances are highly effective against endogenic or exogenic free radicals - which are largely responsible for the acceleration of the ageing process and the genesis of many diseases.

CLAIMS

1. An antioxygen agent having the formula

$$R^1\underset{\underset{NH\text{-}CO\text{-}R}{|}}{CH}\text{-}COOH.\ [H_2N\text{-}A\text{-}(CH_2)_3\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}COOH]_n$$

wherein R represents a saturated or unsaturated hydrocarbon chain having from 4 to 32 carbon atoms, A represents a methylene group or a group of the formula $-\underset{\underset{NH}{\|}}{C}\text{-}NH-$, R^1 is a residue such that $R^1\text{-}\underset{\underset{NH_2}{|}}{CH}\text{-}COOH$ represents a naturally occuring essential animoacid or protein, and n is 0 or 1 if R^1 represents a 2-methylthio-ethyl group or 1 otherwise.

2. An antioxygen agent according to claim 1 which is one of

palmitoylmethionic acid,
caprylylmethionic acid,
lauroylmethionic acid,
butyrylmethionic acid,
oleoylmethionic acid,
lysine palmitoylkeratinic acid,
arginine palmitoylkeratinic acid,
lysine palmitoylcaseinate,
arginine palmitoylcaseinate,
lysine palmitoylcollagenate,
arginine palmitoylcollagenate,
lysine palmitoylcystinate,
arginine palmitoylcystinate,
lysine butyryllysinate,
lysine butyrylcystinate, and
lysine palmitoylhydroxyprolinate.

3. An oil or fat composition containing an antioxygen agent according to claim 1 or claim 2 in an amount effective to protect the oil or fat against oxidative degradation.

4. A cosmetic substance containing an oil or fat composition according to claim 3.

5. A pharmaceutical substance containing an oil or fat composition according to claim 3.

6. A nutriment containing an oil or fat composition according to claim 3.

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