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(54) **DIESTERS A CHAINE COURTE ET PROCEDE D'ELABORATION**

(54) **SHORT CHAIN DIESTERS AND PROCESS FOR THEIR PRODUCTION**

(57) L'invention concerne un procédé d'obtention d'un produit qui comporte un taux élevé de zéaxanthine, lutéine ou un mélange des deux, comme par exemple des diesters d'acides organiques à chaîne courte, de zéaxanthine, de lutéine ou d'un mélange des deux pouvant être utilisé principalement pour la pigmentation de poulets et de jaune d'oeuf, ainsi que comme intermédiaire lors de la synthèse de cantaxanthine (β,β-carotène-4,4'-dione) et d'astaxanthine (3,3'-dihydroxy-β,β-carotène-4,4'-dione) au moyen de la réaction d'extraits obtenus à partir d'oeillets d'Inde (Tagetes Erecta L.) ou d'extraits de plantes renfermant de la lutéine, zéaxanthine ou un mélange des deux dans des proportions quelconques, avec un anhydre propionique ou acétique dans des conditions contrôlées de température et de pression.

(57) Process for producing a product which has a high content of zeaxanthine, luteine or a mixture of both, such as short chain organic acid diesters of zeaxanthine, luteine or a mixture of both, usable essentially for the pigmentation of chicken and the yoke of an egg, as well as an intermediary in the synthesis of cantaxanthine (β,β-carotene-4,4'-dione) and astaxanthine (3,3'-dihydroxy-β,β-caroten-4,4'-dione), by means of the reaction of extracts obtained from French marigolds (Tagetes Erecta L.), or extracts of plants which contain luteine, zeaxanthine or a mixture of both in any proportion, with propionic or acetic anhydride under controlled temperature and pressure conditions.

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(54) Title: SHORT CHAIN DIESTERS AND PROCESS FOR THEIR PRODUCTION

(54) Título: DIESTERES DE CADENA CORTA Y PROCESO PARA SU ELABORACION

(57) Abstract

Process for producing a product which has a high content of zeaxanthine, luteine or a mixture of both, such as short chain organic acid diesters of zeaxanthine, luteine or a mixture of both, usable essentially for the pigmentation of chicken and the yoke of an egg, as well as an intermediary in the synthesis of cantaxanthine (β,β–carotene–4,4'–dione) and astaxanthine (3,3'–dihydroxy–β,β–caroten–4,4'–dione), by means of the reaction of extracts obtained from French marigolds (Tagetes Erecta L.), or extracts of plants which contain luteine, zeaxanthine or a mixture of both in any proportion, with propionic or acetic anhydride under controlled temperature and pressure conditions.

(57) Resumen

Proceso para la obtención de un producto que tiene un alto contenido de zeaxantina, luteina o una mezcla de ambos, como diésteres de ácidos orgánicos de cadena corta, de zeaxantina, luteina o una mezcla de ambos, que puede ser usado principalmente para la pigmentación de pollos y yema del huevo, así como un intermediario en las síntesis de cantaxantina (β,β–caroten–4,4'–diona) y astaxantina (3,3'–dihidroxi–β,β–caroten–4,4'–diona), mediante la reacción de extractos obtenidos de cempasuchil (Tagetes Erecta L.), o extractos de plantas que contengan luteina, zeaxantina o una mezcla de ambas en cualquier proporción, con anhídrido propiónico o acético, bajo condiciones controladas de temperatura y presión.

SHORT CHAIN DIESTERS AND PROCESS FOR MAKING THE SAME

BACKGROUND OF THE INVENTION

A. FIELD OF THE INVENTION

This invention is related to short chain diesters, and more particularly to a process for the obtention of a product with a high content of zeaxanthin, lutein or mixtures thereof, as short chain organic acid diesters of zeaxantine, lutein or mixtures thereof.

B. DESCRIPTION OF THE RELATED ART.

The yellow carotenoids such as the lutein and the zeaxanthin, occur in marigold flowers as mono- or diesters , linked to long chain fatty acids such as palmitic, stearic or myristic acids, among others (Alam, A. U. (1968) *Lipids*, 3(2), 183; Gayle G. (1986) *J. Food Sci.*, 51(4), 1093).

It is assumed that in such chemical structure, the carotenoids are better protected against oxidant processes, so that the flower color is better preserved in nature.

However, in the pigmentation of broilers, it has been shown that the bioavailability of such carotenoid fatty esters is lower than when they are hydrolized, i.e. when they are fed as free carotenoids (Coon, C.N. (1976) *Poult. Sci.*, 55, 841-847).

Applicant's have found that by saponification of the marigold carotenoids and their subsequent linking to short chain organic acids, such as formic, acetic or propionic, acids, etc., an improvement in their bioavailability and a more stable form of the carotenoids, is achieved.

The acetylation of carotenoids, zeaxanthin among others, has been carried out in laboratory scale since decades ago. The reported methodology

specifically refers to a research, for elucidation purposes, about the chemical structures of the carotenoids.

The carotenoid in pure form, zeaxanthin in this case, is dissolved in pyridine, treating it with acetic anhydride and agitation at room temperature to obtain the acetylated derivative after several hours (Liaaen-Jensen, S. and Jensen, A. (1971) *Methods Enzymol.* 23, 586), or in a few minutes if the reagent mixture is maintained under reflux (Alam, 1968).

Another preferred chemical path to obtain the acetylated compound is to dissolve the zeaxanthin in pyridine and benzene to carry on the reaction at 20° C with acetyl chloride, a few minutes later (Bartlett, L. (1969) *J. Chem. Soc.* C, 2538).

In the process acording with the present invention, marigold extracts containing saponified and isomerized carotenoids (Torres, et al. 5,523,494 6/1996, 568/834), are treated directly with acetic anhydride, or propionic anhydride in such a way as to obtain the short chain organic acid diester derivatives of zeaxanthin, lutein or mixtures thereof, present in such extracts.

SUMMARY OF THE INVENTION

It is a main objective of the present invention to provide a product having a high content of zeaxanthin, lutein or mixtures thereof, in the form of short chain organic acid diesters of zeaxanthin, lutein or mixtures thereof, which can be used mainly in the pigmentation of broilers skin and egg yolks.

It is an additional main objective of the present invention to provide a process for obtaining a product with a high content of zeaxanthin, lutein or mixtures thereof, in the form of short chain organic acid diesters of zeaxanthin,

lutein or mixtures thereof, which can be used mainly in the pigmentation of broilers skin and egg yolks.

It is still a main objective of the present invention to provide a process of the above disclosed nature, by reacting short chain organic anhydrides, with saponified extracts containing carotenoids, in an inert atmosphere of carbon dioxide, nitrogen or a mixture of both under controlled conditions of temperature and pressure.

It is another main objective of the present invention to provide a process of the above disclosed nature in which saponified extracts containing carotenoids are treated without the need to add any solvent at all.

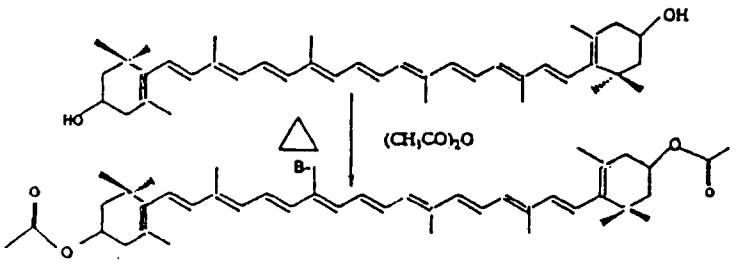
It is also another main objective of the present invention, to provide a process of the above disclosed nature, wherein the product or its formulations can be used for the pigmentation of broiler skin and egg yolk or as a pigmenting agent in aquaculture.

It is also an additional object of the present invention to provide a process of the above disclosed nature wherein the zeaxanthin diester obtained can be used as an intermediate in the synthesis of astaxanthin or cantaxanthin.

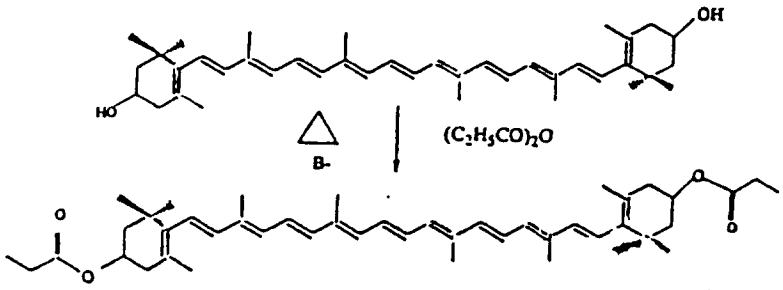
These and other objectives and advantages of the present invention will be apparent to those persons having ordinary skill in the art, from the following detailed description of the invention.

DETAILED DESCRIPTION OF THE INVENTION

The chemical process is carried out according to the following reaction:



ZEAXANTHINE DIACETATE



ZEAXANTHINE DIPROPIONATE

The saponified marigold extracts containing the zeaxanthin, have been obtained according to the procedure reported by Torres, et al. (1996), but any other pigmenting formulation or extract containing zeaxanthin can also be used. Regardless of the raw material employed, it should preferably be moisture free before acetylating with acetic anhydride, or before the treatment with propionic anhydride.

The carotenoid containing substrate is maintained at a temperature in the range of 25°C to 140°C, but preferably between 40°C and 100°C.

When a crude marigold extract containing the saponified and isomerized carotenoids, enters into the reaction with the acetic or propionic anhydride, its residual fatty acids and other present lipids occur as the sodium or potassium salts.

Acetylation reaction

The acetic anhydride is slowly added to the extract in a reaction vessel under agitation, in a proportion from 0.2 to 2.0 parts by weight for one part of the pigmenting concentrate, which is in the form of a sodium or potassium soap (a heavy paste highly soluble in water). The reaction mixture is partially soluble in the acetic anhydride. However, as the acetylation reaction advances, an oily phase separates from the reaction media, mainly composed of acetic acid and sodium or potassium acetates in aqueous solution, from which it is easily separated by decanting. The acetylation vessel wherein the reaction takes place should be kept under an inert atmosphere, in order to avoid the intensive degradation of the xanthophylls. An inert diluent such as ethylene glycol or propylene glycol or an aliphatic or cyclic hydrocarbon can be used to reduce the viscosity of the mixture.

The reaction time depends on the temperature and can be from 6 minutes to 24 hours, but preferably from 4 to 18 hours.

Parameter	Trial 1	Trial 2	Trial 3	Trial 4
Acetic anhydride: extract Ratio	0.5	2.5	1.0	2.0
Reaction time, hrs.	16	16	20	12
Reaction temperature, ° C	90	80	100	60
Pressure, mm Hg	750	760	750	760
Mono-hydroxycarotenoids %	4.2	1.5	1.7	2.8
Di-hydroxycarotenoids %	2.1	1.3	1.3	0.8
Aceto-carotenoids %	88.5	91.8	89.6	90.2

Propionation reaction

Propionic anhydride is slowly added to the extract in a reaction vessel under agitation, in a proportion from 0.2 to 3.0 parts by weight to one part of the pigmenting concentrate, which is in the form of sodium or potassium soap (a heavy paste highly soluble in water). The reaction mixture is partially soluble in the propionic anhydride. However as the propionation reaction advances, an oily phase separates from the reaction media, mainly composed of propionic acid and sodium or potassium propionates in aqueous solution, from which it is easily separated by decanting. The reaction vessel should be kept under an inert atmosphere to avoid degradation.

The reaction time depends on the temperature and can be from 5 minutes to 24 hours, but preferably from 3 to 17 hours.

Parameter	Trial 1	Trial 2	Trial 3	Trial 4
Propionic Anhdride:extract Ratio	0.8	2.5	2.0	3.0
Reaction time, hrs .	6	4	4	3
Reaction temperature, ^{0}C	70	60	80	80
Pressure, mm Hg.	760	780	760	780
Mono-hydroxycarotenoids %	4.1	1.5	1.8	0.7
Di-hydroxycarotenoids %	2.3	1.0	0.6	1.0
Propionate-carotenoids	86.0	90.0	89.0	91.0

The saponified extracts used as the raw material in the above described process, contain approximately 92 % of di-hydroxy carotenoids, and 1-2% of mono-hydroxy carotenoids , quantified according to the AOAC method.

The identification and quantification of the pigments involved were carried out following the HPLC techniques mentioned by Torres, et al.(1996),

as well as by the use of other spectroscopic techniques as IR, UV, ^{1}HNMR, etc., widely used in carotenoids identification.

The end product can be formulated as an aqueous emulsion, or it can be dispersed by means of a carrier to obtain pre-mixtures of a given concentration of zeaxanthin, lutein or mixtures thereof in the form of short chain organic acid diesters, to be used as a pigmenting agent for broilers skin, egg yolks, or shrimps and salmon in aquaculture.

WE CLAIM

1. A process for obtaining organic acid diesters of mono- or polyhydroxilated carotenoids, from saponified extracts containing carotenoids, comprising: reacting 0.2 to 3.5 parts of acetic or propionic anhydride, slowly under agitation, with one part of said saponified extracts containing carotenoids, saponified with sodium or potassium hydroxide, in the absence of solvents, the process proceeding with formation of a member selected from the group consisting of acetic acid and sodium acetate, acetic acid and potassium acetate, propionic acid and sodium propionate, or propionic acid and potassium propionate.

2. The process as claimed in claim 1, wherein the carotenoids are selected from the group consisting in yellow carotenoids, lutein or zeaxhantin.

3. The process as claimed in claim 1, wherein saponified extracts containing carotenoids are obtained from extracts of marigold flowers, marigold meal, yellow corn, yellow corn gluten, or alfalfa.

4. The process as claimed in claim 2, wherein the zeaxanthin is in its free hydrolized form.

5. The process as claimed in claim 1, wherein the lutein or any other hydroxycarotenoid or mixtures thereof are hydrolized.

6. The process as claimed in claim 1, wherein the proportion of acetic anhydride is from 0.2 to 2.0 parts by weight, for one part of extract.

7. The process as claimed in claim 1, wherein the proportion of propionic anhydride is from 0.5 to 3.5 parts by weight for one part of extract.

8. The process as claimed in claim 1, wherein the saponified extract is

moisture free.

9. The process as claimed in claim 1, wherein the saponified extract is obtained from an alkaline reaction, containing residual NaOH, KOH or a mixture of both, or any other alkaline or alkaline-hearth metal hydroxides, as well as organic bases selected from the group consisting of morpholine, ethylamine, diethylamine and ethanolamine.

10. The process as claimed in claim 1, wherein the reaction time is from 5 minutes to 12 hours.

11. The process as claimed in claim 1, wherein the reaction temperature is from 25°C to 140°C.

12. The process as claimed in claim 1, wherein the reaction is carried out in an inert atmosphere of carbon dioxide, nitrogen, or a mixture of both.

13. The process as claimed in claim 1, wherein the reaction product contains from 5% to 90% of zeaxanthin diacetate.

14. The process as claimed in claim 1, wherein the reaction product contains from 5% to 90% of zeaxanthin di-propionate.

15. The process as claimed in claim 6, wherein the proportion of acetic anhydride is from 0.5 to 1.5 parts by weight, for one part of extract.

16. The process as claimed in claim 7, wherein the proportion of propionic anhydride is from 0.5 to 1.5 parts by weight for one part of extract.

17. The process as claimed in claim 1, wherein the reaction time is from about 2 to about 10 hours.

18. The process as claimed in claim 1, wherein the reaction temperature is from about 40°C to about 100°C.