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(54) Title: SOLID MICROCRYSTALLINE GREEN TEA CATECHIN ANTIOXIDANTS FOR LIPIDS

(57) Abstract

The water-soluble and fat-insoluble polyphenolic antioxidants (catechins), recovered from green tea and reduced to a particle size less than 38 microns on their largest dimension, are effective antioxidants in lipid media such as fats, oils, foods, and ingredients of foods without imparting undesirable flavors, aromas, and precipitates, preferably in the form of suspensions or dispersions in a lipid medium in which they are insoluble. Since it is known that tea polyphenols have positive effects on human health, the resulting stabilized lipids can be considered to have nutritional qualities superior to the same lipid stabilized with common synthetic antioxidants. Synergistic effects with other natural antioxidants and with phosphates are also disclosed.

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SOLID MICROCRYSTALLINE GREEN TEA CATECHIN ANTIOXIDANTS FOR LIPIDS

FIELD OF THE INVENTION

Green tea catechins as antioxidants, especially for fats, oils, foods, and ingredients of foods, in the form of less than 38 micron particles, and suspensions thereof in lipid media in which they are insoluble.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 portrays the structures of the four significant catechins contained in and extractable from green tea.

BACKGROUND OF THE INVENTION AND PRIOR ART

Oxidation of fats, vegetable oils, carotenoids and their biologically active derivatives, Vitamin A, and of essential oils and other flavorings results in degradation of their quality, and is deleterious to foodstuffs containing the oxidized products.

The art shows many methods of inhibiting lipid oxidation by adding fat-soluble antioxidants to the substrate. The art does not show the stabilization of fats, oils, fatty foods and ingredients of foods employing green tea catechins in a form effective for such purpose.

Green tea is known to contain significant amounts of four catechins. Their structures are shown in FIG. 1. In the preparation of black or fermented teas, these catechins are partially or totally oxidized as shown by Sanderson, USP 3,812,266. The oxidized catechins are much less effective antioxidants, so that green tea is the preferred source of catechins for the product of this invention.

Hara, in USP 4,840,966, describes the therapeutic use of catechins to reduce blood pressure. They are administered orally in the form of tablets or incorporated into the diet. Other health benefits of green tea catechins are known.

Catechins are very soluble in water, and many patents have issued pertaining to methods of extracting them to make instant tea. Examples of such patents include Mishkin, USP 3,451,823. He describes a method of first extracting the tea with hot water, which will recover the catechins, and then reextracting with highly superheated water under pressure, to degrade and solubilize other tea materials, which may also have antioxidant properties. These undefined substances, of unknown toxicology, are not included in the preferred form of this invention.

Mai, USP 4,839,187, describes a process for making the superheated water extract of Mishkin into a composition effective for the stabilization of lipids. His process starts with black tea, not green tea, and the superheating creates gallic acid, which he considers an important component of his extract. It is not desired in, and not created in, the product and process of this invention, since it is known to develop purple colors in the trace presence of metals. His extract is directly added to the fat to be stabilized in a solid form, or mixed with lecithin. He does not show or suggest any catechin particles of less than 38 microns on their largest dimension or a suspension of solid catechin particles in an oleogenous medium in which they are insoluble, or any method of obtaining such, which are major objects of this invention.

Although water extraction of the catechins is an acceptable method of separating the catechins from the tea, polar organic solvents, such as methanol and ethanol, may

be used. Hara, USP 4,673,530, describes such an extraction using aqueous alcoholic solutions. These aqueous alcoholic extracts complicate the preparation of the preferred form of catechins used in this invention, since it has been found that interfering water-soluble substances are also extracted. These interfering substances are desirably removed if an extract of Hara's type is used. The use of essentially anhydrous solvents is preferred in this invention. The separation of caffeine, which is also present in the aqueous and organic extracts, can be achieved by conventional methods as described in the Hara patent, employing chloroform, leaving the catechins in the water phase and the caffeine in the organic phase. Hara then recovers the catechins from the water using ethyl acetate, without any adjustment of pH, and this solvent is then removed to give a powder.

Alternatively, the caffeine may be removed from the tea leaves by supercritical carbon dioxide extraction, as is done in the preparation of decaffeinated tea leaves. However, the separation of caffeine from the catechins is not necessary or even preferred in this invention, since it does not interfere with the antioxidant activity of the catechins in the lipid. On the other hand, the elimination of the use of chloroform is an object of this invention.

Hara, in USP 4,613,672, describes an elegant method of preparation of purified individual catechins for use in pharmaceutical preparations. These products can be used as aqueous solutions, or as dried powders. He also shows that ethanolic solutions of these powders, when added to lipids, act as antioxidants.

Being insoluble in lipids and other non-polar solvents, concentrated solutions of catechins in vegetable oils cannot be prepared, and an aqueous solution will

simply separate from the lipid to be stabilized. Ethanolic solutions are unstable in use. Likewise, dry powders do not go into solution in oil even with prolonged heating at over 100° C. and agitation, and are inefficient as antioxidants.

OBJECTS OF THE INVENTION

It is an object of the present invention to provide solid antioxidant particles of tea catechins having a particle size of less than 38 microns on their largest dimension, preferably in the form of a suspension or dispersion of such particles in a lipid medium in which the tea catechins are insoluble, a method of producing the same, a method for stabilizing a fat, oil, fatty food, or fatty food ingredient therewith, and such stabilized products. A further object is to provide the foregoing wherein the lipid medium is an edible medium. Another object of the invention is the provision of such products embodying a non-ionic surface-active agent. An additional object is the provision of synergistic combinations of such tea catechin particles or suspension and a natural or synthetic antioxidant. Additional objects of the invention will become apparent hereinafter, and still other objects will be obvious to one skilled in the art to which this invention pertains.

THE INVENTION

Water-soluble and fat-insoluble polyphenolic antioxidants (catechins), recovered from green tea and reduced to a particle size less than 38 microns on their largest dimension, are effective antioxidants in lipid media such as fats, oils, foods, and ingredients of foods without imparting undesirable flavors, aromas, and precipitates,

preferably in the form of suspensions or dispersions in a lipid medium in which they are insoluble. Since it is known that tea polyphenols have positive effects on human health, the resulting stabilized lipids can be considered to have nutritional qualities superior to the same lipid stabilized with common synthetic antioxidants. Synergistic effects with other natural antioxidants and with phosphates are also disclosed.

The present invention provides a form of solid tea catechins which is completely dispersible in oil. Critical to this invention is that the particles be less than 38 microns in size on their largest dimension. It provides maximum utilization of the antioxidant properties of the catechins, even at ambient temperatures, which cannot be achieved using the powdered tea extracts of the present art in the stabilization of lipids.

Therefore, the preferred form of this invention utilizes the water-soluble and fat-insoluble constituents of the tea extract as a lipid antioxidant, and preferably discards the fat-soluble portion of the tea extract. In the less preferred form, the fat-soluble portion may be present, with or without chlorophyll.

The products of this invention are effective in stabilizing foods containing lipids, such as pie crusts, snacks, dressings, meats, pastries, and similar foods, as well as food ingredients such as breadings, flavorings, and colorings.

SUMMARY OF THE INVENTION

My invention then comprises, inter alia, the following, individually or in combination:

A solid green tea catechin which is less than 38 microns in size on its largest dimension, preferably

less than 10 microns on its largest dimension, such a catechin suspended in an oleogenous medium in which it is insoluble, such a

product wherein the medium is an edible medium, and any such catechin or product in combination with a tocopherol, a Labiatae extract, or solid ascorbic acid or phosphate particles of less than 38 micron size on their largest dimension, or a non-ionic surface active agent.

Further, a fat, oil, fatty food or food ingredient substrate stabilized against oxidation with any such catechin, product, or composition, such a

stabilized substrate wherein the substrate includes a carotenoid, and a

method of stabilizing a fat, oil, food, or food ingredient substrate which includes the step of combining the substrate with any such catechin, product, or composition as set forth in the foregoing, and such a

method wherein the substrate includes a carotenoid.

Moreover, a method of preparing solid tea catechin particles of less than 38 micron particle size on their largest dimension or suspensions thereof in a lipid medium in which they are insoluble, which consists essentially of the steps of

a. extracting green tea with a substantially anhydrous lower alkanol,

b. admixing the extract with water,

c. removing alcohol from the aqueous extract,

d. adding a water-immiscible solvent selected from the group consisting of lower-alkyl ketones, lower alkanols, and lower alkyl esters of lower-aliphatic acids, and adding a water-soluble salt, and adjusting the pH of the aqueous phase to a pH between 1 and 6, these latter two steps being conducted simultaneously or in either order,

e. removing the water-immiscible solvent - catechin solution from the aqueous phase and recovering the solid catechin particles therefrom, and

f. reducing the size of the solid catechin particles to less than 38 micron on their largest dimension, such a

method wherein the lower-alkanol employed in step a. is essentially anhydrous methanol, such a

method wherein the water-immiscible solvent employed in step d. is ethyl acetate, such a

method wherein step b. or c. is carried out in the presence of a hydrocarbon solvent and includes the further step of removing the hydrocarbon solvent and the lipids dissolved therein from the aqueous catechin solution, such a

method wherein the solid catechin particles from step f. are suspended in a lipid medium in which they are insoluble, such a

method wherein the lipid medium is an edible medium, such a

method wherein the lipid medium comprises a non-ionic surface-active agent, and such a

method wherein the surface-active agent is selected from lecithin, glycerol mono-oleate, liquid mono- and di-glycerides, acylated mono- and di-glycerides, benzyl alcohol, triacetin, caproic-caprylic acid polyglycerides, and polysorbate.

METHODOLOGY AND DEFINITIONS

An art accepted method of measuring the antioxidant activity of a substance employs the Rancimat™ to ascertain the induction time of a given lipid using a given dose of the antioxidant, generally with 18 liters of air per hour blowing through the fat held at a constant temperature selected for the specific fat. The Rancimat measures conductivity of an aqueous solution which captures the

volatile oxidation products formed as the fat oxidizes. The results are reported as the ratio of the induction time of the test sample to the control, the higher the ratio, the more stable the fat. The results correlate very well with other standard measures of rancidity development, such as the active oxygen method, organoleptic evaluations, and so forth.

Glossary of Terms

This glossary describes abbreviations and other technical terms and apparatus which may sometimes be referred to in one way or another in this specification.

Abbreviation	Technical Term
BHA	butylated hydroxy anisole
BHT	butylated hydroxy toluene
GMO	glycerol mono-oleate
SO	soy oil
SMO	sorbitan mono-oleate
STO	sorbitan trioleate
SMS	sorbitan monostearate
8-1-0	octaglycerol mono-oleate
10-1-CC	decaglycerol mono-capric-caprylate
RM	rosemary extract, especially Herbalox™ product of Kalsec, Inc., Kalamazoo, Michigan

Peroxide Value: This is also a standard test for evaluation of the degree to which an oil has been oxidized.

Labiatae Extract: The solvent extract of a Labiatae herb, and preferably rosemary, sage, or thyme, especially rosemary. The preferable form is that described in Todd USP 4,877,635, and standardized to an antioxidant strength of about twice that of BHT in soy oil, under the standard

Rancimat™ conditions. It is commercially available in the form of Herbalox™.

Rancimat™: An instrument which measures the induction time of an oleogenous substrate, usually at 120 degrees Celsius and at 18 liters of air per hour. This is an accepted methodology for determining relative strengths of preparations of antioxidants. The effectiveness is expressed as the induction time of the sample divided by the induction time of the control, as a percent.

Synergism: As defined in McGraw-Hill Dictionary of Scientific and Technical Terms: "An action where the total effect of two active components is greater than the sum of their individual effects."

Surface-Active Agent: In the context of this specification, it represents a nonionic surface-active agent, especially one taken from the class consisting of:

a. mono and di glycerides of fatty acids,

b. polyglyceride esters of fatty acids,

c. mono and diglyceride esters further esterified with a dibasic organic acid taken from the class consisting of citric, lactic, and tartaric acids,

d. acylated mono and diglyceride esters further esterified with a dibasic organic acid taken from the class consisting of citric, lactic, and tartaric acids,

e. sorbitan esters of fatty acids,

f. propylene glycol esters of fatty acids, and

g. lecithin, and equivalents thereof.

h. caproic-caprylic acid polyglycerides

RM Rosemary Extract: The extract used is Herbalox™, which is a commercial product available from Kalsec, Inc., standardized as to antioxidant activity, and comprising about 20% active antioxidant compounds.

DETAILED DESCRIPTION OF THE INVENTION

The following Examples are given by way of illustration only, and are not to be construed as limiting.

Example 1. Preparation of the preferred form of green tea extract to be used in the lipid antioxidant preparations.

(a) Dried green tea leaves are exhaustively extracted with methanol substantially free of water, preferably less than about 7% to 9%. This is important to the improved process for making the catechins used in this invention. Ethanol or other lower alkanols, which azeotrope with water, are not the preferred solvent, but may be employed. (b) Methanol is removed from the extract, following the addition of sufficient water during the distillation for the purpose of keeping the mass liquid. The extract thus made at this point, if both water and solvent were removed, would be about 30% to 40% catechins, 10% caffeine, and 20% or more fat-soluble substances and pigments, including chlorophyll. (c) The extract is partitioned between the aqueous phase and a hydrocarbon solvent which boils below 200° C., preferably hexane. (d) The solvent layer is removed, the aqueous layer again partitioned against the hydrocarbon solvent to remove traces of lipids, and again separated. (e) The pH of the water layer is then adjusted to an acidic pH between 1 and 6, preferably 2.5 to 4.5, and optimally 3 to 4, and a water-soluble salt, preferably a non-toxic salt such as sodium or potassium chloride, sodium citrate, or sodium sulfate, added to a concentration of at least 0.2%, optimally between 5% and 30%, W/W of the water to salt out the catechins. (f) The catechins are then extracted from the water phase using ethyl acetate or other water-immiscible solvent preferably selected from lower alkanols, lower alkyl ketones, and lower-alkyl lower-

aliphatic acid esters. (g) The ethyl acetate or other water-immiscible solvent solution is used as such, or desolventized to make a powder. These in turn are used to make the lipid antioxidant preparations of this invention. Steps (c) and (d) are essential only when all tea lipids are to be eliminated.

This general process was followed: 100 gms. of green tea was extracted with anhydrous methanol, enough water added to keep the mass liquid, methanol evaporated at a temperature below 80° C to give a thick liquid extract, 90ml of hexane added, the mixture agitated, the water-insoluble hexane phase separated from the water phase, the water phase again extracted with 30 ml of hexane, the hexane phase separated, 10 g of sodium chloride or other suitable salt added to the water layer and the pH adjusted to 3.5 with phosphoric acid, and the aqueous phase then extracted twice with 150 ml of ethyl acetate or other suitable water-immiscible solvent. The ethyl acetate was evaporated at a temperature below 80° C., yielding a dry solid catechin-rich fraction weighing 14.7 gms.

This preferred process differs from the prior art in requiring a substantially anhydrous lower alkanol, e.g., less than about 7% to 9% water being present in the alcoholic solvent, and most preferably less than 5%; the elimination of chloroform by the use of a hydrocarbon solvent, and criticality in adjusting the pH of the aqueous solution prior to ethyl acetate or other water-immiscible solvent extraction to between 1 and 6, and preferably 3 to 4, in the presence of a water-soluble salt for salting the catechins out of the aqueous phase. It goes without saying that the salt addition and pH adjustment can be carried out simultaneously or in either order.

While the foregoing is considered to be the preferred method of preparation of the water-soluble antioxidant fraction, variations suitable for specific equipment will be apparent to one skilled in the art. Although hexane is the preferred hydrocarbon solvent, other aliphatic hydrocarbons, such as heptane, and terpenes such as limonene, are acceptable.

Ethyl acetate can be replaced by other solvents which are immiscible with the aqueous phase, preferably selected from lower alkanols, lower-alkyl ketones, and lower-alkyl esters of lower-aliphatic acids, such as methyl ethyl ketone, acetone, butanol, and other lower aliphatic acid esters of lower alcohols such as isopropanol, e.g., isopropyl acetate, and the like.

Example 2. Preparation of less than 38 micron sized tea catechin solids in a medium in which they are insoluble.

45 g of catechins from the tea solid powder extract, prepared as in Example 1 above by evaporation of ethyl acetate from a solution thereof, were stirred into 270 g of soy oil and placed in a pebble mill. The mill was rolled for 72 hours, by which time the granular tea antioxidant particles were less than 38 microns in size on their largest dimension, and more than 60% less than 10 microns in size on their largest dimension. The homogeneous paste was separated from the pebbles, and was ready for use as such or further diluted with soy oil or other fat or fat-soluble solvent.

Example 3. Preparation of less than 38 micron sized tea catechin solids from a solution of catechins.

150 ml of an ethyl acetate solution containing 25 g catechins was added to 150 g of soy oil, and desolventized.

The desolventized product, containing lumps of catechins and liquid soy oil, was placed in a pebble mill and ground to less than 38 microns in size on the largest dimension of the catechin particles. It had the physical appearance of the product of Example 1.

While pebble milling is a preferred procedure for particle size reduction, since it does not overheat the solids during grinding, other methods of size reduction known to the art are acceptable. Other vegetable and animal oils and fats are as suitable as soy bean oil for suspending the particles of catechins, as well as lipid-soluble non-ionic surface-active agents (See Example 7), for the catechin particles are insoluble in all of these lipids or materials.

It should be noted that my copending application Serial No. 07/717,926, filed June 20, 1991 and now allowed, and my published PCT application WO 93/00015 published January 7, 1993, and the following Preparations as well as my copending application Kseco 31, filed concurrently herewith, respectively show that reduction to less than 38 micron particle size on their largest dimension makes solid ascorbic acid particles and solid phosphate particles effective antioxidants in media in which they are insoluble, whereas they are ineffective in such media when present as larger particles.

* * *

Preparation. Preparation of a suspension or dispersion of less than 38 micron sized phosphate particles in a medium in which they are insoluble.

(a) 318 g of sodium acid pyrophosphate and 1270 g of vegetable oil were added to a pebble mill and ground for 24 hours. The size of the particles in the dispersion was less than 38 microns on their largest dimension. A portion

was withdrawn, and grinding continued until the particles were less than 10 microns on their largest dimension. While essentially all of the particles need to be less than 38 microns in size for this invention to be effective, it is preferred that they be less than 10 microns in size.

(b) The same procedure was used employing an approximately equal mixture of sodium acid pyrophosphate, sodium polyphosphates, and sodium ortho and metaphosphates, with the same results.

(c) A granular sodium acid pyrophosphate was ground in a mortar and pestle, and the powder sieved through a screen to separate particles less than 38 microns in size.

Potassium salts of the phosphates can be substituted for the sodium salts if sodium reduction is an objective.

The above products are representative food-grade phosphates of less than 38 micron particle size on their largest dimension which are effective antioxidants in fats, oils, fatty foods, and ingredients of foods, especially when employed in the form of a suspension in an edible oleogenous medium such as the vegetable oil employed in the foregoing (a).

* * *

Example 4. Use of the Product of Example 1 in lard.

0.5% W/W of the less than 38 micron particle size catechins was added to melted lard, which became hazy. The lard was cooled, and the solid lard had a tan cast but otherwise appeared similar to the original lard. When used as a frying fat in an iron skillet, it did not discolor eggs to a purple cast, as would be expected for gallates. 135 ppm catechins were present in the lard.

The thus stabilized lard was compared for oxidative stability with unstabilized lard, and with lard to which the unground catechin powder of Example 1(g) was added at

the same level of 50 ppm. This comparison utilized the art-accepted Rancimat technique, at an oil temperature of 110° C. The results are shown in Table I.

TABLE I. The effect of reducing catechin solids to less than 38 microns upon their antioxidant strength.

	Induction Time	% increase
Lard	1.95	
Lard + solid catechins powder	5.03	158%
Lard + <38 micron catechins	15.8	710%

Since the temperature of 110° C should dissolve the catechin powder in the oil over a five-hour period, while being vigorously agitated in the oil with air, if it is to dissolve at all, it is clear that little of the catechin powder dissolved and became effective in the oil. At ambient temperature, the powder is simply worthless, whereas the <38 micron sized catechins are highly effective. This demonstrates that it is essential that the particles be reduced to less than 38 microns in size. A further improvement occurs when they are essentially less than 10 microns in size, but reduction to this extent becomes an economic judgment and is not essential.

Example 5. Synergistic mixtures of the <38 micron catechins with other natural antioxidants.

The synergism of the <38 micron size catechins with ascorbic acid is demonstrated by admixing known amounts of each, and their combination, with the lard. The synergism is expressed as the % increase in induction time over the induction time increase if the antioxidants are used by themselves. The results are shown in Table II.

TABLE II. Synergism between catechins and ascorbic acid.

	Induction Time, hours	Increase in Time, hours	% synergism
a. lard	1.95		
b. lard + 50 ppm <38 micron catechins	15.8	13.85	
c. lard + 600 ppm <38 micron ascorbic acid	5.0	3.05	
b+c catechins + ascorbic	21.0	19.05	14

Similarly synergistic combinations with other antioxidants, and especially rosemary and other Labiatae extracts, tocopherols, and solid phosphate particles of less than 38 microns in size, are attainable in the same manner as will be apparent to one skilled in the art.

Thus, additional unexpectedly powerful multiple synergistic effects can be obtained by combining the suspension of <38 micron catechins in vegetable oil with ascorbic acid and phosphates (both of less than 38 microns in size on their greatest diameter) suspended in vegetable oil, Herbalox™, tocopherols, and lecithin. For example, a mixture of 10% microcrystalline catechins (2.5 g), Herbalox™ (3.0 g), tocopherols (2.5 g), 20% W/W microcrystalline phosphates in vegetable oil (5.0 g), and 15% W/W microcrystalline ascorbic acid in vegetable oil (5.0 g) was added to lard at a dose of 0.18% W/W. The increase in induction time, calculated by adding the increase in induction time which would occur if each constituent was added alone, was increased more than 250%, resulting in synergism greater than 150% when the experiment was discontinued.

When lecithin is added to the mixture of antioxidants on an equal weight basis, and the dose in the lard adjusted to reduce the amount of each antioxidant by one-half, the percent synergism is further increased.

Example 6. The effectiveness of the <38 micron catechins in vegetable oils.

When added to coconut oil at a level of 50 ppm catechins, the induction time increases from 6.94 hours to 16.4 hours. In canola oil, which is more readily oxidized, the induction time goes from 3.57 to 5.19 hours at the same dose level. While responses of soy, corn, safflower, and other vegetable oils vary, no vegetable oil has been found in which the <38 micron size particles are not effective antioxidants.

As in the case of animal fats, including marine oils, the development of rancid aromas is inhibited by the <38 micron tea catechins, even when they are added at ambient or lower temperatures and the fat refrigerated.

Example 7. Mixtures of <38 micron sized catechins with non-ionic surface-active agents.

The generally-preferred method of preparation of the catechins is shown in Example 1, and their size reduction in Example 2. In some uses it may be desirable to incorporate a lipid-soluble, non-ionic surface-active agent in the preparation. This can be done before or after grinding. Suitable surface-active agents are lecithin, mono- and di-glycerides, acylated mono- and diglycerides, caproic-caprylic acid polyglycerides, and tartaric acid esters of mono- and diglycerides, as well as polysorbates. The above list is not limiting, but rather preferred.

The teachings of this invention are particularly useful in stabilizing extruded animal foods and snacks, in which a fat is applied to the surface. The enhancement of synergism by the employment of a non-ionic surface-active agent is useful in such applications, as well as in doughs such as frozen pie crusts.

It is clear from the foregoing that the microcrystalline catechins of this invention improve the stability of lipids in broad classes of foods, including snacks, nuts, dressings, breadings, and meats, as well as flavorings such as essential oils and colorings such as those containing oxidizable carotenoids.

Example 8. Stabilization of a carotenoid and food ingredient and food.

A 10% W/W suspension of tea catechins prepared according to Example 1 was mixed with oleoresin paprika at the level of 1.5% to provide 0.1% W/W of tea catechins in the oleoresin. The oleoresin without catechins, and the oleoresin to which they had been added, were plated on dextrose and the dispersions then placed in an oven maintained at 65° C. The oleoresin without catechins lost one-third of its color in 28 hours, whereas that containing the catechins took 72 hours to lose the same amount of its carotenoid color.

Since one hour at 65° C results in approximately as much color loss as one day at ambient temperature, the shelf life is more than doubled, from 28 days to 72 days, a significant commercial improvement, particularly since so little of the green tea catechins are required.

It should be observed that oleoresin paprika contains alpha and beta carotene, lutein, zeaxanthin, capsanthin, and other carotenoids such as apo-carotenals in minor amounts. Therefore the invention will stabilize oxygenated carotenoids as well as hydrocarbons such as beta-carotene.

A dispersion of oleoresin paprika is a common food ingredient, being used in meats, baked goods, breadings, snacks, dressings, and the like, making it desirable to stabilize the color in order to have uniformity of color in

the food. Because of the ability of the catechins to inhibit the development of rancidity in fats and oils, it is particularly desirable to utilize the thus-stabilized carotenoids in an oil medium, for the purpose of introducing the stabilizing effect of the catechins into the food.

In Summary, the present application discloses the preparation of a novel form of solid tea catechins, which are unexpectedly powerful antioxidants in fats and oils in which they are insoluble. Critical to this effectiveness is the reduction of particle size to less than 38 microns. Synergism when used in combination with other antioxidants has been shown. Further, enhancement of the synergism by the incorporation of non-ionic surface-active agents in the catechin suspension is demonstrated. Other general and specific utility of the products and method of the present invention will be apparent to those practicing the art of food stabilization.

* * *

It is thus seen that the present invention provides a novel and advantageous form of solid antioxidant tea catechin particles of less than 38 microns on their largest dimension and antioxidant suspensions or dispersions thereof in an oleogenous or lipidic medium in which they are insoluble, such products having increased antioxidant activity in fats, oils, carotenoids, and fatty foods and food ingredients, especially such materials and products as are exposed to oxidative stress, as well as a method of stabilizing such materials and products against oxidative discoloration, including foods, feeds, and foodstuffs which may encounter oxidative stress stabilized with a product or composition of the invention, and a method of stabilizing a food, feed, foodstuff, flavoring, or coloring with such

a more effective form of catechin antioxidant product or composition of the invention. The stabilization of carotenoid pigments may thus advantageously be carried out. Synergistic effects are obtained by the incorporation of a natural antioxidant in or with such products or compositions, methods, and stabilized products, and the antioxidant effectiveness of the catechin antioxidant, antioxidant compositions, and methods of the invention is further enhanced by inclusion of a natural Labiatae antioxidant, less than 38 micron ascorbic acid or phosphate particles, a tocopherol, or even a synthetic antioxidant such as BHT or BHA therein or therewith, and the antioxidant power and stabilization effectiveness of a composition of the invention may be even further improved by inclusion of a non-ionic surface-active agent therein or therewith. All of the foregoing provide long-awaited solutions to previously-existing oxidation and instability problems not adequately solved by the prior art.

It is to be understood that the invention is not to be limited to the exact details of operation, or to the exact compositions, methods, procedures, or embodiments shown and described, as obvious modifications and equivalents will be apparent to one skilled in the art, and the invention is therefore to be limited only by the full scope which can be legally accorded to the appended claims.

I claim:

- 1 -

An antioxidant composition consisting essentially of solid green tea catechin particles which are less than 38 microns in size on their largest dimension, suspended in an oleogenous medium in which they are insoluble.

- 2 -

A catechin composition of Claim 1, wherein the size of the particles is less than 10 microns on their largest dimension.

- 3 -

A product of Claim 1 wherein the medium is an edible medium.

- 4 -

A product of Claim 2 wherein the medium is an edible medium.

- 5 -

A product of Claim 3 in combination with a tocopherol, a Labiatae extract, or solid ascorbic acid or phosphate particles of less than 38 micron size on their largest dimension, or a non-ionic surface-active agent.

- 6 -

A fat, oil, fatty food or food ingredient substrate stabilized against oxidation with solid green tea catechin particles of less than 38 microns in size on their largest dimension or a composition of any of Claims 1 through 5.

- 7 -

A stabilized substrate of Claim 6 wherein the solid green tea catechin particles are less than 10 microns on their largest dimension.

- 8 -

A stabilized substrate of Claim 6 wherein the substrate includes a carotenoid.

- 9 -

A method of stabilizing a fat, oil, food, or food ingredient substrate which includes the step of combining the substrate with solid green tea catechin particles of less than 38 microns in size on their largest dimension or a composition of any of Claims 1 through 5.

- 10 -

A method of Claim 9 wherein the solid green tea catechin particles are less than 10 microns on their largest dimension.

- 11 -

A method of Claim 9 wherein the substrate includes a carotenoid.

- 12 -

The method of preparing an antioxidant composition of solid tea catechin particles of less than 38 micron particle size on their largest dimension or suspensions thereof in a lipid medium in which they are insoluble, which consists essentially of the steps of

a. extracting green tea with a substantially anhydrous lower alkanol,

b. admixing the extract with water,

c. removing alcohol from the aqueous extract,

d. adding a water-immiscible solvent selected from the group consisting of lower-alkyl ketones, lower alkanols, and lower alkyl esters of lower-aliphatic acids, and

adding a water-soluble salt, and adjusting the pH of the aqueous phase to a pH between 1 and 6, these latter two steps being conducted simultaneously or in either order,

e. removing the water-immiscible solvent - catechin solution from the aqueous phase and recovering the solid catechin particles therefrom, and

f. reducing the size of the solid catechin particles to less than 38 micron on their largest dimension, and

g. suspending the solid catechin particles from step f. in a lipid medium in which they are insoluble.

- 13 -

The method of Claim 12 wherein the lower-alkanol employed in step a. is essentially anhydrous methanol.

- 14 -

The method of Claim 12 wherein the water-immiscible solvent employed in step d. is ethyl acetate.

- 15 -

The method of Claim 12 wherein step b. or c. is carried out in the presence of a hydrocarbon solvent and includes the further step of removing the hydrocarbon solvent and the lipids dissolved therein from the aqueous catechin solution.

- 16 -

The method of Claim 12 wherein the lipid medium is an edible medium.

- 17 -

The method of Claim 16 wherein the lipid medium comprises a non-ionic surface-active agent.

- 18 -

The method of Claim 17 wherein the surface-active agent is selected from lecithin, glycerol mono-oleate, liquid mono- and di-glycerides, acylated mono- and di-glycerides, benzyl alcohol, triacetin, caproic-caprylic acid polyglycerides, and polysorbate.

AMENDED CLAIMS

[received by the International Bureau on 6 September 1994 (06.09.94) ; original claims 1-18 replaced by amended claims 1-13 (3 pages)]

- 1 -

An edible nontoxic antioxidant composition for use in a fat, oil, fatty food or food ingredient substrate, containing an effective antioxidant amount of solid green tea catechin particles which are less than 38 microns in size on their largest dimension, suspended in an edible oleaginous medium in which they are insoluble.

- 2 -

A catechin composition of Claim 1, wherein the size of the particles is less than 10 microns on their largest dimension.

- 3 -

A product of Claim 1 or Claim 2 in combination with a tocopherol, a Labiatae extract, or solid ascorbic acid or phosphate particles of less than 38 micron size on their largest dimension, or a non-ionic surface-active agent.

- 4 -

A fat, oil, fatty food or food ingredient substrate stabilized against oxidation with an effective antioxidant amount of solid green tea catechin particles which are less than 38 microns in size on their largest dimension, either per se or suspended in an edible oleaginous medium in which they are insoluble.

- 5 -

A stabilized substrate of Claim 4 wherein the substrate includes a carotenoid.

- 6 -

A fat, oil, fatty food or food ingredient substrate stabilized against oxidation with an effective antioxidant amount of solid green tea catechin particles which are less than 38 microns in size on their largest dimension, either per se or suspended in an edible oleaginous medium in which they are insoluble, in combination with a tocopherol, or a Labiatae extract, or solid ascorbic acid or phosphate particles of less than 38 micron size on their largest dimension, or a non-ionic surface-active agent.

- 7 -

A stabilized substrate of Claim 6 wherein the substrate includes a carotenoid.

- 8 -

A method of stabilizing a fat, oil, fatty food or food ingredient substrate which includes the step of combining the substrate with an effective antioxidant amount of solid green tea catechin particles which are less than 38 microns in size on their largest dimension, either per se or suspended in an edible oleaginous medium in which they are insoluble.

- 9 -

The method of Claim 8 wherein the substrate includes a carotenoid.

- 10 -

The method of stabilizing a fat, oil, fatty food or food ingredient substrate which includes the step of combining the substrate with an effective antioxidant amount of solid green tea catechin particles which are less than 38 microns in size on their largest dimension, either

per se or suspended in an edible oleaginous medium in which they are insoluble, in combination with a tocopherol, or a Labiatae extract, or solid ascorbic acid or phosphate particles of less than 38 micron size on their largest dimension, or a non-ionic surface-active agent.

- 11 -

The method of Claim 10 wherein the substrate includes a carotenoid.

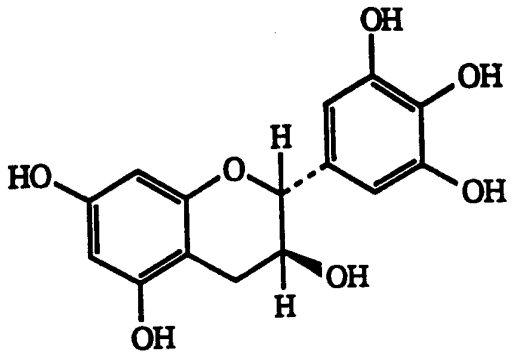
- 12 -

A product of Claim 6 wherein the non-ionic surface-active agent is selected from glycerol mono-oleate, liquid mono- and di-glycerides, acylated mono- and di-glycerides, benzyl alcohol, triacetin, caproic-caprylic acid poly-glycerides, and polysorbate.

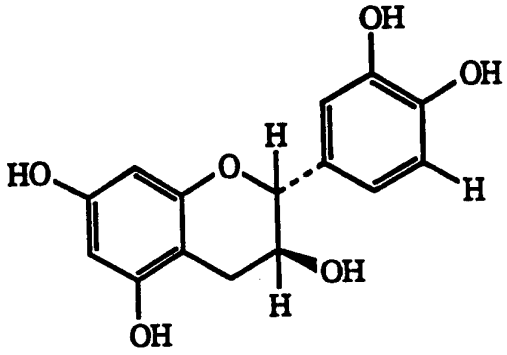
- 13 -

A method of Claim 10, wherein the non-ionic surface active agent is selected from glycerol mono-oleate, liquid mono- and di-glycerides, acylated mono- and di-glycerides, benzyl alcohol, triacetin, caproic-caprylic acid polyglycerides, and polysorbate.

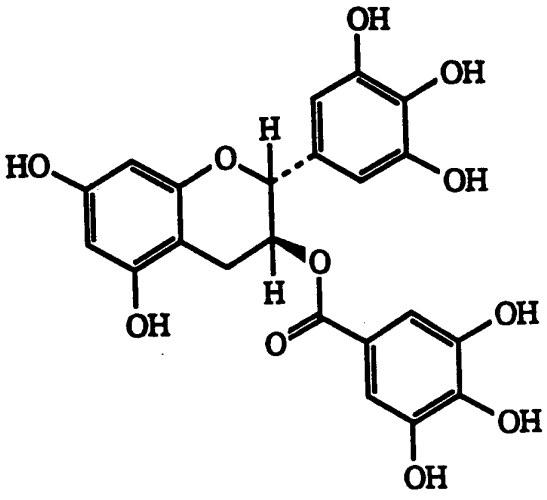
FIG. 1



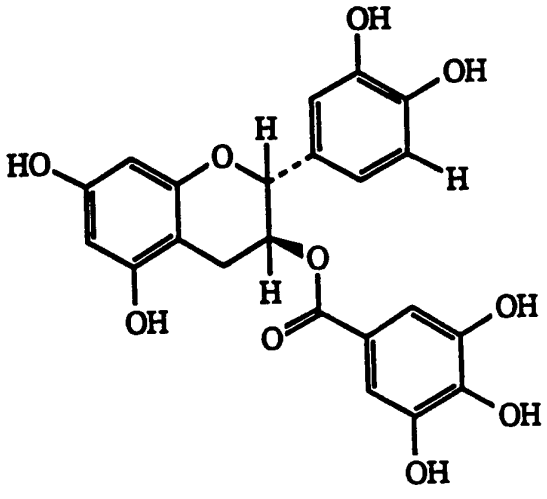
Epigallocatechin (EGC)



Epicatechin (EC)



Epigallocatechingallate (EGCG)



Epicatechingallate (ECG)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US94/03505

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :A23D 9/06; A23L 3/3481
US CL :426/542, 545

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 426/542, 545

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US, A, 4,515,804 (MARTI ET AL.) 07 MAY 1985. SEE COL. 12, LINES 61-66; COL. 18, LINES 36-44; COL. 24, LINES 64-68.	1-18
Y	US, A, 4,613,672 (HARA) 27 SEPTEMBER 1986. SEE COL. 1, LINES 15-61; COL. 3, LINES 44-47, COL. 6, LINES 30-52; COL. 7, LINES 5-32	1-18
Y	US, A, 4,673,530 (HARA) 16 JUNE 1987. SEE THE ABSTRACT AND COL. 1, LINES 30-45.	1-18

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 22 JUNE 1994	Date of mailing of the international search report JUL 07 1994
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