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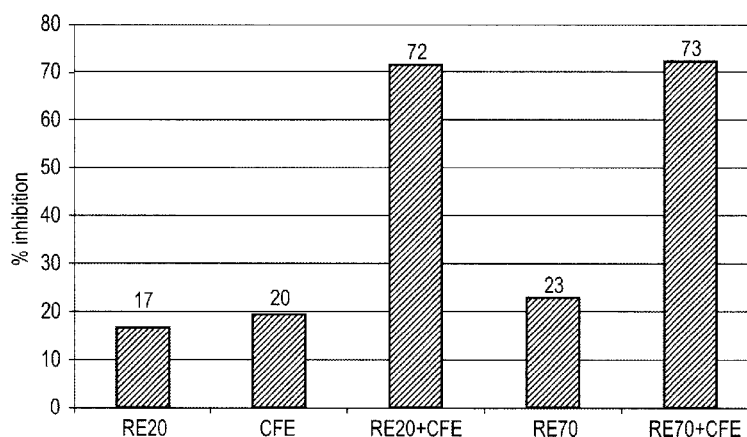
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[Continued on next page]

(54) Title: ANTIOXIDANT COMPOSITION



Percent inhibition of 2,4-heptadienal of five treatments (RE20, CFE, RE20+CFE, RE70, RE70+CFE) compared to unprotected control (CTRL) on the storage time, day 28 (D28).

FIG. 6

(57) Abstract: The present invention provides an anti-oxidant composition comprising (a) an extract obtained from or obtainable from a plant of the Labiatae family comprising carnosic acid and carnosol, and (b) an extract obtained from or obtainable from a plant of the genus Caralluma comprising pregnane glycosides.

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**ANTIOXIDANT COMPOSITION**

The present invention relates to a composition that exhibits an anti-oxidant action.

5 **Background**

Antioxidants are widely used in food products susceptible to oxidative degeneration. An antioxidant is defined by the Food and Drug Administration (21CFR 170.3) as "a substance used to preserve food by retarding deterioration, rancidity, or discoloration due to oxidation". There is an increasing need to develop economical, natural and effective food preservative systems to meet the public demand for convenient, natural, safe, healthy, good quality food products with guaranteed shelf life. To this end spices or plant extracts can be used in food as antioxidants and to impart flavour. One advantage of such extracts is that they are perceived as natural ingredients when compared to chemical antioxidants such as ethylenediaminetetraacetic acid (EDTA), butyl hydroxyanisol (BHA) and butylated hydroxytoluene (BHT).

There are large number of antioxidants known based on naturally occurring plant materials. It is noted that these materials have varying degrees of efficacy. Moreover, the antioxidant levels required to ensure preservation safety may prove uneconomical, or are above levels acceptable due to regulatory and legislation constraints when present in amounts sufficient to offer the required protection.

The present invention alleviates the problems of the prior art.

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In one aspect the present invention provides an anti-oxidant composition comprising (a) an extract comprising phenolic diterpenes obtained from a plant of the *Labiatae* family; and

(b) an extract comprising pregnane glycoside obtained from a plant of the genus *Caralluma*,

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in amounts to provide a synergistic effect.

In one aspect the present invention provides an emulsion containing an anti-oxidant composition comprising

(a) an extract comprising phenolic diterpenes obtained from a plant of the *Labiatae* family; and

35

(b) an extract comprising pregnane glycoside obtained from a plant of the genus *Caralluma*,

in amounts to provide a synergistic effect.

5 In one aspect the present invention provides a foodstuff containing an anti-oxidant composition comprising

(a) an extract comprising phenolic diterpenes obtained from a plant of the *Labiatae* family,

(b) an extract comprising pregnane glycoside obtained from a plant of the genus  
10 *Caralluma*,

in amounts to provide a synergistic effect.

In one aspect the present invention provides a process for preventing and/or inhibiting oxidation of a foodstuff, the process comprising the step of contacting the foodstuff with

15 (a) an extract comprising phenolic diterpenes obtained from a plant of the *Labiatae* family, optionally a plant of the species *Rosmarinus officinalis*, *Salvia officinalis* or mixtures thereof; and

(b) an extract comprising pregnane glycoside obtained from a plant of the genus *Caralluma*, optionally a plant of the species *Caralluma fimbriata*

20 in amounts to provide a synergistic effect.

In one aspect the present invention provides use of

(a) an extract comprising phenolic diterpenes obtained from a plant of the *Labiatae* family, optionally from a plant of the species *Rosmarinus officinalis*, *Salvia officinalis* or  
25 mixtures thereof; and

(b) an extract comprising pregnane glycoside obtained from a plant of the genus *Caralluma*, optionally a plant of the species *Caralluma fimbriata*,

in amounts to provide a synergistic effect;

for preventing and/or inhibiting oxidation of an emulsion or a foodstuff.

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In one aspect the present invention provides a kit for preparing a composition as defined herein, the kit comprising

(a) an extract comprising phenolic diterpenes obtained from a plant of the *Labiatae* family, optionally from a plant of the species *Rosmarinus officinalis*, *Salvia officinalis* or  
35 mixtures thereof; and

2a

- (b) an extract comprising pregnane glycoside obtained from a plant of the genus *Caralluma*, optionally a plant of the species *Caralluma fimbriata*,  
in amounts to provide a synergistic effect;  
in separate packages or containers;
- 5 optionally with instructions for admixture and/or contacting and/or use.

Aspects of the invention are defined in the appended claims.

- 10 The present invention provides a synergistic combination of components for preventing and/or inhibiting oxidation in a material, such as foodstuff. This combination of components allows lower levels of the antioxidants to be used to provide effective action. This is particularly important in food applications where reduction of dosage is desired for commercial and regulatory reasons.

It will be understood by one skilled in the art that by the term 'antioxidant' it is meant a substance which reduces the amount of oxidation over a given period when compared to the oxidation that would occur in the absence of that substance or it is meant a material  
5 which increase the time required for a given amount of oxidation to occur when compared to the oxidation that would occur in the absence of that substance.

Plants of the family *Labiatae* contain several well-known herbs. Extracts from these plants have been shown to have antioxidant and, in some cases, antimicrobial activity  
10 (Nychas & Skandamis, 2003; Smid and Gorris, 1999; Loliger, 1989). Such extracts may be essential oils and oleoresins (extracts with essential oil content used in flavours and fragrances) or "deodorised", extracts that have a high phenolic diterpene content and low level of flavour-inducing compounds.

15 Essential oils are extracted by simple steam distillation of the plant material. The most effective antioxidant compounds in rosemary and sage are reported to be carnosic acid, carnosol and rosmarinic acid (Cuvelier *et al.* 1996). Carnosic acid, a phenolic diterpene ( $C_{20}H_{28}O_4$ ), occurs naturally in leaves of plants of the *Labiatae* family, particularly rosemary and sage, but also thyme and marjoram. Dried leaves of rosemary or sage  
20 contain 1.5 – 2.5% carnosic acid and 0.3 – 0.4% carnosol (US6231896). Carnosol is an oxidative artefact of carnosic acid (Wenkert *et al.* J. Org. Chem 30:2931, 1965). The oxidation takes place in the presence of harvesting in the leaves left to dry in the air and if the leaves are subjected to extraction with solvents. Rosmanol may also be a product of the oxidation of carnosic acid.

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Of the *Labiatae* plant family, rosemary and sage have antioxidant activity in foods that is mainly related to phenolic diterpenes such as carnosic acid and carnosol, as well as other phenolic compounds, including phenolic triterpenes such as betulinic acid, oleanolic acid and ursolic acid; and rosmarinic acid. The phenolic diterpenes, phenolic  
30 triterpenes and rosmarinic acid are distinct from the essential oils and oleoresins that are often used in flavours and fragrances. The high flavour and odour levels of essential oils are not conducive to their use in food.

Plants of the genus *Caralluma*, such as *Caralluma fimbriata*, are also known to have  
35 antioxidant activity. The key phytochemical constituents of plants such as *Caralluma*

*fimbriata* are reported to be pregnane glycosides, flavone glycosides, megastigmane glycosides, and saponins.

One skilled in the art would expect a combination of an extract from the *Labiatae* plant family and an extract from a plant of the genus *Caralluma*, to provide a simple additive antioxidant effect. However, studies described herein have demonstrated synergistic enhancement of antioxidant activity.

For ease of reference, these and further aspects of the present invention are now discussed under appropriate section headings. However, the teachings under each section are not necessarily limited to each particular section.

## PREFERRED ASPECTS

### 15 LABIATAE EXTRACT

As discussed herein one extract used in the present invention is obtained from or is obtainable from a plant of the *Labiatae* family.

20 In one aspect the extract used in the present invention is obtained from a plant of the *Labiatae* family.

It will be appreciated by one skilled in the art that by the term "extract" or "extracts" it is meant any constituent of the plant which may be isolated from the whole plant.

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In one aspect the extract used in the present invention is obtainable from a plant of the *Labiatae* family. It will be appreciated by one skilled in the art that an extract obtainable from a plant may be obtained from a plant or may be isolated from the plant, identified and then obtained from an alternative source, for example by chemical synthesis or enzymatic production. For example the extract may be produced by a eukaryotic or prokaryotic fermentation, by a process of genetic manipulation. The present applicants have recognised that products present in a plant of the *Labiatae* family may synergistically increase the activity of antioxidant material obtained or obtainable from a plant of the genus *Caralluma*. These products may be obtained from any source and will fall within the scope of the present invention.

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The invention comprises use of a combination of an extract from a plant of the *Labiatae* family, such as rosemary (*Rosmarinus officinalis*) and antioxidant material obtained or obtainable from a plant of the genus *Caralluma*, that together give antioxidant activity in a food system. The extracts responsible for synergy in the present invention preferably refer to extracts of the plant family *Labiatae* that have been selectively extracted ("deodorised extracts") to increase their phenolic diterpene content (such as carnosic acid). These deodorised extracts can be distinguished by their high phenolic diterpene content (for example greater than 3.5 wt.%) and their low level (less than 1 wt.%) of flavour-inducing compounds from plant essential oils and oleoresins that are used as flavours or fragrances. Essential oils are typically extracted by simple steam distillation of the plant material.

Essential oils comprise the various essential oils in plants having the odour or the flavour of the plant from which they were extracted. The essential oils are typically terpenoids often comprising monoterpenes. For example an antioxidant type of rosemary extract, which could be described as selectively extracted or deodorised, contains > 3.5% wt.% phenolic diterpenes but less than 1 wt.% essential oils. A non-selective, flavouring extract contains 10–30 wt.% essential oils and a phenolic diterpene content of 2–>3.5wt.%.

An essential oil is commonly described as the volatile ethereal fraction obtained from a plant or plant part by a physical separation process such as distillation or chromatographic separation. Essential oils have also been described as a "group of odorous principles, soluble in alcohol and to a limited extent in water, consisting of a mixtures of esters, aldehydes, ketones and terpenes. Essential oils are typically obtained by distilling plants with water; the oil that separates from distillate usually has highly characteristic odors identified with the plant origin. The resulting mixture of organic compounds was thought, in the days of alchemists, to be the essence of the plant, hence the term "essential oil".

In one preferred aspect the extract is a deodorised extract. Preferably the (deodorised) extract contains from 1.0 to 70 wt.% phenolic diterpenes, preferably 3.5 to 70 wt.% phenolic diterpenes and less than 1 wt.% essential oil. In one aspect the extract obtained from or obtainable from a plant of the *Labiatae* family contains phenolic diterpenes in an

amount of at least 1wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 95 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 90 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 85 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 70 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 50 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 30 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 20 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 15 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 10 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family.

In one preferred aspect the extract is or comprises a phenolic diterpene. Preferably the phenolic diterpene is carnosic acid.

In one aspect the extract obtained from or obtainable from a plant of the *Labiatae* family contains carnosic acid in an amount of at least 1wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 95 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 90 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 85 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 70 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 50 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, , such as in an amount of 1 to 40 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family such as in an amount of 1 to 30 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 25 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 20 wt% based on the weight of extract obtained from or obtainable from a plant of

the *Labiatae* family, such as in an amount of 1 to 10 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family, such as in an amount of 1 to 5 wt% based on the weight of extract obtained from or obtainable from a plant of the *Labiatae* family.

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In one preferred aspect the extract contains flavour-inducing compounds and/or essential oils in an amount of less than 2 wt.% based on the extract. In one preferred aspect the extract contains flavour-inducing compounds and/or essential oils in an amount of less than 1 wt.% based on the extract. In one preferred aspect the extract contains flavour-inducing compounds and/or essential oils in an amount of less than 2 wt.% based on the composition. In one preferred aspect the extract contains flavour-inducing compounds and/or essential oils in an amount of less than 1 wt.% based on the composition.

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Typically flavour-inducing compounds and/or essential oils are camphor, verbenone, borneol and alfa-terpineol.

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In one preferred aspect the combined amount of camphor present in the extract is less than 2 wt.% (preferably less than 1 wt.%, more preferably less than 0.5 wt.%), based on the extract.

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In one preferred aspect the combined amount of verbenone present in the extract is less than 2 wt.% (preferably less than 1 wt.%, more preferably less than 0.5 wt.%), based on the extract.

25

In one preferred aspect the combined amount of borneol present in the extract is less than 2 wt.% (preferably less than 1 wt.%, more preferably less than 0.5 wt.%) based on the extract.

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In one preferred aspect the combined amount of alfa-terpineol present in the extract is less than 2 wt.% (preferably less than 1 wt.%, more preferably less than 0.5 wt.%) based on the extract.

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In one preferred aspect the combined amount of camphor, verbenone, borneol and alfa-terpineol present in the extract is less than 2 wt.% (preferably less than 1.0 wt.%), based on the extract.

In one preferred aspect the extract contain less than 2 wt.% of plant essential oils and/or oleoresins based on the extract. In one preferred aspect the extract contain less than 1 wt.% of plant essential oils and/or oleoresins based on the extract. In one preferred aspect the extract contain less than 2 wt.% of plant essential oils and/or oleoresins based on the composition. In one preferred aspect the extract contain less than 1 wt.% of plant essential oils and/or oleoresins based on the composition.

In one preferred aspect the extract contains essential oils in an amount of less than 2 wt.% based on the extract. In one preferred aspect the extract contains essential oils in an amount of less than 1 wt.% based on the extract. In one preferred aspect the extract contains essential oils in an amount of less than 2 wt.% based on the composition. In one preferred aspect the extract contains essential oils in an amount of less than 1 wt.% based on the composition.

In one preferred aspect the plant of the *Labiatae* family is selected from rosemary, sage, oregano, marjoram, mint, balm, savoury and thyme. In one preferred aspect the plant of the *Labiatae* family is selected from rosemary, sage, oregano, marjoram, mint, balm, and savoury. It will be understood that these name cover all species and varieties of plants known by these names.

In one preferred aspect the plant of the *Labiatae* family is selected from rosemary (*Rosmarinus officinalis* L.), sage (*Salvia officinalis* L.) oregano (*Origanum vulgare* L.), marjoram (*Origanum marjorana* L.), mint (*Mentha* spp.), balm (*Melissa officinalis* L.), savoury (*Satureia hortensis*), thyme (*Thymus vulgaris* L.).

In one preferred aspect the plant of the *Labiatae* family is selected from a plant of the species *Rosmarinus officinalis*, *Salvia officinalis* and mixtures thereof.

In one preferred aspect the plant of the *Labiatae* family is rosemary (*Rosmarinus officinalis*).

In one preferred aspect the composition comprises (a) an extract obtained from or obtainable from a plant of the species *Rosmarinus officinalis*.

In one preferred aspect the composition comprises (a) an extract obtained from a plant of the *Labiatae* family.

5 In one preferred aspect the composition comprises (a) an extract obtained from a plant of the species *Rosmarinus officinalis*, *Salvia officinalis* or mixtures thereof.

In one preferred aspect the composition comprises (a) an extract obtained from a plant of the species *Rosmarinus officinalis*.

10 In a further preferred aspect the phenolic diterpenes, phenolic triterpenes and rosmarinic acid are obtained by chemical synthesis.

Thus in one aspect the present invention provides

- 15 • an anti-oxidant composition comprising (a) carnosic acid, (b) an extract obtained from or obtainable from a plant of the genus *Caralluma*.
- a process for preventing and/or inhibiting oxidation of a foodstuff, the process comprising the step of contacting the foodstuff with (a) carnosic acid, and (b) an extract obtained from or obtainable from a plant of the *genus Caralluma*.
- 20 • use of (a) carnosic acid, and (b) an extract obtained from or obtainable from a plant of the *genus Caralluma*, for preventing and/or inhibiting oxidation of a foodstuff.
- a kit for preparing a composition as defined herein, the kit comprising (a) carnosic acid, and (b) an extract obtained from or obtainable from a plant of the *genus Caralluma*, in separate packages or containers; optionally with instructions for admixture and/or contacting and/or use.
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### CARALLUMA EXTRACT

30 As discussed herein one extract used in the present invention is obtained from or is obtainable from a plant of the *genus Caralluma*.

In one aspect extract (b) is from a plant selected from plants of the species *Caralluma indica*, *Caralluma attenuata*, *Caralluma tuberculata*, *Caralluma edulis*, *Caralluma adscendes*, *Caralluma stalagmifera*, *Caralluma umbellate*, *Caralluma penicillata*,  
35 *Caralluma russeliana*, *Caralluma retrospiciens*, *Caralluma arabica*, *Caralluma lasiantha*,

*Caralluma burchardii*, *Caralluma crenulata* Wall., *Caralluma dummeri*, *Caralluma europaea*, *Caralluma joannis* Maire, *Caralluma somalica*, *Caralluma speciosa* and *Caralluma fimbriata*. In one preferred aspect extract (b) is from a plant of the species *Caralluma fimbriata*.

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In one preferred aspect the composition comprises (b) an extract obtained from a plant of the genus *Caralluma*.

In one preferred aspect the composition comprises (b) an extract obtained from or  
10 obtainable from a plant of the species *Caralluma fimbriata*.

In one preferred aspect the composition comprises (b) an extract obtained from a plant of the species *Caralluma fimbriata*.

15 It will be appreciated by one skilled in the art that by the term "extract" or "extracts" it is meant any constituent of the plant which may be isolated from the whole plant.

In one aspect the extract used in the present invention is obtainable from a plant of the  
20 *genus Caralluma*. It will be appreciated by one skilled in the art that an extract obtainable from a plant may be obtained from a plant or may be isolated from the plant, identified and then obtained from an alternative source, for example by chemical synthesis or enzymatic production. For example the extract may be produced by a eukaryotic or prokaryotic fermentation, by a process of genetic manipulation. The present applicants have recognised that products present in a plant of the  
25 *genus Caralluma* may synergistically increase the activity of antioxidant material obtained or obtainable from a plant of the *Labiatae* family. These products may be obtained from any source and will fall within the scope of the present invention.

The invention comprises use of a combination of an extract from a plant of the *genus*  
30 *Caralluma*, such as *Caralluma fimbriata* and antioxidant material obtained or obtainable from a plant of the *Labiatae* family, that together give antioxidant activity in a food system.

In one aspect the extract from a plant of the *genus Caralluma*, such as *Caralluma*  
35 *fimbriata* is or comprises a glycoside. In one aspect the extract from a plant of the *genus*

*Caralluma*, such as *Caralluma fimbriata* is a glycoside. In one aspect the glycoside is a pregnane glycoside.

In one aspect the extract obtained from or obtainable from a plant of the genus  
5 *Caralluma* contains pregnane glycosides in an amount of at least 1wt% based on the  
weight of extract obtained from or obtainable from the plant of the genus *Caralluma* or of  
the genus *Caralluma*, such as in an amount of at least 2wt% based on the weight of  
extract obtained from or obtainable from the plant of the genus *Caralluma* or of the genus  
10 *Caralluma*, such as in an amount of at least 5wt% based on the weight of extract  
obtained from or obtainable from the plant of the genus *Caralluma* or of the genus  
*Caralluma*, such as in an amount of at least 10wt% based on the weight of extract  
obtained from or obtainable from the plant of the genus *Caralluma* or of the genus  
*Caralluma*, such as in an amount of at least 20wt% based on the weight of extract  
15 *Caralluma*, such as in an amount of at least 30wt% based on the weight of extract  
obtained from or obtainable from the plant of the genus *Caralluma* or of the genus  
*Caralluma*, such as in an amount of 10 to 70 wt% based on the weight of extract  
obtained from or obtainable from the plant of the genus *Caralluma* or of the genus  
*Caralluma*, such as in an amount of 10 to 60 wt% based on the weight of extract  
20 *Caralluma*, such as in an amount of 20 to 60 wt% based on the weight of extract  
obtained from or obtainable from the plant of the genus *Caralluma* or of the genus  
*Caralluma*, such as in an amount of 20 to 50 wt% based on the weight of extract  
obtained from or obtainable from the plant of the genus *Caralluma* or of the genus  
25 *Caralluma*, such as in an amount of 30 to 50 wt% based on the weight of extract  
obtained from or obtainable from the plant of the genus *Caralluma* or of the genus  
*Caralluma*, such as in an amount of 30 to 40 wt% based on the weight of extract  
obtained from or obtainable from the plant of the genus *Caralluma* or of the genus  
*Caralluma*.

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In one aspect the pregnane glycosides are obtained by chemical synthesis.

Thus in highly preferred aspects the present invention provides

- an anti-oxidant composition comprising (a) an extract obtained from or obtainable  
35 from a plant of the *Labiatae* family, (b) pregnane glycosides

- a process for preventing and/or inhibiting oxidation of a foodstuff, the process comprising the step of contacting the foodstuff with (a) an extract obtained from or obtainable from a plant of the *Labiatae* family, and (b) pregnane glycosides.
- use of (a) an extract obtained from or obtainable from a plant of the *Labiatae* family, and (b) pregnane glycosides, for preventing and/or inhibiting oxidation of a foodstuff.
- a kit for preparing a composition as defined herein, the kit comprising (a) an extract obtained from or obtainable from a plant of the *Labiatae* family, and (b) pregnane glycosides, in separate packages or containers; optionally with instructions for admixture and/or contacting and/or use.

### COMPOSITION

It will be understood that the components of the composition utilised in the present invention may be present in any amount to provide an antioxidant effect and in particular (a) extract obtained from or obtainable from a plant of the *Labiatae* family and (b) extract obtained from or obtainable from a plant of the *genus Caralluma*, are present in amounts to provide a synergistic anti-oxidant effect.

In one aspect the ratio of (a) extract obtained from or obtainable from a plant of the *Labiatae* family, to (b) extract obtained from or obtainable from a plant of the *genus Caralluma*, is from 30:1 to 1:30, such as 30:1 to 1:1, such as 1:1 to 1:30, such as 25:1 to 1:25, such as 25:1 to 1:1, such as 1:1 to 1:25, such as 20:1 to 1:20, such as 20:1 to 1:1, such as 1:1 to 1:20, such as 15:1 to 1:15, such as 15:1 to 1:1, such as 1:1 to 1:15, such as 10:1 to 1:10, such as 10:1 to 1:1, such as 1:1 to 1:10, such as 5:1 to 1:5, such as 5:1 to 1:1, such as 1:1 to 1:5, such as 4:1 to 1:4, such as 4:1 to 1:1, such as 1:1 to 1:4. In a preferred aspect the ratio of (a) extract obtained from or obtainable from a plant of the *Labiatae* family, to (b) extract obtained from or obtainable from a plant of the *genus Caralluma*, is from 4:1 to 1:10, such as 4:1 to 1:8, such as 4:1 to 1:5 such as 4:1 to 1:4.

In a preferred aspect the ratio of (a) extract obtained from or obtainable from a plant of the *Labiatae* family, to (b) extract obtained from or obtainable from a plant of the *genus Caralluma*, is from 2:1 to 1:10, such as 2:1 to 1:8, such as 2:1 to 1:5 such as 2:1 to 1:4.

In a preferred aspect the ratio of (a) extract obtained from or obtainable from a plant of the *Labiatae* family, to (b) extract obtained from or obtainable from a plant of the *genus Caralluma*, is from 1:1 to 1:10, such as 1:1 to 1:8, such as 1:1 to 1:5 such as 1:1 to 1:4.



In a preferred aspect the ratio of (a) extract obtained from or obtainable from a plant of the *Labiatae* family, to (b) extract obtained from or obtainable from a plant of the *genus Caralluma*, is from 4:1 to 1:4, such as 2:1 to 1:4, such as 1:1 to 1:4.

5 In one aspect the ratio of (a) active anti-oxidant ingredient obtained from or obtainable from a plant of the *Labiatae* family, to (b) extract obtained from or obtainable from a plant of the *genus Caralluma*, is from 30:1 to 1:30, such as 30:1 to 1:1, such as 1:1 to 1:30, such as 25:1 to 1:25, such as 25:1 to 1:1, such as 1:1 to 1:25, such as 20:1 to 1:20, such as 20:1 to 1:1, such as 1:1 to 1:20, such as 15:1 to 1:15, such as 15:1 to 1:1, such as 1:1 to 1:15, such as 10:1 to 1:10, such as 10:1 to 1:1, such as 1:1 to 1:10, such as 5:1 to 1:5, such as 5:1 to 1:1, such as 1:1 to 1:5, such as 1:2 to 1:5. In a preferred aspect the ratio of (a) active anti-oxidant ingredient obtained from or obtainable from a plant of the *Labiatae* family, to (b) extract obtained from or obtainable from a plant of the *genus Caralluma*, is from 1:1 to 1:10, such as 1:1 to 1:8, such as 1:1 to 1:5 such as 1:2 to 1:5.

15

In one aspect the ratio of (a) active anti-oxidant ingredient obtained from or obtainable from a plant of the *Labiatae* family, to (b) active anti-oxidant ingredient obtained from or obtainable from a plant of the *genus Caralluma*, is from 20:1 to 1:20, such as 15:1 to 1:15, such as 10:1 to 1:10, such as 5:1 to 1:5, such as 4:1 to 1:4, such as 3:1 to 1:3, such as 2:1 to 1:3, such as 1:1 to 1:3.

20

In one aspect the ratio of (a) phenolic diterpene, to (b) pregnane glycosides, is from 20:1 to 1:20, such as 15:1 to 1:15, such as 10:1 to 1:10, such as 5:1 to 1:5, such as 4:1 to 1:4, such as 3:1 to 1:3, such as 2:1 to 1:3, such as 1:1 to 1:3.

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## APPLICATIONS

The antioxidant composition may be utilised in any application in which inhibition of oxidation is required. As discussed herein, usage in foodstuffs is found to be particularly advantageous. In one aspect the present invention therefore provides

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- a process for preventing and/or inhibiting oxidation of a material, the process comprising the step of contacting the material with
  - (a) an extract obtained from or obtainable from a plant of the *Labiatae* family, and
  - (b) an extract obtained from or obtainable from a plant of the *genus Caralluma*.

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- use of

- (a) an extract obtained from or obtainable from a plant of the *Labiatae* family, and
- (b) an extract obtained from or obtainable from a plant of the *genus Caralluma*,  
for preventing and/or inhibiting oxidation of a material.

5 The antioxidant composition can particularly be incorporated into an emulsion. Thus there is provided an emulsion comprising an anti-oxidant composition as described herein. The emulsion may be, for example, a foodstuff or a cosmetic. Preferably the emulsion is a foodstuff.

10 In a further aspect there is provided a foodstuff comprising an anti-oxidant composition as described herein.

### **FOODSTUFF**

15 The composition, process and use of the present invention may prevent and/or inhibit oxidation in any material. However, in view of the problems associated with oxidation of foodstuffs and in view of the particular effectiveness of the present invention in foodstuffs, preferably the composition is a foodstuff or may be added to a foodstuff. It will be appreciated by one skilled in the art that when the present composition is a  
20 foodstuff the essential components of (a) an extract obtained from or obtainable from a plant of the *Labiatae* family and (b) extract from a plant of the *genus Caralluma* must be present in the foodstuff. They may have been provided by one or more means. For example they may have been added in the form of a composition containing the extracts. The components may have been added to the foodstuff sequentially.

25

In one aspect the composition of the present invention is an antioxidant composition suitable for addition to a foodstuff.

Many foodstuffs may be protected by the present invention. Typical foodstuffs are raw  
30 meat, cooked meat, raw poultry products, cooked poultry products, raw seafood products, cooked seafood products, ready to eat meals, pasta sauces, pasteurised soups, mayonnaise, salad dressings, oil-in-water emulsions, margarines, low fat spreads, water-in-oil emulsions, dairy products, cheese spreads, processed cheese, dairy desserts, flavoured milks, cream, fermented milk products, cheese, butter,  
35 condensed milk products, ice cream mixes, soya products, pasteurised liquid egg,

bakery products, confectionery products, fruit products, and foods with fat-based or water-containing fillings. Preferably the foodstuff is mayonnaise.

In one aspect the present composition is dosed in a foodstuff in an amount to provide the  
5 extract obtained from or obtainable from a plant of the genus *Labiatae* in an amount of  
no greater than 5000ppm based on the weight of the foodstuff, such as no greater than  
4000ppm, such as no greater than 3000ppm, such as no greater than 2000 ppm, such  
as no greater than 1000 ppm, such as no greater than 700 ppm, such as no greater than  
500 ppm, such as no greater than 400 ppm, such as no greater than 300 ppm such as  
10 no greater than 200 ppm, such as no greater than 150 ppm, such as no greater than 100  
ppm, such as no greater than 75 ppm, such as no greater than 50 ppm, such as no  
greater than 40 ppm, such as from 1 to 5000ppm, such as from 1 to 4000ppm, such as  
from 1 to 3000ppm, such as from 10 to 3000ppm, such as from 1 to 2000 ppm, such as  
from 1 to 1000 ppm, such as from 1 to 700 ppm, such as from 1 to 500 ppm, such as  
15 from 1 to 400 ppm, such as from 1 to 300 ppm such as from 1 to 200 ppm, such as 1 to  
150 ppm, such as 1 to 100 ppm, such as from 1 to 75 ppm, such as 1 to 50 ppm, such as  
1 to 40 ppm, such as 10 to 300 ppm, such as 10 to 150 ppm, such as 10 to 100 ppm,  
such as 10 to 50 ppm, such as 20 to 50 ppm based on the weight of the foodstuff.

20 In one aspect the present composition is dosed in a foodstuff in an amount to provide  
active anti-oxidant ingredient obtained from or obtainable from a plant of the genus  
*Labiatae* in an amount of no greater than 1000ppm based on the weight of the foodstuff,  
such as no greater than 700 ppm, such as no greater than 500 ppm, such as no greater  
than 400 ppm, such as no greater than 300 ppm such as no greater than 200 ppm, such  
25 as no greater than 150 ppm, such as no greater than 100 ppm, such as no greater than  
75 ppm, such as no greater than 50 ppm, such as no greater than 40 ppm, such as from  
1 to 1000 ppm, such as from 1 to 700 ppm, such as from 1 to 500 ppm, such as from 1 to  
400 ppm, such as from 1 to 300 ppm such as from 1 to 200 ppm, such as 1 to 150 ppm,  
such as 1 to 100 ppm, such as from 1 to 75 ppm, such as 1 to 50 ppm, such as 1 to 40  
30 ppm, such as 10 to 300 ppm, such as 10 to 150 ppm, such as 10 to 100 ppm, such as 10  
to 50 ppm, such as 20 to 50 ppm based on the weight of the foodstuff.

In one aspect the present composition is dosed in a foodstuff in an amount to provide  
phenolic diterpene obtained from or obtainable from a plant of the genus *Labiatae* in an  
35 amount of no greater than 1000ppm based on the weight of the foodstuff, such as no

greater than 700 ppm, such as no greater than 500 ppm, such as no greater than 400 ppm, such as no greater than 300 ppm such as no greater than 200 ppm, such as no greater than 150 ppm, such as no greater than 100 ppm, such as no greater than 75 ppm, such as no greater than 50 ppm, such as no greater than 40 ppm, such as from 1 to 1000 ppm, such as from 1 to 700 ppm, such as from 1 to 500 ppm, such as from 1 to 400 ppm, such as from 1 to 300 ppm such as from 1 to 200 ppm, such as 1 to 150 ppm, such as 1 to 100 ppm, such as from 1 to 75 ppm, such as 1 to 50 ppm, such as 1 to 40 ppm, such as 10 to 300 ppm, such as 10 to 150 ppm, such as 10 to 100 ppm, such as 10 to 50 ppm, such as 20 to 50 ppm based on the weight of the foodstuff.

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In one aspect the present composition is dosed in a foodstuff in an amount to provide carnosic acid in an amount of no greater than 1000ppm based on the weight of the foodstuff, such as no greater than 700 ppm, such as no greater than 500 ppm, such as no greater than 400 ppm, such as no greater than 300 ppm such as no greater than 200 ppm, such as no greater than 150 ppm, such as no greater than 100 ppm, such as no greater than 75 ppm, such as no greater than 50 ppm, such as no greater than 40 ppm, such as from 1 to 1000 ppm, such as from 1 to 700 ppm, such as from 1 to 500 ppm, such as from 1 to 400 ppm, such as from 1 to 300 ppm such as from 1 to 200 ppm, such as 1 to 150 ppm, such as 1 to 100 ppm, such as from 1 to 75 ppm, such as 1 to 50 ppm, such as 1 to 40 ppm, such as 10 to 300 ppm, such as 10 to 150 ppm, such as 10 to 100 ppm, such as 10 to 50 ppm, such as 20 to 50 ppm based on the weight of the foodstuff.

In one aspect the present composition is dosed in a foodstuff in an amount to provide the extract obtained from or obtainable from a plant of the genus *Caralluma* in an amount of no greater than 5000ppm based on the weight of the foodstuff, such as no greater than 4000ppm, such as no greater than 3000ppm, such as no greater than 2000 ppm, such as no greater than 1000 ppm, such as no greater than 700 ppm, such as no greater than 500 ppm, such as no greater than 400 ppm, such as no greater than 300 ppm such as no greater than 200 ppm, such as no greater than 150 ppm, such as no greater than 100 ppm, such as no greater than 75 ppm, such as no greater than 50 ppm, such as no greater than 40 ppm, such as from 1 to 5000ppm, such as from 1 to 4000ppm, such as from 1 to 3000ppm, such as from 1 to 3000ppm, such as from 1 to 2000 ppm, such as from 1 to 1000 ppm, such as from 1 to 700 ppm, such as from 1 to 500 ppm, such as from 1 to 400 ppm, such as from 1 to 300 ppm such as from 1 to 200 ppm, such as 10 to 200 ppm, such as 20 to 200 ppm, such as from 30 to 200 ppm, such as 40 to 200 ppm,

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such as 50 to 200 ppm, such as 50 to 150 ppm based on the weight of the foodstuff.

In one aspect the present composition is dosed in a foodstuff in an amount to provide active anti-oxidant ingredient obtained from or obtainable from a plant of the genus  
5 *Caralluma* in an amount of no greater than 1000ppm based on the weight of the foodstuff, such as no greater than 700 ppm, such as no greater than 500 ppm, such as no greater than 400 ppm, such as no greater than 300 ppm such as no greater than 200 ppm, such as no greater than 150 ppm, such as no greater than 100 ppm, such as no greater than 75 ppm, such as no greater than 50 ppm, such as no greater than 40 ppm,  
10 such as from 1 to 1000 ppm, such as from 1 to 700 ppm, such as from 1 to 500 ppm, such as from 1 to 400 ppm, such as from 1 to 300 ppm such as from 1 to 200 ppm, such as 1 to 150 ppm, such as 1 to 100 ppm, such as from 1 to 75 ppm, such as 1 to 50 ppm, such as 1 to 40 ppm, such as 10 to 300 ppm, such as 10 to 150 ppm, such as 10 to 100 ppm, such as 10 to 50 ppm, such as 20 to 50 ppm based on the weight of the foodstuff.

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In one aspect the present composition is dosed in a foodstuff in an amount to provide glycosides obtained from or obtainable from a plant of the genus *Caralluma* in an amount of no greater than 1000ppm based on the weight of the foodstuff, such as no greater than 700 ppm, such as no greater than 500 ppm, such as no greater than 400 ppm, such  
20 as no greater than 300 ppm such as no greater than 200 ppm, such as no greater than 150 ppm, such as no greater than 100 ppm, such as no greater than 75 ppm, such as no greater than 50 ppm, such as no greater than 40 ppm, such as from 1 to 1000 ppm, such as from 1 to 700 ppm, such as from 1 to 500 ppm, such as from 1 to 400 ppm, such as from 1 to 300 ppm such as from 1 to 200 ppm, such as 1 to 150 ppm, such as 1 to 100  
25 ppm, such as from 1 to 75 ppm, such as 1 to 50 ppm, such as 1 to 40 ppm, such as 10 to 300 ppm, such as 10 to 150 ppm, such as 10 to 100 ppm, such as 10 to 50 ppm, such as 20 to 50 ppm based on the weight of the foodstuff.

In one aspect the present composition is dosed in a foodstuff in an amount to provide  
30 pregnane glycosides in an amount of no greater than 1000ppm based on the weight of the foodstuff, such as no greater than 700 ppm, such as no greater than 500 ppm, such as no greater than 400 ppm, such as no greater than 300 ppm such as no greater than 200 ppm, such as no greater than 150 ppm, such as no greater than 100 ppm, such as no greater than 75 ppm, such as no greater than 50 ppm, such as no greater than 40  
35 ppm, such as from 1 to 1000 ppm, such as from 1 to 700 ppm, such as from 1 to 500

ppm, such as from 1 to 400 ppm, such as from 1 to 300 ppm such as from 1 to 200 ppm, such as 1 to 150 ppm, such as 1 to 100 ppm, such as from 1 to 75 ppm, such as 1 to 50 ppm, such as 1 to 40 ppm, such as 10 to 300 ppm, such as 10 to 150 ppm, such as 10 to 100 ppm, such as 10 to 50 ppm, such as 20 to 50 ppm based on the weight of the  
5 foodstuff.

In one aspect the composition of the present invention is an antioxidant composition suitable for addition to a pet food, i.e. a food suitable for consumption by a domesticated animal. The composition may be applied on, or in, the pet food itself and/or constituent(s)  
10 (e.g. ingredients) of the pet food. For example, the composition may be applied on, or in, a palatant.

### ADDITIONAL COMPONENTS

15 The composition of the present invention or the composition for use in the present invention may contain one or more additional components. However, in some aspects the antioxidant composition of the present invention (suitable for addition to a foodstuff) contains no additional components or contains no additional components that materially affect the properties of the composition. In these aspects the present invention provides

- 20 • an anti-oxidant composition consisting essentially of or consisting of (a) an extract obtained from or obtainable from a plant of the *Labiatae* family, (b) an extract obtained from or obtainable from a plant of the *genus Caralluma*.
- a process for preventing and/or inhibiting oxidation of a foodstuff, the process comprising the step of contacting the foodstuff with a composition consisting  
25 essentially of or consisting of (a) an extract obtained from or obtainable from a plant of the *Labiatae* family, and (b) an extract obtained from or obtainable from a plant of the *genus Caralluma*.
- use of a composition consisting essentially of or consisting of (a) an extract obtained from or obtainable from a plant of the *Labiatae* family, and (b) an extract  
30 obtained from or obtainable from a plant of the *genus Caralluma*, for preventing and/or inhibiting oxidation of a foodstuff.
- a kit for preparing a composition as defined herein, the kit consisting essentially of or consisting of (a) an extract obtained from or obtainable from a plant of the *Labiatae* family, and (b) an extract obtained from or obtainable from a plant of the  
35 *genus Caralluma*, in separate packages or containers; optionally with instructions

for admixture and/or contacting and/or use.

In one preferred aspect the composition further comprises a carrier. Preferably the carrier is selected from propylene glycol, maltodextrin, sugar, salt, ethanol, water, protein, glycerol, medium chain triglyceride (MCT oil), and vegetable oil. It will be understood by one skilled in the art that the components of the present invention, that is (a) the extract obtained from or obtainable from a plant of the *Labiatae* family, (b) the extract obtained from or obtainable from a plant of the genus *Caralluma*, may be blended with the carrier or carriers in any suitable manner. For example, the components of the present invention may be simply mixed with a suitable carrier or carriers.

In one preferred aspect the composition further comprises an emulsifier. Preferably the emulsifier is selected from polyoxyethylene sorbitan esters (polysorbates), polyoxyethylene stearate, mono- and diglycerides of fatty acids, mono- and diglycerides esters further esterified with a dibasic organic acid selected from acetic acid, lactic acid, citric acid and mono- and diacetyl tartaric acid or mixtures thereof, lecithin, polyglycerol esters of fatty acids, polyglycerol polyricinoleate, sucrose esters of fatty acids, sucroglycerides, propylene glycol esters of fatty acids, sorbitan esters of fatty acids, sodium and calcium salt of stearyl-2-lactylate, sodium, potassium, calcium and magnesium salts of fatty acids and ammonium phosphatides.

## **PROCESS**

As discussed herein in one aspect the present invention provides a process for preventing and/or inhibiting oxidation of a foodstuff, the process comprising the step of contacting the foodstuff with a composition comprising (a) an extract obtained from or obtainable from a plant of the *Labiatae* family, and (b) an extract obtained from or obtainable from a plant of the genus *Caralluma*.

In one aspect the extract obtained from or obtainable from a plant of the *Labiatae* family, and the extract obtained from or obtainable from a plant of the genus *Caralluma* are added to the foodstuff together.

In one aspect the extract obtained from or obtainable from a plant of the *Labiatae* family, and the extract obtained from or obtainable from a plant of the *genus Caralluma* are added to the foodstuff sequentially.

5 Thus the present invention provides in one aspect an antioxidant composition which may be added to a range of materials such as food systems and in another aspect a combination of two separate products which may added sequentially to materials such as food products.

10 The present invention will now be described in further detail by way of example only with reference to the accompanying figures in which:-

Figure 1 shows a graph;

Figure 2 shows a graph;

15 Figure 3 shows a graph;

Figure 4 shows a graph;

Figure 5 shows a graph;

Figure 6 shows a graph;

Figure 7 shows a graph; and

20 Figure 8 shows graphs.

The present invention will now be described in further detail in the following example.

### **EXAMPLE**

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In the present example a two-component antioxidant mixture of *Caralluma fimbriata* extract (CFE) and *Rosmarinus officinalis* extract (RE) was investigated in mayonnaise with 80 wt% oil. CFE and RE (two qualities; RE20 and RE70) as well as two-component mixtures (RE20+CFE and RE70+CFE) were tested in mayonnaise manufactured by a  
30 FrymaKoruma batch process.

The dosage was based on total product mayonnaise weight. The dosage of rosemary extracts (76.3ppm RE20 or 27.7ppm RE70) were equivalent to 20ppm total of carnosic acid and carnosol (that is the two rosemary extracts provided equivalent antioxidant  
35 activity). The dosage of *Caralluma fimbriata* extract (CFE) was 100ppm. The experiment



was repeated the day after, that is duplicate experiments of all treatments were carried out.

The combination of CFE and RE was more effective than the individual components and a significant synergistic interaction between CFE and RE was demonstrated by statistical analysis of oxidation parameters (oxygen in sample headspace, conjugated diene hydroperoxides, 2,4-heptadienal and 2,4-decadienal).

The fact that CFE and RE interacts synergistically is novel and provides the following range of functional and economic benefits compared to present commercial natural solutions: high antioxidant activity (increased product shelf-life and consumer satisfaction), low cost-in-use, low impact on taste and low discoloration.

### Antioxidant materials

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**Rosemary extract 70%** was an extract from the leaves of *Rosmarinus officinalis* L. Material reference E030377-107 available from DuPont Nutrition and Biosciences ApS, Denmark.

**Rosemary extract 20%** was an extract from the leaves of *Rosmarinus officinalis* L. Material reference DK12-00776 available from DuPont Nutrition and Biosciences ApS, Denmark.

Contents of the carnosic acid and carnosol were analysed in the rosemary extracts (g/100g extract) by high pressure liquid chromatography (HPLC) according to Thorsen & Hildebrandt (2003) and shown in table 1.

Table 1: Contents (wt%) of carnosic acid and carnosol

Product	Material reference	Carnosic acid (wt%)	Carnosol (wt%)	Total (wt%)
Rosemary extract 70%	E030377-107	70.1	2.3	72.4
Rosemary extract 20%	DK12-00776	20.0	6.2	26.2

**Caralluma fimbriata extract** was an extract from *Caralluma fimbriata* containing min. 30 wt% pregnane glycosides (36 wt% pregnane glycosides determined by gravimetric

30

method). Material reference E070143-148 available from DuPont Nutrition and Biosciences ApS, Denmark.

### EXPERIMENTAL PROCEDURE

5

The following dosage of *Caralluma fimbriata* extract (CFE) and rosemary extract (two rosemary extract qualities; RE20 and RE70) and combinations (RE20+CFE and RE70+CFE) were added to a typical 80% mayonnaise formulation. Mentioned dosages were based on total product weight. The experiment was repeated the day after, i.e. duplicate experiments of all treatments were carried out (table 2).

10

Table 2: Antioxidant treatments and abbreviations used in this report

ID	Sample	Antioxidant treatments
	<i>Trial A</i>	
172A	CTRL	Control, nothing added
173A	RE20	76.3ppm Rosemary extract 20% (RE20)
174A	CFE	100ppm <i>Caralluma fimbriata</i> extract (CFE)
175A	RE20 + CFE	76.3ppm Rosemary extract 20% (RE20) + 100ppm <i>Caralluma fimbriata</i> extract (CFE)
177A	RE70	27.7ppm Rosemary extract 70% (RE70)
178A	RE70 + CFE	27.7ppm Rosemary extract 70% (RE70) + 100ppm <i>Caralluma fimbriata</i> extract (CFE)
	<i>Trial B</i>	
172B	CTRL	Control, nothing added
173B	RE20	76.3ppm Rosemary extract 20% (RE20)
174B	CFE	100ppm <i>Caralluma fimbriata</i> extract (CFE)
175B	RE20 + CFE	76.3ppm Rosemary extract 20% (RE20) + 100ppm <i>Caralluma fimbriata</i> extract (CFE)
177B	RE70	27.7ppm Rosemary extract 70% (RE70)
178B	RE70 + CFE	27.7ppm Rosemary extract 70% (RE70) + 100ppm <i>Caralluma fimbriata</i> extract (CFE)

With respect to the rosemary extracts, additions of RE20 and RE70 were equivalent to 20ppm of carnosic acid plus carnosol (i.e. the two rosemary extracts had equivalent

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antioxidant activity, as shown in table 3). For CFE a dosage of 100ppm extract was added to the mayonnaise equivalent to 36ppm pregnane glycosides (table 4).

Table 3: Dosage of rosemary components (carnosic acid + carnosol)

Material	Material reference	Dosage of extract based on total mayonnaise weight	Content of carnosic acid + carnosol in extract	Dosage of carnosic acid + carnosol in experiment based on total mayonnaise weight
Rosemary extract 70%	E030377-107	27.7ppm	72.4 wt%	20ppm
Rosemary extract 20%	DK12-00776	76.3ppm	26.2 wt%	20ppm

5

Table 4: Dosage of *Caralluma* components (pregnane glycosides)

Material	Material reference	Dosage of extract based on total mayonnaise weight	Content of pregnane glycosides in extract	Dosage of pregnane glycosides in experiment based on total mayonnaise weight
<i>Caralluma fimbriata</i> extract	E070143-148	100ppm	36 wt%	36ppm

The formulation and process conditions for production of 9kg batches are outlined in table 5 and in paragraphs below. CFE, RE20 were added as dry blends together with the dry ingredients. Due to high ethanol and vegetable oil solubility of RE70, this material was pre-dissolved in ethanol and added to oil phase.

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Table 5: Mayonnaise formulation. Grams of ingredients used for production of 9 kg batches on manufacturing day one (trial A) and day two (trial B).

Batch no. & sample ID	172A+B CTRL	173A+B RE20	174A+B CFE	175A+B RE20+CFE	177A+B RE70	178A+B RE70+CFE
Canola oil	7200.00	7200.00	7200.00	7200.00	7200.00	7200.00
Tap water	906.25	905.56	905.35	904.66	906.00	905.10
Salt (sodium chloride)	63.00	63.00	63.00	63.00	63.00	63.00
Sucrose	90.00	90.00	90.00	90.00	90.00	90.00
Potassium sorbate	9.00	9.00	9.00	9.00	9.00	9.00
GRINDSTED® FF 5105	6.75	6.75	6.75	6.75	6.75	6.75
Egg yolk (pasteurised)	405.00	405.00	405.00	405.00	405.00	405.00
Vinegar 10%	270.00	270.00	270.00	270.00	270.00	270.00
Mustard	45.00	45.00	45.00	45.00	45.00	45.00
96% ethanol (mL)	1.000	1.000	1.000	1.000	1.000	1.000
Rosemary extract 70%					0.249	0.249
Maltodextrin	4.000	4.000	4.000	4.000	4.000	4.000
Rosemary extract 20%		0.687		0.687		
Caralluma fimbriata extract			0.900	0.900		0.900
Batch size (gram)	9000.00	9000.00	9000.00	9000.00	9000.00	9000.00

### Process

- 5 1. Dissolve sodium chloride, sugar, potassium sorbate and powder antioxidant blend (if added) in  $\frac{3}{4}$  parts of the water in FrymaKoruma Disho A15 mixer (Romaco FrymaKoruma, Germany), while mixing at 3000 rpm and stirring at 60 rpm in a vacuum of 500 mbar for 1 minute.

2. Make a slurry of GRINDSTED® FF 5105 and approx. 1/100 parts of the canola oil (including pre-dissolved RE70 – if added), and pump the slurry into the water phase at 3000 rpm in a vacuum of 500 mbar and continue mixing for 1 minute.
3. Add egg yolk and the rest of the water, while mixing at 3000 rpm and stirring at 60  
5 rpm in a vacuum of 500 mbar and continue mixing for 3 minutes.
4. Emulsify the rest of the canola oil at 3500rpm and continue mixing for 2 minutes.
5. Add vinegar and mustard while mixing at 3500 rpm and stirring at 60 rpm in a vacuum of 300 mbar for 1 minute.
6. Finally, mixing speed was decreased to 2500 rpm and stirring at 60 rpm in a vacuum  
10 of 300 mbar and held for 30 seconds before each batch before filling (at temperature 25°C).

#### **Filling, storage and sampling**

The mayonnaise was filled into Duma® special containers (Gerresheimer AG, 150 ml HD  
15 Polyethylene - caps LD Polyethylene) with 50g in each and placed at 20°C in a temperature controlled heating cabinet. These containers were used for determination of GC volatiles (2,4-heptadienal and 2,4-decadienal) and conjugated dienes. At each sampling day one container was opened. The mayonnaise sample was manually stirred with spatula for 30 seconds. Two centrifuge tubes (12ml) were filled with 8-10g sample  
20 and stored at minus 18°C (to break emulsion and to halt oxidation until time of analysis). The remaining mayonnaise in Duma® containers was used for sensory analysis on the sampling day.

Further 50g mayonnaise was filled into one glass bottle (volume 115ml) and  
25 subsequently closed with a Teflon® cap. This bottle was placed at 20°C in a temperature controlled heating cabinet and residual oxygen was monitored during storage (repeated analysis of the same bottle).

#### **METHODS**

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##### **Phenolic diterpenes in rosemary extract**

The contents of the phenolic diterpenes (carnosol and carnosic acid) were analysed in rosemary extracts by high pressure liquid chromatography (HPLC) according to Thorsen & Hildebrandt (2003).

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**Peroxide value of canola oil**

The peroxide value was determined for the canola oil by potentiometric titration according to the Official Methods and Recommended Practices of The AOCS, 5<sup>th</sup> Edition, Method: Cd 8-53.

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**Metals in mayonnaises**

The contents of metals (Cu, Fe, Ni and Zn) were analysed for the mayonnaise. The analysis was based on Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) using a Varian Vista MPX (Varian, Palo Alto, CA) according to the Official Methods of Analysis of the AOAC International, 16<sup>th</sup> Edition, Methods: 965.09, 977,29, 985,01, 984,27.

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**Oxygen consumption in mayonnaise**

50 grams ( $\pm 0.1$ g) of freshly produced mayonnaise was filled into a 115ml glass bottle (Mikrolab, Aarhus) and closed with a Teflon® cap. This bottle was placed at 20°C in a temperature controlled heating cabinet and residual oxygen was monitored during storage (repeated analysis of the same bottle) using a Checkmate 9900 Oxygen headspace analyser (PBI-Dansensor A/S, Denmark).

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**Conjugated diene (CD) hydroperoxides in mayonnaise**

The amount of conjugated diene (CD) hydroperoxides was measured by UV-spectroscopy according to internal method (A1987) modified after Fishwick & Swoboba (1977) and Kähkönen *et al.* (1999). Mayonnaise kept at -18°C was allowed to thaw at room temperature for approximately 1-2 hours and then separated in oil/interphase/water phase by centrifugation 2500 rpm/10 min/20°C (Sigma 3-18K). Then 10mg of the upper clear oil phase was added 10 ml isooctane (Sigma-Aldrich, Art. 32291, Steinheim, Germany) and whirl mixed for 5 seconds. Absorption spectra were recorded by scanning the absorbance from 300 to 220nm using a spectrophotometer (Shimadzu UV-1650PC spectrophotometer, Germany) with scan speed: medium, sampling interval: 1nm and a flow cuvette (0.1cm). The spectrophotometer was set to zero with isooctane. The content of conjugated dienes was calculated as the absorbance at 234nm using a molar extinction coefficient of 26000 ( $E_{234 \text{ nm}} = 26000 \text{ l} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ ) to calculate results in millimoles hydroperoxides per kg of oil.

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### **2,4-heptadienal and 2,4-decadienal in mayonnaises**

The amount of 2,4-heptadienal and 2,4-decadienal of the separated oil phase was determined by gas chromatography mass spectrometry with selected ion monitoring (GC-MS-SIM) using an Agilent 6890N GC/Agilent 5973N MSD system. Mayonnaise kept at -18°C until analysis was allowed to thaw at room temperature for approximately 1-2 hours and then separated in oil/interphase/water phase by centrifugation 2500 rpm/10 min/20°C (Sigma 3-18K). 0.5g (+/- 0.1g) of separated oil were added 10ml methanol and internal standard (hexyl hexanoate and 5-methyl-2-hexanone) corresponding to approximately 10 mg/kg of each internal standard. Then the slurry was shaken for 15 minutes on a shaker (Multi Reax, Heidolph, Germany) at 1000 rpm and placed in freezer overnight. An aliquot of the supernatant methanol phase was subsequently transferred to a GC injection vial. A calibration was performed in the range of 0-80 mg/kg by adding a stock solution of 2,4-heptadienal and 2,4-decadienal (mixtures of trans,trans-2,4 and cis,trans-2,4 isomers) and internal standard directly to methanol. In the calculations, the response from the trans,trans-2,4 isomers was added to the response from the cis,trans-2,4 isomers, and reported as the total of 2,4-heptadienal isomers or the total of 2,4-decadienal isomers.

### **20 Sensory evaluation of mayonnaises**

The sensory evaluation was carried out by two oxidation specialists who regularly taste and smell foods to identify the development of oxidation products. The objective was to evaluate sensory characteristics (appearance, aroma, taste and texture) and in particular development of oxidative off-flavour (taste, aroma) during storage.

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### **Statistical analysis of synergistic interaction between CFE and RE**

The basic experiment is a complete 2 by 2 factorial Design of Experiments with factors 'Caralluma fimbriata extract' (absence/presence) and 'Rosemary extract' (absence/presence) comprising a balanced add-on structure which can be used to evaluate both individual main effects and the two-factor interaction.

The study is summarised by 2 storage time measurements (day 14 "D14" and day 28 "D28") of 4 methods (2,4-heptadienal and 2,4-decadienal and conjugated dienes and

oxygen consumption) for all 6 treatments ((CTRL), (RE20), (CFE), (RE20 + CFE), (RE70), (RE70 + CFE)) from table 2.

The data are modelled by a multivariate analysis of variance (50-50 MANOVA) as described by Langsrud (2000, 2002) and the model assumes standardised responses and a two-way model structure for combinations of RE and CFE, with ANOVA contrasts (CFE, RE20, RE70, CFE\*RE20, CFE\*RE70), such that: (1) CFE is the main effect of CFE, (2) RE20 is the main effect of RE20, (3) RE70 is the main effect of RE70, (4) CFE\*RE20 is the interaction between CFE and RE20 and (5) CFE\*RE70 is the interaction between CFE and RE70.

The main effects characterises the corresponding individual effects of singles (i.e. presence vs. absence) whereas the interaction effects characterises the corresponding non-additive effects of compounds (i.e. sum of two-way combination and blank vs. sum of both one-way combinations).

## **RESULTS AND DISCUSSION**

### **Peroxide value in canola oil**

The canola oil (COLZAO™, 190kg drum, AarhusKarlshamn, Denmark) was kept refrigerated and unopened until the day of manufacture of mayonnaise. Each day of manufacture the oil was analysed. The oil had an acceptably low amount of peroxides (0.4 meq/kg oil) as shown in table 6. The values were as expected for commercial canola oil delivered in drums.

*Table 6: Peroxide values (meq/kg) in canola oil used for trial A and trial B.*

Sample ID	Trial	Analytical result (meq/kg)
COLZAO™ canola oil	A	0.4
COLZAO™ canola oil	B	0.4

### **Metals in mayonnaises**

One batch of freshly produced mayonnaise (control batch) was analysed for the content of Cu, Fe, Ni and Zn by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES). Table 7 shows the average of triplicate determinations of metals (mg/kg



mayonnaise). The values were as expected in egg yolk based mayonnaise; egg yolk is the major contributor of metals.

Table 7: Metal contents (mg/kg mayonnaise).

Sample ID	Trial	ppm Cu	ppm Fe	ppm Ni	ppm Zn
CTRL – Day 0	A	0.16	1.80	<0.1	1.30
CTRL – Day 0	B	0.17	1.60	<0.1	1.20

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### **Antioxidant activity of rosemary extract and *Caralluma fimbriata* extract**

#### **Oxygen consumption in sample headspace**

The consumption of oxygen during storage relates to formation of primary oxidation products (hydroperoxides). An antioxidant will delay the uptake of oxygen by the mayonnaise sample. The oxygen content in sample headspace after 1, 14 and 28 days of storage at 20°C is shown in table 8. The standard deviation on means of duplicates in table 8 and subsequent tables was calculated from an ANOVA gauge R&R (ANOVA gauge repeatability and reproducibility). For a graphical view of data see also Figure 1.

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The oxygen was consumed more rapidly in the unprotected control sample (i.e. CTRL) compared to the one-component treated samples (i.e. RE20, CFE, RE70). The two-component treatments (i.e. RE20+CFE and RE70+CFE) demonstrated the strongest antioxidant protection.

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Table 8: Oxygen content (%) in sample headspace after 1, 14 and 28 days of storage at 20°C. Values are mean of duplicates; standard deviation from ANOVA gauge R&R are given in parentheses.

Batch	%O <sub>2</sub> Day 1	%O <sub>2</sub> Day 14	%O <sub>2</sub> Day 28
CTRL	20.4 (0.1)	16.8 (0.5)	8.9 (0.6)
RE20	20.3 (0.1)	18.7 (0.2)	11.3 (1.1)
CFE	20.4 (0.2)	19.2 (0.5)	12.9 (1.5)
RE20 + CFE	20.4 (0.1)	19.5 (0.2)	16.6 (0.6)
RE70	20.4 (0.1)	19.0 (0.1)	13.0 (0.8)
RE70 + CFE	20.4 (0.1)	19.5 (0.1)	17.2 (0.2)

### **Conjugated diene (CD) hydroperoxides development**

Conjugated diene (CD) hydroperoxides relates to formation of primary oxidation products (hydroperoxides). An antioxidant will delay formation of conjugated diene (CD) hydroperoxides in mayonnaise sample. The CD content in mayonnaise after 0, 14 and 28 days of storage at 20°C is shown in table 9. For a graphical view of data see also figure 2.

CD developed more rapidly in the unprotected control sample (i.e. CTRL) compared to the one-component treated samples (i.e. RE20, CFE, RE70). The two-component treated samples (i.e. RE20+CFE and RE70+CFE) demonstrated the strongest antioxidant protection. Further, it appeared that the antioxidant activity of the two different qualities of rosemary extracts (i.e. RE20, RE70) had comparable activity as illustrated when they were added alone (at equal amounts of phenolic diterpenes) and when they were added in combinations with CFE.

*Table 9: Conjugated dienes hydroperoxides ( $\mu\text{mol hydroperoxides/kg oil}$ ) after 0, 14 and 28 days of storage at 20°C. Values are mean of duplicates; standard deviation from ANOVA gauge R&R are given in parentheses.*

Batch	Conjugated dienes Day 0	Conjugated dienes Day 14	Conjugated dienes Day 28
CTRL	13.6 (0.1)	18.6 (0.4)	26.3 (0.2)
RE20	13.6 (0.2)	15.2 (0.4)	22.9 (0.6)
CFE	13.6 (0.1)	15.1 (0.6)	21.8 (1.2)
RE20 + CFE	13.6 (0.1)	14.0 (0.1)	16.9 (0.7)
RE70	13.6 (0.0)	14.7 (0.4)	21.9 (1.9)
RE70 + CFE	13.4 (0.1)	13.8 (0.1)	16.1 (0.1)

### **2,4-heptadienal and 2,4-decadienal development**

Oxidation of oils and fats produces a complex mixture of volatile secondary oxidation products. Snyder *et al.* (1985) found that volatile secondary oxidation products in canola oil was dominated by trans,cis- and trans,trans-2,4-heptadienal, hexanal, nonanal, and the trans,cis- and trans,trans-2,4-decadienals. Jacobsen *et al.* (1999) found that 4 volatile oxidation compounds, namely 3-furaldehyde, 2,4-heptadienal, 2,4-decadienal and ethyl benzene, appeared to correlate to the fishy and rancid off-flavours that developed in mayonnaise. In this experiment 2,4-heptadienal and 2,4-decadienal were

the dominant volatiles in the canola oil based mayonnaises and therefore selected as markers of oxidation.

5 An antioxidant will delay formation of 2,4-heptadienal and 2,4-decadienal in the mayonnaise sample. The 2,4-heptadienal and 2,4-decadienal content in mayonnaise after 0, 14 and 28 days of storage at 20°C is shown in table 10 and table 11. For a graphical view of data see also figure 3 and 4.

10 2,4-heptadienal and 2,4-decadienal developed more rapidly in the unprotected control sample (i.e. CTRL) compared to the one-component treated samples (i.e. RE20, CFE, RE70). The two-component treated samples (i.e. RE20+CFE and RE70+CFE) demonstrated the strongest antioxidant protection. Further, it appeared that the antioxidant activity of the two different qualities of rosemary extracts (i.e. RE20, RE70) have comparable activity as illustrated when they were added alone (at equal amounts of  
15 phenolic diterpenes) and when they were added in combinations with CFE.

The inhibition percentages of the treatments (RE20, CFE, RE20+CFE, RE70, RE70+CFE) were calculated relative to the unprotected control (CTRL) on day 28 (D28) for 2,4-heptadienal and 2,4-decadienal.

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% inhibition is calculated as:

$$((C_{\text{control}} - C_{\text{treatment}}) / C_{\text{control}}) * 100\%$$

where

25  $C_{\text{control}}$  = ppm 2,4-heptadienal or 2,4-decadienal in control

$C_{\text{treatment}}$  = ppm 2,4-heptadienal or 2,4-decadienal in treatment

Figures 6 and 7 are graphical illustrations of the synergy later proven by statistical analysis.

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For % inhibition of 2,4-heptadienal in Figure 6 the one-component treated samples (i.e. RE20, CFE, RE70) provided 17-23% inhibition whereas the two-component treated samples (i.e. RE20+CFE, RE70+CFE) provided 72-73% i.e. more activity than would be expected by adding the two components alone

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For % inhibition of 2,4-decadienal in Figure 7 the one-component treated samples (i.e. RE20, CFE, RE70) provided 22-27% inhibition whereas the two-component treated samples (i.e. RE20+CFE, RE70+CFE) provided 77-80% i.e. more activity than would be expected by adding the two components alone *Table 10: 2,4-heptadienal (mg/kg oil) after 0, 14 and 28 days of storage at 20°C. Values are mean of duplicates; standard deviation from ANOVA gauge R&R are given in parentheses.*

Batch	2,4-heptadienal Day 0	2,4-heptadienal Day 14	2,4-heptadienal Day 28
CTRL	<0.2	11.3 (1.4)	21.0 (4.0)
RE20	<0.2	3.7 (0.5)	17.5 (2.6)
CFE	<0.2	3.3 (0.2)	16.9 (2.6)
RE20 + CFE	<0.2	1.1 (0.3)	5.9 (2.0)
RE70	<0.2	3.2 (1.0)	16.2 (1.2)
RE70 + CFE	<0.2	1.0 (0.0)	5.8 (0.6)

*Table 11: 2,4-decadienal (mg/kg oil) after 0, 14 and 28 days of storage at 20°C. Values are mean of duplicates; standard deviation from ANOVA gauge R&R are given in parentheses.*

Batch	2,4-decadienal Day 0	2,4-decadienal Day 14	2,4-decadienal Day 28
CTRL	<0.2	15.1 (1.1)	35.2 (5.6)
RE20	<0.2	2.8 (0.4)	25.6 (5.4)
CFE	<0.2	2.7 (0.3)	26.2 (5.1)
RE20 + CFE	<0.2	0.8 (0.2)	7.0 (2.9)
RE70	<0.2	3.8 (2.0)	27.5 (4.1)
RE70 + CFE	<0.2	1.1 (0.2)	8.2 (0.4)

### Sensory evaluation

An antioxidant will delay formation of oxidative taste and aroma in the mayonnaise sample. The oxidative taste and aroma in mayonnaise after 0, 14, 21, 28, 42 and 56 days of storage at 20°C is shown in figure 5. With respect to oxidation by sensory analysis, oxidised off-flavour developed faster and increased rapidly in the unprotected control sample compared to treated samples as illustrated in figure 5. In particular the synergistic two-component antioxidant mixtures (i.e. RE20+CFE and RE70+CFE) demonstrated high antioxidant protection against oxidative off-flavour development. This correlated with low oxygen consumption, low CD and low 2,4-heptadienal and 2,4-

decadial development. The treatments did not have an impact on appearance and texture and the plant materials did not contribute with undesirable taste or aroma at tested dosages.

## 5 **Statistical analysis (50-50 MANOVA) of synergism between RE and CFE**

The 50-50 MANOVA focuses on day 28 measurements for the 4 methods. In table 12 is summarised information of ANOVA contrasts for D28 measurements for all 4 methods with format: first is the estimated value, second is the standard error of the estimate in parenthesis and third is an asterisk if the contrast is significant at level 5% (evaluated by a one-sided t-test using the t-statistic estimate/(standard error) with an absolute score of 1.65 as critical value).

Synergy of combination of RE and CFE for D28 measurements is characterised by a negative score for either CFE\*RE20 and/or CFE\*RE70 for methods (2,4-heptadienal and 2,4-decadienal and conjugated dienes) and a positive score for method (oxygen consumption).

From table 12 it is observed that for all 4 methods the combination of RE and CFE is synergistic for D28 measurements in both terms CFE\*RE20 and CFE\*RE70 and significance at level 5% is observed for the 3 methods (2,4-heptadienal and 2,4-decadienal and conjugated dienes).

Figure 8 is a graphical representation of the estimates of interactions CFE\*RE20 and CFE\*RE70 for D28 measurements for all 4 methods and synergy for methods (2,4-heptadienal and 2,4-decadienal and conjugated dienes) is commonly identified by a non-parallel structure of the 2 lines (blue and red) such that the slope of the red line is smaller compared to the blue line.

Table 12. ANOVA contrasts for D28 measurements for the 4 methods (2,4-heptadienal and 2,4-decadienal and conjugated dienes and oxygen consumption).

METHOD	ANOVA				
	CFE	RE20	RE70	CFE*RE20	CFE*RE70
2,4-heptadienal	-26.06 (2.96)*	-14.54 (2.89)*	-16 (2.47)*	-7.42 (2.89)*	-6.28 (2.47)*
2,4-decadienal	-46.9 (5.28)*	-28.84 (4.86)*	-25.74 (4.29)*	-9.58 (4.86)*	-10.2 (4.29)*
conjugated dienes	-16.22 (1.21)*	-8.23 (0.77)*	-10.03 (1.12)*	-1.45 (0.77)*	-1.33 (1.12)
oxygen consumption	13.52 (1.08)*	6.09 (1.01)*	8.37 (0.9)*	1.27 (1.01)	0.13 (0.9)

## CONCLUSION

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In the present example a two-component antioxidant mixture of *Caralluma fimbriata* extract (CFE) and *Rosmarinus officinalis* extract (RE) was investigated in mayonnaise with 80 wt% oil.

10 The combination of RE and CFE is synergistic (and significant at level 5%) on day 28 measurements simultaneous for the 3 methods (2,4-heptadienal and 2,4-decadienal and conjugated dienes).

15 The fact that CFE and RE interacts synergistically provides the following range of functional and economic benefits compared to present commercial natural solutions: high antioxidant activity (increased product shelf-life and consumer satisfaction), low cost-in-use, low impact on taste and low discoloration

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- 30 The invention will be described in further detail in the following numbered paragraphs. The present invention provides:
1. An anti-oxidant composition comprising
    - 35 (a) an extract comprising phenolic diterpenes obtained from a plant of the *Labiatae* family; and
    - (b) an extract comprising pregnane glycoside obtained from a plant of the genus

*Caralluma*;

in amounts to provide a synergistic antioxidant effect.

2. The composition according to Paragraph 1, wherein the composition comprises
  - 5 (a) an extract obtained from a plant of the species *Rosmarinus officinalis*.
3. The composition according to Paragraph 1 or 2, wherein the composition comprises
  - (b) an extract obtained from a plant of the species *Caralluma fimbriata*.
- 10 4. The composition according to any one of the preceding Paragraphs, wherein the weight ratio of
  - (a) the extract obtained from a plant of the *Labiatae* family, to
  - (b) the extract obtained from a plant of the genus *Caralluma*,  
is from 20:1 to 1:20.
- 15 5. The composition according to Paragraph 4, wherein the weight ratio of (a) to (b) is from 1:2 to 1:6.
6. The composition according to any one of the preceding Paragraphs, wherein the
  - 20 (a) phenolic diterpenes obtained from a plant of the *Labiatae* family, to
  - (b) the extract obtained from a plant of the genus *Caralluma*,  
is from 1:1 to 1:40.
- 25 7. A composition according to any one of the preceding Paragraphs, wherein the extract obtained from a plant of the *Labiatae* family, optionally from a plant of the species *Rosmarinus officinalis*, *Salvia officinalis* or mixtures thereof, contains phenolic diterpenes in an amount of at least 1 wt.% based on the weight of the extract.
- 30 8. A composition according to any one of the preceding Paragraphs, wherein the extract obtained from a plant of the *Labiatae* family, optionally from a plant of the species *Rosmarinus officinalis*, *Salvia officinalis* or mixtures thereof, contains carnosic acid in an amount of at least 1 wt.% based on the weight of the extract.
- 35 9. An emulsion comprising an anti-oxidant composition according to any one of



Paragraphs 1 to 8.

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10. A foodstuff comprising an anti-oxidant composition according to any one of Paragraphs 1 to 8.
11. A foodstuff according to Paragraph 10, wherein the (a) extract obtained from a plant of the *Labiatae* family, optionally from a plant of the species *Rosmarinus officinalis*, *Salvia officinalis* or mixtures thereof, is present in an amount of 1 to 4000ppm based on the weight of the foodstuff.
- 10
12. A foodstuff according to Paragraph 10 or 11, wherein the (b) extract obtained from a plant of the genus *Caralluma*, optionally a plant of the species *Caralluma fimbriata*, is present in an amount of 10 to 3000ppm based on the weight of the foodstuff.
- 15
13. A process for preventing and/or inhibiting oxidation of a foodstuff, the process comprising the step of contacting the foodstuff with
- (a) an extract comprising phenolic diterpenes obtained from a plant of the *Labiatae* family, optionally from a plant of the species *Rosmarinus officinalis*, *Salvia officinalis* or mixtures thereof; and
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- (b) an extract comprising pregnane glycoside obtained from a plant of the genus *Caralluma*, optionally a plant of the species *Caralluma fimbriata*; in amounts to provide a synergistic antioxidant effect.
14. Use of
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- (a) an extract comprising phenolic diterpenes obtained from a plant of the *Labiatae* family, optionally from a plant of the species *Rosmarinus officinalis*, *Salvia officinalis* or mixtures thereof; and
- (b) an extract comprising pregnane glycoside obtained from a plant of the genus *Caralluma*, optionally a plant of the species *Caralluma fimbriata*;
- 30
- in amounts to provide a synergistic antioxidant effect; for preventing and/or inhibiting oxidation of an emulsion or a foodstuff.
15. A kit when used to prepare a composition as defined in any one of Paragraphs 1 to 8, or in the use of Paragraph 14, the kit comprising
- 35
- (a) an extract comprising phenolic diterpenes obtained from a plant of the *Labiatae* family, optionally from a plant of the species *Rosmarinus officinalis*, *Salvia*

*officinalis* or mixtures thereof; and

- (b) an extract comprising pregnane glycoside obtained from a plant of the genus *Caralluma*, optionally a plant of the species *Caralluma fimbriata*; in amounts to provide a synergistic antioxidant effect; in separate packages or containers; optionally with instructions for admixture and/or contacting and/or use.

All publications mentioned in the above specification are herein incorporated by reference. Various modifications and variations of the described methods and system of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in chemistry, biology, food science or related fields are intended to be within the scope of the following claims

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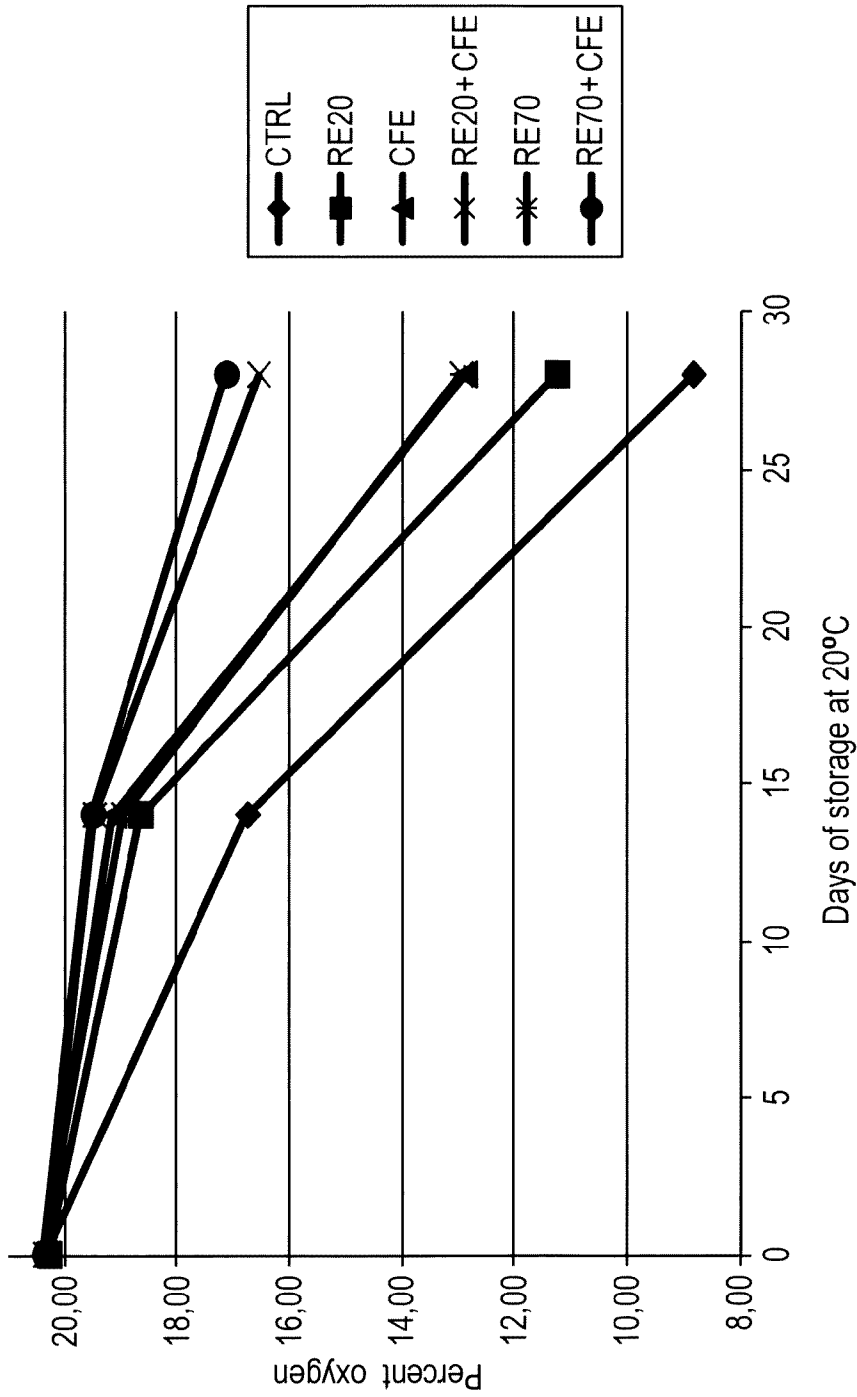
**The Claims defining the invention are as follows:**

1. An anti-oxidant composition comprising
  - (a) an extract comprising phenolic diterpenes obtained from a plant of the *Labiatae* family; and
  - (b) an extract comprising pregnane glycoside obtained from a plant of the genus *Caralluma*;  
in amounts to provide a synergistic antioxidant effect.
2. The composition according to Claim 1, wherein the composition comprises
  - (a) an extract obtained from a plant of the species *Rosmarinus officinalis*.
3. The composition according to Claim 1 or 2, wherein the composition comprises
  - (b) an extract obtained from a plant of the species *Caralluma fimbriata*.
4. The composition according to any one of the preceding Claims, wherein the weight ratio of
  - (a) the extract obtained from a plant of the *Labiatae* family, to
  - (b) the extract obtained from a plant of the genus *Caralluma*,  
is from 20:1 to 1:20.
5. The composition according to Claim 4, wherein the weight ratio of (a) to (b) is from 1:2 to 1:6.
6. The composition according to any one of the preceding Claims, wherein the weight ratio of
  - (a) phenolic diterpenes obtained from a plant of the *Labiatae* family, to
  - (b) the extract obtained from a plant of the genus *Caralluma*,  
is from 1:1 to 1:40.
7. A composition according to any one of the preceding Claims, wherein the extract obtained from a plant of the *Labiatae* family, optionally from a plant of the species *Rosmarinus officinalis*, *Salvia officinalis* or mixtures thereof, contains phenolic diterpenes in an amount of at least 1 wt.% based on the weight of the extract.

8. A composition according to any one of the preceding Claims, wherein the extract obtained from a plant of the *Labiatae* family, optionally from a plant of the species *Rosmarinus officinalis*, *Salvia officinalis* or mixtures thereof, contains carnosic acid in an amount of at least 1 wt.% based on the weight of the extract.
- 5
9. An emulsion comprising an anti-oxidant composition according to any one of Claims 1 to 8.
10. A foodstuff comprising an anti-oxidant composition according to any one of Claims 1 to 8.
- 10
11. A foodstuff according to Claim 10, wherein the (a) extract obtained from a plant of the *Labiatae* family, optionally from a plant of the species *Rosmarinus officinalis*, *Salvia officinalis* or mixtures thereof, is present in an amount of 1 to 4000ppm based on the weight of the foodstuff.
- 15
12. A foodstuff according to Claim 10 or 11, wherein the (b) extract obtained from a plant of the genus *Caralluma*, optionally a plant of the species *Caralluma fimbriata*, is present in an amount of 10 to 3000ppm based on the weight of the foodstuff.
- 20
13. A process for preventing and/or inhibiting oxidation of a foodstuff, the process comprising the step of contacting the foodstuff with
- (a) an extract comprising phenolic diterpenes obtained from a plant of the *Labiatae* family, optionally from a plant of the species *Rosmarinus officinalis*, *Salvia officinalis* or mixtures thereof; and
- 25
- (b) an extract comprising pregnane glycoside obtained from a plant of the genus *Caralluma*, optionally a plant of the species *Caralluma fimbriata*; in amounts to provide a synergistic antioxidant effect.
- 30
14. Use of
- (a) an extract comprising phenolic diterpenes obtained from a plant of the *Labiatae* family, optionally from a plant of the species *Rosmarinus officinalis*, *Salvia officinalis* or mixtures thereof; and
- (b) an extract comprising pregnane glycoside obtained from a plant of the genus *Caralluma*, optionally a plant of the species *Caralluma fimbriata*;
- 35

in amounts to provide a synergistic antioxidant effect;  
for preventing and/or inhibiting oxidation of an emulsion or a foodstuff.

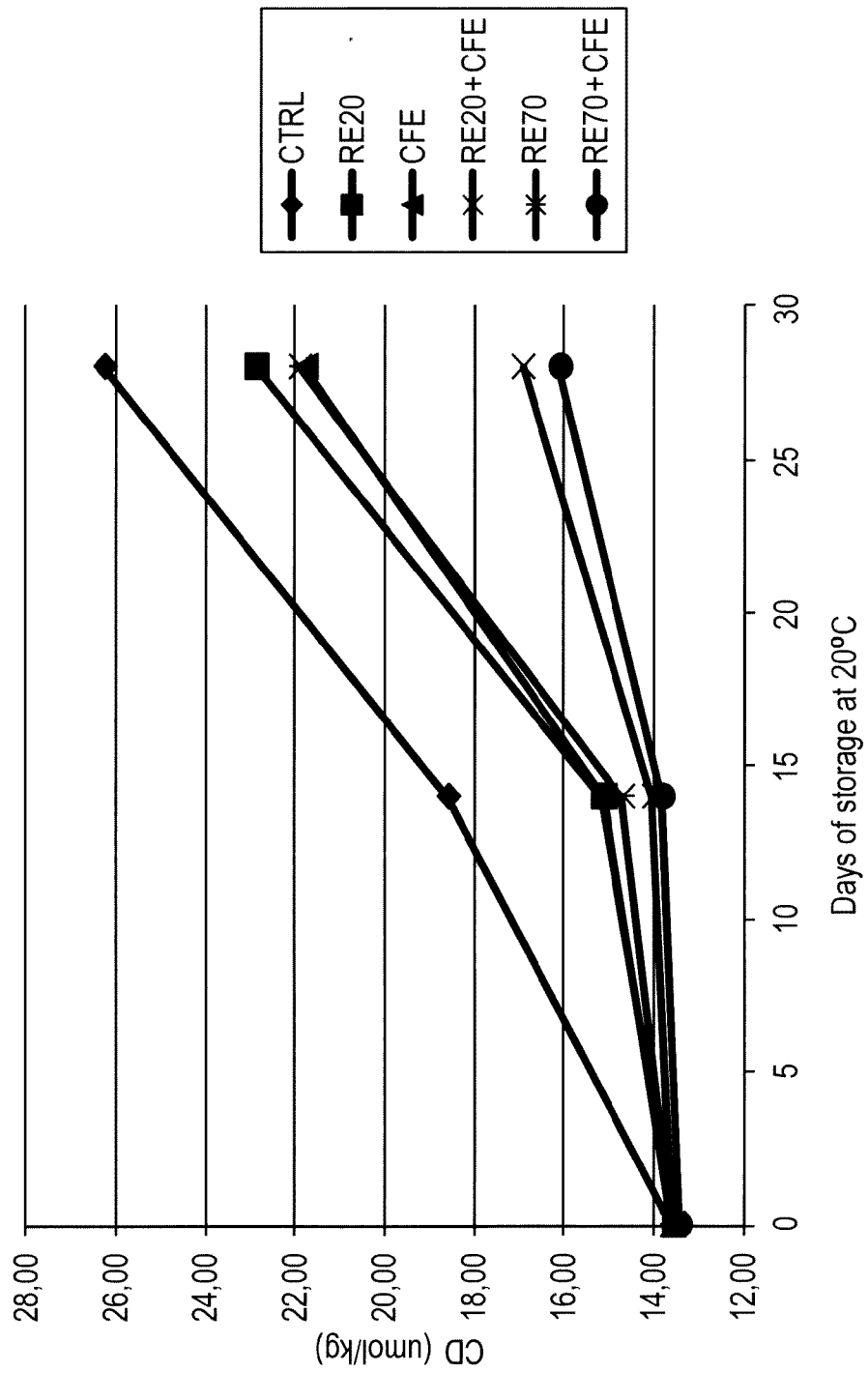
- 5
15. A kit when used to prepare a composition as defined in any one of Claims 1 to 8, or  
in the use of Claim 14, the kit comprising
- (a) an extract comprising phenolic diterpenes obtained from a plant of the  
*Labiatae* family, optionally from a plant of the species *Rosmarinus officinalis*,  
*Salvia officinalis* or mixtures thereof; and
- 10 (b) an extract comprising pregnane glycoside obtained from a plant of the genus  
*Caralluma*, optionally a plant of the species *Caralluma fimbriata*;  
in amounts to provide a synergistic antioxidant effect;  
in separate packages or containers;  
optionally with instructions for admixture and/or contacting and/or use.



Oxygen consumption. Graphical illustration of data in table 8.

FIG. 1

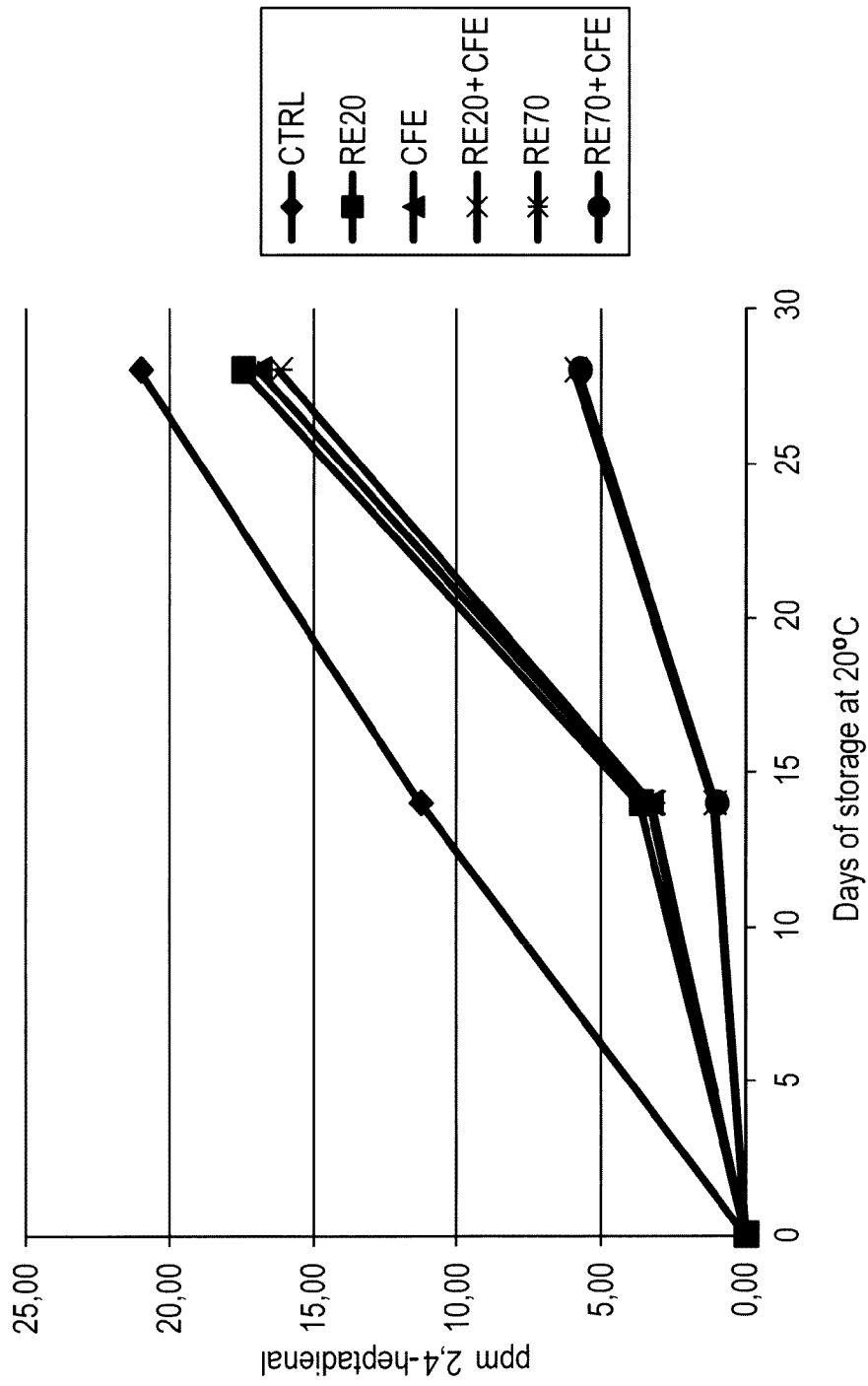




Conjugated dienes hydroperoxides. Graphical illustration of data in table 9.

FIG. 2

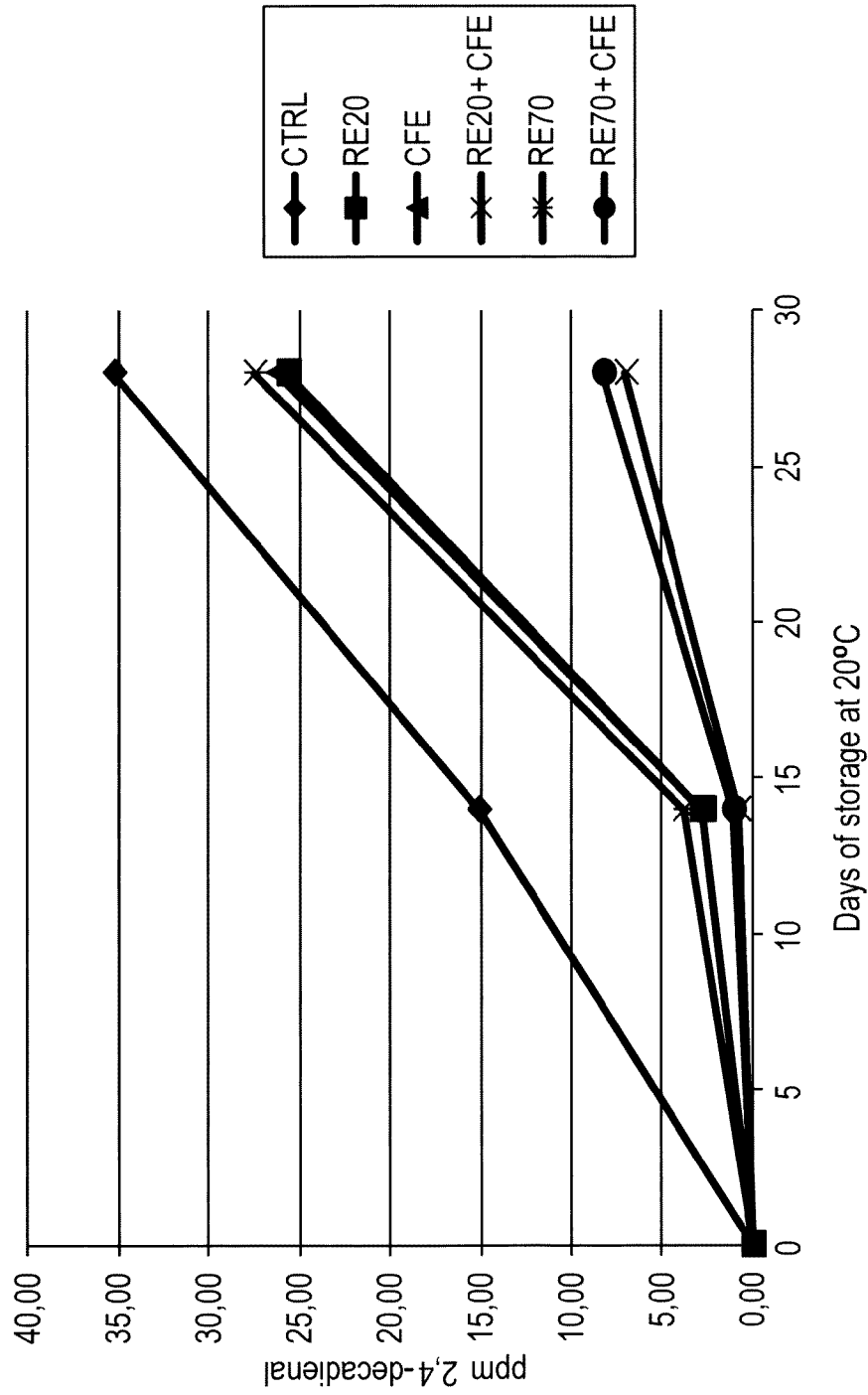
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2,4-heptadienal. Graphical illustration of data in table 10.

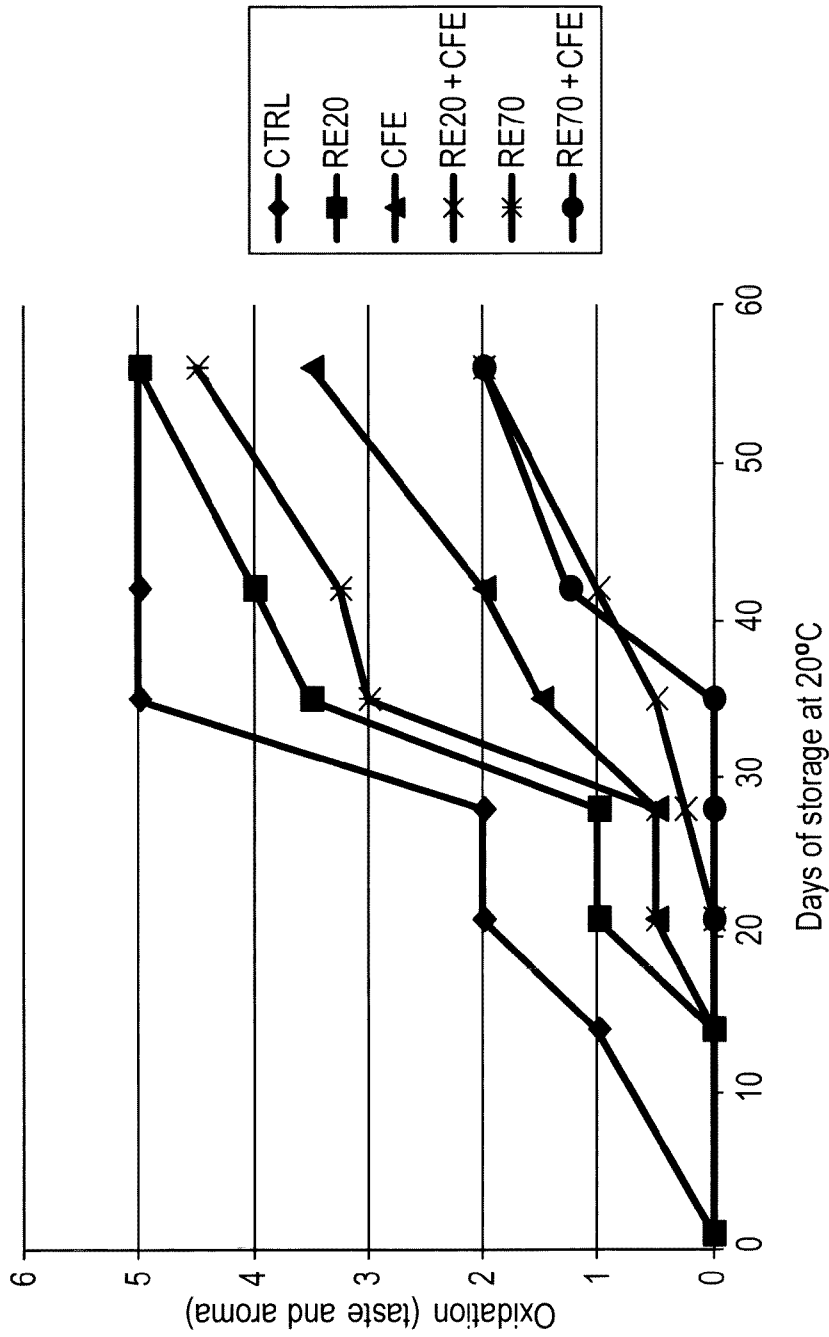
FIG. 3

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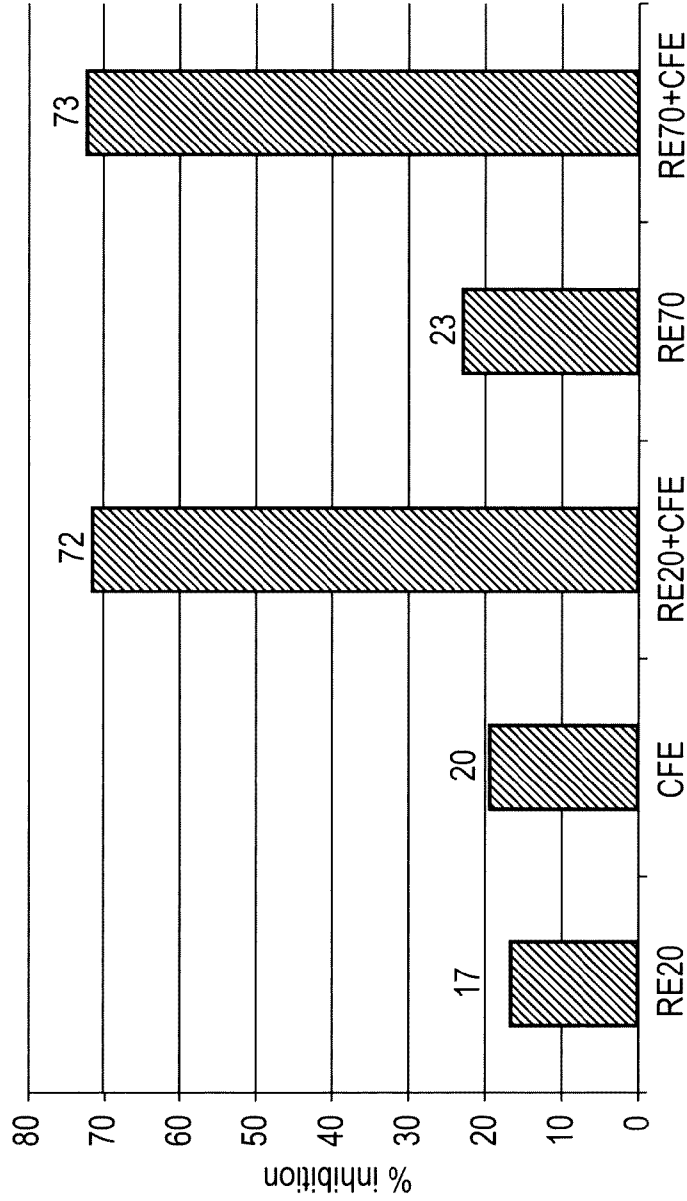
2,4-decadienal. Graphical illustration of data in table 11.

FIG. 4



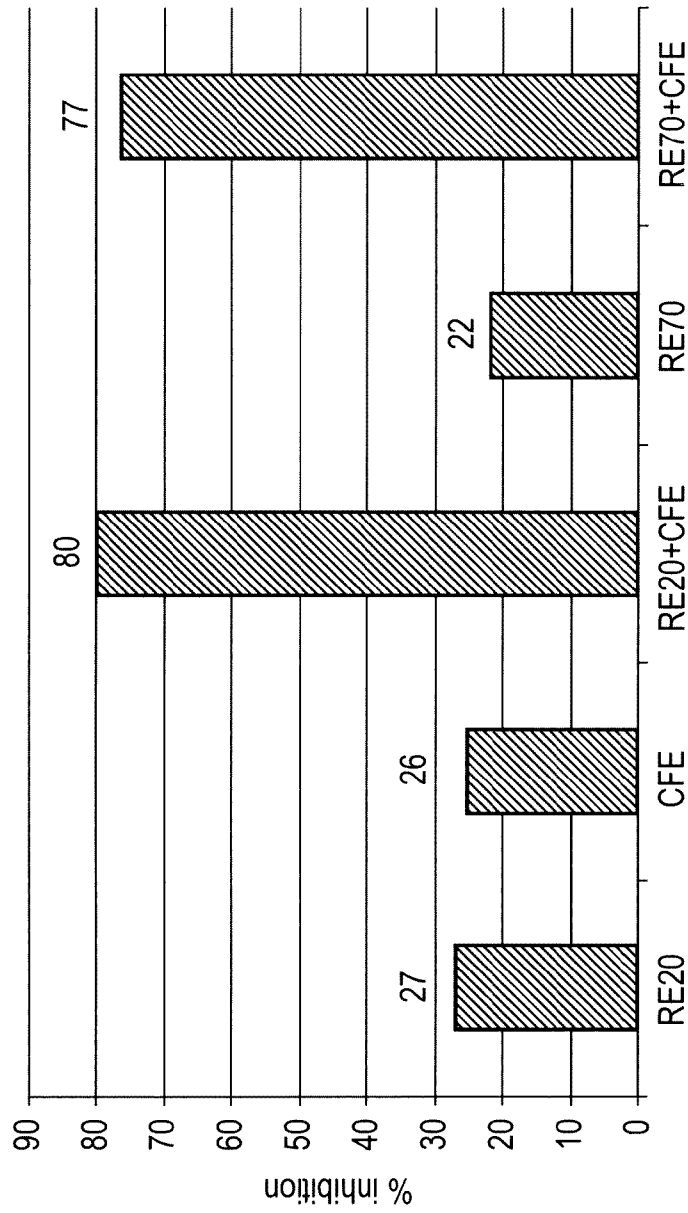
Oxidation (taste and aroma) by sensory analysis. Average of sensory score of A and B samples evaluated by two oxidation specialists. The oxidation was ranked from "0" to "5" ("0" = no oxidation, "1" = low oxidation, . . . "5" = high oxidation).

FIG. 5



Percent inhibition of 2,4-heptadienal of five treatments (RE20, CFE, RE20+CFE, RE70, RE70+CFE) compared to unprotected control (CTRL) on the storage time, day 28 (D28).

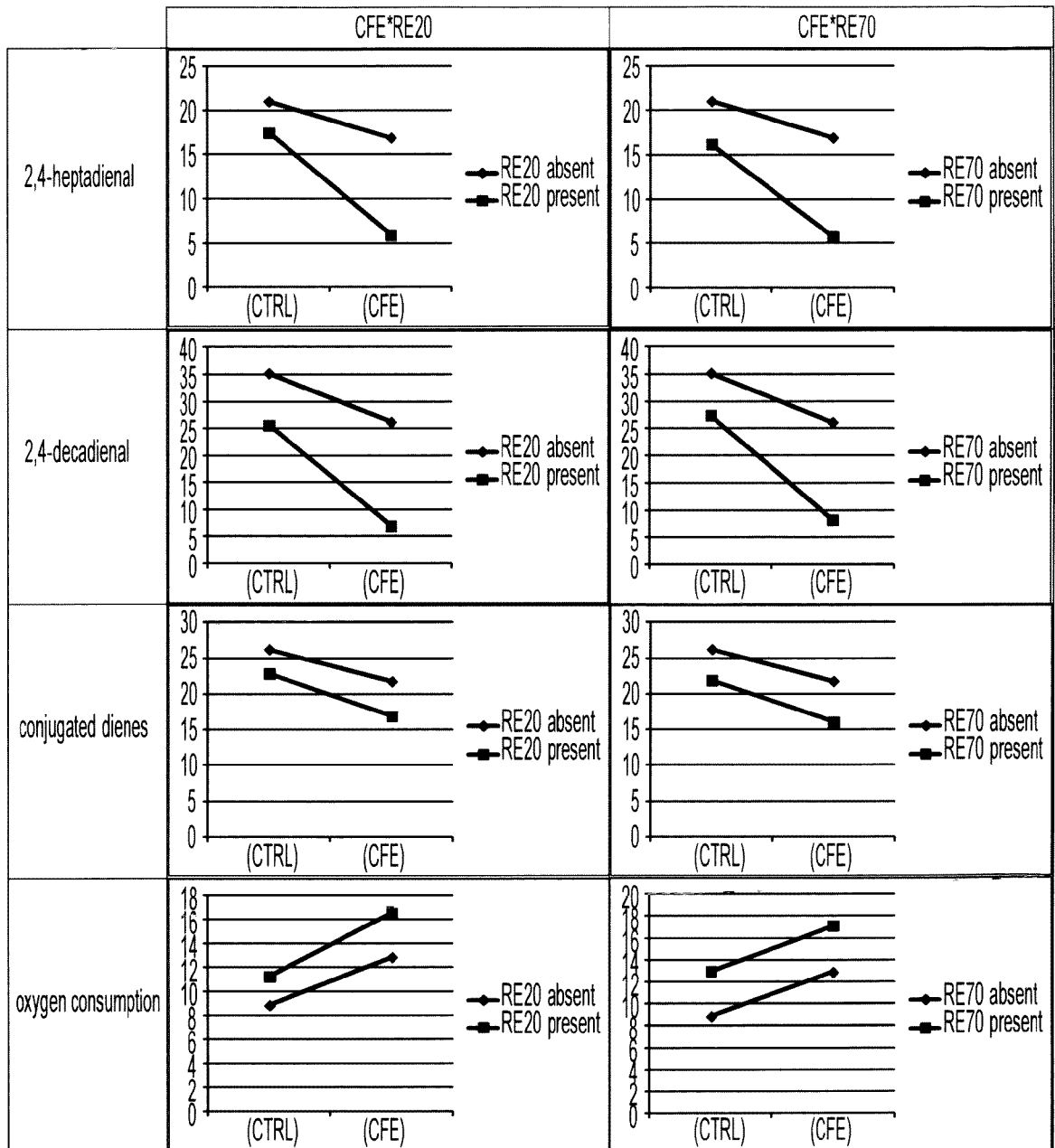
FIG. 6



Percent inhibition of 2,4-decadienal of five treatments (RE20, CFE, RE20+CFE, RE70, RE70+CFE) compared to unprotected control (CTRL) on the storage time, day 28 (D28).

FIG. 7

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Interaction plot of CFE\*RE20 and CFE\*RE70 for D28 measurements for the 4 methods (2,4-heptadienal and 2,4-decadienal and conjugated dienes and oxygen consumption).

FIG. 8