Application to Amend the Australia and New Zealand Food Standards Code.

Addition of the Food Additive Rosemary Extract (INS 392) to Schedule 8 and Schedule 15.

Contents

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Abbreviations Used In this Document

µg	Microgram
bw	Body weight
CCFA	Codex Committee on Food Additives
EFSA	European Food Safety Authority
EU	European Union
FAO	Food and Agriculture Organisation
FCC	Food Chemicals Codex
g	Gram
GMP	Good Manufacturing Practice
JECFA	Joint Expert Committee on Food Additives
Kg	Kilogram
mg	Milligram
MPL	Maximum permitted level
NMT	Not more than
NOAEL	No Observed Adverse Effect Level
TGA	Therapeutic Goods Administration
WHO	World Health Organisation

Executive Summary

An application is made to FSANZ to amend the Food Standards Code to add Rosemary Extract (INS 392) to the following schedules of the FSANZ Food Standards Code:

- Schedule 8: Food additive names and code numbers (for statement of ingredients)
- Schedule 15: Substances that may be used as food additives

This application refers to rosemary extracts prepared using acetone or ethanol solvent extraction and complies with monographs of the JECFA (draft) and the Food Chemicals Codex (FCC 10). The application requests permission for the addition of rosemary extracts in a range of food categories.

Rosemary extracts are derived from Rosmarinus officinalis L. and contain several compounds which have been shown to exert antioxidative functions. Although the entire rosemary (*Rosmarinus officinalis* L.) plant, excluding the woody portions, may be used, it is normally only the leaves, that are commonly used as a culinary herb, flavouring agent and naturally occurring antioxidant. Rosemary extracts are increasingly employed not only to provide flavour but also as natural alternatives to synthetic antioxidants for the stabilisation of oxygen-sensitive foods. The antioxidative function is due to several components in the rosemary extracts, which belong mainly to the classes of phenolic acids, flavonoid diterpenoids and triterpenes

The antioxidative function of rosemary extracts helps to stabilise product formulations thus providing longer shelf-life. Rosemary extracts are naturally derived extracts and thus provide a benefit to consumers seeking more 'natural' ingredients in their food products

Rosemary extract E 392 is approved in the EU additives regulation No. 1129/2011. It was evaluated by the European Food Safety Authority (EFSA) in 2008 (EFSA, 2008) and then again in 2015 to extend it uses to fat based spreads (EFSA, 2015). Rosemary extract is also approved for use as a food additive in Japan, China and Singapore. Rosemary extract is allowed in the United States as a flavour although it appears on some food labels as a preservative. The applicant is pursuing a self-affirmed GRAS position for rosemary antioxidant extract that is based upon the EU and JECFA evaluations

At the Forty-fifth Session of CCFA in 2013 (FAO/WHO, 2013b), it was concluded that although rosemary extract had been assigned an INS number (392), it had not yet been evaluated by Joint FAO/WHO Expert Committee on Food Additives (JECFA). The Committee evaluated rosemary extract at the 82nd meeting at the request of CCFA. The Committee concluded that there are sufficient data to establish an acceptable daily intake (ADI) for rosemary extract prepared according to the specifications established. The Committee established a temporary ADI of 0–0.3 mg/kg bw for rosemary extract, expressed as carnosic acid plus carnosol, on the basis of a NOAEL of 64 mg/kg bw per day, expressed as carnosic acid plus carnosol, the highest dose tested in a short-term toxicity study in rats, with application of a 200-fold uncertainty factor. The overall uncertainty factor of 200 incorporates a factor of 2 to account for the temporary designation of the ADI. The Committee made the ADI temporary pending the submission of studies to elucidate the potential developmental and reproductive toxicity of the rosemary extract under consideration. An additional uncertainty factor to account for the lack of a chronic toxicity study was not considered necessary based on the absence of adverse effects in the short-term toxicity studies at doses up to and including the highest dose tested. The temporary ADI applies to rosemary extract that meets the specifications prepared at the present meeting. The temporary ADI will be withdrawn if the required data are not provided by the end of 2018.

The Committee noted that the dietary exposure estimates for rosemary extract for high consumers in the European and USA populations of 0.09–0.81 mg/kg bw per day (expressed as carnosic acid plus carnosol) may exceed the upper bound of the temporary ADI by up to 2.7-fold (for young

children at the top end of the range of estimated dietary exposures). Based on the conservative nature of the dietary exposure assessments, in which it was assumed that all foods contained rosemary extracts at the maximum use level, the Committee concluded that this exceedance of the temporary ADI does not necessarily represent a safety concern. The Committee requested that data on typical use levels in foods be provided by the end of 2018 in order to refine the dietary exposure estimates.

These studies requested by JECFA are currently being undertaken and will be submitted to JECFA by the end of 2018 as requested. The applicant, however, does not believe that waiting for these additional studies should hold up the progression of this application for a number of reasons. EFSA evaluated rosemary extract in 2008 and again in 2015. On neither occasion did EFSA raise any safety issues. EFSA did not set an ADI, but concluded the use of rosemary extract does not pose a risk to health. Rosemary has been used as an herb in cooking for hundreds of years without any indication that it causes developmental or reproductive toxicity. It has been available, as an antioxidant, in the EU since 2009 and as a flavour, globally, for many decades prior without any indication or report of it causing developmental or reproductive toxicity. However, considering that rosemary extract may have more concentrated levels of constituents than found in rosemary herb, the applicant has sought to address this, while waiting for the additional studies requested by JECFA to be completed, by reducing the number of food categories being sought for the use of rosemary extract as compared to the EU. Further, in some food categories the applicant has reduced the maximum permitted level sought compared to the EU. These measures will reduce the overall exposure of rosemary extract thus providing a further level of safety.

3.1.1 General Requirements

B Applicant Details

(a) Applicant:

KALSEC®, INC.,

(b) Name of Contact Person:

(c) Address:

(d) Telephone Number

(e) Email Address

(f) Nature of Applicants Business

Kalsec® is a supplier of a full line of natural, innovative products and solutions to meet the challenges faced by food and beverage manufacturers throughout the industry and around the world. Their products are derived from natural herbs, spices, vegetables and hops.

(g) Details of other Individuals, Companies or Organisations associated with the Application

The following Regulatory Consultant is involved in the preparation, submission and stewardship of this application:

C Purpose of the Application

The applicant applies to FSANZ to add Rosemary Extract (INS 392) to the following schedules of the FSANZ Food Standards Code:

- Schedule 8: Food additive names and code numbers (for statement of ingredients)

- Schedule 15: Substances that may be used as food additives

D Justification for the Application

Rosemary extracts are derived from Rosmarinus officinalis L. and contain several compounds which have been shown to exert antioxidative functions. Although the entire rosemary (*Rosmarinus officinalis* L.) plant, excluding the woody portions, may be used, it is normally only the leaves, that are commonly used as a culinary herb, flavouring agent and naturally occurring antioxidant. Rosemary extracts are increasingly employed not only to provide flavour but also as natural alternatives to synthetic antioxidants for the stabilisation of oxygen-sensitive foods. The antioxidative function is due to several components in the rosemary extracts, which belong mainly to the classes of phenolic acids, flavonoid diterpenoids and triterpenes

The antioxidative function of rosemary extracts helps to stabilise product formulations thus providing longer shelf-life. Rosemary extracts are naturally derived extracts and thus provide a benefit to consumers seeking more 'natural' ingredients in their food products.

D.1 Regulatory Impact Information

D.1.1 Costs and Benefits of the Application

(a) Cost and Benefits to the Consumer

Rosemary extracts are derived from Rosmarinus officinalis L. and contain several compounds which have been proven to exert antioxidative functions. Rosemary extracts are naturally derived extracts and thus provide a benefit to consumers seeking more 'natural' ingredients in their food products. It is anticipated that rosemary extracts would replace other currently used antioxidants and thus is not anticipated to have any cost impact on the consumer.

(b) Costs and Benefits to Industry

Rosemary extracts are derived from Rosmarinus officinalis L. and contain several compounds which have been proven to exert antioxidative functions. Rosemary extracts are naturally derived extracts and thus offer an alternative to industry who wish to provide g more 'natural' ingredients in their food products. It is anticipated that rosemary extracts would replace other currently used antioxidants and thus is not anticipated to have any cost impact on the industry.

(c) Cost and Benefits to Government

It is not anticipated that the availability of rosemary extract would have any cost impact on Government.

D.1.2 Impact on International Trade

Rosemary extracts are currently approved in a number of countries (EU, China, Japan, Singapore) for the addition to food products. Allowing the use of rosemary extract in Australia and New Zealand would enhance international trade by allowing import and export of products containing rosemary extract.

E Information to Support the Application

E.1 Data Requirements

The information presented in this dossier is a summary of the information submitted to JECFA (JECFA, 2017) and EFSA (EFSA, 2008) (EFSA, 2015). Where possible, we have included in the appendices a copy of the references, however, some unpublished references are not available to the applicant.

E.1.1 Data related to Safety Studies

Please refer to section 3.3.1 Food Additives, B Information Related to Safety of Food Additive.

E.1.2 Data related to surveys on chemicals and other substances in food

Please refer to section 3.3.1 Food Additives, C Information Related to Dietary Exposure of the Food Additive.

E.1.3 Data related to epidemiological / intervention studies in human

Please refer to section 3.3.1 Food Additives, B Information Related to Safety of Food Additive.

F Assessment Procedure

The applicant considers the most appropriate assessment procedure for the application herein relating to the addition of Rosemary Extract (INS 392) to Schedules 8 and 15 of the Australia New Zealand Food Standards Code to be General Procedure, Cost Category Level 2.

G Confidential Commercial Information (CCI)

Not applicable

H Other Confidential Information

Not applicable

I Exclusive Capturable Commercial Benefit (ECCB)

Not applicable

J International and Other National Standards

J.1 International Standards

Following the Twenty-third Session of the Codex Committee on Fats and Oils (CCFO) in 2013 (FAO/WHO, 2013a), CCFO decided to refer to Codex Committee on Food Additives (CCFA) its intention to include "rosemary extract" as an antioxidant in the standard for fish oils, noting that it had not yet been included in the General Standard for Food Additives (GSFA). At the Forty-fifth Session of CCFA in 2013 (FAO/WHO, 2013b), it was concluded that although rosemary extract had been assigned an INS number (392), it had not yet been evaluated by Joint FAO/WHO Expert Committee on Food Additives (JECFA). The Committee evaluated rosemary extract at the 82nd meeting at the request of CCFA.

The Committee concluded that there are sufficient data to establish an acceptable daily intake (ADI) for rosemary extract prepared according to the specifications established at this meeting. The Committee established a temporary ADI of 0–0.3 mg/kg bw for rosemary extract, expressed as carnosic acid plus carnosol, on the basis of a NOAEL of 64 mg/kg bw per day, expressed as carnosic acid plus carnosol, the highest dose tested in a short-term toxicity study in rats, with application of a 200-fold uncertainty factor. The overall uncertainty factor of 200 incorporates a factor of 2 to account for the temporary designation of the ADI. The Committee made the ADI temporary pending the submission of studies to elucidate the potential developmental and reproductive toxicity of the rosemary extract under consideration. An additional uncertainty factor to account for the lack of a chronic toxicity study was not considered necessary based on the absence of adverse effects in the short-term toxicity studies at doses up to and including the highest dose tested.

The Committee noted that the dietary exposure estimates for rosemary extract for high consumers in the European and USA populations of 0.09–0.81 mg/kg bw per day (expressed as carnosic acid plus carnosol) may exceed the upper bound of the temporary ADI by up to 2.7-fold (for young children at the top end of the range of estimated dietary exposures). Based on the conservative nature of the dietary exposure assessments, in which it was assumed that all foods contained rosemary extracts at the maximum use level, the Committee concluded that this exceedance of the temporary ADI does not represent a safety concern. The Committee requested that data on typical use levels in foods be provided by the end of 2018 in order to refine the dietary exposure estimates.

These studies are currently being undertaken and will be submitted to JECFA by the end of 2018 as requested. The applicant, however, does not believe that waiting for these additional studies should hold up the progression of this application for a number of reasons (see Section 3.3.1 Part B.2 (d).).

EFSA evaluated rosemary extract in 2008 and again in 2015. On neither occasion did EFSA raise any safety issues. EFSA did not set an ADI, but concluded the use of rosemary extract does not pose a risk to health

Rosemary has been used as an herb in cooking for hundreds of years without any indication that it causes developmental or reproductive toxicity. It has been available, as an antioxidant, in the EU since 2009 and as a flavor, globally, for many decades prior without any indication or report of it causing developmental or reproductive toxicity. However, considering that rosemary extract may have more concentrated levels of constituents than found in rosemary herb, the applicant has sought to address this, while waiting for the additional studies requested by JECFA to be completed, by reducing the number of food categories being sought for the use of rosemary extract as compared to the EU. Further, in some food categories the applicant has reduced the maximum permitted level sought compared to the EU. These measures will reduce the overall exposure of rosemary extract thus providing a further level of safety.

J.2 Other National Standards or Regulations

United States

Rosemary extract is allowed in the United States as a flavour although it appears on some food labels as a preservative.

The applicant is pursuing a self-affirmed GRAS position for rosemary antioxidant extract that is based upon the EU and JECFA evaluations.

European Union

Rosemary extract (E392) is approved in the EU additives regulation No. 1129/2011. It was evaluated by the European Food Safety Authority (EFSA) in 2008 (EFSA, 2008) and then again in 2015 to extend it uses to fat based spreads (EFSA, 2015). A list of approved uses is provided in Appendix 3.

Japan

Rosemary extract appears as additive #365 in the Japanese Existing Additives List (Appendix 4). There are no specific use limits.

China

China has published a specification that is largely aligned with the EU except that some additional extraction solvents (such as hexane and methanol) are permitted. (GB 1886.172-2016 – Appendix 5A). Uses are published in the attached extracted page from GB2760-2015 (Appendix 5B).

Singapore

Rosemary extract is permitted for use as an antioxidant in foods and beverages in Singapore. Singapore adopts the EU and Codex specification. Permitted uses are listed in the 3rd Schedule in their Food Regulations (Appendix 6).

K Statutory Declaration

See Appendix 10.

L Checklist

See Appendix 11.

3.3.1 Food Additives

A. Technical Information on the Food Additive

A.1 Nature and Technological Purpose of the Additive:

Rosemary extract is intended for use as an antioxidant in various food and beverage applications. Extracts of the rosemary plant can have both flavouring and antioxidative properties, but of late are becoming popular as antioxidant alternatives for the stabilisation of oxygen-sensitive foods. In many cases both functions are utilised within a food; however, extracts can be optimised and marketed primarily for their antioxidant properties. De Raadt et. al. presents a short review of rosemary extract, its properties and uses. (de Raadt, et al., 2015).

Efficacy as an Anti-Oxidant:

Rosemary extract has significant antioxidative activity, mainly contributed from two key antioxidant components belonging to the classes of phenolic acids, flavonoids, diterpenoids and triterpenes, namely, carnosol and carnosic acid. Carnosol and carnosic acid, are phenolic diterpenes, that are responsible for the main antioxidant activity of rosemary extract (Addis & Warner, 1991); (Richheimer, et al., 1996).

There have been several reviews of antioxidants and in particular of rosemary extract regarding their efficacy. A recent review (Carocho, et al., 2018) of food antioxidants looked at the different antioxidant groups, describing their properties, function and applicability, as well as indexing the relevant legislation in order to be a guide for academia and industry. This review concludes rosemary extract is a useful natural antioxidant. Another short overview of the use and effectiveness of rosemary extract is also present by Robbins et. al. (Robbins & Sewalt, 2005).

A number of studies have been conducted over many years elucidating the efficacy of rosemary extract / carnosol / carnosic acid as an antioxidant in a number of different type of foods including meats, nuts, savoury snacks (chips), baked goods / bread, dehydrated potatoes and oils. A summary of these studies are presented below in **Table 1: Efficacy Studies**. These studies show that rosemary extract is an effective anti-oxidant when added to a variety of different foods.

Table 1: Efficacy Studies

Author / Reference	Food Studied	Dose	Conclusion
(Down, et al., 1999)	Meat / Ground Beef	(20-25 ppm carnosic acid + carnosol)	Ground beef with rosemary extract improved meat colour and oxidative stability when stored in commercial display conditions under oxygen permeable film.
(Naveena, et al., 2013)	Meat / buffalo and chicken patties	22.5 ppm carnosic acid phenols	Rosemary extracts added at 22.5 ppm carnosic acid phenols in ground buffalo and chicken patties significantly reduced oxidation in storage at 4ºC for up to 9 days (raw) or 28 days (cooked).
(St. Angelo, et al., 1990)	Meat / beef	5-20 ppm carnosic acid + carnosol	Rosmary extract added to ground beef at 5-20 ppm carnosic acid + carnosol inhibited oxidation and formation of warmed over flavor.
(da Trindade, et al., 2009)	Meat / Beef burgers	10-20 ppm carnosic acid + carnosol	Rosmary extract added to irradiated ground beef alone or in combination with oregano extract at 10-20 ppm carnosic acid + carnosol inhibited oxidation and formation of warmed over flavor
(Keokamnerd, et al., 2008)	Meat / Chicken	20-23 ppm carnosic acid + carnosol	Rosemary extracts in ground chicken thigh meat significantly reduced oxidation and improved color and cooked meat flavour after storage at 3ºC under 80:20 O_2:CO_2 atmosphere for up to 12 days.
(Nissen, et al., 2000)	Meat / Chicken	60 ppm carnosic acid + carnosol	Rosemary extract in dehydrated chicken meat (20 ppm on raw meat basis) significantly reduced oxidation and improved flavour.
(Todd Jr, 2000)	Meat / Various including beef, pork, poultry and fish.	20-200 ppm of carnosic acid + carnosol	Addition of rosemary extract at a level to provide 20-200 ppm of carnosic acid + carnosol is claimed to prevent rapid oxidation and flavour deterioration in irradiated meats.
(Yin, et al., 2016)	Meat / Pork	40-60 ppm carnosic acid	Rosemary extract (5-6% carnosic acid) added to ground pork at a level of 40-60 ppm carnosic acid lowered lipid and protein oxidation and improved cooking yield at 4-10 days storage at 4ºC. Use of rosemary extract also lowered pH of the meat, which may have contributed to a reduction in microbial growth during storage
(Lara, et al., 2011)	Meat / Pork	30-40 ppm carnosic acid	Commercial rosemary extract (estimated 10% carnosic acid) added to ground pork at approx. 30-40 ppm carnosic acid improved oxidative stability and color in storage for up to 6 days at 4ºC. Rosemary extract was superior to lemon balm and BHT treatments.
(Mielnik, et al., 2003)	Meat / Turkey	20-50 ppm carnosic acid	Commercial rosemary extracts added to raw processed turkey at 20-50 ppm carnosic acid reduced lipid oxidation during frozen (-25^{0}C) storage for

Author / Reference	Food Studied	Dose	Conclusion
			up to 7 months.
(Georgantelis, et al., 2007a)	Meat / Pork Sausages	40-50 ppm carnosic acid	Commercial rosemary extract (approx. 20-25% carnosic acid) at a level of 40-50 ppm carnosic acid improved the oxidative and microbiological stability of ground pork under refrigerated (-4ºC) storage for up to 20 days. The impact of rosemary was enhanced when added in combination with chitosan, which may function as a chelator of free iron and water retention aid.
(Georgantelis, et al., 2007b)	Meat / Beef Burgers	40-50 ppm carnosic acid	Commercial rosemary extract (approx. 20-25% carnosic acid) at a level of 40-50 ppm carnosic acid improved the oxidative stability and colour of ground beef under frozen (-18ºC) storage for up to 180 days. The impact of rosemary was enhanced when added in combination with chitosan, which may function as a chelator of free iron and water retention aid.
(Rocío Teruel, et al., 2015)	Meat / Chicken Nuggets	20-25 ppm carnosic acid + carnosol on a whole meat basis	Acetone and methanol extracts of rosemary significantly decreased oxidation of fried chicken nuggets after storage at -18ºC for 9 months. Dose level was 150 mg carnosic acid + carnosol/kg on fat content basis or 20-25 ppm carnosic acid + carnosol on a whole meat basis (assuming 15% fat).
(Grüner-Richter, et al., 2012)	Nuts	10-80 ppm carnosic acid	Pressurized impregnation of unshelled nuts (almond, hazelnut, peanut, walnut, macadamia nut) with 10-80 ppm carnosic acid from commercial rosemary extracts provided dose dependent improvement in lipid oxidative stability (i.e. lower rancidity) under ambient and accelerated (60 & 80ºC) temperatures (2 weeks to 10 months). The oxygen stability and hence the shelf life of nuts was increased with an increasing concentration of carnosic acid.
(Martínez, et al., 2013)	Nuts / Walnut oil	2-15 ppm carnosic acid	Commercial rosemary extract (1.22% carnosic acid) at a level of 2-15 ppm carnosic acid in freshly pressed walnut oil decreased lipid oxidation in dark storage at 25ºC for up to six months but did not protect significantly against light induced oxidation.
(Wang, et al., 2011b)	Nuts / Pine nut oil	50-200 ppm carnosic acid	Purified (92%) carnosic acid at a level of 50-200 ppm improved the oxidative stability of pine nut oil in dark storage at 60ºC and under UV radiation at 25ºC for 4 – 15 days and was more effective than comparable amounts of α-tocopherol or BHT.
(Wambura, et al., 2011)	Nuts / roasted peanuts	Not specified	Rosemary extract added to coatings of cellulosic polymers on roasted

Author / Reference	Food Studied	Dose	Conclusion
			peanuts at an unknown level of carnosic acid + carnosol inhibited peanut oxidation.
(Reblova, et al., 1999)	Savoury Snacks	Rosemary extract 500 ppm to give an estimated level of 50 ppm carnosic acid + carnosol.	Rosemary extract made from acetone or ethyl acetate extract was added to rapeseed oil at 500 ppm to give an estimated level of 50 ppm carnosic acid + carnosol (assuming 10% active compounds). This improved oil quality during frying and the sensory quality of fried potatoes
(Lalas & Dourtoglou, 2003)	Savoury Snacks / Potato Chips	60 ppm carnosic acid + carnosol	Rosemary extracted added to soybean frying oil at 60 ppm carnosic acid + carnosol inhibited oil oxidation, improved oil colour and sensory acceptability of potato chips in repetitive frying cycles at 180ºC. Chips prepared in later cycles and consumed between 0 and 60 days after frying (nitrogen packaged, ambient temperature storage) showed improved sensory acceptability. Oil that was extracted from the fried chips also demonstrated improved oxidative stability. Chips fried in treated oil were lighter in colour after repeated frying cycles. Potato chips fried in the oil with added rosemary extract were more acceptable than chips fried in oil containing no extract until the last frying
(Che Man & Tan, 1999)	Savoury Snacks / Potato Chips	approximately 10 ppm carnosic acid + carnosol	Rosemary extract added to palm frying oil at 10 ppm carnosic acid + carnosol inhibited oil oxidation, improved oil colour and sensory acceptability of potato chips fried in repetitive frying cycles at 180ºC. Rosemary extract significant improved oxidative stability by TBARS analysis during packaged storage up to 14 weeks after frying and outperformed BHA, BHT and sage extract treatments
(Jaswir, et al., 2000)	Savoury Snacks / Potato Chips	20-40 ppm carnosic acid + carnosol	Commercial rosemary extract at 20-40 ppm carnosic acid + carnosol inhibited changes in fatty acid profile of palm oil during repetitive frying relative to control and especially in combination with other antioxidant treatments.
(Urbancic, et al., 2014)	Savoury Snacks / Potato Chips	50 ppm carnosic acid + carnosol	Commercial rosemary extract added to frying oil at 50 ppm carnosic acid + carnosol outperformed tocopherols, tertiary butylhydroquinone and BHA for inhibiting oxidation of sunflower oil during frying of potato chips at 180ºC (4-6 frying cycles). Rosemary extract was also found to significantly inhibit acrylamide formation in the fried chips.
(Chammem, et al., 2015)	Savoury Snacks / Potato Chips	Not specified	Ethanol extract of rosemary slowed frying oil oxidation at 180ºC and improved sensory properties of fried potato chips

Author / Reference	Food Studied	Dose	Conclusion
(Guo, et al., 2016)	Savoury Snacks / Potato Chips	Rosemary extract at 0.12g/kg. Extract contained 8% carnosic acid and 10% carnosol	Ethanol extract of rosemary slowed palm oil oxidation at 165-180ºC in potato chip frying conditions. Under frying condition, the oil with rosemary ethanol extract showed an enhanced stability compared to the oil with synthetic antioxidants. Under accelerated storage condition, rosemary ethanol extract could prevent the palm oil from oxidation the same as the synthetic antioxidants.
(Frutos & Hernandez-Herrero, 2005)	Baked Goods / Bread	500-1500 ppm carnosic acid	This example illustrates the use of rosemary extract to stabilize oils used in baked goods. Commercial rosemary extract was added to a seasoned oil dressing at 500-1500 ppm carnosic acid, which was subsequently used on a freshly baked bread and toasted to 140ºC. Sensory evaluation of the baked/toasted bread with rosemary extracts showed better comparability to fresh samples after 6 and 12 days storage (50ºC).
(Nissen, et al., 2002)	Dehydrated potatoes	200 ppm rosemary extract in the final product	Commercial rosemary extract (10-20% carnosic acid + carnosol) inhibited oxidation of dehydrated potato flakes by a variety of analyses during storage in air at 22ºC for up to one year. Rosemary extract outperformed natural antioxidants from grape skin, green tea and coffee.
(Frankel, et al., 1996)	Fats and emulsions	50 ppm carnosic acid or carnosol	Rosemary extracts and carnosic acid + carnosol, in particular, effectively delayed formation of hexanal in heated (60ºC) corn oil and corn oil in water emulsions
(Tavasalkar, et al., 2012)	Fats and emulsions	0.1% rosemary extract containing 4.8% carnosic acid	Rosemary extract improved the oxidative stability of sunflower oil under accelerated (OSI) and ambient (peroxide value) storage conditions.
(Wang, et al., 2011a)	Fish Oil	Carnosic acid at 100-300 ppm.	Fish oil supplemented with 200ppm carnosic acid exhibited favourable antioxidant effects and is preferable for effectively avoiding oxidation.
(Erkan, et al., 2009)	Fits and emulsions	Carnosic acid or sesamol, were dissolved in methanol and added to oil samples at concentrations of 50-200 ppm	Carnosic acid was found to be a more effective antioxidant than sesamol for sunflower oil.
(Samotyja & Małecka, 2007)	Fat emulsions	40-50 ppm carnosic acid	Rosemary extract added at a level of 40-50 ppm carnosic acid inhibited oxidation in rapeseed oil and rapeseed oil emulsions.

A.2 Information to Enable Identification of the Additive:

Information to enable the identification of rosemary extract, including the chemical structure, the chemical name, the molecular weight and formula, and the common name, are presented below.

Rosemary (Rosmarinus officinalis L) is a small evergreen perennial shrub indigenous to European countries bordering the Mediterranean Sea. Although the entire plant is known to have uses for human applications, it is the extracts of the dried leaves that have common food and medicinal uses. The typical uses of rosemary plant and its extracts have been for its aroma and flavouring properties, in particular as a seasoning (spice) in food preparations, in its dried or fresh form. It is also used in herbal medicinal therapies, although the Therapeutic Goods Administration (TGA) allows Rosmarinus officinalis in topical preparations only.

Rosemary extract has significant antioxidative activity, mainly contributed from two key antioxidant components belonging to the classes of phenolic acids, flavonoids, diterpenoids and triterpenes, namely, carnosol and carnosic acid. Carnosol and carnosic acid, are phenolic diterpenes, that are responsible for the main antioxidant activity of rosemary extract (Addis & Warner, 1991); (Richheimer, et al., 1996). The structural formula for carnosic acid (**Figure 1**) and carnosol (**Figure 2**) are shown below.

The present application refers to rosemary extracts prepared using acetone or ethanol solvent extraction as outlined in the JECFA application and Food Chemicals Codex (FCC) specification (appendix 2). However, it should be noted that the EFSA application also refers to other solvent extraction process (hexane and supercritical carbon dioxide techniques). The principal antioxidative components of the extracts are the phenolic diterpenes carnosol and carnosic acid.

<u>Carnosol:</u>

CAS No: 5957-80-2

Molecular formula: $C_{20}H_{28}O_4$

Chemical Name: 2H-9,4a-(Epoxymethano)phenanthren-12-one, 1,3,4,9,10,10ahexahydro-5,6-dihydroxy-1,1-dimethyl-7(1- methylethyl), (4aR-(4aα,9α,10aβ))-

<u>Carnosic acid:</u>

CAS No: 3650-09-7

Molecular formula: $C_{20}H_{28}O_4$

Chemical Name: 4a(2H)-Phenanthrenecarboxylic acid, 1,3,4,9,10,10ahexahydro-5,6-dihydroxy-1,1-dimethyl-7-(1-methylethyl)-, (4aR-trans)-

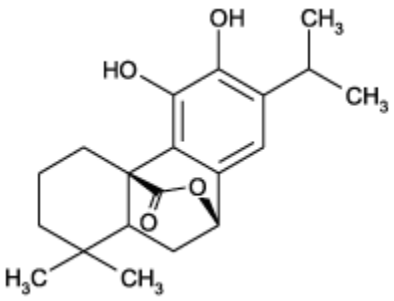

Figure 2: Chemical Structure of Carnosol in Rosemary Extract

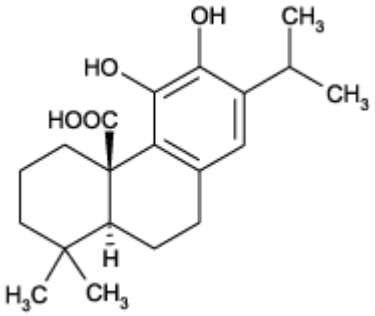

Figure 2: Chemical Structure of Carnosic in Rosemary Extract

In addition to antioxidants, rosemary extract also contains several reference volatiles that are responsible for its flavour and odour. The components of reference volatiles include 1,8-cineol (eucalyptol), camphor, borneol, verbenone, and bornyl acetate. While rosemary extract will contain both volatile and antioxidant compounds, they may be produced such that their antioxidative function is enhanced. Therefor the rosemary extract used principally for their antioxidant properties are selectively increased, and characterised by their carnosol and carnosic acid content (antioxidant), ratio with their key volatile components (flavour). The final levels of the total antioxidant component are adjusted by the choice of solvent extraction method used, in addition to post-processing deodorisation techniques. Rosmarinic acid, a depside of caffeic acid, and hydroxyhydrocaffeic acid is another identified compound in rosemary extracts from other solvent-based procedures that have shown complementary antioxidant activity (JECFA, 2016). It has also been reported that the primarily extracted oleoresins have some antioxidant activity.

A.3 Information on the Chemical and Physical Properties of the Additive:

Rosemary extract is a beige to light brown powder. It is insoluble in water, however soluble in oil and is frequently sold as a liquid in vegetable oil or other compatible carriers.

A.4 Information on the Impurity Profile:

Possible impurities of rosemary extract are (i) residues of solvents (i.e., ethanol and acetone) used in the manufacturing processes, (ii) any inorganic impurities and heavy metals, and (iii) any pesticide residues from the plant raw material. Studies have shown that antioxidant activity of rosemary extract under simultaneous storage and thermal stress; depend directly on the concentration of phenolic diterpenes (Schwarz & Ternes, 1992). Differences in rates of degradation of individual phenolic diterpenes at different temperatures were obtained. Studies have also showed that the degradation of carnosic acid and carnosol in ethanol increased with temperature, with the formation of some unique degradation products with exposure to light (JECFA, 2016). The major degradation products of carnosol were rosmanol, epirosmanol and epirosmanol ethyl ether. In addition to these, 11-ethoxy-rosemanol semiquinone, was identified as an indirect degradation product of carnosol at the highest storage temperature. The degradation of carnosic acid was identified to occur via an intermediate quinone, namely carnosic acid quinone. Carnosic acid quinone was identified to further degrade to carnosol. Another major degradant of carnosic acid was identified as 5,6,7-10-tetrahydro-7-hydroxy-rosmariquinone.

The JECFA and FCC specifications include limits for the level of the residual solvents, namely, acetone or ethanol, to not more than 50 mg/kg or 500 mg/kg, respectively. Since the commercial products are further processed and diluted with food-grade carriers, the levels of residual acetone and ethanol are expected and demonstrated to be well below the limit set, in the finished product. Results from analyses of residual levels of acetone are included in batch analysis below (**Table 4**).

The applicant also analyses the dried rosemary leaves starting material for potential pesticide residues periodically and confirms that levels do not exceed published tolerance limits provided for rosemary.

A.5 Manufacturing Process:

Rosemary extracts are prepared by extraction from dried rosemary leaves. The present application refers to production processes using solvent extraction by ethanol or acetone, as also submitted to

JECFA and EFSA. Solvent extraction using hexane and supercritical carbon dioxide are also described in the EFSA opinion.

Rosemary extract is produced from ground dried rosemary leaves using one of two extraction solvents, namely, food-grade ethanol or acetone. The resulting mixture is separated from the dried leaves by concentration and/or precipitation. This is followed by filtration to remove the leaves residue, vacuum-based solvent evaporation, drying and sieving to obtain a fine powder of native extract of rosemary. The final product in commerce is obtained after the native extracts are further processed down-stream and may include deodorisation, and decolourisation steps with food-grade excipients. The native extract is a green colour powder, and the final product in commerce can be a fine beige powder or liquid after dilution with suitable food-grade carriers.

Chemical Characterisation

Composition

The final product consists of a limit of not less than 5% weight per weight (w/w) for total carnosic acid and carnosol content in the acetone and ethanol extracts of rosemary respectively. **Table 4** provides batch analyses for three native commercial batches to support the consistency of the manufacturing processes.

Analysis of rosemary extract show presence of tannins, polyphenols, polysaccharides, triterpenic acids, volatiles, phenolic diterpenes, as well as some protein matter and lipophilic substances. Studies have shown that the concentration of carnosic acid and carnosol in the final extract will be affected by the starting composition of the dried rosemary leaves, as well as the extraction process (JECFA, 2016). While the total content of carnosic acid and carnosol is variable depending on the particular extract of rosemary, the composition when the intended function is antioxidant comprises not less than 90% of the total of the two phenolic diterpenes. The total carnosic acid and carnosol levels of the two diterpenes in native rosemary extract using acetone, ranges from approximately 17 to 53 %w/w, while the levels of the two principal antioxidant components in native rosemary extract using ethanol ranged from approximately 16-49 %w/w. However, rosemary extract for use as antioxidant is not commercialised as such; rather, the carnosol and carnosic acid content is standardised to range from >5% to 25 %w/w by the addition of appropriate food-grade excipients and carriers (e.g., silicon dioxide, DATEM, Propylene glycol, Polysorbate 80, monoglycerides of fatty acids, sucroesters of fatty acids, lecithin, glycerol, gum arabic, modified starch, maltodextrin, vegetable oil, or medium chain triglyceride (MCT) oil).

In order to further distinguish between the components of flavouring and antioxidant, a ratio of key antioxidants level, i. e. total percent of carnosic acid and carnosol, to the reference volatiles present in each of the extracts is calculated. The reference volatiles that mainly contribute to the distinct aroma and flavour of rosemary are borneol, bornyl acetate, camphor, 1,8-cineol (eucalyptol), verbenone. The total % of carnosic acid and carnosol to the total % of reference volatiles: (-)-borneol, (-)-bornyl acetate, (-)-camphor, 1,8-Cineole (eucalyptol) and verbenone is not less than 15.

In addition to the active antioxidant principles and the reference volatiles, rosemary extract may also contain residual organic material from the rosemary plant such as proteins, lipids, resins and waxes, carbohydrates, and inorganic constituents. Levels of organic components in the dried rosemary leaves prior to and after the extraction processes demonstrate their low levels and/or absence in the final products for commerce.

Identity specifications include solubility, in addition to antioxidant/reference volatiles ratio. Purity specifications include limits for residual extraction solvents, of NMT 50 mg/kg of acetone, and 500 mg/kg of ethanol; a limit for lead at NMT 2 mg/kg, and arsenic at NMT 3mg/kg, and a limit for moisture levels (loss on drying) at < 5%, in addition to method of assay of the native extract.

Data on the analysis of the composition of the rosemary extracts was provided to EFSA (EFSA, 2008), including those used in the safety studies. A detailed analysis of single samples of the various extracts has been conducted to show the various components present. Secondly, consecutive batches have been analysed to show reproducibility.

To provide a direct comparison between the extracts and dried rosemary, data was submitted to EFSA (EFSA, 2008) which shows the analytical profile for all samples adjusted to 10% carnosol plus carnosic acid content (**Table 2**). The results show the comparability of the solvent extracts when characterised on the basis of the key active principals carnosol plus carnosic acid. Reference key volatiles are very low compared to dried rosemary. Similar analytical results for consecutive batches of D74 and F62 indicate reproducibility of these two extraction procedures. HPLC fingerprints for rosemary dried leaves, and extracts D74, F62, ARD, AR and RES reveal reproducibility of consecutive batches and also indicate the patterns of the different extracts to be comparable with carnosic acid and carnosol being major components (**Table 2**).

Table 2: HPLC fingerprints for rosemary dried leaves, and extracts D74, F62, ARD, A and RES

Parameter	Unit	Dried Leaves	Extract D74	Extract F62	Extract AR	Extract ARD	Extract RES
Phenolic diterpenes							
Carnosic acid	mg/g	15-25	240-260	155-175	30-50	60-80	110-150
Carnosol	mg/g	1-2	35-45	15-17	20-30	18-22	30-50
Triterpenic acids							
Betulinic acid	mg/g	10-15	130-145	85-110	15-30	55-65	60-80
Sum oleanic + ursolic acid	mg/g	20-35	130-150	185-220	35-45	130-170	90-120

D74 – supercritical carbon dioxide extraction; F62 – acetone extraction; AR- partially deodorised ethanol extraction; ARD - deodorised ethanol extraction; RES – hexane and ethanol extraction

Possible Impurities (including degradation products)

Possible impurities of rosemary extract are (i) residues of solvents (i.e., ethanol and acetone) used in the manufacturing processes, or their degradation products, (ii) any inorganic impurities and heavy metals, and (iii) any pesticide residues from the plant raw material. Studies have shown that antioxidant activity of rosemary extract under simultaneous storage and thermal stress depend directly on the concentration of phenolic diterpenes (Schwarz & Ternes, 1992). Differences in rates of degradation of individual phenolic diterpenes at different temperatures were obtained. Studies have also showed that the degradation of carnosic acid and carnosol in ethanol increased with temperature, with the formation of some unique degradation products with exposure to light (JECFA, 2016). The major degradation products of carnosol were rosmanol, epirosmanol and epirosmanol ethyl ether. In addition to these, 11-ethoxy-rosemanol semiquinone, was identified as an indirect degradation product of carnosol at the highest storage temperature. The degradation of carnosic acid was identified to occur via an intermediate quinone, namely carnosic acid quinone. Carnosic acid quinone was identified to further degrade to carnosol. Another major degradant of carnosic acid was identified as 5,6,7-10-tetrahydro-7-hydroxy-rosmariquinone.

Results from analyses of residual levels of acetone and ethanol from the two manufacturing processes for both the native and commercial rosemary extract are provided in **Table 4** . The specifications proposed by the sponsor limit the level of the residual solvents, namely, acetone or ethanol, to not more than 50 mg/kg or 500 mg/kg, respectively. Since the commercial products are further processed and diluted with food-grade carriers, the levels of residual acetone and ethanol are expected and demonstrated to be well below the limit set, in the finished product.

Analyses of the dried rosemary leaves starting material is also carried out for potential pesticide

residues periodically and confirms that levels do not exceed published tolerance limits provided for rosemary.

A.6 Specification for Identity and Purity:

Proposed specifications are provided in **Table 3** which are based on the FAO / WHO Rosemary Extract (Tentative) Monograph provided in Appendix 1 and the Food Chemical Codex specification for Rosemary Extract provided in Appendix 2.

Table 3: Proposed specifications for antioxidant rosemary extracts

Parameter:	**Requirement**
Description:	Beige to light brown powder
Assay	Not less than 5% of the total carnosic acid and carnosol
Solubility	Insoluble in water; soluble in oil
Antioxidants/Reference Volatiles Ratio	Total % of carnosic acid and carnosol /Total % of reference volatiles: (-)-borneol, (-)-bornyl acetate, (-)-camphor, 1,8-Cineole (eucalyptol) and verbenone: not less than 15
Loss on drying:	Not more than 5%
Residual solvents:	Acetone: Not more than 50 mg/kg Ethanol: Not more than 500 mg/kg
Arsenic:	Not more than 3 mg/kg
Lead:	Not more than 2 mg/kg

The identity assay for rosemary extract is intended to define the final products so as to differentiate the extract intended for use as antioxidant from its use as flavouring. The distinct feature of the product of commerce intended for use as antioxidant is the total content of the phenolic diterpenes, carnosic acid and carnosol, the principal constituents responsible for the antioxidative properties. The identity of the products of commerce is also verified by visual inspection and solubility.

The purity of the final product of commerce is established by determination of loss on drying, levels of residual extraction solvents (acetone and ethanol), and presence of inorganic matter, including arsenic and lead. At the 14th meeting, the Committee evaluated the safety of acetone and ethanol and concluded that use of either as an extraction solvent needed to be limited only by good manufacturing practice (GMP). The Committee also noted that residues resulting from their use under conditions of GMP are unlikely to have any significant toxicological effects (JECFA, 1970). The specifications for arsenic and lead are NMT 3 mg/kg and NMT 2 mg/kg, consistent with JECFA's limits for heavy metals in food additives (JECFA, 2002).

Table 4 below provides analytical results of 3 typical batches of rosemary extract tested in accordance with the JECFA and FCC specification.

Table 4: Analytical Results for 3 Batches of Rosemary Extract tested in Accordance with JECFA Specification

Parameter	JECFA Specification	Results		
		Lot 787567K	Lot 742769K	Lot 736387K
Solubility	Soluble in oil< Soluble in water	Complies	Complies	Complies
Loss on drying	<5%	<1%	<1%	<1%
Arsenic	<3mg/kg	<0.5mg/kg	<0.5mg/kg	<0.5mg/kg
Lead	<2mg/kg	<0.5mg/kg	<0.5mg/kg	<0.5mg/kg
Carnosic acid (CA) and carnosol (C) concentration	>5%	17.3%	13.2	11.1
%(CA+C)/% reference volatiles	>15	350	720	50 -135
Residual Solvents:				
Acetone	<50 mg/kg	<30mg/kg	<30mg/kg	<30mg/kg
Ethanol	<500 mg/kg	<50mg/kg	<50mg/kg	<50mg/kg

A.7 Information for Labelling:

Rosemary extracts are antioxidants as well as having flavour and odour properties enhancers when added to various food products. Rosemary extracts have been assigned the INS number of 392. Rosemary extracts will be labelled under its functional class, antioxidant, either as antioxidant (392) or antioxidant (rosemary extract).

A.8 Analytical Method for Detection

Analytical methods are based on the FAO/WHO Rosemary Extract (Tentative) Monograph provided in Appendix 1 and the Food Chemical Codex (FCC) specification for Rosemary Extract provided in Appendix 2.

The proposed assay method for the determination of carnosic acid and carnosol content in the rosemary extract is based on high-performance liquid chromatography coupled with a diode-array detector and is consistent with the HPLC method published in the monograph for rosemary extract in the FCC (FCC, 2016)

Two methods are used for the determination of levels of the reference volatiles used to calculate the antioxidants/reference volatiles ratio - gas chromatography coupled with mass spectrometry detection (GC-MS), and gas chromatography with flame ionisation detection (GC-FID). The GC-MS method was accepted in the specifications monograph.

The analytical method proposed to assay for residual solvents in rosemary extract is described in the Vol. 4, General Methods, Organic Components, Residual Solvents. All other methods in the specifications for rosemary extract, namely, solubility, loss on drying, and heavy metal analysis are standard methods, published in the Combined Compendium of Food Additive Specifications FAO JECFA Monographs 1, Vol 4 (JECFA, 2006).

A.9 Potential Additional Purposes of the Food Additive when Added to Food

Rosemary is a common herb used commonly in cooking. Extracts of the rosemary plant, in addition to their antioxidative properties, can also be used for flavouring. When used as flavours, it is more common to use the herb than flavouring substances containing extracts of rosemary (personal communication with Givaudan, Firmenich).

B Information related to the safety of the food additive

The information presented in this section is a summary of the information submitted to JECFA (JECFA, 2017). Where possible, we have included in the appendices a copy of the references, however, some unpublished references are not available to the applicant.

B.1 Information on the toxicokinetics and metabolism of the food additive and, if necessary, its degradation products or major metabolites

The disposition of carnosic acid (purity 98% and 91%) in male rats was determined following intravenous and oral gavage administration. In a study (Yan, et al., 2009), the plasma levels of carnosic acid following oral administration (90 mg/kg bw) revealed an apparent elimination half-life of 962 minutes, which was approximately 14 times longer than the apparent elimination half-life following intravenous administration (68 minutes). This result indicates that the terminal slope in the oral plasma concentration–time curve is not truly representative of the elimination process. It suggests that the rate-limiting step is likely the absorption of carnosic acid from the gastrointestinal tract and not its elimination from plasma. Orally administered carnosic acid (90 mg/kg bw) was detected in stomach, liver and small intestine at maximum concentrations of 1871, 16 and 34 µg/g, respectively, but it was not detected in other tissues with high blood flow, such as heart, kidney and lung (Zuo, 2008). It has been. reported that the time to peak concentration (Tmax) of carnosic acid in plasma following oral dosing was around 126 minutes, and the absolute bioavailability was calculated to be 65% (Yan, et al., 2009). A similar Tmax (137 minutes) was observed and a bioavailability of around 40% (Doolaege, et al., 2011). No evidence for enterohepatic circulation of carnosic acid was observed in either pharmacokinetic study following intravenous administration (Yan, et al., 2009), (Doolaege, et al., 2011).

Incubation of human and rat liver microsomes with carnosic acid resulted in similar metabolic profiles, providing evidence that carnosic acid undergoes similar biotransformation in the two species (Song, et al., 2014). It is reported (Zuo, 2008), (Song, et al., 2014) that carnosic acid is extensively metabolized in rats, with four metabolites detected in bile and faeces and an additional 15 detected in urine. Evidence indicates that carnosic acid can be oxidized to carnosol and further metabolized via glucuronidation and methylation reactions (Song, et al., 2014). The predominant metabolite of carnosic acid was glucuronidated carnosic acid. It has been reported that 15.6 ± 8.2% of carnosic acid administered orally was recovered in the faeces of rats over a 24-hour period post-administration (Doolaege, et al., 2011).

To identify the metabolites formed, a commercial rosemary extract (571 mg/kg bw, equivalent to 230 mg/kg bw expressed as carnosic acid) was administered to rats by gavage following a 24-hour fast. These rats had received the same extract in their diet for 2 weeks prior to the gavage administration. Carnosic acid was detected in plasma after 25 minutes, and this was considered to be the Tmax. The maximum plasma concentration for the main conjugate of carnosic acid, carnosic acid glucuronide, was reported at the last sampling time of 800 minutes. The most abundant metabolites quantified in plasma were the 5,6,7,10-tetrahydro-7-hydroxyrosmariquinone and carnosic acid 12-methyl ether. Nine major metabolites were identified in the liver. Small quantities of carnosic acid 12-methyl ether and carnosic acid (1.9–4.0 µg/g) were detected in brain tissue. Several metabolites of carnosic acid indicative of both glucuronidation and methylation were identified following the oral administration of a commercial rosemary extract to rats (Romo Vaquero, et al., 2013). These results were consistent with the metabolic profile elucidated for carnosic acid following oral administration to rats (Song, et al., 2014).

In summary, oral bioavailability for carnosic acid has been estimated to be 40–65%, characterized

by relatively slow absorption from the gastrointestinal tract. In vitro, similar metabolic profiles of carnosic acid have been observed using human and rat liver microsomes. In vivo, carnosic acid is extensively metabolized by direct glucuronidation and/or methylation reactions, as well as oxidation of carnosic acid to carnosol. Additional metabolites of carnosic acid and carnosol can undergo further glucuronidation, oxidation and/or methylation reactions, with several metabolites identified in liver, urine and faeces of rats.

Hepatic enzyme induction was reported in primary cultures of human hepatocytes following exposure of the cells to carnosic acid, as evidenced by up regulation of CYP2B6 and CYP3A4 mRNA levels in a concentration-dependent manner (Dickman, et al., 2012). In female rats treated with supercritical carbon dioxide extract of rosemary (33% weight per weight [w/w] carnosol plus carnosic acid content) at a dose equal to 195 mg/kg bw per day, total hepatic microsomal P450 content was increased by approximately 1.5-fold compared with controls following a 13-week treatment period; similar minimal increases were observed in levels of hepatic CYP2A, CYP2C11, CYP2E1 and CYP4A activity. No induction of activities associated with CYP1A, CYP2B or CYP3A was noted. This enzyme induction was observed to be reversible following a 4-week treatment-free period (JECFA, 2017). Elevated liver enzyme activity (glutathione S-transferase [GST] and quinone reductase) was also observed in mice and rats fed commercial extracts of rosemary in the diet at concentrations of up to 10 000 mg/kg (equivalent to up to 900 and 500 mg/ kg bw per day for mice and rats, respectively) for 2–4 weeks (Singletary, 1996), (Singletary & Rokusek, 1997)], but not for carnosol (Singletary, 1996).

B.2 Information on the toxicity of the food additive and, if necessary, its degradation products and major metabolites

A range of studies on acute toxicity, short-term toxicity and genotoxicity were evaluated in the safety assessment of rosemary extract by JECFA and EFSA. A summary is provided below.

(a) acute toxicity

Rosemary extracts and an isolated extract constituent, carnosic acid, have low acute oral toxicity in rats and mice. The oral median lethal dose (LD50) was greater than 2000 mg/kg bw for rosemary extracts administered by gavage to rats (Anadon, et al., 2008), (EFSA, 2008) and was 7100 mg/kg bw for carnosic acid administered by gavage to mice (Wang, et al., 2012).

(b) short-term toxicity

Short-term studies (14–90 days) investigating the toxicity of five different solvent extracts of rosemary (acetone, ethanol, deodorized ethanol, supercritical carbon dioxide and hexane-ethanol) administered in the diet were assessed in rats. Rats were administered extracts of rosemary in the diet at dose levels ranging between 26 and 400 mg/kg bw per day. Depending on the type of extract, the carnosic acid and carnosol content ranged from 5% to 33%, and the rats were exposed to carnosol and carnosic acid at a dose range of 3–69 mg/kg bw per day (JECFA, 2017).

In the 90-day studies conducted with solvent extracts of rosemary, a common observation was an increase in relative liver weight in treated animals compared with controls (10–21%). These observations in the liver were also associated with centrilobular hypertrophy, cytoplasmic characteristics of increased glycogen storage and increases in smooth endoplasmic reticulum. As no changes in clinical chemistry or any morphological features of liver damage were observed in the same studies, the JECFA Committee concluded that the observed hepatic changes are consistent

with a common adaptive response of rodent livers and are not adverse. Slight bile duct hyperplasia was observed in high- dose rats after 4 weeks of exposure to the hexane-ethanol extract (JECFA, 2017). The bile duct hyperplasia decreased with increasing duration of exposure and was not associated with any increase in blood bilirubin or enzyme markers indicative of biliary obstruction or hepatocyte damage. The JECFA Committee concluded that the observed bile duct hyperplasia in high-dose rats was not adverse.

NOAELs for each of these short-term studies were identified as the highest dose tested based on an absence of adverse effects. The highest NOAEL expressed as carnosic acid plus carnosol in the 90-day studies was 64 mg/kg bw per day.

(c) long-term toxicity and carcinogenicity

No chronic toxicity studies conducted with extracts of rosemary are available.

(d) reproductive toxicity & (e) developmental toxicity

Studies examining the potential reproductive or developmental toxicity of extracts of rosemary have not been conducted with any of the five solvent-based extracts. In a reproductive study conducted with a hydro-alcoholic (70% ethanol:30% water) extract of rosemary (Nusier, et al., 2007), significant effects related to reduced reproductive organ weights and sperm parameters were observed in male rats at a rosemary extract dose of 500 mg/kg bw per day in water. The relevance of this reproductive study was questioned by the JECFA Committee, as none of the commercial extracts used in the short-term feeding studies is soluble in water, and significant compositional differences between aqueous and solvent-based extracts of rosemary would be expected (Berdahl & McKeague, 2015). In the short-term toxicity studies conducted for each of the solvent-based extracts, no treatment- related adverse effects in reproductive organs of male or female rats were observed at doses up to 180–400 mg/kg bw per day, the highest doses tested, equivalent to approximately 20–64 mg/kg bw per day expressed as carnosol and carnosic acid, depending on the type of extract (JECFA, 2017). In a developmental toxicity study (Lemonica, et al., 1996), a water-based rosemary extract at a dose of 130 mg/kg bw per day caused no significant effects on preimplantation or post-implantation loss or on the number of variations or malformations in term fetuses. Again, both EFSA and JECFA questioned the relevance of this study given the significant compositional differences likely present between the water- and solvent-based extraction methods.

The JECFA Committee concluded that there are sufficient data to establish an ADI of 0.3 mg/kg-bw/day for rosemary extract. This ADI is based on NOAEL of 64 mg/kg-bw/day carnosol + carnosic acid (highest dose studied in multiple 90-day trials) and an uncertainty factor of 200. The Committee, however, made the ADI temporary pending the submission of studies to elucidate the potential developmental and reproductive toxicity of the rosemary extract under consideration. These studies are currently being undertaken and will be submitted to JECFA by the end of 2018 as requested.

The applicant, however, does not believe that waiting for these additional studies should hold up the progression of this application for the following reasons:

- The Committee established a temporary ADI of 0–0.3 mg/kg bw for rosemary extract, expressed as carnosic acid plus carnosol, on the basis of a NOAEL of 64 mg/kg bw per day, expressed as carnosic acid plus carnosol, the highest dose tested in a short-term toxicity study in rats, with application of a 200-fold uncertainty factor (UF). The overall UF of 200 incorporates a factor of 2 to account for the temporary designation of the ADI. This extra level of conservatism is a cautionary factor while waiting for the submission of data addressing developmental and

reproductive toxicity. However, even with this additional UF in place the mean exposure estimates summarized by JECFA indicate all populations except the most conservative, upper-bound estimates for children are below the temporary ADI.

- JECFA did not consider necessary an additional uncertainty factor to account for the lack of a chronic toxicity study based on the absence of adverse effects in the short-term toxicity studies at doses up to and including the highest dose tested.
- While there was a consistent finding of increased liver weight across 90-day studies, this resolved after washout and was concluded that this was a metabolic adaptive response and not an adverse effect. 90-day studies concluded no adverse effects up to highest doses tested – including sex organ tissue weights and histology.
- One Reproductive and one Developmental study exist, however both utilized uncharacterized materials produced in manner significantly different than industry standards. The NOAEL from this study is 250 mg/kg-bw/day rosemary extract – carnosol content undefined. The developmental study used a single dose (130 mg/kg-bw/day rosemary extract – carnosol content undefined) and while the authors concluded there was evidence of effect, the results were small and NOT statistically significant. EFSA decided not to include these studies in their opinion whereas JECFA questioned their relevance while requesting further clarity, hence the additional studies.
- While it is possible an effect could be found, as in the one reproductive study which indicated reduced male sex organ weight and function, this effect was NOT seen in 90-day studies (reduced organ weight). This study was available to EFSA during its review in 2008. EFSA concluded the material was significantly dissimilar and therefore did not consider it in their Opinion.
- EFSA did not set an ADI, but concluded the use of rosemary extract does not pose a risk to health. EFSA has reviewed rosemary extract in 2008 and again in 2015. On both occasions EFSA did not have any safety concerns with rosemary extract.
- Based on the 90-day study results and difference in material from previous reproductive study, the applicant anticipates these additional studies to have little to no effect on the NOAEL.

Rosemary has been used as a herb in cooking for hundreds of years without any indication that it causes developmental or reproductive toxicity. In addition, rosemary extract has been available, as an antioxidant, in the EU since 2009 and as a flavor, globally, for many decades prior without any indication or report of it causing developmental or reproductive toxicity. However, considering that rosemary extract may have more concentrated levels of constituents than found in rosemary herb, the applicant has sought to address this, while waiting for the additional studies requested by JECFA to be completed, by reducing the number of food categories being sought for the use of rosemary extract as compared to the EU. Further, in some food categories the applicant has reduced the maximum permitted level sought compared to the MPL in the EU. These measures will reduce the overall exposure of rosemary extract thus providing a further level of safety.

(f) genotoxicity

The genotoxicity potential was assessed for the supercritical carbon dioxide, ethanol and hexane-ethanol extracts of rosemary and the two primary constituents, carnosic acid and carnosol, in prokaryotic and eukaryotic test systems in vitro (EFSA, 2008) (JECFA, 2017) and in two in vivo assays (JECFA, 2017), (Gaiani, et al., 2006). The results did not indicate a genotoxic concern. No studies evaluating the genotoxicity potential of acetone extract of rosemary were identified; however, the JECFA assessment concluded that genotoxicity data for the acetone extract of rosemary were not

considered necessary, based on the absence of significant differences noted in the compositions of the solvent-based extracts or in the toxicological observations from the short-term toxicity studies.

(g) special studies, such as neurotoxicity or immunotoxicity

Not applicable.

Observations in humans

Published studies in which humans were administered commercial extracts of rosemary (extraction method not provided) reported that consumption of a single dose (0.32 mg/kg bw expressed as carnosol plus carnosic acid) (Samman, et al., 2001) or repeated doses (0.13 mg/kg bw per day expressed as carnosol plus carnosic acid) for 21 days (Sinkovic, et al., 2011) was not associated with adverse effects in young healthy individuals. In addition, rosemary (and its constituents) has a long history of consumption as part of the normal human diet as a seasoning herb.

B.3 Safety assessment reports prepared by international agencies or other national government agencies, if available

Rosemary extract has been reviewed by both the European Food Safety Authority (EFSA) and Joint FAO/WHO Expert Committee on Food Additives (JECFA). A summary of their safety assessments is presented below.

JECFA

JECFA established a temporary ADI of 0–0.3 mg/kg bw for rosemary extract, expressed as carnosic acid plus carnosol, on the basis of a NOAEL of 64 mg/kg bw per day, expressed as carnosic acid plus carnosol, the highest dose tested in a short-term toxicity study in rats, with application of a 200-fold uncertainty factor. The overall uncertainty factor of 200 incorporates a factor of 2 to account for the temporary designation of the ADI. The Committee made the ADI temporary pending the submission of studies to elucidate the potential developmental and reproductive toxicity of the rosemary extract under consideration. An additional uncertainty factor to account for the lack of a chronic toxicity study was not considered necessary based on the absence of adverse effects in the short-term toxicity studies at doses up to and including the highest dose tested. The temporary ADI applies to rosemary extract that meets the specifications outlined in Appendix 1.

The Committee noted that the dietary exposure estimates for rosemary extract for high consumers in the European and USA populations of 0.09–0.81 mg/kg bw per day (expressed as carnosic acid plus carnosol) may exceed the upper bound of the temporary ADI by up to 2.7-fold (for young children at the top end of the range of estimated dietary exposures). Based on the conservative nature of the dietary exposure assessments, in which it was assumed that all foods contained rosemary extracts at the maximum use level, the Committee concluded that this exceedance of the temporary ADI does not necessarily represent a safety concern.

EFSA

Following a request from the European Commission, the Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) was asked to deliver a scientific opinion on the safety in use of rosemary extracts when used as an antioxidant (EFSA, 2008).

This EFSA opinion refers to rosemary extracts prepared using several solvent extraction techniques as follows (note: the present application is for the ethanol and acetone extracts only):

- F62: rosemary extract produced from dried rosemary leaves by acetone extraction,
- D74: rosemary extract prepared by extraction of dried rosemary leaves by means of supercritical carbon dioxide,
- AR: rosemary extract prepared from a partially deodorised ethanolic extract of rosemary,
- ARD: extract prepared from a deodorised ethanolic extract of rosemary,
- RES: extract which is a decolourised and deodorised rosemary extract obtained by a two-step extraction using hexane and ethanol.

The principal antioxidative components of the extracts are the phenolic diterpenes carnosol and carnosic acid.

Four of the five rosemary extracts considered in this opinion, (D74, AR, ARD, and RES) were tested for genotoxicity. Several in vitro genotoxicity studies were performed in both prokaryotic and eukaryotic test systems and an in vivo mouse micronucleus test performed with rosemary extract RES. The Panel concluded that these do not give rise to safety concerns with respect to genotoxicity of the rosemary extracts.

Antioxidant rosemary extracts have low acute and sub-chronic toxicity in the rat. Sub-chronic studies on all five solvent extracts (D74, AR, ARD, F62, RES) reveal that the only effect at high doses of these rosemary extracts is a slight increase in relative liver weight. This effect has been shown to be reversible and may be the result of Phase I and II enzyme induction. The effect was not accompanied by increases in plasma levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (AP). Considering the low magnitude, reversibility and the nature of the hepatic changes, and the absence of increases in plasma ALT, AST and AP, the Panel concludes that the minor increase in the liver weight reported, accompanied by minimal centrilobular hypertrophy and microsomal enzyme induction, represent an adaptive response and are not of toxicological concern.

Overall, the 90-day feeding studies in rats with the different rosemary extracts tested, including AR, ARD, RES and D74, reveal NOAEL values in the range of 180 to 400 mg extract/kg body weight/day equivalent, depending on the carnosol and carnosic acid content of the respective extracts, to 20-60 mg /kg bw /day of carnosol plus carnosic acid.

The toxicological data on the rosemary extracts were insufficient to establish a numerical ADI, because the toxicity data set does not provide reproductive toxicity studies or a long term study. On the other hand, the existing data, including the absence of effects in the 90-day studies on reproductive organs and lack of genotoxicity, do not give reason for concern.

Dietary exposure to carnosol plus carnosic acid has been estimated for adults and pre-school children (aged 1.5 to 4.5 years) and amounts to mean values of respectively 0.04 and 0.11 mg carnosol plus carnosic acid/kg bw/day, 0.10 and 0.20 mg carnosol plus carnosic acid/kg bw/day at the 95th percentiles and 97.5th percentile values of 0.12 and 0.23 mg carnosol plus carnosic acid/kg bw/day.

The Panel noted that the margin between the NOAEL range in the 90-day rat studies with all five extracts of 180 to 400 mg extract/kg bw/day equivalent to 20-60 mg/kg bw/day of carnosol plus carnosic acid, and the dietary exposure estimates for adults would amount between 500- 1500 for the mean intake values, between 200-600 for the 95th percentile values and between 167-500 for the 97.5th percentile values. For pre-school children these margins would amount to respectively at least 182-546, 100-300 and 87-261. The Panel notes that these margins of safety are worst case estimates since the NOAELs from the different studies were generally the highest dose levels tested, and that the estimates of dietary exposure were conservative.

Therefore, the Panel was of the opinion that the margin of safety is high enough to conclude that dietary exposure resulting from the proposed uses and use levels are not of safety concern.

The Panel notes that to achieve these levels of dietary exposure, high level consumers would need to select a diet that was entirely composed of foods containing rosemary extracts for those food categories in which it was permitted. In reality not all processed foods will contain added antioxidants and it seems unlikely that these extracts would be used at the maximum usage level in all the proposed food in each category or that some consumers would systematically always choose all foods containing rosemary extract.

Based on the margins of safety identified, the Panel concluded that the use of rosemary extracts at the proposed uses and use levels would not be of safety concern.

In 2015, EFSA published an opinion on an extension of use of extracts of rosemary (E 392) in fat-based spreads (EFSA, 2015). Following a request from the European Commission, a refined exposure assessment was carried out based on the maximum permitted levels (MPLs) authorised in Annex II of Regulation (EC) No 1333/2008 for extracts of rosemary (E 392) and the extension of its use in fat-based spreads at the levels proposed by the applicant of 30 mg/kg and 100 mg/kg. This was not covered by the previous re-evaluation of the safety of extracts of rosemary (E 392) as a food additive performed by EFSA in 2008. In that previous opinion, it was noted that, whilst the data were insufficient to establish a numerical ADI, the margin of safety was high enough to conclude that dietary exposure resulting from the proposed uses and use levels was not of safety concern. In providing a scientific opinion on the safety of the proposed extensions of use, the ANS Panel has decided that a comparison of the exposure resulting from the current uses and use levels with the exposure resulting from these additional proposed uses would be sufficient to address the safety of extracts of rosemary. The Panel concluded that, since the two additional uses for rosemary extracts in fat-based spreads would not change the estimated exposure to the food additive compared with the already approved permitted uses in any part of the population, the conclusions made by the AFC Panel in 2008 regarding safety remain valid. Therefore, the Panel considered that it is unlikely that there is a safety concern with the current permitted uses together with the additional proposed extension of uses compared with the current permitted uses alone.

C Information Related to the Dietary Exposure of the Food Additive

C.1 A list of the food groups or foods proposed to contain the food additive, or changes to currently permitted foods

Please refer to **Table 5: Proposed Food Uses and Maximum Permitted Levels** below for proposed foods and maximum proposed levels to contain the food additive.

C.2 The maximum proposed level or the concentration range of the food additive for each food group or food, or the proposed changes to the currently permitted levels

Please refer to **Table 5: Proposed Food Uses and Maximum Permitted Levels** below for proposed foods and maximum proposed levels to contain the food additive.

Table 5: Proposed Food Uses and Maximum Permitted Levels

Category No.	Description	Proposed MPL (mg/kg)*	Included Foods	Excluded Foods	Comments / Examples
2.1	Edible oils essentially free of water	50	Fish oil and algal oil only		
2.2.2	Oil emulsions (<80% oil)	75	Margarines (solid and liquid) only		
4.3.4	Fruit and vegetable spreads including jams, chutneys and related products	50	Nut butters and nut spreads only		
5.4	Icings and frostings	20	Icings, frostings, glazings and fillings		
6.3	Processed cereals and meal products	50	Grain bars, breakfast bars, breakfast cereals only		
6.4	Flour products (including noodles and pasta)	10	Flour based snacks (examples: pretzels, fritters, crackers etc)	Not pasta or noodles	
7.2	Biscuits, cakes and pastries	40	Cookies, pancakes, waffles, sweet pastries (rolls, doughnuts, muffins)		
8.2	Processed meat, poultry and game products in whole cuts or pieces	1.5 37.5	Meat with a fat content not higher than 10%, excluding dried sausages Meat with a fat content > 10%, excluding dried sausages		
8.2.3	Dried Meat	37.5			
8.3.2	Sausage and sausage meat containing raw, unprocessed meat	50	Dried sausages only		

12	Salts and condiments	40		Not condiment sauces such as ketchup, mayonnaise, mustard or relishes.	
20.2.04	Sauces and toppings (including mayonnaises and salad dressings)	50			Examples: Dipping sauces, gravy, salad dressings, BBQ and chili sauce, marinades
20.2	Foods not included in items 0 to 14 - Food other than beverages	50	Processed nuts only		
20.2	Foods not included in items 0 to 14 - Food other than beverages	20	Potato chips, including starch based snacks from roots and tubers, pulses and legumes		

***Based on whole food, expressed as the sum of carnosol and carnosic acid**

C.3 For foods or food groups not currently listed in the most recent Australian or New Zealand National Nutrition Surveys (NNSs), information on the likely level of consumption

Not applicable.

C.4 The percentage of the food group in which the food additive is proposed to be used or the percentage of the market likely to use the food additive

Based on the experience in the EU, where rosemary extract has been available for use as an antioxidant since 2009, it is estimated that typical us levels is about 50% of the MPL except in the categories of fats and oils, where the use level is probably around 75-80%.

According to the Innova database[1], less than 2% of products contain rosemary extract in both the EU (Appendix 7) and the USA (Appendix 8). The data presented in appendices 7 and 8 are broken down by category and by country for the EU. A definition of the product categories used in the Innova database is provided in Appendix 9.

It should be noted that the TGA has listed rosemary oil and Rosmarinus officinalis as a permitted ingredient in complementary medicines for topical preparations only.

C.5 Information relating to the use of the food additive in other countries, if applicable

Estimates of dietary exposure to rosemary extract as an antioxidant for populations in Europe and the USA were available to the JECFA from the sponsor and EFSA (EFSA, 2008, 2015). The Committee noted that the estimates for these two population groups are considered to be conservative estimates of dietary exposure, in that it is assumed that all food products within a food category contain rosemary extract at the maximum permitted level of use. The highest estimates of dietary exposure to rosemary extract for consumers in European populations were observed for toddlers (0.09–0.44 mg/kg bw per day) at the mean level of exposure and for children aged 4–9 years (0.25–0.81mg/kg bw per day) at the 95th percentile exposure (expressed as carnosol plus carnosic acid). The highest estimates of dietary exposure to rosemary extract for consumers in the population in the USA were for infants and young children aged 0–3 years, using food consumption data from the United States NHANES 2011–2012 in conjunction with the EU maximum permitted level of use for rosemary extract; the estimates (expressed as carnosol plus carnosic acid) were 0.18 mg/kg bw per day at the mean level of exposure and 0.40 mg/kg bw per day at the 90th percentile exposure. Two main contributors to dietary exposure for European populations were fine bakery wares (6.5–57.8%) and processed meat (8.1–63.4%) across all age groups. No information on the main contributing food groups in the USA was reported. Rosemary is also consumed as a seasoning, but use levels vary dramatically according to taste, and the Committee concluded that this contribution need not be further considered because of the conservative nature of the assumptions applied in the assessments for rosemary extract. Therefore, the JECFA Committee concluded that the overall dietary exposure estimates for high consumers in all age groups (95th percentile exposure in the EU and 90th percentile exposure in the USA) ranging from 0.09–0.81 mg/kg bw per day should be used for the safety assessment of rosemary extract.

[1] The Innova Database is an online, cutting-edge food and beverage product database that collect the latest data from more than 70 countries. www.innovadatabase.com/Home/Index

C.6 For foods where consumption has changed in recent years, information on likely current food consumption

Not applicable.

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