Food and Agriculture Organization of the United Nations

82nd JECFA - Chemical and Technical Assessment (CTA), 2016

ROSEMARY EXTRACT

Chemical and Technical Assessment (CTA)

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1. Summary

This Chemical and Technical Assessment (CTA) summarises data and information on rosemary extract submitted to the 82nd meeting of the Joint FAO/WHO Expert Committee on Food Additives (Committee) by Intertek Scientific and Regulatory Consultancy, in a dossier dated December 21, 2015 (Intertek, 2015 upon request by the 47th Codex Committee on Food Additives (CCFA). At the present meeting, the Committee was asked to evaluate all data necessary for the assessment of safety, dietary intake and specifications related to the use of rosemary extract (INS 392) as an antioxidant in a variety of food categories. This document discusses published information relevant to rosemary, rosemary extract, the production methodologies, and specifications.

The data presented for the safety assessment by 82nd JECFA is specific to rosemary extract prepared using acetone or ethanol as the extraction solvents. The extract is produced consistent with current Good Manufacturing Practices (cGMP) and Hazards Analysis and Critical Control Points (HACCP) principles. The rosemary extract is subject to selective extraction to increase the content of carnosic acid and carnosol, relative to the aromatic composition such as borneol, bornyl acetate, camphor, 1,8-cineol (eucalyptol) and verbenone (reference volatiles). Depending on the production method used, the rosemary extracts may also be subject to a deodorisation step, in order to reduce the levels of reference volatiles.

The rosemary extract has been reviewed at European Food Safety Authority (EFSA), and meet appropriate food-grade specifications as laid down under Commission Regulation (EU) No 231/2012 of 9 March 2012 laying down specifications for food additives listed in Annexes II and III to Regulation (EC) No 1333/2008 (EFSA, 2008).

2. Description

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.

Rosemary extract has significant antioxidative activity, mainly contributed from two key antioxidant components belonging to the classes of phenolic acids, flavonoids and diterpenoids, namely, carnosol (CAS No 5957-80-2, molecular formula $C_{20}H_{28}O_4$) and carnosic acid (CAS No 3650-09-7, formula $C_{20}H_{28}O_4$). 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 carnosol and carnosic acid are shown in Figure 1.

In addition to antioxidants rosemary extract also contain 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 (Scarpati & Oriente, 1958). It has also been reported that the primarily extracted oleoresins have some antioxidant activity.

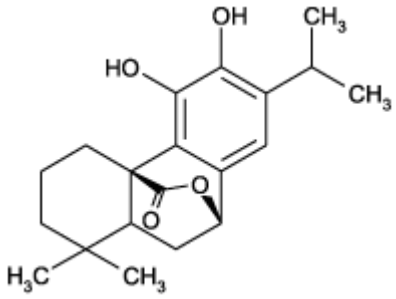
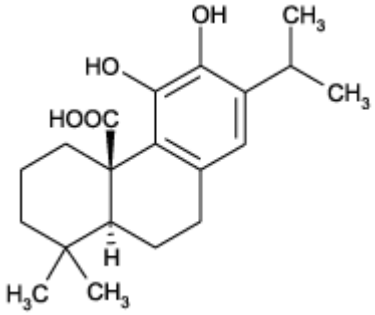


Figure 1: Carnosol



Carnosic acid

3. Method of Manufacture

The rosemary extract are 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 beige colour powders, and the final product in commerce can be a fine green powder or liquid after dilution with suitable food-grade carriers.

4. Chemical Characterization

4.1 Composition

The final product consist 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. The sponsor provided non-consecutive batch analyses for the two native extracts and the two commercial products 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 (Birtic et al). 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 by the sponsor. 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 expected to be not less than 15.

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

Identity specifications proposed by the sponsor 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 $\leq$ 5%, in addition to method of assay of the native extract.

4.2 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 et al. (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 (Y. Zhang et al, 2012). 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 sponsor provided results from analyses of residual levels of acetone and ethanol from the two manufacturing processes for both the native and commercial rosemary extract, respectively. 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.

The sponsor also analyses the dried rosemary leaves starting material for potential pesticide residues periodically and confirms absence of any pesticide residue in the final commercial product.

4.3. Analytical methods

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 extract of rosemary in the FCC (FCC, 2014).

Two methods were proposed for the determination of levels of the reference volatiles used to calculate the antioxidants/reference volatiles ratio, and they were, 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).

4.4. Rationale for proposed specifications

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).

5. Functional uses

5.1. Technological function

The rosemary extract is intended for use as 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.

5.2 Food categories and use levels

Rosemary extract is proposed by the sponsor for use as antioxidants in various food and beverage applications, as summarized in Table 1 In all applications the actual use level will vary according to the fat content of the food and the nature and intended use of the fat. The use-levels presented in Table 1 are considered maximum levels required to achieve the desired antioxidant, but there are instances when the use levels will be limited by the concurrent flavouring (and colouring) properties of the extract.

Table 1: Proposed food uses for rosemary extract (INS No. 392) for incorporation in the General Standard for Food Additives of the Codex Alimentarius Commission

Food Category No. and Food Category[a]		Maximum Proposed Use- Level for rosemary extract (mg/kg)[b]
01.0 Dairy products and analogues, excluding products of food category		
01.5.1	Milk powder and cream powder (plain) Milk powder for vending machines Dried milk for the manufacturing of ice creams	200* 30
02.0 Fats and oils, and fat emulsions		
02.1.2	Vegetable oils and fats	
	Fats and oils for the professional manufacturing of heat treated foodstuffs	50*
	Vegetable oils (excluding virgin and olive oils) and fat where content of polyunsaturated fatty acids is higher than 15 % w/w of the total fatty acid, for the use in non-heat treated food products	30*
	Frying oil and frying fat, excluding olive oil and olive pomace oil	50*
	Algal oils	50*
02.1.3	Lard beef, tallow, fish oil, and other animal fats	50*
02.2.2	Fat spreads, dairy fat spreads and blended spreads Spreadable fats with a fat content less than 80 %	100*
04.0 Fruits and vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds		
04.2.2.2	Dried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds Only seaweed based fish roe analogues Dehydrated potato products	200 200
04.2.2.5	Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed purees and spreads (e.g., peanut butter) Only nut butters and nut spreads	200*
05.0 Confectionery		
05.3	Chewing gum	200
05.4	Decorations (*e.g.*, for fine bakery wares), toppings (non-fruit) and sweet sauces Sauces only	100*
06.0 Cereals and cereal products, derived from cereal grains, from roots and tubers, pulses, legumes and pith or soft core of palm tree, excluding bakery wares of food category 07.0		
06.4 Pastas and noodles and like products (e.g. rice paper, rice vermicelli, soybean pastas and noodles)		

Food Category No. and Food Category[a]		Maximum Proposed Use-Level for rosemary extract (mg/kg)[b]
	Filling of stuffed dry pasta	250*
07.0 Bakery wares		
07.1.2	Crackers, excluding sweet crackers	200*
07.2	Fine bakery wares (sweet, salty, savoury) and mixes	200*
08.0 Meat and meat products, including poultry and game		
08.1.2	Fresh meat, poultry, and game, comminuted	150*^, 15‡
08.2.1	Non-heat treated processed meats , poultry and game products in whole pieces or cuts Dehydrated meat Non-heat treated processed meat	150 150*^, 15‡
08.2.2	Heat treated processed meats , poultry and game products in whole pieces or cuts Dehydrated meat	150*^, 15‡ 150
08.3.1	Non-heat treated processed comminuted meat, poultry, and game products	
08.3.2	Heat-treated processed comminuted meat, poultry, and game products	
	Dried sausages	100
	Dehydrated meat	150
09.0 Fish and fish products, including molluscs, crustaceans, and echinoderms		
09.2	Processed fish and fish products, including molluscs, crustaceans, and echinoderms	150*^, 15‡
09.3	Semi-preserved fish and fish products, including molluscs, crustaceans, and echinoderms	150*^, 15‡
09.4	Fully preserved, including canned or fermented fish and fish products, including molluscs, crustaceans, and echinoderms	150*^, 15‡
10.0 Eggs and egg products		
10.2	Egg Products	200
12.0 Salts, spices, soups, sauces, salads, protein products		
12.2.2	Seasonings and condiments	200*
12.4	Mustards	100*
12.5	Soups and broths	50
12.6	Sauces and like products	100*
13.0 Foodstuffs intended for particular nutritional uses		
13.6	Food supplements	400
Food Category No. and Food Category[a]		Maximum Proposed Use- Level for rosemary extract (mg/kg)[b]

15.0 Ready-to-eat savouries		
15.1	Snacks – potato, cereal, flour or starch based (from roots and	50*
15.2	Processed nuts, including coated nuts and nut mixtures (with e.g.	200*
15.3	Snacks-fish based	50*

[a] Food category system (Annex B) of the GSFA of the Codex Alimentarius Commission *[CODEX STAN 192- 1995]* (Codex Alimentarius Commission, 2015)
[b] Expressed as sum of carnosol and carnosic acid.
* Based on fat content.
^ Products with a fat content higher than 10%.
¥ Products with a fat content not higher than 10%.

6. References

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