Short Review of Extracts of Rosemary as a Food Additive

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Authors’ contributions

This work was carried out in collaboration between all authors. Authors HV and SW designed the work. Author PdR conducted the literature research, analysed the data and wrote the first version. Authors HV, SW and EV were responsible for subsequent reviewing and scientific editing, while author PdR was the primary responsible for final content. All authors read and approved the final manuscript.

ABSTRACT

Extracts from Rosmarinus officinalis L., more commonly known as rosemary, have been approved for use in the EU as food additive E932 under Regulation 1333/2008 of the European Parliament and the Council. Rosemary extracts are currently widely used to increase shelf life of food products. Rosemary extracts are characterised by two reference antioxidant compounds, carnosol and carnosic acid. This characterization allows for differences in rosemary extracts. Four approved production methods, as described by Commission Regulation 231/2012, produce rosemary extracts with different compositions and antioxidant activity. This results in difficulties to compare scientific data and to assess the safety of approved rosemary extracts. Based on unpublished studies for each of the four approved extract types, EFSA concluded that “the proposed uses and use levels would not be of safety concern”. Yet, gaps in knowledge still exist for the approved extracts as many different rosemary extracts are used in scientific research.

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1. INTRODUCTION

Oxidative degradation by free radicals in foods results in a reduction of quality, odour, and texture, but this process can be delayed by the addition of antioxidants. In the food industry, both synthetic and natural antioxidants are being used to improve the storage stability of foods. The benefit of synthetic antioxidants originates from the high level of activity in comparison with natural anti-oxidants [1]. However, some synthetic antioxidants have shown potential adverse effects. For example, butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) have been extensively used as synthetic antioxidants in foods. However, after research showed a potential carcinogenic effect [2,3] regulations regarding these synthetic antioxidants became restricted. Recent research however contradicts this by showing a potential anti-carcinogenic effect [4,5]. As a consequence of the contradiction and the general consumer request for natural products, the use of natural antioxidants has increased in recent years.

*Rosmarinus officinalis* L., more commonly known as rosemary, belongs to the Lamiaceae family of herbs and is a popular spice originating from Mediterranean areas. Traditionally it was used for flavour and aromatic reasons, but after the discovery of the antioxidant properties of rosemary extracts the European Rosemary Extract Manufactures Group (EREMG) applied for the authorization by the European Commission of rosemary extracts as a food additive. Subsequently, the European Food Safety Authority (EFSA) evaluated the safety of rosemary extracts in 2008. After the assessment of EFSA rosemary extracts were approved as a food additive and specified for use in the EU. Henceforth the extracts were formally labelled “E392 extracts of rosemary”. However, some gaps in knowledge were present during the assessment. To assess the current state of knowledge in relation with EU legislation a literature search was performed using PubMed with “rosemary extracts” “extracts of rosemary”, and “*Rosmarinus officinalis* L extracts” as search terms in combination with section specific search terms.

2. CHEMICAL STRUCTURE

Commission Regulation 231/2012 specifies approved food additive in Regulation 1333/2008, and defines rosemary extracts as: “Extracts of rosemary contain several components, which have been proven to exert antioxidative functions. These components belong mainly to the classes of phenolic acids, flavonoids, diterpenoids. Besides the antioxidant compounds the extract can also contain triterpenes and organic solvents extractable material specifically defined in the following specification”. More specifically, carnosol (CAS No 5957-80-2) and carnosic acid (CAS No 3650-09-7) (Fig. 1) are identified as the reference antioxidant compounds in extracts of rosemary, and should not comprise less than 90% of the total phenolic diterpenes in the extract.

![Fig. 1. Chemical structure of carnosol C_{20}H_{28}O_{4} (A) and carnosic acid C_{20}H_{26}O_{4} (B). Based on Senorans et al. [7].](image)

Commission Regulation 231/2012 only mentions carnosol and carnosic acid specifically as reference compounds, as a consequence all other antioxidant compounds in rosemary extracts can vary. Table 1 illustrates the findings from Almela et al. [6] on the compounds present in rosemary extracts. Although, many compounds have been identified, there still remain unidentified substances. Senorans et al. [7] found several non-identified compounds using a liquid chromatographic and mass spectrometric analysis. Furthermore, both studies concluded that the exact composition for rosemary extracts was influenced by the extraction and purification process used. Additionally, the composition of active components in the final extract was found to be dependent on which parts of the rosemary plant were used in the extraction process, and whether or not they were dried [8]. Furthermore, evidence exists that the composition of rosemary essential oil is influenced by the harvesting time [9]. Although, rosemary essential oil and
rosemary extracts are different, both are produced from the leaves. Therefore rosemary extracts composition might also be influenced by harvest time, however there is no evidence to either support or contradict this.

### 3. MANUFACTURING

Within the EU, food additives, in accordance with Regulation 1333/2008, need authorization prior to their use in foodstuffs. Article 12 of this Regulation limits the production of a food additive to the specified production methods as included in the Community list of approved food additives. Significant changes in the production method, starting materials or a change in particle size requires additional authorization.

In accordance with Commission Regulation 231/2012 extracts of rosemary can be produced from dried leaves of rosemary using four methods. The first method utilizes acetone to dissolve the active components from rosemary leaves. After filtration the solvent is evaporated and the residue is dried and sieved by conventional food processing methods. The second method is based on supercritical extraction substances at a temperature and pressure above its critical point. As a result the supercritical fluid can effuse through solids like a gas and dissolve materials from this solid similarly to a liquid. In this manufacturing method supercritical CO₂ is used to dissolve the active components. In the second step of the manufacturing process the extract is separated from the supercritical CO₂ [10]. Ethanol extracts of rosemary constitute the third manufacturing method. This method is similar to the first mentioned method, but ethanol is used as a solvent and deodorizer [10]. The fourth and last approved method for the manufacturing of rosemary extracts is also based on a two-step process. Extracts of rosemary are produced in accordance with the third method; subsequently the deodorized ethanol rosemary extracts are decolorized with a hexane extraction [10]. Regardless of the method used, the extracts can be combined with, by Regulation 1333/2008 approved, food grade carriers or standardized to a specific carnosol plus carnosic acid content.

The first two manufacturing methods produce rosemary extracts with similar compositions. However, supercritical fluid extraction produces extracts with a higher antioxidant concentration than organic solvents [11]. Furthermore, supercritical fluids have a higher diffusion coefficient, lower viscosity, lower surface tension for rapid penetration, and are more selective in extraction substances [12]. Additionally, the supercritical extraction method provides the possibility to remove flavour from the rosemary extract, although this characteristic of the method is not specifically mentioned in Regulation 231/2012 [13]. Aside from a different composition the third method produces an odourless extract and the fourth method produces both an odourless and colourless extract.

### Table 1. Main compounds identified in rosemary extracts. Based on Almela et al. [6]

<table>
<thead>
<tr>
<th>Type of compound</th>
<th>Chemical name</th>
<th>Related compounds</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenolic acids</td>
<td>Rosmarinic acid</td>
<td>Methyl Carnosate (C₂₀:COOCH₃)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Carnosol (δ-lactone bridge C₂₀: C₇)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Episorskaran (δ-lactone bridge C₂₀-C₇: C₆:OH)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rosmarin (γ-lactone bridge C₂₀-C₆: C₇:OH)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Epirosmanol (γ-lactone bridge C₂₀-C₆: C₇:OH)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Epirosmanol-methylether (γ-lactone bridge C₂₀-C₆: C₇:OH)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rosmadial (lactone bridge C₂₀-C₁₁: C₆: C₇: CHO)</td>
</tr>
<tr>
<td>Diterpenes</td>
<td>Carnosic Acid</td>
<td>6-hydroxyxuteol 7-glucoside</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Genkwanin (7-methyl ether)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4&quot;-methoxysystematricherin (4&quot;-7-dimethyl ether)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Homoplataginin (6-methyl ether, 7-glucoside)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Scutellarein (6-hydroxy)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4”,5,7,8-Tetrahydroxyflavone (8-hydroxyapigenin)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cirsimaritin (6,7-dimethyl ether)</td>
</tr>
<tr>
<td>Flavones</td>
<td>Luteolin</td>
<td>6-hydroxyxuteol 7-glucoside</td>
</tr>
<tr>
<td></td>
<td>Apigenin</td>
<td>Genkwanin (7-methyl ether)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4&quot;-methoxysystematricherin (4&quot;-7-dimethyl ether)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Homoplataginin (6-methyl ether, 7-glucoside)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Scutellarein (6-hydroxy)</td>
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<tr>
<td></td>
<td></td>
<td>4”,5,7,8-Tetrahydroxyflavone (8-hydroxyapigenin)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Cirsimaritin (6,7-dimethyl ether)</td>
</tr>
</tbody>
</table>
In 2002, Ibanez et al. [14] reported on the use of subcritical water for the extraction for rosemary extracts. In short, subcritical water (H₂O at a temperature and pressure below its critical point) is used as a solvent, and in a second step the extracts of rosemary are separated from the subcritical water. It was concluded that this extraction method produces extracts with similar antioxidant activity as supercritical fluid extraction, however the extract composition was different. The petitioner specifically excluded water extracts within its application. Subsequently, water based extracts have not been assessed by EFSA and are not incorporated in Regulation 321/2012.

4. USES IN FOODS

4.1 Function in Foods

Unless intentionally removed, the extracts of rosemary still contain flavourings and can be used to improve the flavour of products, however rosemary extracts are currently widely used to increase shelf life of food products due to the high antioxidant activity of its main components [15,16]. Research on the antioxidant activity of the compounds in rosemary extract in foods is primarily focussed on carnosic acid. The potency of carnosic acid in comparison to the other compounds in rosemary extracts in soybean oil was found to be more than twice as high based on Rancimat measurements [17]. Furthermore, the same study also found higher antioxidant activity for carnosic acid in comparison to the synthetic antioxidants BHT and BHA, whereas tertiary butylhydroquinone (TBHQ) was found to surpass rosemary extracts in antioxidant activity. Zhang et al. [18] found the similar results when comparing BHT, BHA, and TBHQ with carnosic acid based on peroxide values, TBARS assays, free fatty acid measurements, and p-anisidine values in sunflower oil. However, antioxidant activity of rosemary extracts seems to depend on the medium. Frankel et al. [19] measured the antioxidant activity of both carnosol and carnosic acid in a corn oil emulsion and bulk oil using hydroperoxide and hexanal formations and found reduced antioxidant activity in the emulsion system. Furthermore, antioxidant activity at pH 7 was lower compared to activity at pH 4 and 5. Koleva et al. [20] found a similar difference between bulk oil measurements and emulsion oil measurements for rosmaric acid.

The aforementioned findings suggest that rosemary extracts might not increase shelf life in all products, but evidence exist for several food categories. Using the TBARS assay, Sebranek et al. [21] found similar antioxidant activity for an unspecified rosemary extracts as BHA and BHT in precooked frozen pork sausages, but in raw frozen pork sausages rosemary extracts was found to be more effective than BHA and BHT in preventing increased TBARS values, and in preventing loss of red colour. Moreover, Nassu et al. [22] investigated the efficacy of a commercial albeit unspecified rosemary extract in fermented goat sausage using a TBARS assay and found a significant difference in oxidation with 0.050% w/w extract compared to controls. Using peroxide values according to the AOCS (1989) method, Frutos et al. [23] found significant protection against oxidation in bread with an oil, garlic and parsley dressing when using a 4 g/l concentration of rosemary extracts (carnosic acid (20–30%), rosmarinic acid (0–1%) and rosmanol (0.5–1.5%)). Despite the possible differences in the extract composition, these studies depict the efficacy of rosemary extracts in prolonging oxidative degradation in actual food products, however no evidence was found for the approved rosemary extracts.

4.2 Dietary Exposure

In 2008 EFSA published its opinion on the use of extracts of rosemary as a food additive [24]. As part of this opinion, expected dietary exposure was assessed based on consumption in the UK. The UK National Dietary and Nutrition Survey for adults [25] and pre-school children [26] were used as the main data sources. Despite the exclusion of rosemary as flavouring agent, a conservative estimate, based on the assumption of maximum usage levels in all proposed foods, was made. The potential mean exposure to carnosol plus carnosic acid from all proposed uses was estimated at 0.04 mg/kg bw/day for adults and 0.11 mg/kg bw/day for pre-school children. At the 90th, 95th and 97.5th percentile the expected intake was estimated at 0.08, 0.10 and 0.12 mg/kg bw/day for adults. For pre-school children this was estimated at 0.18, 0.20 and 0.23 mg/kg bw/day respectively.

5. LEGAL STATUS IN EU AND BEYOND

With the introduction of the legislative Package on Food Improvement Agents in December 2008 also the regulation of food additives in the European Union was reformed. Regulation 1333/2008 of the European Parliament and the Council, introduced one regime for the use of
food additives in the Union, food colours sweeteners and the remaining food additives that until then were regulated in separate Directives (respectively Directive 94/36/EC, Directive 94/35/EC and Directive 95/2/EC). In accordance with Regulation 1333/2008 rosemary extracts can only be used in the approved food categories, in food additive preparations, and in food grade carriers. For rosemary extracts the maximum levels of use for each approved food category ranges from 30 mg/l or mg/kg for fats and oils essentially free from water till 400 mg/l or mg/kg for food supplements. A more detailed description is shown in Table 2. For colour, beta-carotene, and lycopene preparations maximum level of 1000 mg/kg is allowed in the preparation and 5 mg/kg for the final product. Additionally, rosemary extracts may be added to all food flavourings with a maximum of 1000 mg/kg. These levels are expressed as the sum of carnosic acid and carnosol rather than the dosage of the whole rosemary extract. Regulation 1333/2008 also specifies labelling requirements, as a result extracts of rosemary can either be labelled with the E-number “E392” or “antioxidant: rosemary extract”.

Besides a maximum level of use, the purity of food additives is also specified by EU legislation (Commission Regulation 231/2012). Acetone extracts of rosemary should contain more than 10% w/w of carnosol plus carnosic acid and should not contain more than 500 mg/kg acetone. Supercritical extracts should contain more than 13% w/w and no more than 2% ethanol. Deodorized ethanolic extracts should contain more than 5% w/w and no more than 200 mg/kg ethanol. Decolorized and deodorized hexane and ethanol extracts should contain more than 5% w/w, and not more than 25 mg/kg hexane and/or 500 mg/kg ethanol. Moreover, all rosemary extracts should have an antioxidant/volatiles ratio higher than 15.

In its assessment, EFSA concluded that “the proposed uses and use levels would not be of safety concern” even though EFSA could not establish a numerical acceptable daily intake due to the lack of reproductive and development toxicity data [24]. On a global scale, rosemary extracts have not yet been evaluated by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). In March 2013 the Codex Alimentarius Commission expressed its intention to include rosemary extracts as an antioxidant in the standard for fish oils. However, to date rosemary extracts (internationally labelled as INS 392) has not been included in the Codex General Standard for Food Additives (GSFA) [27].

Within the United States (US) food additives can be used if one of three conditions are met. The Federal Code of Regulations 21 U.S.C. 321 – Definitions: Generally states that food additives as defined by that code are allowed when the additive has either received premarket approval from the Food and Drug Administration (FDA), has a Generally Recognized As Safe (GRAS) status, or was consumed in significant quantities prior to January, 1, 1958. Natural extracts of rosemary have received a GRAS status (Federal Code of Regulations 21 U.S.C. 182.20), which has been affirmed by the FDA [29]. Subsequently, rosemary extracts is listed in the ‘Everything added to food in the United States (EAFUS)’ database. As a result the use of rosemary extracts as a food additive is allowed in the US based on the general recognition of safety, rather than a safety assessment. Furthermore, no clear characterisation of natural extract of rosemary is described by US legislation.

6. BIOLOGICAL AND TOXICOLOGICAL DATA

6.1 Absorption, Distribution, Metabolism, and Excretion

Providing a complete description of the absorption, distribution, metabolism and excretion is difficult due to the complex nature of rosemary extracts. However, Vaquero et al. [30] attempted to establish a complete metabolic profile for all major bioactive components of rosemary extracts in Zucker rats. A commercial rosemary extract was used containing carnosic acid (38.9±1.7%), carnosol (6.5±0.1%), and methyl carnosate (6.9±0.6%). Other compounds or the production method were not specified. This study reported that after oral administration of 100 mg rosemary extract traces of both carnosol and carnosic acid were found in the small intestine after 25 minutes. After 13.3 hour large quantities of both carnosol and carnosic acid were present in the small intestine, caecum and colon. In total twenty-seven metabolites were found following the intake of rosemary extract by Vaquero et al. [30].
Table 2. Detailed description of the approved food categories, maximum levels, and restriction for the use of extracts of rosemary as stated in EU regulation 1333/2008 [28]

<table>
<thead>
<tr>
<th>Food category</th>
<th>Maximum level (mg/l or mg/kg) *</th>
<th>Restrictions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dehydrated milk as defined by Directive 2001/114/EC</td>
<td>200</td>
<td>Only milk powder for vending machines</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>Only dried milk for manufacturing of ice cream</td>
</tr>
<tr>
<td>Fats and oils essentially free from water (excluding anhydrous milkfat)</td>
<td>30</td>
<td>Only vegetable oils (excluding virgin oils 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</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>Only fish oil and algal oil; lard, beef, poultry sheep and porcine fat; fat and oils for the professional manufacture of heat-treated foods; frying oils and frying fat, excluding olive oil and pomace oil</td>
</tr>
<tr>
<td>Vegetable oil pan spray</td>
<td>50</td>
<td>Only fats and oils for the professional manufacture of heat-treated foods</td>
</tr>
<tr>
<td>Nut butters and nut spreads</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Processed potato products</td>
<td>200</td>
<td>Only dehydrated potatoes products</td>
</tr>
<tr>
<td>Chewing gum</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Decorations, coatings and fillings, except fruit-based fillings</td>
<td>100</td>
<td>Only sauces</td>
</tr>
<tr>
<td>Fillings of stuffed pasta (ravioli and similar)</td>
<td>250</td>
<td>Only in fillings of stuffed dry pasta Period of application: From 25 December 2012</td>
</tr>
<tr>
<td>Fine bakery wares</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Non-heat-treated processed meat</td>
<td>100</td>
<td>Only dried sausages</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>Excluding dried sausages</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>Only dehydrated meat</td>
</tr>
<tr>
<td>Heat-treated processed meat</td>
<td>100</td>
<td>Only dried sausages</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>Excluding dried sausages</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>Only dehydrated meat</td>
</tr>
<tr>
<td>Processed fish and fishery products including molluscs and crustaceans</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td>Processed eggs and egg products</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Seasonings and condiments</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Mustard</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Soups and broths</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Sauces</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Potato-, cereal-, flour- or starch-based snacks</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Processed nuts</td>
<td>200</td>
<td></td>
</tr>
<tr>
<td>Food supplements supplied in a solid form including capsules and similar forms, excluding chewable forms</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>Food supplements supplied in a liquid form</td>
<td>400</td>
<td></td>
</tr>
<tr>
<td>Food supplements supplied in a syrup-type or chewable form</td>
<td>400</td>
<td></td>
</tr>
</tbody>
</table>

* Extracts of rosemary as the sum of carnosol and carnosic acid
Furthermore, nine metabolites, including carnosol and carnosic acid, were found in varying concentrations in the plasma, liver and brain. Vaquero et al. [30] also reported on the time course of carnosic acid and carnosol in plasma. Carnosic acid reached the maximum peak concentration of 26.6 \( \mu \text{M} \) after 0.4 hour and the last measurable concentration was found after 1.7 hour. The clearance of carnosol had not started at the end of the measurements, as such no maximum peak concentration was found (after 13.3 hours the concentration was 18.2 \( \mu \text{M} \)). For both carnosic acid and carnosol the limit of detection was 1 \( \mu \text{M} \) (0.3 ppm) and the limit of quantification was 3 \( \mu \text{M} \) (1 ppm). In contrast, Doolaege et al. [31] found a maximum peak concentration for carnosic acid of 0.035 mg/ml after 2.3 hour using a lower dosage (limit of detection and limit of quantification were both 0.005 mg/ml). Additionally, traces of carnosic acid were found in the liver and muscle tissue.

Only the metabolism of rosmarinic acid has been evaluated in humans. Baba et al. [32] reports a peak concentration in plasma after 0.5 hour, which is followed by an increase of methylrosmarinic acid with a peak concentration after 2 hour. Furthermore, 75% of the ingested rosmarinic acid is excreted in the urine as methylated rosmarinic acid, caffeic acid, ferulic acid, and m-coumaric acid after 6 hours.

6.2 Acute, Short Term, Subchronic Toxicity, and Chronic Toxicity

Based on unpublished data from the petitioner EFSA concluded that two versions of ethanol extraction extracts can be considered to be of low acute toxicity [24]. This assessment was based on the acute oral administration by gavage of 8.5/10 g/kg bw and 24/28.5 g/kg bw for female and male rats respectively and the daily administration by gavage of 4.5/5 g/kg bw and 11.8/14.1 g/kg bw for females and males respectively during five days. No mortality was reported in any of the experiments, however both extracts showed an increase in liver weight for both sexes and increase in fatty liver.

In lower concentrations however, no adverse effects have been reported. In the acute oral safety study of Anadon et al. [33], Winstarrats were orally given a single dose of 2000 mg/kg bw of rosemary extract. For this study a supercritical fluid extraction method was used to obtain the rosemary extract. During the two-week observation period no abnormal signs, changes in behaviour, or body weight were observed. After two weeks no histological, hematological, or organ weight changes were found. Moreover, Fahim et al. [34] found no adverse effect using a daily oral dose of 0.15 g / 100 g bw ethanol extract for three weeks, and even reports a potential hepatoprotective effect for the extract compared to a reference compound. However, no acute toxicity studies have been performed with acetone or hexane rosemary extracts.

For the assessment of short-term and sub-chronic toxicity ESFA used unpublished data from five 13-week experiments provided by the petitioner [24]. For two ethanol extracts and a hexane extract administered dosages ranged from 500 or 1000 till 5000 mg/kg diet, for which the latter is equal to 400 mg/kg bw/day rosemary extract. Furthermore, an acetone extract was administered at 3800 mg/kg diet and a supercritical CO2 extract was administered at 300,600 & 2400 mg/kg diet. Aside from some palatability issues, no mortality or any clinical effects were found in any of the studies. The only notable effect was a dose dependent increase in liver weight, which was only significant at the highest dose groups for all types of rosemary extracts. Upon further investigation, using a supercritical CO2 extract at dose of 2400 mg/kg diet, a 1.5-fold increase in liver enzymes cytochrome P450 and several CYP2 enzymes were found. However, these effects were shown to be reversible after a 28 day recovery period. Using an ethanol extract, a similar increase was found, however, this change was not significant. Moreover, the increase in liver weight was accompanied by minimal centrilobular hypertrophy and microsomal enzyme induction. Furthermore, using a hexane extract a reduction in hepatic DNA content was found for the 5000mg/kg diet group suggesting hypertrophy rather than hyperplasia. The changes in liver weight, caused by centrilobular hypertrophy and microsomal enzyme induction, are generally not considered a toxic response. Additionally, the low magnitude, reversibility, and no increases in plasma levels of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase lead the EFSA panel to conclude that the increase in liver weight represents an adaptive response rather than hepatotoxicity [24]. No further effects were reported for any of the studies and the NOAEL range was determined at 180 till 400 mg extract/kg bw/day, which is equivalent to 20-60 mg/kg bw/day of carnosol plus carnosic acid. Currently, no published data is present to support or contradict these findings.
6.3 Genotoxicity, Carcinogenicity, Reproductive and Developmental Toxicity

At the time of evaluation by EFSA unpublished studies were used to evaluate the mutagenicity of rosemary extracts [24]. Several extracts were tested with the Ames test using different strains of S. typhimurium. Based on these tests ESFA concluded that ethanol and hexane-ethanol rosemary extracts were not mutagenic. Another study showed no changes in mutation frequency for a hexane-ethanol rosemary extract in a human lymphoblastoid cell line. Additionally, it was concluded, based on another study, that hexane-ethanol rosemary extracts do not increase mutagenicity in vivo in mice. Genotoxicity of an ethanol rosemary extract was further assessed with an in vitro chromosome aberration test using human lymphocytes. No significant damage occurred in the chromosomes and it was concluded that no genotoxic effect was found. No studies have investigated the genotoxicity of supercritical CO2 and acetone rosemary extracts.

Currently, there are no published studies to report a genotoxic effect for rosemary extract, however several studies suggest rosemary extracts to affect enzymes related to carcinogenicity. Offord et al. [35,36] and Debersac et al. [37] reported that several different rosemary extracts affect several phase I and II enzymes involved with carcinogenicity/genotoxicity, like cytochrome P450 1A1 (decreased) and glutathione-S-transferase (increased). However, the extracts used differ from the approved extracts. Mohebati et al. [38] replicated these findings with one of the main components, carnosol, present in the approved extracts. Furthermore, several other authors reported on potential protective properties against carcinogenicity/genotoxicity of different rosemary extracts through different pathways [39-44]. Due to the difference in composition between rosemary extract it is unclear whether similar effect are also present for the approved extracts.

A rosemary extract (carnosic acid 88%, carnosol 12%) was also identified as anti-mutagenic by Minnunni et al. [45] by preventing reactive oxygen species from reacting with genetic information in the Ames test with strain TA102. Furthermore, Slamenova et al. [46] reported on the protective properties of an ethanol rosemary extract to DNA by scavenging for both OH radicals and singlet oxygen. No long term studies for carcinogenicity have been performed for any of the rosemary extracts.

Only two published studies investigated reproductive and developmental toxicity. The effect of rosemary extract on fertility after 63 days was examined by Nusier et al. [47]. Two ingestion levels were used, 250 and 500 mg/kg/day, of which the 500 mg/kg/day group showed a significant decline in spermatogenesis, and a decrease in sperm motility and density. However, these results were found using an extract made by a 70% ethanol and water 30% extraction resulting in an extract of a polar nature. Therefore EFSA concluded that these results were not applicable to the proposed extracts under assessment. Lemonica et al. [48] comparededaily dosage of 26 mg of a 30% w/w rosemary extract with a saline solution on the incidence of abortion and/or interference with normal development in Winstarrats. In contrast to most other studies, a water based rosemary extracts was made from leaves, flowers and stems, which was administered by gavage prior and during pregnancy. Due to the production method of the rosemary extract it suffers from the same problem as the aforementioned study. Nevertheless, no differences were found post-implantation or in the development of the foetuses, however a non-significant difference was found in implantation loss.

Based on the histopathology conducted in previously mentioned 13-week studies EFSA was able to conclude that at the tested dose levels do not affect the male reproductive system. As mentioned before, due to the lack of reproductive and developmental toxicity or long-term studies EFSA was not able to establish an acceptable daily intake.

7. OTHER DATA

Rosemary was also found to have potential antimicrobial, antifungal, and antiviral effects albeit mainly as essential oil not as extract. De Melo et al. [49] investigated the antimicrobial effects of rosemary essential oils for active packaging. A significant difference in microbial activity was found for 50% w/w rosemary essential oil. Similarly to the antioxidant activity of rosemary extracts, the antimicrobial effects were reported to be strongly dependent on the composition of the medium [50]. Rosemary oil and rosemary extracts have several compounds in common, however no evidence exist to
support an antimicrobial effect of rosemary extracts. Furthermore, significant differences in the biological control of fungi in foodstuffs were found for low concentrations of an unspecified rosemary extract by Centeno et al. [51], this was also reported for rosemary essential oils [52].

8. DISCUSSION AND CONCLUSION

Rosemary is a spice that was traditionally used as a flavour and aromatic agent. In 2008 EFSA published its opinion regarding the safety as a food additive, stating: “the proposed uses and use levels would not be of safety concern” [24], and subsequently the European Commission approved the use of rosemary extracts in 2010 [28]. Despite the approval under Regulation 1333/2008 and further specification under Commission Regulation 231/2012, extracts of rosemary are not yet fully characterised. The characterisation based on the reference compounds leaves room for different compositions. These differences in composition are partially restricted by Commission Regulation 231/2013 that only allows production of rosemary extracts from dried rosemary leaves. However, the different approved production methods still result in different composition and antioxidant activity [10]. Moreover, the characterisation of rosemary extracts is different outside the EU and research using rosemary extracts are not bound to these restrictions. This results in difficult to compare scientific evidence and difficulty to assess the safety of approved rosemary extracts.

Despite the differences in composition, the use of rosemary extracts has shown antioxidant properties in several food categories. However, this effect might not be present in all foods, since the antioxidant activity of one of the major components of rosemary extracts, carnosic acid, was found to be medium dependent [19,20]. From a functional perspective, the room for different compositions might allow the optimization of antioxidant activity of the rosemary extract for a specific medium or food. However, this depends on the properties of the other components of the extract, which are currently unknown.

No new evidence is present to suggest adverse effects, but some discrepancies still exist. The unpublished data suggests an increase in total cytochrome P450 content, however Offord et al. [35,36] reports the inhibition of cytochrome P4501A1 mRNA expression and decreased enzyme activity in human bronchial epithelial cells. In contrast, Debersac et al. [37] did not find any changes for cytochrome p450 in hepatic tissue of Wistar rats. The reason for this contradiction is difficult to identify due to differences in test materials and extract composition. Additionally, both studies report a strong induction of phase II enzymes, particularly glutathione-S-transferase, and quinone reductase. These studies and others that show similar protective effects tend to show a large diversity in extract compositions, potential mechanism, and effect magnitude [39-44]. Many studies also focus solely on carnosol or carnosic acid. While there is no reason for concern, the lack of knowledge about other components than carnosol and carnosic acid and possible combined effects of extract components are clear.

The increase in liver weight was not seen as an adverse effect; as such the NOAEL range for all five extracts was determined at 20-60 mg/kg bw/day of carnosol plus carnosic acid. Based on the estimated dietary exposure, this results in a safety margin for the 95th percentile between 200-600 for adults, and 100-300 for pre-school children. Furthermore, no lowest observed adverse effect level was determined. However, the effect of the rosemary extract from Nusier et al. [47] on spermatogenesis and sperm motility and density raise some concern. Although the extracts are considered to be different, both are produced from the leaves of rosemary and could contain many of the same compounds. However even if it is considered as an adverse effect, it was only present above the intake of 60mg/kg bw/day carnosol plus carnosic acid. Leaving a greater margin of safety than for the increase in liver weight effect. Therefore there is no reason for concern, however further research is needed to ensure this adverse effect, and any other effect on reproduction, development or otherwise are not present for the approved rosemary extracts. The indications for other potential functional properties of rosemary extracts only strengthens the need for further investigation. Additionally, further specification, characterisation of rosemary extracts might also help fill the knowledge gaps.

Despite the uncertainty created by the compositional differences between rosemary extracts and the gaps in knowledge, there is no evidence to suggest a direct need for re-evaluation of approval. Moreover, since rosemary extracts have been approved by Directive 2010/69/EU in 2010, it will not be re-evaluated under the process started by
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Competing Interests

Authors have declared that no competing interests exist.

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