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Invited Review

In vitro toxicological evaluation of essential oils and their main compounds used in active food packaging: A review

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ABSTRACT

Essential oils (EOs) and their main constituent compounds have been extensively investigated due to their application in the food industry for improving the shelf life of perishable products. Although they are still not available for use in food packaging in the market in Europe, considerable research in this field has been carried out recently. The safety of these EOs should be guaranteed before being commercialized. The aim of this work was to review the scientific publications, with a primary focus on the last 10 years, with respect to different *in vitro* toxicological aspects, mainly focussed on mutagenicity/genotoxicity. In general, fewer genotoxic studies have been reported on EOs in comparison to their main components, and most of them did not show mutagenic activity. However, more studies are needed in this field since the guidelines of the European Food Safety Authority have not always been followed accurately. The mutagenic/genotoxic activities of these substances have been related to metabolic activation. Therefore, in *vivo tests* are required to confirm the absence of genotoxic effects. Considering the great variability of the EOs and their main compounds, a case-by-case evaluation is needed to assure their safe use in food packaging.

1. Introduction

The safety and organoleptic properties of food, such as perishable products, may be altered due to several reactions, which include microbial spoilage or oxidation processes. These reactions decrease the value of the food because of the destruction of substances with beneficial properties (fatty acids, vitamins or proteins); production of off-flavours and odours; and, in the worst case, production of foodborne illnesses related to unsafe food intake (Sanches-Silva et al., 2014). New trends in consumer demands and internationalization of markets have caused changes in the retail and distribution practices, resulting in an increased distribution distance and longer storage time. Hence, the improvement in the shelf life of perishable products has become a large challenge for the food packaging industry worldwide (Vermeiren et al., 1999).

To improve food packaging and extend the optimal quality and freshness of perishable products, the food industry has developed a new packaging system called "active packaging" (Sung et al., 2013). Active food contact materials are "materials that are intended to extend the shelf-life or to maintain or improve the conditions of packaged food" (EFSA, 2009, http://www.efsa.europa.eu/en/efsajournal/doc/1208.pdf). They are designed to deliberately incorporate components that would release or absorb substances into or from the packaged food or the environment surrounding the food (European Commission, 2004). These components, which would be incorporated into the packaging matrix, include both synthetic and natural substances. Nevertheless, consumers have become more conscious about potential health problems associated with synthetic preservatives and are interested in ingredients from natural sources (Amorati et al., 2013; Bahram et al., 2012). In this sense, the use of essential oils (EOs) in active packaging has become a good alternative for improving the shelf life of perishable products (Sacchetti et al., 2005).

Essential oils are the secondary metabolites of plants, which have been studied due to their flavour and fragrance for flavouring foods, drinks and other goods. Additionally, EOs have been traditionally used to extend the shelf life due to their antimicrobial/antifungal activity (Bajpai et al., 2012; Bakri and Douglas, 2005) or their antioxidant properties (Roby et al., 2013; Teixeira et al., 2012). Currently, their relatively safe status, properties and acceptance by consumers have piqued the interest of industries (Sacchetti et al., 2005). However, the amount of substances added to food will determine the acceptability of products because strong flavours might result in palatability problems. In this sense, the use of EOs as preservatives in food has been limited because high concentrations are required to reach sufficient activity (Hyldgaard et al., 2012). Active

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packaging, where volatile compounds originate in a preservative atmosphere, is a good alternative to the direct incorporation of EOs because this method enables a slow release of the agent onto the food surface, maintaining an adequate concentration of the active compounds to preserve the quality of the food and avoiding the undesirable effects produced by the direct addition of high levels of EOs (Kuorwel et al., 2011; Nerín et al., 2006). Antimicrobial packaging has attracted substantial attention from the food industry, and many EOs and their main compounds can be incorporated in active packaging as antimicrobial agents (Hyldgaard et al., 2012; Seow et al., 2014). Moreover, the antioxidant properties of this active packaging have been reviewed in the literature (Eça et al., 2014; Sanches-Silva et al., 2014).

Many EOs, such as clove, oregano, thyme, nutmeg, basil, mustard or cinnamon, are categorized as GRAS (Generally Recognized as Safe) by the US Food and Drug Administration (FDA) (Manso et al., 2014) but there are regulatory limitations on the accepted daily intake of the EOs, and a daily intake survey should be available for the evaluation of the safety. Only a limited range of EO components such as linalool, thymol, eugenol, carvone, cinnamaldehyde, vanillin, carvacrol, citral, and limonene have been accepted as flavouring agents in Europe, all of which are considered to present no health risk to consumers, at the estimated intake level (Hyldgaard et al., 2012). However, in the case of active packaging, the ingredients allowed to be used for this purpose have not been published so far in Europe. Currently, a community list of authorized substances is being established, taking into account the European Food Safety Authority (EFSA) opinion on each substance. The extent of toxicological data needed in order to be included in this list depends on the expected migration into food and it is necessary to perform at least 3 mutagenicity studies *in vitro* (EFSA, 2008a). In non-European countries such as the USA, Japan and Australia, the use of this technology is approved (Dainelli et al., 2008). Due to the market globalization, consumers could be exposed to these substances worldwide. There is therefore a need for more toxicity and exposure data on EOs and their ingredients used for food contact materials in order to confirm their safety (Evandri et al., 2005).

The general biological effects of EOs used in pharmaceutical, sanitary, cosmetic, agricultural and food industries have been reviewed by Bakkali et al. (2008), including some toxicological assessment. Taking into account all these considerations, this review aims to compile evidence on the *in vitro* toxicological aspects, mainly mutagenicity and genotoxicity of the EOs, and their major compounds, that are used in active food packaging. To achieve these objectives, a thorough review of the scientific literature on this topic from the last 10 years was performed; most of the works were published from 2008 (approximately 60%).

2. Main active compounds present in essential oils

Essential oils are low molecular weight, volatile mixtures that are biosynthesized in various organs of plants (Bajpai et al., 2012). In general, the components of EOs are divided into two groups according to their biosynthetic origin, compounds from terpene origin and aromatic compounds (Bakkali et al., 2008). The main terpenes are the monoterpenes (C10) and sesquiterpenes (C15), but hemiterpenes (C5), diterpenes (C20), triterpenes (C30) and tetraterpenes (C40) also exist. The monoterpenes are the most representative molecules, constituting 90% of the EOs and allowing for a great variety of structures. In this way, terpene compounds comprise alcohols, aldehydes, carbures, ketones, esters, ethers, peroxides and phenols (Bakkali et al., 2008). Aromatic compounds occur less frequently than the terpenes and its constituents comprise aldehydes, alcohol, phenols, methoxy derivates and methylene dioxy compounds (Bajpai et al., 2012; Bakkali et al., 2008).

Essential oils can contain approximately 20–60 components at substantially different concentrations, which are characterized by two or three major components (main compounds) at fairly high concentrations (20–70% or even up to 85%) compared to other components present in trace levels (Bakkali et al., 2008; Burt, 2004). Table 1 shows the main plants containing EOs used for food packaging. Generally, these major components determine the biological properties of the EOs, both the beneficial effects (antibacterial, antifungal, antioxidant, etc.) and the toxicological profile of the substances. Therefore, both the beneficial and prejudicial aspects of EOs should be considered in order to ensure human safety. It is also important to note that the composition of EOs can vary considerably between subspecies of the plant from which they are extracted, origin and harvest time (Smith et al., 2005), and also depending on differences in the cultivation, vegetative stage and growing season of the plants (Vagi et al., 2005). Between different species, the carvacrol content may change considerably from 22.0% in *Origanum compactum* to 64.3% in *Origanum onites* or even exhibit different chemical composition, such as the case for β-caryophyllene and α-thuyene, which are present in *O. compactum* and not detected in *O. onites* (Bostancioglu et al., 2012; Mezzoug et al., 2007). For these reasons also the toxicity of EO extracts can vary based on the above-mentioned differences, which is very important to bear in mind for risk assessors. It is important to highlight that the quantitative and qualitative composition of EO extracts can be modified (in general, larger quantitative changes rather than qualitative ones) depending on the pre-treatment, processing technology and extraction processes.

As already mentioned above, the use of EOs in active packaging in Europe is not yet a reality. Moreover, to our knowledge, no data are available about the use of active packaging containing EOs in non-EU countries. However, the EOs that are more frequently studied to be used for this purpose are oregano, (*Origanum vulgare*), rosemary (*Rosmarinus officinalis*), green tea (*Camellia sinensis*), cinnamon (*Cinnamomum zeylanicum*), and clove (*Eugenia caryophyllata*) because of their antimicrobial, antibacterial, antifungal, and antioxidant properties. In this regard, the EOs from "oregano" plants are characterized by the high content of carvacrol (64–22%), a component of particular importance, and also by thymol (19–1.4%) (Bostancioglu et al., 2012; Llana-Ruiz-Cabello et al., 2014a; Mezzoug et al., 2007). Carvacrol (5-isopropyl-2-methylphenol) and its isomer, thymol (2-isopropyl-5-methylphenol), are the major compounds in thyme, marjoram, and savoury EOs (Al-Bandak and Oreopoulou, 2007; De Vincenzi et al., 2004). Carvacrol and thymol are commonly used due to antimicrobial effects on bacteria, fungi, and yeast (Bakkali et al., 2008; Burt, 2004; Lambert et al., 2001; Zhou et al., 2007), as well as insecticidal and antioxidant effects (Akalin and Incesu, 2011). Several studies have reported on the use of carvacrol in packaged food, such as baked chicken, wrapped (Du et al., 2012) or refrigerated fresh fish and meat (Guarda et al., 2011, 2012). Thymol has been incorporated, by direct dissolution, into soy protein isolate-based film to package olive oil (Hu et al., 2012). Both carvacrol and thymol are suitable for their microencapsulated use in a polymer matrix for fresh food preservation (Guarda et al., 2011). Linalool, the main constituent of various EOs (69.2–2.91%), such as basil, cilantro, green tea, lavender and oregano, has potential use in low-density polyethylene-based films with its antimicrobial component that can enhance the quality and safety of cheeses (Suppakul et al., 2008). Linalool has been evaluated by EFSA as a flavouring substance and it has been classified in category A (flavouring substance, which may be used in foodstuffs) (EFSA, 2011a). Moreover, it is stable at relatively high temperatures; therefore, it may have the potential to be incorporated into polymers and used in antimicrobial packaging (AM) (Suppakul et al., 2008). Cinnamon EO from the plant *Cinnamomum zeylanicum* and cinnamaldehyde (with a concentration of 85% in cinnamon EO) have high antioxidant,