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Control of microbial growth in bakery products fortified with polyphenols recovered from olive mill wastewater

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HIGHLIGHTS

- Polyphenols from olive mill wastewater were assayed as antimicrobial agents in bread and rusks.
- Ascorbic acid, tocopherols mixture and α-tocopherol were also tested.
- 200 mg of olive polyphenols/Kg flour extended the preservation of both products.
- Emulsification of olive polyphenols enhanced their antimicrobial effect.
- The study reveal the opportunity of fortifying bakery products with olive polyphenols.

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ABSTRACT

Polyphenols recovered from olive mill wastewater and other natural antioxidants [ascorbic acid, tocopherols mixture (α-,β-,γ- and δ-) and α-tocopherol] were mixed in different concentrations with bread and rusks, stored over a period of 20 days and 12 weeks, respectively, and assayed periodically to different microbiological assays (i.e. *Total coliforms*, *Yeasts-Moulds* and *Bacillus spp.*). Antioxidants were able to induce antimicrobial properties in bakery products and subsequently prolong their shelf life. The optimal concentration of olive polyphenols was this of 200 mg /Kg flour, whereas their emulsification enhanced antimicrobial effect. The formulation of 500 mg α-tocopherol /Kg was also able to extend the shelf life of the bread up to 15 days. Ascorbic acid and tocopherols mixture had did not affect importantly to the overall bread preservation in both assayed concentrations (500 and 1000 mg/Kg). Concerning rusk preservation, α-tocopherol, tocopherols mixture and ascorbic acid were effective at 600 mg/Kg. The results of the study reveal the possibility of polyphenols derived from olive mill wastewater and other natural antioxidants (e.g. α-tocopherol, tocopherols mixture and ascorbic acid) as an antimicrobial agent in foods that undergo oxidative deterioration during cooking (i.e. bakery products).

1. Introduction

Bakery products comprise an important part of a balanced diet and that is why a wide variety of such products can be found on food market shelves. Like many processed foods, bakery products are subjected to physical, chemical and microbial spoilage. Physical and chemical spoilage limits the shelf life of low and intermediate moisture bakery products, whereas

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microbial spoilage is the concern in high moisture products ($a_w > 0.85$) (Smith et al., 2004). Indeed, the most common type of microbial spoilage is mould growth that contributes to the shortening of products' shelf life (Guynot et al., 2003). In wheat based bakery products, deteriorations have been attributed to the following fungi genus: *Penicillium, Aspergillus, Cladosporium and Neurospora*. A less common factor is the deterioration caused by bacteria such as *Bacillus subtilis* and *Bacillus licheniformis* (Pateras, 2007; Saranraj and Geetha, 2012). Microorganisms may be present in raw materials, developed in the product or contaminate it during processing and packaging, i.e. after its removal from the oven. Likewise, baking process accelerates the formation of primary lipid oxidation products and peroxides, leading to the development of rancidity and off-flavour in the final product.

Bread producers focus on the control of bread firming and microbial growth rate, allowing the production of bread with a shelf life up to 4 weeks (Jensen et al., 2011). The addition of antioxidants is known to delay or inhibit deterioration of food products and benefit consumer by prolonging their shelf life (Lindley, 1998). Besides, antioxidants have antimicrobial activities, too (Krishnan et al., 2014). Primary synthetic antioxidants such as butylated hydroxy anisole and butylated hydroxy toluene have been used in the food industry (especially in oil, fats, meat and bakery products) over the last 50 years (Rojas and Brewer, 2008; Nanditha and Prabhasankar, 2009). These compounds are rather heat stable, whereas they undergo bactericidal rather than bacteriostatic activities (Gailani et al., 1980). Nevertheless, despite their superior efficacy and high stability, there is an increasing concern about their safety. For instance, experimental studies have shown that some of the applied synthetic antioxidants have toxigenic, mutagenic and carcinogenic effects in animals (Nanditha and Prabhasankar, 2009).

On the other hand, the addition of natural antioxidants that exist inherently in foods and sound healthier to the consumers (Ramarathnam et al., 1995; Kiokias et al., 2008). Although they lack of efficiency compared to the synthetic ones, natural antioxidants are also able to reduce contamination from pathogens and enhance the shelf life of bakery products (Nanditha and Prabhasankar, 2009). Tocopherols, ascorbic acid and polyphenols are among the antioxidants found in natural bioresources (Boskou, 2006; Galanakis et al., 2010a). For example, tocopherols have been used alone or in combination with ascorbic acid to protect fats from lipid peroxidation and baked products (i.e. crackers, pastry and sugar-snap cookies) from rancidity. However, both antioxidants undergo deterioration during baking process (Park et al., 1997). Deterioration of tocopherols can take place even during the non-anticipated storage of flour and thus affecting the preservation of bakery products, too (Wennermark and Jagerstad, 1992). On the other hand, polyphenols derived from fruit sources, such as mango (Vergara-Valencia et al., 2007) and apple (Sivam et al., 2011), have been implemented in bakery products in order to protect them from rancidity. Polyphenols are also susceptible during heating, but this has been observed at higher baking temperatures (i.e. up to 240 °C) (Davidov-Pardo et al., 2011; Ross et al., 2011).

Olive mill wastewater contains high amounts of polyphenols, whereas their recovery and commercial implementation has today been achieved (Galanakis, 2012; Rahmanian et al., 2014). Indeed, more than four companies around the world produce polyphenol rich products from olive mill wastewater (Galanakis and Schieber, 2014) with the final purpose of merchandising them as natural preservatives in foodstuff. In literature, there are numerous investigations denoting the antioxidant, antimicrobial (i.e. against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*) and other bioactivities of olive polyphenols (Boskou, 2006; Obied et al., 2007; Galanakis et al., 2010b; Sivam et al., 2011). However, to the best of our knowledge, there are no investigations reporting their direct implementation in foods to control microbial growth. In addition, the studies investigating the effect of ascorbic acid, tocopherols and polyphenols on microbial growth in bakery products are scarce. Following this consideration, the scope of the current study is to investigate the efficiency of olive polyphenols and other natural antioxidants to inhibit microbial growth of bakery products during storage. In particular, polyphenols from olive mill wastewater, ascorbic acid, pure α-tocopherol and tocopherols mixture were added at different concentrations in two kind of bakery products: bread and rusks. The activity of antioxidant formulations was tested using the $ABTS^+$ radical scavenging assay. The produced products were stored over a period of 20 days and 12 weeks, respectively, and assayed periodically to different microbial determinations.

2. Experimental

2.1. Materials

Extra virgin olive oil was supplied by a local producer (Chania, Greece), whereas granulated sugar (EBZ), salt (KALLAS), wheat flour (Rivercote) and yeast (Morfat) were obtained by the local market. Lundolive P1100 powder was obtained from Phenoliv AB (Lund, Sweden), containing 10% olive polyphenols per weight (50% of which is hydroxytyrosol) and 50% maltodextrin (powder carrier) according to manufacturer. The recovery process was the one described by Galanakis et al. (2018). D-a-tocopherol (97%) was supplied from Alfa Aesar (Karlsruhe, Germany), whereas tocopherols mixture (Tocoblend L50 IP) was purchased by Vita Blend (Wolvega, The Netherlands). Ascorbic acid (95%) was obtained from Sigma-Alrich (Germany). According to the manufacturer, tocopherols mixture is comprised of 9%–20% D-α-tocopherol, 1%–4% D-β-tocopherol, 50%–65% D-γ-tocopherol and 20%–35% D-δ-tocopherol. Emulsifiers (Tween-20 and Span-80) were purchased from Merck (Darmastadt, Germany). Buffered Peptone Water and Dextrose Tryptone Agar for microbiological analysis were purchased from LAB M Limited (Lancashire BL9, United Kingdom), whereas Trypton Bile X-Gluc Agar, Chloramphenicol Glucose Yeast Extract Agar and Violet Red Bile Agar were purchased from Biolife, Milano, Italia.