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Application of experimental design and response surface methodology to optimize the procedure to obtain a bactericide and highly antioxidant aqueous extract from orange peels

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ABSTRACT

In recent years there is a growing demand for healthy and safe food, without added preservatives or synthetic antioxidants. In this regard, plant extracts have promising future due to their high contents of bioactive compounds, especially in phenolic and polyphenolic compounds that exhibit antimicrobial and antioxidant activities. For this reason, the plant extracts could form the basis of many applications, including the preservation of fresh and processed foods. In this work, the methodology to obtain orange peel extracts with high bioactive properties is presented. To adjust the extraction conditions, experimental design and response surface methodology were applied to establish the optimum value of all the experimental variables that have influence in the extraction system. Total phenolic contents and antioxidant activity values were used as response parameter. The peel extracts, obtained with the proposed methodology, exhibited a high content of phenolic compounds, high antioxidant and antimicrobial activity, against *Escherichia coli* and *Listeria innocua* (*in-vitro* experiments). Finally, to demonstrate the feasibility of the peel extracts, they were used as natural additive in apple juice reducing significantly juice browning and the microbial load for both bacteria.

1. Introduction

The healthy benefits associated with the regular consumption of vegetal products have been reported extensively during the last few decades. The healthy properties of vegetal products are due to the presence of secondary metabolites with high antioxidant activity. Phenolic and polyphenolic compounds are likely the most abundant phytochemicals present in plants (Dreosti, 2000). Among them, flavonoids constitute the main subgroup (Hertog, Feskens, Hollman, Katan, & Kromhout, 1993), and it has been extensively reported that they have a high antioxidant activity (El-Seedi et al., 2012; Pokorny, Yanishlieva, & Gordon, 2001). Positive correlations between the ingestion of these active compounds through the diet with a reduction of the incidence of some chronic diseases are reported in different works (Ugartondo, Mitjans, Lozano, Torres, & Vinardell, 2006; Urquiaga & Leighton, 2000).

On the other hand, there is a growing interest in using bioactive compounds, extracted directly from plants and vegetal products, as natural additives in processed food, instead of using synthetic chemical preservatives (Delgado-Adámez, Gamero, Valdés, & González-Gómez, 2012a; Pokorny & Parkányiová, 2004), that might have some toxic or carcinogenic problems (Moure et al., 2001). In fact, the use of highly antioxidant plant extracts, containing significant amount of phenolic compounds, have been proven to be adequate to provide oxidative stability when they are used as food preservatives (Shi, Noguchi, & Niky, 2001) avoiding, in that way, the used of not natural chemical compounds. Antioxidant additives are incorporated in foodstuff formulas to avoid or retard oxidation processes due to internal or external factors (Madrid & Cenzano, 2000). Furthermore, antioxidant preservatives can inhibit or avoid oxidative reactions preventing oxidative decay (Shi et al., 2001), such as the browning processes observed in fresh cut fruit or raw fruit products that limit the marketability of these types of food products. Another important factor that limits the shelf life of fruit fresh cut product is due to microbial spoilage, mostly by the development of fermentation processes, causing unfavorable effects on quality and safety (Tauxe, 1997). With this regard, natural

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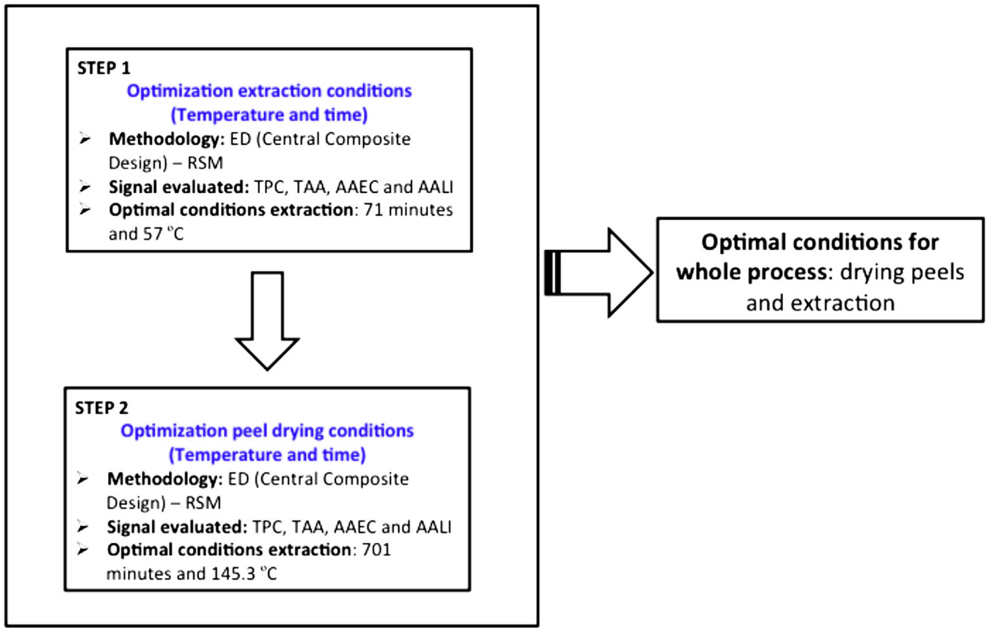

Fig. 1. Workflow scheme followed to optimize the processes to obtain microbice and highly antioxidant extracts from orange peel by means of experimental design (ED) and response surface methodology (RSM).

extracts could be a feasible alternative, since it has been observed that they exhibit antimicrobial activity (Delgado-Adámez et al., 2012a, Delgado-Adámez, Fernández-León, Velardo-Micharet, & González-Gómez, 2012b) due to their high contents of terpenoids, tannins, quinones, phenolic acids and polyphenols (Calvo et al., 2006; Lee & Lee, 2010).

The applicability of natural extracts increases when they are prepared in aqueous media instead of using organic solvents. Thus, the procedure to obtain extracts from plant material is a crucial factor in order to preserve their antioxidant and antimicrobial activity. The extraction efficiency of phenolic compounds relies on different factors that have a direct influence in the raw material conditioning and the extraction process; among these factors, temperature and time of extraction are the variables that have the strongest influence in the whole process (Pinelo, Rubilar, Sineiro, & Nunez, 2004). The establishment of these conditions must consider not only the chemical compounds of the resulting extracts, but also they have to be fixed to obtain the highest antioxidant and bactericide activity.

Therefore, the aim of this research is to establish the procedure to obtain highly antioxidant and microbicide aqueous extracts from orange peels and assess their suitability as natural antioxidant additive and preservative when added to foodstuff. In order to establish the operation conditions of the extraction process, an Experimental Design (ED) together with Response Surface Methodology (RSM) are proposed in this research work. ED-RSM is an effective statistical technique for optimizing complex processes. Instead of varying one variable at a time and keeping the rest constant, ED-RSM reduces the number of experimental trials needed to evaluate multiple parameters and their interaction; thus less laborious and time-consuming than other approaches (Esbensen, 2009, pp. 362–447). The selection of the optimal values was based on the total phenolic content, the antioxidant and antimicrobial activities against *Escherichia coli* and *Listeria innocua*, always taking into account the viability of the processes. The results from this experiment showed that the orange peel extract, when applied in an apple juice at different concentrations, reduced the juice browning, due to the antioxidant activity of the extract, and this extract also decreased the microbial load for both bacteria.

2. Material and methods

2.1. Material

Plant material: The study carried out in this research was performed with orange peels (*Citrus sinesis* (L.) Osbeck, cv. Valencia Late). Oranges were produced in Huelva, in the south-west of Spain. The fruits were hand harvested at full maturity and they were transported under refrigeration to our laboratory. In the INTAEX laboratory, the fruits were washed in a 100 mg L^{-1} NaClO solution (adjusted to pH 6.5 with citric acid) for 2 min. The peel was mechanically removed and the resulting peels were frozen at −20 °C until used.

Chemicals: All chemicals used in this research were purchased from Sigma–Aldrich SA Madrid (Spain). The solvents used were obtained from Fisher-Scientific Spain Madrid (Spain) and were at least of analytical grade.

2.2. Bacterial strains

Bacterial cultures used in this study were obtained from the Spanish type culture collection (CECT) of Valencia University. The following bacterial strains were used in the screening of the antimicrobial spectrum of the extracts: *E. coli* 45 and *L. innocua* 910.

2.3. Experimental design and response surface methodology

Experimental Design (ED) and Response Surface Methodology (RSM) are proposed for designing and optimizing the independent variables that affect the process to obtain aqueous extracts from orange peels. The process has been designed in two steps (Fig. 1): firstly, the experimental conditions that affect the extraction were optimized, and secondly the conditions that affect the drying process of orange peels. The Unscrambler Software Version 9.8 (Camo