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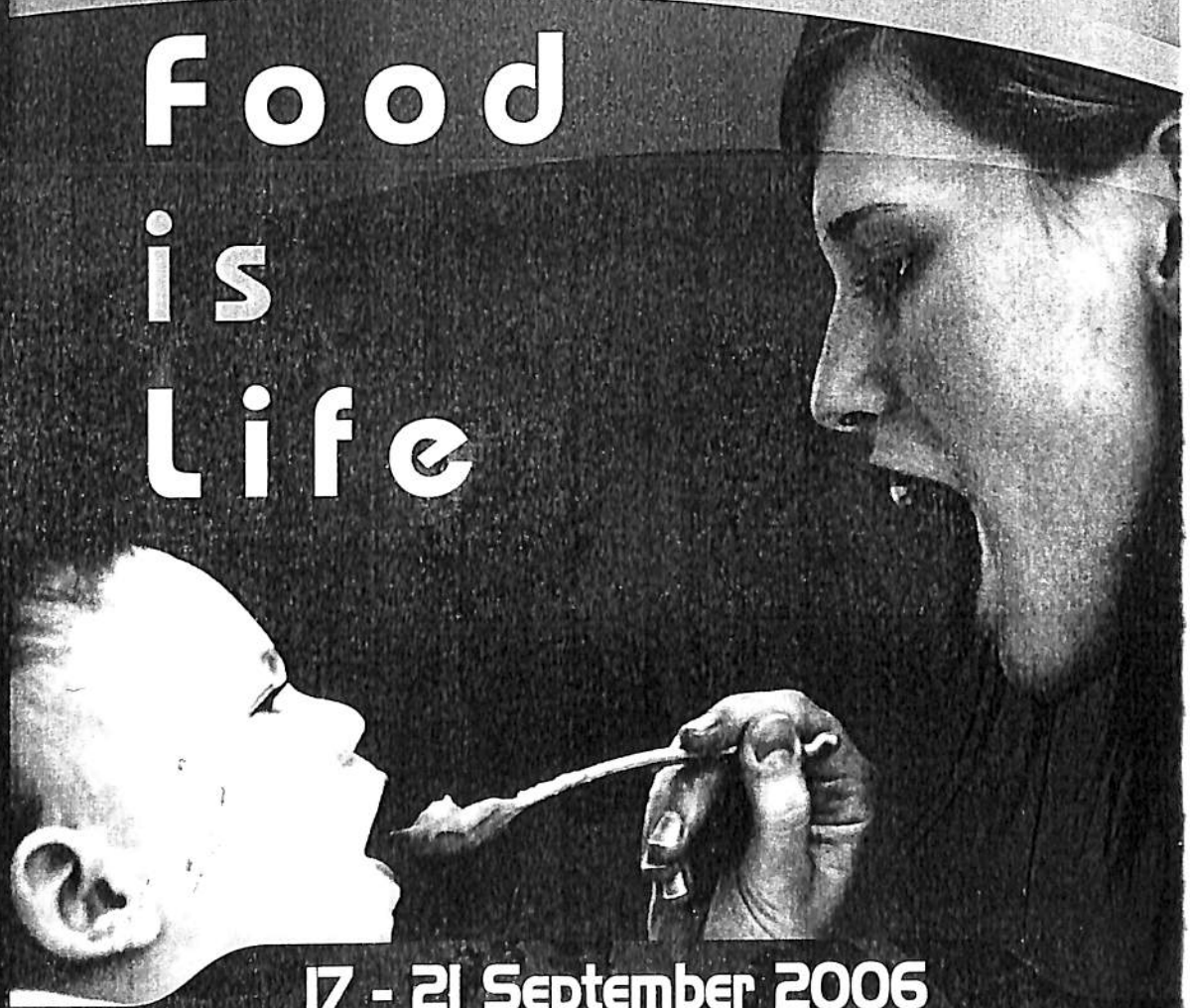

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OPTIMISATION OF PHENOLICS EXTRACTION METHOD IN HAZELNUT SHELL

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Abstract
Agricultural and industrial residues are an attractive source of natural antioxidants. Shell and skin of diverse nuts such as pistachio, almond and peanut have been already studied as a phenolic compounds source. By-products derived from industrial processing of hazelnut (*Corylus avellana* L.) represent about 50% of the fruit in which shell constitutes the major component. However, there is scarce information regarding hazelnut by-products utilisation.
The purpose of this work was to assess the optimum conditions for an extraction of phenolics in hazelnut shell at laboratory scale. Nuts of Tonda Gentile delle Langhe variety was used for this study. The phenolics in the shell extracts were determined spectrophotometrically according to the Folin-Ciocalteu method and expressed as milligrams of gallic acid equivalents (GAE) per gram of hazelnut shell. Two central composite designs (CCD) were developed to optimize the phenolics extraction method. For each CDD, solvent composition and extraction time were chosen as independent parameters. In a first series of repeated batch extractions, the solvent contained different methanol percentages in distilled water at pH 4. In a second series, methanol was substituted by ethanol. For both CCD's, a second degree polynomial equation was obtained and expressed as surface plots using response surface methodology.
The highest phenolic content was found with the ethanol-water mixture: 6.67 mg GAE g^{-1} at the extraction conditions of 55.7% of ethanol and 108.7 min.

Keywords: *Corylus avellana* L.; nut shell; phenolic content; CCD; RSM; by-products valorisation

Introduction

The food and agricultural products processing industries generate substantial quantities of phenolics-rich by-products, which could be valuable natural sources of antioxidants. Today there is strong evidence that polyphenols play a role in prevention of age-related diseases including cardiovascular disease and cancer (Balasundram et al., 2006). Shell and skin of diverse nuts such as pistachio (Goli et al., 2005), almond (Pinelo et al., 2004), and peanut (Yu et al., 2005) have been studied as a phenolic compounds source. Nut shells represent the major constituent from hazelnut industrial processing and signify a huge amount of discarded material available at very low cost. Some publications have dealt with antioxidant activity and phenolic constituents of hazelnut kernel and its brown skin and its green leafy cover (Alasalvar et al., 2006). No information is available about the phenolic content inside the hard shell of the hazelnut. Due to the complex nature of phytochemical presents, there is not a single universal method or extraction solvent system for the phenolics inside fruit or vegetables material. Therefore the purpose of this work was to apply a response surface methodology (RSM) to predict optimum conditions for an extraction of phenolics inside hazelnut shell. Central composite design (CCD) was used to investigate the effects of two independent variables, namely solvent composition (%) and extraction time (min), on phenolic content.

Methods

Extraction protocol

Tonda Gentile delle Langhe hazelnut variety shells were ground. Each shell powder sample (0.5 g) was macerated with 5 ml of extraction solvents in a capped glass tube on an agitating plate at a constant stirring rate at 20-22 °C. Extraction solvents used were methanol, ethanol and distilled water acidified with chlorhydric acid (pH 4). After, the extracts were centrifuged and then filtered on 0.45 µm membrane. Each solvent extraction was carried out in triplicate.

Determination of phenolic content

The amount of phenolics was assayed spectrophotometrically by means of the Folin-Ciocalteu method, as modified by Singleton and Rossi (1965). The phenolic content was expressed as gallic acid equivalent (GAE mg g^{-1} of shell).

Experimental design

Two factorial 2^2 central composite designs were developed to optimise the phenol extraction method. Solvent composition (X_1, %) and extraction time (X_2, min) were chosen for the independent variables. In a first series of repeated batch extractions, the solvent contained different methanol percentages in distilled water at pH 4. In a second series, methanol was substituted by ethanol. Extraction time varied between 30 and 150 min. The second degree polynomials equations were calculated with Statistica software version 7.0 (Statsoft Inc., Tulsa, OK, USA) and expressed as surface plots using RSM in order to visualise the relationship between the response and experimental levels of each factor and to deduce the optimum conditions. A statistical analysis system was used to predict models through regression analysis (R^2) and analysis of variance (ANOVA).

Results and conclusion

The obtained relationships between mathematical and experimental results led to optimal extraction conditions. The regression analysis for both two responses indicated that the results were highly significant ($p<0.001$). Correlation coefficients of the models were 0.9845 for methanol and 0.9911 for ethanol. For the methanol–water mixture, the maximum phenolics content was 6.00 mg GAE g^{-1} at the extraction conditions of 67.1% of methanol and 81 min. The highest phenolics content was found with the ethanol-water mixture : 6.67 mg GAE g^{-1} at the extraction conditions of 55.7% of ethanol and 108.7 min. In summary this work revealed that the hazelnut hard shell could potentially be considered as a valuable source of natural phenolics.

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