LWT - Food Science and Technology 70 (2016) 24–32

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Combined design as a useful statistical approach to extract maximum amount of phenolic compounds from virgin olive oil waste

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ARTICLE INFO

Article history:
Received 26 August 2015
Received in revised form
9 February 2016
Accepted 11 February 2016
Available online 13 February 2016

Keywords:
Modeling
Response surface methodology
Mixture design
Byproduct
Functional ingredient

ABSTRACT

Combined design composed of mixture design and response surface methodology (RSM) was firstly applied for simultaneously optimization of extraction conditions (solvent concentration and ultrasonic conditions) to obtain maximum amount of phenolic compounds from virgin olive oil waste. Various water:methanol (0–1000 mL/L) mixtures were used as solvent under different ultrasonic temperature (30–60 °C), amplitude (20–100%) and time (10–30 min) conditions. Phenolic yields of the extracts ranged from 1.6 to 45 mg/g. Generally, increase of time, temperature and amplitude raised extraction yield; however, at elevated temperature and amplitude levels, phenolics were degraded. Optimum ultrasonic condition varied depending on the solvent concentration, that is reason of why combined design is necessary. Optimum water:methanol concentration was 499:511 mL/L; optimum ultrasonic temperature, amplitude and time was 60 °C, 13.71% and 21.05 min, respectively. Findings highlighted that combined design has potential to be used in many areas of food industry for optimization of many parameters with low experimental points.

1. Introduction

Humans living in different regions of the world have formed their own cuisine and consumption styles for ancient times. Mediterranean area is a rich part of the world, which can be characterized by high consumption of olive oil as well as legumes, grape and orange. This area alone constitutes 98% and 97% of worldwide olive tree lands and total olive oil production, respectively (Alcaide & Nefzaoui, 1996). Health promoting effects of olive oil have been well demonstrated and it is well known that olive oil is protective against cardiovascular and inflammatory diseases, obesity and cancer (Harwood & Yaqoob, 2002).

Virgin olive oil is considered as the most valuable product manufactured from the crushed olive fruit without any refining process that allows the oil ensuring its original composition. Therefore, it is known as a natural source of healthy unsaturated fatty acids and other micronutrients such as antioxidants. As the olive oil is one of the most important items and has a huge production level in the world, a considerable amount of waste is formed as a result of virgin oil production especially in Mediterranean area. Therefore, the olive oil producers are facing serious problems about processing or recycling of those wastes (Azbar et al., 2004). Olive wastes are considered as rich sources of certain valuable constituents such as phenolic antioxidants and can be used as a potential raw material for extraction of those compounds. Researchers have expended their effort for increasing extraction yield due to tendency of people to consume food products with high functional properties. For this purpose, different treatments such as ultrasonic process have been investigated. Ultrasonication is used in the solid–liquid extraction process in order to increase extraction yields, reduce extraction time (Cheung, Siu, & Wu, 2013; Peralta-Jiménez & Cañizares-Macías, 2013) and lower the solvent consumption (Wang & Weller, 2006).

Determination of optimum ultrasonic conditions and solvent combination is desired for increasing effectiveness of the ultrasonic or any other treatments. For this aim, different modeling techniques have been tested. In this respect, response surface methodology (RSM) is a widely used statistical technique for evaluation of the effects of process variables and their interactions (Liyana-Pathirana & Shahidi, 2005). It has been successfully used for

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http://dx.doi.org/10.1016/j.lwt.2016.02.029
0023-6438/

optimization of the food processing conditions such as temperature, time and solvent concentration (Masmoudi, Besbes, Chaabouni, Robert, Paquot, Blecker, et al., 2008; Toker & Dogan, 2013; Toker, Dogan, Ersöz, & Yilmaz, 2013; Wang, Sun, Cao, Tian, & Li, 2008; Wang et al., 2007). Mixture design is another technique used for optimization of ingredients found in the formula considering alternative ingredients (Iop, Silva, & Beleia, 1999). Mixture design was tested for solvent optimization for extraction of phenolic compounds from persimmon fruit (Karaman et al., 2014). When considering optimum ultrasonic conditions they can differ based on the solvent type and/or solvent combination and it is necessary to optimize process factors for extraction solvent combinations. For this aim, mixture design and RSM can be combined to determine the effects of extraction processing factors. In this study, therefore, combined design a combination of RSM and mixture design, was used for obtaining many detailed information about relationship between many independent and dependent factors at specified conditions. In the RSM part of the combined design, D-optimal design type was used. The variation resulted from coefficients in the model can be minimized by using D-optimality (Anonymous, 2015). By using combined design, the simultaneous optimization of mixture and factor responses could be possible, which gains favor in many aspects in terms of raw material usage, time, cost etc. since experimental points can be remarkably reduced by using this design.

To the best of our knowledge, there has not been any study in the literature focused on the application of combined design for optimization of extraction conditions in the food industry. Therefore, in the present study, extraction parameters including solvent (methanol and water concentration), extraction temperature, extraction time and amplitude levels were optimized using combined design methodology in order to determine the optimum extraction conditions to extract phenolics from virgin olive oil mill waste.

2. Materials and methods

2.1. Materials

Dried virgin olive oil mill waste (Moisture content: 59 g/kg; Ash content: 97 g/kg; Oil content: 34 g/kg; Protein content: 75 g/kg) was obtained in a powder form from Kale Naturel Limited Company, Balıkesir, Turkey. The production process of the material was acquired from the company. Briefly, following removal of the seeds, the olives were pressed and then the residual part was dried with spray drier to obtain waste powders. Folin-Ciocalteu's phenol reagent, methanol, gallic acid and Na_2CO_3 were obtained from Merck (Darmstadt, Germany). All chemicals used in this research were analytical grade.

2.2. Establishment of the combined design

As mentioned in the introduction part, combined design is composed of mixture design and RSM. Mixture components were selected as methanol and water with the concentrations ranging between 0 and 1000 mL/L. Categoric process factors in the RSM part were determined as ultrasonic temperature, amplitude and time, which were between 30 and 60 °C, 20–100% and 10–30 min, respectively. D-optimal design was performed in the RSM part of the combined design. The response was selected as total phenolic contents (TPC) of the extracts obtained under conditions designated using the combined design. Totally, 40 experimental points were obtained by the design as presented in Table 1. The ratio of maximum TPC to minimum TPC was 28.15; therefore, the data were transformed using natural logarithm function. Design Expert software (Version 7 Stat-Easy Co., Minneapolis, MN, USA) was used to model the total phenolic concentration of the extracts as a function of mixture components' concentration (methanol and water) and process factor (temperature, amplitude and time) levels. A quadratic model type was used in response part of the design. And linear type model was performed in the mixture part. By using backward elimination technique non-significant parameters ($p > 0.1$) were removed from the established model. Contour and 3D surface plots were drawn using the software under the predefined conditions. Optimization was carried out by maximizing the TPCs of the extracts.

2.3. Extraction of phenolics and determination of total phenolic content (TPC)

Phenolic extraction was performed at the experimental points presented in Table 1. In the extraction process, the variables of solvent were selected as water and methanol and those of ultrasonic conditions were ultrasonic temperature, amplitude and time. One g of the waste powder was weighed into a test tube and incorporated with 10 mL of the solvent. Then the mixture was finely stirred using a vortex. Extraction process was performed using an ultrasonic water bath (Daihan, WUC-D10H, Seoul, South Korea) under the extraction conditions described in Table 1. After the process, the mixture was filtered using a filter paper. The filtrate was used for determination of the TPC.

TPCs of the filtrates were determined according to the modified method described by Singleton, Orthofer, and Lamuela-Raventós (1999). Briefly, 0.4 mL of a 2–40 fold diluted extract was mixed with 2 mL of diluted Folin-Ciocalteu's phenol reagent (diluted 1:10 with distilled water) and 1.6 mL of Na_2CO_3 (75 g/L). The tubes were allowed to stand for 1 h in a dark place at room temperature. Various gallic acid solutions diluted 20 to 100 were prepared for drawing the calibration curve and absorbance of the samples was measured at 760 nm using a spectrophotometer (Shimadzu UV-1800, Kyoto, Japan). TPC was expressed as mg/g (mg gallic acid equivalent (GAE) per 1 g of the olive waste powder).

3. Results and discussion

3.1. Phenolic concentration of the extracts obtained from virgin olive oil waste

The TPCs of the olive oil waste extracts were shown as mg GAE in Table 1. TPCs of the extracts ranged from 1.6 to 45 mg/g, indicating that olive oil waste could be considered as a rich source of natural phenolics. As seen from the results extraction solvent and ultrasonic process conditions markedly influenced the phenolic yield that was extracted from olive oil waste. TPC of the virgin olive oil mill waste was in conformity with the previous studies. TPC was reported to be 5.27–10.01 g GAE/L by Leouifoudi et al. (2014) and 1196.24–3961.79 mg/L in the study of Giuffre, Sicari, Piscopo, and Louadj (2012).

3.2. Establishment of combined design to maximize phenolic extraction yield

As mentioned above, mixture design and RSM were combined in order to observe simultaneous effects of the independent mixture and process factors. Determination coefficient of the established model was 0.9734, indicating that the model could be used to predict TPC of the sample based on concentration of mixture components (methanol and water) and ultrasonic process factor (temperature, time and amplitude) levels. Adj-R^2 value was determined to be 0.9529 very close to R^2 implying that all