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Optimization of extraction of phenolic compounds from wheat using response surface methodology

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Abstract

The optimum conditions for the extraction of crude phenolics from whole grain and bran of soft and hard wheat were determined using response surface methodology (RSM). A face-centered cubic design (FCD) was used to investigate the effects of three independent variables, namely solvent composition (%), extraction temperature (°C) and time (min) on the response, total antioxidant activity (TAA). The independent variables were coded at three levels and their actual values selected on the basis of preliminary experimental results. The FCD consisted of 14 experimental points and three replications at the center point. Data were analyzed using design expert and statistical analysis system software. A second-order polynomial model was used for predicting the response. Regression analysis showed that more than 89% of the variation was explained by the models. Canonical analysis of surface responses revealed that the stationary surface was a saddle. The optimal conditions forthe TAA obtained using ridge analysis were 54%, 61 °C, 64 min and 49%, 64 °C, 60 min, for whole grain and bran of soft wheat, respectively. Under the optimum conditions the corresponding predicted response values for TAA were 56.5 and 63 TE. The crude phenolics were extracted under optimum conditions to check the validity of the model. The values were 54.7 ± 3.2 and 61.3 ± 1.9 TE, for whole grain and bran of soft wheat, respectively; A similar trend was observed for TAA of hard wheat. The experimental values agreed with those predicted, thus indicating suitability of the model employed and the success of RSM in optimizing the extraction conditions.

Keywords: Response surface methodology; Extraction of phenolics; Optimization of extraction; Total antioxidant activity; Canonical and ridge analyses

1. Introduction

Phenolic compounds are common dietary phytochemicals found in fruits, vegetables and grains. Epidemiological evidences have suggested that food phenolics may have protective effects against degenerative diseases (Mazza, 2000). Most of the beneficial characteristics of phenolic compounds have been ascribed to their antioxidant activity which is a fundamental property important to life (Rice-Evans, Miller, & Paganga, 1997). Andreasen, Landbo, Christensen, Hansen, and Meyer (2000) have reported on the positive effects of higher intake of whole grain foods in lowering the risk of coronary heart disease. It has also been suggested that adults would gain appreciable protection from coronary heart disease by consuming the recommended three servings of whole grains daily (Andreasen, Christensen, Meyer, & Hansen, 2000). Thus, whole grains, rich in fiber and phytochemicals, are among the healthiest foods that individuals may consume and render a wide variety of health benefits (Andreasen et al., 2001). Plants and plant extracts have been used in traditional cures and herbal remedies for centuries throughout the world.

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Recently there has been a renewed interest in secondary plant metabolites because of their potential preventive effects on the chronic diseases such as cardiovascular disease and cancer (Rowland, 1999). Hence, isolation, identification and quantification of phytochemicals in foods and evaluation of their potential health benefits have been in focus: However, in vitro and animal studies have shown that the action of some chemicals are likely to be achieved only at doses much higher than those that can be obtained from eating plants (Rowland, 1999). Thus, the extraction of the active ingredient is essential if they are to be of prophylactic or therapeutic value in human subjects (Rowland, 1999).

Many factors such as solvent composition, extraction time, extraction temperature (Wettasinghe & Shahidi, 1999), solvent to solid ratio (Cacace & Mazza, 2003a) and extraction pressure (Cacace & Mazza, 2002), among others, may significantly influence the extraction efficacy. In general, optimization of a process could be achieved by either empirical or statistical methods; the former having limitations toward complete optimization. The traditional one-factor-at-a-time approach to process optimization is time consuming. Moreover, the interactions among various factors may be ignored hence the chance of approaching a true optimum is very unlikely. Thus, one-factor-at-a-time procedure assumes that various parameters do not interact, thus the process response is a direct function of the single varied parameter. However, the actual response of the process results from the interactive influence of various variables. Unlike conventional optimization, the statistical optimization procedures allows one to take interaction of variables into consideration (Haaland, 1989).

Response surface methodology (RSM), originally described by Box and Wilson (1951), enables evaluation of the effects of several process variables and their interactions on response variables. Thus, RSM is a collection of statistical and mathematical techniques that has been successfully used for developing, improving and optimizing processes (Myers & Montgomery, 2002). Response surface methodology has been successfully used to model and optimize biochemical and biotechnological processes related to food systems (Cacace & Mazza, 2003b; Parajo, Santos, Dominguez, & Vazquez, 1995; Senanayake & Shahidi, 1999; Senanayake & Shahidi, 2002; Telez-Luis, Moldes, Alonso, & Vazquez, 2003; Vasquez & Martin, 1998) including extraction of phenolic compounds from berries (Cacace & Mazza, 2003a, 2003b) and evening primrose meal (Wettasinghe & Shahidi, 1999), anthocyanins from black currants (Cacace & Mazza, 2003a) and sunflower hull (Gao & Mazza, 1996) and vitamin E from wheat germ (Ge, Ni, Yan, Chen, & Cai, 2002), among others.

The extraction and purification of phytochemicals from natural sources is needed, since these bioactives are often used in the preparation of dietary supplements, nutraceuticals, functional food ingredients, food additives, pharmaceutical and cosmetic products (Gao & Mazza, 1996). In this study, optimization of experimental conditions that results in the highest antioxidant activity of crude wheat phenolic extracts was conducted. Whole grain and bran of soft and hard wheat were extracted with a number of polar solvents and their total antioxidant activity (TAA) determined and optimum experimental conditions derived using RSM.

2. Materials and methods

2.1. Materials

Whole grain and bran of commercial soft (70% Canadian eastern soft red spring and 30% Canadian eastern soft white winter) and hard (90% Canadian western hard red spring and 10% Canadian eastern hard red winter) wheat mixtures were obtained from the Robin Hood Multifoods Inc. plant in Saskatchewan through their head office in Markham, ON. The compounds 1,1-diphenyl-2-picrlthydrazyl (DPPH), 2,2′-azino-di[3-ethylbenzthiazoline sulfonate(ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), ferulic acid and Folin–Ciocalteu phenol reagent were purchased from Sigma–Aldrich Chemical Co. (St. Louis, MO). All other chemicals and solvents were purchased from Fisher Scientific (Nepean, ON) and were of ACS grade or better.

2.2. Preparation of samples

Whole grain wheat and its bran were ground in an electric grinder (Black and Decker Canada Inc., Brockville, ON) to obtain a fine powder. All samples tested were defatted by blending the ground material with hexane (1:5 w/v, 5 min, ×3) in a Waring blender (Model 33BL73, Waring Products Division, Dynamics Corp. of America, New Hartford, CT) at ambient temperature. Defatted wheat samples were air dried for 12 h and stored in vacuum packaged polyethylene pouches at −20 °C until used for further analysis.

2.3. Selection of appropriate extraction conditions

The initial step of the preliminary experiment was to select an appropriate extraction medium for wheat phenolics. Three different solvent systems, namely ethanol, methanol and acetone were examined. Crude phenolic compounds from whole wheat and bran of soft and hard wheat were extracted using a series of extraction media varying in the range of 0–100% (v/v; water/ethanol, methanol or acetone). The crude phenolic ↑ extracts were prepared by extracting the ground wheat samples (6 g) with 100 ml of solvent for 20 min at 80 °C. Based on total antioxidant activity (TAA), determined