HOT PRESSURIZED FLUID EXTRACTION OPTIMIZATION OF POTATO PEEL USING RESPONSE SURFACE AND THE TAGUCHI METHOD

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Abstract: Potato peel is considered an industrial by-product mainly composed of complex carbohydrates, phenolics, and glycoalkaloids. In this study, the Taguchi method to design a Response Surface Methodology (RSM) for the responses of total carbohydrates and phenolics was used. Different processing parameters were evaluated (40-140 bar, 140-240^{o}C, 1-5 mL/min of flow rate, and 2-16 min of static holding time). The results showed the optimal conditions as a Pareto Front for total carbohydrates (1300 mg glucose equivalent/g potato peel) and phenolics (52 mg gallic acid equivalent/g potato peel) obtained at 89 bar, 240^{o}C, 1 mL/min, and 15 min of static holding time. These values had deviations <15% of the predicted values using the Taguchi method. Therefore, the RSM based on the Taguchi method is a reliable method to find the optimal conditions for hot pressurized fluid extraction.

Keywords: carbohydrates, extraction, phenolics, Taguchi method.

1. Introduction

By-products resulting from agriculture and food processing companies are abundant biomasses and represent cheap sources of phytochemicals. Among such are husk, hull, and peels, like potato peel that is a by-product from the peeling process. Extraction of phenolics from potato peel using hot pressurized fluid (HPF) technology have been conducted at various temperatures and a constant pressure [1,2]. Singh and Saldaña [1] removed total phenolics with methanol in a solid-liquid (S-L) batch extraction (0.46 mg/g wet potato peel) and with subcritical water (sCW) extraction (0.2 mg/g dry potato peel). Wijngaard et al. [2] reported similar total phenolics extraction with aqueous ethanol in the S-L batch extraction (3.94 mg/g dried potato peel) and with subcritical water ethanol extraction (3.68 mg/g dried potato peel).

For the optimal removal of phytochemicals from crop biomass, Response Surface Methodology (RSM) based on central composite design is commonly used. The RSM determines optimum conditions for one response using the significant factors with a polynomial fitting on the response. The accuracy of the RSM is affected by the limitations on the number of experimental data points, noise of the data (experimental errors not normally distributed), and inadequacy of the quadratic fitting model [3]. The noise of the data can increase in experiments with highly sensitive responses, such as the temperature on the sCW extractions, thus requiring the use of replicates in the experimental design. These limitations explain the results reported by Wijngaard et al. [2], who obtained slightly higher extraction of phenolics using the S-L batch extraction than with the sCF extraction. An alternative experimental design is the Taguchi method [3] that determines the optimal conditions of the factors without the use of a fitting model. In a multiresponse experiment, such as the sCW extraction of phytochemicals, measurements of several responses are obtained for each set of parameters. Optimum conditions for a multiresponse experiment are not global because conditions that are optimal for one response may be not adequate for the other responses. However, the desirability function approach is the most widely used method in the industry to calculate the optimum conditions of multiresponse experiments [3].

The main objective of this study was to evaluate the effect of pressure, temperature, flow rate, static holding time and pH for the sCF extraction of total phenolics and total carbohydrates from potato peel. The results obtained with the one variable at a time method were compared with those obtained with the Taguchi method experimental design.

2. Materials and Methods

2.1 Materials and sample preparation

Red potato was obtained from a local supermarket Superstore (Edmonton, AB, Canada). The water used was millipore water with pH of 6.7. Glucose and gallic acid standards, with purity ≥ 96%, were obtained from Sigma Aldrich (St. Louis, MO) and Fisher Scientific (Fair Lawn, NJ), respectively. Potato peel was freeze dried for 3 days using a Vertis freeze drier (Gardiner, NY) and milled in a Fritsch mill model #14-4050 (Idar-Oberstein, Rhineland-Palatinate, Germany) using four different sieves (20, 30, 40 and 50 mesh). The retained material with an average particle size of 0.43 mm was used for the extraction experiments.

2.2 Total phenolics

The Folin-Ciocalteu method was used to determine total phenolics content, following the methodology proposed by Singleton and Rossi [4]. For the analysis of the extracts, a calibration curve using six concentrations of gallic acid was first performed. The final results were expressed as milligrams of gallic acid equivalents per gram of dried potato peel (mg GAE/g pp).

2.3 Total carbohydrates

The methodology of Dubois et al. [5] was used to determine total carbohydrates content. The standard curve for total polysaccharides used glucose solutions ranging from 0.5 to 10 mg/g solution. The final results were expressed as milligrams of glucose equivalents per gram of dried potato peel (mg GE/g pp).

2.4 Subcritical water extraction

Figure 1 shows the equipment used for the sCW extraction of phytochemicals from potato peel. This system can operate up to 250 bar and 300 °C. All experiments were carried out using the same extraction cell (0.025 m i.d. × 0.10 m length) with two filters of 20 μm syntherized stainless steel for the inlet and outlet. Freeze dried potato peel (0.5 g) was loaded into the extraction vessel. The solvent was first degassed and then delivered with the pump at a constant flow rate to the preheating section and to the extraction vessel preloaded with potato peel. The pressure of the system was adjusted to the desired operating condition by using the back-pressure regulator. An optimal static holding time of 15 min was maintained constant before starting the extractions. The temperature of the system was monitored by thermocouples type K. The sCW system is equipped with a cooling unit that uses cold water at 20^{o}C to prevent thermo-degradation. The extracts were stored at -20°C for further analysis. Duplicates for each extraction experiment and analysis were performed.

3. Experimental Design

The optimization of the sCW extraction was first studied with the use of a factorial design and one variable at the time method. The results were then compared to the optimized conditions obtained by the Taguchi method. This method used parameters with discrete values (categorical parameters) to describe the behavior of the experiments. The L9 orthogonal array was selected in the Taguchi method to analyze the variables at three levels. An advantage of this experimental design to evaluate four parameters is that it required only nine experiments.

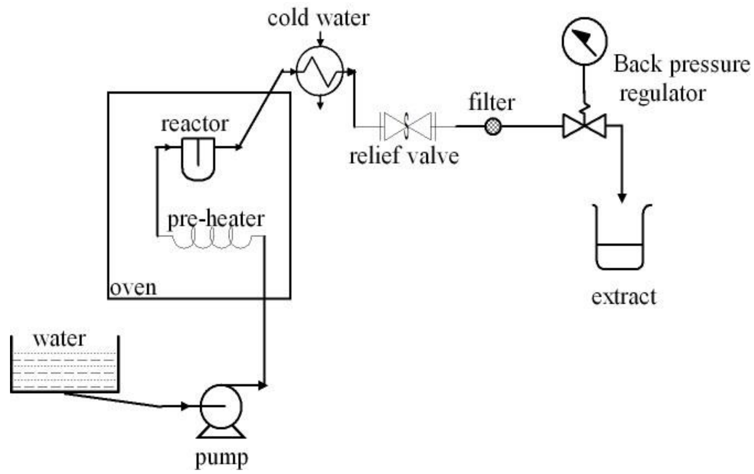


Figure 1. Subcritical water extraction system.

4. Results and discussion

The pressures used in this study guaranteed liquid state and prevented vapor formation [6]. The experiments were performed using 60 mL of solvent (pure water, acidic or alkaline water), allowing a variable extraction time as a function of the flow rate used. The maximum relative standard deviations for the replicates of the experiments for total phenolics and carbohydrates were 8 and 17%, respectively. The colorimetric method for carbohydrates determination reported higher deviations in the repeatability.

4.1 Effect of static holding time

Two static holding times of 5 and 15 min were evaluated for the sCW extraction of phytochemicals at 160°C and 80 bar (Fig. 2). The yield of phenolics (Fig 2.a) and carbohydrates (2.b) extraction using 15 min are higher than using 5 min of static holding time. Then, the static holding time of 15 min was selected due to its high yield and this static holding time was used for further sCW extractions.

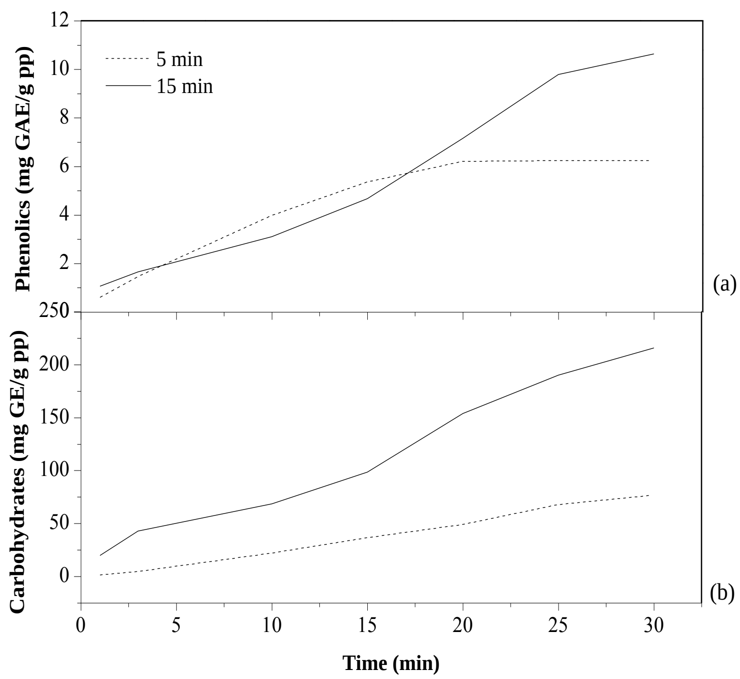


Figure 2. Total phenolics (a) and total carbohydrates (b) of extracts obtained after the sCW extraction at 160°C, 80 bar, 2 mL/min, 30 min of extraction and static holding times of 5 min (¨) and 15 min (line).

4.2 Effect of pressure and flow rate

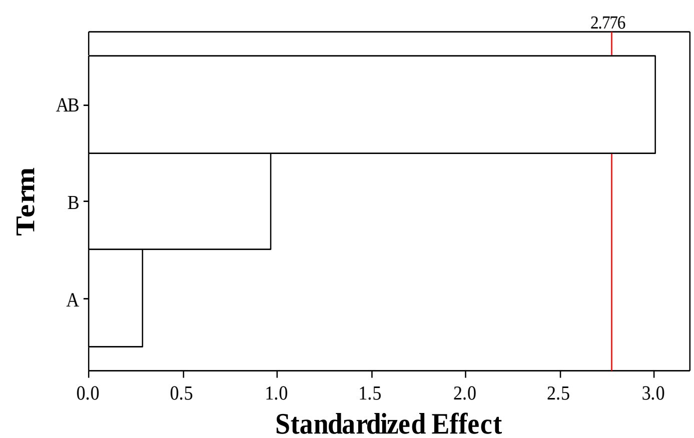
Table 1 shows the factorial design to evaluate the influence of pressure (40 and 180 bar) and flow rate (1 and 6 mL/min) for the sCW extraction of total phenolics and total carbohydrates from potato peel at 160^{o}C and 15 min of static holding time. Total carbohydrates were better extracted using 40 bar than using 180 bar at a constant flow rate of 1 or 6 mL/min. However, no extraction trend was observed for total phenolics.

Table 1. Effect of pressure and flow rate on the sCW extraction of phytochemicals at 160^{o}C, 15 min of static holding time and 60 mL of water.

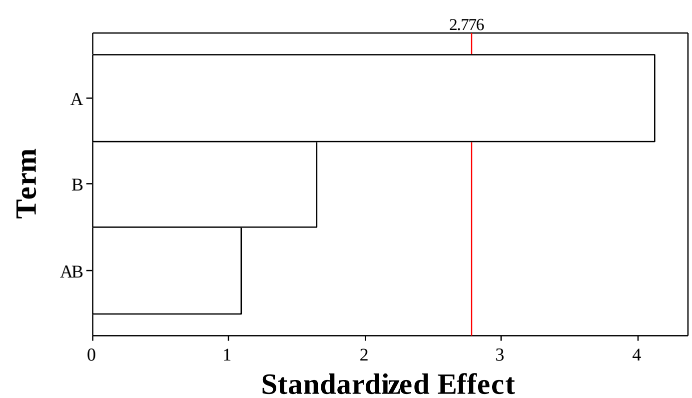
Pressure (bar)	Flow rate (mL/min)	Total phenolics (mg GAE/g pp)	Total carbohydrates (mg GE/g pp)
40	1	25.20	307.20
180	1	19.90	119.71
40	6	18.80	211.37
180	6	24.91	102.10

GAE: Gallic acid equivalent, pp: potato peel, GE: Glucose equivalent.

Figure 3 shows the pareto chart of standardized effects of pressure, flow rate and their interaction for the sCW extraction of total phenolics and total carbohydrates from potato peel. The results confirmed that pressure had a significant effect on the extraction of total carbohydrates while the interaction of pressure and flow rate had a significant effect on the extraction of total phenolics



(a)



(b)

Figure 3. Pareto chart of standardized effects for total phenolics (a) and total carbohydrates (b) of the sCW extracts obtained at 160^{o}C and 15 min of static holding time.
A: Pressure (40-180 bar), B: Flow rate (1-6 mL/min), AB: Interaction of pressure and flow rate.

4.3 Effect of temperature and pH

The increase of temperature increased the extraction of phytochemicals as shown in Fig. 4. The acidic or alkaline water media increased the extraction of total phenolics up to 220°C. Pure sCW (pH 7) was better for the extraction above 220°C (Fig. 4a). This trend indicates possible degradation of phenolics in acidic or alkaline sCW at temperatures over 220°C. Fig. 4b shows that the extraction of total carbohydrates was high in acidic sCW media and low in alkaline sCW media at all temperatures investigated. Also, the maximum extraction of total carbohydrates was obtained at 240°C. Hydrolysis of polysaccharides, such as hemicellulose, is favored in acidic water media than in pure or alkaline water media.

Figure 4 also shows the values obtained by High Performance Liquid Chromatography (HPLC) analysis for the main phenolics and carbohydrates in potato peel extracts. These values (e.g. xylose and flavonoids) increased as the extraction temperature increased. A similar extraction trend was observed using values obtained from the colorimetric method, but these values overestimated the amounts of total phenolics and carbohydrates. The overestimation obtained with the colorimetric method is related to the dark extract solution color and the presence of interfering compounds for total carbohydrates and phenolics analysis, such as xylose and flavonoids [5,7] and reducing compounds, respectively [4]. However, the colorimetric method is simple and fast and therefore was used for this study.

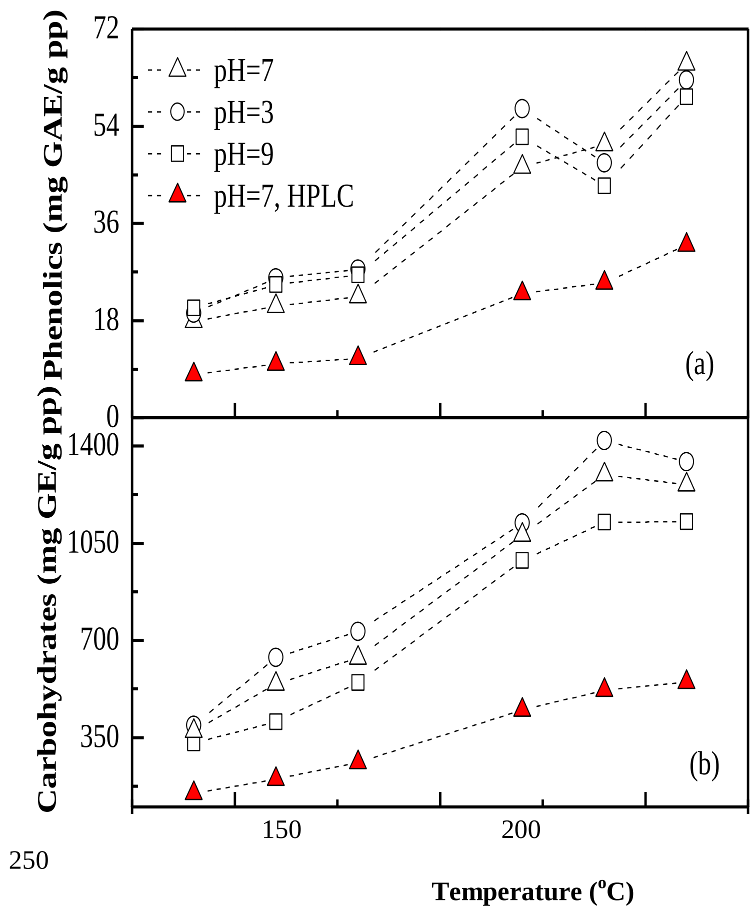


Figure 4. Total phenolics (a) and total carbohydrates (b) obtained using sCW extraction at 110 bar, 2 mL/min, and 30 min of extraction. Dots are used for visual help. Gallic acid equivalent (GAE), Glucose equivalent (GE), $FeSO_4$ equivalent ($FeSO_4$), and potato peel (pp).

4.4 Taguchi method

The main parameters of the sCW extraction (pressure, temperature, static holding time and flow rate) were evaluated for a potato peel extract with 60 mL of water using the Taguchi method [3] as shown in Table 2. The influence of the parameters on the sCW extraction is shown in Fig. 5.

Table 2. Orthogonal array of the Taguchi method to evaluate the effect of processing conditions of the sCW extraction using 60 mL of water.

sCW processing conditions				**Extract**	
Pressure (bar)	**T (^oC)**	**SHT (min)**	**Flow rate (mL/min)**	**Total phenolics (mg GAE/g pp)**	**Total carbohydrates (mg GE/g pp)**
40	140	2	1	11.07	346.68
40	190	9	3	25.60	568.17
40	240	16	5	42.84	992.45
90	140	9	5	9.73	345.42
90	190	16	1	29.89	950.62
90	240	2	3	50.42	1276.16
140	140	16	3	12.72	465.84
140	190	2	5	17.06	469.27
140	240	9	1	45.39	1106.58
Predicted conditions					
90*	240	16	3	52.72	1381.76

Temperature (T), Static holding time (SHT), Gallic acid equivalent (GAE), Glucose equivalent (GE), Ferric Reducing/Antioxidant Power (FRAP), $FeSO_4$ equivalent ($FeSO_4$), potato peel (pp). *Predicted conditions for maximum extraction of total phenolics and total carbohydrates.

The increase of temperature up to 240^{o}C increased the extraction of phytochemicals. The best pressure for the extraction of total carbohydrates and total phenolics was 90 bar. The static holding time (SHT) between 2 and 16 min showed no influence on the sCW extraction of phytochemicals, and flow rates lower than 3 mL/min increased the extraction of phytochemicals. The analysis with the Taguchi method was similar to the one-variable-at-a-time method, except that the use of high pressures increased the extraction of total phenolics and decreased the extraction of total carbohydrates. These contradictory results suggest that the Taguchi method lacks to represent well the main effects of the parameters due to the influence of significant interaction effects, such as pressure-flow rate.

Following the analysis of the results from the Taguchi method, the maximum extraction conditions of total phenolics and total carbohydrates were calculated as 90 bar, 240^{o}C, 16 min of SHT, and 3 mL/min of flow rate. These conditions predicted total phenolics corresponding to 52.72 mg GAE/g potato peel, and total carbohydrates corresponding to 1381.76 mg GE/g potato peel. The high value of total carbohydrates predicted is due to overestimation of the colorimetric method as discussed earlier. These values contrasted with those of total phenolics and total carbohydrates reported in Fig. 4, with differences lower than 4 and 21%, respectively. Therefore, the Taguchi method can be used with confidence for a fast prediction of the optimal conditions of the sCW extraction.

In addition, a method is proposed to increase the advantages of the Taguchi method. The orthogonal array was used to design the RSM using a polynomial equation that fitted each response value. Then, the models obtained for total carbohydrates and total phenolics were optimized using a multi-objective genetic algorithm to obtain the Pareto Frontier [8], as shown in Fig. 6. Also, the mean squared error (mse) of the curves for total phenolics ($3.06E\text{-}01*P - 1.70E\text{-}01*T + 1.69E\text{-}01*SHT - 1.37*FR - 1.77E\text{-}03*P^2 + 1.38E\text{-}03*T^2$) and carbohydrates ($1.49E\text{+}01*P - 5.40*T + 7.58*SHT - 4.96E\text{+}01*FR - 8.03E\text{-}02*P^2 + 3.37E\text{-}02*T^2$) is reported in Figure 6. The extractions of total phenolics and total carbohydrates had a linear trend (Fig. 6), thus challenging the extraction of high amounts of phenolics with low amounts of carbohydrates. Fig. 6 also shows the predicted extraction by the Taguchi method as point A, value that is similar to the values obtained from the Pareto Frontier. When comparing points A, B and C to the sCW data of Fig. 4, similar deviations lower than 4 and 24% were observed for total phenolics and carbohydrates, respectively.

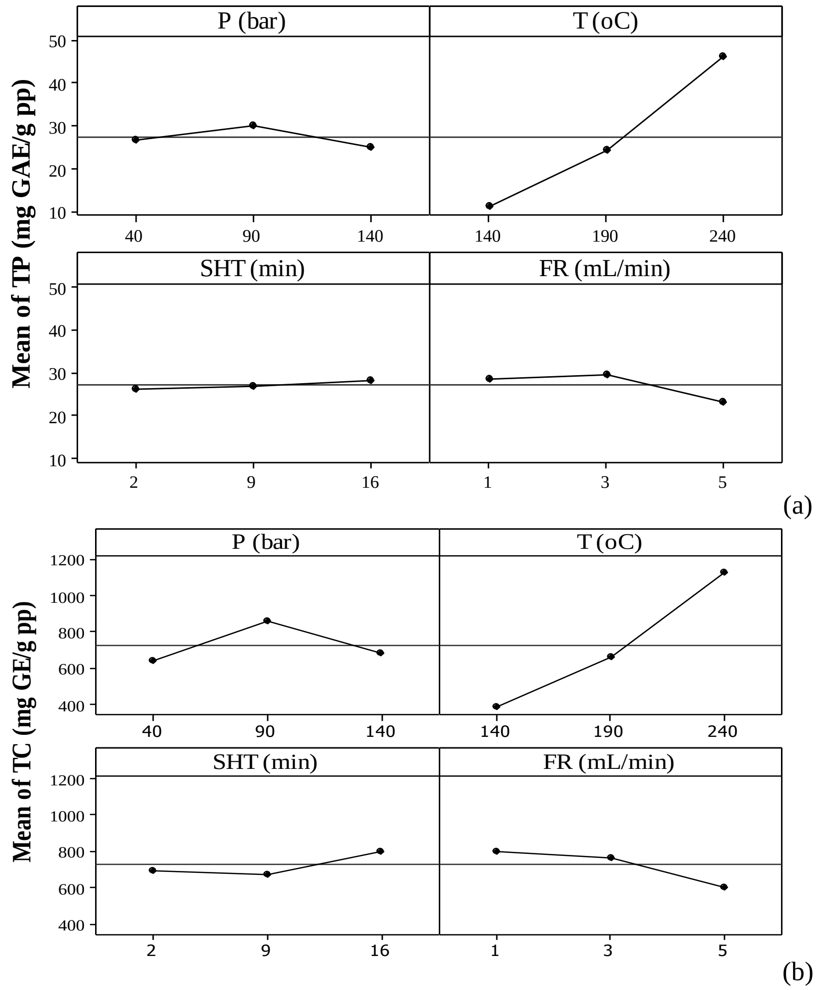


Figure 5. Main effect plots for the extraction of total phenolics (a) and total carbohydrates calculated by the Taguchi method. Pressure (P), Temperature (T), Static holding time (SHT), Gallic acid equivalent (GAE), Glucose equivalent (GE), potato peel (pp).

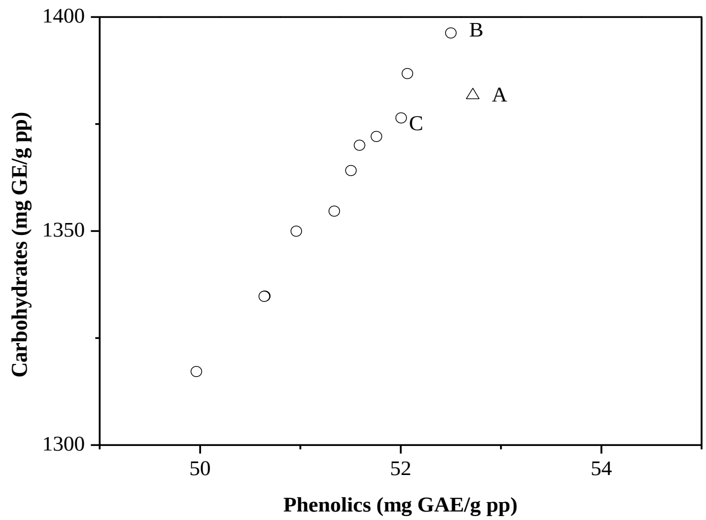


Figure 6. Optimal extraction rates for total phenolics and carbohydrates calculated using the Taguchi method (Δ) and Pareto Frontier (○) for the RSM[1] of the orthogonal array. Point A (90 bar, 240°C, 16 min, and 3 mL/min), point B (89 bar, 240°C, 15 min, and 1 mL/min), and point C (90 bar, 240°C, 13 min, and 1 mL/min). [1]Phenolics (mg GAE/g pp) mse = 3.1, and carbohydrates (mg GE/g pp) mse = 84.2.

5. Conclusions

Phytochemicals, such as carbohydrates and phenolics were successfully extracted from potato peel using subcritical water. Pressure was a statistically significant parameter that influenced the yield of extraction of carbohydrates and phenolics from potato peel. The use of pressures above 80 bar decreased the extraction of carbohydrates, but increased the extraction of phenolics, facilitating the separation of these two compounds. The flow rate and static holding time showed no significant influence on the sCW extraction. Temperatures over 220^{o}C favored the maximum extraction of phenolics and carbohydrates. Acidic sCW increased the extraction of carbohydrates up to 260^{o}C, but pure sCW over 220^{o}C was a better fluid for the extractions. The Taguchi method can be used with confidence for the optimization of the sCW extraction and the Response Surface Methodology obtained from this method can be used to visualize the performance of the optimization.

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