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Extraction of Bioactive Compounds from Yellow Onion Peels: Taguchi-SAW Hybrid Optimization

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ABSTRACT

The aim of this study was to obtain an extract rich in bioactive components from yellow onion peels, which are generally considered waste material. Accordingly, a three-factor three-level Taguchi (L9) experimental design with three factors, namely ethanol concentration (A; 50%, 75%, 100%), extraction temperature (B; 30, 40, 50 °C), and sonication time (C; 10, 20, 30 min) was used to optimize the ultrasound-assisted extraction process of onion peel powders. Through Taguchi optimization, the optimum extraction conditions were determined as $A_2B_3C_2$ to obtain the extract with the highest total phenolic matter (TPM) content and antioxidant activity (DPPH (%)). In addition, the extract produced under $A_2B_1C_2$ conditions was found to be the richest in total monomeric anthocyanin (TMA) content with the highest level of color pigments. In order to determine the overall optimization conditions and to reduce the three-response optimization process to a single response, the simple sum weighting (SAW) method was used as a multi-criteria decision-making method. As a result of the optimization, it was concluded that an extract rich in bioactive components with optimal TPM and TMA contents and DPPH (%) value could be obtained as a result of sonication at 30 °C for 20 min to onion peel powders mixed with a solvent containing 75% ethanol ($A_2B_1C_2$). The extraction conditions of bioactive components from yellow onion peels were successfully optimized by the Taguchi-SAW hybrid optimization method.

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Introduction

Biomass waste is recognized as one of the significant global issues throughout the world and is becoming increasingly important in developing countries due to its detrimental impact on the environment. Fruit and vegetable waste and associated by-products are produced in substantial quantities through industrial processes, posing a serious environmental threat when not appropriately managed. However, these waste materials are rich in bioactive compounds known for their potential health benefits. In recent years, considerable efforts have been made to develop techniques for the efficient utilization of fruit and vegetable waste (Pal and Jadeja, 2019).

Onions (*Allium cepa* L.), a member of the Allium family, are not only known for their delicious taste but also serve as a valuable source of various beneficial compounds. Onions, particularly their peels, are rich in phenolic compounds and exhibit antioxidant properties (Bordin Viera et al., 2023). Red, yellow, and white onions contain significant amounts of anthocyanins, which are responsible for the coloration of onion peels. These anthocyanins have found applications in treating various diseases, including cancer, atherosclerosis, diabetes, and cardiovascular disease (Jeya Krithika et al., 2022).

Bioactive components are used as additives in foods due to their positive effects on human health. These functional compounds, which are already used in various applications such as pharmaceuticals, cosmetics, and textiles, are usually obtained by extraction from different parts of plants (stems, leaves, bark, seeds, fruits, etc.). Studies on different extraction methods have focused on the separation of these components in order to increase extraction efficiency, reduce costs, and maximize profits. Ultrasound (US) technique is an alternative to supercritical fluid extraction and microwave-assisted extraction for the extraction of active substances from plant samples. This technique is economical, environmentally friendly, and time-saving compared to conventional extraction methods. The US disrupts cell tissues in a short time, accelerating mass transfer and thus increasing extraction efficiency (Alves Filho et al., 2021).

Taguchi method is one of the optimization techniques that can reduce the number of experiments, improve product quality, and determine design solutions. Taguchi method, which is generally used in single response optimization, can be integrated with multi-criteria decision-making methods and applied to obtain optimal conditions for multiple responses (Güldane, 2023). Taguchi-PROMETHEE (Crnjac et al., 2019), Taguchi-TOPSIS (Singh et al., 2011), and Taguchi-AHP (Salari et al., 2019) hybrid optimization techniques have been applied successfully in various process operations. Pal and Jadeja (2019) applied the Taguchi method in single response optimization to maximize polyphenolic antioxidant extraction from onion peel.

In the literature, there is no study in which the Taguchi-SAW method was applied in multiple response optimization for the extraction of bioactive compounds from yellow onion peels. In this study, it was aimed to determine the optimal extraction conditions of yellow onion peels by ultrasonically assisted extraction method. Extraction conditions, ethanol concentration (A; 50%, 75%, 100%), extraction temperature (B; 30, 40, 50 °C), and sonication time (C; 10, 20, 30 min) were optimized by Taguchi-SAW hybrid optimization method to maximize total phenolic and total monomeric anthocyanin contents, and DPPH radical scavenging activity.

Materials and Methods

Materials

The waste onion peels used in this study were obtained from a local grocery store in the Pamukova region of Sakarya. The chemicals used in the analysis were of analytical purity and were obtained from Merck (Germany).

Extraction of Bioactive Compounds from Yellow Onion Peels

Onion peels were washed with distilled water and dried in an oven at 40 °C for 4 hours (Santos and Martins, 2022). After cooling the onion peels to room temperature, they were ground with a blender. Then, 1 g of the powdered samples was transferred to a beaker and 10 ml of 96% (v/v) ethanol was added at different concentrations (50, 75, and 100%). The pH of the mixtures was adjusted to 2.0 using 0.1N HCl (Santos and Martins, 2022). The samples were sonicated for different times (10, 20, and 30 min) in a 150 kW ultrasonic water bath (CALISKAN, LAB ULT 4045, China) set at various temperatures (30, 40, and 50 °C). The mixtures were then filtered through a water tromb using filter paper (Whatman no:1). The filtrates were stored at 4°C until analysis.

Experimental Design

Taguchi method was used in the optimization of bioactive component extraction from onion peel. Minitab 19.0 software was utilized in the planning of the study. Experiments were carried out according to Taguchi L9 experimental design which consists of 3 factors and 3 levels (Table 2). Ethanol concentration (50-100%), extraction temperature (30-50 °C), and sonication time (10-30 min) were selected as control parameters (Table 1). Literature data was used to determine the extraction factors and the levels of each extraction factor.

Taguchi Optimization Method

Taguchi method is an optimization technique that is characterized by determining the optimum levels of extraction parameters with a minimum number of experiments. This procedure is specifically preferred for optimizing a single process characteristic. In the Taguchi technique, optimal parameters are obtained by comparing the S/N ratios corresponding to each test parameter. In this study, the "larger is better" (Equation 1), which corresponds to the maximum levels of S/N ratios for each of the responses was preferred since the aim was to obtain an extract rich in bioactive components (Roy, 2010).

$$S/_N = -10log\left[\frac{1}{n}\sum_{i=1}^{n}\frac{1}{y_{ij}^2}\right] \quad (1)$$

Table 1. Control parameters and levels

Symbol	Factors	Unit	Level 1	Level 2	Level 3
A	Ethanol concentration	%	50	75	100
B	Sonication temperature	°C	30	40	50
C	Sonication time	min	10	20	30

Table 2. Taguchi L9 orthogonal design, experimental results, and S/N ratio values

Run	Factors and levels			Responses					
	A	B	C	TPM (mg GAE/g)		TMA (mg C3G/100 g)		DPPH (%)	
				Mean	S/N	Mean	S/N	Mean	S/N
1	50	30	10	29.54	29.41	10.75	20.63	47.53	33.54
2	50	40	20	30.91	29.80	7.86	17.91	62.46	35.91
3	50	50	30	33.29	30.45	12.31	21.81	64.43	36.18
4	75	30	20	33.36	30.47	17.63	24.92	61.24	35.74
5	75	40	30	32.49	30.24	9.30	19.37	53.67	34.59
6	75	50	10	33.83	30.59	10.30	20.26	58.71	35.37
7	100	30	30	31.05	29.84	9.56	19.61	45.98	33.25
8	100	40	10	24.80	27.89	10.70	20.58	36.88	31.34
9	100	50	20	33.12	30.40	9.80	19.83	46.08	33.27

SAW Method

Simple additive weighting (SAW) method was used for multiple response optimization in the extraction of bioactive components from onion peels. In this approach, the runs are scored between 0 and 1, and the sample equal to or closest to 1 is considered as the most ideal sample. The SAW method involves 4 steps (Muddineni et al., 2017):

- Construction of decision matrix (D_{mxn}) (Equation 2)

$$D_{mxn} = \begin{Bmatrix} x_{11} & x_{12} \cdots & x_{1n} \\ x_{12} & x_{22} \cdots & x_{2n} \\ \vdots & \vdots & \vdots \\ x_{m1} & x_{m2} \cdots & x_{mn} \end{Bmatrix}, \quad (2)$$

- Normalization of the parameters using Equation 3

$$n_{ij} = \frac{r_{ij}}{\max(r_{ij})} \quad (3)$$

- Calculation of the weighted decision matrix using Equation 4. The weighting value for each parameter was determined by principal component analysis (PCA). The details of this method are described by Guldane and Dogan (2022).

$$A_j = \sum w_i x_{ij} \quad (4)$$

- The sample with the highest score is identified as the ideal.

Analysis

Total phenolic matter (TPM)

The TPM content of onion peel extracts was determined using the Folin-Ciocalteu spectrophotometric method described by Pal and Jadeja (2019). Firstly, 1 ml onion peel extract, 5 ml distilled water, and 0.5 ml Folin Ciocalteu reagent were added to the tubes and vortexed. After 5 minutes, 1.5 ml of 20% sodium carbonate (Na_2CO_3) and 2 ml of distilled water were added to the tubes and kept in the dark for 2 hours and the absorbance at 760 nm wavelength was measured in a spectrophotometer (Shimadzu UV-1240, Japan). The phenolic content of the samples was calculated using the calibration curve equation prepared using standard gallic acid solutions (25-500 mg/L). The total phenolic content of the extracts was calculated as mg GAE/g extract. These measurements were performed in 3 replicates and the results were given as the average.

Total monomeric anthocyanin (TMA)

The total anthocyanin content of onion peel extracts was determined according to the pH differential method reported by Stoica et al. (2020). This method is based on the absorbance values obtained spectrophotometrically at different pH ranges. Buffer solutions of pH 1.0 prepared with potassium chloride and pH 4.5 prepared with sodium acetate were used to prepare the dilutions of the prepared extracts. The absorbance values of the samples were measured in a UV-VIS spectrophotometer at 520 and 700 nm wavelengths. The TMA content of the samples was calculated by Equation 6 in terms of cyanidin-3-glycoside (C3G) equivalents based on the absorbance differences determined by Equation 5. Results are expressed as mg C3G/ 100 g sample.

$$Abs = (A_{520\,nm} - A_{700\,nm})_{pH\,1.0} - (A_{520\,nm} - A_{700\,nm})_{pH\,4.5} \quad (5)$$

$$TMA\ \left(\frac{mg}{100\ g}\right) = \frac{Abs \times M_w \times DF \times 10}{\varepsilon \times l} \quad (6)$$

where M_w= 449.2 g/mol for cyanidin-3-glucoside (C3G); DF= dilution factor; 1000= factor for conversion from g to mg; ε= 26.900 L/mol x cm, molar extinction coefficient for C3G; l= path length in cm.

Antioxidant activity (DPPH(%))

The total antioxidant activity of onion peel extracts was determined by using the radical scavenging activity of the extracts against DPPH radical according to the method proposed by Viera et al. (2023). For this purpose, 0.5 ml of ethanolic extract was added to 2.5 ml of 0.1 mM DPPH solution. The mixture was shaken gently by hand and kept at room temperature in the dark for 30 minutes. The absorbance of the samples was determined by measuring at 517 nm wavelength. The percent radical scavenging activity values of the samples were expressed as DPPH (%). The results were calculated using Equation 7.

$$DPPH(\%) = \frac{(A_0 - A_{sample})}{A_0} \times 100 \quad (7)$$

Results and Discussion

Taguchi Optimization

Experimental studies were carried out according to the L9 experimental design (Table 2) to determine the influence of extraction parameters on the responses in ultrasound-assisted extraction of onion peel. The studies were carefully conducted to determine the optimal extraction process for each response variable. Ultrasonic extraction aimed to maximize the total phenolic matter, total monomeric anthocyanin content, and antioxidant activity of the extracts. Therefore, the individual optimization of all three responses was based on the “larger is better” criterion to determine the optimal conditions.

Effect of Process Parameters on Total Phenolic Matter (TPM)

The TPM content of onion peel extracts varied between 24.80 and 33.83 mg GAE/g (Table 2). Viera et al. (2023) stated that the TPM content of red onion peels varied between 117.50 and 822.87 mg GAE/g. JK et al. (2022) also reported that red onion was richer in bioactive compounds than yellow one. The main effect plot for total phenolic content during ultrasonic extraction of bioactive phenolic compounds is given in Figure 1. From the response plot, it can be seen that the phenolic content of extracts obtained with 75% ethanol is higher than those extracted with 50% and 100% ethanol. Also, it can be concluded from Figure 1 that the TPM contents of the samples extracted at 50°C were higher than the others. The positive effect of temperature on TPM content can be attributed to keeping the extraction temperature at a moderate level. Short-term (10 min) ultrasound treatment had little effect on the phenolic content of the extracts. Increasing the sonication time to 20 min significantly increased the TPM content of the extracts. However, longer (30 min) ultrasound treatment had no significant effect on the TPM content of the extracts.

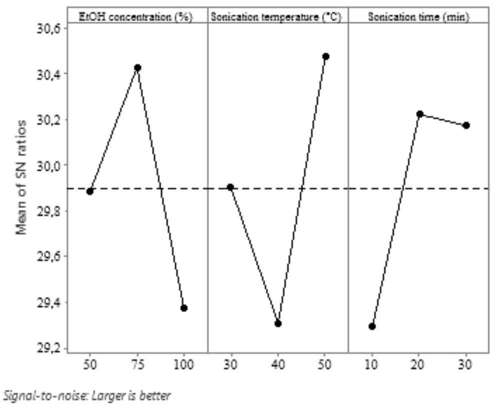

Figure 1. Main effect plot for total phenolic matter (TPM)

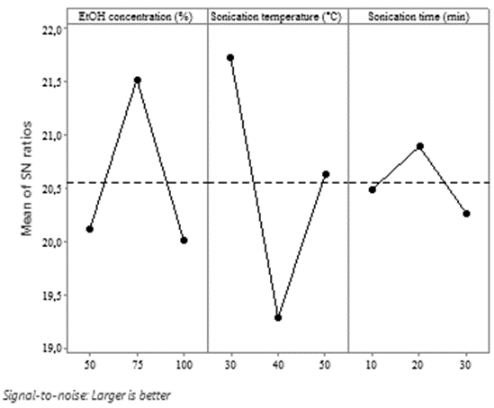

Figure 2. Main effect plot for total monomeric anthocyanin (TMA)

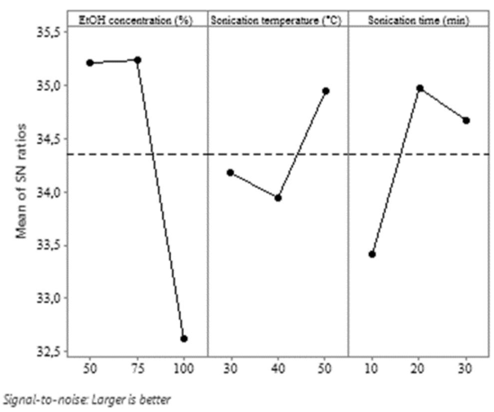

Figure 3. Main effect plot for DPPH (%)

The S/N ratios response table in the optimization study carried out to maximize the TPM content of onion peel extracts is given in Table 3. The optimal levels of each parameter are indicated in italics. As a result, the optimum process parameters for higher phenolic extraction were determined as $A_2B_3C_2$. This means that the optimal combination of process variables for better extraction performance is 75% for ethanol concentration, 50°C for extraction temperature, and 20 min for sonication time. Furthermore, according to delta values, extraction temperature was also found to be the most effective parameter for phenolic extraction, followed by ethanol concentration and sonication time, respectively.

Effect of Process Parameters On Total Monomeric Anthocyanin (TMA)

The TMA content of onion peel extracts varied between 7.86 and 17.63 mg C3G/100g sample (Table 2). Stoica et al. (2022) reported that the TMA content of red onion peels varied between 45-143 mg C3G/100 g. It was observed that the anthocyanin content of yellow onion peels was lower than that of red onion peels. The main effect graph of TMA content in ultrasonic extraction of onion peel is given in Figure 2. According to the graph, it is clear that the anthocyanin content of extracts containing 75% ethanol is significantly higher than the other solvents. Viera et al. (2023) reported that the TMA content of red onion peel extracts increased as the concentration of ethanol in the solvent increased from 20% to 80%. They also reported that the maximum TMA content was obtained at 60% and 80% ethanol concentrations. This result is in agreement with our study. However, it can be concluded that TMA contents are low in samples extracted at higher temperatures. The effect of sonication time on the extraction of color substances from onion peel was similar to that of TPM extraction. The ultrasound application for 20 min resulted in the highest yield of TMA extraction.

Taguchi method was performed for the TMA content extracted from onion peel and the S/N ratios response table is presented in Table 4. In the table, the optimal levels of each parameter are indicated in italics. Parameter levels with higher average S/N ratios can be expressed as optimum process parameters $A_2B_1C_2$ for TMA content. In terms of TMA content, it can be concluded that extracts sonicated for 20 min at 30 °C with 75% ethanol-containing solvent (Example 4) have the maximum TMA content. However, extraction temperature, which had the highest delta value (2.431) in TMA extraction, was found to be the most effective parameter in the extraction of color substances, followed by ethanol concentration and sonication time, respectively.

Effect of Process Parameters on Antioxidant Activity

The total antioxidant activities of the extracts obtained from ultrasonic extraction of onion peel were determined based on the DPPH radical scavenging activity of the extracts and expressed as DPPH (%). The DPPH radical scavenging activities of the samples ranged from 36.88% to 64.43% (Table 2). The S/N ratios average plot for DPPH (%) values of the samples extracted according to Taguchi L9 experimental design is given in Figure 3. In the S/N ratios response graph, it was determined that the antioxidant activities of the samples extracted at 75% ethanol concentration were higher than the other samples. DPPH (%) values of the samples extracted at high temperatures (50 °C) were also higher. Increasing the sonication time to 20 min significantly increased the DPPH (%) value of the extracts. Also, there was no positive effect of a longer ultrasonication time on the antioxidant activity of the samples observed.

The S/N responses of Taguchi optimization performed to maximize the antioxidant activities of onion peel extracts are given in Table 5. The data in the table shows that the extraction conditions for the maximum DPPH (%) value were found as $A_2B_3C_2$.

Table 3. S/N ratio response table for total phenolic matter

Level	A	B	C
1	29.89	29.90	29.29
2	30.43	29.31	30.22
3	29.38	30.48	30.17
Delta	1.05	1.17	0.93
Range	2	1	3

*Italic values indicate optimal levels.

Table 4. S/N ratio response table for total monomeric anthocyanin (TMA)

Level	A	B	C
1	20.12	21.72	20.49
2	21.52	19.29	20.89
3	20.01	20.63	20.26
Delta	1.51	2.43	0.63
Range	2	1	3

*Italic values indicate optimal levels.

Table 5. S/N ratio response table for DPPH (%)

Level	A	B	C
1	35.21	34.18	33.42
2	35.24	33.95	34.97
3	32.62	34.94	34.68
Delta	2.62	0.99	1.56
Range	1	3	2

Table 6. SAW results for optimization of bioactive component extraction

Sample	Normalized values			Weighted normalized values			Results	
	TPM	TMA	DPPH	TPM (0.419)*	TMA (0.146)*	DPPH (0.436)*	Score	Range
1	0.873	0.610	0.738	0.366	0.089	0.322	0.776	7
2	0.914	0.446	0.969	0.383	0.065	0.423	0.871	4
3	0.984	0.698	1.000	0.412	0.102	0.436	0.950	2
4	0.986	1.000	0.950	0.413	0.146	0.414	0.974	1
5	0.961	0.528	0.833	0.402	0.077	0.363	0.843	5
6	1.000	0.584	0.911	0.419	0.085	0.397	0.902	3
7	0.918	0.542	0.714	0.385	0.079	0.311	0.775	8
8	0.733	0.607	0.572	0.307	0.089	0.250	0.645	9
9	0.979	0.556	0.715	0.410	0.081	0.312	0.803	6

*Weight values determined by principal component analysis (PCA).

The optimum extraction variables for the extract with higher antioxidant activity were determined as 75%, 50 °C, and 20 min for ethanol concentration, extraction temperature, and sonication time, respectively. The most effective process parameter on DPPH (%) values was found to be ethanol concentration. This parameter was followed by sonication time and extraction temperature, respectively.

Multi-Response Optimization Through SAW Method

In the extraction of bioactive substances from onion peel, there were no common optimization conditions for the responses. $A_2B_3C_2$ for TPM, $A_2B_1C_2$ for TMA, and $A_2B_3C_2$ for DPPH (%) were found to be the optimum conditions. In these conditions, the sample providing the best-targeted properties among the available specimens was determined by the SAW method and the results are given in Table 6. The results show that sample 4 ($A_2B_1C_2$), which has the highest score (0.974) in the ranking, represents the optimal extraction process for all three responses.

Conclusion

In this study, extraction conditions were optimized by the Taguchi-SAW hybrid method for an extract rich in phenolics and anthocyanins and high antioxidant activity from onion peel rich in bioactive components. Individual responses in the extraction process were successfully optimized by Taguchi optimization. Multiple response optimization was also performed successfully with the SAW ranking method. It is recommended to verify the success of this hybrid technique by comparing it with alternative optimization methods such as the response surface method, genetic algorithm, and fuzzy logic.

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