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OPTIMIZATION OF ULTRASOUND-ASSISTED PROTEIN EXTRACTION FROM WATERMELON SEEDS: TAGUCHI APPROACH

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Abstract: Nowadays, there has been a growing interest in finding alternative protein sources for both the food industry and nutritional purposes. Protein experts have recently focused on investigating watermelon seeds, which are not only a food processing waste but also contain high-quality proteins. Therefore, this study aimed to achieve maximum protein extraction from watermelon seeds using an ultrasound-assisted extraction process. The study investigated the effects of pH (A; 7—11), sonication temperature (B; 30—60 °C), and sonication time (C; 5—15 min) on protein recovery to develop a Taguchi model. Through optimization, the optimal conditions for maximum protein recovery (85.81%) within the range of process variables were found to be 11 pH, 45 °C sonication temperature, and 10 min sonication time ($A_3B_2C_2$). An analysis of variance (ANOVA) revealed that pH and sonication temperature significantly influenced the protein extraction process ($P<0.05$). The optimized extraction conditions resulted in a remarkable improvement (56.79%) in protein recovery compared to the initial process parameters ($A_1B_1C_1$). This study demonstrates the effectiveness of the proposed extraction model for obtaining proteins from high-protein seed sources.

Keywords: ANOVA, Protein recovery, Sonication, pH, Optimization

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1. Introduction

In recent years, consumers have become very interested in products derived from healthy and natural plant sources. Plant-based proteins are the most preferred protein sources due to their safety and the presence of antioxidant peptides that promote human health (Liu et al., 2017). On an industrial scale, there is a growing trend towards plant-derived proteins from agro-industrial wastes due to a number of challenges such as the increasing cost of animal proteins and concerns about food safety (Fatima et al., 2023). Moreover, this approach offers solutions to several critical issues such as environmental pollution, waste accumulation, and disposal expenses (Gadalkar and Rathod, 2020). Thus, by-products from fruit and vegetable processing waste possess the potential to serve as innovative, cost-effective, and non-traditional sources of protein (Firatligil-Durmus and Evranuz, 2010).

Watermelon is an iconic fruit of the summer months, typically grown in warm climate regions. While the juice and pulp of watermelon are utilized in human consumption and the production of non-alcoholic beverages such as juices and nectars, the peel and seeds of this fruit are generally considered waste materials. Notably, the seeds present a potential source of protein and oil. Due to their high arginine, glutamic acid, aspartic acid, and leucine content, the seed proteins are classified as high-quality proteins (Wani et al., 2006; Wani et al., 2011). Various solvents (i.e., hot water, NaOH, HCI, NaCI, and sodium bis(2- ethylhexyl) sulfosuccinate) and extraction techniques (conventional batch extraction, microwave, ultrasound-assisted extraction, mechanochemical-assisted extraction, enzyme-assisted extraction, and liquid-phase pulsed discharge) have been studied for protein extraction from watermelon seeds (WS) (Wani et al., 2006; Wani et al., 2008; Wani et al., 2011; Gadalkar and Rathod, 2020; Behere et al., 2021; Liu and Xi, 2021; Qin et al., 2021). However, these studies often rely on one-factor-at-a-time (OFAT) and response surface methodology approaches to optimize protein extraction from WS. The extraction of seed proteins poses a notable challenge in selecting a suitable strategy and optimization method. Conventional extraction techniques and OFAT optimization can be time-consuming and costly (Guldane and Dogan, 2022). Therefore, the use of ultrasonic-assisted extraction technology, which reduces costs and is an environmentally friendly and time-saving method, has gained prominence (Fatima et al., 2023). Sonication equipment generates sound energy with very high-frequency sound waves (> 16 kHz), which cannot be perceived by the human ear. This US energy causes compression and solubilization in tissues through the cavitation effect, leading to the rapid removal of targeted components from biological structures (Biswas and Sit, 2020). Gadalkar and Rathod (2020) employed an ultrasound-assisted extraction for protein recovery from WS. They used the OFAT technique to optimize extraction conditions, including pH (7-12), mass-to-solvent ratio (1:20-1:60 (w/v)), temperature (30-60°C), ultrasound power (30-120 W), and frequency (25 and 40 kHz). However, this optimization method is time-consuming and may not consider the potential interaction between extraction parameters.

Taguchi method (TM) has found applications in a wide range of fields for optimizing processes and predicting experimental datasets (Dimou et al., 2009; Ayoubi-Feiz et al., 2019; Güldane and Doğan, 2020; Pathak et al., 2020; Kannan and Thangaraju, 2022). TM has proven to be particularly effective in addressing challenges within the engineering industry. TM is capable of optimizing process variables with a much smaller number of experiments compared to the traditional OFAT technique. It identifies optimum process conditions by utilizing the signal-to-noise ratio (SNR), aiming for a higher SNR value to achieve maximum efficiency. The main objective of Taguchi optimization is to minimize the influence of noise (N) factors that adversely affect the process (Güldane, 2023).

Research on the utilization of the TM for enhancing production processes in food applications has been limited. Furthermore, a notable research gap exists in the current literature regarding the optimization of the protein extraction process from plant seeds, particularly utilizing the TM approach. This study aims primarily to improve the recovery of protein from WS through ultrasound-assisted extraction, employing the TM. To achieve this goal, the study evaluates the impact of process parameters, including pH, sonication temperature, and sonication time, on protein recovery, considering SNR values obtained from Taguchi L9 (3^3) orthogonal experimental design.

2. Materials and Methods

2.1. Materials

The watermelons with no commercial value (i.e., damaged and/or left in the watermelon field after harvest), were obtained from a local producer in Pamukova, Sakarya (Türkiye). As soon as the watermelon arrived at the laboratory, the peels were removed using a knife. The remaining part was then cut into small pieces, crushed using a blender (Kenwood KM070, UK), and transferred to a beaker. Distilled water was added to the beaker in a 1:1 (w/w) ratio, thoroughly mixed, and allowed to stand for 10 min to allow the seeds to settle at the bottom. Subsequently, the top portion was removed, and the remaining kernels were collected. This process was repeated until no watermelon residue remained in the samples. Finally, the clean seeds were spread on filter paper on the laboratory bench and left to dry for 24 hours. The dried samples were then packed and stored under dry conditions at room temperature. The chemicals utilized in protein analysis were of analytical grade and purchased from Merck, (Germany).

2.2. Methods

2.2.1. Preparation of watermelon seeds for analysis

The method proposed by Wani et al. (2006) was employed with some modifications to prepare the WS samples for protein extraction. Initially, the watermelon seed oil was removed through the Soxhlet extraction procedure. Briefly, the WS was ground with a blender apparatus (Kenwood KM070, England). The resulting sample was then mixed with hexane in a ratio of 10:1 (v/w) and subjected to extraction in a Soxhlet extractor for 12 hours. Following this defatting process, the seeds were dried in an oven at 40 °C for 24 hours. After drying, the seeds were ground and pulverized. The resulting defatted seed powder was packed into polyethylene bags and stored at +4°C until analysis.

2.2.2. Protein extraction process

An ultrasound-assisted extraction technique was employed to extract proteins from defatted WS. The sonication process was carried out in an ultrasonic water bath (ÇALIŞKAN, İstanbul, Türkiye) with a constant frequency of 40 kHz. Briefly, 5 g of defatted seed powder was dispersed in 200 mL of distilled water. The pH was adjusted to 7, 9, or 11 using 0.1M NaOH. Subsequently, the samples were then subjected to sonication at different temperatures (30, 45, and 60 °C) for varying durations (5, 10, and 15 min). Following ultrasound treatment, the mixture was filtered using Whatman no:1 filter paper. The filtered solution was then centrifuged at 10000g for 15 min at +4 °C. The resulting supernatant was employed to determine the total nitrogen content using the Kjeldahl method. The protein recovery (PR) was calculated using Equation 1.

$$\text{PR (\%)} = \frac{\text{protein content in the extract}}{\text{protein content in defatted seed powder}} * 100 \qquad (1)$$

2.2.3. Experimental design and statistical analysis

The experimental design for protein extraction from WS was carried out using MINITAB 19.0 software. Unlike Design Expert software, MINITAB allows for the selection of process variables, thereby minimizing the influence of uncontrollable factors. This step is crucial in the optimization process (Pathak et al., 2020).

The extraction parameters and their corresponding levels are provided in Table 1. To optimize the process variables, an L9 (3^3) orthogonal matrix was employed, involving three factors and three levels, as shown in Table 2. To maximize protein recovery, the S/N ratio of the experimental results was assessed using Minitab software, applying the "larger the better" criteria (Equation 2).

$$\frac{S}{N} = -10log\left[1/R\sum_{f=1}^{R} 1/{y_i}^2\right] \qquad (2)$$

where R indicates data points and y_i refers to ith data point value.

An analysis of variance (ANOVA) was performed on the Taguchi experimental test results to determine the statistical significance of the control parameters in the ultrasonic-assisted protein extraction process. The Fischer test (F-value) and the associated probability of the F-value (P-value) were utilized to assess the significance of the selected parameters in Taguchi methodology (Table 1 and 2).

3. Results and Discussion

3.1. Taguchi Optimization

Taguchi optimization technique evaluates the experimental results based on the SNR values (Taguchi, 1986). In the ultrasonic-assisted extraction of proteins from WS, a Taguchi L9 design matrix was employed to maximize the PR by selecting the “Larger the better” option. The mean experimental results and corresponding SNR values are presented in Table 2. The results show that the highest protein content was obtained when the process variables (pH, sonication temperature, and sonication time) were set at 11, 45 °C, and 5 min, respectively ($A_3B_2C_1$). In contrast, the lowest extraction efficiency was observed in Run 1 ($A_1B_1C_1$). Moreover, the influence of each extraction parameter on PR was investigated through average SNR values for the levels of the extraction parameters, as outlined in Table 3. Delta values, representing the difference between the maximum and minimum SNR values for the respective levels of each process variable, indicate their influence on the extraction process (Bose et al., 2013). Observations from Table 3 revealed that the alkalinity of the extraction medium had the highest delta value (Δ=2.46) and was the most influential process factor affecting the PR from WS, compared to the other two parameters. Following pH, sonication temperature (Δ= 0.96) and sonication time (Δ= 0.42) were identified as important factors affecting the extraction process.

Figure 1 illustrates the mean SNR graph for the protein extraction process. By considering the “Larger the better” criteria, the primary objective of the TM is maximize the SNR values of the control parameters. The data in Figure 1 suggests that the optimum process parameters for PR from WS were found to be a pH of 11, a sonication temperature of 45 °C, and a sonication time of 10 min ($A_3B_2C_2$). The results also revealed a positive relationship between an increase in pH and PR. Hence, there was an improvement of about 52% in PR when the pH of the extraction medium increased from 7 to 11 as shown in Table 2. A similar trend of improved PR with increasing pH was reported by Gadalkar and Rathod (2020), who attributed the improvement to the increased surface negative charge on proteins resulting from the dissociation of acidic groups with increasing alkalinity. In addition, sonication temperature was also a significant role in protein extraction.

Table 1. Extraction parameters and their levels in Taguchi optimization

Factor	Symbol	Level 1	Level 2	Level 3
pH	A	7	9	11
Sonication temperature (°C)	B	30	45	60
Sonication time (min)	C	5	10	15

Table 2. Taguchi L9 (3^3) design matrix, test results, and SNR values

Run	Factors			Protein recovery (%)	SNR (dB)
	A	B	C		
1	7	30	5	54.73	34.76
2	7	45	10	66.03	36.39
3	7	60	15	61.48	35.77
4	9	30	10	68.93	36.77
5	9	45	15	73.35	37.31
6	9	60	5	70.33	36.94
7	11	30	15	76.73	37.70
8	11	45	5	83.17	38.40
9	11	60	10	81.45	38.22
Mean (η_m)				70.69	

Table 3. Response table for SNRs (dB) of protein recovery (%)

Parameters	1	2	3	Delta (Δ)	Rank
A	35.64	37.01	38.11	2.46	1
B	37.37	37.37	37.13	0.96	2
C	37.13	37.13	36.93	0.42	3

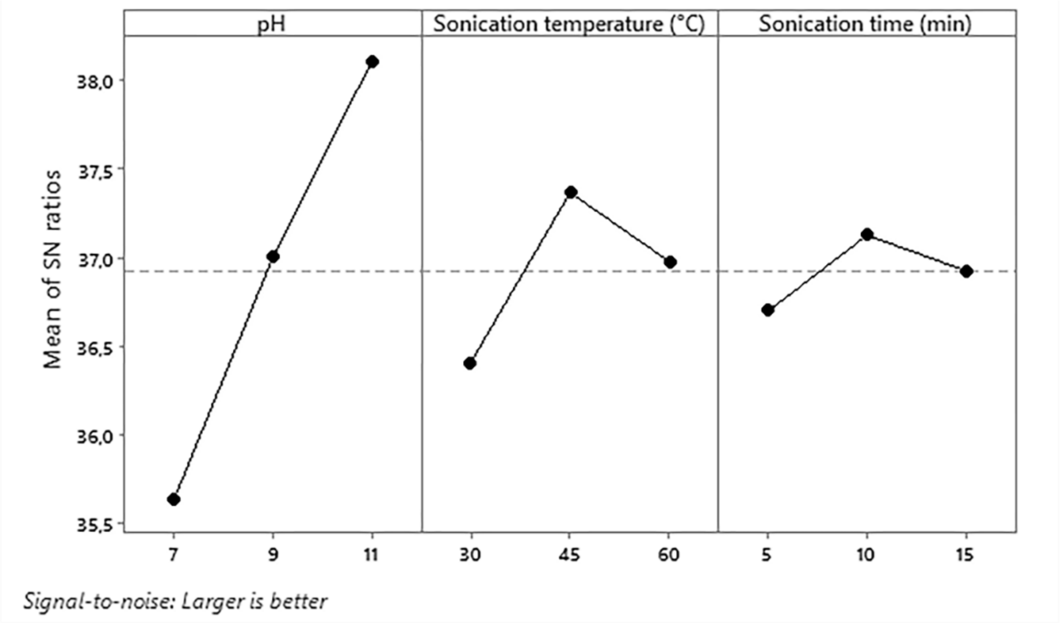

Figure 1. The S/N ratio response graph for total protein recovery.

The SNR of the PR value increased from 34.76 to 38.40 as the sonication temperature increased from 30 to 45 °C. However, a further increase in process temperature to 60 °C, led to a notable decrease in SNR of PR. This observation may be attributed to partial protein denaturation resulting from the combined effect of temperature and sonication. Wani et al. (2006) reported an optimal temperature of 40 °C for protein extraction from WS, which aligns well with our findings. Figure 1 also highlights the potentially detrimental impact of prolonged ultrasound treatment in the protein extraction process. Extended sonication may lead to increased interactions between denatured proteins and other components within the defatted seed structure.

The ANOVA results confirmed the significance of process factors in protein extraction from WS. As shown in Table 4, pH exhibited the highest F-value (178.69) and the lowest *P*-value (0.006), highlighting its role as the most influential process factor affecting PR. Following this, sonication temperature also played a vital role (F-value = 25.31; *P*-value = 0.038). Thus, the influence of these two process variables on the protein extraction process was found statistically significant within a 95% confidence interval. However, it was observed that sonication time had the least significant impact on PR (P<0.05). A similar observation regarding the insignificant effect of extraction time on PR from red pepper seeds was reported by Firatligil-Durmus and Evranuz (2010). These findings were further supported by percent contribution values, with pH having the highest contribution at 85.72%, followed by sonication temperature and sonication time contributing 12.14% and 1.66%, respectively. The percentage contribution of error was calculated to be 0.48, indicating that the impact of non-process parameters on PR is minimal.

3.2. Confirmation Experiments

Validation tests were performed to confirm the correlation between the observed and predicted response values for the PR process using optimal extraction parameters ($A_3B_2C_2$) and to identify whether an improvement was achieved compared to the initial process conditions ($A_1B_1C_1$). The predicted response value was calculated using Equation 3 with the optimal levels of process factors:

$$\eta_0 = \eta_m + \sum_{i=1}^{j} (\eta_i - \eta_m) \tag{3}$$

Table 4. ANOVA results for Taguchi optimization

Source	DF	SS_f	MS	F-Value	*P*-Value	Contribution (%)
pH	2	582.336	291.168	178.69	0.006*	85.72
Sonication temperature (°C)	2	82.480	41.240	25.31	0.038*	12.14
Sonication time (min)	2	11.282	5.641	3.46	0.224	1.66
Error	2	3.259	1.629			0.48
Total (SS_T)	8	679.357				

S= 1.27620, R-sq= 0.9952, R-sq(adj)= 0.9808 and R-sq(pred)= 0.9029, * significant (P<0.05).

Table 5. Results of confirmation experiments

	Initial process parameters	Prediction	Experiment
Factors	$A_1B_1C_1$	$A_3B_2C_2$	$A_3B_2C_2$
Protein recovery (%)	54.73 ± 0.02^b	85.39 ± 0.12^a	85.81 ± 0.14^a
S/N ratio	34.36 ± 0.03^b	38.76 ± 0.09^a	38.80 ± 0.05^a
Improvement (%)			56.79

Values are means ± standard deviation. a-b refers to the significant differences between the values in the same line (P<0.05).

where η_m was the overall average of the mean values or S/N ratio, η_i was the average value corresponding to optimal levels, and j was the number of experiments (Güldane, 2023).

The predicted and experimental test results for the optimal process conditions ($A_3B_2C_2$) and the results for initial process parameters ($A_1B_1C_1$) are presented in Table 5. The variation between the predicted and observed values for tPR was found to be within the range of a 95% confidence level. Furthermore, a substantial enhancement of 56.79% was achieved under the optimal extraction conditions compared to the initial process parameters.

4. Conclusion

This study aimed to optimize an ultrasound-assisted technique for extracting high yields of protein from defatted watermelon seeds, which are rich in high-quality proteins and have the potential to be utilized as valuable industrial waste. The Taguchi approach was employed to model protein recovery from watermelon seeds, using process variables such as pH, sonication temperature, and sonication time. The experiments were designed using a Taguchi L9 (3^3) orthogonal matrix. The Taguchi optimization process resulted in a remarkable increase (> 50%) in protein recovery rate compared to the initial process parameters. However, ANOVA analysis revealed that the pH variable had the most significant contribution to this improvement among other process variables. These results demonstrated that the Taguchi model can be effectively employed to optimize the process variables in protein extraction from fruit seeds.

Author Contributions

The percentage of the author contributions is presented below. The author reviewed and approved the final version of the manuscript.

	M.G.
C	100
D	100
S	100
DCP	100
DAI	100
L	100
W	100
CR	100
SR	100
PM	100
FA	100

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The author declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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