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Impact of particle size on HCN detoxification in wild apricot kernels

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Abstract

Wild apricot kernels are rich in carbohydrates, proteins, and lipids, but also high in amygdalin, which hydrolyzes to form hydrogen cyanide (HCN), a deadly compound. The purpose of this study was to lower HCN levels in wild apricot kernels by soaking them at various kernel diameters (0.34 mm to 11.66 mm) and periods (0.34 h to 11.66 h) under controlled circumstances. The soaking process was done at 30 ± 2°C, with water replaced every hour and a water-to-kernel ratio of 10:1. These conditions were optimized using Design Expert software and results indicate significantly reduced hydrogen cyanide (HCN) levels while maximizing nutritious content can be obtained. Breaking the kernels into smaller sizes (7.88 mm) and soaking for 10 hours lowered HCN levels to 36.13 mg/100 g while maintaining excellent nutritional content (21.16% protein, 47.75% fat, and 21.22% carbs) and yield (96.45%). The improved soaking process provides an effective and practical way for detoxifying wild apricot kernels while retaining their nutritional value.

Key words: Apricot kernels, amygdalin, detoxification, soaking, size reduction

Introduction

Wild apricot or 'Chullu' in Uttarakhand is an under utilised fruit plant of hilly regions of India which is highly nutritious (Raj *et al.*, 2012; Desser, 2015). These kernels contain carbohydrates, proteins and fats which make them a possible food product. However, the presence of amygdalin a cyanogenic glucoside that when hydrolysed releases hydrogen cyanide (HCN) is a health hazard. HCN toxicity results in effects such as dizziness, headaches, and respiratory paralysis, so detoxification of these kernels is crucial for safe consumption (Pritchard, 2007; Barbhai *et al.*, 2024).

Different methods of detoxification such as thermal treatment, enzymatic inactivation and soaking have been used to minimize the level of HCN in apricot kernels (Bolarinwa, 2013; Desser, 2015). Of these, soaking is effective because HCN is soluble in water. Therefore, the purpose of this study is to establish the impact of kernel size reduction and soaking duration on the detoxification process to determine the most efficient method of reducing HCN content without compromising the nutritional value of the kernels.

Improving processes is critical for increasing productivity, accuracy, and cost-effectiveness in both research and production. In this scenario, tools like Design Expert are extremely beneficial since they offer extensive statistical and experimental design skills that go beyond traditional methodologies. These technologies reduce the need for time-consuming and resource-intensive trial-and-error methods by examining several variables and their interactions at the same time. They allow researchers to swiftly identify ideal settings while maintaining precision and reliability. Furthermore, Design Expert encourages the use of methodologies like Response Surface Methodology (RSM) to define factor correlations, forecast outcomes, and validate results (Mahour et al., 2024). This not only speeds up process development but also eliminates resource waste, making such techniques indispensable in modern optimization research.This study aimed at establishing the effect of kernel size reduction on the effectiveness of HCN removal in wild apricot kernels during soaking and its effect on the nutritional quality of kernels. Which kernel size, soaking time and water to kernel ratio are most appropriate for maximizing HCN reduction and maximizing the nutritional value of wild apricot kernels through statistical analysis using Design Expert software?

Materials and methods

Fruit kernels: The primary raw material used in this investigation was wild apricot kernels. These kernels were sourced from local farmers in the Tehri-Garhwal district of Uttarakhand and transported to the Food Testing Laboratory in the Department of Food Science and Technology at the College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, U.S. Nagar district, Uttarakhand.

Chemicals: The entire range of chemicals that were used for various chemical analysis were obtained from HiMedia Laboratories Pvt. Ltd., Mumbai and SD Fine Chem. Ltd. Mumbai. All the chemicals used, were of AR grade.

Experimental design and statistical analysis: Layout and analysis of data was carried out using the software, DESIGN EXPERT 7.0.0 (Stat-Ease, Inc. 2005, Minneapolis, United States). Central Composite Rotatable Design was chosen as it was most appropriate for our objective. A total of 13 treatment combinations / runs were made for the experiment. Each of the 13 treatment runs were conducted in triplicates. A three level factorial design was used (Table 1). The data recorded w.r.t. the dependent variables were analyzed and the response functions were developed using

multiple regression. ANOVA was used to analyze the models. Minimization of the HCN levels and maximization of the yield, protein, fat and carbohydrate levels was the criteria selected for optimization of the process. The validity of the model fitting and development of a highest order equation was done to find out the individual and combined effects of all independent variables on the dependent variables. Optimization was done based on high desirability levels and predicted solutions were compared with the actual values.

Table 1. Range and levels of independent variables for broken kernel experiment

Factor symbol	Independent variable	Units	Minimum	Maximum
A	Kernel size	mm	0.34	11.66
B	Soaking time	h	0.34	11.66

Experimental setup: Wild apricot kernels were cut into specified sizes (approximately measured in length) using a sharp stainless steel knife. The kernels were then soaked in water for varying durations (Fig. 1). The soaking process was conducted at a fixed water temperature of 30 ± 2 °C and a water-to-kernel ratio of 10:1. Hot water soaking was avoided to minimize production costs and prevent undesirable nutrient losses due to leaching. Instead, soaking in room-temperature water was evaluated. Kernels with sizes ranging from 0.34 mm to 11.66 mm were soaked for durations between 0.34 and 11.66 hours. To maximize detoxification, the soaking water was replaced every hour.

Fig. 1. Broken wild apricot kernels of different sizes used for trials

Analysis of the detoxified kernels

Hydrocyanic acid: Hydrocyanic acid (HCN) in raw and detoxified kernels was determined using the AOAC (2000) method. Ground kernels (20 g) were hydrolyzed in 200 mL distilled water for 2 hours. The mixture was steam-distilled, collecting 150-160 mL distillate in 0.625 N NaOH, then diluted to 250 mL. A 100 mL aliquot was titrated with 0.02 M silver nitrate ($AgNO_3$) in the presence of ammonium hydroxide (NH_4OH) and potassium iodide (KI). HCN forms a soluble complex [$Ag(CN)_2$], with turbidity indicating the endpoint. HCN content was calculated and expressed as mg/100 g of kernels.

HCN (mg/100g)=[(Titer value (mL) x1.08 x Volume made up (mL))/(Weight(g) of kernel taken x Vol.(mL)of distillate taken for titration)] x 100

Crude protein: Crude protein in raw and detoxified kernels was determined via the Kjeldahl method, involving digestion, distillation, and titration to measure nitrogen content. A moisture-free sample (0.2–0.3 g) was mixed with 3-4 g catalyst (K_2SO_4 + $CuSO_4$, 5:1) and digested in 10 mL concentrated H_2SO_4 at 420 °C for 90 minutes until a clear green solution formed. After cooling, the sample was distilled using NaOH to release ammonia, which was absorbed in boric acid (4%). The ammonia solution was titrated with 0.1 N HCl to determine nitrogen content. A blank was run, and crude protein was calculated using the obtained titre value.

Nitrogen (%) = [(14.01x0.1x (TV -BV) x 100) /(W x 1000)]

Where, 14.01 – Molecular weight of ammonia; 0.1 – Normality of HCl; TV – Titre value; BV – Blank value; W – Sample weight

Crude protein (%) = Nitrogen (%) x 6.25

Protein content was calculated by multiplying the nitrogen content by a factor of 6.25 and expressed as a percentage.

Crude fat: Crude fat in wild apricot kernels (raw and detoxified) was estimated using the Soxhlet extraction method. Approximately 2 g of ground sample was placed in a cellulose thimble within a pre-weighed beaker. Ether was used as the solvent, with boiling at 90 °C followed by solvent evaporation at 150 °C. Fat adhering to the thimble was washed and collected. The beaker with extracted fat was weighed, and the fat content was calculated.

Crude fat(%) = [(W_2-W_1)/W] x 100

Where, W = Weight of sample, W_2 = Weight of beaker after evaporation of solvent, W_1 = Weight of empty beaker

Total carbohydrate: Carbohydrate content in wild apricot kernels was determined using the phenol-sulfuric acid method (Sadasivam and Manickam, 2004). Ground kernels (100 mg) were treated with 5 mL 2.5N HCl and boiled for 3 hours, then neutralized with sodium carbonate and diluted to 100 mL. The filtrate was analyzed. Standards were prepared using dextrose (100 µg/mL), and after adding phenol and concentrated H_2SO_4, absorbance at 490 nm was measured. Samples (0.1 and 0.2 mL) were processed similarly, and carbohydrate content was calculated using the standard curve.

Total carbohydrate(%) = [(mg of glucose)/(Volume of test sample)]x 100

Results and discussion

Model fitting and adaptness of experimental data: The experimental data were analyzed using a second-order polynomial equation for each response, including only significant terms ($P < 0.05$). Analysis of variance for all the responses is presented in Table 3. To assess the relationship between experimental and predicted data, an R^2 value greater than 0.85 was considered acceptable (Dobhal *et al.*, 2023). The R^2 values for HCN, crude protein, crude fat, total carbohydrate, and yield were 0.98, 0.98, 0.99, 0.99, and 0.94, respectively. The reliability of the experimental data was determined by the coefficient of variation (CV), where lower CV indicates higher reliability (Mehra *et al.*, 2024). The observed CV values were 10.19% for HCN, 0.57% for crude protein, 0.25% for crude fat, 2.29% for total carbohydrate, and 5.82% for yield. The model for HCN, crude protein, crude fat, total carbohydrate and yield respectively was highly significant, with F-values of 137.03, 447.45, 3764.92, 158.55 and 22.93.

Effect of detoxification on HCN, nutritional content and yield of wild apricot kernels

HCN: The HCN content in treated kernels ranged from 6.75 mg/100 g (Run 10) to 121.5 mg/100 g (Run 11), with the highest HCN level found in kernels of 10 mm size soaked for 2 hours (Table 2). In contrast, soaking 2 mm kernels for 10 hours resulted in the lowest HCN value (6.75 mg/100 g). The regression

Table 2. Responses of process conditions on detoxification of broken kernels

Run	Independent variables		Dependent variables				
	Factor A Kernel size (mm)	Factor B Soaking duration (h)	HCN (mg/ 100g)	Crude protein (%)	Crude fat (%)	Total carbo-hydrate (%)	Yield (%)
1	10.00	10.00	58.725	20.04	46.78	21.36	95.58
2	6.00	11.66	19.17	21.48	48.88	19.56	87.81
3	6.00	6.00	37.8	20.45	44.97	20.4	92.44
4	11.66	6.00	106.65	20.99	40.18	21	98.95
5	6.00	6.00	37.8	20.45	44.97	20.4	92.44
6	0.34	6.00	10.8	16.51	34.61	10.26	42.01
7	6.00	6.00	37.8	20.45	44.97	20.4	92.44
8	6.00	6.00	37.8	20.45	44.8	20.4	92.44
9	2.00	2.00	13.5	19.45	43.72	17.28	81.93
10	2.00	10.00	6.75	18.19	38.85	12.6	64.91
11	10.00	2.00	121.5	20.61	43.69	21.44	96.21
12	6.00	6.00	37.8	20.45	44.97	20.4	92.44
13	6.00	0.34	81.675	22.93	49.96	21.32	97.69

*HCN and nutritional content in raw kernel (a) HCN: 253.8 ± 2.70 mg/ 100 g (b) Protein: 20.92 ± 2.46 % (c) Fat: 45.34 ± 5.53 % (d) Total carbohydrate: 21.75 ± 0.22 %

coefficients were determined using Design Expert software, yielding the following second-order polynomial equation for the response "HCN" in the broken kernel detoxification experiment.

$$Y = 37.80 + 36.94A - 19.74B - 14.01AB + 9.35A^2 + 5.20B^2$$

Where, Y is the response *i.e.,* HCN (mg/100g) and A and B are coded values of the test variables kernel size and soaking duration respectively.

In the equation, coefficients A and B represent the individual effects of kernel size and soaking duration, respectively, while AB denotes their interaction, and A^2 and B^2 are second-order terms indicating the squared effects. The equation indicates that kernel size had a significant positive impact on HCN levels in broken kernels, whereas soaking duration had a significant negative impact. This implies that smaller kernel sizes and longer soaking durations reduce HCN levels. Additionally, the positive effect of kernel size was more pronounced than the negative effect of soaking duration. However, the interaction between these factors significantly reduced HCN content.

Fig. 2 (A) shows that reducing kernel size from 10 mm to 2 mm resulted in a steady decline in HCN levels. Conversely, HCN levels decreased with increasing soaking duration from 2 to 10 hours. It is estimated that soaking 2 mm kernels for 8 hours can reduce HCN to approximately 8.64 mg/100 g, while soaking 1 mm kernels for 6 hours can lower it further to about 6.32 mg/100g.

These observations align with Tunçel *et al.* (1994) who examined the influence of particle size, soaking temperature, and soaking duration on cyanide release from apricot kernel glycosides, observing complete glycoside removal within 0.5 hours for finely ground kernels (<1 mm). The removal of toxic constituents can be further enhanced by periodically replacing the soaking water. Similarly, it was also reported that changing the water every 4 hours and replacing it with fresh water reduced HCN content from 801.32 ± 77.53 mg/kg to 29.46 ± 1.63 mg/kg, achieving 96.3% detoxification within 8 hours (Desser, 2015). Additionally, 97% detoxification was achieved by soaking peeled apricot kernels under flowing water (43 mL/s, 5°C) for 8 hours, with a further reduction to 98.4% after 24 hours of treatment (Desser, 2015).

Crude protein: The regression coefficients for the response "protein" in the broken kernel detoxification experiment is as follows:

$$Y = 20.45 + 1.20A - 0.49B + 0.16AB - 1.16A^2 + 0.82B^2$$

Where, Y is the response *i.e.,* crude protein (%) and A and B are coded values of the test variables kernel size and soaking duration respectively.

The equation indicates that kernel size had a significant positive impact on the protein content of broken kernels, whereas soaking duration had a significant negative impact. This suggests that smaller kernel sizes and longer soaking durations reduce crude protein content. Moreover, the positive effect of kernel size was more than twice as strong as the negative effect of soaking duration.

Fig.2 (B) reveals that reducing kernel size from 10 mm to 2 mm resulted in a steady decline in crude protein levels in wild apricot broken kernels. Similarly, crude protein levels decreased as soaking duration increased from 2 to 10 hours. Notably, soaking kernels smaller than 1 mm for approximately 6 hours could reduce protein content to below 17%.

Crude fat: The regression coefficients were determined using Design Expert software, resulting in the following second-order polynomial equation for the response "fat" in the broken kernel detoxification experiment.

$$Y = 44.94 + 1.97A - 0.41B + 1.99AB - 3.81A^2 + 2.21B^2$$

where, Y is the response *i.e.,* Fat (%) and A and B are coded values of the test variables kernel size and soaking duration respectively

Positive coefficients indicate a synergistic effect of the variable, while negative coefficients represent an antagonistic effect on the response, *i.e.*, crude fat. The equation suggests that kernel size had a significant positive impact on crude fat content, whereas soaking duration had a significant negative impact. This implies that smaller kernel sizes and longer soaking durations reduce crude fat content. Additionally, the positive effect of kernel size was more than twice as strong as the negative effect of soaking duration. Fig.2 (C) shows that reducing kernel size from 10 mm to 2 mm caused a steady decline in crude fat content in wild apricot broken kernels. Conversely, fat levels also decreased with increasing soaking duration from 2 to 10 hours.

Total carbohydrate: The regression coefficients for the response "total carbohydrate" in the broken kernel detoxification experiment are as follows:

$$Y = 20.40 + 3.51A - 0.91B + 1.15AB - 2.35A^2 + 0.054B^2$$

Where, Y is the response *i.e.,* total carbohydrate (%) and A and B are coded values of the test variables kernel size and soaking duration, respectively.

Positive coefficients indicate a synergistic effect of the variable, while negative coefficients represent an antagonistic effect on the response, *i.e.*, total carbohydrate. The equation shows that kernel size had a significant positive impact on the total carbohydrate content of wild apricot broken kernels, while soaking duration had a significant negative impact. This suggests that smaller kernel sizes and longer soaking durations reduce total carbohydrate content. Additionally, the positive effect of kernel size was approximately three times greater than the negative effect of soaking duration.

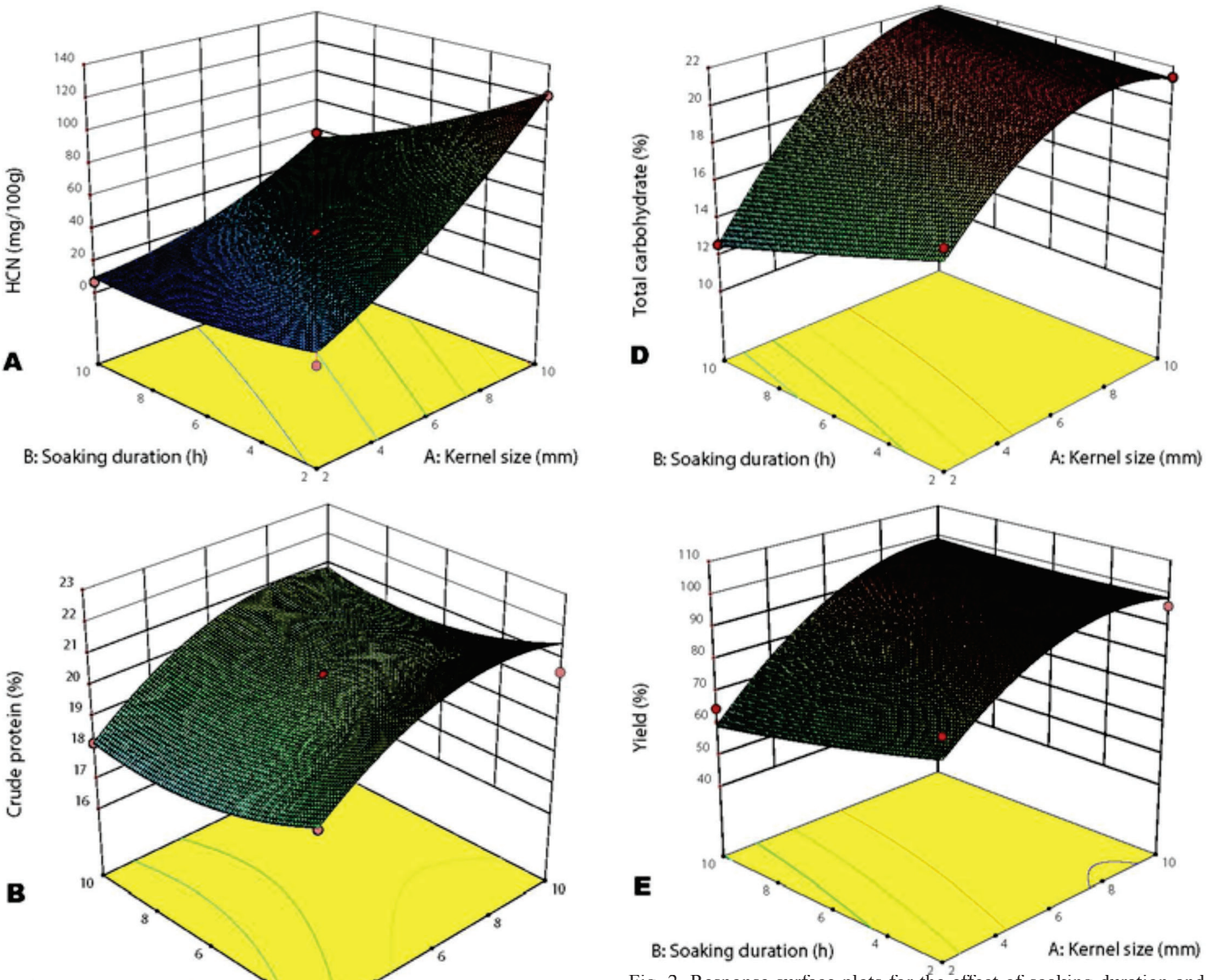

Fig. 2. Response surface plots for the effect of soaking duration and kernel size on A. HCN, B. Protein, C. Fat, D. Total carbohydrate, E. Yield

Fig.2 (D) shows that as kernel size decreased from 10 mm to 2 mm, the total carbohydrate content of wild apricot broken kernels steadily declined. Similarly, longer soaking durations (2 to 10 hours) also reduced total carbohydrate levels. The impact of kernel size was more significant, leading to a greater reduction in carbohydrate content. Therefore, kernel sizes larger than 4 mm are recommended to minimize carbohydrate loss and retain 18–20% total carbohydrate in the treated kernels.

Yield: The regression coefficients for the response "yield" are in the equation below:

$$Y = 92.44 + 15.68A - 3.95B + 4.10AB - 10.22A^2 + 0.92B^2$$

Where, Y is the response *i.e.,* yield (%) and A and B are coded values of the test variables kernel size and soaking duration respectively.

Positive coefficients indicate a synergistic effect of the variable, whereas negative coefficients signify an antagonistic effect on the response, *i.e.*, yield. The equation reveals that kernel size had a significant positive impact on the yield of wild apricot broken kernels, while soaking duration had a notable negative impact. This suggests that smaller kernel sizes and longer soaking

Table 3. Analysis of variance for all the responses for detoxification of wild apricot kernel

Source	F-value					*P*-value				
	HCN	Crude protein	Crude fat	Total carbo-hydrate	Yield	HCN	Crude protein	Crude fat	Total carbo-hydrate	Yield
Model	137.03	447.45	3764.92	158.55	22.93	<0.0001	< 0.0001	< 0.0001	< 0.0001	0.0003
A (Kernel size, mm)	481.34	869.09	2916.63	521.97	77.22	<0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
B (Soaking duration, h)	137.45	145.98	128.17	34.72	4.90	<0.0001	< 0.0001	< 0.0001	0.0006	0.0624
AB	34.60	7.46	1484.84	27.96	2.64	0.0006	0.0293	< 0.0001	0.0011	0.1486
A^2	26.81	707.04	9452.72	203.26	28.51	0.0013	< 0.0001	< 0.0001	< 0.0001	0.0011
B^2	8.29	356.10	3170.82	0.11	0.23	0.0237	< 0.0001	< 0.0001	0.7540	0.6470
Std dev.	4.76	0.12	0.10	0.43	5.05					
CV (%)	10.19	0.57	0.24	2.29	5.82					
R^2	0.98	0.97	0.99	0.99	0.94					
Adequate precision	36.712	81.331	219.451	37.668	14.590					

durations lead to reduced yield. Additionally, the positive effect of kernel size was over three times greater than the negative effect of soaking duration. Analysis of the data in Fig. 2 (E) shows that reducing the kernel size from 10 mm to 2 mm led to a consistent decrease in the yield of wild apricot broken kernels. Similarly, extending the soaking duration from 2 to 10 hours also resulted in a reduction in yield.

From the above data it can be observed that reducing kernel size negatively impacted yield and nutritional content, with protein, fat, and carbohydrate levels decreasing. The lowest nutrient levels and yield were observed in 0.34 mm kernels soaked for 6 hours (Run 6), indicating a significant loss of both mass and nutrients. On the other hand, the highest nutritional values and yield were observed under specific conditions: crude protein (21.48%) at 6 mm size and 11.66 hours of soaking, total carbohydrate (21.44%) at 10 mm size soaked for 2 hours, and crude fat (49.96%) at 6 mm size soaked for 0.34 hours. The highest yield (97.69%) was achieved with kernels of 6 mm size soaked for 0.34 hours. Hence, optimization was based on achieving a higher level of detoxification and yield with limited loss of nutrients.

Optimization: Optimization was conducted to find the best combination of process variables using DESIGN EXPERT version 7.0.0. The main objective was to maximize yield while minimizing HCN levels, with the highest priority given to these factors. Nutritional parameters were also optimized for better nutrient retention. The optimal solution was selected based on these criteria.

The data in Table 4 show that the optimized solution recommended by the design software was a kernel size of 7.88 mm soaked for 10 hours. Under these conditions, the predicted results were 36.13 mg/100 g HCN, 21.16% crude protein, 47.75% crude fat, 21.22% total carbohydrate, and 96.45% yield. The combined desirability for all factors was 0.845, which was considered sufficient for optimization, as desirability values above 0.80 are typically regarded as favorable. A comparison between the predicted and actual responses obtained under the optimized process conditions shows that they were in close agreement, confirming the accuracy of the conclusions drawn (Table 4).

Table 4. Predicted and observed values of various parameters in broken kernel experiment

Response	Predicted	Observed / Actual
HCN (mg/ 100g)	36.1366	38.95
Crude protein (%)	21.1648	20.76
Crude fat (%)	47.7493	47.83
Total carbohydrate (%)	21.2228	20.46
Yield (%)	96.454	91.70

This study aimed to optimize the detoxification process of wild apricot kernels by varying kernel sizes and soaking durations showed that a kernel size of 7.8 mm and a soaking duration of 10 hours were optimal for detoxification, ensuring the kernels met safe HCN consumption limits. Further research is needed for complete detoxification and clinical safety testing.

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