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DETOXIFICATION PROCESS FOR CYANOGENIC GLUCOSIDE 'AMYGDALIN' FROM WILD APRICOT KERNEL

Neha Rawat*, S.K. Sharma, Anil Kumar, C.S. Chopra, Divya Gaur and Deepa Saini
Department of Food Science and Technology, G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India
*Corresponding author Email: rawatneha1225@gmail.com,
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ABSTRACT

Wild apricot kernels are rich in nutrients and can be potential raw material for food product utilization. However, presence of cyanogenic glucoside amygdalin which leads to cyanide toxicity when hydrolyzed during crushing or grinding and induces bitter taste to the kernels as well prevents the further use of these kernels. The present study aimed at removal of amygdalin from wild apricot kernels. Considering the fact that amygdalin and HCN are water soluble, soaking was used as a method for detoxification. Design expert software was used to make experimental plan and optimization, and a central composite rotatable design was used. Two independent variables taken were Soaking duration (3- 9 hr) and water replacement time (20- 60 min). Optimization was done on the laid criteria and best results were achieved after 8 hours of soaking (7.97 hours) with change of water after every 60 minutes, reducing HCN and amygdalin levels to 3.37 mg/100 g and 56.56 mg/100 g, respectively. This method achieved a 97.6% detoxification while retaining essential nutrients in the kernels.
Keywords **:** Amygdalin, wild apricot kernels, cyanogenic glucoside, detoxification, HCN toxicity

Introduction

Among the various agro-wastes generated during postharvest operations, seeds represent a significant portion. Wild apricot (*Prunus armeniaca*) seed kernels are valuable by-products, rich in several bioactive compounds that contribute to their nutraceutical, medicinal and cosmetic potential (Cherif *et al*., 2024; Tamta *et al*., 2024; Kumari *et al*., 2024). These kernels are packed with dietary protein, essential amino acids, fatty acids, fiber, minerals and vitamins making them beneficial for consumption (Alajil *et al*., 2022; Nausad *et al*., 2024). The diverse range of bioactive compounds found in wild apricot seeds gives them notable antitumor, anti- obesity and antidiabetic properties (Barbhai *et al*., 2024; Makrygiannis *et al*., 2024; Hamid, *et al*., 2024;).

Wild apricot kernels contain amygdalin, a cyanogenic glycoside known for its anti-carcinogenic properties (Wang *et al*., 2022; Todorova, 2023). This compound is also present in the seeds of peaches, apples, almonds and cherries. While amygdalin itself is non-toxic, it is metabolized in the body into prunasin and glucose by the enzyme amygdalin lyase. Prunasin is further broken down into mandelonitrile and glucose by prunasin lyase and mandelonitrile is then converted into benzaldehyde and hydrogen cyanide (HCN) by hydroxynitrile lyase. The released cyanide can inhibit cellular respiration by binding to cytochrome oxidase, disrupting electron transfer in the respiratory chain and blocking oxygen utilization, leading to cell hypoxia and lactic acidosis, which can be fatal. According to the European Food Safety Authority, the daily oral dose of HCN for an adult weighing 50- 60 kg is 0.5-3.5 mg/ kg body weight (EFSA, 2016). One gram of amygdalin can release 59 mg of HCN, and if fully hydrolyzed, 500 mg of amygdalin can produce 180 mg of HCN, which is lethal to an adult (EFSA, 2016). These values serve as rough guidelines to assess the toxicity of HCN in cases of amygdalin overdose. Regulations are crucial to mitigate the risk of cyanide toxicity from consuming foods and beverages containing cyanogenic glycosides, such as amygdalin.

The toxicity of wild apricot kernels poses challenges for their use in food products. It has also been observed that not only due to toxicity, but the presence of amygdalin also hampers the taste of the kernels and consequently the taste of the products made from it as more is the amygdalin content more bitter is the kernel (Hamid *et al*., 2023; Zhang *et al*., 2024). To unlock their potential for value addition, it is essential to remove these cyanogenic glycosides. Various methods can be employed to detoxify foods containing cyanogenic glycosides. Processes such as crushing, grating, grinding, soaking, fermenting, or drying plant materials can reduce cyanide toxicity. These techniques may help remove water-soluble glycosides or activate specific plant or microbial enzymes that convert cyanogenic glycosides into cyanide, which evaporates before consumption (Saini *et al*., 2021). Additionally, in hot aqueous solutions, amygdalin can undergo isomerization alongside enzymatic degradation. For instance, heating D-amygdalin in boiling water for approximately three minutes can transform it into neoamygdalin, an epimer of amygdalin (Bolarinwa, 2014).

Currently, there is no standardized procedure for detoxifying apricot kernels on a commercial scale that is universally applicable to all apricot cultivars. This underscores the need to evaluate various detoxification techniques tailored specifically for wild apricot kernels. A related study reported achieving up to 92% detoxification of HCN in wild apricot kernels (Gaur, 2017). However, this study did not quantify the amygdalin content, making it impossible to establish a clear relationship between HCN levels and amygdalin concentration. Thus, the current study was done with an objective to detoxify wild apricot kernels with simultaneous estimation of both amygdalin and HCN levels which could provide a deeper understanding of the toxicity in apricot kernels along with exploring the effect of detoxification on the nutritional quantity of the kernels. Such insights would be instrumental in developing a reliable detoxification method and establishing comprehensive guidelines for the complete detoxification of apricot kernels.

Materials and Methods

Fruit kernels

The primary raw material for the experiment consisted of wild apricot kernels, sourced from local farmers in the Tehri-Garhwal district of Uttarakhand. These kernels were transported to the Food Testing Laboratory at the Department of Food Science and Technology, College of Agriculture, GBPUAT, Pantnagar. The kernels were utilized in split portions as needed for the experiments.

Chemicals

The analytical chemicals used in the study were sourced from HiMedia Laboratories Pvt. Ltd., Mumbai, and SD Fine Chem. Ltd., Mumbai. All chemicals utilized were of Analytical Reagent (AR) grade.

Experimental design

A total of 13 experiments, including 5 center points, were designed using a two-level factorial Central Composite Rotatable Design (CCRD) to ensure suitability for this study. The independent variables selected were A (Soaking duration, hours) and B (Time of water replacement, minutes), with their respective levels detailed in Table 1. A second-order polynomial equation (Eq. 1) was employed to evaluate the effects of these independent variables on the response, utilizing DESIGN EXPERT 11.0.0 software (Stat-Ease, Inc., 2021, East Hennepin Ave., Suite 480, Minneapolis, MN 55413, United States). Optimization focused on minimizing the content of amygdalin and HCN in the kernels while retaining key nutrients, including proteins, carbohydrates, and fats. Response surface plots were developed to illustrate the individual and interactive effects of the process variables. Optimal factor values were determined through numerical optimization of the process responses. To minimize experimental error, the experiments were conducted in a randomized order. The overall methodology of the study is summarized in Figure 1.

Kernel detoxification experiment

Approximately 75 g of kernels were subjected to various treatment combinations as per the experimental design. The kernels were soaked in water at a kernel-to-water ratio of 1:10, maintained at a temperature of 80°C, for durations ranging from 3 to 9 hours. During this process, the soaking water was replaced at intervals of 20 to 60 minutes, depending on the specific treatment. Given that both amygdalin and HCN are water-soluble, they were expected to leach into the soaking water during the detoxification process. The replaced water from each interval was subsequently analyzed to determine its HCN and amygdalin content.

Hydrocyanic acid

Hydrocyanic acid (HCN) in raw and detoxified kernels was analyzed using the AOAC (2000) method. Amygdalin in kernels was hydrolyzed to release HCN, which was extracted by steam distillation. About 20 g of ground kernels were hydrolyzed in 200 mL water for 2 hours, and 150–160 mL distillate was collected

into 0.625 N NaOH. The solution was diluted to 250 mL, and 100 mL was titrated with 0.02 M $AgNO_3$ in ammonical media using KI as an indicator till visualization of permanent turbidity, as end point. The HCN content in the samples was determined using the following formula and expressed as mg per 100 g of kernel:

$$\text{HCN (mg/100g)} = \frac{\text{Titre value (mL)} \times 1.08 \times \text{Volume made up (mL)}}{\text{Weight (g) of kernel taken} \times \text{Vol. (mL) of distillate taken for titrat ion}} \times 100$$

Table 1: Range and levels of independent variables for apricot kernel detoxification experiment

Factor symbol	Independent variable	Units	Minimum	Maximum	Levels	
					Coded	Actual
A	Soaking duration	hours	1.75	10.24	-1	3
					0	6
					1	9
B	Time of water replacement	min.	11.71	68.23	-1	20
					0	40
					1	60

Amygdalin content

Amygdalin content was determined following the method of Bolarinwa *et al.* (2016). A standard amygdalin solution (1000 µg/mL) was prepared, and a standard curve was generated by measuring absorbance at 256 nm for varying concentrations. For kernel samples, 4 g of ground kernels were refluxed with 100 mL ethanol for 20 minutes. The extract was filtered, dried, treated with diethyl ether, and left overnight to precipitate amygdalin. The precipitate was dissolved in water, filtered, and analyzed at 256 nm. Concentrations were calculated using the standard curve

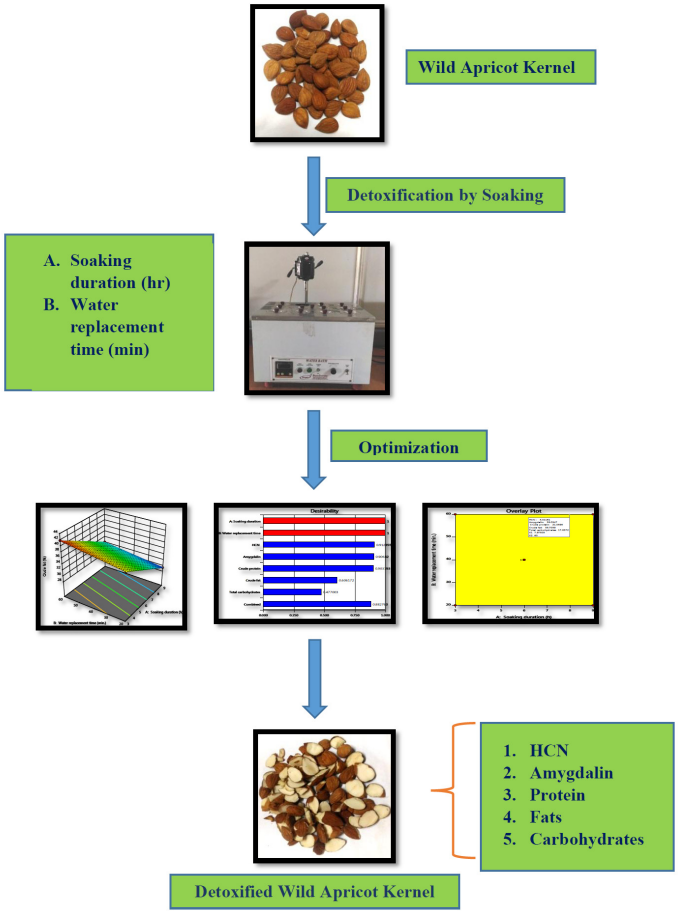

Fig. 1 : Experimental methodology for the study.

Crude protein

The crude protein content of raw and detoxified kernels was determined using the Kjeldahl method, which quantifies reduced nitrogen (NH_2 and NH) in the sample. The procedure involves digestion, distillation, and titration. During digestion, nitrogenous compounds in wild apricot kernels are converted into ammonium sulfate by boiling with concentrated sulfuric acid. The ammonium sulfate was decomposed with sodium hydroxide, releasing ammonia, which was absorbed in neutral boric acid and titrated with 0.1 N HCl. To analyze, 0.2 g of finely ground kernel was mixed with 3 g of catalyst (K_2SO_4, 5:1) in a digestion tube (DTL), and 10 mL concentrated H_2SO_4 was added. The tubes were heated in a digestion unit at 350–420 °C for 90 minutes until a clear green solution indicated complete digestion. After cooling, the distillation unit was set up with 4% boric acid and 40% sodium hydroxide solutions. The digested sample, diluted with 30 mL distilled water, underwent automated distillation. Ammonia collected in boric acid was titrated with 0.1 N HCl to determine protein content.

The titre value of sample and blank was observed and noted down for calculation using the following formula.

$$\text{Nitrogen } (\%) = \frac{14.01 \times 0.1 \times (TV - BV) \times 100}{W \times 1000}$$

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

Nitrogen content when multiplied by a factor of 6.25 gave the crude protein content in the wild apricot kernels.

Crude fat

Crude fat in wild apricot kernels, comprising triglycerides, phospholipids, essential oils, sterols, and fat-soluble pigments, was extracted using a Soxhlet apparatus (Ranganna, 1997). Finely ground, oven-dried samples (2 g) were placed in cellulose thimbles within extraction beakers, pre-weighed and coded. Petroleum ether was used as the solvent. The Soxhlet extraction was run at 70 °C for 90 minutes, with 10 °C water circulation via a chiller. Solvent recovery followed at 150 °C, and residual solvent was evaporated in a hot air oven at 70 °C for one hour. Beakers were reweighed, and crude fat content was calculated using the following formula:

$$\text{Crude fat } (\%) = \frac{W_2 - W_1}{W} \times 100$$

where,

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

Total carbohydrate

The carbohydrate content of wild apricot kernels was determined using the phenol-sulfuric acid method (Sadasivam and Manickam, 2004). In this method, glucose is dehydrated to hydroxymethyl furfural in a hot acidic medium, reacting with phenol to form a yellow-brown product. Finely ground kernel samples (100 mg) were hydrolyzed with 2.5 N HCl in a boiling water bath for three hours, neutralized with sodium carbonate, and diluted to 100 mL. A glucose stock solution (1000 µg/mL) was prepared, diluted to 100 µg/mL, and used to create a standard curve by measuring absorbance at 490 nm. Test samples (0.1–0.2 mL) underwent the same procedure, and carbohydrate content was calculated using the standard curve.

$$\text{Total carbohydrate } (\%) = \frac{\text{mg of glucose}}{\text{Volume of test sample}} \times 100$$

Detoxification

Detoxification of wild apricot kernels was assessed based on (i) HCN content, (ii) amygdalin content, and (iii) the overall potential toxicity. The calculations were performed using formulae as given below:

$$\text{Detoxification based on HCN } (\%) = \frac{\text{HCN present in raw kernels - HCN present in detoxified kernels}}{\text{HCN present in raw kernels}} \times 100$$

$$\text{Detoxification based on amygdalin } (\%) = \frac{\text{Amygdalin present in raw kernels - Amygdalin present in detoxified kernels}}{\text{Amygdalin present in raw kernels}} \times 100$$

$$\text{Overll Detoxification } (\%) = \frac{\text{Toxic principles present in raw kernels - Toxic principles left in detoxified kernels}}{\text{Toxic principles present in raw kernels}} \times 100$$

$$\text{Toxic principles (mg/100 g)} = \text{HCN content (mg/100 g)} + [59 \times \text{amygdalin contents (g/100 g)}]$$

It was assumed that both the HCN naturally present in the kernels and the HCN released from the complete hydrolysis of amygdalin (1 g of amygdalin releases 59 mg of HCN) contribute to toxicity. Therefore, overall potential toxicity and detoxification were computed to evaluate the effectiveness of the treatments.

Statistical Analysis

Data analysis was performed using DESIGN EXPERT 11.0.0 (Stat-Ease, Inc., Minneapolis, MN, USA), which was also used for graph plotting. Multiple regression analysis was applied to fit the experimental data into a model, preferably of the highest order. Model adequacy was evaluated using ANOVA, considering lack of fit (LOF) and adequate precision as key criteria.

Results and Discussion

The amygdalin content in wild apricot kernels is a primary factor contributing to their potential cyanide toxicity. Hydrolysis of amygdalin during physical processes like chewing, crushing, or grinding releases HCN, with 1 g of amygdalin producing 59 mg of HCN upon complete degradation (EFSA, 2016). Both amygdalin and HCN, being water-soluble, can be reduced through washing, soaking or boiling. Soaking, as the most practical method, was selected for detoxifying wild apricot kernels in this study. Kernels were soaked in hot water (80°C, kernel-to-water ratio 1:10) for varying durations, with periodic water replacement. Treated kernels and soaking water were analyzed to evaluate detoxification in terms of amygdalin and HCN reduction. Results (Table 2) shows HCN levels in treated kernels ranged from 2.53 to 12.59 mg/100 g, with the lowest recorded after 10.24 hours of soaking with 40-minute water replacement intervals (Run 1) and the highest after 1.75 hours with the same replacement interval (Run 7). Amygdalin content ranged from 42.33 to 221.8 mg/100 g, correlating to the same runs. The most effective detoxification (10.24 hours) also resulted in higher nutritional losses. Crude fat content varied from 28.69% (Run 1) to 42.34% (Run 7), crude protein from 20.29% to 22.42% and carbohydrates from 17.27% to 19.85%. Longer soaking durations and more frequent water replacement increased nutrient losses, likely due to leaching at high temperatures.

Soaking in water at different temperatures has been evaluated for detoxification of kernels in previous studies also. After 3 min. of heating in boiling water, D-amygdalin undergoes isomerization and can be converted to neoamygdalin which is an epimer of amygdalin (Bolarinwa *et al.,* 2014). *Chuli* and New Castle variety kernels having 72 mg HCN per 100 g initially when immersed in water for 30 min. was completely detoxified. However same results were obtained when 20 per cent salt solution was used for soaking for about 30 minute (Kamboj, 2002). Also, when 0.1 per cent sodium thiosulphate was used for soaking, complete removal of HCN was observed in 5 min. This may be due to the action of inherent enzymes (β-glucosidase) which causes hydrolysis of amygdalin into HCN and consequently get solubilised in water due to water soluble nature (Do *et al*., 2007).

The findings of this study indicate that while a soaking duration of 10.24 hours achieves maximum detoxification, it also results in significant nutritional losses, which is unfavorable for further processing of the kernels. Therefore, optimization is necessary to balance maximum detoxification with minimal nutritional loss. All responses were evaluated using Response Surface Methodology (RSM), leading to an optimized solution with high desirability.

Effect of detoxification on the HCN content of detoxified wild apricot kernels

The analysis of variance (ANOVA) for hydrocyanic acid in response to various process variables is summarized in Table 3. The model's F-value of 30,171,703.46 indicates its statistical significance, which is desirable for the experiment. P-values below 0.0500 denote significant model terms. In this case, the terms A, B, AB, A^2 and B^2 are significant, confirming that both process variables: soaking duration and frequency of water replacement play a significant role in the model.

The regression equation coefficients were calculated using Design Expert software, resulting in the following second-order polynomial equation for the response "HCN" in the apricot kernel detoxification experiment.

$$Y = 4.40 - 3.32A + 0.7819B - 0.4975AB + 1.75A^2 - 0.0175B^2$$

where, Y is the response i.e., HCN (mg/100 g) and A and B are coded values of the test variables soaking duration and water replacement time respectively.

The positive coefficients indicate a synergistic effect of the variable, while negative coefficients suggest an antagonistic effect on the response, in this case, total HCN content. From the equation, it can be concluded that soaking duration has a significant negative impact on the total HCN content of wild apricot kernels, while water replacement frequency has a significant positive effect. This implies that longer

soaking durations and shorter (more frequent) water replacement times lead to a reduction in total HCN content. Moreover, the negative impact of soaking duration is approximately three times greater than the positive effect of water replacement time.

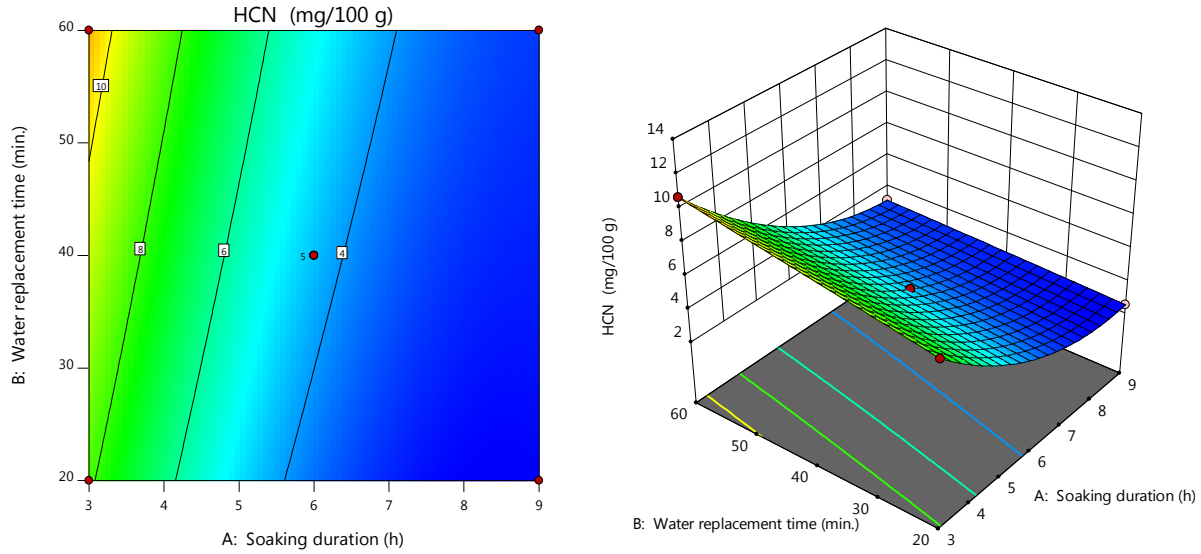

Fig. 2 : Effect of soaking duration and water replacement time on HCN content of detoxified apricot kernels

The data in Fig.2 reveals that as soaking duration increased from 1.75 hours to 10.24 hours, and water replacement time decreased from 60 minutes to 20 minutes, there was a consistent reduction in the HCN content of the kernels. The effect of soaking duration was more significant, leading to a greater decrease in cyanide content. Therefore, longer soaking durations combined with shorter water replacement intervals should be chosen to effectively reduce HCN content in the treated kernels.

Table 2 : Responses of process conditions on detoxification of apricot kernels

Run	Independent variables		Dependent variables				
	Factor A Soaking duration (h)	Factor B Water replacement time (min)	HCN (mg/100g)	Amygdalin (mg/100 g)	Crude protein (%)	Crude fat (%)	Total carbohydrates (%)
1	10.24	40	2.53*	42.33*	20.29*	28.69*	17.27*
2	6	40	4.40	75.30	21.70	35.50	18.60
3	6	11.71	3.26	51.61	21.22	33.54	18.62
4	9	60	3.10	53.05	20.71	32.06	17.52
5	6	40	4.40	75.30	21.70	35.50	18.60
6	6	40	4.40	75.30	21.70	35.50	18.60
7	1.75	40	12.59**	221.80**	22.42**	42.34**	19.85**
8	6	40	4.40	75.30	21.70	35.50	18.60
9	9	20	3.21	55.10	20.50	29.30	17.75
10	6	68.28	5.47	97.98	21.49	37.47	18.43
11	3	60	10.73	193.00	22.20	41.81	19.42
12	3	20	8.17	138.12	22.02	38.88	19.48
13	6	40	4.40	75.30	21.70	35.50	18.60

Note: * and ** represent minimum and maximum value for the responses

Table 3 : ANOVA of response surface quadratic model for HCN in detoxified apricot kernels

Source	SS	Df	MS	F-value	p-value Prob>F
Model	115.63	5	23.13	3.017E+07	< 0.0001
A	88.02	1	88.02	1.148E+08	< 0.0001
B	4.89	1	4.89	6.382E+06	< 0.0001
AB	0.9900	1	0.9900	1.292E+06	< 0.0001
A^2	21.30	1	21.30	2.780E+07	< 0.0001
B^2	0.0021	1	0.0021	2779.54	< 0.0001
Residual	5.365E-06	7	7.665E-07		
Lack of Fit	5.365E-06	3	1.788E-06		
Pure Error	0.0000	4	0.0000		
Cor Total	115.63	12			

A: Soaking duration, B: water replacement time, df: Degrees of freedom, SS: Sum of squares, MS: Mean sum of squares, LOF: Lack of fit

Effect of detoxification on the Amygdalin content of detoxified wild apricot kernels

The regression equation coefficients were determined using Design Expert software, resulting in the following second-order polynomial equation for the response "amygdalin content" in the wild apricot kernel detoxification experiment.

$$Y = +75.30 - 58.94\,A + 16.40\,B - 11.04\,AB + 31.58\,A^2 - 0.2519\,B^2$$

where, Y is the response i.e. amygdalin (mg/100 g) and A and B are coded values of the test variables soaking duration and water replacement time respectively.

The positive coefficients indicate a synergistic effect of the variable, while the negative coefficient suggests an antagonistic effect on the total amygdalin content. From the equation, it can be concluded that soaking duration significantly reduced the total amygdalin content in detoxified wild apricot kernels, while water replacement time had a significant positive effect. This suggests that longer soaking durations combined with shorter (or more frequent) water replacement times would reduce the amygdalin content in detoxified apricot kernels. Additionally, the negative impact of soaking duration was more pronounced than the positive effect of water replacement time.

Examination of the data in Fig. 3 reveals that as soaking duration increased from 1.75 h to 10.24 h and water replacement time decreased from 60 min to 20 min, there was a consistent reduction in the amygdalin content of the kernels. It is evident that soaking duration had a more significant impact, leading to a greater decrease in amygdalin content. Therefore, longer soaking durations combined with shorter water replacement intervals should be chosen to effectively remove amygdalin from wild apricot kernels.

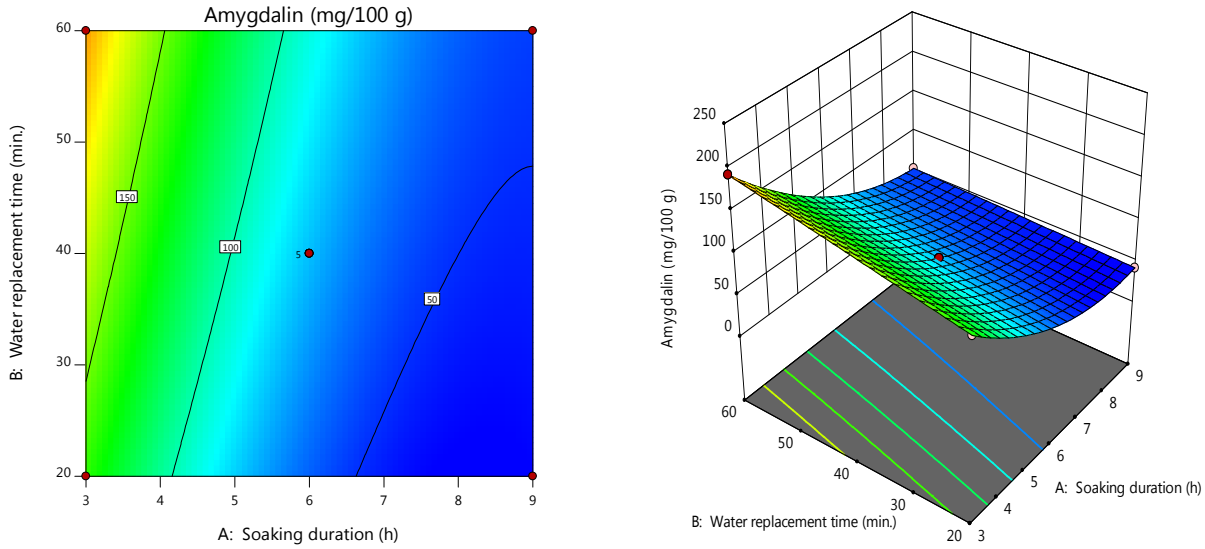

Fig. 3 : Effect of soaking duration and water replacement time on amygdalin content of detoxified apricot kernels

Effect of detoxification on the protein content of detoxified wild apricot kernels

The regression equation for the effect of different variable on the response "crude protein" of wild apricot kernel detoxification experiment is as follows:

$$Y = 21.70 - 0.7528\ A + 0.0965\ B + 0.0075\ AB - 0.1719A^2 - 0.1719\ B^2$$

where, Y is the response i.e. crude protein (%) and A and B are coded values of the test variables soaking duration and water replacement time respectively.

The equation suggests that soaking duration had a significant negative impact on the crude protein content of apricot kernels, while water replacement time had a significant positive effect. This implies that longer soaking durations with shorter water replacement intervals would reduce the crude protein content of wild apricot kernels. Therefore, optimizing shorter soaking durations with more frequent water replacement is essential to achieve desired detoxification while retaining an optimal amount of crude protein. Analysis of the data in Fig. 4 shows that as soaking duration increased from 1.75 h to 10.24 h and water replacement time decreased from 60 min to 20 min, there was a gradual decline in crude protein content. It is evident that soaking duration had a more pronounced effect, leading to a greater reduction in protein content. Thus, selecting shorter soaking durations and longer water replacement times would help minimize crude protein loss during detoxification.

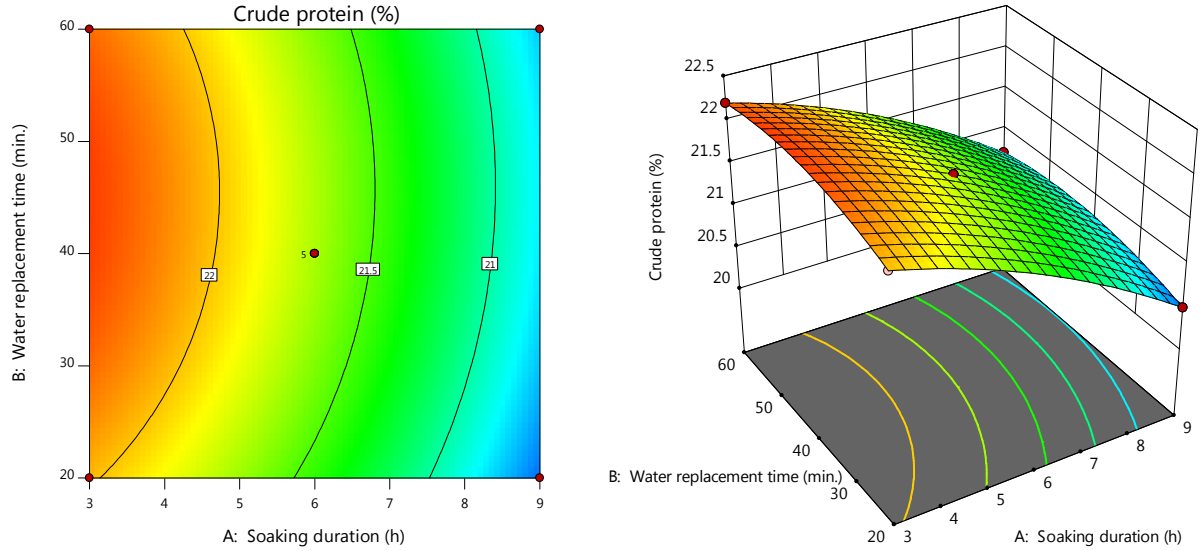

Fig. 4 : Effect of soaking duration and water replacement time on crude protein content of detoxified apricot kernels

Effect of detoxification on the crude fat content of detoxified wild apricot kernels

The regression equation for the response crude fat of apricot kernel detoxification experiment is as follows:

$$Y = 35.50 - 4.83A + 1.41B - 0.0425AB + 0.0081A^2 + 0.0031B^2$$

where, Y is the response i.e. crude fat (%) and A and B are coded values of the test variables soaking duration and water replacement time respectively.

The positive coefficients suggest a synergistic effect of the variable, while a negative coefficient indicates an antagonistic effect on the response, i.e., crude fat content. Based on the equation, it can be concluded that soaking duration had a significant negative impact on the fat content of apricot kernels, while water replacement time had a significant positive effect. This indicates that longer soaking durations and more frequent water replacements resulted in greater losses of crude fat.

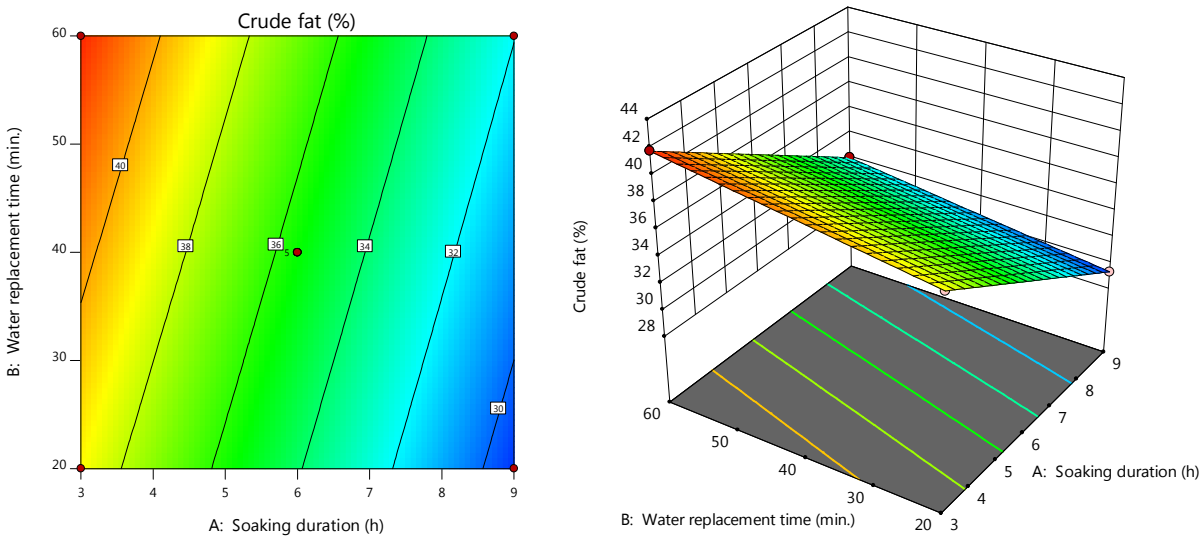

Fig. 5 : Effect of soaking duration and water replacement time on crude fat content of detoxified apricot kernels

Effect of detoxification on the total carbohydrate content of detoxified wild apricot kernels

The regression equation for the effect of detoxification on the response "total carbohydrate" of kernel detoxification experiment is as follows:

$Y = +18.6 - 0.9098A - 0.0698\ B - 0.0425\ AB - 0.0200\ A^2 - 0.0375\ B^2$

where, Y is the response i.e. total carbohydrate (%) and A and B are coded values of the test variables soaking duration and water replacement time respectively.

The above equation indicates that soaking duration significantly reduced the total carbohydrate content in wild apricot kernels, and frequent water replacement also had a similar negative effect. Similarly, Fig 6 suggests that prolonged soaking and frequent water replacement lead to greater carbohydrate loss in detoxified kernels. Therefore, the process should be optimized to minimize carbohydrate losses while achieving the desired outcome.

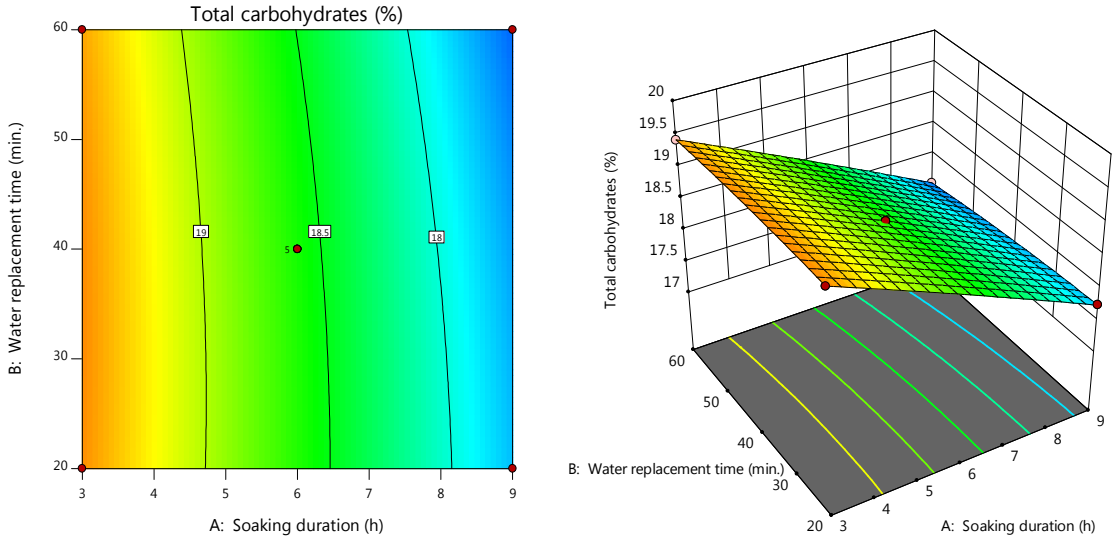

Fig. 6 : Effect of soaking duration and water replacement time on total carbohydrate content of detoxified apricot kernels

Detoxification percentage

Different combinations of soaking duration and water replacement time resulted in over 90% detoxification across all treatments (Table 4). The highest detoxification rate (98%) for both HCN and amygdalin was achieved by soaking the kernels for 10.24 hours with water replaced every 40 minutes. In

contrast, soaking for 1.75 hours with the same water replacement frequency resulted in approximately 90% detoxification. While a 10.24-hour soaking duration seems optimal for detoxification, the associated nutrient losses necessitate further optimization to balance maximum detoxification with minimal nutrient depletion.

Table 4: Detoxification in wild apricot kernels

Run	Factor A Soaking duration (h)	Factor B Water replacement time (min)	HCN		Amygdalin	
			Content in treated kernels (mg/100g)	Detoxification (%)	Content in treated kernels (mg/100 g)	Detoxification (%)
1	10.24	40	2.53	98.1	42.33	98.22
2	6	40	4.4	96.7	75.3	96.9
3	6	11.71	3.26	97.5	51.61	97.8
4	9	60	3.1	97.7	53.05	97.8
5	6	40	4.4	96.7	75.3	96.9
6	6	40	4.4	96.7	75.3	96.9
7	1.75	40	12.59	90.7	221.8	90.9
8	6	40	4.4	96.7	75.3	96.9
9	9	20	3.21	97.6	55.1	97.7
10	6	68.28	5.47	95.9	97.98	96
11	3	60	10.73	92.0	193	92.1
12	3	20	8.17	93.9	138.12	94.3
13	6	40	4.4	96.7	75.3	96.9

*HCN and amygdalin in raw kernels 135.5 mg/100 g and 2450 mg/100 g

Optimization

Optimization was performed to identify the most favorable combination of process variables that align with the desired conditions for the study. The DESIGN EXPERT 11.0.0 software was utilized to determine the optimal process parameters. Specific goals were established for each independent variable and response to guide the optimization process and achieve the best possible outcomes.

Given that prolonged soaking durations resulted in nutrient losses, the primary goal of this study was to minimize HCN and amygdalin levels. These parameters, being critical to the detoxification process, were assigned the highest priority (5). Nutritional parameters were targeted for maximization to achieve optimized results with maximum nutrient retention. Soaking duration was set within a range, while water replacement time was targeted at 60 minutes, as frequent water changes were deemed impractical for industrial applications. Based on these criteria, optimization was conducted, and various solutions were generated. The most suitable solution was selected according to the specified criteria (Table 5).

Figures 7 and 8 illustrate that the optimized conditions suggested by the design software included a soaking duration of 7.97 hours with water replacement every 60 minutes. Under these conditions, the predicted outcomes were 3.41 mg/100 g HCN, 59.05 mg/100 g amygdalin, 21.05% crude protein, 33.70% crude fat, and 17.85% total carbohydrates. The combined desirability score for these parameters was 0.882, indicating the suitability of the optimization. A desirability score above 0.80 is generally considered acceptable for selecting optimized solutions.

According to existing literature, soaking ground kernels overnight reduced amygdalin content from an initial 118 µmol/g to 30 µmol/g, while soaking followed by cooking at 100 °C in an open pan for 30 minutes further reduced it to 28 µmol/g (Nout *et al.*, 1995). Another study reported that soaking apricot kernels at 80 °C for 5 minutes with water changes every 4 hours decreased HCN content by 96.3%, from 801.32 ± 77.53 mg/kg to 29.46 ± 1.63 mg/kg in 8 hours, and 97.1% after 24 hours (Desser, 2015). Similarly, blanched and peeled kernels kept under flowing water (43 mL/s; 5 °C) for 8 and 24 hours showed HCN reductions of 97.0% and 98.4%, respectively (Desser, 2015). Whole and broken kernels detoxified through soaking at a water-to-kernel ratio of 10:1 at 80 °C for 4 hours achieved 92% detoxification (Gaur, 2017). These variations in detoxification outcomes may be attributed to differences in genetic and environmental factors affecting the raw materials. To confirm the effects of process variables under optimized conditions, fresh experiments were

conducted, and predicted and actual outcomes were compared. Data in Table 6 show that the predicted and actual results closely matched.

Table 5 : Goals and assigned importance for optimization of variables for detoxification of apricot kernels

Factor	Unit	Goal	Lower Limit	Upper Limit	Importance
A: soaking duration	h	in range	3	9	3
B: water replacement time	min	target = 60	20	60	5
HCN	mg/100 g	minimize	2.53	12.59	5
Amygdalin	mg/100 g	minimize	42.33	221.8	5
Crude protein	%	maximize	20.29	22.42	3
Crude fat	%	maximize	28.69	42.34	1
Total carbohydrates	%	maximize	17.27	19.85	1

Table 6 : Predicted and observed values of various parameters in apricot kernel detoxification experiment

Response	Predicted	Observed / Actual
HCN (mg/ 100 g)	3.41	3.37
Amygdalin (mg/100 g)	59.05	56.56
Crude protein (%)	21.05	21.74
Crude fat (%)	33.70	35.00
Total carbohydrate (%)	17.85	18.20
Detoxification based on HCN (%)	-	97.5#
Detoxification based on amygdalin (%)	-	97.6#
Overall detoxification (%)	-	97.6#

Using formula given in section 2.10

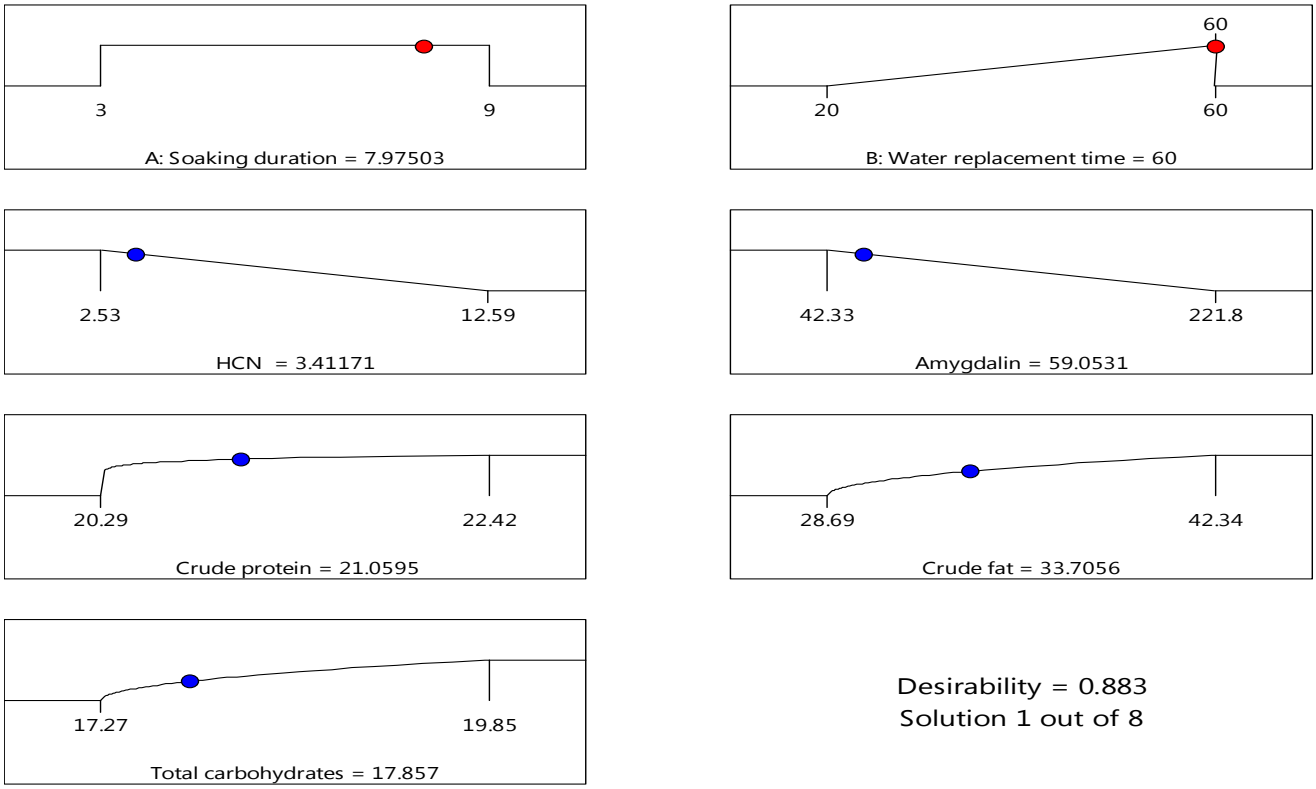

Fig. 7 : Optimized process points in the form of ramps for detoxification of wild apricot kernels

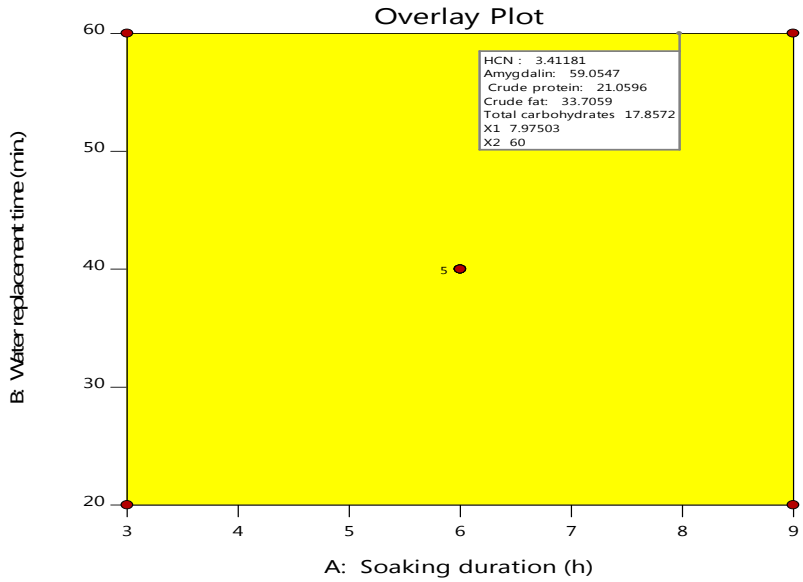

Fig. 8 : Overlay plot indicating optimized conditions for detoxification of wild apricot kernel

Summary and Conclusion

In conclusion, wild apricot kernels can be effectively detoxified by soaking them in a kernel-to-water ratio of 1:10 at 80 °C for approximately 8 hours (7.97 hours), with water replacement every 60 minutes. This treatment reduces the HCN and amygdalin levels to 3.37 mg/100 g and 56.56 mg/100 g, respectively, achieving about 97% detoxification for both compounds. After detoxification, the kernels retain approximately 21.74% crude protein, 35% crude fat, and 18.20% total carbohydrates. According to the European Food Safety Authority (2016), the lethal HCN dose for humans ranges from 0.5 to 3.5 mg per kg of body weight. For an average adult weighing 50 kg, the safe intake limit is about 25 mg of HCN. Consuming 25 g of detoxified kernels would result in an HCN intake of 0.84 mg, which is well within the safe limit. Furthermore, the amygdalin content in 25 g of detoxified kernels would amount to 14.14 mg, which, if fully hydrolyzed, could produce 0.82 mg of HCN (considering that 1 g of amygdalin yields 59 mg of HCN upon complete hydrolysis). Thus, the total HCN intake from consuming 25 g of detoxified kernels, including both present HCN and that released from amygdalin, would be 1.66 mg, safely below the 25 mg limit for a 50 kg adult.

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