Biological Forum – An International Journal **15(2): 541-548(2023)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Standardization of Pretreatment for the Development of the Edible Flour from the Indian Horse Chestnut (*Aesculus indica* Colebra.)

Jyoti Rani*, N.S. Thakur, Abhimanyu Thakur and Pavneet Kaur
Department of Food Science and Technology,
Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan (Himachal Pradesh), India.

(Corresponding author: Jyoti Rani)*
(Received: 27 December 2022; Revised: 07 February 2023; Accepted: 10 February 2023; Published: 16 February 2023)
(Published by Research Trend)

ABSTRACT: *Aesculus indica* Colebr. which is popularly known as Indian horse chestnut. It is a good source of starch but contains toxic compounds such as saponins which make it a bitter and unsuitable for consumption. To utilize the starch source for edible purposes, saponins were first reduced to an acceptable level by pretreating the crushed mass of Indian horse chestnut. Studies were undertaken to remove the saponins content from the horse chestnut mass by pre-treatments. Prescribed methods were used to evaluate the results at Dr. YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. Various physcio-chemical characteristics of nut and flour were determined like moisture, crude protein, fiber, fat, total carbohydrate, ash content, saponins, reducing sugars and total sugars. Nuts after dehulling were crushed/grated into a mass which was treated with various treatments. Pre-treatments were used to remove the saponin content of the horse chestnut mass. After dehulling, the nuts were grated into a mass and then treated with various treatments. The grated mass can be blanched for 4 min for followed by soaking in water for 6 hours at 60°C while replacing the water in three cycles of every 2 hours. Same pretreated mass was further soaked in ethanol: water solvent (30:70) for 6 h at 60°C while replacing the water in three cycles of every 2 hours. Then, further ultrasound assisted extraction was done with 30 per cent ethanol in water for 15 minutes at 50 ± 1°C and further soaking can be performed at 60°C while replacing the solvent mixture in three cycles of 2 h. After all the previous sequential treatments, the mass was dried in mechanical cabinet drier at 60 ± 2°C. In the end of all pretreatments the formation of froth was negligible and saponins content could be reduced 6.50 ± 0.04 to 0.88 g/100g in the treated mass.

Keywords: Anti- nutrition, blanching, Indian horse chestnut, pre-treatment, saponins.

INTRODUCTION

Indian horse chestnut (*Aesculus indica* Colebr.) belongs to the family *Hippocastanaceae* and in India it is generally known as *bankhor* (Mishra *et al*., 2018). Its generic name, *Aesculus* has come from Latin word "esca" which means food. It is found in the temperate regions of Europe, Asia, north western Himalayas and North America (Santapau and Henry 1973) and its trees are distributed in hilly area and temperate region from Kashmir to Nepal at an elevation between 900 to 3,600 meters above mean sea level. In India, it is found in Kashmir, Himachal Pradesh and Uttaakhand. In Himachal Pradesh, its trees are widely distributed in the various districts like Solan, Shimla, Sirmour, Kullu, Kangra, Mandi and Chamba. The flowering season of this nuts tarts from May to June and nuts are harvested in the month of October and November. The seeds are smooth and shiny but sometimes they are dark and wrinkled. Each single seed being present in hard shining black capsule and each capsule bears single lime white cotyledons). This nut is rich sources of carbohydrate, sugars, proteins, lipids, minerals and fibers besides rich source of saponins which is an antinutritional factor (Singh *et al*., 2003; Parmar and Kaushal 1982). Its oil is used to cure rheumatism and also applied to wounds (Sharma and Sehgal 1992). However its use is restricted because of high amount of saponins it contains. These anti-nutritional factors are chemical compounds that are synthesized in natural foods and/or feedstuffs through normal species metabolism and various mechanisms (for example, inactivation of some nutrients, diminution of the digestive process, or metabolic utilization of food/feed) which have an anti-nutritional effect (Soetan and Oyewol 2009). These anti-nutritional factors are secondary metabolites which have been shown to be biologically active (Shanthakumari *et al.,* 2008). These chemicals have been found to be beneficial to human and animal health when consumed in appropriate amounts. The antinutrtional factors in Indian horse chestnut have been classified as toxic compounds, popularly known as saponins and many of these

compounds exhibited strong hemolytic activity. In high concentrations, they have a bitter taste and strong astringency which are the limiting factor for their use. They have previously been recognized as antinutrient constituents due to their negative effects such as growth impairment and decreased food intake due to the bitterness and throat-irritating activity. The removal of saponins has been the subject of many scientific studies. Over the past many years for the removal of saponins various "wet" or "dry" techniques or a combination of both have been used. The dry techniques like extrusion, roasting and mechanical abrasion for the removal of saponins and maintenance of the nutritional quality of the treated nuts have been tried in the past. Wet technique like rinsing or soaking the nuts in water have been found more efficient to get rid of saponins (El Hazzam *et al.,* 2020). Blanching is one of the unit operations for removing antinutritional factors. Nwosu (2010) have observed that asparagus beans blanched for 8 minutes reduced saponins from 0.42 to 0.36 per cent, whereas, the same worker recorded 97.66 per cent decrease in saponins content after 8 minutes of blanching in Oze seeds by Nwosu (2011). Khokhar and Chauhan (1989) have reported that moth bean soaked in a mixed mineral salt solution also removed a significant amount (30-36%) of saponins, whereas, soaking in plain water reduced only 9-18 per cent. The reduction of the saponins as high as 29.0 per cent after the soaking process in faba bean has been reported by Sharma and Sehgal (1992). However, solvent based techniques like conventional and green technologies also being used presently. The green technologies like ultrasound-assisted, microwave-assisted, and accelerated solvent extractions are energy efficient, involve the safer use of chemicals, use of renewable feedstock, and prevent the pollution (Cheok *et al.,* 2014). Yuliana *et al.* (2014) have observed that 100 per cent methanol was optimal to extract the saponins from klerek fruits.

Fig. 1. Indian horse chestnut.

Hadidi *et al.* (2022) have reported optimal extraction of saponins in different conditions of extraction time (2.84 h), extraction temperature (76.80°C), ultrasound power (112.0 w) and ethanol concentration (78.20 per cent) and the resulting yield of total saponins as 1.61 per cent. Combinations of treatments were used to extract the most saponins from the sample because complete saponin removal was not possible and treated mass turning it into edible flour. This edible flour can have many applications for the development of various products since it is a rich source of starch.

MATERIALS AND METHODS

Indian horse chestnuts harvested at optimum maturity were procured from Kasauli area of district Solan (HP) and brought to the Department of Food Science and Technology, UHF, Nauni, Solan (Himachal Pradesh) for conducting the studies. Dehulling of nuts was done manually with the help of stainless steel knife. Dehulled nuts were further grated with the help of mechanical grater and this grated mass was used further for standardization of pretreatments.

Physical characteristics. The colour of nuts, kernel and flour was observed visually by comparing with colour cards of Royal Horticulture Society, London. Vernier callipers were used to measure the size of nuts in terms of length and diameter and expressed in millimeter (mm) whereas, weight of nuts was measured by single pan weighing balance, volume was measured in volumetric cylinder and expressed in gram. The nuts per capsule were counted individually and average number of nuts per capsule were calculated.

Chemical characteristics. Moisture content, ash, crude protein, fiber, fat, total carbohydrate and ash content of nut were determined according to the method of AOAC (2009). Sugar and starch content was observed by the method of AOAC (2009).For pH estimation, a digital pH meter (CRISON Instrument, Ltd, Spain) was used. Total phenols content was determined by Folin-Ciocalteu procedure given by Singleton and Rossi (1965) in which absorbance was measured at 765 nm. Oxalate content was determined according to the method given by Chinma and Igyor (2007). Phytate content was determined according to the method given by Ohlander *et al*. (1978) and saponins content was determined according to the method Vanillin-Sulphuric Acid method given by Hiai *et al.* (1976). Peroxides activity was determined according to the method given in (Ranganna, 2009).

Froth. Percentage of froth was calculated by dividing the length of froth in beaker by total length of froth and water in beaker with the following formula:

$$\text{Froth (\%)} = \frac{\text{Length of froth (cm)}}{\text{Total length of froth and water (cm)}} \times 100$$

Removal of saponins by blanching and soaking at varying temperatures. The grated mass after blanching was soaked in tap water in the ratio of 1:20 in a graduated beaker. This mixture was kept at varying temperature (Room temperature, 50, 60, 70 and 80°C). After two hours of soaking the whole mixture was agitated vigorously with magnetic stirrer for 10 minutes. The water of the mixture was replaced with

the same quantity of water by syphoning technique and this process was repeated every 2 hours till the three cycles were completed. This mixture was agitated for 10 minutes at the end of 3rd cycle. The height of the froth in mixture after the agitation was noted down immediately in the beaker. The concentration of saponins in wet mass was also observed at the end of 3rd cycle.

Table 1: Detail of treatments for removal of saponins by blanching and soaking at varying temperatures.

Treatment Symbols	Treatment
T_1	Control
T_2	Soaking in water at RT
T_3	Blanching followed by soaking in water at RT
T_4	Soaking in water at 50°C
T_5	Blanching followed by soaking in water at 50°C
T_6	Soaking in water at 60°C
T_7	Blanching followed by soaking in water at 60°C
T_8	Soaking in water at 70°C
T_9	Blanching followed by soaking in water at 70°C
T_{10}	Soaking in water at 80°C
T_{11}	Blanching followed by soaking in water at 80°C

Removal of saponins by soaking in the varying combinations of ethanol and water. Best treatment of previous experiment (Table 1) was further soaked in the mixture of ethanol and water different concentration in the ratio of 1:20. This mixture was kept at varying temperature (Room temperature, 50, 60, 70 and 80°C).After two hours of soaking the whole mixture was agitated vigorously with magnetic stirrer for 10 minutes. The water of the mixture was replaced with the same quantity of water by syphoning technique and this process was repeated every 2 hours till the three cycles were completed. This mixture was agitated for 10 minutes at the end of 3rd cycle. The height of the froth in mixture after the agitation was noted down immediately in the beaker. The concentration of saponins in wet mass was also observed at the end of 3rd cycle.

Table 2: Detail of treatments for removal of saponins by soaking in the varying combinations of ethanol and water.

Treatment Symbols	Treatment
T_1	Soaking in ethanol- water (10:90) at RT
T_2	Soaking in ethanol- water (10:90) at 50°C
T_3	Soaking in ethanol- water (10:90) at 60°C
T_4	Soaking in ethanol- water (10:90) at 70°C
T_5	Soaking in ethanol- water (10:90) at 80°C
T_6	Soaking in ethanol- water (20:80) at RT
T_7	Soaking in ethanol- water (20:80) at 50°C
T_8	Soaking in ethanol- water (20:80) at 60°C
T_9	Soaking in ethanol- water (20:80) at 70°C
T_{10}	Soaking in ethanol- water (20:80) at 80°C
T_{11}	Soaking in ethanol- water (30:70) at RT
T_{12}	Soaking in ethanol- water (30:70) at 50°C
T_{13}	Soaking in ethanol- water (30:70) at 60°C
T_{14}	Soaking in ethanol- water (30:70) at 70°C
T_{15}	Soaking in ethanol- water (30:70) at 80°C

Removal of saponins by ultrasound assisted and microwave assisted extraction techniques. Best treatment of previous (Table 2) was treated further by advanced techniques of extraction. Treated mass of horse chestnut was mixed with a mixture of best combination of ethanol and water in the ratio of 1:20 and treated in ultrasonic bath having 40 KHz frequency at varying time period. In other lot, grated mass was treated in microwave oven at 2.45GHz frequency at varying time period. All the treated material in different treatment combinations was soaked at varying temperature (40 and 60°C). After two hours of soaking the whole mixture was agitated vigorously with magnetic stirrer for 10 minutes. The water of the mixture was replaced with the same quantity of water by syphoning technique and this process was repeated every 2 hours till the three cycles were completed. This mixture was agitated for 10 minutes at the end of 3rd cycle. The height of the froth in mixture after the agitation was noted down immediately in the beaker. The concentration of saponins in wet mass was also observed at the end of 3rd cycle.

Table 3: Detail of treatments for removal of saponins from already treated mass by ultrasound assisted and microwave assisted extraction techniques.

Treatment Symbols	Treatment
T_1	Control (Best treatment from Table 2)
T_2	Ultrasonic treatment for 5 min and soaking at 40°C
T_3	Ultrasonic treatment for 10 min and soaking at 40°C
T_4	Ultrasonic treatment for 15 min and soaking at 40°C
T_5	Ultrasonic treatment for 5 min and soaking at 60°C
T_6	Ultrasonic treatment for 10 min and soaking at 60°C
T_7	Ultrasonic treatment for 15 min and soaking at 60°C
T_8	Microwave treatment for 2 min and soaking at 40°C
T_9	Microwave treatment for 4 min and soaking at 40°C
T_{10}	Microwave treatment for 6 min and soaking at 40°C
T_{11}	Microwave treatment for 2 min and soaking at 60°C
T_{12}	Microwave treatment for 4 min and soaking at 60°C
T_{13}	Microwave treatment for 6 min and soaking at 60°C

Statistical analysis. Completely Randomized Design was used to analyze the statistical data on physicochemical characteristics before and during storage (Cochran and Cox, 1957). OPSTAT was used to analyze the data pertaining to the sensory evaluation of the samples using the Randomized Block Design (RBD) using one factor, two factor, and three factor analysis of variance (ANOVA).

RESULT AND DISCUSSION

Physical characteristics. The number of nuts per capsule, weight of capsule, nut and kernel per nut of Indian horse chestnut were recorded (Table 4) as 1.00±0,27.72±1.53 g,26.29±1.02 g, 22.88±0.95 g, respectively. Whereas, length, diameter and volume of nut were 2.74±0.13 cm, 4.51±0.13 cm, and 29.11±1.84 mL, respectively. The visual colour of nut was found to be Brown group (200 C), whereas, the visual colour of the kernel was observed as Yellow orange group (18 B). These findings are within the range as have been reported by Thakur *et al.* (2015); Parmar and Kaushal (1982).

Chemical characteristics. The data pertaining to chemical characteristics of Indian horse chestnut have been presented in Table 5 which indicate that its L*, a*, and b* values were observed as 90.95±0.14, 2.99±0.01and 14.86±0.05, respectively. The moisture content in the nut was recorded as 58.44±0.25 per cent, whereas, the reducing and total sugars content in the nut was found as 3.92±0.07 and 4.89±0.11per cent, respectively. The crude protein and fat content in the same were recorded as 5.77±0.06 per cent and 2.62±0.11 per cent, respectively. Whereas, total phenols and crude fiber in the nut were found to be 494.16±3.83 mg/100g and 0.68±0.05 per cent, respectively. The starch content in nut was found as 27.43±0.33 per cent and total carbohydrates as 31.20±0.57 per cent in the nut. The pH was recorded as 6.50±0.12 and ash content as 1.97±0.04 per cent in the nut. The oxalate, phylate and saponins in the nut were recorded as 1.64±0.02 µg/g, 0.20± 0.01 µg/g and 6.50±0.04 g/100g respectively. The energy value in the nut was recorded as 171.22±0.58kcal/100g. Present, results are within the range as reported by Thakur *et al.* (2015); Rafiq *et al.* (2016); Čukanović *et al.* (2011) in the horse chestnut.

Table 4: Physical characteristics of Indian horse chestnut.

Parameters	Mean ±SE*
No. of nuts per capsule	1.00±0
Weight of capsule (g)	27.72±1.53
Weight of nuts (g)	26.29±1.02
Weight of the kernel per nut (g)	22.88±0.95
Size of nuts: Length(cm) Diameter(cm)	2.74±0.13 4.51±0.13
Volume of nuts (mL)	29.11±1.84
Visual colour of the nut	*Brown group (200 C)
Visual colour of the kernel	*Yellow orange group (18 B)

*SE= Standard Error; *Colour and colour card number of Royal Horticulture Society, London

Table 5: Chemical characteristics of horse chestnut and flour.

Parameters		Nut (Mean±SE)
Colour values	L*	90.95±0.14
	a*	2.99±0.01
	b*	14.86±0.05
Moisture (%)		58.44±0.25
Reducing sugars (%)		3.92±0.07
Total sugars (%)		4.89±0.11
Crude protein (%)		5.77±0.06
Fat (%)		2.62±0.11
Crude fiber (%)		0.68±0.05
Total phenol (mg/100g)		494.16±3.83
Starch (%)		27.43±0.33
Carbohydrates (%)		31.20±0.57
Ash (%)		1.97±0.04
pH		6.50±0.12
Oxalate (µg/g)		1.64±0.02
Phytate (µg/g)		0.20±0.01
Saponins (g/100 g)		6.50±0.04

Effect of pretreatments. To remove the saponins content from the horse chestnut mass, various pre-treatments were followed and best treatments were selected on the basis of froth and saponins percentage. Frothing test was used to determine the presence of saponins and it is a qualitative test. Saponins have the tendency to make the stable froth after the vigorously shaken the sample in the liquid.

Blanching followed by soaking (at 60^{o}C) treatment of horse chestnut mass removed saponins the most which might be due to the leaching of most saponins. Blanching might have contributed towards the release of saponins by softening the texture of mass and further soaking ultimately contributed towards the movement of saponins from mass to solvent (water) since saponins are water soluble compounds. Data of blanching and water soaking treatment (Table 6) revealed that froth percentage reduced from 31.88 to 26.10 per cent and saponins content of grated mass of horse chestnut could be reduced from 6.51 to 4.81 g/100g in T_7. Patel *et al.* (2018) has observed the decrease in saponins content from 16 µg/g to 5.14 µg/g in bathua leaves after 3 minutes of blanching. Shi *et al.* (2009) have reported that soaking of navy bean seeds in water resulted in a considerable drop in saponin B level. They also observed that with the increase in the amount and length of soaking water removed the saponins B upto 6.3 per cent after 12 hours of soaking.

Further best selected treatment used in the next treatment where different concentration of ethanol: water was used as solvent during soaking (Table 7). Highest reduction in froth and saponins content was found in the T_{13} where froth percentage reduced from 19.55 to 13.79 per cent and saponins from 4.33 to 2.88 g/100g. Extraction yield percentages obtained 78 % from ethanol: water (1:10) soaking of Soapnut (Sapindus Mukorossi) has been observed by Kose and Bayraktar (2016).

Further this best selected treatment used next treatment (Table 8) where ultrasound and microwave assisted technique used for different time for the removal of saponins from the mass. In the best selected treatment, formation of froth was negligible and saponins content could be reduced 2.88 to 0.88 g/100g at the end of the treatment (T_7). The removal of saponins from mass under these conditions might be due to the crushing of the cell wall which led towards the release of saponins compounds into the solvent. This might have happened because of the phenomenon of acoustic cavitation which results because of the alternating high pressure/low pressure cycles when ultrasound waves travel through a liquid (solvent). He *et al.* (2022) have reported that extraction of saponins from Polygonatum by UAE technique (ethanol concentration of 85 per cent (*v*/*v*), an extraction time of 75 min, an extraction temperature of 50°C) had highest extraction yield. Ultrasonic solvent extraction (USE) used for extraction of saponins from cow cockle seeds by using different concentration of solvent like water, methanol (50%, 80%, and 100%) and ethanol (50%, 80%, and 100%) and highest yield obtained in 50 per cent ethanol (Ozlem *et al.,* 2007).

Table 6: Removal of saponins by blanching and soaking of horse chestnut mass at varying temperature.

Treatments	Froth (%)	Saponins (g/100g)
T_1	31.88	6.51
T_2	29.54	6.36
T_3	28.61	6.05
T_4	29.26	6.32
T_5	27.63	5.62
T_6	27.29	5.38
T_7	**26.10**	**4.81**
T_8	27.30	5.41
T_9	26.11	4.82
T_{10}	27.34	5.42
T_{11}	26.13	4.84
$CD_{0.05}$	0.22	0.07

Table 7: Removal of saponins from horse chestnut mass by soaking in the varying combinations of ethanol and water.

Treatments	Froth (%)	Saponins (g/100g)
T_1	19.55	4.33
T_2	16.08	4.04
T_3	15.30	3.33
T_4	15.81	3.78
T_5	15.85	3.81
T_6	15.10	4.06
T_7	14.50	3.67
T_8	14.28	3.13
T_9	14.29	3.44
T_{10}	14.32	3.48
T_{11}	14.75	3.80
T_{12}	14.38	3.44
T_{13}	**13.79**	**2.88**
T_{14}	13.86	2.90
T_{15}	13.87	2.92
$CD_{0.05}$	0.73	0.06

Table 8: Removal of saponins from already treated mass by ultrasound assisted and microwave assisted extraction technique.

Treatments	Saponins (g/100g)	Froth (%)
T_1	2.88	13.79
T_2	1.11	-
T_3	1.00	
T_4	0.98	**-**
T_5	1.01	
T_6	0.99	-
T_7	**0.88**	-
T_8	1.39	-
T_9	1.10	
T_{10}	1.00	-
T_{11}	1.19	-
T_{12}	0.99	-
T_{13}	0.98	-
$CD_{0.05}$	0.07	-

Means negligible froth

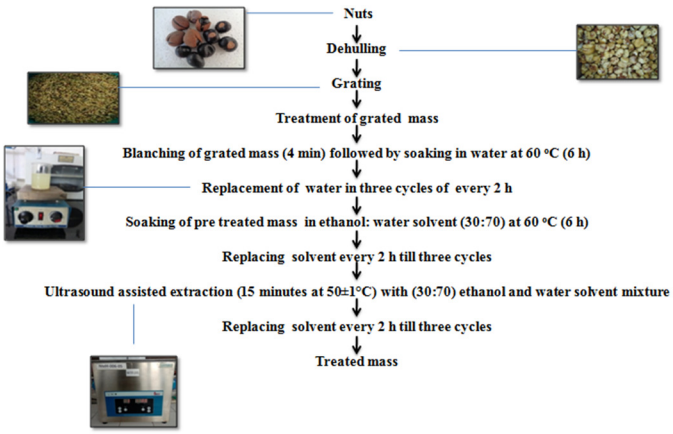

Fig. 2. Unit operations for the removal of saponins from Indian horse chestnut.

Thakur *et al.* (2015) used different pre-treatments to make the Indian horse chestnut edible, but they used the traditional method for removing saponins, which was a time-consuming process and saponin removal was also inefficient. In this study, we combined treatment and green technology to remove the most saponins from Indian horse chestnut and make the nut edible.

CONCLUSIONS

To remove saponins content from horse chestnut mass (grated materials), various pre-treatments like blanching, cooking, ethanol soaking and ultrasound assisted extraction were followed and best treatments were selected on the basis of froth formation and amount of saponins. Pretreatments such as blanching, soaking and ultrasound and microwave extraction techniques were standardized. The best selected treatments were then evaluated further based on maximum saponins removal and minimum froth percentage. In the end, a treatment with low saponin content and unstable froth was used to make the flour.

FUTURE SCOPE

The starch content of Indian horse chestnut is high, but due to saponins, it is not used further in food. As a result, the starch used in the preparation of the supplemented product should first be edible. In this study, we use different pretreatment methods to make this Indian horse chestnut grated mass edible while removing the most saponins. Edible grated mass flour can be used in the food industry to make a variety of supplemented products.

Acknowledgement. I would like to thank the Department of Food Science and Technology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh.
Conflict of interest. None.

REFERENCES

AOAC International (2009). Official Methods of Analysis of AOAC International, 18thed

Cheok, C. Y., Salman, H. A. K. and Sulaiman, R. (2014). Extraction and quantification of saponins: A review. *Food Research International*, *59*, 16-40.

Chinma, C. E. and Igyor, M. A. (2007). Micronutrients and anti-nutritional contents of selected tropical vegetables grown in South East, Nigeria. *Nigerian Food Journal*, *25*(1), 111-116.

Cochran, W. G. and Cox, G. M. (1957). Experimental Designs. *John Wiley & Sons*; New York, USA.

Čukanović, J., Ninić-Todorović, J., Ognjanov, V., Mladenović, E., Ljubojević, M., and Kurjakov, A. (2011). Biochemical composition of the horse chestnut seed (*Aesculus hippocastanum* L.). *Archives of biological sciences*, *63*(2), 345-351.

El Hazzam, K., Hafsa, J., Sobeh, M., Mhada, M., Taourirte, M., El Kacimi, K. and Yasri, A. (2020). An insight into saponins from Quinoa (*Chenopodium quinoa* Willd): A review. *Molecules*, *25*(5), 1059.

He, S., Wang, X., Chen, J., Li, X., Gu, W., Zhang, F., ... & Yu, J. (2022). Optimization of the Ultrasonic-Assisted Extraction Technology of Steroidal Saponins from Polygonatum kingianum Collett & Hemsl and Evaluating Its Quality Planted in Different Areas. *Molecules*, *27*(5), 1463.

Hadidi, M., Rostamabadi, H., Moreno, A. and Jafari, S. M. (2022). Nanoencapsulation of essential oils from industrial hemp (*Cannabis sativa* L.) by-products into alfalfa protein nanoparticles. *Food Chemistry*, *386*, 132765.

Hiai, S., Oura, H. and Nakajima, T. (1976). Color reaction of some sapogenins and saponins with vanillin and sulfur1c acid. *Planta Medica*, *29*(02), 116-122.

Khokhar, S. and Chauhan, B. M. (1986). Antinutritional factors in moth bean (*Vigna aconitifolia*): varietal differences and effects of methods of domestic processing and cooking. *Journal of Food Science*, *51*(3), 591-594.

Kose, M. D. and Bayraktar, O. (2016). Extraction of saponins from soapnut (*Sapindus Mukorossi*) and their antimicrobial properties. *World Journal of Research and Review*, *2*(5), 262952.

Mishra, M. L., Sood, S. and Shukla, U. N. (2018). Phyto-nutritional and mineral composition of Indian Horse Chestnut (*Aesculus indica*) seeds. *Journal of Pharmacognosy and Phytochemistry*, *7*(1), 2159-2162.

Nwosu, J. N. (2011). The effects of processing on the anti-nutritional properties of'oze'(*Bosqueia angolensis*) seeds. *The Journal of American Science*, *7*(1), 1-6.

Ohlander, R., Price, K. and Reddy, D. R. (1978). Picture segmentation using a recursive region splitting method. *Computer graphics and image processing*, *8*(3), 313-333.

Parmar, C. and Kaushal, M. K. (1982)**.** *Aesculus indica*. In: *Wild Fruits*. Kalyani Publishers, New Delhi, India. pp.6-9.

Patel, B., Prakash, S., Singh, M. K., Kumar, A., Kumar, M., Shukla, S. and Dixit, P. (2018). Effect of bio-regulator treatment, wounding and growing media on survival and vegetative growth of stem cutting in lemon (*Citrus limon* Burm.). *IJCS*, *6*(6), 2154-2158.

Rafiq, S. I., Singh, S., & Saxena, D. C. (2016). Effect of heat-moisture and acid treatment on physicochemical, pasting, thermal and morphological properties of Horse Chestnut (*Aesculus indica*) starch. *Food Hydrocolloids*, *57*, 103-113.

Ranganna, S. (2009). Hand book of analysis and quality control of fruit and vegetable products. 4th edn.Tata McGraw Hill, New Delhi1112p.

Santapau, H. and Henery, A. H. (1973). A dictionary of the flowering plants in India. Publication and Information Directorate CSIR, New Delhi. pp.144-148.

Shanthakumari, S., Mohan, V. R. and de Britto, J. (2008). Nutritional evaluation and elimination of toxic principles in wild yam (*Dioscorea spp*.). *Tropical and Subtropical Agroecosystems*, *8*(3), 319-325.

Sharma, A. and Sehgal, S. (1992). Effect of processing and cooking on the antinutritional factors of faba bean. *Food chemistry*, *43*(5), 383-385.

Shi, J., Xue, S. J., Ma, Y., Li, D., Kakuda, Y. and Lan, Y. (2009). Kinetic study of saponins B stability in navy beans under different processing conditions. *Journal of food engineering*, *93*(1), 59-65.

Singh, B., Katoch, M., Ram, R. and Aijaz, Z. (2003). A new antiviral agent from Indian Horse Chestnut (*Aesculus indica*). European Patent Specification. *International publication No. WO*, *79795*.

Singleton, V. L. and Rossi, J. A. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American journal of Enology and Viticulture*, *16*(3), 144-158.

Soetan, K. O. and Oyewole, O. E. (2009). The need for adequate processing to reduce the anti-nutritional factors in plants used as human foods and animal feeds: A review. *African Journal of food science*, *3*(9), 223-232.

Thakur, N. S., Kumar, P. and Joshi, V. K. (2015). Improvement of traditional methods for the development of edible flour from Indian horse chestnut (*Aesculus indica*). *International Journal of Food and Fermentation Technology, 5***,**169-176.

Yuliana, M., Truong, C. T., Huynh, L. H., Ho, Q. P. and Ju, Y. H. (2014). Isolation and characterization of protein isolated from defatted cashew nut shell: Influence of pH and NaCl on solubility and functional properties. *LWT-Food Science and Technology*, *55*(2), 621-626.

How to cite this article: Jyoti Rani, N.S. Thakur, Abhimanyu Thakur and Pavneet Kaur (2023). Standardization of Pretreatment for the Development of the Edible Flour from the Indian Horse Chestnut (*Aesculus indica* Colebra.). *Biological Forum – An International Journal, 15*(2): 541-548.