Avrupa Bilim ve Teknoloji Dergisi
Sayı 19, S. 757-762, Ağustos 2020

Araştırma Makalesi

www.ejosat.com ISSN:2148-2683

EuropeanJournal of ScienceandTechnology
No19, pp. 757-762, August2020

ResearchArticle

The Effects of Soaking and Autoclaving on Chemical and Nutritional Composition of the Horse Chestnut (*Aesculus hippocastanum*) Seed

Yusuf Konca[1*], Selma Büyükkılıç Beyzi[1]

[1*]Erciyes Üniversitesi, Ziraat Fakültesi, Zootekni Bölümü, Kayseri, Türkiye (ORCID: 0000-0002-6231-1512)
[1]ErciyesÜniversitesi, Ziraat Fakültesi, Zootekni Bölümü, Kayseri, Türkiye (ORCID: 0000-0002-4622-0645)

(First received 2 June 2020 and in final form 26 August 2020)

(**DOI:** 10.31590/ejosat.747017)

ATIF/REFERENCE: Konca, Y., & Büyükkılıç Beyzi, S. (2020). The Effects of Soaking and Autoclaving on Chemical and Nutritional Composition of the Horse Chestnut (*Aesculus hippocastanum*) Seed. *European Journal of Science and Technology*, (19), 757-762.

Abstract

The aim of this study is to determine the nutrient composition of the horse chestnut (*Aesculus hippocastanum*) seed and to determine the differences of these nutrients with some technological processes. For this purpose, the horse chestnut seed was divided into 3 groups as 1) raw, 2) soaking in cold water for 24 hours and 3) autoclaving at 121 °C for 60 minutes. The obtained groups were then analyzed under two groups as shelled and unshelled. The obtained data were analyzed in general linear model (GLM). As a result, the effect of the treatments applied on horse chestnut seed on dry matter (DM), crude protein (CP), Ether extract (EE), crude ash (CA), cellulose fractions and metabolic energy values were insignificant, while the tannin ratio decreased significantly in the autoclaved group. When horse chestnut seeds are compared with shell and without shell; while the content of DM, CP, EE and CA did not change, the starch content increased, acid detergent fiber, neutral detergent fiber and hemicellulose contents decreased in the shell less horse chestnut seed. The tannin ratio was significantly lower in the shelled horse chestnut. In addition, as a result of mineral analysis of raw horse chestnut, P, K, Ca, Na, Cl, Si, Al, Ni, Cr, Rb, Zn, Fe, Mg and S contents were; 0.257%, 1.106%, 0.27%, 0.146%, 0.061%, 0.055%, 0.023%, 0.016%, 0.005%, 0.003%, 0.006%, 0.08%, 0.056% and 0.117%, respectively. The technological processes applied were effective in reducing the tannins content, or can be used without shell for the purpose of reducing the tannins content.

Keywords: Tannin, Mineral, Shell Ratio, Technological Processes

Atkestanesi (*Aesculus hippocastanum*) TohumundaIslatmaveOtoklavEtmeninBesinMaddeKompozisyonuÜzerineEtkileri

Öz

Bu çalışmada atkestanesi (*Aesculus hippocastanum*) tohumunun besin madde kompozisyonunu belirlemek ve yapılan bazı önişlemlerin besin maddelerine etkisini belirlemek amacıyla yapılmıştır. Bu amaçla çalışma atkestanesi tohumunun 1) ham hali, 2) soğuk suda 24 saat ıslatılması ve 3) 121 °C'de 60 dk otoklav edilmesi şeklinde 3 gruba ayrılmıştır. Elde edilen gruplar daha sonra kabuklu ve kabuksuz olarak iki grup altında analiz edilmiştir. Elde edilen veriler general lineer modelde (GLM) analiz edilmiştir. Sonuç olarak atkestanesi tohumunda uygulanan işlemlerin kuru madde (KM), ham protein (HP), ham yağ (HY), ham kül (HK), selüloz fraksiyonları ve metabolik enerji değerlerine etkisi önemsiz bulunurken, tanen oranı otoklav edilen grupta önemli derecede azalmıştır. Atkestanesi tohumu kabuklu ve kabuksuz olarak karşılaştırıldığında; KM, HP, HY ve HK oranı değişmezken, kabuksuz atkestanesinde nişasta oranı artmış, ADF, NDF ve hemiselüloz oranı azalmıştır. Tanen oranı ise kabuksuz atkestanesinde önemli derecede daha düşük bulunmuştur. Ayrıca ham atkestanesinin mineral madde içerikleri: P, K, Ca, Na, Cl, Si, Al, Ni,Cr, Rb, Zn, Fe, Mg ve S sırasıyla; %0.257, %1.106, %0.27, %0.146, %0.061, %0.055,%0.023, %0.016, %0.005, %0.003, %0.006, %0.08, %0.056 ve

[*]Corresponding Author: Erciyes Üniversitesi, Ziraat Fakültesi, Zootekni Bölümü, Kayseri, Türkiye, ORCID: 0000-0002-6231-1512, sbuyukkilic@erciyes.edu.tr

%0.117 olarak bulunmuştur. Sonuç olarak uygulanan teknolojik işlemlerin tanen içeriğinin azaltılmasında etkili olduğu veya tanen içeriğinin azaltılması amacıyla kabuksuz olarak da kullanılabileceği belirlenmiştir.

AnahtarKelimeler: Tannin, Mineral, Kabuk Oranı, Teknolojik İşlem

1. Introduction

Reducing feed input costs in animal husbandry is a very important issue, and for this purpose, demand and studies for alternative feed sources have been increasing in recent years. Horse chestnut is a seed tree species belonging to the genus *Aesculus* (Hippocastanaceae). The seeds provide abundant resources for wildlife and especially pasture breeding. Horse chestnut (*Aesculus hippocastanum*) is a fast-growing tree species found especially in the temperate regions and Balkans of Asia. The average height of the trees is 22.5 m and it gives a huge amount of seeds every year. The seeds are covered with a capsule and each capsule contains a single seed (Rafiq et al., 2016). The seeds are about 3.5 cm in diameter, with a round, hard brown-black shell on the outside and white cotyledons on it (Parmar and Kaushal, 1982). Flour is obtained from seeds and used in making halva (Rajasekaran& Singh, 2009). In addition to playing an important role in food and non-food applications, it is well known for its medical value (Chakraborthy, 2009; Kaur et al., 2011). Seeds are eaten by wild animals and cattles, however the seeds are lethal, if consumed without processing or raw, due to the presence of anti-nutrients like saponin and tannin (Mishra et al., 2018). So, the seeds must be decorticoid, crushed and soaked with repeatedly changing water, to remove its poisonous substance and then dried under sunlight(Mishra et al., 2018). Fats obtained from seeds are also important components in human and animal diet. However, anti-nutritional factors found in many tree species are also found in horse chestnut. Tree species usually contain saponins as tannins and plant secondary metabolites, which are anti-nutritional factors (Shimada 2001). The fact that tannins are especially water-soluble,and the higher content of tannins is known to inhibit the activity ofthe digestive enzymes such as a-amylase, trypsin, chymotrypsin and lipase. Thus, interfere with the digestion andabsorption of dietary proteins, carbohydrates, minerals andother nutrients, such as vitamin B12 (Vijayakumari et al.2007; Doss et al. 2011). However,if correctly dosed, could possibly increase the digestibility andefficiency of nutrient utilization, because they mightescape ruminal digestion, thereby decreasing ruminallosses, and become available for digestion and absorption in the small intestine (Tabke et al., 2017).Saponins, a steroid or triterpenoid glycoside group, these compounds affect protein and mineral utilization (Francis et al., 2001, Pashwar, 2005). In addition, these glycosides may decrease palatability, digestibility, or metabolism (Pashwar, 2005).

Performing nutrient analysis of a feed source is the first criterion to determine nutritional properties. However, in order to use the feed sources better and safely, it is necessary to undergo some technological or physical processes besides some detailed analyses. Techniques such as heating and/or autoclaving have been proposed as ways of eliminating anti-nutritional factorsand improving the nutritional value of feed ingredients. The aim of this study is: (1) to determine the nutrient components of horse chestnut (*Aesculus hippocastanum*) and (2) to determine the effect of technological processes on the nutrient components.

2. Material and Method

2.1. Seed Material

Horse chestnut seeds, which are fully ripe from the trees in the promenade areas in Kayseri(38°43'32.4"N 35°28'59.4"E), were collected and rotten or diseased seeds were planted. Then, the seeds were washed under drinking water and the dirt on the surface was removed and air dried and used as the basic raw material for the study.

2.2. Technological processes

Seeds are divided into three groups as 1) raw material, 2) soaking in water at room temperature for 24 hours and 3) autoclaving at 121 ° C for 60 minutes. The material obtained by these methods was divided into 2 parts again and some of them were prepared for analysis by separating the shell in its natural state and the remaining part by the shells.

2.3. Determination of nutrient composition

2.3.1. Proximate analysis

Dry matter (DM), crude protein (CP), crude ash (CA), ether extract (EE) and crude fiber (CF) analyzes were performed according to the methods specified in AOAC (1989). Neutral detergent fiber (NDF) and acid detergent fiber (ADF) analyzes were performed according to Van Soest and Robertson (1991) and Goering and Van Soest (1970), respectively. Starch content analyzes was determined according to Ewers polarimetric method (European Economic Community, 1972).

Metabolic energy (ME): The ME calculation from raw nutrients in concentrate feed was calculated using the formula below according to Alderman (1985).

$$\text{ME (Mcal/Kg DM)} = (2816+15.63\ \%CP +15.89\ \%EE^2 – 9.89\ \%EE \times \%CF -28.2\ \%CA)/1000$$

2.3.2. Determination of tannin content

6 ml of tannin solution was added on 0.01 g of feed sample. Boiled in a beaker with water for 1 hour. After boiling, 3 ml of the sample was taken and a spectrophotometer with a wavelength of 550 nm was read. The readings made without feed sample are

defined as blind reading. The results were calculated by replacing them with the formula. Tannin solution (for 100 ml): 5ml HCl, 95 ml N Butanol, 0.05 g Iron Sulphate ($FeSO_4 7H2O$) (Makkar et al., 1995).

$$\text{Tannin (\%)} = \frac{0.584 \times (\text{Sample value} - \text{Blank value})}{\text{Sample weight}} \times 0.1$$

2.3.3. Determination of shell content

In order to determine the shell ratio, seeds weighing enough to constitute 20% of the total sample were randomly selected and 10 seeds selected from them were weighed and recorded in their raw form. The shell of these seeds was removed, and the shell and shell-less seed were re-weighed, and the shell ratio was determined with the help of the formula below.

$$\text{Shell content (\%)} = [(\text{seed weight} \times \text{shell-less seed weight}) / \text{shell weight}] \times 100$$

2.3.4. Determination of mineral content

Approximately 0.5 g of shelled seed sample was taken and 10 ml of nitric + perchloric acid mixture was added on them, and approximately 1 ml of sample was wet burned until thick. After the combustion process, the prepared solutions are diluted with distilled water and read in the ICP OES spectrophotometer (Inductively Couple Plasma spectrophotometer) (Perkin-Elmer, Optima 4300 DV, ICP / OES, Shelton, CT 06484-4794, USA) in the seed P, K, Ca, Na, Cl, Si, Al, Ni, Cr, Rb, Fe, Zn, Mg and S contents were determined (AOAC, 2005).

2.4. Statistical analysis

The data obtained from the technological processes treatments were analyzed in the general linear model (GLM). The lowercases in the table are significant in shelled or shell-less, the uppercases are the significance in the treatment's groups (technological processes). The data were analyzed in SPSS 9.05 package program. Duncan multiple comparison test was used to determine the difference between the treated (technological processes treatments) groups.

3. Results and Discussion

The effects of technological processes to horse chestnut seeds on dry matter, crude protein, ether extract, crude ash, crude fiber, starch contents, metabolic energy, and tannins content of with and without shell are given in Table 1. The effects of the technological processes on DM, CP, EE, CA, CF, ADF, NDF, hemicellulose, starch and ME values in horse chestnut were found insignificant ($P > 0.05$), however, the tannins content decreased in horse chestnut seeds autoclaved ($P < 0.05$). It was observed that the contents of DM, CP, EE and CA were not affected in the shelled and unshelled seeds, but the cellulose fractions were significantly affected. While starch content increased in shelled horse chestnut seed, ADF content decreased ($P < 0.05$) and NDF content did not change ($P > 0.05$). The tannin content was significantly lower in the shell-less horse chestnut ($P < 0.1$). In a study, the contents of DM, CP, EE and CA in horse chestnut were found as 12.71%, 6.78%, 3.27% and 3.16%, respectively (Syed et al., 2016). In another study, DM, CP, EE and CA contents were reported as 10.71%, 6.78%, 3.27% and 3.16%, respectively (Rafiq et al., 2016). Compared to the current study results, it was found higher in dry matter ratio, while protein and ash ratio was lower; ether extract content was found similarly. These differences are thought to be due to the variety or agronomic differences of horse chestnut seed. In a study by Shimada (2001), it was determined that horse chestnut seed contains 0.4% tannins and has higher saponin content and the current study is similar in terms of tannins.In present study, tannin content decreased with autoclaving treatment but, the soaking treatment was not decreased. In a study recommended that for removing its tannin substance in horse chestnut, soaked for overnight in water for 10-12 days with repeatedly changing water, and then dried under sunlight (Mishra et al., 2018). Soaking treatment were effective inreducing tannin content which possibly was contributed dueto the water-soluble nature of tannins (Sharma et al., 2017). Similar results were reported by Gurumoorthi and Uma (2011).High temperature treatments i.e. autoclaving, and extrusion were observed to be most efficient treatments for reduction of tannins (Sharma et al., 2017).Also, Shimelis et al. 2007) have concluded that autoclaving as best treatment for reduction of antinutrients. In food and feed, it has been reported that anti-nutritional factors can be eliminated or reduced by applying various pre-treatments such as soaking, heat treatments and use of chemicals (Kaur et al., 2012).In a study, it was reported that the saponin content decreased between 3-65% (Duhan et al., 2001) by applying the soaking process of legumes and the content of saponin decreased significantly (81-84%) in soaked, peeled and autoclaved beans (Sharma et al., 1992). Shah et al. (2016) exposed to infrared and microwave radiation in order to increase the quality of horse chestnut seed. Using microwave energy as a heating medium is an effective method used in reducing anti-nutritional factors due to its economically superior and shorter processing time (Kaur et al., 2012).

Table 1. The Effect of Technological Processes to Horse Chestnut on Shelled (SL) and Shell-Less (SLS) Horse Chestnut Nutrient Composition

	Technological processes						SEM	P
	Raw		Soaking		Autoclaving			
Item	**SL**	**SLS**	**SL**	**SLS**	**SL**	**SLS**		
DM, %	93.03	93.03	93.04	93.06	93.03	93.01	0.04	NS
CP, %	5.42	5.83	5.47	5.69	5.48	5.78	0.48	NS
EE, %	3.32	3.39	3.31	3.39	3.33	3.39	0.08	NS
CA, %	2.29	2.26	2.22	2.21	2.23	2.26	0.04	NS
CF, %	2.91	0.89	2.91	0.73	2.79	0.7	0.97	NS
HEM, %	6.77^{a}	4.80^{b}	6.31^{a}	4.53^{b}	6.09^{a}	4.87^{b}	0.96	NS
ADF, %	10.44^{a}	2.45^{b}	10.97^{a}	2.60^{b}	11.25^{a}	2.42^{b}	2.84	NS
NDF, %	17.21^{a}	7.25^{b}	17.28^{a}	7.13^{b}	17.34^{a}	7.29^{b}	2.44	NS
Starch, %	24.27^{b}	36.40^{a}	22.06^{b}	33.09^{a}	23.17^{b}	34.20^{a}	3.78	NS
ME, Mcal/kg DM	2.92	3.00	2.92	3.00	2.92	3.00	10.25	NS
Tannin, %	0.43^{a}	0.06^{A}	0.44^{a}	0.04^{A}	0.32^{b}	0.01^{B}	0.78	*

DM: dry matter, CP: crude protein, EE: ether extract, CA: crude ash, HEM: hemicellulose, ADF: acid detergent fiber, NDF: neutral detergent fiber, ME: metabolic energy, P: significance NS: non significance; SL: shelled seed, SLS: shell-less seed, SEM: standard error of means.

The shell ratio and nutrient composition of horse chestnut are given in Table 2. As a result, it was found that while the protein content was lower in the shell, it did not contain starch and had high levels of ADF and NDF. In the current study, the shell ratio was determined as to be 12.4%, while in a study conducted by Syed et al. (2016) was found to be 14.29%. In addition, it can be said that the whole content of tannins in horse chestnut is found in the shell.

Table 2.Nutrient Contents of Horse Chestnut Seed Shell

Item	**%**
Dry matter	89.64
Crude protein	3.78
Ether extract	2.53
Crude ash	2.22
Crude fiber	17.80
Hemicellulose	18.86
ADF	65.66
NDF	84.52
Starch	Not detected
Metabolic Energy, Mcal/kg DM	2.47
Tannin	5.2
Shell ratio of horse chestnut seed	12.4

In present study, the mineral composition of raw horse chestnut seed is given in Table 3. While the highest value was seen in potassium in terms of macro mineral substances, the highest value among micro minerals was observed in iron element. In a study conducted by Mishra et al. (2018), Ca, Mg, Zn and Fe contents of horse chestnut were found to be 0.25, 0.002, 0.003 and 0.032%, respectively. When these values are compared with the current study results, the Ca contents are similar; Mg, Zn and Fe contents were higher in the current study. These differences are thought to be due to the variety or agronomic differences of horse chestnut seed.

Table 3.Raw horse chestnut seed mineral composition

Minerals*	**%**
Phosphorus, P	0.257
Potassium, K	1.106
Calcium, Ca	0.270
Sodium, Na	0.146
Chlorine, Cl	0.061
Silicon, Si	0.055
Aluminium, Al	0.023
Nickel, Ni	0.016
Chrome, Cr	0.005
Rubidium, Rb	0.003
Zinc, Zn	0.006
Iron, Fe	0.080
Magnesium, Mg	0.056
Sulphur, S	0.117

* Raw horse chesnut shelled seed

4. Conclusions and Recommendations

In the study, the nutrient composition of horse chestnut was determined, and it was detected that the technological processes applied were effective in reducing the tannins content, which is an anti-nutritional factor. In addition, it was evaluated that the seeds could be used without shell for the purpose of reducing the tannins content. It has also been concluded that when examined in terms of nutrient content, it can be used as an alternative feed source for animal feeding, but *invitro* and *invivo* studies are also needed to obtain clear results.

References

Alderman, G. (1985). Prediction of the energy value of compound feeds. Recent advances in animal nutrition, 1985, 3-52.

AOAC. (2005). Official Method 922.02, Plants Preparation of Laboratory Sample, in Horwitz, W., Latimer, G.W. (Eds.): Official Methods of Analysis. 18th edn. Chapter 3, AOAC-International Suite 500, 481. North Frederick Avenue, Gaitherburg, Maryland 20877-2417, USA, pp.1-2.

AOAC. (1989). Association of Official Analytical Chemists (A.O.A.C). Official Methods of Analysis, 15th ed., Vol. 1. AOAC, Washington, DC.

Chakraborthy, G. S. (2009). Evaluation of Immunomodulatory action/activity of *Aesculus indica*. International Journal of Pharma Technological Research, 1, 132-134.

Dearing, M. D. (1997). Effects of Acomastylisrossii tannins on a mammalian herbivore, the North Americanpika, Ochotonaprinceps. Oecologia, 109, 122-131.

Duhan, N., Saroj K. & Bishnoi, A. (2001). Saponin content and trypsin inhibitor activity in processed and cooked pigeon pea cultivars. International Journal of Food Sciences and Nutrition, 52(1), 53-59.

European Economic Community. 1972. Determination of starch. Polarimetric method. Official Journal ofEuropean Communities, Brussels, Belgium. p. 6.

Gershenzon, J. & Croteau, R. (1991). Terpenoids. In: Herbivores; Their Interaction with Secondary Plant Metabolites (eds G. A. Rosenthal& M. R. Berenbaum) pp. 165–220. Academic Press Inc., San Diego, California.

Goering, H. K. & Van Soest, P. J. (1970). Forage fiber analyses (apparatus, reagents, procedures, and some applications). USDA Agr Handbook.

Gurumoorthi, P. & Uma, S. (2011). Heat-stable and heat-labile antinutritional profile in Mucunapruriens var utilis: effected bygermination. International Food Research Journal, 18(4), 1421–1426.

Francis, G., Makkar, H. P. & Becker, K. (2001). Antinutritional factors present in plant-derived alternate fish feed ingredients and their effects in fish. Aquaculture, 199(3-4), 197-227.

Kaur, L., Joseph, L. & George, M. (2011). Phytochemical analysis of leaf extract of *Aesculus indica*. International Journal of Pharmacy and Pharmaceutical Sciences, 3, 232-234.

Kaur, S., Sharma, S., Dar, B. N. & Singh, B. (2012). Optimization of process for reduction of antinutritional factors in edible cereal brans. Food Science and Technology International, 18(5), 445-454.

Makkar, H. P. S., Blummel, M. & Becker, K. (1995). Formation of complexes betweenpolyvinyl pyrrolidones or polyethylene glycols and their implication ingas production and true digestibility in vitro techniques. British Journal of Nutrition, 73,897-913.

Mishra, M. L., Sood, S. & 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.

Pashwar, F. (2005). Anti-nutritional factors in oil seeds as aflatoxin in ground nut. Digitalverlag GmbH, 1-8.

Parmar, C. & Kaushal, M. K. (1982).*Aesculus indica*. Wild fruits, Kalyani Publishers, New Delhi, India.

Rafiq, S. I., Singh, S. & Saxena, D. C. (2016). Physical, physicochemical and anti-nutritional properties of Horse Chestnut (*Aesculus indica*) seed. Journal of Food Measurement and Characterization, 10(2), 302-310.

Rajasekaran, A. & Singh, J. (2009). Ethnobotany of Indian horse chestnut (*Aesculus indica*) in Mandi district, Himachal Pradesh. Indian Journal of Traditional Knowledge, 8, 285-286.

Shah, U., Gani, A., Ashwar, B. A., Shah, A., Wani, I. A. & Masoodi, F. A. (2016). Effect of infrared and microwave radiations on properties of Indian Horse Chestnut starch. International Journal of Biological Macro Molecules, 84, 166-173.

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

Sharma, P., Kaur, A. & Kaur, S. (2017). Nutritional quality of flours from guar bean (Cyamopsistetragonoloba) varieties as affected by different processing methods. Journal of Food Science and Technology, 54(7), 1866-1872.

Shimada, T. (2001). Nutrient compositions of acorns and horse chestnuts in relation to seed-hoarding. Ecological Research, 16(4), 803-808.

Shimelis, E., Meaza, M. & Rakshit, S. (2007). Physico-chemical properties, pasting behavior and functional characteristics of flours andstarches from improved bean (Phaseolus vulgaris L.) varietiesgrown in East Africa. Agricultural Engineering International CIGR E-J 8, 5–15.

Syed, I. R., Sukhcharn, S. & Saxena, D. C. (2016). Evaluation of physical and compositional properties of horse-chestnut (*Aesculus indica*) seed. Journal of Food Processing and Technology, 7(3).

Tabke, M. C., Sarturi, J. O., Galyean, M. L., Trojan, S. J., Brooks, J. C., Johnson, B. J. & Thompson, A. J. (2017). Effects of tannic acid on growth performance, carcass characteristics, digestibility, nitrogen volatilization, and meat lipid oxidation of steers fed steam-flaked corn–based finishing diets. Journal of Animal Science, 95(11), 5124-5136.

Van Soest, P. J., Robertson, J. D. & Lewis, B. A. (1991). Methods for dietary fibre, neutral detergent fibre and non-starch polysaccharides in relation to animal nutrition. Journal of Dairy Science, 74, 3583-3597.