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Physicochemical Properties of Black Walnut (*Juglans nigra L.)* in the Western Mid-hills of Nepal

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A R T I C L E I N F O

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A B S T R A C T

The black walnut (*Juglans nigra L.)* is an underutilized fruit in the mid-hills of Nepal. However, the physico-chemical properties of Nepalese black walnut are almost unavailable in the literature. Four walnut growing districts namely Baitadi, Dadeldhura, Jumla and East Rukum selected and the black walnut collected at commercial maturity from the local markets. The physical properties were assessed following the IPGRI descriptors, whilst chemical composition by the proximate method across the location. The energy content estimated from the fat, protein and carbohydrate values obtained from the proximate content. The nut weight, kernel weight, nut length, nut diameter were found significantly affected by site, while the effect was similar for the kernel ratio (19-23%). A strong positive correlation (r^2 = 0.58) was found between moisture and nut diameter, between moisture and nut weight (r^2 = 0.52) and between nut weight and kernel weight (r^2 = 0.64). The moisture content, crude protein and total ash content were unaffected by site, whilst the crude fibre, crude fat and soluble carbohydrate content were differed by location of sample collection. The proximate revealed the very best fat or oil content in Dadeldhura district (53.05%), the highest crude fibre in East Rukum district (7.26%), whilst the carbohydrate was found highest in the Jumla (24.69%). The low carbohydrate content in walnut of Dadeldhura might be a hint for the drought tolerance genotype of black walnut. It's still early to conclude the location effect on black walnut quality without a detailed analysis of environmental and genotypic studies.

Introduction

The walnuts in Nepal are mainly found within the north-west mountainous region at the range of 1000 – 4000 m a.s.l. The walnuts in Nepal are believed to be the descendants of the Iranian descendants and are believed to be extending to the higher elevations in the Karnali province. Likewise, the indigenous hard-shelled walnuts (black walnut) are found around the Mahabharat hills of Nepal and mainly exist in naturally grown forests (Acharya *et al.*, 2009). Two types of walnut exit in Nepal are indigenous hard shell (black walnut) walnut and soft shell (Persian walnut). The black walnut (*Juglans nigra L.*) is a large tree (Rodriguez *et al*., 1989), that contains edible nuts (Woeste *et al*., 2002), and a hard black shell with the stronger flavoured kernel (Parvin *et al*., 2015). The trees are 25-35m tall with a trunk up to 2 m in diameter and lateral crown (Igara *et al.*, 2017). It is an economic plant widely cultivated for the production of nuts ranking third in nut production after cashews nut and almonds (Farsi *et al.*, 2018). In the case of Nepal, it is a minor and underutilized fruit and has a sacred value (Acharya, 2006). The primary use of the walnut tree in Nepal is harvesting of its fruit

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(Kamal *et al.*, 2009), which has significant export potential. The recent epidemiologic study shows the walnut consumption greatly reduces cardiovascular mortality (Anderson *et al.*, 2018).

Black walnuts are a multipurpose species providing valuable timber, high-quality edible nut and is attractive to wildlife (Woeste *et al*., 2002). It has a high nutritional value (Ghanbari *et al*., 2018), as contains a high amount of protein, fat, vitamins and minerals (Savage, 2001; Özcan, 2009). Therefore, it is classified as a strategic species for human regarding its nutritional value and is included in the FAO list of prior plants (Gandev, 2007). It may deserve to be considered as food to alleviate malnutrition, especially in developing countries (Taha and Al-wadan, 2011; Rana *et al*., 2015) because of enrichment with high value of nutrients (Chatrabnous *et al.*, 2018) and its antioxidant properties (Ghasemi *et al*., 2011 and Jahanbani *et al*., 2016). There were limited attempts in Nepal to find out the nutritive value of black walnut. Farmers are still unaware of the nutritive value of both of its nut and oil, therefore, few efforts have been applied in the cultivation of walnut in Nepal. Although the demands of walnut have been increasing day by day in the national and international market, farmers are not encouraged to grow this high value crop in a commercial scale (Bhattarai and Tomar, 2009). The walnuts (*Juglans* spp.) are ecologically and commercially important trees, however, past and current research findings on walnut ecophysiology is lacking, (Gauthier and Jacobs, 2011) especially in terms of the physico-chemical properties. These properties might be different according to location of production (Ehteshamnia *et al*., 2010) as differences in physiochemical properties due to variation in ecological locations has been reported in different plants (Roozban *et al.*, 2005; Shojaeiyan *et al.*, 2018; Etehadpour and Tavassolian, 2019). It is worths to mention that, not only diverge physiochemical properties but also genetic variation have been reported in walnuts that are originated from different ecological regions (Elmeer *et al*., 2019). The present study was conducted with an objective to find the effect of location of production on the physical and chemical properties of black walnut in the western mid- hills of Nepal.

Materials and Methods

Sampling sites

Walnuts were collected from four districts namely East Rukum, Dadeldhura, Baitadi and Jumla of Nepal at the. All the sampling sites were above 2000 m in altitude (see Table 1). From each district, a site at 2000 m and above 2500 m were randomly selected and four samples at each site per district (eight samples per district) were kept for further study (Fig.1.)

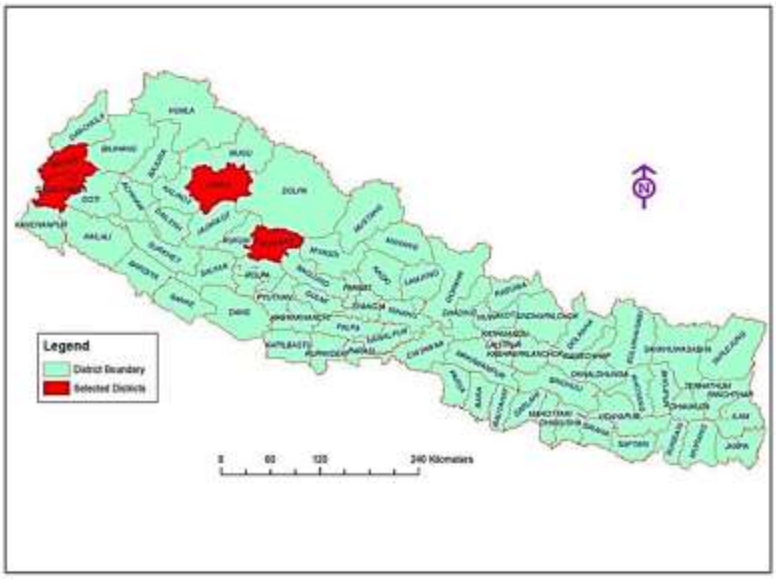

Fig. 1. Map of Nepal, showing the walnut sample districts (namely Baitadi, Dadeldhura, Jumla and Rukum).

Sampling of black walnut fruits

The walnut fruits were collected from the market sites in between 2000m to 2600 m asl. The fruits were harvested at commercial maturity in the farmer's field. While sampling it was for sure that the walnuts available in the local markets were supplied by the local farmers respectively.

Climatic features of the black walnut collection sites

The pattern of rainfall and temperature was variable in the districts studied. The lowest minimum temperature was in Jumla district about -6.2^{o}C. The maximum temperature was highest in Dadeldhura district (about 33 ^{o}C). The details of the climate data are shown in Table 1.

Table 1. Climatic features of the walnut collecting sites.

District	Rainfall (mm)	Temp max (^{o}C)	Temp min (^{o}C)	Temp Avg(^{o}C)	District altitude, (m. asl)	Sampling altitude (m .asl)
Rukum	0-751.8	30.7	3.3	13.6	800-6000	2000-2600
Dadeldhura	0-1343.6	32.7	3.6	18.0	432-2500	2000-2500
Baitadi	12-360.0	26.3	3.4	19.8	390-2950	2000-2500
Jumla	1-210.5	25.8	-6.2	19.5	915-4679	2000-2550

Source: CBS, 2019.

Measurement of the physical properties

Physical properties included nut weight, kernel weight, dry matter, nut length, nut diameter, kernel ratio and shell percentage were analyzed according to IPGRI (1994). The nut weight and kernel weight were measured by digital balance by and the nut length and nut diameter was measured by a Vernier Calliper. The kernel ratio and shell percentage were also measured as given below.

Kernel ratio(%) = kernel wt.(g) / nut wt(g) × 100 eq. 1

The shell% was measured by the following formula.

Shell% = nut weight – kernel weight / nut weight × 100 eq. 2

Measurement of Chemical Properties

The proximate analysis was done in the Animal Nutrition Laboratory of the Agriculture and Forestry University (AFU), Rampur, Chitwan following AOAC (1997) for the following parameters.

Moisture Content

One gram of the sample was weighed into a dried weighted crucible. The sample was put into a moisture extraction oven at 105^{0} c and was heated for 3 hours. The dried sample was put into a desiccator and allowed to cool and reweighed. The process was repeated until constant weight obtained. The difference in weight was calculated as the percentage of moisture content.

Ash Content

One gram of the sample was weighed into a crucible heated in a moisture extraction oven for 3 hours at 100 ^{0}C before being transferred into a muffle furnace at 550 ^{0}C until it turned white and free of carbon. The sample was then removed from the furnace and cooled in a desiccator to room temperature and reweighed immediately. The weight of the residual ash was then calculated as Ash content.

% Ash content = wt. of ash / wt. of original sample × 100 eq. 3

Crude Protein

The micro Kjeldahl method described by AOAC (1997) was used. One gram of the sample was mixed with 10ml of H2SO4 acid in a heating tube. One tablespoon of selenium catalyst was added to the tube and mixture heated inside a fume cupboard. The digest was transferred into distilled water. Ten millilitres (10 ml) portion of the digest was mixed with an equal volume of 45% NaOH solution and poured into Kjeldahl distillation apparatus. The mixture was distilled and the distillate collected into 4% boric acid solution containing 3 drops of methyl red indicator. A total of 50ml distillate was collected and titrated as well. The titration was duplicated and the average value was taken. The nitrogen content was calculated and multiplied with 6.25 to obtain the crude protein.

$\% \text{ Nitrogen} = 100 \times N \times 14 \times Vf \times T / 100 \times Va$ eq. 4

N = Normality of titrate

Vf = Total volume of the digest

T = Titrate value

Va = Aliquot of volume distilled

Fat Content

One gram of the sample was loosely wrapped with a filter paper and put into a thimble which was fitted to a dried weighed clean round bottom flask. The flask contained 120ml of petroleum ether. The sample was heated with a heating mantle and allowed to reflux for 5 hours. The heating was then stopped and spent sample kept and later weighed. The difference in weight was taken as the mass of fat and expressed as percentage fat content of the sample as given:

$\% \text{ Fat or Oil content} = W2-W1 / W3 \times 100$ eq. 5

W1 = Weight of the empty extraction flask

W2 = Weight of the flask and oil extracted

W3 = Weight of the sample

Crude Fiber

One gram of sample and one gram of asbestos was put into 200 ml of 1.25 % of H_2SO^4 and boiled for 30 minutes. The solution and the content were then poured into Buchner funnel equipped with a muslin cloth and secured with an elastic band. This was allowed to filter, the residue was then put into 200ml boiled sodium hydroxide (NaOH) and boiling continued for 30 minutes. It was then transferred to the Buchner funnel and filtered and later washed twice with petroleum ether. The residue obtained was put in a clean dry crucible and dried in the moisture extraction oven to a constant weight. The dried crucible was removed, cooled and weighed. The difference in weight (i.e. loss in ignition) was recorded as crude fibre and expressed as the percentage of crude fibre as given:

$\% \text{ Crude fibre} = W1-W2 / W3 \times 100$ eq. 6

W1 = Weight of sample before incineration

W2 = Weight of sample after incineration

W3 = Weight of the original sample

Soluble carbohydrate content:

The total soluble carbohydrate content was calculated as the weight difference between 100 % and the summation of other proximate parameters as a nitrogen-free extract (NFE) as shown in the following:

$\% \text{ Carbohydrate (NFE)} = 100- (M + P + F1+ A + F2)$ eq. 7

M =moisture,

P = protein,

F1 = Fat,

A = Ash and

F2 = Crude fiber.

Energy content determination:

Energy content (kcal/100 g) was determined by using the following formula as follow;

$\text{Energy} = (\text{Fat} \times 9) + (\text{protein} \times 4) + (\text{NFE} \times 4)$ eq. 8

Data analysis

Data were analyzed by one way ANOVA model using R and the mean difference was set by Duncan's Multiple range Test (DMRT) at p=0.05 level of significance. The following statistical model was used to predict each parameter:

$Y_{ij} = \mu + \sigma_i + \varepsilon_{ij}$ eq. 9

Where Y_{ij}= output of individual observation for parameters.

μ = Overall mean for parameter Y.

ε_{ij} = Random error .

Further, the correlation was analyzed for the moisture content, nut length, nut weight, and nut diameter and kernel weight.

Results

Physical properties

The fresh weight, nut length and nut diameter were found affected by the site (Table 2). The nut weight was found highest in East Rukum (15.49g), followed by Jumla (13.41g). The lowest nut weight was found in Dadeldhura (10.35g) which was at par with Baitadi (10.85g). The highest nut length was found in East Rukum district (39.55mm) and lowest in the Jumla district (32.57mm). The nut length of walnut from Dadeldhura (34.58 mm) was found to be highest which was similar to Baitadi district (35.48 mm). And the highest nut diameter was found in East Rukum (31.14 mm) which was as par with Jumla (31.02 mm) followed by Dadeldhura (29.96mm), whilst the lowest nut diameter was found in Baitadi (25.82mm) respectively. The highest dry matter was found in East Rukum (3.15g) followed by Jumla (2.98g) and Dadeldhura (2.52g). The lowest dry matter was found in Baitadi (2.09g). The highest kernel weight was found in East Rukum (3.21g) followed by Jumla (3.03g) and Dadeldhura (2.57g) and the lowest was found in Baitadi (2.14g).

Table 2. Physical characteristics of indigenous walnut collected from different district of Nepal.

Site	Nut weight (g)	Kernel weight (g)	Dry matter (g)	Nut length (mm)	Nut diameter (mm)	Kernel ratio (%)	Shelling percentage (%)
Rukum	15.49[a]	3.21[a]	3.15[a]	39.55[a]	31.14[a]	20.67	79.33
Dadeldhura	10.35[c]	2.57[ab]	2.52[ab]	34.58[b]	26.96[b]	24.81	75.19
Baitadi	10.85[c]	2.14[b]	2.09[b]	35.48[b]	25.82[b]	19.97	80.03
Jumla	13.41[b]	3.03[a]	2.98[a]	32.57[c]	31.02[a]	22.59	77.41
SEM	0.45	0.14	0.14	0.68	0.66	1.02	1.02
p-value	<0.001	<0.05	<0.05	<0.001	<0.001	0.33	0.27

Note: The different superscripts i.e. a, b, c in the same column indicated significance difference (p<0.05) of the means across the site according to the statistical model.

Chemical properties

The details of the chemical properties of the black walnut collected from four mid-hills of Nepal has been shown in Table 3. The crude fibre, fat or oil content and carbohydrate of walnuts collected from the different district was found to be significant (Table 3). However, the highest fat (or oil content) was found in Dadeldhura district (53.05%) which was as par with East Rukum (50.00%). The lowest fat was found in Jumla (45%), whilst it was the lowest in Dadeldhura (about 53%). The highest crude fibre content was found in East Rukum (7.26%) followed by Dadeldhura (6.95%) which was as par with Jumla (6.83%), whilst the lowest was found in Baitadi (6.56%). The carbohydrate content was found highest in Jumla (24.69%) which was as par with Baitadi (24.16%) followed by East Rukum (20.21%). The lowest carbohydrate was found in Dadeldhura (17.73%).

Table 3. Chemical composition of Nepalese black walnut collected from different district of Nepal.

Site	Moisture content (%)	Crude protein (%)	Crude fibre (%)	Fat or oil content (%)	Ash content (%)	Carbohydrate (%)	Energy (kcal/100g)
Rukum	1.67	18.56	7.28[a]	50.00[ab]	2.28	20.21[bc]	807.57
Dadeldhura	1.82	18.48	6.95[b]	53.05[a]	1.98	17.73[c]	757.84
Baitadi	1.92	18.45	6.58[c]	46.70[bc]	2.20	24.16[ab]	825.40
Jumla	1.67	19.72	6.83[b]	45.00[c]	2.08	24.69[a]	807.60
SEM	0.05	0.21	0.07	0.94	0.07	0.91	10.36
p-value	0.07	0.08	<0.001	<0.01	0.06	<0.05	0.09

Note: The different superscripts i.e. a, b, c in the same column indicated significance difference (p<0.05) of the means across the site according to the statistical model.

Correlation between physical properties

The details of the correlation between some of the physical attributes of the black walnut are shown in Table 4. The moisture content of the kernel has a significant positive correlation with the nut weight (r^2=0.52) and nut diameter (r2=0.58) respectively. Likewise, the nut weight was positively correlated with nut length (r^2=0.54) and nut diameter (r2=0.87) It was also found that the kernel weight was also positively correlated with nut diameter (r^2=0.61) and nut weight (r^2=0.64) respectively (Table 4).

Table 4. Correlation analysis of the physical parameters of the black walnut sampled from the four western mid-hills of Nepal.

	Moisture	Nut length	Nut diameter	Nut weight	Kernel weight
Moisture	1				
Nut length	0.064	1			
Nut diameter	0.58*	0.20	1		
Nut weight	0.52*	0.54*	0.87***	1	
Kernel weight	0.13	0.27	0.61*	0.64***	1

Note: * indicated significance at p<0.05, *** indicated the significance at p<0.001.

Discussion

Physical properties

The differences in the nut weight among four districts might be due to the soil physical and chemical properties. These parameters are lacking to describe the cause and effect relationships of nut parameters and site in the present study. The experiment conducted by Ramos *et al* (1978) had shown that the mild moisture stress in the early nut growing period even some soil moisture available in the root zone reduces the nut weight. The genotype differences are one of the major causes of the difference in kernel weight (Mirzabea, *et al*., 2014; Hassankhah *et al*., 2017). The variation in nut weight might be due to the characteristics of parent shoot because nut weight depends on it (Kelc *et al.*, 2007). The results are in line with the findings of Akca and Sen (1995) who also reported 13.07g as the nut weight of the promising walnut genotype. The results are in conformity with the finding of Özkan and Koyuncu (2005), who also reported nut length range from 37.75mm to 29.72mm and nut diameter range 31.12 to 27.68mm of different walnut genotypes grown in Turkey. The nut weight was positively correlated with kernel weight, nut length, nut diameter which is in agreement with the findings of Kelc *et al.* (2007). The kernel ratio was about 19-23% in the present study which was about 15-50 %.

The slight variation in altitude did not contribute for kernel percentage in the present study, which however was reported significant in the Iranian case (Ehteshamnia *et al*., 2009). The trait differences in physical properties of walnut might be associated to the walnut tree diversity, which further genotypic and the phenotypic nomenclature. However, in the author's current knowledge, this is ever a first report documenting the multisite effect on nuts physical and chemical properties in Nepal.

Chemical properties

The variation in the fat content of walnut from one district to another was found. It may be due to the soil fertility status of the walnut growing land. The fat content of walnut from East Rukum district was high due to the reason of high potassium content in the soil. The fat or oil content of walnut can vary from 52% to 72 % which depends on the location and rate of irrigation (Dogan *et al.*, 2005, Aryapak & Ziarati, 2014). And the maximum oil is accumulated in the late summer (Ramos *et al.*, 1978). So, the variation in the oil content among four districts might be due to the location, inadequate irrigation and any stress during the oil accumulation period. The results are in agreement with the findings of Igara *et al*., 2017 who also reported fibre content was found 6.62% in the cultivars of walnut. The variation in the chemical composition of walnut was found among four districts because it is affected by environmental factors such as climate, geographical origin, methods of cultivation (Gharibzahedi *et al.*, 2011) irrigation regime (Mohebi, 2019), the walnut cultivar (Golzari *et al.*, 2013), the genetic makeup (Karimi *et al*., 2010; Karimi *et al*., 2014; Ghanbari, 2018) and phenotypic diversity (Khadivi-Khub, et.al., 2015). The positive correlation between the nut dimensions and kernel weight was also reported in the earlier studies in Iran (e.g. Ebrahimi, *et al*., 2015; Khadivi-Khub *et al*., 2015).

Besides the beneficial nutritive value, walnut trees have the value to protect the environment (Vahdati *et al.*, 2018); and they are basically good in the deteriorated lands. However, in the present study, the data on bearing habit (Rezaee *et al.*, 2009) is not available, which might have influenced the physico-chemical properties. Likewise, the effect of soil properties and other abiotic stressors are lacking in order to understand the differences in physicochemical parameters of black walnuts sampled from different sites in the present study. Although, the low carbohydrate content in walnut of Dadeldhura in the present study gives a hint for the drought tolerance (Heidari, 2019).

Conclusions

The results of this research showed that walnuts that are growing in four different mid-hills districts of Nepal are characterised with different physical and chemical properties. The black walnut found in Nepal would have a greater genotypic diversity as it is shown by the physio-chemical differences in a similar range of altitude of the sampling sites. For instance, the walnuts found in Rukum were a superb source of crude fibre, highest nut weight, kernel weight, dry matter, and nut length and nut diameter respectively. Likewise, in the drought-tolerant types of walnut, the fat or oil content is higher in Dadheldhura while kernel weight is low. It can be said that the physico-chemical properties of black walnuts vary in different environments, which might be controlled by genetic factors. It is not reasonable to conclude the effect of the location on physical and chemical properties of black walnut in the present study without the detailed information on soil-related parameters and diversity of the walnut (genotype and phenotype) known.

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