EVALUATION OF THE SEEDS OF *LATHYRUS SATIVUS* FOR SEED WEIGHT, ß-ODAP, ANTINUTRITIONAL CHARACTERISTICS AND CONSUMPTION HABIT IN ETHIOPIA

ABSTRACT

Grass pea (*Lathyrus sativus* L.) is one of the most important food legumes in countries like Bangladesh, India and Ethiopia. The legume is nutritious, rich in protein (28-32%) and contains good quantities of essential amino acids. Consumption of *Lathyrus sativus* seeds has been associated for more than 2000 years with neurolathyrim caused by the nearotoxin ß-ODAP (ß-N-Oxalyl-L-α, ß-diaminopropionic acid) present in the seeds. In the present study, *Lathyrus sativus* seed samples collected from the traditional grass pea growing areas in the country were analyzed for characteristics such as crude protein (CP), ß-ODAP, catechin equivalents (CE), trypsin inhibitor activity (TIA), 100 seed weight, seed color and consumption habit of grass pea seeds. The CP content ranged from 272.9 g/kg $^{-1}$ to 319.8 g/kg $^{-1}$ dry matter, the lowest and highest CP being observed in samples collected from Grar Jarso and Akaki, respectively. There were higher variations between samples in ß-ODAP levels. On average, ß-ODAP level in samples from Akaki was about twofold higher than those from Asgido. CE, which detects simple flavonoids as well as condensed tannins, was not more than 7.66 g/kg^{-1} dry matter in any sample. The TIA content did not differ significantly between the samples. The correlation coefficients indicated that, ß-ODAP, CE, TIA and 100 seed weight were positively correlated ($p<0.05$) with CP. In contrast, CE and TIA were negatively correlated ($p<0.05$) with ß-ODAP. The dietary interviews revealed that grass pea has diversified uses in Ethiopia both as human food and feed or crop. The habit of consuming *shiro wot* (the Ethiopian traditional spiced soup-like dish), *kik wot* (sauce made of dehusked split seeds) *kollo* (roasted whole seeds), *nifro* (boiled whole seeds) or *kitta* (unlearned flat bread) prepared from grass pea alone is significantly higher ($p<0.05$) than the habit of consuming those food items prepared from grass pea mixed with other legumes. Further studies are warranted to understand the reasons that influenced food choices of the housewives for establishing respective ways to improve healthy consumption habits.

Key words: consumption habit, *Lathyrus sativus*, ß-ODAP, tannins, trypsin inhibitor activity.

FRENCH

RÉSUMÉ

Le petit pois (Lathyrus sativus L.) est l'une des légumineuses alimentaires les plus importantes dans des pays tels que le Bangladesh, l'Inde et l'Ethiopie. Les légumineuses sont nutritives, riches en protéines (28-32%) et elles contiennent de

bonnes quantités d'acides aminés essentiels. La consommation des graines de Lathyrus sativus a été associée pendant plus de 2000 ans au neurolathyrime causé par le néarotoxine ß-ODAP (ß-N-Oxalyl-L-α, l'acide ß- diaminopropionique) présent dans les graines. Au cours de la présente étude, des échantillons de graines de Lathyrus sativus collectés à partir des régions où pousse le petit pois traditionnel dans le pays ont été analysés pour trouver des caractéristiques telles que les protéines à l'état brut (CP), ß-ODAP, des équivalents de catéchine (CE), l'activité inhibitrice de trypsine (AIT), le poids de la graine 100, la couleur de la graine et les habitudes liées à la consommation des graines de petits pois. Le contenu de CP variait entre 272.9 g/kg $^{-1}$ et 319.8 g/kg $^{-1}$ de matière sèche, le niveau le plus bas et le plus élevé de CP étant observés dans des échantillons collectés à Grar Jarso et à Akaki, respectivement. Il y avait des variations plus élevées entre les échantillons dans les niveaux de ß-ODAP. En moyenne, la teneur en ß-ODAP dans les échantillons d'Akaki était presque le double de celle des échantillons d'Asgido. CE, qui détecte de simples flavonoïdes et des tanins condensés, ne constituait pas plus de 7.66 g/kg^{-1} de matière sèche dans n'importe quel échantillon. La teneur en AIT ne différait pas beaucoup entre les échantillons. Les coefficients des corrélations indiquaient que les poids des graines de ß-ODAP, CE, AIT et 100 avaient des corrélations positives ($p<0.05$) avec CP. Par contre, CE et AIT avaient une corrélation négative ($p<0.05$) avec ß-ODAP. Les interviews sur les régimes alimentaires ont révélé qu'en Ethiopie le petit pois est utilisé de manières diversifiées à la fois en tant qu'aliment humain et en tant que ration de nourriture ou culture. L'habitude de consommer le shiro wot (un plat traditionnel éthiopien épicé qui ressemble à de la soupe), le kik wot (sauce faite de graines cassées et décortiquées), le kollo (des graines entières rôties), le nifro (des graines entières bouillies) ou le kitta (un pain plat désappris), préparés à partir de petit pois uniquement, est beaucoup plus répandue ($p<0.05$) que l'habitude de consommer ces denrées alimentaires préparées à partir du petit pois mélangé avec d'autres légumineuses. Des études plus approfondies s'imposent pour comprendre les raisons qui ont influencé les choix des aliments chez les femmes au foyer pour établir les moyens respectifs d'améliorer des habitudes alimentaires saines.

Mots-clés: habitude alimentaire, Lathyrus sativus, ß-ODAP, tanins, activité inhibitrice de trypsine.

INTRODUCTION

Lathyrus sativus **L**. or grass pea (khesari in India and Bangladesh, guaya in Ethiopia) has been cultivated in South Asia and Ethiopia for over 2500 years and is used as food and feed. The legume is one of the many crops that have its primary genetic diversity in Ethiopia [1]. Grass pea is valued for its high protein content (26-32%), high degree of adaptability under extreme conditions, disease resistance and low input requirement for its cultivation. Despite its tolerance to drought, grass pea is not affected by excessive

rainfall and can be grown on land subjected to flooding, including very poor soils and heavy clays [2].

In Ethiopia, grass pea is the third most important pulse crop after faba bean and chick pea [3]. The legume is planted in the off-season (in September/ October) on residual moisture in the combisol and vertisol soils at altitudes ranging from 1600-2200 meter above sea level, across the different administrative regions of the country [4]. The cultivation of the legume is mainly concentrated in the north west (50.04%), the central (16.3%) and the north east (12.8%) and northern regions of the country. Of these regions, Shoa, Gojam and Gondar are responsible for more than 70% of the total production of the crops (5).

For human consumption, grass pea is utilized as boiled (*nifro*) or roasted (*kolo*) whole seed, *shiro* (powdered seeds). *Shiro* is used in the preparation of *shiro wott*, a traditional Ethiopian sauce. Grass pea flour increasingly is being used to adulterate legume flours such as chickpea or dry peas (6).

In common with those of other grain legumes, grass pea seeds contain a variety of anti-nutritional factors. The presence of trypsin inhibitors, tannins, phytic and β-ODAP has also been established in selected Ethiopian grass pea germ plasm collections (7). Beta-oxalyl-diamino-propionic acid described in short as β-ODAP or as BOAA (beta-oxalyl-amino-alanine) is a neurotoxic secondary metabolite present in the legume *Lathyrus sativus*. The neurotoxic non-protein amino acid causes irreversible spastic paraparesis (paralysis) of the legs known as neurolathyrism, when it is consumed as a major portion of the diet over a three-to-four month period. Neurolathyrism, characterized by nervous disorders such as hyper-irritability, muscular rigidity, weakness and paralysis of the leg muscles and convulsions leaves the patients crippled for life [8].

Neurolathyrism is a widespread problem and occurs in recurrent epidemics in the north, north east and central parts Ethiopia following heavy consumption of grass pea seeds. Although sporadic cases of neurolathyrism also appear in normal years, the disease is highly prevalent in times of food shortages following flooding or famine. The most recently reported epidemic of neurolathyrism occurred in three districts of north east Ethiopia following a drought where more than 2000 people were affected in one sub-district alone [9].

Most studies on the quality of *Lathyrus sativus* in Ethiopia have centered mainly on its agronomic characters, content of β-ODAP in selected germ plasm collections and prevalence of neurolathyrism [5, 7, and 9]. Information on proximate composition and anti-nutritional components of grass pea cultivated by farmers is, however, scanty. The purpose of this investigation was, therefore, to evaluate the proximate composition, mineral contents and anti-nutritional components of local grass pea land-races collected from farmers' fields.

MATERIALS AND METHODS

Sample collection and preparation

Grass pea seed samples were collected from the traditional grass pea producing provinces of the country: east Gojam, north Gonder, central Tigray, north and south Wello, north, east and west Shoa. Two or more districts each from the grass pea producing provinces were randomly selected for the survey. Then, two peasant associations each were randomly selected from the identified districts. A systematic sampling choosing every second farmer was done in each of the selected peasant associations until total fifteen farmers were selected for the study which brings the number of farmers selected to thirty in every district.

Sample preparation

A total of 450 seed samples each weighing one kilogram, were obtained from selected farmers. The seeds were screened by removing stones and defective seeds and were oven-dried at 60±2 ^{0}C for 48 hours to a constant weight. The dried seeds were finely ground in an Udy Cyclone mill with 0.5 mm sieve (Udy Corporation, Fort Collins, CO, USA) immediately before analysis.

Chemical analysis

Grass pea seed samples were analyzed for proximate composition by AOAC (1995) methods [10]. Crude protein was calculated using the factor 6.25. The carbohydrate values were calculated by difference and energy values were calculated using the physiological fuel values of carbohydrates, protein and fat.

Mineral concentrations in ash samples were analyzed using a Varian SpectrAA PLUS (Varian Techtron Pty Ltd, Mulgrave, Victoria, Australia) Atomic Absorption Spectrophotometer equipped with an air-acetylene burner and a metal cathode-hollow lamp. Phosphorus in the ash samples was determined colorimetrically according to the method of Chen *et al*. [11].

For phytate determination the samples were extracted in 0.2M HCl with continuous shaking for 3 hours in a mechanical shaker at room temperature. Phytic acid in the extract was estimated colorimetrically [12].

Concentration of β-ODAP in the seeds was measured spectrophotometrically using the *o*-phtalaldehyde method of Rao as modified by Briggs *et al*. [13, 14]. The modification involved the two times extraction of 0.5 g of the flour with 60% ethanol followed by hydrolysis of the flour with 3M KOH in boiling water bath for 30 minutes. After centrifugation for 15 minutes, an aliquot (250 µl) of the hydrolysate was diluted with 750 µl water and reacted with 2 ml *o*-phthalaldehyde (Sigma, St. Louis, MO, USA) reagent. The mixture was incubated at 35 ^{0}C for 2 hours before measuring the absorbance at 425 nm. The β-ODAP standard curve was calibrated using DAP.HCl (Sigma).

Tannins expressed as catechin equivalent (CE), were determined by the modified Vanillin-HCl procedure [15, 16]. The major modification of the method was the correction for the background absorbance of the samples obtained at 500 nm by subtracting the blanks prepared without vanillin.

Trypsin inhibitor activity (TIA) was assayed according to the method of Kakade *et al*. [17].One trypsin inhibitor unit (TIU) was defined as the amount of inhibitor which caused 10% inhibition of trypsin in 10min under the described assay condition. Trypsin and the substrate benzoyl-DL-arginine-*p*-nitroaniline hydrochloride (BAPNA) (Sigma, Sigma Chemical Company, St. Paul, MO) were used. The results are presented as trypsin inhibitor units (TIU) per gram of sample. Seed coat color was evaluated by visual observation. All samples were analyzed as duplicate and were presented as group mean ± standard deviation (SD) of individual districts. Differences between districts were analyzed by one-way analysis of variance (ANOVA). All statistical analyses were performed using the SPSS, version 10.0 (SPSS Inc., Chicago, IL) statistical package. P-values less than 0.05 were considered significant.

RESULTS

The results of proximate analysis are presented in Table 1. Protein content ranged from 27.29% in Girar Jarso to 31.98% in Akaki. There were no significant differences in protein content by area of cultivation. Ash and crude fiber concentrations ranged from 3.56 and 1.2% in Gubalafto and Abasoqotu to 8.62 and 4.14% in Axum and Bahirdar Zuria, respectively. However, crude fiber and ash content was significantly different by area of cultivation. In contrast, the fat, carbohydrate and energy contents did not vary significantly by area of cultivation.

The content of minerals in seed samples are given in Table 2. Calcium, magnesium, phosphorus and iron in the seed samples were generally higher than the corresponding manganese, copper and zinc levels. Highest concentration of copper was obtained in seed

samples from Wolonkomi. The levels of calcium ranged from a low 82.01±11.79 mg/100 g in Becho to a high 118.97±23.48 mg/100 g in Axum whereas, magnesium and phosphorus values ranged from a low 98±17 mg/100 g and 242±27 mg/100 g in Gerado and Girar Jarso to a high 178 ±89 mg/100 g and 432±93 mg/100 g in Akaki, respectively. Zinc content ranged from 2.74±0.5 mg/100 g in Dembia to 4.52 mg/100 g in Axum, a significant variation. The results also indicated that the contents of minerals significantly varied ($p<0.05$) between areas of cultivation. In the present study, all the grass pea seeds were characterized by a gray-mottled to black pigmentation of the seed coats.

Levels of ß-ODAP in the samples of grass pea seeds collected from the survey areas ranged from 518 in Asgido to 1001 mg/100 g in Akaki on dry seeds weight (Table 3). The ß-ODAP concentration in *Lathyrus sativus* from Akaki was nearly twofold higher than that in Asgido and Girar Jarso. Thus, a large range of variability in ß-ODAP exists in the grass pea seeds obtained from the different survey locations. These differences, however, were significant ($p<0.05$).

Concentrations of tannins in the samples ranged from 558 to 766 mg/100 g. Tannins were highest in samples collected from Dembia (766 mg/100 g) and the lowest in samples collected from Gimbichu. The tannin levels were generally found to vary with the intensity of pigmentation, with darker seed coats generally giving higher levels of tannins.

Trypsin inhibitor activity (TIA) values varied from 15.53 to 18.99TIU/ mg with the highest trypsin inhibitor activity detected in samples from Asgido and the lowest in Habro, respectively. However, the variation in TIA was within narrow ranges as investigated by area of cultivation. Phytic acid content of grass pea seed samples ranged from 547.27 mg/100 g in Asgido to 1008.57 mg/100/ g in Akaki. The phytic acid content of all samples differed significantly ($p<0.05$) by area of cultivation.

DISCUSSION

The analytical results presented clearly indicate the potential of *Lathyrus sativus* in Ethiopia as possible additional sources of protein and carbohydrates. For example, the mean protein content of *Lathyrus sativus* (29.7± 1.5 %) compares favorably with that reported for the more conventional legumes such as *Glycine max* and *Lupinus* species and surpasses those reported for *Pisum*, *Vicia* and *Phaseolus* species [18]. In addition to the high protein content, the good quality of the amino acid profile is well documented [19].

In addition to being important source of protein and calories, grass pea is rich in minerals. The seeds have a higher concentration of magnesium and phosphorus followed by calcium. The ratio of calcium to magnesium ranges from 1:0.53 to 1:1.02 and that of calcium to phosphorus ranges from 1:0.22 to 1: 0.43. Although not much data are

available for contents of minerals in grass pea foods from Ethiopia or other countries, results of the present study indicate that the levels of minerals obtained falls within the usual range for most grain legumes [20].

The major drawback to widespread use of *Lathyrus sativus* as a dietary protein source is the inherent presence of the potent neurotoxin ß-ODAP. The present study indicates a high variability in ß-ODAP levels by area of cultivation. Our results further evidence that the *Lathyrus sativus* seeds are characterized as high neurotoxic varieties in which the level of ß-ODAP is quite variable, between 0.518 to 1.001 mg/100 g seed weight. These values are 2.6 to 5 times higher than the level presumed safe for human consumption [21]. Such variability of the level of ß-ODAP in seeds of *Lathyrus sativus* samples is in agreement with similar previous reports [7**,** 22]. Although the concentrations of ß-ODAP reported in this paper are higher than the results previously reported, it must be emphasized that ß-ODAP levels, even within the same species, may be influenced by environment and agronomic practices.

The major physiological effect of trypsin inhibitor is to cause the enlargement of the pancreas and secretion of excessive amounts of pancreatic enzymes much of which is lost to the animal in feces. The TIA values in the present study are comparable to those previously reported for selected Ethiopian grass pea germ plasm collections and seeds cultivated in Poland [7, 23]. Our findings are, however, in contrast to previous reports that grass pea seeds were characterized by very high trypsin inhibitor activity [24].

The high content of phytate in food samples is of nutritional significance since phytates form complexes with minerals such as iron, zinc, calcium and magnesium can make the mineral content of a food inadequate, especially for children. Of the grass pea samples evaluated in this study, samples collected from Akaki, Habro and Gimbichu, all with 1% or more phytic acid, have the potential to interfere with the availability of minerals.

Tannins have been claimed to affect adversely protein digestibility from plant-based diets (28). In the present study, the concentration of tannins was generally high in all the samples analyzed and similar to values reported for grass pea germ plasm [7]**.** Tannin levels in our study were found to generally vary with the intensity of pigmentation, with the darker seed coats generally giving higher levels of tannins consistent with the work of Deshpande and Campbell [24]. These workers also observed that the color of flower in *Lathyrus sativus* is highly correlated with the seed color, the blue pink or red colored flowers usually produce speckled, colored seeds, whereas the white flowers are associated with white to creamy yellow seeds. The blue flowered varieties are concentrated in south-west Asia and Ethiopia whereas grass pea types with white or cream seeds are rarely found in accessions from Ethiopia or from the Indian sub-continent. Blue flowered varieties are also reported to exhibit tolerance to abiotic factors such as drought, water-logging, heavy soils, high and low pH, poor soil, and biotic factors [25].

CONCLUSION

This study clearly indicates the high potential of *Lathyrus sativus* in the provision of high levels of protein, carbohydrates and minerals for humans. The main limitation is the presence of various anti-nutritional factors, particularly the neurotoxin ß-ODAP which could greatly undermine the potentials of an otherwise very tasty, nutritious, easily cultivated and hardy crop in Ethiopia and other countries. The present study also provided very important data that may be utilized in breeding for low toxin cultivars with better nutritional qualities from the local land-races.

TABLES

Table 1

Proximate composition in grass pea seeds[ab]

Study province	Study district	Protein %	Ash %	Fat %	Crude fiber %	Carbohydrates %	Energy Kcal/100g
South Wollo	Gerado	28.17±0.89	1.32±0.17	1.42±0.08	5.31± 0.12	63.78±1.14	380.59±8.12
	Abasoqotu	29.38±0.37	1.28±0.11	1.47±0.07	4.64 ±0.33	63.23±0.55	381.67±3.48
	Assigido	27.82±0.35	2.33±0.42	1.29±0.05	7.41± 0.18	61.15±0.82	367.49±4.68
North Wollo	Gubalafto	28.07±0.17	1.87±0.51	1.33±0.04	3.56± 0.54	65.17±0.72	384.93±3.56
	Habro	30.23±0.29	3.63±0.95	0.93±0.03	6.23 ±0.37	58.98±1.27	365.31±624
Central Tigrary	Axum	31.03±1.13	3.17±0.82	1.38±0.11	8.62 ±0.93	55.80±2.05	359.74±12.72
North Gonder	Dembia	28.51±1.15	2.91±0.47	1.29±0.01	4.19 ±0.26	55.60±1.63	360.05±11.12
	Gonder Zuria	29.92±0.96	31.7±0.79	0.97±0.08	4.75 ±0.45	61.19±1.83	373.67±11.16
East Gojam	Adet	31.18±1.27	1.82±0.38	0.98±0.03	6.83± 0.95	59.28±1.68	369.81±11.80
	Bahirdar zuria	29.08±0.81	4.14±1.20	0.92±0.01	5.33± 0.55	60.53±2.03	366.72±11.40
North Shoa	Girar Jarso	27.29±0.13	3.87±1.45	1.13±0.03	7.24± 0.39	60.47±1.61	361.21±6.96

West Shoa	Wolonkomi	30.39±1.28	2.33±0.87	1.27±0.04	6.47±0.76	60.79±219	347.47±13.86
	Becho	29.93±0.87	1.95±0.18	1.23±0.05	5.22 ±0.19	61.67±1.10	372.47±7.88
East Shoa	Gimbichu	31.14±0.89	2.87±0.61	1.17±0.02	7.15 ±0..89	57.67±1.52	365.74±9.64
	Akaki	31.98±1.25	3.181.27	1.17±0.02	8.48 ±0.98	55.05±2.57	359.91±15.58

[a]N=30. [b]Values expressed on dry weight basis as means ± standard deviation.

Table 2

Mineral and trace elements contents of grass pea (mg/100g) [ab]

Study province	Study district	Calcium	Phosphorus	Magnesium	Iron	Manganese	Copper	Zinc
South Wollo	Gerado	99.64±15.81	247.61± 63.18	98.21±17.09	5.92±0.56	1.25±0.12	0.88±0.04	3.71±0.35
	Abasoqotu	110.15±856	410.54± 81.79	140.45±25.11	4.69±0.40	1.31±0.27	0.83±0.05	3.63±0.17
	Asgido	100.03±10.12	233 .92±29.51	116.39±42.17	5.88± 1.89	1.29±0.19	0.78±0.07	3.01±0.68
North Wollo	Gubalafto	94.33±13.78	297.38 ±54.61	132.8±16.14	5.25±0.48	1.34±0.05	0.78±0.20	3.11±0.60
	Hubro	100.95±11.46	429.59±94.43	157.12±46.21	6.94±0.99	1.81±0.11	0.93±0.09	3.74±0.74
Central	Axum	116.97±23.	395.79±47.2	168.19±69.	5.88±054	1.78±0.27	0.95±0.08	4.52±0.46

Tigray		48	8	35				
North Gonder	Dembia	87.84±12.84	283.48±23.61	113.55±53.19	6.21±1.59	1.26±0.15	0.85±0.10	2.74±0.50
	Gonder Zuria	84.21±22.66	319.34±77.58	109.47±26.33	5.45±0.27	1.16±0.14	0.89±0.06	2.88±0.28
	Adet	112.36±20.42	394.41± 83.54	128.9±37.28	5.28±0.58	1.25±0.20	0.87±0.18	3.60±0.41
East Gojam	Bahirdar Zuria	87.00±7.12	275.37± 43.72	119.48±45.73	5.37±1.27	1.26±0.08	0.98±0.09	3.93±0.48
North Shoa	Girar Jarso	89.80±13.65	242.26 ±27.17	98.24±29.18	4.64±0.46	1.21±0.11	0.87±0.11	3.01±0.31
West Shoa	Wolonkomi	90.26±11.95	399.59±52.63	139.59±59.23	6.84±1.44	1.55±0.11	1.23±0.30	3.76±0.19
	Becho	82.01±11.79	357.85±63.21	151.62±48.74	8.74±256	1.68±0.13	1.05±0.21	3.43±0.15
	Gimbichu	98.89±1652	379.89± 32.36	162.26±73.53	633. ±1.80	1.53±0.12	0.87±0.09	3.32±0.75
East Shoa	Akaki	94.13±18.97	432.65± 93.33	178.75±89.15	5.03±0.62	1.69±0.18	0.89±0.09	3.62±0.45

[a]N=30. [b]Values expressed on dry weight basis as means ± standard deviation.

Table 3

Anti-nutritional constituents and seed color of grass pea[ab]

Survey province	Survey district	Seed coat color	ß-ODAP mg/100g	TIA TIU/mg	Phytic acid mg/100g	Tannins mg/100g
South Wollo	Gerodo	Gray	674.21±14.08	16.08±0.22	693.19±23.10	578.64±42.35
	Abasoqotu	Black	836.14±11.47	16.88±0.32	883.47±73.42	734.27±61.13
	Assgido	Black	518.29±24.15	15.76±0.23	547.63±17.32	724.48±53.25
North Wollo	Gubba Lafto	Black	638.38±17.11	15.62±0.17	584.51±24.27	719.39±27.41
	Habro	Gray-molted	831.23±14.09	17.81±0.34	1041.37±79.14	452.53±19.72
Central Tigray	Axum Zuria	Black	891.31±39.13	17.71±0.41	994.73±63.36	756.67±37.12
North Gonder	Dembia	Gray-black	636.27±13.13	16.61±0.28	558.42±37.23	866.29±56.64
	Gonder Zuria	Gray	782.34±29.12	17.19±0.37	712.87±54.31	596.73±21.19
East Gojam	Adet	Black	830.41±15.13	18.87±0.54	894.41±67.45	784.62±42.13
	Bahirdar.Zuria	Black	698.29±22.07	16.68±0.25	669.92±45.13	714.23±34.49
North Shoa	Girar Jarso	Black	587.15±16.06	15.53±0.18	635.39±17.27	762.81±51.17
West Shoa	Wolonkomi	Black	737.27±49.17	17.71±0.22	823.35±63.32	742.95±43.49
	Becho	Black	756.38±37.14	16.61±0.16	717.57±37.21	776.78±55.57
East Shoa	Gimbichu	Gray-white	821.45±24.16	18.92±0.49	1098.62±58.15	458.25±19.18
	Akaki	Gray-mottled	1001.49±72.13	18.99±0.61	1008.89±29.42	518.31±21.22

[a]N=30. [b]Values expressed on dry weight basis as means ± standard deviation.

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