ORIGINAL ARTICLE

THE CHEMICAL COMPOSITION, PROTEIN FRACTIONS, MINERAL CONTENTS AND NUTRITIONAL QUALITY OF SOME AMARANTH GRAINS COLLECTED FROM SOUTH WESTERN ETHIOPIA

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ABSTRACT

BACKGROUND: *The prevalence of malnutrition among children in Ethiopia is very high. To reduce the prevalence of malnutrition, one of the possible remedies could be to search for under utilized food sources with high protein and energy contents. The aim of this study was to investigate the nutrient composition of amaranth grains cultivated in southwestern Ethiopia.*

METHODS: *Five different samples of amaranth grains were collected randomly from open markets in Goldiya, Maji and Yeki, southwest Ethiopia in Jan 1997 and evaluated for their proximate composition, minerals contents, lysine content and protein fractions, available carbohydrates and crude fat characteristics using standard methods.*

RESULTS: *The protein content ranged from 13 to15.1 g%, crude fibre 4.3 to10 g %, and lysine from 4.6 to 6.1 g/16g N and was significantly different from each other ($p< 0.05$). The crude fat content ranged from 7 to 8% and ash 3.1 to 4.0%. Calcium content ranged from 121 to198, iron 13 to 22, zinc 2.7 - 5.5, phosphorous 487 to 673, sodium 9 to 19 and potassium 478 to 581 mg% showing a significant variation ($p<0.05$). Glutelin is the most abundant while prolamin the least of protein fractions in all the samples ($p< 0.05$) with albumin to glutelin ratio varied from 0.81 to 1.14.*

CONCLUSION: *The present results indicate that amaranth seeds are superior in nutritional qualities and may offer unique opportunities to complement other cereals and root crops and alleviate the high prevalence of protein energy malnutrition in Ethiopia.*

KEY WORDS: *Amaranth grain, protein quality, protein fractions, calcium, mineral, malnutrition.*

INTRODUCTION

Malnutrition remains one of the public health problems among preschool children worldwide. About 200 million children under five years of age are undernourished (1). Malnutrition leads to health problems including stunted growth, weakened

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resist to infection and impaired intellectual development (2). Inadequate diet and infectious diseases are the major causes of malnutrition contributing to the high prevalence of child morbidity and mortality in developing countries (3-5). It has been estimated that about 12 million children under five years of age die annually due to infectious disease and malnutrition of which malnutrition alone contributes to more than half of the causes (6). Ethiopia is no exception: nutritional deficiencies and infectious diseases are the leading health problems in the country (7). This is thought o be due primarily to inadequate intake of protein and energy as well as impaired bioavailability of micronutrients, which is exacerbated by increased need due to vulnerability to infections. One of the possible olutions to the problem would be to diversify protein sources utilizing less exploited but igh quality food sources that are locally vailable.

Amaranths are broad-leafed non-grass lants that produce significant amounts of dible cereal-like grains. Amaranth (family *maranthaceae)* is an under exploited plant with exceptionally nutritive value (8). Many pecies of the genus Amaranths are best nown as opportunistic weeds which have a ong history of use as leafy vegetables and re gathered as subsistence food in many arts of the world (9). Only three species, *A. ruanthus* (L.), *A. hypochondriacus* (L.) and *caudatus* (L.) are the main cultivated ecies that produce large seed heads loaded ith edible seeds that are consumed by mans as a grain (10-11).

Amaranths have been reported to be ique in their ability to grow vigorously, sist drought, heat, pests and their ability to apt to environments that are inhospitable conventional cereal crops (12). The seeds hough barely bigger than a tef seed (0.9 – mm in diameter) occur in massive mbers to a plant and are pale-white, golden, pink, red or dark-brown colored. The protein contents of the amaranth grains are reported to be high with their amino acids well balanced and are close to those of animal origin products (13, 14).

Although grain amaranths are native to the Americas, currently many countries of Asia and Africa grow amaranths as grain and vegetable crops. For example, a dark seeded strain of *A. cruentus* (L.) is commonly cultivated as a vegetable in west Africa (15). In Kenya, milled amaranths flour is added to maize-based gruel (9). In Ethiopia, little is known about the grain amaranth. The cultivation and usage has generally been limited to western and southwestern part of the country by the Neolithic tribes (16). In these localities, the grain amaranth is used to produce porridge (local traditional diet) and a fermented thin porridge or gruel known as *borde*.

Although the distribution of amaranth species used as cereal grains and their nutrient composition has been reported for several countries, no such investigation have bas been carried out in Ethiopia. The purpose of the present investigation was therefore to study the nutrient composition of amaranth grains used as food in southwestern part of Ethiopia.

MATERIALS AND METHODS

Sample collection

A total of 5 different amaranth grain samples grown in Bench-Maji zone and Gambella were collected randomly from the local markets in Jan 1997 and the study extended for five years. Three pale-white seeded, one black and one red seeded were obtained for the study. The samples were packed in plastic bags of which the opening was sealed using a lighted candle, labeled and transported to the Ethiopian Health and Nutrition Research Institute (EHNRI). They were planted in June 1997 in plots ($3m^2$/

plot), assigning two replicates per sample collected. All samples were planted in one locality in garden setting at EHNRI. The plants were neither irrigated nor fertilized. The seeds were harvested in October 1997 when they were fully matured. Threshing was done by hand and then wind-winnowed. Seeds were cleaned to remove the remaining chaff and dirt and stored at 4^0C in sealed container until analysis.

Analytical Methods

The amaranth grain samples were ground to flour using a Cyclotec mill (Tecator AB, Haganas, Sweden) to pass a 0.5 mm mesh screen. Moisture, protein, ether extract (fat), and crude fibre, were determined according to The Association of Official Analytical Chemists, AOAC (17); total carbohydrates were calculated by difference. The nitrogen determined by Kjeldahl method was converted to crude protein by multiplying by a factor of 6.25. Lysine content was determined by the method of Sadasivan and Maniciman (18). Samples of (ca 2 g) of the flour was ashed at 550^0 C and dissolved in 6 N HCl. Minerals concentration, (calcium, iron, zinc and copper) in samples digested were determined using an atomic absorption spectrophotometer (Varian Spectra A 10/20 Plus, Varian Australia Pty., Ltd., Mulgrave, Vic. 3171, Australia) following the method of Osborne and Vooget (19). For the determination of calcium, lanthanum chloride (1% w/v) was added to both standard and samples to suppress interferences from phosphorous. The sodium and potassium in the sample digest was determined using flame photometery while total phosphorous was analysed colorimetrically by the method of Fiske and Subboarrow (20). Iodine value, acid value and saponification value of the ether extracts were estimated according to America Oil Chemists Society (Official and Tentative Methods), AOCS (21).

Solubility of the proteins fractions were estimated according to Osborne and Mandle as described by Cagampang *et al.* (22). Weighed quantity of amaranth flour was subjected to successive solvent extraction (1:30 w/v) with distilled water, 10% NaCl, 80% ethanol and 0.2% NaOH to obtain albumin, globulin prolamin and glutelin fractions, respectively. True protein was estimated as follows: non-protein nitrogen (NPN) was quantified as the nitrogen in the supernatant recovered after having precipitated the protein solution by means of trichloroacetic acid (24% w/w) followed by filtration. True protein was the difference between crude nitrogen by Kjeldahl and NPN, times 6.25.

Total soluble sugars were extracted with hot 80% aqueous ethanol. After evaporating the contents in vacuo, the residue was dissolved in water and made up to a known volume. Total soluble sugars were estimated by the phenol-sulphuric acid method (23). Starch and available carbohydrates were estimated colorimetrically using anthrone reagent as described by McReady et al. (24). Reducing sugars were estimated by the method of Nelson (25).

Data analysis:

The data were statistically analysed using standard methods as described by Snedecor and Cochran (26). Descriptive data are expressed as means (SD). Significance was set at p less than 0.05. One-way analysis of variance (ANOVA) was used and differences between means identified by the student t-test. All analysis were done with SPSS version 8.00

RESULTS

The protein content ranged from 15 to 18% with an average of 17% (Table 1). The average ether extract (fat) content was 7.5% with values ranging from 7 to 8% while those for crude fibre varied significantly ($p < 0.05$) from 4.9 to 11.5% with an average of 7%. Amaranth grain of pale - white color from Gldiya exhibited the highest protein (18%) and fat (8%) contents ($p<0.05$) than all other samples studied.

The seed proteins were fractionated as albumin, globulin, prolamin and glutelin based on Osborne and Mandle classification (Table 2). Glutelin, being 35% was the major protein fraction followed by albumin (29%), and globulin (28%) and prolamin (9.4%) among the samples analyzed. The albumin to glutelin ratio varied from 0.81 to 1.14. Values expressed as percentage for albumin content ranged from 22.8 to 31.5%. The pale-white seeded amaranth from Goldiya had the lowest albumin content (22.8%). The amount of prolamin extracted varied from 8.1 to 11% with pale-white seeded amaranth from Maji and Goldiya exhibiting the highest value.

The efficiency of extraction varied from 76 to 88% in the five samples.

Total nitrogen in amaranth seeds varied between 2.4 and 2.9% whereas non-protein nitrogen (NPN) as presented in the samples varied between 0.18 and 0.31% (Table 3). The proportion of non-protein nitrogen was low in all the samples studied but the pale-white seeds from Maji contained a significantly ($p<0.05$) higher NPN. On the other hand, when expressed as percentage of nitrogen, NPN of the amaranth seeds varied between 6.7and 10 % and showed a lower but appreciable positive correlation with the percentage of the total nitrogen.

All the amaranth samples studied contained a high amount of lysine, ranging from 4.6 to 6.8 g/16g N (Table 3). The pale-white seeded amaranth grain from Maji contained higher lysine (6.8 ± 0.5g/16g N) compared with red (5.2± 0.2g/16g N) and black (4.6± 0.3g/16g N) seeded amaranth grains collected from Maji and Goldiya respectively.

Calcium content ranged between 121 and 198 mg/100g with a significant ($p<0.05$) variation among the samples studied (Table 4). Similar variations in iron (13 to 22 mg/100g), zinc (2.7 to 5.3 mg/100g), phosphorous (487 to 673 mg/100g), sodium (9 to 39mg/100g) and potassium (478 to 581 mg/100g) contents were also observed. The black seeded amaranth grains contained the highest amount of calcium and sodium whereas the pale- white seeded grains from Yeki contained the least amount of calcium, zinc and potassium.

Table 1. Proximate composition of amaranth grains (g/100 g, dry basis)

Origin	Color	Food energy (kcal)	Protein (g)	Fat (g)	Total carbohydrates (including fibre, %)	Crude fibre (g)	Ash (g)
Maji	Red	393.5	17.3 ± 0.3	7.1 ± 0.2†	71.9 ± 2.6	6.5 ± 0.8	4.0 ± 0.5
Goldiya	Black	378.3	16.6 ±0.3	7.5 ± 0.5	70.4 ± 3.2	11.5 ± 1.2†	3.3 ± 0.2
Maji	Pale- white	402.4	18.2 ± 1.5†	8.0 ± 0.5†	65.2 ± 2.6	6.3 ± 0.6	3.1 ± 0.4
Goldiya	Pale -white	407.6	15.0 ± 0.3	8.0 ± 0.5†	72.9 ± 2.8	4.9 ± 0.3†	3.2 ± 0.2
Yeki	Pale-white	398.7	17.6 ± 0.4	7.1 ± 0.4†	71.8 ± 2.9	6.0 ± 0.5	3.2 ± 0.2

*Data are expressed as mean ± SD of six replicates; Significant difference between the samples, $^{\dagger}p<0.05$

Table 2. Protein fractions of amaranths grain (g/100g, dry basis)

Origin	Colour	Protein fraction				Total	Crude protein content	Extraction efficiency (%)
		Albumin	Globulin	Prolamin	Glutelin			
Maji	Red	4.0 ±0.4 (29.6)	3.8 ± 0.5 (28.1)	1.1 ± 0.1 (8.1)	4.6 ± 0.6 (34.1)	13.5 ± 0.4	17.3±0.3	78.5
Goldiya	Black	4.6± 0.5† (31.5)	4.0 ±0.4 (27.4)	1.3 ± 0.1 (8.9)	4.7 ± 0.3 (32.2)	14.6 ± 0.3	16.6±0.3	88.0
Maji	Pale-white	3.8± 0.3† (27.3)	3.7 ± 0.6 (26.6)	1.5 ± 0.2 (10.8)	4.9 ± 0.8† (35.3)	13.9 ± 0.5	18.2±1.5	76.4
Goldiya	Pale-white	2.9 ±0.2† (22.8)	3.6 ± 0.2 (28.3)	1.4 ± 0.1 (11.0)	4.8 ± 0.5† (37.8)	12.7 ± 0.3	15.0±0.3	84.7
Yeki	Pale-white	4.1± 0.4† (29.1)	3.9 ± 0.2 (27.7)	1.2 ± 0.1 (8.5)	4.9 ± 0.5† (34.8)	14.1 ± 0.3	17.6±0.4	80.1

*Data are expressed as mean ± SD of six replicates. Figure in parenthesis are percentage; Significant difference between the samples, $^{\dagger}p<0.05$

Table 3. Non-protein nitrogen (NPN), true protein and lysine content of amaranths grain (g/100g, dry basis)

Origin	Color	N (%)	NPN (%)	True protein (%)	Lysine	
					g (%)	g /16 g N
Maji	Red	2.77 ± 0.05	0.23 ± 0.01	16.7± 0.7	0.90 ± 0.03	5.5 ± 0.2
Goldiya	Black	2.66 ± 0.05	0.22 ± 0.02	15.2 ± 0.5	0.76 ± 0.05	4.6 ± 0.3†
Maji	Pale-white	2.91 ± 0.12	0.31 ± 0.01†	15.0 ± 0.6	1.11 ± 0.09	6.8 ± 0.5†
Goldiya	Pale-white	2.41 ± 0.05	0.18 ± 0.01†	14.0 ± 0.3	0.83 ± 0.11	5.1 ± 0.5
Yeki	Pale-white	2.82 ± 0.06	0.19 ± 0.02	16.0 ± 0.4	0.97 ± 0.04	6.0 ± 0.7

*Data are expressed as mean ± SD of six replicates; N = Nitrogen; NPN = non-nitrogen protein; Significant difference between the samples, $^{\dagger}p<0.05$

Table 4. Minerals and trace elements content of amaranths grain (mg/100g, dry basis)

Origin	Colour	P	Ca	Fe	Zn	Cu	Na	K
Maji	Red	540 ± 7	164 ± 10	22 ± 4†	5.3 ± 0.5†	4.3 ± 0.2	11 ± 2	581 ± 9†
Goldiya	Black	487± 9†	198 ± 10†	21 ± 5	3.9 ± 0.5	4.0 ± 0.5	19 ± 3†	570 ± 10
Maji	Pale-white	548± 8	154 ± 8	19 ± 2	4.4 ± 0.3	3.9 ± 0.2	9 ± 2†	552 ± 8
Goldiya	Pale-white	530 ±10	154 ± 10	13 ± 3†	3.5 ± 0.2	4.0 ± 0.3	12 ± 1	568 ± 10
Yeki	Pale-white	673± 12†	121 ± 6†	20 ± 6	2.7 ± 0.4†	4.0 ± 0.2	11 ± 1	478 ± 8†

*Data are expressed as mean ± SD of six replicates; Significant difference between the samples, $^{\dagger}p<0.05$

The total carbohydrate content of amaranth grains investigated varied between 65 and 71% (Table 5). Available carbohydrates and starch content varied between 54 to 68% with starch is the most abundant and major carbohydrate constituent of amaranth grains. The total soluble sugars content was small and varied from 2.9 to 5.4% among the samples. High amounts of total and available carbohydrates, starch and soluble sugars were observed in pale -white amaranth grains obtained from Goldya.

High acid value (1.26 ± 0.07 mg KOH/100g) was noted for pale-white seeded grain from Goldiya and the least (0.73 ±0.04 mg KOH/100g) from the seed samples obtained from Yeki (Table 6). The iodine index of total acids, varied between 118 and 149 mg/100g samples with the pale-white grain sample obtained from Goldya being the highest value.

.**Table 5**. Carbohydrates components of amaranths grain (g/100g, dry basis)

Origin	Color	Carbohydrates		Starch	Sugars	
		Total (including fibre, %)	Available (%)		Soluble (mg/100g)	Reducing (mg/100g)
Maji	Red	71.9 ± 2.6	66.2 ± 2.6	62.8 ± 2.3	4.4 ± 0.7	12.4 ± 0.7
Goldiya	Black	70.4 ± 3.2	59.3 ± 3.1	56.5 ± 2.4	2.9 ± 0.7	14.4 ± 1.3
Maji	Pale-white	65.2 ± 2.6	59.3 ± 1.5	54.2 ± 1.5	4.8 ± 0.8	16.7 ± 1.0
Goldiya	Pale-white	72.9 ± 2.8	67.7 ± 3.0	61.7 ± 2.7	5.2 ± 1.0	21.4 ± 2.4
Yeki	Pale-white	71.8 ± 2.9	65.8 ± 3.4	60.5 ± 2.8	5.4 ± 0.8	15.4 ± 1.6

*Data are expressed as mean ± SD of six replicates; significant difference between the samples $^{+}p<0.05$

Table 6. Characteristics of fat isolated from amaranths grain (dry basis)

Origin	Color	Acid value (mg KOH/g)	Saponification value (mg KOH/g)	Iodine value (g I/100 g)
Maji	Red	1.00 ±0.06	209.4 ± 4.1†	118.7 ± 2.6†
Goldiya	Black	0.88 ± 0.03	178.8 ± 3.5†	143.9 ± 3.2
Maji	Pale-white	0.88± 0.05	193.4 ± 2.9	148.2 ± 4.0
Goldiya	Pale- white	1.26 ±0.07	194.7 ± 4.4	149.0 ± 4.6†
Yeki	Pale-white	0.73 ± 0.04	183.9 ± 2.9	133.3 ± 2.5

*Data are expressed as mean ± SD of six replicates; Significant difference between the samples, $^{\dagger}p<0.05$

DISCUSSIONS

Amaranth is one of the rare plants whose leaves are eaten as vegetable while the seeds are used as cereals (11). Amaranth grains are not only good sources of protein but are also rich in lysine, which is a limiting amino acid in most cereal grains. Results of the present study indicated that the protein content of amaranth grains are higher than the conventional varieties of wheat, maize, rice and other popular grains (27). However, the protein content of the amaranth seed samples investigated in this study was in close agreement with values reported by Becker, *et al* (28). Thus amaranth grain may provide a significant amount of protein to the daily diet and serves as a good potential protein source. It is also interesting to note that the crude fibre content (7g/100) of all amaranth grains studied is on average twice that of in other conventional cereals like wheat (3.3g/100g), maize (3.1g/100g) and sorghum (2.8g/100g). A salient fact is that the pale-white seeded amaranth from Goldya had significantly (p<0.05) lower and higher crude fat contents than samples from Maji red and Yeki pale-white. This variability observed may be attribute to both genetic and environmental factors among the samples studied.

The NPN content of amaranth grain flour also increased with nitrogen content. The large NPN of amaranth seeds may nfluence the estimated protein by 6 – 12% and consequently leading to an erroneous estimation of protein intake from diet prepared from this grain. It is evident from hese results that all nitrogen present in the maranth seeds are not associated with seed protein suggesting that the NPN has to be aken into account if total protein content is o be measured accurately.

The nutritional quality of amaranth as food is determined by the amino acid make up of its protein. Lysine is the most miting essential amino acid in most major ereal proteins (29). Compared with other ereals, amaranth seed grains contained ouble the lysine content of wheat and arley, triple that of maize and sorghum nd equal to the amount in milk. The esent study also confirmed that amaranth ains contained high level of lysine, which akes it a good source of protein quality. These characteristics indicate that amaranth could be a good supplement for maize and other cereal grains. In effect, the lysine content of the different amaranth samples as a percent of protein was found to be associated with its protein content. A high positive correlation between lysine and protein contents was observed. This finding was in agreement with the results previously reported (10,30).

Protein fractionation studies on the five samples of amaranth grain protein revealed that increased protein content in different samples is associated with an increase mainly in glutelin fraction of the grain (Table 2). The proportions of albumin and globulin were almost equal, while that of albumin and glutelin varied significantly with a ratio of 0.81 to 1.14. A significant (p<0.05) but higher proportion of glutelin than the other protein fractions was observed further indicating that glutelin is the major storage protein extracted. In selected amaranth species a value of 20.7% albumin, 19.2% globulin, 2.2% prolamin and 44.4% glutelin have been reported whereas albumin and globulin fractions in sorghum seed flour fractions constituted 8-14% and 31-40%, respectively (31, 32).

In the diets of the developing countries, cereals, roots and tubers serve as the major source of calorie, which are obtained in the form of carbohydrates. As observed, the principal source of calories in amaranth grains was carbohydrate, which contained 70 to 77% of the total calories. Other carbohydrates are simple sugars present as glucose, sucrose and fructose in amounts that vary from 0.1 - 3 % and their contribution to available carbohydrates is low. The accumulations of simple sugars were not obtained in significant amount suggesting that most soluble carbohydrates were not hydrolysed.

Mineral and trace elements are important dietary nutrients. Calcium, zinc

and iron are usually deficient in the diets of low-income people, particularly the vulnerable group of the society, the infants, pre-school children and pregnant and lactating women. The content of minerals observed in our study are consistent with values reported by other similar studies but generally higher than that observed in conventional grains (33). Becker *et al* have reported that the calcium and iron contents of amaranth grains were more than five – fold higher than that of wheat (28). In the present study, the pale-white seeded grains contained about three times as much iron, 1.5 times as much copper and the same quantity of zinc that in wheat (33). The high iron content of amaranth is beneficial especially to those who suffer from a certain degree of anaemia. The high phosphorous and calcium content of amaranth grains may jeopardise the bioavaiability of iron and zinc.

Amaranth's high protein and fat content make it a significant resource in human feeding (8). The values for fat content of amaranth grain samples obtained in the present study are much higher than the content in conventional cereals but are similar to those published by other authors (11, 28). Fat isolated from all five amaranth grains samples is characterised by its high acid value which is an indication of large free fatty acid content and high iodine value, which is an index of unsaturation. Studied elsewhere indicated that the fatty acid composition of amaranth oil is similar to that of wheat germ, oat and rice bran oil in that it contains around 77% unsaturated fatty acids and is rich in linoleic acid (28, 34).

In conclusion, amaranth grains are rich in protein, lysine and high in fat contents. Because of such characteristics, utilization of grain amaranth as inexpensive substitute for animal protein foods would contribute to the high prevalence of protein-energy malnutrition in Ethiopia. Incorporation of amaranth grain flour into home-prepared weaning diets for child feeding would significantly improve the nutritional quality of such diets. In addition, grain amaranth can be used to complement other cereals and root crops as a supplement for added nutritional value in traditional foods in Ethiopia.

ACKNOWLEDGEMENTS

The study was financially supported by the Ethiopian Health and Nutrition Research Institute. The technical assistance of Ato Mengistu Gebre-Tsadik is gratefully acknowledged.

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