Evaluation of Methods for Roots Processing on Removal of Anti-nutritional Factors of Selected Cultivars of Cassava (*Manihot esculenta C.*) Grown in Ethiopia.

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Abstract

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Locally grown cassava cultivars were subjected to study the effect of boiling, sun-drying and fermentation processing methods on removal of anti-nutritional factors. The root product flours were analyzed for anti-nutritional factors using standard methods. The data generated were statistically analyzed using statistical package for social scientists (SPSS). Out of the three processing techniques, fermentation of grated cassava roots for 72 hours sufficiently reduced HCN content to safe level (<10 ppm, WHO) of human consumption. The unprocessed cassava root of Gamo cultivar (48.00 ppm) belonged to the category of sweet or non-toxic. Whereas, cultivars of Hayik (78.07 ppm), 28 (83.70 ppm), 44/72-NW (129.20 ppm) and Koree (159.00 ppm) were found to be moderately toxic. Cultivars of 192 (211.17 ppm) and 5538-19 (247.20 ppm) belonged to highly bitter (highly toxic) cultivar too. Reduction in phytate and tannin levels were highest for sun-dried followed by fermented and boiled flours. However, reduction in oxalate contents were highest for fermented followed by boiled and sun-dried flours. The study reveals that the effect of processing methods found to be significant (P<0.05) on removing anti-nutritional factors.

Key words: Cassava, cyanide, phytate, processing methods

INTRODUCTION

Ethiopia with its diverse agro-ecologies and suitable environments, allows the growth of numerous root and tuber crops many parts of the country's smallholder farmers. Cassava (*Manihot esculenta C.*) plant is exotically was introduced to Ethiopia at the middle of nineteenth century (Desalegn, 2007). Cassava is known in Ethiopia in different names such as "Muka Furno (Oromifa)", "Yenchet Boye (Welayitigna)", and "Tesike/Mogo (Koreegna)". Cassava is an essential part of the diet for more than half a billion people in the world, important carbohydrate supply and source of income for farmers in several African countries (Nweke, 2004). However, cassava in

Ethiopia is considered as less important, and was planted at the backyards and farm borders as fences. Moreover, consumption and processing of cassava in the country is in a primitive stage as compared to many African countries.

It is known that cassava-based diets have been associated with two neurological disorders: tropical ataxic neuropathy (TAN) and Konzo. Both occur to people whose diets are largely restricted to high-cyanide cassava. When raw cassava or inadequately processed cassava was consumed different symptoms of health problem happened to the consumer (Banea-Mayambu, et al., 1990). Apart from HCN cassava contains anti-nutrients like tannin, oxalate and phytate which inhibit the absorption of minerals to the body (Paredes-Lopeze, et al., 2006).

For Ethiopians, the consumption of cassava as food is of immense importance and regarded as the food security crop for millions of people. Therefore, this calls for methods for enhancing the nutrient content at the same time reducing the anti-nutrients without adversely affecting the acceptability of the crop. Cassava-based traditional food products could become even more important in feeding additional segments of the increasing Ethiopian population in the future. This study investigates the effect of common processing methods on nutritional quality of cassava roots cultivars grown in Ethiopia.

MATERIALS AND METHODS

Source of materials and study areas

The study cassava samples were collected from Agricultural Research Centre: Western (Jimma), Northern (Hayik) and Southern (Amaro) parts of Ethiopia. The seven cultivars of cassava roots were 12 to 14 months old, and with accession number/local name of 28, 192, 5538-19, 44/72-NW; Gamo (red skin) and Koree (white skin); and Hayik (red skin), taken from Western, Southern and Northern parts of the country, respectively. The cultivars were selected on the bases of most released varieties and highly grown areas of the country. The cassava roots were manually harvested, packed into a sack, and transported within one day to the laboratory of the Centre for Food Science and Nutrition (CFSN), Addis Ababa University (AAU). These studies were carried out in two laboratories. Sample preparation by processing techniques were performed in the CFSN laboratory, AAU. While, all chemical analyses were done in the Ethiopian Public Health Institute (EPHI) former EHNRI laboratory, AA, Ethiopia.

Sample preparation by three common processing methods

Sun-drying

Undamaged and uniformly matured raw fresh cassava tubers were taken and then washed with potable water to remove dirt. The tubers were peeled and manually cut in to pieces on chopping board with a stainless steel knife. The pieces were sun-dried to less than 12 % moisture content (db) using the procedure of Gomez, et al. (1984). The dried chips were ground into flour by electrical grinder (Mouliex, A2424A, France) and then the flour was sieved by 40 mesh size (450 µm) stainless steel sieve (W.S. Tyler Co., Member, Ohio, USA) packed into polyethylene bags and stored in a cool and dry environment away from sunlight until analysis.

Boiling

Using methods described by Cooke and Maduagwu (1978), the raw fresh cassava roots were peeled, sized and placed into stainless steel pan and boiled for about 45 minutes. The cooked cassava roots were crushed and then sun-dried to less than 12 % moisture content. Furthermore, the dried cassava roots were ground into flour using electrical grinder (Mouliex, A2424A, France), sieved by 40 mesh size (450 µm) stainless steel sieve (W.S. Tyler Co., Member, Ohio, USA), packed in polyethylene bags and stored in a similar manner like sun-dried flour until analysis.

Fermentation

Cassava fermentation was followed the Nigerian traditional cassava roots processing methods (FAO, 1998) with slight modification. Six Kg of each of the tuber roots from the seven cultivars were sorted, peeled, washed with tap water, cut into smaller pieces and grated by electrical grinder. The grated pulps were put into a 1000 mL measuring cylinder and the cylinder was covered with aluminium foil and allowed to ferment naturally (spontaneously) at ambient temperature for 72 hours. After 72 hours of fermentation, the paste was spread over the tray and sun-dried to less than 12 % moisture content (db). The sun-dried paste was milled by electrical grinder (Mouliex, A2424A, France), sieved by 40 mesh size (450 µm) stainless steel sieve (W.S. Tyler Co., Member, Ohio, USA), packed and stored in similar manner like other samples.

Chemicals and Reagents

All chemicals and reagents used in laboratory analyses were of analytical grade or A.C.S. reagents.

Anti-nutritional Factors Determination

Total Cyanide Content

The total cyanide content of the raw cassava roots sample was analyzed using hydrolysis according to the procedure of Bradbury, et al. (1991) and the flour sample was analyzed by Picrate kit (Bradbury, et al., 1999). The absorbance of the solution was measured at 510 nm and the total cyanide content in ppm was calculated by multiplying with 396.

Phytate Content

Phytate content of the unprocessed and processed cassava flour samples were determined according to the procedure of Latta and Eskin (1980) as phytic acid.

Tannin Content

The tannin content of the unprocessed and processed flour of cassava root was determined according to Maxson and Rooney (1972).

Oxalate Content

The oxalate content was determined using the method of Ukpabi and Ejidoh (1989) which involves digestion, oxalate precipitation and permanganate titration.

Statistical Analysis

Analysis of the different parameters was computed using statistical package for social scientists (SPSS, ver. 16). Data were analyzed using two-way analysis of variance (ANOVA) based on the standard method for factorial experiment as described by Steele and Torrie (1980). Differences between means was found using Duncan's Multiple Range Test ($P < 0.05$).

RESULTS AND DISCUSSION

Effect of common processing methods on anti-nutritional factors

Total cyanide content

The result Table 1 shows the total cyanide values for the marginal means were 136.62, 39.91, 17.44 and 4.93 ppm, for the raw, boiled, sun-dried and fermented flours, respectively. The highest and lowest level of the cell means total cyanide obtained for boiled 5538-19 cultivar (90.33 ± 0.25 ppm) and fermented Gamo cultivar (1.09 ± 0.07 ppm), respectively. The total cyanide levels of commonly processed cassava roots flour was significantly varied ($P<0.05$) to each other. The Interaction of cultivars and processing methods, cultivars and processing effects are found to be significant ($P<0.05$). The pattern of total cyanide reduction was observed in the fermentation, followed by sun-dried and boiled. Of the three processing methods, fermentation significantly reduced the cyanide content ($P<0.05$) having the value below the recommended safe level value (10ppm) HCN, dwb (WHO 1991). This limit has been questioned because it was established with HCN as gas through inhalation instead of ingestion. Ramalho, et al. (2007) reported an experiment in which it is used linamarin extracted from cassava and given orally to mice. In such experiment the lethal dose (LD_{50}) was 324.86 ppm linamarin for body weight, a value three times higher than that recommend by WHO. The present study result of all fermented cassava roots flour is not associated with acute toxicity. Furthermore, it was observed to be below the lethal dose for humans' intake by mouth (0.5-3.5 mg/kg body weight for a 60 kg adult) which amount to 30-210 mg HCN (Montgomery, 1980; Solomonson, 1981). While in other processing methods the total cyanide cell means levels are above 10 ppm except for the two cultivars 192 (sun drying=8.27 ppm) and Gamo (sun-drying=4.60 ppm; boiling=8.23 ppm) which are less than recommended value (10ppm) of WHO. This might be due to the soil condition and genotypic difference. The cyanide level of the seven cultivars of sun-dried results (4.60 - 26.84 ppm) are in between for the 5 genotypes oven dried results observed value (8.33 - 28.8 ppm) reported by Charles, et al. (2005).

The total cyanide content of unprocessed cassava roots of the seven cultivars was ranged from 48.00 to 247.20 ppm. The unprocessed cassava roots of Gamo cultivar (48.00 ± 1.10 ppm) which belong to category of sweet, which is defined as having low cyanide content value (<50 ppm), and thus is non-toxic. The cassava roots having cyanide content of 50 to 200 ppm belongs to the bitter (moderately toxic) categories such as cultivars of Hayik (78.07 ± 0.16 ppm), 28 (83.70 ± 0.40 ppm), 44/72-NW (129.20 ± 0.18 ppm) and Koree (159.00 ± 1.07 ppm). Cultivars of 192 (211.17 ± 1.04 ppm) and 5538-19 (247.20 ± 0.40 ppm) belong to highly bitter (highly toxic) cultivar, according to the cassava toxicity category (Bourdoux, 1982). The seven unprocessed cultivars result show high variability of HCN content in cassava roots, this might be due to genotypic difference and others. The maximum and minimum percentage reduction was observed to be for fermented cultivars of Gamo and 5553-19 (98 %), and boiled cultivar Hayik (51 %), respectively (Figure1).

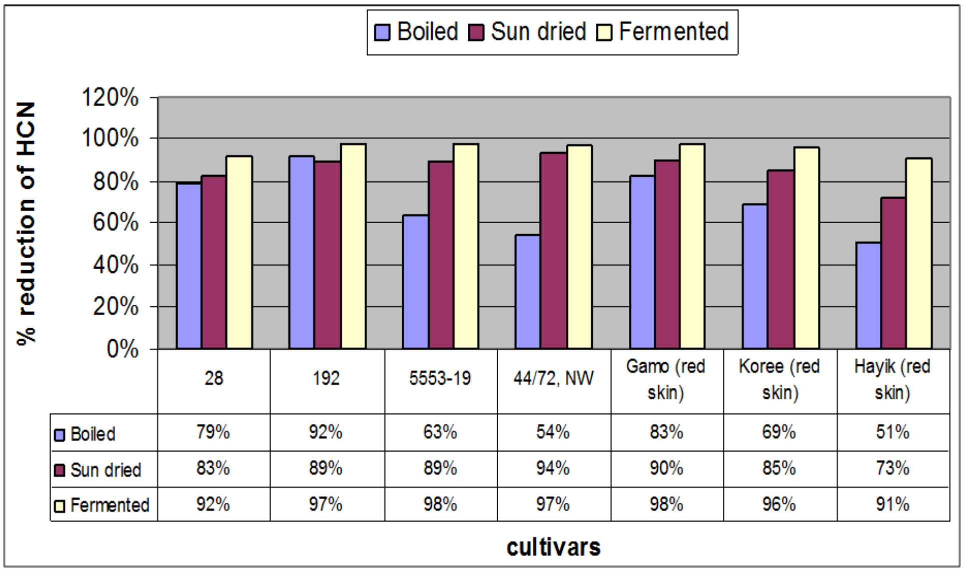

Figure 1. Effect of processing techniques on percentage reduction of total cyanide in cassava root flours.

Phytate, tannin and oxalate levels

Phytate The phytate, tannin and oxalate levels obtained from seven cassava roots cultivars and common processing methods are indicated in Table 2. The interaction effect due to processing methods and cultivars were found to be significant (P<0.05). Similarly, the cultivars and processing effects were significant (P<0.05). The marginal means of phytate levels of processed cassava roots flour was found to be in the values of 655.15, 403.80 and 425.80 mg/100 g, for the boiled, sun-dried and fermented flours, respectively. The marginal means of processing methods over levels of cultivars are significantly (P<0.05) different with the mean for processed sun-dried (403.80 mg/100 g) being the lowest followed fermented and boiled. The mean phytate level was highest for boiled cultivar of Gamo (910.66 mg/100 g) followed by Koree (818.70 mg/100 g) and the lowest belongs to fermented cultivar 44/72-NW (108.57 mg/100 g). The marginal means of cultivars over levels of processing methods are significantly (P<0.05) different with the cultivar Hayik (269 mg/100 g) being the lowest. The cell mean phytate content of processed cassava flour was significantly different (P<0.05) from one another except for the phytate content of boiled (44/72-NW) and sun-dried (Hayik). The complexing of phytate with nutritionally essential elements and the possibility of interference with protolytic digestion have been suggested as responsible for anti-nutritional activity. One of the factors is the presence of phytate, which is negatively charged phosphate compound that binds minerals and inhibits absorption (Howarth, et al., 2001). The variability of phytate content in cassava roots is not only due to cultivars factors but also it might be due to the total phosphorous content, found in soil and fertilizers, which can influence the phytic acid concentration (Maga, 1980). The phytate contents of seven unprocessed cassava roots varieties presented in this investigation are found to be higher than the phytate content values (253 - 624 mg/100 g) of cassava roots reported by Oke (1990); Edeogu and Ekuma (2007). Among the processing methods, sun-drying and fermentation are appeared to be effective to reduce the phytate levels, when compared to the boiling. The decrease in the phytate content of the fermented cassava flour for all the seven cultivars could possibly be attributed to the secretion of the enzyme phytase. This enzyme is capable of hydrolyzing phytate, thereby decreasing the phytate content of the cassava flour (Nwokoro, et al., 2005)**.** The high content of phytate of nutritional significance is lowering the availability of many other essential dietary minerals. Thus, reduction of phytate is expected to enhance the bioavailability of dietary minerals of the cassava (Siddhuraju and Becker, 2001).

Table 1. Total cyanide levels in seven cassava cultivars roots of raw and processed in to three different methods (in ppm).

[1]Results are mean values of three replicates ± SD, *****- Interactions significant at P < 0.05, [1] means with the same letters within a

Tres[1, 2]	Cultivars*										
	28	192	5538-19	44/72-NW	Gamo (red skin)	Koree (white skin)	Hayik (red skin)	Marginal means	P of cultivar	P of Proc. method	P of cultivar x Proc. method
Raw	**83.7 ± 0.40d**	**129.2 ± 0.18d**	**247.2 ± 0.4d**	**211 ± 0.104d**	**48 ± 1.10d**	**159 ± 1.07d**	**78.07 ± 0.16d**	**136.61**	**	**	**
Boiled	**17.58 ± 0.56c**	**16.26 ± 0.06c**	**90.33 ± 0.25c**	**59.17 ± 0.20c**	**8.23 ± 0.18c**	**49.77 ± 0.75c**	**38 ± 0.12c**	**39.90**			
Sun-dried	**14.20 ± 0.74b**	**8.27 ± 0.41b**	**26.78 ± 0.82b**	**23.34 ± 0.33b**	**4.60 ± 0.41b**	**23.45 ± 0.65b**	**21.45 ± 0.58b**	**17.44**			
Fermented	**6.57 ± 0.30a**	**3.39 ± 0.18a**	**5.19 ± 0.15a**	**5.37 ± 0.49a**	**1.09 ± 0.07a**	**6.20 ± 0.11a**	**6.70 ± 0.43a**	**4.93**			
Marginal means	**30.51**	**50.01**	**92.37**	**64.03**	**15.48**	**59.60**	**36.05**				

column are not significantly different with a<b<c<d (P>0.05), [2] tres - treatments/processing methods, * - accession number/name of cultivars,******- Interactions significant at P<0.05.

Tannin The results shown in Table 2 signify that the tannin level of processed cassava roots cultivars of boiled, fermented and all of the sun-dried flours was obtained below the detectable limit. However, the marginal means of tannin level of boiled three cultivars (28, 192 and 5538-19) and fermented two cultivars (192 and 5538-19) of cassava roots flour were found to be 4.37 and 4.16 mg/100 g, respectively. In those cultivars, tannins were not completely removed during fermentation and boiling, which might be due to the genotypic difference, environmental or combination effects. The mean tannin level of unprocessed cassava root flours was found to be 66.89 mg/100 g. The observed tannin content of unprocessed cassava root is different from that reported (Sarkiyayi and Agar, 2010), whose values are 0.40 and 0.6 mg/100 g for sweet and bitter cassava, respectively. The tannin content of the study is lower than that of rice value (513 - 572 mg/100 g) reported by Saikia (1999). The presence of tannins can cause browning problems in both fresh food and processed products, and they act as anti-nutritional factor by provoking an astringent reaction in the mouth there by making the food unpalatable, they also form complex with proteins, precipitate proteins in the gut, inhibits digestive enzymes and microorganisms (Oboh and Elusiyan, 2007). This has nutritional implication for both human and livestock in that there is damage to the intestinal tract through absorption of tannic acid toxicity in the gut, also interference with the absorption of iron and a possible carcinogenic effect (Onimawo and Akubor, 2005). **Oxalate** The result Table 2 shows the marginal means of raw and processed cassava roots flour oxalate levels were found to be in the values of 27.16, 17.75, 18.13 and 10.28 mg/100 g for the raw, boiled, sun-dried and fermented flours, respectively. The effects of cultivars and processing methods were found to be significant ($P<0.05$). Similarly, the interaction of cultivars and processing methods was significant ($P<0.05$). The three common processing techniques are found to be effective in reducing the oxalate levels of seven cassava root cultivars. The ingestion of oxalic acid cause series health problem due to formation of calcium oxalate which is insoluble at physiological pH and can be deposited in the brain and kidney tubules. The lethal dose for oxalates in adults is estimated to be 143 - 428 mg/kg (Libert and Franceschi, 1987). The highest marginal means oxalate levels reduction are found to be for fermented followed by boiled and sun-dried flours. The possible reason to the observed high reduction in oxalate level is due to both fermentation and boiling cause considerable cell rupture and facilitate the leakage of soluble oxalate into fermenting and cooking water (Albihn and Savage, 2001). These average oxalate level variation observed is much higher than the range reported in previous study value (1.35 - 2.88 mg/100 g) for leaves (Correa, 2000; Wobeto, et al., 2007). The processed cultivars flour has shown significant ($P<0.05$) variability in cell means oxalate levels except for the cultivar Hayik (13.04 ± 0.06 mg/100 g). Whereas, the highest and lowest cell means oxalate levels found were 30.63 ± 0.21 and 19.81 ± 0.26 mg/100 g flour in Koree and 192 unprocessed cassava cultivars, respectively.

Table 2. Raw and processed cassava root cultivars flour phytate, tannin and oxalate levels in mg/100 g.

Pars[1]	Tres[2]	Cultivars*										
		28	192	5538 -19	44/72-NW	Gamo (red skin)	Koree (white skin)	Hayik (red skin)	Marginal means	P o C	Po Pm	P o C x Pms
Phytate	Raw	724.98 ± 23.80d	741.84 ± 44.10d	871.53 ± 23.15d	622.24 ± 11.10c	1087.38 ± 21.68d	.868.33 ± ;.25d	711.34 ± 0.69c	803.95	**	**	**
	Boiled	547.74 ± 26.47c	506.80 ± 28.47a	.154.55 ± :1.35a	526.13 ± 28.75b	910.66 ± 0.40c	818.7 ± 1.56c	121.47 ± 0.54a	655.15			
	Sun-dried	137.76 ± 0.02a	569.82 ± 7.00b	225.88 ± 35.14b	535.21 ± 9.60c	713.32 ± 0.57b	521.70 ± 0.51b	122.91 ± 0.21b	403.80			
	Fermented	518.38 ±103.56b	720.41 ± 0.00c	745.57 ± 14.01c	108.57 ± 2.10a	611.79 ± 0.21a	153.78 ± 0.60a	122.13 ± 0.97b	425.80			
Marginal means		482.22	634.72	749.38	448.04	830.79	590.63	269.46				
Tannin	Raw	73.54 ± 0.00b	74.18 ± 4.40c	87.15 ± 2.31c	62.22 ± 1.10	47.74 ± 1.09	57.83 ± 0.11	65.58 ± 0.41	66.89	**	**	**
	Boiled	14.59 ± 0.47a	3.88 ± 0.97a	12.12 ± 0.37a	Nd[C]	Nd[C]	Nd[C]	Nd[C]	4.37			
	Sun-dried	Nd[C]	Nd[C]	Nd[C]	Nd[C]	Nd[C]	Nd[C]	Nd[C]	-			
	Fermented	Nd[C]	4.82 ± 1.60b	24.32 ± 4.03b	Nd[C]	Nd[C]	Nd[C]	Nd[C]	4.16			
Marginal means		22.03	20.83	30.81	15.56	11.93	14.46	16.39				
Oxalate	Raw	27.84 ± 0.50d	19.81 ± 0.26d	26.70 ± 0.29d	29.99 ± 0.24d	25.11 ± 0.28d	30.63 ± 0.21d	30.05 ± 0.34b	27.16	**	**	**
	Boiled	13.61 ± 0.22b	10.63 ± 0.21b	21.20 ± 0.21c	24.34 ± 0.00b	12.49 ± 0.40a	29.00 ± 0.01c	13.04 ± 0.06a	17.75			
	Sun-	16.31 ±	17.99 ±	16.03 ±	27.29 ±	18.15 ±	18.01 ±	13.07 ±	18.13			

dried	0.24c	0.24c	0.25b	0.26c	0.16c	0.05a	0.08a				
Fermented	7.14 ± 0.51a	4.14 ± 0.53a	3.35 ± 0.02a	3.18 ± 0.00a	15.04 ± 0.08b	26.00 ± 0.12b	13.10 ± 0.11a	10.28			
Margina means	16.22	13.09	16.62	21.20	17.70	27.91	17.31				

[1]Results were mean values of triplicate determination (dwb) ± SD, PoC - P of cultivar, PoPm - P of processing methods, PoC x Pms – P of cultivar x processing methods, [1]means with the same letters within a column of respective group of parameters are not significantly different with a<b<c<d (P>0.05),
[2] Trets-treatments, [1]Pars-parameters, *- accession number of cultivars, Nd[C] -below detectable limit (Nd taken as=0 value), ****-** Interactions significant (P < 0.05).

CONCLUSIONS AND RECOMMENDATION

All processing methods enhanced the availability of nutrients in cassava by decreasing the anti-nutritional factors including cyanide, phytate, tannin and oxalate. Among the anti-nutritional factors analyzed, the low content of tannins in the three processing techniques of the seven cassava root cultivars is one good advantage for consumers of cassava in terms of inhibitory effect of tannin on nutrient availability. Out of the three common processing methods, fermentation of the cassava roots flour sufficiently reduced toxic cyanide (HCN) content to a safe level of human consumption (<10 ppm, FAO) compared to other methods. Organized public information regarding removal of anti-nutritional factors by processing methods should be promoted to consumer.

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