

NUTRIENTS AND ANTINUTRIENTS COMPOSITION OF RAW, COOKED AND SUN- DRIED SWEET POTATO LEAVES

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ABSTRACT

Traditional indigenous vegetables are the most economically efficient source of micronutrients in terms of both land required and production costs per unit. Promotion of production and consumption of such micronutrient-rich foods will improve intakes, the overall diet, and health status. This study aimed to determine nutrient (iron, calcium, vitamin A and ascorbic acid) and anti-nutrient (oxalates and polyphenols) contents in raw, cooked and dried sweet potato leaves. Two varieties of sweet potatoes, which were identified as commonly grown for leaves consumption were analyzed at Department of Food Technology, Sokoine University of Agriculture and at the Government Chief Chemist Laboratory Tanzania. The analysis included proximate, nutrient (ascorbic acid, carotenoids, iron and calcium) and anti-nutrient (oxalate and polyphenols) composition. The purple midrib sweet potato leaves were further analyzed for nutrient and anti-nutrient retention after cooking (with and without lemon) and open sun-drying (with and without salting). There was no significant difference ($P>0.05$) between the two varieties in crude protein, crude lipid and moisture content. The purple midrib sweet potato leaves had significantly ($P<0.05$) higher ash, crude fibre, carotenoids, calcium and iron contents while the green midrib sweet potato leaves had significantly ($P<0.05$) higher ascorbic acid content. The polyphenols were about 4 times higher in the purple midrib sweet potato leaves (22.16%) as compared to that of the green ones (5.28%), which had significantly higher oxalate levels (3730 mg/100g). Drying with salt and cooking with lemon reduced polyphenols significantly ($p<0.05$), with retention of 42% and 56% respectively; while cooking with lemon lowered significantly the oxalate levels. The traditional methods of cooking SPL with addition of lemon is advantageous because it reduces polyphenols while retaining higher levels of minerals, β carotene and vitamin C. Drying with salt results in a nutritionally and organoleptically good product, hence, drying with salt and cooking with addition of lemon is encouraged. Since the sweet potato leaves are harvested more than once before the plant is uprooted, further studies are recommended to assess whether there is variation in nutrient and anti-nutrient contents in consecutive harvests.

Key words: Sweet potato leaves, nutrients, antinutrients

INTRODUCTION

Sweet potato (*Ipomoea batatas* L) is grown as a food crop in most parts of the world, mainly for root production. Consumption of leaves as a vegetable is only practiced in some parts of Africa [1, 2, 3]. In Tanzania, sweet potato leaves (SPL) are among common traditional food crops. Although the leaves are popular only in some regions, in Tanga, they are commonly grown in the backyard garden or along the river valleys mainly for the local market and household consumption. Most of the consumed and marketed leaves are young tops from specific varieties grown only for leaves.

Though nutritious, SPL have been considered a poor man's salad [2,4]. Sweet potato leaves are more available for a longer period of the year because the plant is less sensitive to drought, tolerant to heavy rains, needs low inputs, and grows in a wide range of ecological zones with a short time to cook. They are the most economically efficient source of micronutrients [5]. Sweet potato leaves are known to contain high potassium-sodium ratio and can, therefore, be used in low salt diets. They are also known to increase the body's capacity to eliminate sodium salts and increasing the amount of urine produced, hence suitable for individuals suffering from high blood pressure [4]. A study in China reported that consumption of SPL modulates various immune functions [6]. Promotion of production and consumption of such micronutrient-rich foods cannot only improve intakes but also the overall diet and health status. Some communities in Tanga region are practising limited preservation of SPL by direct sun-drying. Nutrient loss and antinutrient removal depends on methods of processing and storage.

Although SPL are known to contain appreciable amounts of minerals, protein and vitamins [1, 3,7], the values reflect an average of more than one variety and the data available are mainly for the raw leaves. Cooking and preservation methods for leafy vegetables in East-African countries are variable. Depending on the type of vegetable, they can be boiled and the water discarded before addition of onions, oil or groundnuts. They can be chopped, then stewed directly or mixed with other foods [8]. The cooking practices may result in high losses of both nutrient and antinutrient contents.

Most studies assess nutrient composition of traditional vegetables and only a few considered the antinutritional contents. Nutrient and antinutrient contents in foods are known to vary according to the variety, cooking, and preservation techniques [4]. There is a need to determine composition of the specific varieties, which are grown for leaves to step up promotion of SPL use in the country. This study was done to determine the nutrient and antinutrient levels of raw, cooked and dried sweet potato leaves (*matembele*) with purple (PMSPL) and green (GMSPL) midrib.

MATERIALS AND METHODS

Survey

A cross-sectional survey was done in Ngamiani and Chumbageni divisions in Tanga region, where 243 randomly selected households were interviewed on the common vegetables eaten in the household, preparation methods, cooking and preservation techniques. Additionally, focus group discussion (FGD) was held to get an insight of people's perception of different vegetables eaten.

Production of sweet potato leaves

The leaves used in this study were grown at Nguvumali, (the area known to be one of the main suppliers of SPL to the city market) towards the end of the short rains in December, 2007. The stems of both the PMSPL and the GMSPL, used as mother plants, were bought from the city market. All leaves were removed. The remaining stems were randomly planted on the same plot. Cow dung manure was spread evenly before planting. The vegetables were watered once a day in the evening. Harvesting was done 45 days after planting by cutting about 30 cm off the stem, which had an average of five good soft leaves. Although SPL can be harvested more than once, the analysed samples were from the first harvest

Preparation and processing of samples

GMSPL and PMSPL were subjected to laboratory analyses. The PMSPL was used for preparation of cooked and preserved samples. Cooking was done with and without addition of lemon juice and preservation was done by direct sun-drying with and without salt. All methods were commonly practiced in the area. The leaves were harvested from the same plot that was planted at the same time.

Cooking of the leaves

Purple midrib sweet potato leaves were sorted and prepared for cooking. During sorting, the thin outer skin and part of the petiole were removed. Then, 150 grams of whole fresh-sorted leaves with a part of the petiole were washed using tap water and the excess water drained. About 15 grams of finely chopped onion were put in an aluminium cooking pot containing 15 ml of sunflower cooking oil and cooked on Tanelec electric plate (made in CARMATEC Arusha, Tanzania) at medium heat. The onions were left to cook until they turned brown/yellow. About 150 g of the washed leaves and 1 medium-sized tomato cut into small pieces were put on top of cooking vegetables and covered. The vegetables were mixed every minute and a small amount of normal iodized salt was added. The pot was covered and mixing repeated two times and then about 20 ml of lemon juice was added. The pot was covered and cooking continued for one more minute. Another lot was cooked in the same way but no lemon juice was added. Total cooking time was five minutes, counted immediately after putting the vegetables in the pan.

Drying of the leaves

The methods used for drying the vegetables were the commonly used (as documented during the survey) for preservation of SPL. Purple midrib sweet potato leaves were sorted and 500g were spread on trays and exposed to sunlight for about six hours in a day (10.00 a.m. – 4.00 p.m.) for two days. The other lot of 500g was rubbed with 10mg of salt and left to dry in the sun for two days. The samples were packed in labelled polyethylene bags and analysed within a day to avoid storage changes of sensitive nutrients.

Chemical analyses

Raw and dried SPL were transported (about 8 hours) in closed black polythene bags to Government Chief Chemist Laboratory, Dar-es-salaam for oxalate and total carotenoid analysis and others to Sokoine University of Agriculture (SUA), Morogoro, for proximate composition, nutrient and antinutrient analyses (iron, calcium, polyphenols, and vitamin C). The cooking was done in the laboratory and the cooked samples were immediately analysed.

Proximate analysis

For proximate composition, AOAC methods were applied [9]. Determination of moisture content involved weighing five grams of a sample (on Mettler P162 weighing balance) and drying in an oven at 105°C to constant weight. The dried samples were cooled in a desiccator and weighed. The moisture content was determined and the dry matter was calculated as the difference from 100. Crude fibre was determined using dilute acid and dilute alkali hydrolysis. Crude protein was determined using micro-kjeldahl method, whereby the percentage nitrogen was calculated on a dry matter basis. The amount of nitrogen was then multiplied by a nitrogen-protein conversion factor of 6.25 to get the crude protein content of the samples. Total lipids were determined by Soxhlet continuous extraction method while total ash was determined by ashing in a muffle furnace at 550°C SPL obtained after drying the vegetables in porcelain ashing dishes, which had previously been dried in an air oven at 98°C - 100°C and cooled. The soluble carbohydrates were determined by subtracting the sum percentages of ash, protein, fibre, dry matter and lipid from 100%.

Analyses for minerals and vitamins

Carotenoids were determined as carotene equivalent using acetone-hexane solvent mixture (1:9) according to AOAC [9]. The method involved extraction and pigment separation. Concentration of carotene was read directly from the Pye Unicam (8700 series SP-100 UV/visible spectrophotometer, at 436 nm after proper calibration of instrument with standard solution of pure β -carotene in acetone-hexane mixture. Ascorbic acid was determined by titration with indophenol solution [10]

Mineral elements (iron and calcium) were determined using AOAC procedure [9]. Each mineral was specifically analysed using Unicam 919 atomic absorption spectrophotometer at the Soil Science Department, Sokoine University of Agriculture.

The device was operated at standard conditions using volume and slit width and wavelength specific for each element.

Determination of antinutrient content

Oxalate content in fresh, cooked and dried vegetable was determined by calcium oxalate precipitation [8]. The method involved titration of acidic aqueous extracts of the sample with a standard solution of potassium permanganate. Polyphenols in fresh, cooked and dried vegetable were analysed as total soluble polyphenols using Folin-Denis method and absorbance read at 760 nm using Cesil CE 2021 spectrophotometer [11].

Data analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 7.0 computer package. Descriptive statistics were used to determine the measures of central tendency. Analysis of variance (ANOVA) was performed and differences between the two vegetable samples were considered statistically significant at 95% confidence interval. Means were separated using Least Significant Differences (LSD) test.

RESULTS

Proximate composition of raw SPL

The two varieties differed significantly ($p < 0.05$) with respect to crude fibre, soluble carbohydrates and total ash (Table 1). The other parameters were not significantly different ($p > 0.05$). The crude fibre was higher in PMSPL (21.48%) than in GMSPL (16.01%).

Vitamin, mineral and antinutrient contents in raw SPL

Significant differences ($p < 0.05$) were noted in the content of nutrients between the two varieties (Table 2). Ascorbic acid content was significantly higher ($p < 0.05$) in GMSPL than in PMSPL. The rest of the analysed nutrients were significantly higher ($p < 0.05$) in PMSPL than in GMSPL. The level of oxalate in GMSPL was significantly higher ($p < 0.05$) than in PMSPL. The polyphenols content in PMSPL (22.16%) was four times higher than in GMSPL (5.28%).

Effect of drying on ascorbic acid, carotenoids, iron, calcium, oxalates and polyphenols

The retention of nutrients (ascorbic acid, carotenoids, iron and calcium) and antinutrients (polyphenols and oxalates) when SPL were sun dried with and without rubbing them with salt is shown in Table 3. Carotenoids content of the vegetables dried with salt and those without salt were not different ($p > 0.05$). The carotene content of raw SPL was significantly higher ($p < 0.05$) than that of SPL dried after salting (32 mg/100g dmb) and those dried without salting. There was a significant ($p < 0.05$) reduction of carotenoids content in drying but the loss was not significant ($p > 0.05$) between the two methods. Drying resulted in high loss of ascorbic acid,

which was 60% when the vegetable was dried with salt and 85% without salt. This apparent loss was statistically significant ($p < 0.05$).

There was a significant difference ($p < 0.05$) in iron content between the raw SPL and vegetables dried before salting. There was a slight increase in iron content in the SPL, which were dried after salting. The calcium content before salting was significantly higher than that after salting and significantly lower than that of raw vegetable (Table 3). Significantly higher retention of polyphenols and oxalates was observed when SPL were dried with salt as compared to those without.

Effects of cooking on ascorbic acid, carotenoids, iron, calcium, oxalates and polyphenols

Sweet potato leaves cooked with lemon had similar ascorbic acid content as fresh (Table 4). The ascorbic acid content of SPL cooked without lemon was significantly lower than that of raw vegetable with retention of 65%. Carotenoids content was significantly lower in SPL cooked with lemon than in those cooked without lemon. Cooking with lemon had a significant effect ($p < 0.05$) on the iron content but not calcium. Polyphenol content decreased when the vegetables were cooked.

DISCUSSION

Proximate composition

The proximate composition of the two varieties of SPL is comparable to that reported for other indigenous vegetables like cowpea leaves and amaranthus [8,12,13]. They were also within the range reported for SPL [2]. The value of protein, fat, fibre and soluble carbohydrates were slightly higher than those reported by Huls and others, [14] for vegetables consumed in Bangladesh. The protein of SPL is known to contain high amounts of the amino acids lysine and tryptophan, which are lacking in cereals, hence can complement the cereal-based staple diets in the area. The levels of nutrients in food depend on natural sources of variation in the species, nature of the soil, genetic diversity, seasonality (such as amount of moisture and sunlight available during critical times of growth), and stage of maturity and effect of processing [3, 15]. The variations in nutrients in the two varieties of SPL could be explained partly by these highlighted causes.

Vitamins and minerals

The levels of ascorbic acid in the two varieties were slightly lower than those reported for other indigenous vegetables such as amaranths and cowpea leaves [8, 12, 13]. However, it was higher than the values reported for the indigenous leafy vegetables consumed in Cote d'Ivoire (22.67 mg/100 g) [16] and in Tanzania (15 to 249 mg/100g) [17]. Maeda and Salunkhe reported that SPL contain 1374 mg/100g of ascorbic acid, a variety whose identity was, however, not given [7]. The low level of ascorbic acid observed may be due to variation in agronomic and soil conditions. The analysed SPL were blanched, while in this study the leaves were harvested about eight hours before analysis and they were not blanched. Blanching would have resulted in

higher levels of ascorbic acid as it is protective to deteriorative changes as long as leaching losses are prevented. Tropical leafy vegetables lose significant amounts of ascorbic acid rapidly immediately after harvesting. Based on this generalisation, the determined concentration of the vitamin C of fresh leaves depends much on the time lapse from harvesting to actual chemical assay. This is because ascorbate is oxidised by exposure to oxygen and degraded by enzymatic activities [15]. Although ascorbic acid was lower in SPL, the level was much higher than that of exotic vegetables. This supports efforts to promote consumption of SPL.

Total carotenoid content were slightly higher than values reported by Mosha and others [18]. In some Indian vegetables, carotenoid content varied from 36.6 to 238 mg/100gm [19]. Analysis of different varieties of SPL in China showed that beta carotene was the most dominant carotenoid in SPL varieties [20]. It is, therefore, possible that the content of provitamin A is high in this vegetable. High amount of carotenoids in PMSPL may be attributed to its purple colour and being more dark green compared to the GMSPL.

Low iron and calcium in GMSPL was also reflected in significant difference in the ash content (Table 1). Since the two varieties were grown on the same plot, the observed differences were due to variety or genetic constitution. The calcium levels for both varieties were higher than those reported in the Tanzania food composition tables [21]. This could be attributed by varietal differences, stage of growth, type of soil and mode of harvest (whether first or second harvesting). Iron content was much lower than that for the *C. taro* and *C. tridens* as reported by Prabhu and Barret [22] to be 38.8 mg /100g. Sheela *et al.* [23] reported an iron content range from 3.6 to 37.3 mg/100g in green leafy vegetables consumed in Southern Karnataka.

Levels of oxalates and polyphenols

The oxalate level in GMSPL is disproportionately higher than its calcium level. Since oxalic acid interferes with absorption of calcium from other components of a meal depending on the calcium-oxalate ratio in the food that is the source of the oxalic acid [15], it is possible that there may be free oxalate in GMSPL, which can bind calcium from other foods and render it unavailable. The oxalate content of SPL was lower than that for amaranthus grown in Tanzania (3383-4333 mg/100g) and in Kenya (5830mg/100g) [12, 13]. Some Indian vegetables were found to contain extremely higher oxalate values ranging from 5138 to 12576 mg/100g DWB [24].

Polyphenol content in PMSPL (22.16%) was higher than in GMSPL (5.28%). This may be due to presence of other flavour and colouring pigments in PMSPL shown by purplish colour on the petiole and midrib, which was not present in GMSPL. This was noted because the pigment of raw leaves of PMSPL appears dark green compared to that of GMSPL, which is medium green. Although high polyphenols can combine with protein and make it unavailable, research by Islam revealed that they have an advantage of reducing oxidative stress in the body as they act as antioxidants, hence

reducing the chances of getting diseases, such as cancer and cardiovascular problems [25].

Effect of drying on nutrients and antinutrients

Drying of SPL resulted in high losses of ascorbic acid (Table 3). A similar observation has been reported by Maeda and Salunkhe [7] for vegetables consumed in Tanzania, whereby when dried in open sunshine, cassava leaves, SPL and cowpea leaves retained only 5.5, 2.5 and 10.6% of the vitamin, respectively. The vegetables dried with salt retained their colour, which was reflected in the high level of ascorbic acid retention. Salt could have reduced enzymatic breakdown of ascorbic acid during drying, hence higher retention compared to the leaves dried without salt.

Carotenoids retention was 61 and 47% for the SPL dried with and without salt, respectively. Higher retention was observed when leafy vegetables were dried in open sun [18]. Since carotenoids are affected by ultraviolet light; the loss could be reduced if the vegetable was dried in improved solar driers. Generally, it is known that direct exposure of vegetables to sunshine as is often practiced in the tropics, results in marginal retention of vitamins due to increased oxidation by ultraviolet light. However, the extent of loss of vitamins depends on the type of vegetable.

There was a slight but significant increase in iron when the vegetables were dried with salt, which may be due to contamination from salt. The lower retention of calcium when the vegetables were dried with salt may probably be due to cation exchange, whereby some calcium is replaced by sodium and consequently lost in any water that could have been drawn by salt from the vegetables. Polyphenols decreased when the vegetables were traditionally dried. The noted decrease in polyphenols after drying may be due to enzymic reaction and possibly reaction with proteins.

Effects of cooking on nutrients and antinutrients

Higher levels of ascorbic acid in those vegetables cooked with lemon may be because pH was lowered by the lemon juice. Ascorbic acid was reported to be fairly stable under acidic medium [26]. Additionally, lemon juice containing ascorbic acid might have caused the observed results. It was observed that vegetables were most often cooked covered, such that oxidative losses were possibly minimized. The values were higher than reported in other studies for tropical vegetables consumed in Tanzania that leafy vegetables may lose between 30 and 93% of ascorbic acid during cooking [21, 27]. However, in other studies, vegetables were cooked in large volumes of water and sometimes the water was drained. They were also cooked for a longer time compared to cooking of SPL. Moreover, SPL were cooked without chopping. Generally, chopping is known to facilitate loss of vitamin C during cooking as it increases enzymatic activities [15].

The observed significant decrease in carotenoids may be due to degradation during heat processing. Lower retention when the leaves were cooked with lemon than when cooked without could be due to isomerization, which occurs at pH 4.5 or less [28].

Mosha reported an increase in total carotenoids in cooked pumpkin and sweet potato leaves [18]. Similar results were reported by other researchers [17]. However, other studies reported a slight but insignificant increase of carotenoids in cooked leafy vegetables [18], while others found that carotene is unaffected by most cooking methods but small amount may be lost during frying [29].

Imungi and Potter [8] reported a slight increase in iron content of cowpea leaves after cooking from 38.8 to 43.8 mg/100g. A loss of 34.3% for amaranthus and up to 11% for *Cassia taro* and *Chorchorus tridens* was observed [12, 22]. The reason for high calcium retention may be high levels of oxalate such that most of the calcium was in bound form and was therefore not soluble. Calcium loss of 28% and 29% was reported for cooked cowpea leaves and amaranthus, respectively [8, 12]. The main route for loss of minerals from cooked vegetables would be leaching into the cooking water, which was not used in this study with SPL.

The results show that after cooking, the level of oxalate was lower than that reported to be of health concern, which was 2 to 5 g per day for population consuming low calcium [30]. Since the level of calcium in cooked SPL was much higher, compared to the level of oxalate, the risk of calcium deficiency due to consumption of SPL may not be expected. Polyphenolic levels decreased by 44 and 19% in the SPL cooked with and without lemon, respectively. This implies that phenolics, being organic compounds can be affected by pH.

CONCLUSION

Fresh SPL consumed in Tanga contains high levels of protein, carotenoids, vitamin C, calcium, and iron. The traditional methods of cooking SPL with addition of lemon is advantageous because it reduces polyphenols while retaining higher levels of minerals, β carotene and vitamin C. Oxalates and polyphenols are also high. Drying with salt results in a nutritionally and organoleptically good product, hence the use of improved solar dryers could result in a better product with high nutrient retention. Higher levels of ascorbic acid and β -carotene are retained when the leaves are rubbed with salt before drying. The PMSPL were relatively better nutritionally compared to GMSPL. Drying with salt and cooking with addition of lemon is encouraged. Further studies would be important to determine the effect of growing conditions including weather (season) on nutrient, antinutrient content, and eating quality, as well as the variation of nutrients for the different harvests, considering that leaves are harvested several times.

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Table 1: Proximate composition of PMSPL and GMSPL*

Variety	Moisture content (%)	Dry matter (%)	Crude protein (%)	Total Ash (%)	Crude fibre (%)	Total lipid (%)	Soluble carbohydrates (%)
GMSPL	85.63±0.35 ^a	14.37±0.35 ^a	26.37±0.085 ^a	12.87±0.035 ^a	16.01±0.25 ^a	3.11±0.97 ^a	41.62±0.66 ^a
PMSPL	86.28±0.36 ^a	13.72±0.36 ^a	37.06±2.76 ^a	20.41±0.17 ^b	21.48±0.67 ^b	3.27±0.28 ^a	17.76±1.92 ^b

* Values reported in table are mean ± SE of duplicate determinations

The values in a column with different superscript letters are significantly different (p<0.05)

Table 2: Levels of nutrients and antinutrients in raw SPL *

Variety	Ascorbic acid (mg/100gm)	Carotenoids (mg/100gm)	Iron (mg/100gm)	Calcium (mg/100gm)	Oxalates (mg/100gm)	Polyphenols (%)
GMSPL	303.58 ±0.81 ^a	44.18±0.77 ^a	15.22±0.28 ^a	3457±181 ^a	3730.50± 4.50 ^a	5.28± 0.1 ^a
PMSPL	273.17±0.15 ^b	53.32±0.26 ^b	17.48±0.13 ^b	4255±31 ^b	2901.505± 4.50 ^b	22.16± 0.04 ^b

*Mean ± SE of duplicate determinations

Values in a column with different superscript letters are significantly different (p<0.05).

Table 3: Retention of mineral, vitamins and antinutrients contents in dried SPL*

Variety	Ascorbic acid (mg/100g)	Carotenoids (mg/100g)	Iron (mg/100g)	Calcium (mg/100g)	Oxalates (mg/100g)	Polyphenols (%)
Raw	273.18±0.15 ^a	53.32±0.26 ^a	17.43±0.13 ^a	4255±31 ^a	2901.5±4.5 ^a	22.16 ± 0.04 ^a
Dried with Salt	108.56±0.11 ^b	32.44±1.57 ^b	19.15±0.19 ^b	2752±20 ^b	2502 ±4.5 ^b	9.21 ±0.02 ^b
Percentage retention	40	41	110	65	86	42
Dried without Salt	41.44±0.04 ^c	28.38±1.75 ^b	16.55±0.11 ^c	3393±43 ^c	2635 ± 16.5 ^c	21.25 ± 0.28 ^a
Percentage retention	15	53	0.5	80	91	96

*Mean ± SE of duplicate determinations

Values in a column with different superscript letters are significantly different (p<0.05).

Table 4: Retention of minerals, vitamins, oxalates and polyphenols in cooked SPL*

Variety	Ascorbic acid (mg/100g)	Carotenoids (mg/100g)	Iron (mg/100g)	Calcium (mg/100g)	Oxalates (mg/100g)	Polyphenols (%)
Raw	273.18±0.15 ^a	53.32±0.26 ^a	17.43±0.13 ^a	4255±31 ^a	2901.5±4.5 ^a	22.16 ± 0.04 ^a
Cooked with lemon	274.86±0.11 ^a	43.90±0.25 ^b	16.14±0.16 ^b	4046±92 ^a	1282±4.0 ^b	10.29 ± 0.02 ^b
Percentage retention	100.6	82	93	95	44	56
Cooked without lemon	178.06±0.07 ^b	48.21±0.55 ^c	17.00±0.01 ^a	3964±92 ^a	1348 ± 1.5 ^c	17.92 ± 0.05 ^c
Percentage retention	65	90	98	93	46	81

*Mean ± SE expressed on dry matter basis of duplicate determinations

Values with different letters in a column are significantly different (p<0.05)

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