Asian Journal of Agriculture and Food Sciences (ISSN: 2321 – 1571)
Volume 04 – Issue 05, October 2016

The Effect of Processing on the Antinutrients in Selected Legumes in Botswana

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ABSTRACT---- ***Edible legumes particularly grain legumes or pulses are important sources of protein in many developing countries. However, this protein may not be available for digestion because of antinutritional factors such as condensed tannins, enzyme inhibitors, and others. Two local varieties of cowpeasVignaunguiculata (tswana and black-eyed) were obtained from the Botswana Agricultural Marketing Board in Gaborone, Botswana. Processed and unprocessed samples of Tylosemaesculentum (marama) and the two local cowpea varieties were analysed for condensed tannins and trypsin inhibitors. The marama beans were roasted in an oven at 80 ºC for 30 minutes, and the cowpeas were boiled for one hour.Condensed tannin was measured using the butanol-HCl method. For the condensed tannin contents, the results (%) for the unprocessed samples were: marama 0.381, black-eyed 0.111 and tswana 0.381 respectively. After processing, the condensed tannin results (%) were: marama 0.223, black-eyed 0.050 and tswana 0.032. After processing, the condensed tannin contents (%) were reduced by 41%, 55% and 92% for marama, black-eyed and tswana beans, respectively.The trypsin inhibitor activity was determined using benzoyl-DL-arginine-p-nitranilide hydrochloride method. The trypsin inhibitor activities (TIU/g dry matter) for the raw legumes were: marama 27.8, black-eyed17.4, and tswana 17.3. After roasting of the marama bean and boiling of the cowpeas, the following results were obtained:marama 26.4, black eye 8.4 and tswana 8.0. The decrease in trypsin inhibitor activity was reduced by 5%, 52% and 54% for marama, black-eyed and tswana beans respectively. The results showed that processing reduced the antinutrients in the legumes studied by varying amounts. In order to benefit from the high protein contents in these legumes, they have to be well cooked in order to reduce or completely destroy the antinutrients. This would improve on the digestibility of the protein in these legumes. The cultivation and increased consumption of these legumes would positively contribute to food security in the southern Africa region.***

Keywords: legumes, protein, antinutrients, processed, unprocessed

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1. INTRODUCTION

Edible legumes particularly grain legumes or pulses are important sources of protein in many developing countries.They are important also in the cropping system because of their ability to fix nitrogen in the soil and so increase the overall soil fertility. They belong to the family Leguminosae. For most pulses,the protein content ranges between 19 and 25% with an average value of about 22% (Nwokolo, 1996).].However, this protein may not be available for digestion because of antinutritional factors such as condensed tannins, enzyme inhibitors, and others.

Condensed tannins (CT) are polyphenolic substances which are widely distributed in plants particularly legumes. They interact with protein and starch to form complexes which are resistant to enzymatic hydrolyses (Agugo et al. 2013, Aberoumand, 2012), thus reducing the bioavailability of nutrients.Trypsin inhibitors reduce the availability of the enzyme trypsin which breaks down protein during digestion. Processing such as cooking and roasting help to reduce or eliminate antinutrients(Aberoumand, 2012, Apata & Ologhobo, 1997).

Cowpea is an important food crop in Botswana, and it is widely consumed as a main meal and is prepared by boiling. However it was reported that it contains antinutritional factors (Plahar et al. 1997).This study investigated the effect of processing on the tannin and trypsin inhibitor contents in two local varietiesof *Vigna uniguiculata* (L).Walp (cowpea, Figure 1) and in *Tylosema esculentum*(Burch) A. Schreib; marama bean (Figure 2).

Tswana Black eye
Figure 1. Black eye and tswana cowpeas [6].

Marama bean Marama bean plant with beans
Figure 2. Marama bean and marama bean plant [7].

Marama beanis a wild perennial, underutilised legume, indigenous to the Kalahari desert region of Southern Africa, where agricultural practices are a challenge(Monaghan & Hallovan, 1995). It is an important food source for the people of the Kalahari area in Botswana.The seeds are contained in pods that dehisce when dry. These beans are dark brown in colour(Fig 2) and weigh 2-3 g each. Each bean has a hard inedible outer shell with an edible two-lobed seed inside (National Research Council Marama, 2006). They are usually roasted and eaten as a snack. When roasted, marama beans taste like roasted cashew nuts or chest nuts. They contain trypsin inhibitors that may be destroyed by roasting (Mmonatau, 2005).The seeds contain a high amount of crude protein and oil, 34.1% and 33.5% respectively(Amarteifio & Moholo, 1998).The protein of the edible seeds is largely lysine and relatively S-amino acid, while the oil of the seeds is largely oleic (49%) and linoleic acid (24%) (Jackson et al. 2013).

The composition of marama bean is similar to that of peanuts and soybeans. Ithas a protein content of 29.6-41.8%and oil content of 32.1-45.3%(Monaghan & Hallovan, 1995). They can be a useful source of protein in the diet. Even though these legumes are consumed there is limited information on the effect of processing on the anti-nutrients. This study investigated the effect of boiling on the condensed tannin and trypsin inhibitor contents of two local varieties of cowpeas; black-eyed and tswana in Botswana. In addition, the effect of roasting on the condensed tannin and trypsin inhibitor contents in marama bean was determined. The results of this study will provide useful information to consumers, food scientists and nutritionists.

2. METHODOLOGY

Plant Samples

Two local varieties of cowpeas, tswana and black-eyed, were obtained from the Botswana Agricultural Marketing Board in Gaborone, Botswana. Fifty grams of each sample was ground using a coffee mill to a particle size of 20mesh and stored in airtight plastic containers.Mature dried seeds of marama beans were collected from the Ngwaketse district, Southern part of Botswana. The dehulledmarama bean seeds(100 g)were defatted using hexane. The dried defatted sampleswere ground using a coffee mill to a particle size of twenty mesh and stored in airtight plastic containers.All the samples were stored in a refrigerator at 4 ºC until required for analysis.

Condensed Tannin Determination

Condensed tannin was measured using the butanol-HCl method (Amarteifio & Moholo, 1998) and absorbance readings were taken using Ultra violet/visible spectrophotometer (Spectronic 20D Milton Roy) at 550 nm. Condensed tannin was calculated using the leucocynadin equivalent formula:

A550nm x 78.26 x dilution factor/ % dry matter, where A550 nm is the absorbance of each sample at 550 nm.

Trypsin Inhibitor Activity Determination

The trypsin inhibitor activity(TIA) wasdetermined using benzoyl-DL-arginine-p-nitranilide hydrochloride method as described by Kakade et al. (1974). One trypsin unit (TU) is defined as an increase of 0.01 absorbance unit at 410 nm. Trypsin inhibitor activity is the number of TU inhibited (TIU) per gram of dried sample.

The Effect of Processing on Condensed Tannin and Trypsin Inhibitor Activity

To determine the effect of processing,each variety of cowpeas (500 g) was boiled for one hour. The samples were drained and dried in an oven at 55 ºC for 20 hours and then ground into a flour. Marama beans (500 g)in the hard shell were roasted at 80ºC for 30 minutes with constant stirring in an open earthenware pot containing 1 kg of fine sand to prevent charring and uneven distribution of heat. The samples were sieved from the sand and allowed to cool. The shells were removed and the nuts defatted and then ground into a powder.The cowpeas and marama bean samples were kept in the fridge at 4ºC and used for the analysis for trypsin inhibitor and condensed tannins.

Statistical Analysis

For each parameter, the samples were analysed in triplicate and the mean calculated. The data was subjected to the analyses of variance (ANOVA) and Duncan's Multiple Range Test was used to separate the mean values ($p \leq 0.05$).

3. RESULTS

Condensed Tannin Determination

Table 1 gives the details of the CT contents in cowpeas and marama beans. The CT contents for cowpeas were reduced by 55% and 92% for black eye and tswana respectively, after boiling for one hour. Before boiling, the CT contents were 0.111% and 0.381% and after boiling they were 0.05% and 0.032% for black eye and tswana respectively.

Trypsin Inhibitor Activity Determination

The results for the trypsin inhibitor activity (TIA) for the legumes analysed are shown in Table 2. The TIA for the unprocessed cowpeas were similar ($p > 0.05$) but different from that of the marama bean. The marama beans had more TIA (27.8 TIU/g dried sample) than black eye and tswana;17.4 and 17.3 respectively. For the cowpeasboiling reduced the TIA by 52% and 54% respectively.

4. DISCUSSION

The results of this study showed that the unprocessed tswana cowpeas and the marama beans had more condensed tannins (CT) than the unprocessed black-eyed beans. This confirms the observation by Plahar et al. (2013) and Tibe et al. (2007) that CT contents increased with the intensity of the seed coat colour. However from the results obtained, the study demonstrated that processing such as boiling can reduce the CT considerably. Boiling seemed to have the same effect on the trypsin inhibitor activity (TIA) in the cowpea varieties. Marconi et al. (1993) reported a range of 9.01-25.9 TIU/mg sample for 22 raw cowpea varieties grown in Nigeria. The results (17.3-17.4 TIU/g dried sample) for the two raw cowpeas variety analysed in the study are lower than those cited by Marconi et al. [1993). Roasting reduced the TIA in marama by 5%. Maruatona (2008) stated that dry heating of marama beans at 150 ºC for 20 minutes reduced the level of TIA from 251 TIU/mg flour to 3 TIU/mg flour. In this study, the level of TIA was reduced from 27.8 to 26.4 TIU/g of dried sample. The difference in reduction of TIA may be due to many factors such as the difference in temperature and the location from which the samples were collected. Apata et al. (1997) found out that roasting of legumes is less effective in destroying TIA than autoclaving or cooking. They recommended that for the complete inactivation of TIA the legumes should be boiled before roasting. The results of this study indicate that boiling or roasting of the samples was more effective in reducing the CT levels than the TIA. Therefore, other processing methods that can completely destroy trypsin inhibitors should be investigated. Differences obtained when compared to the results of other researchers may be due to many factors including the different varieties analysed and environmental conditions such as soil fertility and climate.

5. CONCLUSION

In order to benefit from the high protein contents in these legumes, they have to be well cooked in order to reduce or completely destroy the antinutrients. This will improve on the digestibility of the protein in these legumes. The cultivation and increased consumption of these legumes would positively contribute to food security in the southern Africa region.

6. ACKNOWLEDGEMENT

The authors are grateful to the Botswana College of Agriculture for providing the research funds for this project.

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Table 1: Mean condensed tannin (% leucocyandin equivalent) for the unprocessed and processed cowpeas and marama beans.

Legume	Unprocessed	Processed
Black eye	0.111^{b}	0.050^{b}
Tswana	0.381^{a}	0.032^{b}
Marama	0.381^{a}	0.223^{a}

Means with the same letters in a column are not significantly different ($p>0.05$)

Table 2:Mean trypsin inhibitor activity (TIU/g of dried sample) in unprocessed and processed cowpeas and marama beans

Legume	Unprocessed	Processed
Black eye	17.4^{b}	8.4^{b}
Tswana	17.3^{b}	8.0^{b}
Marama	27.8^{a}	26.4^{a}

Means with the same letters in a column are not significantly different ($p>0.05$)