Effect of soaking and germinating techniques on the macronutrient content of *Hyptis spicigera* Lamiaceae seeds in Morobo County, South Sudan

Grace Ayite Banja¹,²*, Kiative-Mokua Beatrice¹ and Okoth Judith Kanensi¹

¹Jomo Kenyatta University of Agriculture and Technology, Department of Food Science and Technology, P. O. Box 62000-00200, Nairobi, Kenya.
²College of Community Studies and Rural Development, Department of Community Studies. University of Juba. Republic of South Sudan.

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This research work discusses the macronutrient content of raw and processed seed flour of Black Beni sesame (*Hyptis spicigera* Lam) seed. Although, the plant is well known for its insecticidal, herbal and medicinal properties, its seed would be used effectively to address the food insecurity and malnutrition in south Sudan, because of its high macronutrient content. The *Hyptis* seed were soaked and germinated; raw seed served as control. The seed were milled into flour and their proximate compositions were analyzed. Data were subjected to analysis of variance, mean were separated by Duncan multiple range test at 5% significance level using Genstat Release 14.1 Software. Composition analysis was done on dry weight basis. Crude fiber content of raw *H. spicigera* Lam seed was 18.2%, 26.0% for soaked seed and 26.0% for seed soaked for 5 h and germinated for 72 h on dry weight basis. Fat content of *H. spicigera* Lam seed was 34.1% for raw seed, 50.4% for seed soaked for 15 h and 48.6% for seed soaked for 20 h and germinated for 24 h. Carbohydrate content of raw *H. spicigera* Lam seed was 24.1%, 29.7% for seed soaked for 20 h and 39.5% for seed soaked for 10 h and germinated for 24 h. Protein content of raw *H. spicigera* Lam seed was 16.0% and 17.6% for seed soaked for 10 h and germinated for 48 h. Soaked and germinated seed had significantly high crude fiber, fat and protein content as compared to the raw seed (P value < 0.05). In conclusion, soaking and germination appears to be a promising processing method of enhancing the proximate composition of *H. spicigera* Lam seed.

**Key words:** Effects, soaking, germination, macronutrient content, *Hyptis spicigera* seed.

INTRODUCTION

Food insecurity is deteriorating and has become a global concern (Olanipekun et al., 2015). More than two million people were under severe food shortage (Harvey and Rogers-Witte, 2007; World Vision, 2014) exposing them to high rate of malnutrition in Southern Sudan (Ministry of Health, 2009). Sorghum is the main staple food of South Sudan and the local production does not meet the overall demand, as a result supplies to other staple grains are imported from Uganda and Sudan (World Food Programme, 2015).

*Hyptis spicigera* Lam plant which belongs to the family Lam is commonly found in the bush lands of Southern Sudan (Othira et al., 2009). The plant is generally known as bushmints, Black Beni-Seed or Sesame in English (Ladan et al., 2011). Locally, *H. spicigera* Lam plant is called "Kinu" in Bari Language. The plant has been used as food especially among the Kakwa tribe from Yei in South Sudan (Kakwa org. (2015). *H. spicigera* Lam is a strong erect aromatic herbaceous annual plant with approximately 0.5 - 1 m in height and seeds are crowded in quadruplets (Uraku et al., 2015). The seeds are tiny with brown or black color (Ladan et al., 2011).

In Guinea, central and eastern Africa, *H. spicigera* seeds are cultivated and the oleaginous seeds are eaten.

*Corresponding author. E-mail: ayitena@gmail.com.*
like sesame (Noudjou et al., 2007). The seeds are used for oil production whereas the leaves are eaten as vegetables and spices (Ladan et al., 2011). *H. spicigera* seeds are roasted and pounded, then used for preparing other food (Agea et al., 2014). It is also used in the treatment of several kinds of illnesses such as upper respiratory tract infections, diarrhea, headache, pneumonia, fever and cholera (Baba et al., 2012). The quality of foodstuffs may be enriched by processing. Processing methods, such as soaking, sprouting and cooking have been reported to improve the nutritional and functional properties of plant seeds (Kaijhausa et al., 2014; Ikuomola et al., 2013). These processing techniques may potentially avail macronutrients for easy absorption, hence increasing the utilization of *H. spicigera* Lam seeds and this potentially promotes food security.

Researchers have been done on volatile oils, insecticidal, pharmacological and biological activity of *H. spicigera* Lam seeds (Aja et al., 2015; Barbosa et al., 2013; Ladan et al., 2011), however, there is limited information on the macronutrient content and the effect of processing methods on *H. spicigera* Lam seeds in South Sudan. The objective of this paper is to determine the effect of soaking and germinating on the macronutrient content of *H. spicigera* Lam (Black beni/bushmint) seed.

**MATERIALS AND METHODS**

**Collection and preparation of sample**

*H. spicigera* Lam seeds were obtained from Morobo market, in Morobo County, South Sudan. The seeds were placed in a polythene bag, closed and transported to Jomo Kenyatta University of Agriculture and Technology (JKUAT) food biochemistry lab. The seeds were carefully cleaned to remove any foreign materials and divided in three portions. The first portion was ground raw seeds, second soaked and the third was germinated. The samples were then ground to fine flour and kept in polyethylene bags in a (cold room) at 10°C for further analysis. All chemicals used in this study were of reagent grade (Figure 1).

**Sample preparation**

**Production of raw seed flour**

Five hundred grams (500 g) of *H. spicigera* Lam seeds were ground into fine flour using a Kenwood grinding mill and stored in polyethylene bags in a (cold room) at 10°C for further analysis (Kanensi et al., 2011; Makinde and Akinoso, 2013).

**Production of soaked seed flour**

Five hundred grams (500 g) of *H. spicigera* Lam seeds were placed in a plastic bowl and soaked in distilled water in seed to water ratio of 1:2 (w/v) for 5, 10, 15, 20, and 24 hours respectively. Soaking was carried out at room temperature. The remnant of water left after soaking was discarded. The soaked seeds were dried in an oven at 50°C until constant weight, then the dried sample was milled into fine flour using a Kenwood grinding mill and stored in polyethylene bags in a (cold room) at 10°C for further analysis (Kanensi et al., 2011; Makinde and Akinoso, 2013).

**Production of germinated seed flour**

The seeds were weighed in clean gauze then steeped in distilled water into seed of water ratio 1:2 (w/v) for 5 h, 10 h, 15 h, 20 h and 24 h at room temperature. After steeping, the seeds were germinated for 24, 48 and 72 h in the dark at room temperature respectively. The germinated seeds were dried in an oven at 50°C until constant weight, the dried sample was milled into fine flour using a Kenwood grinding mill and stored in polyethylene bags in a (cold room) at 10°C for further analysis (Kanensi et al., 2011; Makinde and Akinoso, 2013).

**Proximate determination of raw and processed seed flour**

Association of analytical communities (AOAC, 1995) method was used to determine protein, ash, crude-fibre, fat and moisture in processed and raw samples. The protein content was determined by digestion using micro-Kjeldahl method where total Nitrogen was determined; crude protein was calculated by the formula N x 6.25; fat content was determined by the continuous solvent extraction method using soxhlet apparatus; crude fibre was determined gravimetrically; total ash content was determined by furnace incineration; carbohydrate was determined by difference.

**Determination of carbohydrate**

The carbohydrate content was determined by calculation (by difference) according to (Uraku et al., 2015) calculation:

\[ \% \text{carbohydrate} = 100 - (\% \text{moisture} + \% \text{crude fibre} + \% \text{ash} + \% \text{crude fat} + \% \text{crude protein}) \]

**Data analysis**

The results obtained were analyzed using Genstat Release 14.1. One way analysis of variance was used to determine the significant difference between the treatments and separation of mean was done using
RESULTS AND DISCUSSION

The effect of soaking and germination on the proximate composition of Hyptis spicigera seeds are given in Figure 2 and Table 1.

Duncan's Multiple Range Test (DMRT). Significant difference was accepted at P<0.05.

The carbohydrate content of the soaked and germinated seeds was significantly higher as compared to the raw seeds. The carbohydrate content of soaked and germinated seeds was in the range of 14.6% - 39.5%. Seeds soaked for 10 hours and germinated for 24 h had a carbohydrate content of 39.5% which was significantly higher at (p < 0.05) compared to the carbohydrate content of the raw seeds which was 24.1%. The increase in carbohydrate content is due to the
Table 1. Mean proximate composition of raw and germinated seed flours of Black beni (Hyptis spicigera Lam) on dry weight basis.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Moisture%</th>
<th>Ash%</th>
<th>Fibre %</th>
<th>Fat %</th>
<th>Protein%</th>
<th>CHO%</th>
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</thead>
<tbody>
<tr>
<td>Raw seeds</td>
<td>4.71±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.83±0.13&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.2±0.71&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.1±0.49&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.04±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>24.09±0.75&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soaked 5h germ 24h</td>
<td>1.31±0.20&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.15±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.3±1.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>42.6±4.28&lt;sup&gt;d&lt;/sup&gt;</td>
<td>12.7±0.27&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>18.8±3.99&lt;sup&gt;bcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soaked5h germ 48h</td>
<td>1.18±0.50&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.03±0.03&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.3±1.24&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>42.2±0.74&lt;sup&gt;d&lt;/sup&gt;</td>
<td>13.2±0.55&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>20.1±2.26&lt;sup&gt;abcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soaked 5h germ 72h</td>
<td>2.22±0.12&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>2.96±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.6±2.06&lt;sup&gt;c&lt;/sup&gt;</td>
<td>46.2±6.33&lt;sup&gt;de&lt;/sup&gt;</td>
<td>11.1±5.99&lt;sup&gt;b&lt;/sup&gt;</td>
<td>14.6±10.6&lt;sup)b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soaked 10h germ 24h</td>
<td>1.91±0.09&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>2.72±0.23&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.1±1.59&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.1±0.18&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>13.1±0.28&lt;sup&gt;abcd&lt;/sup&gt;</td>
<td>39.5±3.36&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soaked10h germ 48h</td>
<td>2.03±0.13&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.20±0.02&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.3±1.41&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>38.7±2.00&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>17.6±1.36&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>17.9±0.22&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>Soaked10h germ 72h</td>
<td>2.38±0.08&lt;sup&gt;def&lt;/sup&gt;</td>
<td>3.23±0.17&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.0±1.53&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>34.9±1.72&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>9.65±0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>29.8±3.34&lt;sup&gt;de&lt;/sup&gt;</td>
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<td>Soaked15h germ 24h</td>
<td>2.66±0.15&lt;sup&gt;def&lt;/sup&gt;</td>
<td>3.11±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.4±0.28&lt;sup&gt;a&lt;/sup&gt;</td>
<td>39.3±0.67&lt;sup&gt;abc&lt;/sup&gt;</td>
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<td>25.5±1.23&lt;sup&gt;bcde&lt;/sup&gt;</td>
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<tr>
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<td>2.88±0.03&lt;sup&gt;ef&lt;/sup&gt;</td>
<td>2.29±0.85&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.8±0.44&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>41.2±1.65&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>12.6±0.26&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.2±0.70&lt;sup&gt;abcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soaked15h germ 72h</td>
<td>0.98±0.06&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.45±0.31&lt;sup&gt;b&lt;/sup&gt;</td>
<td>22.6±0.73&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>42.2±1.59&lt;sup&gt;cde&lt;/sup&gt;</td>
<td>14.8±0.70&lt;sup&gt;b&lt;/sup&gt;</td>
<td>16.9±1.67&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soaked20h germ 24h</td>
<td>0.75±0.04&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2.44±0.16&lt;sup&gt;b&lt;/sup&gt;</td>
<td>18.4±0.52&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>48.6±1.96&lt;sup&gt;eg&lt;/sup&gt;</td>
<td>10.9±0.35&lt;sup&gt;c&lt;/sup&gt;</td>
<td>18.8±2.09&lt;sup&gt;abcd&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soaked20h germ 48h</td>
<td>2.59±1.03&lt;sup&gt;d&lt;/sup&gt;</td>
<td>2.70±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.7±2.45&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>34.0±0.42&lt;sup&gt;a&lt;/sup&gt;</td>
<td>14.0±0.44&lt;sup&gt;bd&lt;/sup&gt;</td>
<td>28.9±3.09&lt;sup&gt;de&lt;/sup&gt;</td>
</tr>
<tr>
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<td>2.06±0.07&lt;sup&gt;bcd&lt;/sup&gt;</td>
<td>2.44±0.33&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17.5±2.93&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>33.0±0.10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>10.5±0.05&lt;sup&gt;b&lt;/sup&gt;</td>
<td>34.5±2.74&lt;sup&gt;ef&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soaked24h germ 24h</td>
<td>2.15±0.17&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>3.16±0.11&lt;sup&gt;b&lt;/sup&gt;</td>
<td>19.3±0.09&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>36.6±2.69&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>12.3±0.45&lt;sup&gt;b&lt;/sup&gt;</td>
<td>26.4±2.48&lt;sup&gt;bcde&lt;/sup&gt;</td>
</tr>
<tr>
<td>Soaked24h germ 48h</td>
<td>3.01±0.19&lt;sup&gt;g&lt;/sup&gt;</td>
<td>2.11±0.72&lt;sup&gt;b&lt;/sup&gt;</td>
<td>20.7±1.12&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>39.1±0.74&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>13.7±0.41&lt;sup&gt;b&lt;/sup&gt;</td>
<td>21.3±1.06&lt;sup&gt;abcd&lt;/sup&gt;</td>
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<td>Soaked24h germ 72h</td>
<td>1.39±0.21&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>1.03±0.29&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.2±0.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>37.5±2.05&lt;sup&gt;abc&lt;/sup&gt;</td>
<td>15.8±1.84&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>27.1±3.49&lt;sup&gt;bde&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Germ: Germination, h: Hours. Values are mean of triplicate determinations ± S.D. Mean in the same column followed by the same superscript are not significantly different at p<0.05.

extensive conversion of storage lipids to soluble carbohydrate after the onset of germination (Eastmond & Graham, 2001). Therefore, soaked and germinated H. spicigera Lam seeds can potentially be a good source of energy for consumers especially young children (Olanipekun et al., 2015).

The protein content was significantly (p < 0.05) lower in the soaked and slightly high in the germinated flour compared to the raw seeds. The protein content ranges of soaked and germinated flours were 11.3% - 16.4% and 9.65% - 17.6% respectively in comparison to 16.0% of the raw seeds. Acipa et al. (2013) reported 0.04% for protein, which is very low compared to the result obtained in this study. This deviation could be attributed to variety of the seed and type of soil. Protein is the building material for all body parts, such as muscle, brain, blood, skin, hair, nails, bones and body fluids (Agea et al., 2014).

The raw sample was significantly lower in fat compared to the soaked and germinated samples. The fat content of the germinated samples increased with increase in germinating time that ranged from 33.0% – 48.6% respectively. Soaking the seeds for 15 h resulted in the rise of fat content of the flour from 34.6% to 50.4%. The high fat content of the seeds gave the flour an oily and compacted appearance instead of a smooth powdery appearance (Kajihausa et al., 2014). The high fat content of the samples might be due to the very high oil content of the seeds.

The raw sample was slightly lower in crude fibre at a value of 18.2 % compared to the sample soaked for 10 hours, which ranged from 24.3% – 26.0%, while the samples soaked (5 h) and germinated (72 h) ranged from 21.3% – 22.6%. Crude fibre is an indication of the roughage/bulkiness of the sample and its presence in the diet serves in reducing constipation by increasing bowel movement (Abiodun and Adegbite, 2012).

The moisture content of the processed flour significantly (p < 0.05) decreased with an increase in soaking and germinating time, ranging from 3.01% - 0.75% and 3.55%-0.06% of seeds soaked (20hrs); germinated (24hrs) and soaked (24hr) as compared with the control 4.71% respectively. The low moisture content of the seed is an indication of the high dry matter content.
and possible long shelf-life (Agea et al., 2014).

The ash content of the processed seed flour had high ash content than the raw sample (2.82%). It ranged from 1.78% - 3.08% in the soaked seed flour and 1.03% - 3.23% in the germinated flour. The seeds soaked for 10 hours and germinated 72 h obtained the highest ash content. This agrees with the findings in Rekha et al. (2007), who reported that the ash content rose to 3.23% in cowpea germinated for 72 h. The rise in ash content might be due to loss of non-mineral dry matter during germination.

**Conclusion**

This study has shown that *H. spicigera* Lam (raw and processed) seed flour are good sources of macronutrients, especially carbohydrate, proteins and fat. Soaking and germinating have varying effect on the nutritional composition of the seed when compared to the raw seed. The seed is high in fat and can be a good source of oil. It has been noticed that germination improved the carbohydrate content of the seeds. The principle behind the increase in carbohydrate is the glyoxylate cycle which is operative in germinating oil seeds. Germinating the seed is a good process to maintain most the nutrients. The seed can be used in value addition and product development which can help to alleviate food insecurity problems in south Sudan and reduce prevalence of malnutrition. There is a great need for more research to be carried out on the other nutritional properties of the seed for its full utilization.

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