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Comparative study on proximate and antinutritional factors of dehulled and undehulled fermented Lyon bean (*Mucuna cochinchinensis*)

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Abstract

The impact of fermentation times and temperatures on the proximate composition and anti-nutrient content of dehulled and un-dehulled *Mucuna cochinchinensis* flour were investigated. Dehulled and un-dehulled Lyon beans were subjected to fermentation at 30° C and 45°C for 24, 48 and 72 hrs respectively and the fermented seeds were processed into flour. Results obtained revealed that the fermentation time/day had an effect on the proximate composition of the beans causing an appreciable increase in the crude protein (from 22.19 - 36.41%) and fat content (from 4.94 - 10.79%) of the fermented *Mucuna* beans samples when compared with results of the unfermented samples (22.19 and 4.94%) while there was a decrease in carbohydrate (from 57.34 - 40.85%) of the fermented beans. Anti-nutrients (oxalate, tannin and phytate) contents of the seeds decreased significantly (from 1.708-0.316 mg/g, 1.071-1.353%, 1.868-0.515%) as fermentation time and temperature also increased. This study has revealed that fermentation for 72 hrs at 45°C was most effective in increasing the nutritional content of the beans while also reducing the anti-nutrient content to a minimum level.

1. Introduction

In different parts of the world today, legumes are some of the most necessary foods consumed. The seeds contain at least twice the amount of protein present in cereals and the profile of the essential amino acids shows that it is more adequate. These legumes range from those usually consumed such as cowpea, soybean and groundnut to the underutilized ones such as African yam bean, Lyon beans and Lima beans (Ndidi *et al*., 2014).

Lyon beans (*Mucuna cochinchinensis*), one of the many species of *Mucuna* in the family *Leguminosae*, is an underutilized tropical legume grown in many parts of the world. It is nutritionally comparable to other legumes such as soybeans because of their similar contents of protein, fat and other nutrients (Adebowale *et al*. 2005). It contains approximately 28.7% protein, 6.12% ether extract, 3.82% crude fibre, 3.97% ash, 50.3% nitrogen free extract and 4.42kcal/g energy. It is used as a minor food crop in parts of Nigeria as well as Asia (Tuleun *et al*., 2008).

The need for cheap sources of protein for developing countries has led many individuals to research potentials of underutilized legumes to supply valuable nutrients of which Lyon beans are one but its utilization in food forms may be hindered as a result of the anti-nutritional factors present in the plant. These anti-nutritional factors have been reported to interfere with nutrient absorption and also cause a reduction in nutrient intake, digestion and utilization (Popova and Mihaylova, 2019). Consumption of food containing anti-nutritional factors may cause some specific symptoms in the human body such as nausea, rashes, bloating, and nutritional deficiencies among others (Essack *et al*., 2017).

In order to improve the nutritional content and reduce the anti-nutrient contents of Mucuna species, some common processing methods have been used such as soaking in portable water, heating in water, alkaline or acid solutions at elevated temperatures, germination, roasting, dehulling and fermentation (Esenwah *et al.,* 2010).

Fermentation has proven over the years to be one of the most effective processing methods to practically limit anti-nutritional factors of legumes (Onwurafor *et al*., 2014; Bello and Udo, 2017; Iheke *et al*., 2017). The knowledge of the optimum temperature-time combination to achieve the fermentation of the beans will give a clear profile about how to eliminate anti-nutrients and increase the utilization of this under-utilized crop. Therefore, this study was carried out to

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determine the optimum fermentation temperature-time combination than could effectively reduce or eliminate anti-nutritional factors in *Mucuna cochinchinensis.*

2. Materials and methods

2.1 Fermentation and preparation of the seed flour

Samples of *Lyon beans* were purchased from Iddo market, Yaba, Lagos, Nigeria. Lyon bean was sorted to discard damaged or immature seeds. The seeds were portioned in two parts. The first part was further portioned into two. One portion was milled whole, sieved using a 0.5 µm mesh size, packaged in zip lock bags and stored at 4°C until further analysis. The second portion was dehulled, milled, sieved using 0.5 µm, packaged in zip lock bags and stored at 4°C until further analysis. The second part was further divided into twelve portions; the first six portions used as un-dehulled and the second six were dehulled for the fermentation process (Table 1). The seeds were soaked separately in sterile water for 10hrs, drained, and fermented in a perforated polyethylene bag at 30°C and 45°C for 24 hrs, 48 hrs and 72 hrs respectively. Fermentation was carried out in a Gallenkamp Incubator (INC200 Plus Series) and temperature range applicable to each sample was kept constant. The fermented seeds were dried in a cabinet dryer (D28, Nigeria) at 60°C, milled, sieved using sieve with mesh size 0.5 µm, packaged in zip lock bags and stored at 4°C until further analysis. The unfermented beans were used as control samples (modified method of Udensi *et al*., 2006).

Table 1. Sample codes with fermentation time and temperature combinations

Sample codes	Treatment
$WUMC_1$	Whole unfermented *mucuna bean*
$DUFMC_2$	Dehulled unfermented *mucuna bean*
$UFMC_3$	Un-dehulled Fermented at 30°C for 24 hrs
$UFMC_4$	Un-dehulled fermented at 30°C for 48 hrs
$UFMC_5$	Un-dehulled Fermented at 30°C for 72 hrs
$UFMC_6$	Un-dehulled Fermented at 45°C for 24 hrs
UFMC7	Un-dehulled fermented at 45°C for 48 hrs
$UFMC_8$	Un-dehulled Fermented at 45°C for 72 hrs
$DFMC_9$	Dehulled fermented at 30°C for 24 hrs
$DFMC_{10}$	Dehulled fermented at 30°C for 48 hrs
$DFMC_{11}$	Dehulled fermented at 30°C for 72 hrs
$DFMC_{12}$	Dehulled fermented at 45°C for 24 hrs
$DFMC_{13}$	Dehulled fermented at 45°C for 48 hrs
$DFMC_{14}$	Dehulled fermented at 45°C for 72 hrs

2.2 Determination of proximate composition

Proximate constituents (moisture, ash, crude fat, crude fibre, and protein) contents were assessed as per AOAC (2005), and carbohydrates were determined by difference.

2.3 Anti-nutritional content

Selected anti-nutrients that were determined were phytic acid, oxalic acid and tannin contents. Phytic and Oxalic acid contents were determined using the AOAC (2005) procedure while the method described by Kirk and Sawyer (1998) was used to determine tannin content.

2.4 Statistical analysis

Data obtained were subjected to analysis of variance (ANOVA) using SPSS version 21.0 and the differences between significant mean values were evaluated at $p < 0.05$ probability level using Duncan's Multiple Range Test.

3. Result and discussion

3.1 Proximate composition

The results of the proximate content of unfermented and fermented beans are presented in Table 2. Crude protein and carbohydrate have been attributed to be the major proximate constituents of legume samples. The protein content determined ranged between 19.97±0.08 and 36.41±0.025. The 36.41±0.25 obtained from the sample (dehulled fermented at 30°C for 72 hrs) was higher than reports by other researchers of some other common legumes with a range of 18.5 to 21.9% (Costa *et al.,* 2006; Mugendi *et al.,* 2010). The protein content of Lyon bean was found to be within the range reported for cowpea at 29.3% (Tresina and Mohan, 2013). As a result of this high crude protein content of the samples investigated, it is expected that Lyon beans will be useful as an alternative protein source.

The moisture content of the Lyon beans ranged between 5.10±0.11 and 10.62±0.23 with dehulled fermented at temperature 45°C for 48 hrs having the least and raw unfermented Lyon bean seeds having the highest. Percentage fat content determined ranged between 4.06±0.15 and 10.44±0.69 with dehulled fermented Lyon bean seed having the least and unfermented Lyon bean seed the highest. This observed rise in the fat content may be because of soaking the seeds for 10 hrs which caused the breakdown of the protein-lipid or carbohydrate-lipid bonds which has been reported to give rise to easy fat extraction of legumes (Omafuvbe *et al.,* 2004).

Crude fibre content of all sample ranged between 0.79±0.08 to 4.83±0.21 with the unfermented Lyon bean seed having the highest and dehulled fermented Lyon bean the least. The variation in the results observed in the dehulled fermented beans may be due to total

Table 2. Proximate composition of the Lyon beans samples

Sample	Moisture Content (%)	Protein (%)	Fat (%)	Crude Ash (%)	Crude Fiber (%)	Carbohydrate (%)
WUMC1	10.62±0.23^{a}	22.19±2.06^{b}	4.94±0.11^{d}	3.53±0.27^{c}	1.40±0.22^{e}	57.34±2.23^{ab}
DUFMC2	10.5±0.18^{b}	26.66±0.30^{c}	8.66±0.26^{a}	5.10±0.05^{bc}	1.22±0.10^{d}	47.81±0.52^{e}
UFMC3	9.22±0.08^{c}	22.43±0.52^{a}	10.09±0.05^{b}	4.39±0.09^{d}	3.91±0.04^{ac}	49.98±0.51^{b}
UFMC4	8.83±0.28^{ab}	24.84±0.26^{ab}	10.44±0.69^{c}	3.32±0.05^{cd}	2.16±0.08^{bc}	50.43±1.36^{ac}
UFMC5	8.76±0.30^{ac}	32.69±0.51^{bc}	7.56±0.13^{c}	5.29±0.07^{a}	3.20±0.16^{ae}	42.51±0.57^{be}
UFMC6	7.83±0.21^{be}	22.91±0.17^{d}	10.07±0.08^{ac}	3.78±0.05^{b}	2.67±0.08^{bd}	52.75±0.33^{d}
UFMC7	7.78±0.08^{bc}	22.10±0.05^{ac}	8.00±0.18^{bc}	3.07±0.08^{ab}	4.83±0.21^{a}	54.23±0.52^{c}
UFMC8	6.77±0.21^{be}	35.52±0.84^{ad}	7.49±0.34^{ad}	3.10±0.04^{cd}	4.33±0.09^{c}	42.80±1.34^{ac}
DFMC9	8.05±0.25^{ae}	32.65±0.34^{bd}	4.06±0.15^{ab}	4.44±0.20^{ac}	0.91±0.03^{ce}	49.90±0.07^{bd}
DFMC10	9.22±0.26^{ae}	19.97±0.08^{ae}	7.67±0.12^{bd}	3.42±0.06^{cd}	1.45±0.13^{de}	58.29±0.54^{de}
DFMC11	8.79±0.21^{d}	36.41±0.25^{be}	8.33±0.45^{ae}	4.85±0.23^{e}	0.79±0.13^{ce}	40.85±0.35^{ac}
DFMC12	12.71±0.28^{ad}	29.12±0.47^{cd}	9.27±0.14^{be}	4.76±0.18^{ae}	0.29±0.11^{ce}	43.86±1.17^{ad}
DFMC13	5.10±0.11^{e}	24.69±0.21^{ae}	5.66±0.14^{c}	3.35±0.21^{be}	3.38±0.16^{be}	57.82±0.83^{cd}
DFMC14	7.46±0.26^{bd}	31.47±0.23^{e}	10.79±0.31^{cd}	3.66±0.18^{ce}	0.90±0.08^{cd}	45.73±0.61^{a}

Mean values with different letters in a column are significantly different at 95% confidence level (p-value < 0.05).

breakdown of the endosperm of the legume which normally houses the fibrous material of the beans. The crude fibre content in both fermented and unfermented samples was significantly different from the dehulled and fermented samples lower than unfermented samples.

Crude ash content of the investigated Lyon bean ranged between 3.07±0.08 and 5.10±0.05. Results were similar to reports in the literature for mucuna varieties/ species (4.78 – 5.30%) (Kala and Mohan, 2010). It was within the range (3.4 – 4.0%) reported for beans but lower than values (9.8%; 10.4%) reported for chickpeas and peas (Costa *et al*., 2006; Tresina and Mohan, 2013). The carbohydrate content was found to range 40.85±0.35 to 58.29±0.35. Significant differences (P < 0.05) were observed between the results of carbohydrate in the fermented and unfermented samples.

Results for dehulled Lyon bean seeds were observed to be higher than that of un-dehulled beans and this could be attributed to the effect of the soaking and dehulling processing methods which have been reported to improve the nutritional content of legumes (Oghbaei and Prakash, 2016).

3.2 Anti-nutritional content

Results of the anti-nutritional content of unfermented and fermented beans are presented in Table 3. The anti-nutrients content of the fermented beans flour was observed to be lower than that of the unfermented beans flour samples. Oxalate content ranged between 0.316±0.001 mg/g to 1.708±0.005 mg/g with dehulled fermented at 45°C for 48 hrs having the least and the highest raw unfermented Lyon beans. There was no significant difference between undehulled fermented at 30°C for 24 hrs and undehulled fermented at 45°C for 24 hrs ($UFMC_3$ and $UFMC_6$) but there was a significant difference among the remaining samples.

Table 3. Result of the anti-nutrients of the Lyon beans samples

Sample	mg/g Oxalate	% Phytic acid	%Tannin
WUMC1	1.708±0.005^{a}	1.971±0.060^{e}	1.868±0.052^{b}
DUFMC2	1.548±0.005^{b}	1.787±0.054^{a}	1.814±0.050^{c}
UFMC3	1.426±0.004^{cd}	1.691±0.051^{e}	1.697±0.047^{d}
UFMC4	1.387±0.004^{c}	1.503±0.046^{b}	1.658±0.046^{a}
UFMC5	1.233±0.004^{ab}	1.490±0.045^{be}	1.638±0.045^{de}
UFMC6	0.777±0.002^{cd}	1.456±0.044^{c}	1.550±0.043^{ab}
UFMC7	0.630±0.002^{ac}	1.701±0.052^{ab}	1.608±0.045^{bc}
UFMC8	0.546±0.002^{be}	1.496±0.046^{bc}	1.388±0.038^{cd}
DFMC9	0.440±0.001^{bc}	1.435±0.044^{ac}	1.300±0.036^{ad}
DFMC10	0.424±0.001^{d}	1.408±0.043^{e}	1.280±0.035^{bd}
DFMC11	0.411±0.001^{ce}	1.399±0.043^{cd}	0.868±0.024^{e}
DFMC12	0.330±0.001^{ce}	1.385±0.042^{ae}	0.618±0.017^{ce}
DFMC13	0.316±0.001e	1.378±0.042^{de}	0.579±0.016^{ae}
DFMC14	0.321±0.001^{de}	1.353±0.041^{e}	0.515±0.014^{ae}

Mean values with different letters in a column are significantly different at 95% confidence level (p-value < 0.05).

The phytic acid content ranged between 1.971±0.060 to 1.353±0.041% with dehulled fermented seeds having the least and the raw *mucuna* seed having the highest. The high phytate content (1.353±0.041) of the dehulled fermented Lyon bean samples could be as a result of their high prevalence in the cotyledons. In legumes, phytates have been reported to bind multivalent cations thereby reducing the bioavailability of mineral elements present in foods. They were also known to form complexes with protein and starch, thereby inhibiting the enzymatic digestion of starch and protein (Oatway *et al.,* 2001).

The tannin content ranged from 1.868±0.052 to 0.515±0.014 with dehulled fermented *mucuna* seed at 45°C for 72 hrs having the least and the raw Lyon bean seed having the highest. There was no significant difference between dehulled fermented *mucuna* bean at 45°C for 48h and at 45°C for 72 hrs but there is significant difference among all other samples.

As fermentation time increased, the anti-nutrients content reduced. The dehulled fermented Lyon beans flour samples were found to have lower values for anti-nutrients compared with the un-dehulled fermented Lyon beans flour samples. It has been reported that combining several pre-processing treatments such as soaking, dehulling, fermentation and milling, reduces anti-nutrient content in legumes. This also indicates that the anti-nutrients are more concentrated in the hull (Oghbaei and Prakash, 2016).

4. Conclusion

This research work has shown fermentation significantly increased the protein and fat contents while also reducing the anti-nutrients content of the flour. The optimal fermentation time and temperature for Lyon beans flour with good nutritional properties as well as reduced anti-nutrients content were at 45°C for 72 hrs. This study confirms dehulling and fermentation improved the nutritional quality of Lyon beans flour and this can enhance the utilization of the beans.

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