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EFFECT OF BOILING AND SOAKING DURATIONS ON THE PROXIMATE COMPOSITION, RICIN AND MINERAL CONTENTS OF UNDECORTICATED CASTOR OIL SEEDS *(RICINUS COMMUNIS)*

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ABSTRACT: Boiling and soaking methods for different time durations were employed in processing castor oil seeds *(Ricinus communis*) to improve its nutritive value. These processing methods were boiling for 0, 10, 20 and 30 minutes, where part of the samples from each lot were further soaked for 0, 24, 48, 72 and 96 hours before sun drying for four days, the beans were then milled, and a representative sample of the milled (castor oil seeds) from each of the processing methods along with a raw sample of the beans were taken to the laboratory for ricin, minerals and proximate analysis according to A.O.A.C. (1995). There were statistically evaluated using a 4x5 factorial format in a completely randomized design. Results indicated that boiling and soaking the castor oil seeds for different time durations affected the proximate composition, mineral and ricin component of the test ingredient. Boiling the seeds for more than 10 minutes resulted in significant ($P<0.05$) decrease in crude protein, ether extract and ricin content of the seeds, but with overall improvement in the Nitrogen Free Extract (NFE) of the test ingredient. Soaking the seeds for different time durations brought about decrease in the crude protein content, but became only significant at 72 and 96hours of soaking. NFE also showed significant improvement at 72 and 96 hours of soaking the seeds. Among the minerals only calcium and magnesium were affected by boiling durations, with a significant ($P<0.05$) decrease at 30 minutes of boiling. The interaction effect showed significant ($P<0.05$) decrease of crude protein content in 10 minutes boiled seeds that were soaked for 48 hours. 30 minutes boiled seeds that were soaked for 48 hours showed a significant ($P<0.05$) decrease in crude fibre content. Ether extract decreasing effect was significant ($P<0.05$) with seeds boiled for 10 minutes and soaked for 48 hours. Ash decreasing effect was significant ($P<0.05$) with 30 minutes boiled and 48. 72 and 96 hours soaked seeds. NFE improved significantly ($P<0.05$) from 20 minutes boiled, followed by 72 hours soaked seeds. Calcium and Magnesium component of the seeds values depressed significantly ($P<0.05$) with seeds that were subjected to 30 minutes boiling followed by 48, 72 and 96 hours of soaking. The ricin content of the seeds significantly($P<0.05$) reduced with seeds boiled for 10 minutes followed by 0, 24, 48, 72 and 96 hours, and continued to reduce as boiling and soaking duration increased, with seeds that were boiled for 30 minutes and soaked for 96 hours having no ricin content. With appreciable level of crude protein (22.08%) and Nitrogen free extract (51.07%) and highest percentage reduction in ricin-the most potent toxin in castor oil seed, the seeds boiled for 30 minutes followed by soaking for 96 hours are therefore recommended as a treatment method for castor oil seed, as feedstuff in livestock feed.

Keywords: Ricin, castor oil seed, boiling and soaking.

INTRODUCTION

Conventional feedstuffs especially protein ingredients in Nigeria are becoming increasingly scarce and expensive. This is attributed to the rising needs of man for the same livestock feedstuffs (Durunna *et al.,* 1999). As a result of these, animal nutritionists are searching alternatives which should be readily available, easy to procure and process into usable forms, have a comparable cost advantage and must not be a stable item for human consumption (Okonkwo and Adikpe, 1988: Oyebiye *et al*., 2007)

One of the envisaged alternative feedstuffs is castor oil seed bean (COSB). It belongs to the family of Europhorbiaceae (Spurge family) and grows naturally over a wide range of soils that are deep and well drained. Under favorable condition it yields about 20-25 bushels (363kg-454kg) of seed per acre (Hill, 1982: Simpson and Ogarzarly, 1986). It grows virtually everywhere in the southern part of Nigeria and possesses as much agronomic and nutritional potentials just as the conventionally used legumes. In most parts of the southern states, the seed products are used as seasoning agents popularly known as "Ogiri" in Ibo vernacular. "Ogiri" is gotten by first boiling the seed to remove the seed coat, it is then ground and tied in small bits in leaves in which it is fermented for 2-3 days to produce the "Ogiri" (Okorie *et al.*, 1985).

Several workers have reported on the proximate and chemical composition of castor oil seeds, Okorie *et al.*, 1985 gave the values of crude protein of 32.18%, 11.40% crude fibre, 15.50% ether extract, 31.40% for nitrogen free extract, 0.008% calcium and 0.26% phosphorus. Harnold (2002) reported 21-48% for crude protein, the wide range was said to be dependent on the extent of decortications. The seeds, the leaves and stems of castor plant are poisonous to people, animals and insect, one of the main toxic protein is ricin. Ricin is an extremely toxic phytotoxin found in castor oil plant. It is believed to have cytostatic and then cytotoxic effects in the cells (Lugmer, 1952, Mise *et al.,* 1971). Ricin is an alkaloid, a potent goitrogen (Pahuja *et al.,* 1978). The toxin is said to exhibit antitrypsin action as found in some legumes like soybean (Clark and clark, 1975).

As the result of the presence of ricin, a potent poison contained in raw castor oil seeds, studies on the feeding value of the meal have been limited, and however, several researchers on the detoxification of the bean through various means have provided many encouraging results (Ensminger, 1996). However, many methods of detoxifying the meal end up destroying the nutrient content of the meal. Wet heat treatments are known to be effective means of inactivating ricin (Harnold, 2002, Nsa and Ukachukwu, 2009).

The objective of this study is to determine and compare the nutritive value of castor oil seeds subjected to different durations of boiling and soaking using their proximate composition, minerals and ricin contents.

MATERIALS AND METHODS

Processing of seeds

Castor oil seeds (*Ricinus communis*) were purchased from open market in Ogoja Local Government Area of Cross River State, Nigeria. The processing methods adopted were as follows: for the boiled castor oil seeds, about 15 liters of water was first brought to boiling (100^{o}C) in a 30 liter aluminum cooking pot and about 2000g of the beans were poured into the water and covered. From the moment the beans were poured into the water, the specific time for boiling was taken. The beans were boiled for 10, 20 and 30 minutes. Samples from each lot were further soaked for 24, 48, 96 and 72 hours respectively. The beans were not stirred neither was the water changed for the duration of the soaking periods. All the processed seeds were sundried for 5 days and then milled. Representative samples of the milled castor oil beans from each of the processing methods along with a raw sample were taken to the laboratory for analysis.

Analytical procedure

Proximate composition, mineral constituents and ricin content

Determination of proximate composition of the sample was by AOAC (1995) procedure: Crude protein content of the sample was determined by Kjeldahl method: Twenty milligram of sample was digested with concentrated tetraoxosulphate VI acid using Lithium tetraoxosulphate VI as catalyst. This oxidized the organic matter and the nitrogen present into ammonium tetraoxosulphate VI $(NH_4)_2.SO_4$. Excess caustic soda (NaOH) was added and the mixture distilled, expelling N_2 and NH_3, which were absorbed in 5.0ml of tetraoxosulphate VI acid. The excess acid was determined by titrating with 0.11M NaOH. The percentage crude protein was estimated by multiplying the titre with 6.25 and expressed as percentage of weight of sample before digestion.

The ether extract or lipid content of the sample was determined using Soxhlet extractor. Five grams of the milled sample was weighed and placed into a dry Soxhlet thimble suspended in a beaker. The beaker was placed in soxhlet condenser attached to a flask containing sufficient ether to fill the thimble. Heat was supplied to the flask by means of electric hot plate so as to keep the ether gently boiling. The ether vapour passes up the side tube of the extractor to the reflux condenser where it is condensed and returned back into the sample in a thimble. When the thimble is practically full, the ether is returned to the flask by an automatic siphoning device carrying with some of the fat from the sample. The siphoning process was terminated after the 24^{th} time, just before the next lot of ether entered the point of siphoning over. The flask was dried of in the air and the fat washed out of the soxhlet flask by chloroform. It was dried and weighed again. The percentage ether extract was estimated by expressing the weight of lipid as percentage of sample weight before extraction.

The crude fibre (Structural carbohydrate) was determined by defatting 20 grams of the sample with petroleum ether. The sample was boiled first with 1.25% dilute tetraoxosulphate VI acid and washed with distilled water. The same sample was again boiled with 1.25% potassium hydroxide (KOH) with boiling lasting for 30 minutes. The insoluble residue was separated by filtration, washed, dried, weighed and ashed. The loss of weight resulting from ashing was expressed as percentage of the sample weight before ashing. The crude ash was determined by igniting muffled furnace of a temperature of 600^{o}C. The ashing was terminated on formation of white ash from the sample. The ash formed was cooled in a desicator and weighed. The weight after ashing is expressed as a percentage crude ash of the sample.

Minerals

The mineral elements selected for determination were Potassium, Calcium, Magnesium, Iron and Phosphorus. Determination of Potassium was by flame photometry (AOAC, 1995). Calcium, Magnesium and Iron contents were determined by atomic absorption spectrophotometer as laid down by AOAC (1995) while the determination of Phosphorus was by Ammonium molybdate method as modified by Fiske and Sorbbarow (1925), using Hydroquinone as a reducing agent.

Ricin

Ricin was extracted and isolated by methods similar to Mise *et al.* (1971). The potent ricin component was extracted from the ground castor seed meal with methanol under reflux for 24 hours.

Statistical analysis

All data were subjected to a two-way analysis of variance using a 5x4 factorial format in a completely randomized block design and significantly different treatment means were separated using Duncan Multiple Range Test at 0.05 probability level (Duncans, 1955).

RESULTS AND DISCUSSION

Boiling and soaking of castor oil seeds for various time durations affected the proximate composition, mineral content and ricin content of the castor oil seed (*Ricinus communis*), Table-1

Table1. Effect of boiling duration on the proximate composition, minerals and ricin content of undecorticated castor oil seeds (*Ricinus communis)*

Components	Control Raw	Duration of boiling(in minutes) 10	20	30
% Crude protein(CP)	30.82[a]	30.46[a]	26.65[b]	24.76[c]
% Crude fibre(CF)	11.42	11.37	11.33	11.20
% Ether extract(EE)	20.72[a]	19.73[b]	18.97[c]	18.44[c]
% Ash	5.54	4.87	4.82	4.61
% NFE	31.16[c]	33.55[c]	38.23[b]	40.87[a]
Ricin(mg/100g)	4.37[a]	0.66[b]	0.21[c]	0.08[d]
Calcium(mg/100g)	0.74	0.72	0.71	0.71
Magnesium(mg/100g)	0.41[a]	0.36[ab]	0.35[ab]	0.31[b]
Iron(mg/100g)	0.02	0.02	0.02	0.02
Phosphorus(mg/100g)	0.13	0.09	0.08	0.08

Values in the row differently superscripted are significantly different at 5% level

Table 2. Effect of soaking duration on the proximate composition, minerals and ricin content of undecorticated castor oil seed (*Ricinus communis.)*

Components	Control raw	Soaking duration (in hours) 24	48	72	96
%Crude protein	30.96[a]	29.76[a]	27.80[ab]	26.11[b]	26.53[b]
% Crude fibre	11.39	11.34	11.32	11.31	11.29
%ether extract	19.92	19.75	19.58	19.19	18.92
% Ash	5.25	5.04	4.98	4.82	4.73
% NFE	32.48[b]	34.09[ab]	35.38[ab]	37.83[a]	39.97[a]
Ricin(mg/100g)	1.53	1.45	1.37	1.22	1.08
Calcium(mg/100g)	0.77	0.73	0.72	0.71	0.68
Magnesium(mg/100g	0.44	0.36	0.35	0.33	0.31
Iron(mg/100g)	0.02	0.02	0.02	0.02	0.02
Phosphorus(mg/100g)	0.13	0.10	0.09	0.08	0.07

Values in the row differently superscripted are significantly different at 5%level

Percentage Crude protein (CP)

Boiling of castor oil seeds up to 10 minutes resulted in non-significant decrease in CP, but boiling above this minutes resulted in significant ($P<0.05$) decrease in the CP with 30 minutes boiled seeds having the least significant ($P<0.05$) CP content. Soaking durations also brought about decrease in CP, which was only significant with seeds soaked for 72 and 96 hours. The interaction effect revealed a general decrease in the crude protein as the duration of boiling and soaking increased but became significant ($P<0.05$) in seeds boiled up to 10 minutes followed by 48 hours of soaking. The least value (22.08 %) was obtained for seeds boiled for 30 minutes followed by soaking for 96 hours. The observed decrease, as boiling and soaking duration increased could be attributed to some solubilisation and leashing out of nitrogenous substances into the water. This accounts for the longer duration of boiling and soaking, the more the lost of CP.

This explains the lowest value of 22.08 for 30 minutes boiled seeds that were further soaked for 96 hours, and highest value of 30.82 % for raw seeds that were not treated. Iyayi and Egharevba (1998), Nestares *et al.* (1996), Mbajunwa (1995), Akinmutimi, 2007 have all reported similar decrease, when they worked with different legumes. They attributed it to the loss of soluble or proteinous parts of their seeds into cooking water. The values obtained even after exposing the seeds to boiling and soaking durations compared favorably with other legumes like cooked Lima bean, boiled jack bean and boiled sword bean of crude protein content of 21.50%, 25.88% and 25.83%, respectively (Akinmutimi, 2004b) the crude protein values also fell within the value of a protein supplement as prescribed by Olomu, (1995).

Percentage Crude fibre(CF)

There was a general non-significant ($P<0.05$) decrease in CF values as the duration of boiling and soaking increased. The interaction effect showed a significant ($P<0.05$) decrease in CF value only with seeds boiled for 30 minutes followed by soaking for 72 and 96 hours. The reduction in crude fibre levels as duration of boiling increased could be due to softening and subsequent loss of hard coat of some of the seeds in course of boiling and decanting of water. The testa is high in fibre; loss of it means decrease in CF. This observation is in line with the report of Akinmutimi (2004b), Akinmutimi (2007), Nsa *et al.* (2010) who found same for *Mucuna utilis, Mucuna pruriens* and castor oil seeds respectively. Depending on the class and species of animal, a level of fibre is needed to facilitate digestion, and sometimes needed for dilution of concentrate feeds. However, the CF values obtained upon subjecting the seeds to the treatment methods are still high as grain seed for monogastric animals, dehulling the seed or little or no other fibre source should be used when using the seeds in is in non-ruminant feed. High fibre has been found to contribute to reducing feed intake in chickens (Abdelsamie *et al*., 1983, Ani and Okorie, 2004)

Percentage Ash

Boiling durations and soaking durations showed a non- significant ($P>0.5$) decrease in ash content as both durations increased. The interaction effect showed a significant ($P<0.05$) decreased at after 30 minutes of boiling followed by 48, 72 and 96 hours of soaking, 20 minutes of boiling, followed by 0, 24, 48, 72 and 96 hours of soaking, 30 minutes of boiling followed by 0, 24, 48, 72 and 96 hours of soaking. The reduction in the ash content as boiling and soaking durations increased could be attributed to the leaching of the mineral elements into the water. This agrees with the findings of Udedibie and Mba (1994), Ukachukwu and Obioha (2000), who all attributed it to the dehulling effect of boiling treatment which must have predisposed the seeds to some kind of leaching of some of its mineral elements.

Percentage Ether Extract (E.E)

The EE decreased as duration of boiling increased, but only became significant ($P<0.05$) at 20 and 30 minutes of boiling the seeds. Soaking durations did not have any significant ($P>0.05$) effect on the EE content of the seed, the interaction effect, showed significantly ($P<0.05$) decrease of seeds boiled for 30 minutes of followed by 24, 48, 72 and 96 hours of soaking. The observation could be attributed to solubilisation and leaching of oil in the water. This observation is in agreement with that of Udedibie *et al.* (1996), Balogun *and Fetuga (1986*); Bawa *et al.(* 2003) who worked on *Mucuna utilis* and Soybean respectively. The least value of oil still left in the seed was far higher than the range of values for conventional protein sources such as soybean meal (1.5%) and groundnut meal (3.2%) (Olomu, 1995), and can impose problem of rancidity to rations if the seed is not defatted or antioxidant added.

Percentage Nitrogen Free Extract (NFE)

Boiling and soaking durations of castor oil seeds resulted in significant (P<0.05) improvement in the NFE of the seeds. The value of NFE appreciated as duration of boiling and soaking increased, of which 30 minutes boiled seeds that were further soaked for 96 hours having the highest value of 43.96 %.

This observation could be attributed to the loss of testa in the boiling water which conversely led to increase in NFE, which is mainly the more digestible component of the test ingredient. The improvement in the NFE should also be due to the lower levels of other proximate components (crude protein, crude fibre, ash and ether extract) this should normally increase the level of NFE which is obtained by subtracting other components from 100.

Minerals (mg/100g)

Apart from iron, there was a general decrease in the mineral content of the seed as durations of boiling and soaking increased.

Calcium, Phosphorus and Magnesium (Ca, P and Mg)

Calcium values decreased (P>0.05) as duration of boiling and soaking increased. Also there was anon-significant decrease in the Phosphorus values as boiling and soaking durations increased. Magnesium level followed similar trend, but significant (P<0.05) decreased was noticed for seeds that were boiled for 30 minutes followed by soaking for 24, 48, 72 and 96 hours. By soaking and heating similar decreases were recorded for jackbean (Esonu *et al.,* 1998) and for *Mucuna utilis* (Iyayi and Egharevba, 1998). The decrease could be due to leaching out of minerals into water.

Iron

Iron was not affected by the boiling and soaking duration neither the interaction effect, an indication that iron was not leached in the cause of boiling or soaking. This agreed with the report of UKorebi (2002) where iron content of *Milletia obanensis* remained unchanged during boiling or soaking.

Ricin (mg/100g)

Generally, processing significantly (P<0.05) reduced the ricin content of the seeds. The reduction increases as the duration of boiling and soaking increases. Boiling durations had more effect on the ricin content than soaking durations. It varied from 0.0g/kg for seeds that were boiled for 30 minutes followed by soaking for 96 hours to 4.46g/kg of the raw seeds. 10 minutes boiling resulted in drastic drop in ricin content up to 84.50%, the remaining boiling durations had statistically similar values that were significantly (P<0.05) lower than 10 minutes boiled seeds. The 0% ricin content of 30 minutes boiled seeds followed by 96hours of soaking is an indication that boiling followed by soaking for 96 hours can completely eliminate ricin content in castor oil seeds. Wet heat treatments are known to be effective means of inactivating ricin (Harnold, 2002, Nsa, 2008).

CONCLUSION

With appreciable value of 22.22% for crude protein and Nitrogen value of 55.5%, highest reduction in ricin content for the seeds boiled for 30 minutes followed by 96 hours soaking, this treatment method is therefore recommended.

Table 3. Interaction effect of boiling and soaking on the proximate composition, minerals and ricin contents of undecorticated castor oil seed *(Ricinus communis*)

Treatment combination									
	% Crude Protein	%crude fibre	% Ether extract	%Ash	% NFE	Ricin (mg/100g)	Ca (mg/100g)	Mg (mg/100g)	Iron (mg/100g)
B_0S_{24}	30.82[a]	11.42a	20.72[a]	5.54a	31.50[c]	4.37[a]	0.74	0.41[a]	0.02
B_0S_{48}	30.79[a]	11.42a	20.69a	5.54a	31.56[c]	4.19[a]	0.74	0.41[a]	0.02
B_0S_{72}	30.73[a]	11.41a	20.69a	5.51a	31.66[c]	4.14[a]	0.71	0.41[a]	0.02
B_0S_{96}	30.61[a]	11.41a	20.69[a]	5.48a	31.81[c]	4.03[a]	0.69	0.40[a]	0.02
$B_{10}S_0$	30.53[a]	11.37a	19.73[a]	4.87ab	33.55[bc]	0.66[b]	0.72	0.36[ab]	0.02
$B_{10}S_{24}$	30.46[a]	11.37a	19.73[a]	4.86ab	33.58[bc]	0.61[b]	0.73	0.36[ab]	0.02
$B_{10}S_{48}$	30.31[a]	11.35a	19.06ab	4.86ab	33.77[bc]	0.58	0.72	0.35[ab]	0.02
$B_{10}S_{72}$	28.15[b]	11.35a	19.05ab	4.86ab	36.02[b]	0.44[b]	0.71	0.34[ab]	0.02
$B_{10}S_{96}$	28.13b	11.34a	19.03ab	4.85ab	36.05[b]	0.37[b]	0.67	0.3a1[b]	0.02
$B_{20}S_0$	26.65bc	11.33a	18.97ab	4.82ab	38.23b	0.21[b]	0.71	0.35a[b]	0.02
$B_{20}S_{24}$	26.18bc	11.30a	18.97ab	4.79ab	38.76[b]	0.18[b]	0.73	0.33a[b]	0.02
$B_{20}S_{48}$	25.17bc	11.28a	18.88ab	4.79ab	39.88[b]	0.16[b]	0.71	0.33a[b]	0.02
$B_{20}S_{72}$	24.93c	11.23a	18.68ab	4.73ab	40.43b	0.13[b]	0.70	0.31a[b]	0.02
$B_{20}S_{96}$	24.84[c]	11.21a	18.61ab	4.71ab	40.63b	0.11[b]	0.67	0.31a[b]	0.02
$B_{30}S_0$	24.76c	11.20a	18.44ab	4.61ab	40.88b	0.008[b]	0.71	0.30a[b]	0.02
$B_{30}S_{24}$	23.44cd	11.18a	15.32b	4.59ab	45.47ab	0.03b	0.72	0.30a[b]	0.02
$B_{30}S_{48}$	22.67d	11.16a	15.29b	3.05b	47.83ab	0.01[b]	0.71	0.23[b]	0.02
$B_{30}S_{72}$	22.41d	9.13b	14.96b	3.02b	49.52a	0.01[b]	0.70	0.22[b]	0.02
$B_{30}S_{96}$	22.08d	9.10b	14.21b	2.91b	51.70a	0.00[b]	0.67	0.22[b]	0.02

Values in the column differently superscripted are significantly different at 5% level.
B-represents boiling and the subscripts indicate duration of boiling in minutes
S-represents soaking and the subscripts indicate duration of soaking in hours

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