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Effects of Processing Methods on Anti-Nutrient Levels of Ricinus Communis L

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ABSTRACT

The anti-nutritional values in raw, autoclaved, boiled, fermented, soaked and toasted castor oil seeds (Ricinus communis L.) collected from Ado-Ekiti metropolis, Nigeria were 3CSCA$_{30}$ and CSCA$_{40}$) for autoclaving; 40, 50 and 60 min (CSCB$_{40}$, CSCB$_{50}$ and CSCB$_{60}$) for boiling; 48, 72 and 96 h (CSCF$_{48}$, CSCF$_{72}$ and CSCF$_{96}$) for fermenting; 48, 72 and 96 h(CSCS$_{48}$, CSCS$_{72}$ and CSCS$_{96}$) for soaking and 20, 30 and 40 min (CSCT$_{20}$, CSCT$_{30}$ and CSCT$_{40}$) for toasting. The raw sample which served as control was tagged CSCR$_{00}$. The 15 processed and the raw samples were decorticated, after the wet samples (except toasted) had been sundried for four days, ground and then representative samples were taken to the laboratory for anti-nutrients determination according to AOAC (2006), Abeza et al., (1968), Reddy et al., (1982) and Wang et al., (1996) (tannin, oxalate, phytate and lectin respectively). Samples exhibited significant difference ($p<0.05$) compared to the control sample and among one another in terms of all the parameters examined. There was reduction in phytate, oxalate, tannin and lectin with increase in temperature of all the treatment methods adopted. Owing to the drastic reduction in virtually all the parameters examined, castor seed sample autoclaved for 30 min (CSCA$_{30}$) was considered best level among others. It was therefore recommended for fish feed formulation.

Keywords: *Castor oil seed, R. communis, phytate, oxalate, tannin and lectin.*

INTRODUCTION

Castor oil plant (*Ricinus communis* L.) is a species of flowering plant in the spurge family, Euphorbiaceae. It is indigenous to the south eastern Mediterranean Basin, Eastern Africa, and India. Castor seed is the source of castor oil, which has a wide variety of uses. The seeds contain between 40% and 60% oil that is rich in triglycerides, mainly ricinolein. The seed contains ricin, a toxin, which is also present in lower concentrations throughout the plant. It is a fast-growing, suckering perennial shrub which can reach the size of a small tree, around 12 m or 39ft (Devendra and Mukhtar, 2014). It is easy to cultivate, early maturity, underutilized and grow all the year round (Akande *et al.*, 2011).

The major challenges to the use of this spurge plant as animal feed is the presence of toxic and anti-nutritional constituents. There are two categories of anti-nutrients viz, the protein and non-protein types. The non-protein category includes alkaloids, tannins, phytic acid, saponins, and phenolics, while protein category includes trypsin inhibitors, Chymotrypsin inhibitors, lectins, and antifungal peptides (Fereidoon, 2014). Anwa *et al*., (2007) reported high quantities of these substances do inhibit maximum use of the nutritive values embedded in legumes seed. Redondo *et al*., (2014) reviewed biological activities of tannins and animal responses to dietary tannins with a focus on animal nutrition and production. Schlesinger *et al*., (2014) reported tannins and phytic acids are more heat stable, but can be reduced by dehulling, soaking, or germination.

Lectins are glycoproteins found in all food plants such as seeds, legumes and grains (Atli, 2017) which possess an affinity for binding with specific sugar molecules and are characterized by their ability to combine with carbohydrate membrane receptors (Fereidoon, 2014).Ricin/lectin, an anti-nutritional compound in castor bean limits its use in animal diets, hence the need to determine the best treatment method for its detoxification and nutrient availability (Annongu and Joseph, 2008). Considering the escalating cost of aqua-feed

which accounts for 60-70% of production cost in fish farming (Balogun, 2013), attempts have been made to use castor seed as an alternative protein source in livestock feeds such as rat (Akande *et al*., 2011), rabbits (Adedeji*et al*., 2006) broiler chickens (Ani and Okorie, 2013 and Mustapha *et al*., 2015), cockerels (Oso*et al*., 2011) and layers (Olayemi *et al*., 2006, Adedeji, 2013). However, growth of fish would be suppressed due to poor intake of feed (Adebayo, 2017) if castor seed used in formulating its feed is not subjected to proper processing techniques such that the anti-nutrients embedded in it are reduced to the minimum.

Attempts have also been made to investigate the effects of processing methods such as boiling, soaking, fermentation and toasting on proximate contents of castor seed (Nsa *et al*., 2011; Ishiwu *et al*., 2015). Geetanjali *et al*,(2018) reported cooking, among other methods, is the activity by which food is prepared for consumption through heat application (either dry heat or moist heat). It involves a wide range of methods based on various customs and traditions, ease of resource availability, and affordability. There is death of information on detailed investigation of anti-nutritional contents of castor seed, particularly phytate, oxalate, tannin and lectin, using several levels of treatments (autoclaving, boiling, fermenting, soaking and toasting with three different levels for each method) simultaneously. This study therefore aims at evaluating anti-nutritional factors inherent in castor seed cake.

Materials and Methods

Study Area

The study was conducted at the Fishery Laboratory of the Department of Fisheries and Aquaculture Management, Faculty of Agricultural Sciences, Ekiti State University, Ado-Ekiti. Ado-Ekiti is in the Western tropical rain forest region of Nigeria, latitude 7.67ºN and longitude 5.25ºE and at an altitude of 431m above sea level. The mean average annual rainfall of Ado-Ekiti is about 1800mm. The mean monthly temperature is about 28ºC while mean monthly relative humidity is about 65% (Akinyemi *et al*., 2013) being in Ekiti State.

Experimental Set Up

The following experiments and analysis were carried out namely

- Detoxification of the raw *Castor* seeds (CS) using different treatment methods (boiling, roasting, soaking autoclaving and fermentation)
- Anti-nutrients determination of both the raw and processed castor seeds.

Sample Collection/Identification

Castor seeds from dehiscence mature capsules of the plants were fetched within Ado-Ekiti metropolis, Nigeria, and used for this research. The plant capsule and seed samples were identified at the Herbarium of the Department of Plant Science and Biotechnology, Ekiti State University, Ado-Ekiti.

Sample Preparation

The collected seed samples were sorted, screened and distributed into six batches based on different treatment methods namely: (i) raw seed, (ii) boiled, (iii) toasted, (iv) soaked, (v) autoclaved and (vi) fermented, respectively. For each of the treatments, apart from raw seeds, 3 levels/ranges were worked upon in order to ensure proper investigation (Balogun, 2011).

Processing of Untreated (Raw) Seeds

Two hundred grams (200g) of raw castor seeds were washed, decorticated, sundried, ground and used for proximate analysis and anti-nutrients determinations.

Boiling

According to Balogun *et al*., (2005), castor seeds are best detoxified by boiling between 40-60 min at 100ºC. 2kg of castor seed samples were boiled at 100ºC using tap water at the ratio of 1kg to 10 l of water in a 15 l metal cooking pot for duration of 60 min (Vadivel and Pugalenthi, 2007). A portion (600g) of the original seed samples was removed from the boiling water with a sieve at 40, 50 and 60 min intervals respectively using a stopwatch while the boiling continues (Balogun, 2011). Samples were sun-dried separately to a constant weight; decorticated, ground, oil extracted and packed in air tight polythene bags for the subsequent analysis.

Toasting

Toasting time ranges for castor seed as reported by Okorie and Anugwa (1987) and Nsa*et al*., (2013) between 20 and 30 min at 140ºC respectively. Toasting was done in this study at 20, 30 and 40 min using Okorie and Anugwa (1987) method. 2kg of raw sample of dry raw castor oil bean in a medium of sand was put in open pan at 140°C for 40 min. The beans and sand were stirred constantly to avoid charring using a hand shovel. The temperature of the sand medium was monitored using a 150°C

Celcius thermometer. Six hundred gram (600g) sample was removed at 20, 30 and 40 minutes intervals then spread separately and allowed to cool on clean trays placed on concrete slabs. The samples were deorticated, ground, defatted to form cake and then stored separately in tightly sealed and labeled polythene bags.

Soaking in Water

According to Nsa *et al*., (2011), the minimum and maximum duration of soaking CS ranges between 48-96 h. This study soaked castor seed at 48, 72 and 96 h. 2kg of Raw *RC* samples were soaked in a bowl containing tap water at room temperature (30 ± 2ºC) in seed to water ratio of 1:10(w/v) at the rate of 5kg to 10l (Vadivel and Pugalenthi, 2007). The samples were removed at the rate of 600g with a sieve at 48, 72 and 96 hours respectively and then spread separately on clean trays to sundry. Decorticating, grinding and oil extraction to enhance cake formation followed accordingly.

Autoclaving

According to Alagbaoso *et al*, (2015) the minimum and maximum minutes of autoclaving for castor seed ranges between 20-40 min. In this study, autoclaving of CS at 121ºC was carried out for 20, 30 and 40 min in order to examine the best level. 2kg raw seeds sample were parboiled for 2 minutes in water at 60ºC in order to ease dehuling. 600g was removed at each time interval. Samples were sun-dried separately to a constant weight, decorticated and oil extracted. The samples were then packed in air tight polythene bags for the subsequent proximate analysis.

Fermentation

According to Ishiwu *et al*., (2015), the minimum and maximum duration of fermentation of CS ranges between 48-96 h. This study fermented castor seed at 48, 72 and 96 h. 2kg of raw castor seed sample was used for the fermentation technique. Slightly warm water (60ºC) was poured on the seeds and then covered in an air-tight container (Ologhobo *et al.,* 1993). This allowed natural fermentation to take place; 600g was collected at the expiration of each time (48, 76 and 96 hour) interval were taken to the Laboratory of the Department of Fisheries and Aquaculture, Ekiti State University, for anti-nutrients determinations.

Determination of Anti-Nutrients

The sixteen samples of differently processed seeds were taken to Food Science Laboratory Faculty of Science, Ekiti State University, Ado-Ekiti for the determination of anti-nutritional factors. The anti-nutrients that were determined included Tannin, Total Oxalate and Phytic acid (Phytate).

Determination of Tannin

Tannin was determined using the standard method described by AOAC (1990). Two grams of the dried sample was boiled with 300 ml of distilled water. This was diluted in a standard volumetric flask and filtered through a non-absorbent cotton wool. A volume of 25 ml of the infusion was measured into a two- liter porcelain dish and titrated with 0.1N $KMnO_4$ (0.1N $KMnO_4$ was standardized against 0.1N oxalic acid) until the blue solution changed to green; then few drops of 0.1N $KMnO_4$ was added. The titer was multiplied by 0.0066235 to obtain the amount of tannin in the sample. The equation is given thus: 0.1N oxalic acid = 0.0066235 g tannin.

Determination of Total Oxalate

The total oxalic acid of the powdered samples was determined as described by Abeza *et al.* (1968), weighing of 2 grams of the sample into a 250 ml flask. Then, 190 ml distilled water and 10 ml of 6M hydrochloric acid were added. The mixture was warmed for 1 h on boiling water bath, cooled, transferred into 250 ml volumetric flask, diluted to volume and filtered. Four drops of Methyl red indicator were added followed by concentrated ammonia until the solution turned faint yellow. It was then heated to 100ºC, allowed to cool, and then filtered to remove precipitate containing ferrous ions. The filtrate was boiled and 10 ml of 5 % Calcium chloride was added with constant stirring. It was then allowed to stand overnight. The mixture was filtered through Whatman No 40 filter paper. Then the precipitate was washed several times with distilled water and transferred to a beaker and 5 ml of 25 % sulphuric acid was added to dissolve the precipitate. The resultant solution was maintained at 80ºC and titrated against 0.5 % potassium permanganate until the pink color persisted for approximately one min. A blank was also run for the test sample from the amount of $KMnO_4$ used, the oxalate content of the unknown sample was calculated using the equation below:

1 ml of $KMnO_4$ = 2.24 mg oxalate

Determination of Percentage Phytate

A known weight of each ground sample was soaked into 100ml of 2% HCl for 5 h and filtered. Twenty-five cubic centimeters (25cm^3) of the filtrate was taken into a conical flask and

five cubic centimeters ($5cm^3$) of 0.3% ammonium thiocyanate solution was added. The mixture was titrated with a standard solution of$FeCl_3$ until a brownish – yellow color persisted for 5 min (Reddy *et al.*, 1982).

The concentration of the $FeCl_3$ was 1.04% W/V

Calculation mole ratio of Fe to phytate = 1:1

Concentration of phytate phosphorous= $\frac{Titre\ value \times 0.064}{1000 \times Weight\ of\ sample}$

Phytic acid content was calculated on assumption that it contains 28.20% phosphorus by weight.

Determination of Lectin

The extracts of the various beans were screened for the presence of lectin by hem agglutinating assay and was carried out by the method of Wang *et al*, (1996), as described by Odekanyin and Kuku, (2014). Hem agglutination experiments were performed in a 96-well U-shaped microtitre plate. PBS (100 µl) was delivered sequentially into wells arranged in rows (each row contained 12 wells). Extract (100 µl) was added into the first well and a serial dilution was done by transferring 100 µl of the diluted sample in a particular well into the next well containing 100 µl PBS until the last 24th well.

Aliquots (50 µl) of the 2% red blood cells suspension were added to each well and the microtitre plates were left undisturbed for 2 h. The titre value was taken as the reciprocal of the highest dilution of the extract causing visible hem agglutination. Specific activity is the number of hem agglutination units (HU) per mg protein (HU)/mg). HU of hem agglutinating Titre of 2^3=8.Determination of protein by Bradford method (1976) was used to determine the protein concentration using Bovine Serum Albumin (BSA) as the standard. The reaction mixture consists of 0.2ml of the enzyme solution and 1.0 ml of Bradford reagent optical density was read at 595 nm.

Statistical Analysis

Data obtained were subjected to a one-way analysis of variance (ANOVA) to determine the significance of the variations among parameters examined at ($P<0.05$). Means obtained were separated using Duncan's multiple range tests (DMRT) with the aid of SPSS version 20

Results

The anti-nutritional composition of the boiled castor seed at 0, 40, 50 and 60 min time intervals are presented in Table 1. The mean values recorded in terms of phytate, oxalate, tannin and lectin contents are significantly different ($p>0.05$) from one another. However, the mean phytate, oxalate, tannin and lectin were highest in the control, 0.42±0.02, 8.58±0.02, 10.54±0.06 and 7.85±0.006 respectively; but lowest in 60 min boiled, 0.20±0.00, 4.63±0.03, 5.78±0.03 and 0.49±0.01 respectively.

Table1. *Anti-nutritional composition (mg/100g) of boiled Castor seed at different time intervals*

		Treatments		
Parameters	**$CSCR_{00}$(Control)(00 min)**	**$CSCB_{20}$ (40 min)**	**$CSCB_{30}$(50 min)**	**$CSCB_{40}$(60 min)**
Phytate(mg/g)	$0.42±0.02^{d}$	$0.34±0.01^{c}$	$0.29±0.01^{b}$	$0.20±0.00^{a}$
Oxalate(mg/g)	$8.58±0.02^{d}$	$6.65±0.15^{c}$	$5.75±0.15^{b}$	$4.63±0.03^{a}$
Tannin(mg/g)	$10.54±0.06^{d}$	$7.64±0.04^{c}$	$6.08±0.03^{b}$	$5.78±0.03^{a}$
Lectin(HU)/mg)	$7.85±0.06^{c}$	$0.059±0.004^{a}$	$0.074±0.004^{a}$	$0.49±0.01^{b}$

Values shown are means ± standard deviation. Means with different letters along the same column are significantly different at $p<0.05$

The anti-nutritional composition of the soaked castor seed at 0, 48, 72 and 96 h time intervals are presented in Table 2. The mean values recorded in terms of phytate, oxalate, tannin and lectin contents are significantly different ($p>0.05$) from one another. However, the mean phytate, oxalate and tannin and were highest in the control, 0.42±0.02, 8.58±0.02 and 10.54±0.06 respectively; lectin was highest in seeds soaked for 48 h (11.87±0.02). The mean values lowest in castor seed cake soaked at 96 h for phytate, tannin and lectin (29±0.01, 8.42±0.02 and 6.83±0.03 respectively) and at 72 h for oxalate, 7.83±0.03.

Table2. *Anti-nutritional composition (mg/100g) of soaked Castor seed at different time intervals*

		Treatments		
Parameters	**$CSCR_{00}$(Control) (00 h)**	**$CSCS_{48}$(48 h)**	**$CSCS_{72}$(72 h)**	**$CSCS_{96}$(96 h)**
Phytate(mg/g)	$0.42±0.02^{d}$	$0.33±0.01^{c}$	$0.30±0.00^{b}$	$0.29±0.01^{a}$
Oxalate(mg/g)	$8.58±0.02^{d}$	$8.16±0.04^{c}$	$7.83±0.03^{a}$	$7.89±0.04^{b}$
Tannin(mg/g)	$10.54±0.06^{d}$	$8.73±0.03^{c}$	$8.46±0.04^{b}$	$8.42±0.02^{a}$
Lectin(HU)/mg)	$7.85±0.06^{d}$	$11.87±0.02^{c}$	$7.57±0.03^{b}$	$6.83±0.03^{a}$

Values shown are means ± standard deviation. Means with different letters along the same column are significantly different at $p<0.05$

The anti-nutritional composition of the toasted castor seed at 0, 20, 30 and 40 min time intervals are presented in Table 3. The mean values recorded in terms of phytate, oxalate, tannin and lectin contents are significantly different ($p>0.05$) from one another. However, the mean phytate, oxalate and tannin were highest in the control, 0.42±0.02, 8.58±0.02 and 10.54±0.06 respectively; lectin was highest in seeds soaked for 20 min (14.22±0.04).The values lowest in 40 min toasted, 0.16±0.00, 2.35±0.0, 8.30±0.10 and 0.08±0.01 respectively.

Table3. *Anti-nutritional composition (mg/100g) of toasted Castor seed at different time intervals*

		Treatments		
Parameters	**$CSCR_{00}$ (Control) (00 min)**	**$CSCT_{20}$ (20 min)**	**$CSCT_{30}$(30 min)**	**$CSCT_{40}$ (40 min)**
Phytate(mg/g)	0.42 ± 0.02^{d}	0.28 ± 0.00^{c}	0.21 ± 0.01^{b}	0.16 ± 0.00^{a}
Oxalate(mg/g)	8.58 ± 0.02^{d}	5.70 ± 0.10^{c}	4.40 ± 0.10^{b}	2.35 ± 0.05^{a}
Tannin(mg/g)	10.54 ± 0.06^{d}	8.38 ± 0.08^{c}	8.34 ± 0.04^{b}	8.30 ± 0.10^{a}
Lectin(HU)/mg)	7.85 ± 0.06^{d}	14.22 ± 0.04^{c}	4.10 ± 0.03^{b}	0.08 ± 0.01^{a}

Values shown are means ± standard deviation. Means with different letters along the same column are significantly different at $p<0.05$

The ant-inutritional composition of the fermented castor seed at 0, 48, 72 and 96 h time intervals are presented in Table 4. The mean values recorded in terms of phytate, oxalate, tannin and lectin contents are significantly different ($p>0.05$) from one another. However, the mean phytate, oxalate, tannin and lectin were highest in the control, 0.42±0.02, 8.58±0.02, 10.54±0.06 and 7.85±0.06 respectively; but lowest in castor seed cake fermented at 96 h for phytate and oxalate (0.34±0.00and 6.29±0.01 respectively) and at 72 h for tannin, 8.72±0.02 and at 48 h for lectin.

Table4. *Anti-nutritional composition (mg/100g) of fermented Castor seed at different time intervals*

		Treatments		
Parameters	**$CSCR_{00}$(Control) (00 h)**	**$CSCF_{48}$(48 h)**	**$CSCF_{72}$ (72 h)**	**$CSCF_{96}$ (96 h)**
Phytate(mg/g)	0.42 ± 0.02^{d}	0.40 ± 0.00^{c}	0.37 ± 0.01^{b}	0.34 ± 0.00^{a}
Oxalate(mg/g)	8.58 ± 0.02^{d}	6.75 ± 0.05^{c}	6.36 ± 0.04^{b}	6.29 ± 0.01^{a}
Tannin(mg/g)	10.54 ± 0.06^{d}	8.61 ± 0.01^{b}	8.54 ± 0.02^{a}	8.72 ± 0.02^{c}
Lectin(HU)/mg)	7.85 ± 0.06^{d}	4.26 ± 0.01^{a}	5.49 ± 0.01^{b}	7.45 ± 0.01^{c}

Values shown are means ± standard deviation. Means with different letters along the same column are significantly different at $p<0.05$

The anti nutritional composition of the autoclaved castor seed at 0, 20, 30 and 40 min time intervals are presented in Table 5. The mean values recorded in terms of phytate, oxalate, tannin and lectin contents are significantly different ($p>0.05$) from one another. However, the mean phytate, oxalate and tannin were highest in the control, 0.42±0.02, 8.58±0.02 and 10.54±0.06 respectively; highest in seeds autoclaved for 20 min, 14.22±0.04 but lowest in castor seed cake autoclaved at 30 min, 0.18±0.00 and 2.41±0.01 respectively and at 40 min for tannin and lectin (6.14±0.04 and 0.08±0.01).

Table5. *Anti-nutritional composition (mg/100g) of autoclaved Castor seed at different time intervals*

		Treatments		
Parameters	**$CSCR_{00}$(Control) (00 min)**	**$CSCA_{20}$ (20 min)**	**$CSCA_{30}$(30 min)**	**$CSCA_{40}$(40 min)**
Phytate(mg/g)	0.42 ± 0.02^{d}	0.23 ± 0.01^{b}	0.18 ± 0.00^{a}	0.26 ± 0.02^{c}
Oxalate(mg/g)	8.58 ± 0.02^{d}	3.13 ± 0.03^{b}	2.41 ± 0.01^{a}	3.76 ± 0.04^{c}
Tannin(mg/g)	10.54 ± 0.06^{d}	6.46 ± 0.04^{c}	6.23 ± 0.03^{b}	6.14 ± 0.04^{a}
Lectin(HU)/mg)	7.85 ± 0.06^{c}	0.22 ± 0.01^{b}	0.004 ± 0.001^{a}	0.021 ± 0.003^{a}

Values shown are means ± standard deviation. Means with different letters along the same column are significantly different at $p<0.05$

DISCUSSION

Gemede and Ratta (2014), Singh *et al.*, (2015) and Atli (2017), reported antinutrients are compounds that reduce the absorption, optimum utilization or metabolism of nutrients from the digestive system and producing deleterious health consequences in man and animals particularly among those that their diets largely are on grains and legumes. They can be eliminated almost completely when subjected to some simple processing methods such as soaking, fermentation, boiling, healing and combination of methods. In

this study, the following submissions were made in respect of the results obtained from the various processing methods adopted on castor seeds:

PHYTATE

Phytate values in all the treatments are significantly different ($p< 0.05$) from one another. It is highest in control castor seed sample, 0.42±0.02 and least in 40 min toasted samples, 0.16±0.00. The result shows that all the treatments (boiling, soaking, toasting, fermenting and autoclaving) significantly reduced ($p< 0.05$) the phytate level in castor seed cake as the timing of each treatment increased. This corroborate the report of Sade (2009), Nissar *et al*., (2017), Agbugui *et al*., (2010) and Ayegba *et al*., (2016) who observed reduction of phytate through roasting of wheat and barley, fermentation of sourdough, boiling of *Bauhinia monandra* and soaking of Moringa leaves respectively.

Augustine and Anitha (2014) also reported phytate reduction in green leafy vegetables commonly consumed in India while subjected to boiling. Reduction of phytate in castor seeds subjected to various treatments recorded in this work is an indication that autoclaving, toasting and boiling (in the order of performance) can be recommended as efficient processing methods for phytate-rich plant materials. Phytic acid, the hexaphosphate ester of myo-inositol, is a major phosphorus storage constituent of most cereals, legumes and oilseeds (Paul, 1989 and Nissar*et al*., 2017).

OXALATE

Oxalate is a diprotic acid which carries two negative charges at neutral pH hence less amenable to nucleophilic attack by a thiamin pyrophosphate (TPP) cofactor (Marcus *et al*., 2016). In this study oxalate values in all the treatments are significantly different ($p< 0.05$) from one another. It is highest in control castor seed sample, 0.42±0.02 and least in 40 min toasted samples, 0.16±0.00. The results indicated that all the processing methods adopted have significant reduction effect ($p< 0.05$) on the oxalate content of CSC. However the trend of reduction effects differ from one treatment to another, but the percentage of reduction was much higher in autoclaving, boiling and toasting ranged between 32% (reduction % of 5.75±0.15 value recorded against $CSCB_{50}$ compared with control value) and 72.61% (reduction % of 2.35±0.05 value recorded against $CSCT_{40}$ compared with control value). This is in tandem with the report of Chai and Liebman (2005), Massey, (2007), Jack (2013)and Augustine and Anitha (2014) which indicated there was a significant loss of oxalate in almost all the test vegetablesviz, red swiss chard, spinach, rhubarb, beets, carrots, Brussels sprouts, broccoli, potato and green leafy vegetables subjected to boiling which ranged from 30 to 87%.Nonetheless, toasting of castor seed at 40 min level appeared to be more efficient method in reducing its oxalate content followed by autoclaving at 30 min level.

TANNIN

It is expedient that tannins in any plant materials to be used for animal feed be made to undergo a process of reduction because these substances are astringent, bitter plant polyphenols that either bind and precipitate or shrink proteins and interfere with iron absorption through a complex formation with iron when it is in the gastro-intestinal lumen which decreases the bioavailability of iron (King-Thom *et al*., 1998 and Praveen and Kumud, 2012).In this work, tannin values in all the treatments are significantly different ($p< 0.05$) from one another. It is highest in control castor seed sample, 10.54±0.06 and least in 60 minutes boiled samples, 5.78±0.03. In general, the processing methods adopted in this study reduced significantly the tannin content of castor seed cake. However, boiling and autoclaving have much more reduction effect on the seeds. The values recorded for soaked seeds are in contrast with the report of Ayegba*et al*., (2016) who reported 35% reduction of tannin on Moringa leaves (40.35 values for processed leaves against 62.23 raw value). In this work however, the average tanning reduction in soaked castor seeds are between 17.17 to 20.1% (8.73±0.03and 8.42±0.02 respectively for $CSCS_{48}$and $CSCS_{96}$as against the control value of 10.54±0.06 (100%).

On the other hand, result of tannin values in this work were in tandem with the report of Agbugui *et al*., (2010) as it was found to reduce significantly as the boiling periods increased for *Bauhinia monandra* seeds from 6.03mg/g to between 2.02 to 0.68mg/g. Similar low tannin content was recorded in Indian green leafy vegetables subjected to boiling (Augustine and Anitha (2014).

LECTIN

Lectin values in fermentation, soaking and toasting treatments on castor oil seed are significantly different ($p< 0.05$) among the levels for each. There were no significant differences ($p> 0.05$) between 30 min and 40 min autoclaved seeds and 50 min and 60 minutes boiled seeds. Being

lectin category (Olsnes, 2004) the value reported by Nsa *et al*., (2011) for ricin in soaked castor seeds is higher than the raw seeds. The difference observed may be attributed to differences in geographical distribution and variety (Ani and Okorie, 2008). In their (Nsa *et al*., 2011) stated report however, boiling durations had more effect on the ricin content than soaking durations up to 84.50%, which is in tandem with the result observed in this work where lectin content reduced to virtually 0% at all the levels of boiling and autoclaving. With the result observed 30 minutes autoclaving was therefore considered the best level for lectin reduction in castor seeds.

CONCLUSION

Based on the results of this study in terms of lowest levels of reduction of the anti nutrient contents of castor oil seed cake, boiled castor seed at 60 min, autoclaving at 30 min level and toasting at 40 min level were considered best owing to the drastic reduction in the various contents across the board. However, the lowest toxic lectin value recorded in the 30 min autoclaved seeds makes it the best among the three. In general, the results recorded in this study makes the seed a better supplement for fish feed formulation.

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