EFFECT OF DETOXIFICATION ON NUTRITIONAL AND ANTI NUTRITIONAL CONTENTS OF CASTOR SEED (*Ricinus communis*)

BY

AJAO, SHARAFADEEN

MATRIC NO. 2006/24049EA

DEPARTMENT OF AGRICULTURAL AND BIORESOURCES ENGINEERING

FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA.

FEBRUARY, 2012

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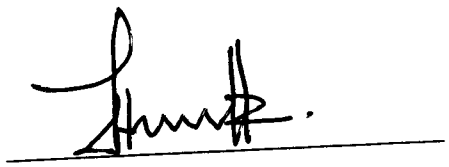
BEING A FINAL YEAR PROJECT REPORT SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENT FOR THE AWARD OF BACHELOR OF ENGINEERING (B.ENG) DEGREE IN AGRICULTURAL AND BIORESOURCES ENGINEERING, FEDERAL UNIVERSITY OF TECHNOLOGY, MINNA, NIGER STATE.

FEBRUARY, 2012

DECLARATION

I hereby declare that this project work is a record of a research work that was undertaken and written by me. It has not been presented before for any degree or diploma or certificate at any university or institution. Information derived from personal communications, published and unpublished work were duly referenced in the text.



02/03/2012

AJAO, SHARAFADEEN

Date

CERTIFICATION

This is to certify that this project entitled "Effect of Detoxification on Nutritional and Anti nutritional Content of Castor Seed *(Ricinus Communis)*" by Ajao, Sharafadeen meets the regulations governing the award of the degree of Bachelor of Engineering (B. ENG.) of the Federal University of Technology, Minna, and it is approved for its contribution to scientific knowledge and literary presentation.

02/03/2012

Engr. Dr. (Mrs.) Z.D. Osunde

Supervisor

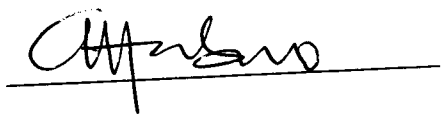
Date

02/03/2012

Engr. Dr. P. A. Idah

Head of Department

Date



22-02-2012

External Examiner

Date

DEDICATION

This project is dedicated to Almighty ALLAH for His infinite mercy and favor, the source of my strength and sustainer of my life, all I am have been by his grace from the beginning to this point of reporting and to the future of my career. It is also dedicated to my parents, guardians and to individuals who has assisted me on my academics studies. Thank you all.

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ABSTRACT

In this research work, the effect of detoxification on nutritional and anti nutritional contents of castor seed were studied. The detoxification methods adopted were soaking and ammonia treatment. The studied nutritional contents were; crude protein, crude fibre, carbohydrate, lipids and ash, while the anti nutrients were; phytate, saponnins and tannins. The results on nutritional contents reveals that percentage ash value are; 3.33%, 2.5%, 4%, for un-detoxified, detoxified by soaking and detoxified by ammonia treatment, crude protein values are; 31.09%, 31.50%, 30.34%, crude fibre values are; 3.33%, 5.33%, 4.67% and carbohydrate values are;5.57%, 4.58%,6.99%. The analysis on the anti nutritional values revealed how well the anti nutrient is removed when the seed is detoxified. For saponins, soaking was effective compare to ammonia which left some trace of the anti nutrient. While, tannins was absent when the seed was detoxified with soaking and ammonia. In phytate, soaking was effective in removing the anti nutrient as there were trace amount left when treated with ammonia.

The results confirm the high oil content of the raw seed and reveal the potential for their utilization in commercial vegetable oil production, having about 56.67% oil. Thus, the protein value also shows that the treatment do not really affect the protein, but show the value obtained for soaking and ammonia are within range with that of raw seed. The changes noticed in anti nutritional contents shows that soaking as method of treatment shows no trace of saponin, tannins and phytate, which encouraged its utilization for detoxification of castor seed for animal feed.

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CHAPTER ONE

1.0 INTRODUCTION

With the rising cost of conventional feed stuffs resulting from increased competition between man and farm animals for orthodox food stuffs, novel feedstuffs are becoming increasingly in use today and research into their utilization are currently gaining priority. In developing countries of the world, Nigeria inclusive, wide variety of ingredients have been tested and are being used in feeding livestock either solely or in combination with others in various feed formulation. Thus, chemical evaluation and nutritional assessment of detoxified and un detoxified castor seeds is being considered in the present work as follow up to the quest for cheaper and available alternative feedstuffs for livestock production.

1.1 Background to the study

The castor plant (*Ricinus communis L.)*, belonging to the plant family of *Euphorbiaceae*, is grown as annual crops in temperate and perennials in tropical climates. In Nigeria, it is grown in the Northern and middle belts where the weather is favourable, but not at the commercial scale despite the abundant land, ecological and climatical condition which are favourable to its cultivation. Castor seed is presently planted, harvested and fermented for usage as condiments in soup in Southern-eastern Nigeria (Enujiugha *et al.*, 2001). Naturally, it is highly toxic due to the presence of ricin, a water-soluble glycoprotein concentrated in the seed endosperm but present in lower amounts in the rest of the plant and reputed to be one of the most poisonous of the naturally occurring compounds. All animals (livestock and pets) are vulnerable. Ricin is the most lethal of the toxins found in castor bean, of less concentration anti nutrients in the seeds are;

saponins, tannins, phytic acid, etc. Thus, any attempt to detoxify castor bean residue should be aimed at removing ricin (Anandan *et al.*, 2004)

Castor bean is a high-yield oil seed crop producing around 50% oil by weight, out yielding soybean and cottonseed in U.S. production schemes (Weiss, 2000). The oil from the seed is one of the few naturally occurring glycerides with high purity, since the fatty acid portion is nearly 90% of ricinoleic acid (Akpan *et al.*, 2006). The oil is not only a naturally occurring resources; it is also inexpensive and environmentally friendly. Relative to other vegetable oil, it has a good shelf life. The oil is used industrially as coating fabrics and other protective coverings, in the manufacture of high grade lubricant, polish, waxes carbon paper, candle, crayons and biodiesel (Lyon, 2007).

The production of castor bean in Africa is very low and the contribution to the world market is very small with Ethiopia, Tanzania and South Africa leading in production. The crop is also grown in Sudan, Kenya, Angola, Madagascar and Uganda (Gobin *et al.*,2001). Castor seed production in Nigeria had been low, with more concentration in the south eastern states and the neighboring Kogi and Benue states.

1.2 Statement of problem

Detoxified castor seed meal has been used as a feed for livestock, but because of the presence of ricin and other toxins de-oiled castor seed cake is seldom used as a livestock feed (Aganga and T shwenyane, 2003). Thus, the most outbreaks of poisoning in animals result from being fed with improperly detoxified castor bean meal. Castor cake has not found a place as protein supplement due to its toxicity and currently is used as organic fertilizer.

1.3 Objective of the study

The general objective of this project work is to determine the effect of detoxification on nutritional and anti nutritional properties of castor seed. Thus, The specific objectives are as follows;

1. Detoxifying castor seed bean using different methods
2. To assess some selected nutritional (Crude protein, Crude fibre, Ash, Carbohydrates, Ether extract) and anti nutritional (Tannins, Saponins, Phytates) properties of detoxified and un detoxified castor seed
3. To Identify the effect of detoxification on the nutritional and anti nutritional properties studied

1.4 Justification of study

The study on the effect of detoxification on nutritional and anti nutritional properties of castor seed is an attempt to provide objective measurement resulting in more meaningful data that will help processing the seed as a result help in castor meal production. Thus, the study also reveals the important nutrients in the seed and ways of eliminating some anti nutrients presents.

1.5 Scope of study

The project is centered on the effect of detoxification of castor seed on the outcome on the nutrients –Crude protein, Crude fibre, Ash, Carbohydrate and Ether extracts, and anti nutrients contents- Tannins, Saponins and phytates - so as to serve the need of man and animal. Thus, ricin as major anti nutrient was not determined in this project work, due to unavailability of places to carry out the test within the country.

CHAPTER TWO

2.0 LITERATURE REVIEW

Castor plant (*Ricinus Communis*) from which castor beans and oil are subsequently derived grows naturally over a wide range of geographical regions and may be activating under a variety of physical and climatic regions. The plant is most common in tropical countries, it is probably indigenous to some areas in the south eastern Mediterranean region (Phillips and Martyn, 1999).Castor seed have been found in ancient Egyptian tombs dating back to 4000 B.C., and the oil was used thousands of years ago in wick lamps for lighting (Weiss, 1983). To many people castor plant is just an overgrown undesirable weed, and yet it produces one of nature's finest natural oils (Armstrong, 2000.).

The plants are very common along stream banks, river beds, bottom lands, and just above any hot area where the soil is well drained and with sufficient nutrients and moisture to sustain the vigorous growth (Armstrong, 2000.).

The toxic ricin is not transferred to the oil fraction during extraction but remains in the seed cake, the seed cake must be detoxified before use as an animal feeds. The seeds are fermented and detoxified to obtain a seasoning agent (Uzogara *et al.*, 1990) called ogiri-igbo by the Ibos of the southeast zone of Nigeria; they are used as a condiment in soups, salads and stews for rice and yam meals. The condiment has been reported to enhance food flavours, thereby making the food palatable and appetizing (Onyeike, 1999).

Most outbreaks of poisoning in animals result from them being fed with improperly detoxified castor bean meal (Cooper and Johnson, 1984). A number of physical and chemical methods for detoxifying castor seed meal have been investigated and have been received (Anandan *et al.*,

2004). Nutritionally, the seeds contain 5.1-5.6% moisture, 29-31% protein, 45.6-50.6% oil, 3.1-7.0% NFE, 23.1-27.2% crude fibre, and 2.0-2.2% ash. The oil-cake from crushing whole seeds contain, 9.6% moisture, 6.5% oil, 20.5% protein, 49% total carbohydrate and 15% ash (CSIR, 1948-1976). Hence there are several anti nutritional factors associated with castor bean, the principal among these are ricin and ricinine, of less importance are phytates, tannins, saponins etc.

2.1 Reproduction

The seed capsules are covered with weak spines. The capsules is composed of three sections or carpels which split apart at maturity. Each section (carpel) contains a single seed, and as the carpel dries and splits open, the seed is often ejected with considerable force. The seeds are shiny brown with darker streaks or spots and resemble a bloated tick (Little, 1974).

Source, Armstrong, 2000

Plate 2.0 Reproduction of castor seed

2.2 The seed

The shiny seeds of castor plants are a little larger than pinto beans and have very beautiful and intricate designs. At one end is a small, spongy structure called the caruncle, which aids in the absorption of water when the seeds are planted. Like human faces, finger prints or the spots on a leopard, no two seeds have exactly the same pattern. They are unquestionably among the most deadly seeds on earth, and it is their irresistible appearance that makes them so dangerous (Armstrong, 2000).

2.3 Uses of castor bean seed

2.3.1 Industrial uses

It is used in the manufacture of pharmaceuticals and cosmetics and as a laxative (cosmetic ingredient Review Expert Panel, 2007), in the texile and leather industries, and for manufacturing plastics, fibres soap, printing inks and lubricants (Weiss, 2000)

2.3.2 Castor in soil

The residue obtained from castor cake, help enhances the fertility of the soil without causing any damages. It help to neutralized the detrimental effect of chemical fertilizer. They are excellent fertilizer because of high contents of nitrogen(6.4%), phosphoric acid (2.55%) and potash (1%) (Weiss, 2000).

2.3.3 Castor oil in food

In some parts of Nigeria (south east and part of west) do extract oil after processing from the seed and can be used to prepare vegetable meal (egusi), thus the meal source obtained from the oil seed could traditionally referred to as ''ogiri-igbo" (Weiss, 2000)

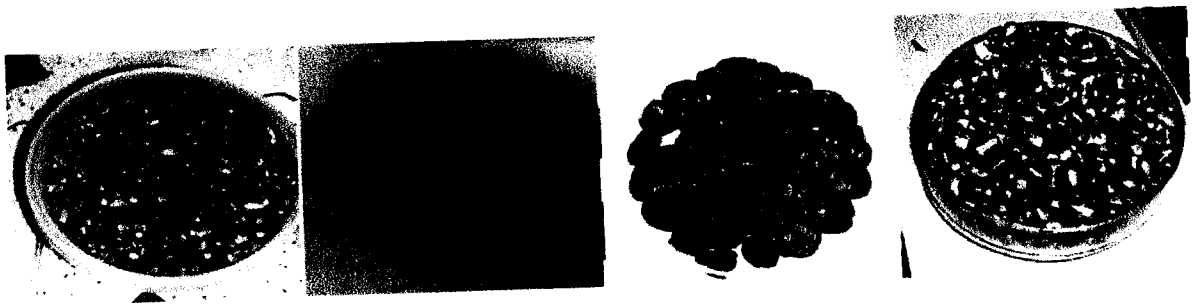
contain a toxic protein which he named *Ricin*. According to the 2007 edition of the Guinness book of world records, the plant is the most poisonous in the world, if ingested symptoms may be delayed up to 36hours but commonly begin within 2-4hours, these includes; burning sensation in the mouth and throat, abdominal pains, drop in blood pressure, diarrhea etc. Unless treated death could be expected to occur within 3-5years.

2.4.1 Poisonous plant part (Castor plant)

The ricin content is the highest in the seed, although, a small fraction of toxin is contained in the leaves. Swallowing a seed without chewing prevents the release of the toxin because of the hard seed coat. However, chewing the seed allows release of the water soluble chemical and poisoning can occur (Cooper and Johnson, 1984).

2.4.2 Poisoning mechanism of castor bean

It causes clumping (agglutination) and breakdown (hemolysis) of red blood cells, hemorrhaging in the digestive tract, and irreparable damage to vital organs such as the liver and kidneys (Lewis and Elvin.1977). It is most toxic when taken intravenously or inhaled as fine particles (Wiley and Oeltmann, 1991). The agglutination of red blood cells has been attributed to a powerful hemagglutinin in castor bean called Ricinus communis agglutinin(RCA) not ricin (Robertus, 1991). Ricin is a potent cytoxin but a weak hemagglutinin (Wiley and Oeltmann, 1991). Poisoning by ingestion of castor beans is due to ricin, not RCA, because RCA does not penetrates the intestinal wall and does not affect red blood cells unless given intravenously. If RCA is injected into the blood, it will cause the red blood cells to agglutinate and burst due to hemolysis (Wiley and Oeltmann, 1991).



a) Jatropha seed b) Rubber seed c) Karanj seed d) Castor seed

Plate 2.1 Different Oil Seed

2.3.4 Castor Bean motor oil

The superior "oiliness" of castor oil and its ability to "cling" to very hot moving parts make it an outstanding racing oil for high performance engines. In fact, it is the basic ingredient of Castrol-R racing motor oil for high speed automobile and motorcycle engines. Castor oil is a popular fuel additive for two cycle engines, and imparts a distinctive aroma to the exhaust of these engines. Castor wax, a hard wax produced by the hydrogenation (chemical combination with hydrogen) of pure castor oil, is used in polishes, electrical condensers, carbon paper, and as a solid lubricant (Weiss, 2000).

2.3.5 Fruit flavors from castor oil

It is the source of several synthetic flower scents and fruit flavors (esters), such as jasmine, apricot, peach, plum, rose, banana, and lemon. The chemicals (esters) responsible for these flavors and aromas are obtained from ricinoleic acid, one of the important ingredients of natural castor oil. In fact, ricinoleic acid comprises about 90% of the total triglyceride fatty acids of Castor oil (weiss, 2000)

2.4 Toxicity of castor seed

The toxicity of castor bean was discovered by Stillmark, 1889. Who also stated that the seed

2.5 Selection and definition of some nutritional components of castor meal

The properties below were chosen for this study, these help to determine the suitability of castor seed in various processes and applications. The definition of these properties is therefore paramount

2.5.1 Crude protein

The word protein is derived from a Greek word, 'proten' and means 'holding the first place'. Proteins literally hold the first place in the architecture and machinery of all living things. Without them no life can exist. No plant can grow or trap sunlight; nobody can be born on reared unless proteins have been made (Mottram, 1979).

2.5.2 Crude Fibre Content

Crude fibre is a chemical entity. It is the reminant after plant material or more precisely plant always has been treated with hot concentrated H_2SO_4, alkalis and alcohol; this is the cellulose and lignin portion of the plant material. In order words, crude fibre is a complex mixture of indigestible compounds derives mainly from plant cell walls. It consist mainly of polysaccharides, particularly cellulose fibres. Its bulk stimulates the movement of through the gut. These are evidence that fibre helps to reduce blood cholesterol levels and the risk of bowel cancer and gallstone (Taylor *et. al.* 1992).

2.5.3 Carbohydrates

These are a group of compounds that contain the elements carbon, hydrogen and oxygen with hydrogen and oxygen being present in the same proportions as in water. Carbohydrates are found in food either as sugar or as starches and glycogen. These later materials are long straight or

branched chain of the many sugar molecules joined. The chemical nature of sugar determines their properties, their functions in living tissues and how starches are formed and broken down. The sugar includes the monosaccharide, disaccharides and polysaccharides. Glucose and fructose are the monosaccharide that are nutritionally important. Sucrose, lactose and maltose are the disaccharides of nutrient importance. The only polysaccharides of major nutrient importance are starches and glycogen because they can be digested in the human gut. (Mottram, 1979)

2.5.4 Ash Content

These are inorganic compounds, which appear in food analysis i.e. they are substances left behind, when the carbon, hydrogen and nitrogen (organic compounds) have all been burnt off by excess oxygen. In order words, ash of a biological material is an analytical term for the inorganic residue that remains after the organic matter has been burnt off. (NRC, 1990).

2.5.5 Moisture Content

Moisture content is the amount of water present per given weight of sample. In order words, moisture content is the loss in weight of the sample during drying. Moisture is removed in other to know the storage ability of the product, by so doing, improve the shelf life and eliminate or reduce oxidative rancidity, microbial activities and other infestations

2.6 Selection and definition of some anti nutritional components of castor meal

They are substances generated in natural feed stuff by the normal metabolism of species and by different mechanisms exert effects contrary to optimum nutrition. Anti nutrients in plants seems to be as a way of storing nutrients or as a means of defending their structure and reproductive elements (Harborne, 1989).

2.6.1 Ricin

It occurs in castor beans, which have been reported to cause poisoning in all class of livestock. Due to ricin, deboiled castor seed cake (CP 35%) is seldom used as a livestock feed. However, the mature leaves of ricinus communis have been found suitable for two sheeps (Behl *et al.* 1986). Ricin is found in the meal or cake after the oil has been extracted. Those who occasionally take castor oil may be assured that ricin does not occur in the pure oil. When a gram of ricin is compared with equivalent weights of other toxic substances, it turns out to be one of our deadliest natural poisons. It has been estimated that, gram for gram, ricin is 6,000 times more poisonous than cyanide and 12,000 times more poisonous than rattlesnake venom (Robertus, 1991).

2.6.2 phytates

Phytic acid (known as phytate when in the salt form) is the principal storage form of phosphorus in most plants seeds. Phosphorus in phytate form is in general not bio available to non-ruminant animals because those animal lack the digestive enzyme phytase, which is require to separate phosphorus from phytate molecule (Duffus, 1991).

2.6.3 Saponins

They are characterized by a bitter taste and thus, when present in high concentrations would reduce plant palatability in livestock (Cheeke and shull, 1985). The adverse effects of saponins can be overcome by repeated washing with water which makes the feed more palatable by reducing the bitterness associated with saponins (Duffus., 1991).

2.6.4 Tannins

They are phenolic substances associated with toxic and anti-nutritional effects including reduced food/intake, growth reduction and improved nutrient absorption (Butler *et al.*, 1986). Several studies indicate that tannin-rich leaves in combination with concentrates rations, could be fed to animals without any adverse effect (Raghavan, 1990), this happens because animals consume protein in excess of their requirement from the concentrate and therefore, the anti nutritional effects of tannins were masked.

2.7 Treatment / detoxification

Some methods reported for he detoxification of the cake includes; treatment with ammonia, caustic soda, lime and heat etc. (Anandan *et al.*, 2004). In addition, some people in the parts of south-east Nigeria have long developed a method for treating and detoxifying the unextracted seed (fermentation) that is subsequently used as food seasoning known as ''Ogiri Igbo'' (Odunfa, 1985).

2.7.1 Physical treatment

- **Soaking:** Castor cake (1000 g) was steeped in 10 l water (1:10) at three different time intervals of 3, 6 and 12 h. The cake was filtered using a muslin cloth, air dried and stored at 4 °C for further evaluation.
- **Steaming:** Powdered castor (1000 g) cake was moistened with water to have moisture content of 150 g/kg in the cake. It was spread on a muslin cloth in a perforated dish and steam was allowed to pass through it for 30 and 60 min. A pressure cooker was used for the purpose with out closing the outlet by removing the knob to produce the steam with out any pressure. The treated cake was air dried and stored at 4 °C.

- **Boiling:** Castor cake (1000 g) steeped in 10 l of water was boiled at 100 °C for 30 and 60 min. The water was poured off and the sample filtered through a muslin cloth, air dried and stored at 4 °C for further evaluation.
- **Autoclaving:** The castor cake samples each weighing 1000 g were autoclaved at 15 psi for 30 and 60 min. The cake was allowed to dry at room temperature before storing.
- **Heating:** The castor cake samples of 1000 g each were subjected to dry heat at different time and temperature combination in a hot air oven. The different combinations that were tried were 100 °C for 30 min and 120 °C for 25 min.

2.7.2 Chemical treatment

- **Ammonia treatment**: Ammonia solution (25 ml/l) was added to castor cake samples (each weighing 1000 g) at the rate of 30 and 50 ml to have a concentration of 7.5 and 12.5 ml of ammonia per kg of castor cake sample. The treated samples were kept in airtight plastic containers for 7 days. The samples were then air dried and used for analysis.
- **Formaldehyde treatment:** A formaldehyde solution of 400 g/l was added to each of 1000 g of castor cake samples. The samples were subjected to treatment at 5 and 10 g/kg on protein basis by adding 5 and 10 ml of the solution to the respective samples. The treated samples were thoroughly mixed and kept in airtight containers for a period of 7 days. The samples were air dried and used for analysis.
- **Lime treatment:** The castor cake samples of 1000 g each were mixed with calcium hydroxide solutions in a ratio of 3 g/ml. Calcium hydroxide concentration were 10, 20

and 40 g/kg. The treated samples were left over night (8 h) and the sun dried lime treated samples were then used for analysis.

- **Sodium chloride treatment:** Sodium chloride was used at 0.25, 0.5 and 1.0N strength to treat the castor cake samples. The cake samples of 1000 g each were initially mixed with sodium chloride solution in a ratio of 3 g/ml. The treated samples were left over night and sun dried.
- **Sodium hydroxide treatment:** Sodium hydroxide at 0.18, 0.38 and 0.75N strength was used to treat the cake. Cake samples of 1000 g each were mixed with sodium hydroxide solution at the rate of 3 g/ml. The treated cake was left overnight and sun dried for further studies.

2.7.3 Traditional method

Fermention: The seeds are first dehulled and boiled in water for about 18hour. The boiled seeds are cooled and wrapped together with banana leaves and allowed to ferment in the fire place for about five days. The fermentated seeds are then mashed by pounding using a morter and pestle. This was followed by addition of ash from burnt palm kernel husk which gives it a dark colour. The dark mashed product is allowed to mature for a further period of five days, after which, it is packaged for sale. It is believed that most of the detoxification takes place during fermentation and it leads to elimination of the toxic factors (Odunfa,1985).

2.8 Adverse effect of castor seed on livestocks

2.8.1 Ruminant

Detoxified castor bean meal has been fed to lactating dairy cows over a 14-month period without signs of adverse effects (Robb *et al.*, 1974). Diets containing 10 and 20% castor bean meal, with

or without 0.5% added oil, were compared with control diets containing 10% cottonseed meal and 0.5% cotton seed oil, no abnormal production or fertility conditions related to castor bean meal were noted in the cows.

2.8.2 Poultry

Growing chicks (150 days old) were fed non-roasted castor beans at 10% of the diet or roasted castor beans at 10, 15 or 20% of the diet for 6 weeks (Okorie and Anugwa, 1987). Roasting was performed to destroy ricin, feed intake, growth rate, feed conversion ratios and mortalities were monitored. Significantly, reduced food intake weight gain, increased feed conversion ratio were found at all diets with castor beans.

CHAPTER THREE

3.0 MATERIAL AND METHODS

3.1 MATERIALS

3.1.1 Source of material

The castor seed for this study was purchased from a market at Oja-Oba market in Ilorin, Kwara State. All the experiments were carried out at department of Animal Production, Fishery Technology and Biochemistry of the, Federal University of Technology, Minna.

3.2 Equipment and reagent

3.2.1 Equipment

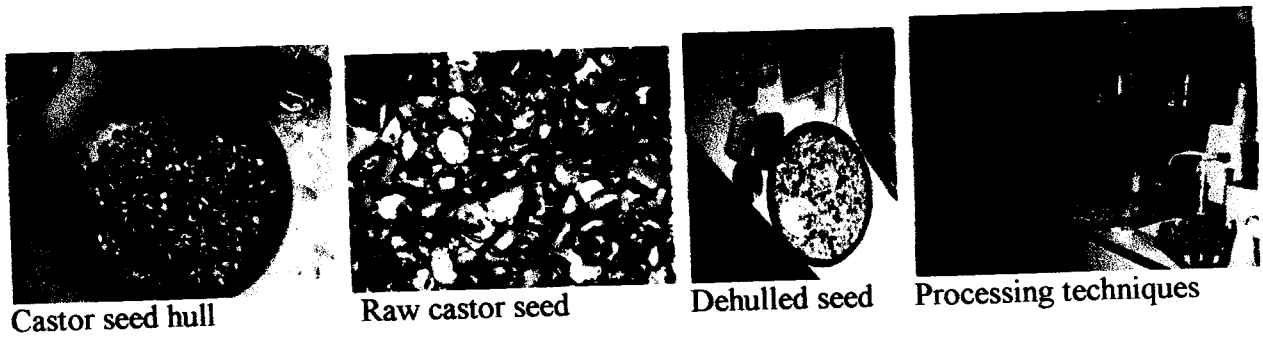
- Mechanical convection oven
- Burette
- Desiccator
- Muffle furnace
- Measuring cylinder
- Pipette
- Weighing balance
- Crucible
- Conical flask
- Petri dish
- Blender
- Pestle

3.2.2 Reagents

- Ferric chloride
- Ammonium thiocyanate
- Ammonia solution
- Olive oil
- concentrated H_2SO_4
- Sodium hydroxide
- ethanol
- petroleum ether
- HCl
- Filter paper
- N-hexane
- Distilled water

3.3 Sample preparation

The dehulled seed weighing 450g were used for all the experiments. The seed were sorted to remove some foreign materials and broken seeds, de-shelled manually by cracking with pestle to gain access to the creamy endosperm. The seed is divided into three parts with each parts further separated into three sample, each weighing 50g. Thus, the first part was not detoxified, second part was detoxified by soaking and third detoxified by ammonia treatment. After detoxification, the samples were milled separately and used for analysis.



Castor seed hull | Raw castor seed | Dehulled seed | Processing techniques

Plate 3.0: Sample preparation

3.4 METHODS

The proximate analysis, Phytochemical test, and subsequent detoxification methods were carried out on the sample (castor seed).

3.4 METHODS OF DETOXIFICATION OF CASTOR SEED

3.4.1 Physical treatment

Soaking: Castor cake sample weighing 50g was steeped/soaked in 50ml of distilled water (0.5lit) at three consecutives portions inside a plastics container for 12h. The cake was filtered using a muslin cloth, then was air dried and stored at 4°c. The sample were milled and stored in a plastic container for further analysis.

3.4.2 Chemical treatment

Ammonia treatment: Ammonia solution of 1.25ml in 50ml of distilled water was added to castor samples (each weighing 50g) at the rate of 2.5ml for each sample prepared to have a concentration of ammonia per gram of castor cake sample. The treated samples were kept in

airtight plastic containers for 7 days. The samples were then air dried, milled and used for analysis.

3.5 DETERMINATION OF NUTRITIONAL CONTENTS

The procedure outline in the AOAC, (2000) were used to carried out all the nutritional analysis on both the un-detoxified and detoxified castor seed.

3.5.1 Crude protein

0.5g (aliquot) of each samples were weighed into 100ml kjeldhl flask and three tablets of selenium were added. To this 25ml concentrated sulphuric acid were added. The flask and it content were heated gently for digestion in the fume cupboard, until the liquid was clear and free from black/brown colour. They are allowed to cool and diluted with distilled water to make to 100ml. 5ml boric acid was put into the conical flask, the digested sample was dissolved with NaOH solution and placed under the stoppered portion of condenser. The solution was allow to distilled until the purple solution turned green. The distillation was titrated against 0.1m HCL to obtained a grayish blue colour.

A blank titration was carried out, percentage nitrogen $= \frac{T.v \times M.a \times 0.014 \times 10}{w} \times 100$

Percentage protein=(N×6.25). where; T.V = titre value, M.A = molar acid, W = sample weight

3.5.2 Carbohydrate

This was calculated by difference: Fat, crude fibre, protein, ash were subtracted from 100 as;

% carbohydrate = 100 – % (ash + protein + fibre + oil). From the calculation of carbohydrate, moisture content is excluded, because the samples used is dried after treatment.

3.5.3 Crude fibre

About 2g of the sample with petroleum ether was reflux for 30 minutes with 200ml solution containing 1.25g of sulphuric acid per 100ml of solution, the solution was filtered through linen cloth on a fluted funnel. The mixture was washed with boiling water until the washings are no longer acid. The residue was transfer to a beaker and boiled for 30 minutes with 200ml of solution containing 1.25g NaOH per ml. The final residue was filter and dried in oven, it was later weighed and percentage crude fibre was calculated

$$\% \text{ crude fibre} = \frac{W_2 - W_3}{W_1} \times 100$$

Where W_1 weight of sample

W_2 weight of crucible and sample

W_3 weight of crucible and Ash

3.5.4 Crude fat

250ml clean boiling flask was dry in oven at 105-110°c for about 30min. and transfer into a dessicator to cool. 2g of each samples were weighed with filter paper into the thimble. The extraction was carried out using petroleum ether of 300ml (boiling point 40-60°c), the thimble plugged with cotton wool and the extraction was continuously for 6 hours. At the end of the extraction, the solvent was removed by evaporation on the water bath and the remaining part in the flask dried in the oven and cool in the dessicator. The flask was reweighed and percentage fat calculated as:

Percentage oil content= $\frac{weight\ of\ Extracted\ fat}{weight\ of\ sample} \times 100$

3.5.5 Ash content

The ash content of this biological material is analyzed for the inorganic residue that remains after the organic matter has burnt off in a furnace at high temperature of 550^0C. Some sample of material is weigh into a crucible of known weigh, and place into the furnace, till it is completely ash and later placed in the dessicator to cool, then the weight is taken again and calculated in percentage.

Percentage ash content = $\frac{Total\ weight\ of\ extracted\ ash}{weight\ of\ sample} \times 100$

3.5.6 STATISTICAL ANALYSIS

Proximate composition analysis of castor seed was replicated three times. Data obtained on nutritional contents were analysed by computing for the mean value, standard deviation, and coefficient of variation from the values and if the difference on values obtained on replicate were small the standard deviation gives a smaller value, thus, result to increase in coefficient of variation value, all were presented in the table.

3.6 QUALITATIVE ANALYSIS TEST FOR ANTI-NUTRIENTS CONTENTS (tannins and saponins) OF CASTOR SEED

3.6.1 Preparation of the extracts using different solvents (distilled water, n-hexane and ethanol)

100ml of the solvent were added to 20g of each grounded samples (Nine samples) of detoxified and un detoxified castor seed in a conical flask. The mixture was stirred and covered. It is

allowed to stand for 24hrs and filtered using sterile whatmann No1 filter paper. The bright yellow filterate (extracts) is concentrated to 10ml on a water bath. It is cooled and stored in refrigerator for further analysis. (Mshelia *et al.*, 2000)

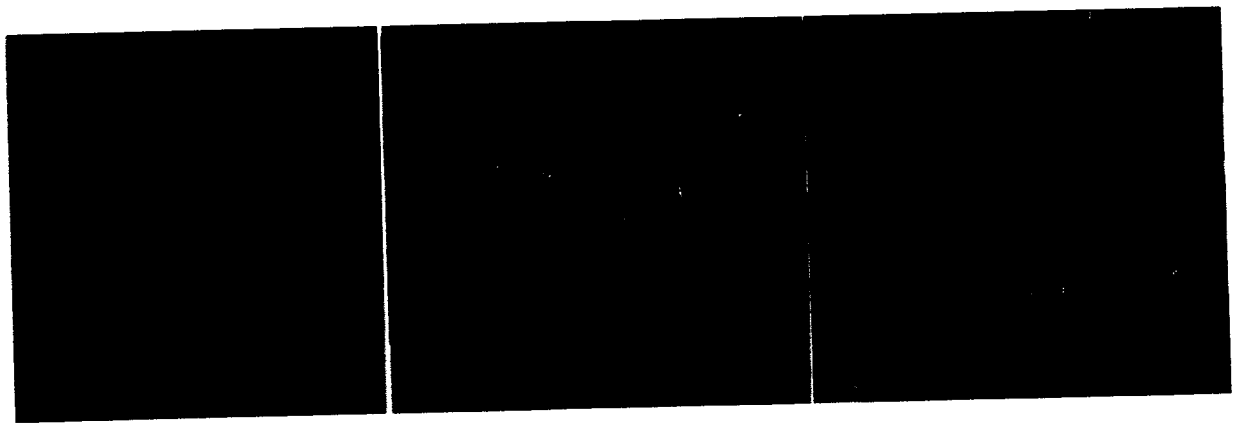


Plate 3.1: Extraction of the extracts of castor seed using distill, n-hexane and ethanol as solvent

3.6.2 Tannins: The presence of tannins can cause browning or other pigmentation problems in both fresh food and processed products. Thus, qualitative test is carried out by method outline by Sofowora, 2009 . About two drops of 5% $FeCl_3$ was added to $1cm^3$ of the extracs. A greenish precipitate, blue black or green observed indicated the presence of tannins in all extracts.

3.6.3 Saponins

This was determined using emulsion test as given by Sofowora, 2009. About five drops of Olive Oil was added to $3cm^3$ of the extracts in a test tube and the mixture was vigorously shaken. A stable emulsion formed in each extract tested, indicated the presence of saponins.

3.6.4 Phytic acid

Determined by method of Young and Greaves (1971) based on the ability of standard ferric chloride to precipitate phytate in dilute HCL extracts of the sample. The phytin content in the seed is determined by titrating it against standard iron chloride ($FeCl_3$) solution containing 0.00195g until a brownish yellow colour persists for 5min. to confirm for the presence of phytate content

Plate 3.2 Distilling protein using markham distillation apparatus

Plate 3.3 Boiling of castor seed sample for determining of crude fibre

CHAPTER FOUR

4.0 RESULT AND DISCUSSION

4.1 Presentation of Result

Table 4.1 Proximate Analysis Result Treated and Un Treated Castor Seed

Procesing Techniques	Crude protein (%)	Crude fibre (%)	Ash (%)	Lipids (%)	Carbohydrate (%)
Un-detoxified	31.09	3.33	3.33	56.67	5.57
	(0.27,0.867)	(1.15,34.641)	(1.04,31.225)	(1.53,2.696)	(3.44,61.773)
Detoxified (soaking)	31.50	7.33	2.50	55.33	4.58
	(0.80,2.553)	(4.62,62.984)	(0.50,20.000)	(2.02,3.652)	(3.24,70.619)
Detoxified (Ammonia)	30.34	4.67	4.00	54.00	6.99
	(1.33,4.380)	(1.15,24.73)	(0.87,21.651)	(1.32,2.450)	(1.92,27.619)

Values in the bracket are the standard deviation and coefficient of variation

Table 4.2 Qualitative Result Test for Phytochemical Content of Treated and Un treated Castor Seed

Table 4.2 Qualitative result test for tannins

Processing	Distilled water	N-hexane	Ethanol
Un detoxified	+	++	++
Soaking	++	–	+
Ammonia	–	–	++

Table 4.3 Qualitative result test for Saponins using distil, n-hexane and ethanol as solvent extraction

Processing	Distilled water	N-hexane	Ethanol
Un detoxified	++	++	+
Soaking	++	–	–
Ammonia	+	–	++

Table 4.4 Qualitative result test for phytate

Processing	Extracts
Undetoxified	++
Soaking	–
Ammonia	+

Key: + =trace/ faint

++ = present

- = absent

4.2 Discussion of Results

A very vital but not surprising observation from the proximate analysis results is the high concentration of nutrients both in the raw and the treated. The crude protein content of the detoxified castor seed is comparatively higher than that of some conventional protein feed stuff such as spent grain (18%) (Enujiugha, 2001). Protein values were; 31.09, 31.50%, 30.34 for raw, detoxified by soaking and ammonia. Thus, the raw castor seed confirmed the high oil content of 56.67%, for detoxified by soaking (55.33%) and ammonia (54.00%) and reveal it potential for their utilization in commercial vegetable oil production, the detoxification by soaking and ammonia do not show much effect on protein content and lipid as their values are within range compare with that of raw seed. Literature has revealed the relative low moisture of processed castor seed (Enuijiugha, 2001) claims a good advantages for it shelf life in storage. The ash content is relatively low, about 3.33% in raw, 2.50% in detoxified by soaking and ammonia giving the highest value of 4%, which show that ammonia treatment has effect on the ash content. The crude fibre content was low, which make it ideal for poultry as the raw yield 3.33%, soaking (7.33%) and ammonia treatment yield 4.66%, thus, both the treatment has effect on the fibre content. The nitrogen free extracts is more in ammonia treatment (6.90%) and less in soaking and un treated (5.57% and 4.58%).

Castor seed has also been found to contain some anti nutrients such as phytic acid, saponins and tannins which are typical of most legumes and oil seeds (Enuijiugha, 2001). From the qualitative

analysis test, it is observed that the justification of whether the anti nutrients are present or absent do not only depends on methods detoxifications employed but also to the solvents used for extraction (distilled water, n-hexane and ethanol) in both tannins and saponins, where phytic is carried out separately. The results of anti nutrients screening present in castor seed for tannins and saponins, shows that n-hexane is more effective as the solvents used for extraction, compare to distilled water and ethanol which are not effective in this project work. In table 4.2, the result shows tannins is not present, soaking and ammonia treatment has effect on tannins by reducing it using n-hexane as solvent of extraction, but there is trace of tannins when treated with soaking using distilled water as solvent. Hence, Table 4.3, shows that soaking and ammonia treatment show much effect on saponnins, by eliminating it from the seed compare to the raw seed where saponins is present. Thus, from the qualitative result test obtained for phytate content from the table 4.4 above, it reveals that soaking method is most efficient in removing the anti nutrients, compared to treatment with ammonia, where there is trace of phytate.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

In the course of this study, it has been established that some valuable nutritional content of raw castor seed has not been altered much by the treatments used to detoxify the seed. The result on nutritional contents reveals that percentage crude protein are; 31.09%, 31.50%, 30.34%, and lipids; 56.67%, 55.33%, 54%, for un treated, treated by soaking and ammonia. The result for lipid shows that the treatment did not really have effect to some extent compare to the raw castor seed. Thus, the percentage high oil content obtained in both raw and treated castor bean seed revealed the seed as a good source of vegetable oil. The value of ash content detoxified by ammonia, shows that ammonia has effect on the ash in castor seed, also soaking method shows effect on the fibre content, which is more compare to raw castor seed. The carbohydrate values of the two treatment used are within range to the raw seed. Hence, the use of soaking and ammonia in treating castor seed has reported by Anadan *et al.*, 2004, has reduced the anti nutrients and apparently affect some nutritional contents (ash and crude fibre). Thus, the detoxification method is outrightly efficient, and has actually reduced the level of the toxic substances in the seed. It can be concluded that if the detoxification process is carried out in the right manner, then the nutritional properties can be explored with no threat of being poisoned.

5.2 Recommendation

- For effective detoxification and utilization of castor seed, it is imperative to remove the seed coat to arrive at more suitable product

- Castor seed (meal) can be recommended as a source of cheap quality protein for both human and animal having been discovered the toxic substances can be detoxified

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APPENDICES

APPENDIX 1: CALCULATIONS

Calculation on how the tables in Chapter four were gotten are as follows:

Ash Content

Percentage ash content = $\frac{W_3 - W_1}{W_2 - W_1} \times 100$

Where W_1 is the weight of the crucible

W_2 is the weight of the crucible and the sample material

W_3 is the weight of the crucible and the ash content

Table 2 ash content calculation

Sample	W_1	W_2	W_3	%Ash content
$T^1$1R1	25.65	27.65	25.74	4.50
T^1R2	24.27	26.27	24.32	2.50
T^1R3	25.79	27.79	25.85	3.00
T2R1	25.01	27.01	25.33	2.50
T2R2	26.44	28.44	26.48	2.00
T2R3	28.50	30.50	28.56	3.00
T3R1	25.31	27.31	25.38	3.50
T3R2	25.47	27.47	25.06	5.00
T3R3	38.10	40.10	38.17	3.50

W_2 weight of the filter paper and dried sample

W_3 weight of the filter paper and sample

Table 4 Lipids contents calculation

Sample	W_1	W_2	W3	%Lipid contents
$T^1$1R1	0.81	2.81	1.65	58.00
T^1R2	0.80	2.80	1.65	57.00
T^1R3	0.77	2.77	1.67	55.00
T2R1	0.80	2.80	1.74	53.00
T2R2	0.82	2.82	1.69	56.50
T2R3	0.71	2.81	1.68	56.50
T3R1	0.77	2.77	1.72	52.50
T3R2	0.81	2.81	1.71	55.00
T3R3	0.77	2.77	1.68	54.50

Crude protein

This can be calculated with the expressions below:

% Protein= 6.25 × % Nitrogen,

$$\% N = = \frac{T.V \times N \times 0.014 \times 10 \times 100}{0.5}$$

6.25 is the common factor used for most food and food mixture

T.V, is the titre value after titration

100, is the prepared solution of H_2SO_4, Thus 20ml sulphuric acid make up to 100ml

0.5, sample weight (W)

Table 5 Crude protein calculation

Sample	W	T.V	%N	% Crude protein
$T^1$1R1	0.5	1.78	4.98	31.15
$T^1$1R2	0.5	1.79	5.01	31.33
$T^1$1R3	0.5	1.76	4.93	30.80
T2R1	0.5	1.76	4.93	30.80
T2R2	0.5	1.79	5.01	31.33
T2R3	0.5	1.85	5.18	32.38
T3R1	0.5	1.80	5.04	31.50
T3R2	0.5	1.75	4.90	30.63
T3R3	0.5	1.65	4.62	28.89

Carbohydrate content

% carbohydrate = 100 - % (ash + protein + fibre + oil)

Table 6 carbohydrate calculation

Sample	Values
$T^1$1R1	2.35
$T^1$1R2	5.17
$T^1$1R3	9.20
T2R1	3.70

T2R2	8.17
T2R3	1.88
T3R1	6.50
T3R2	5.37
T3R3	9.11

From the carbohydrate formular, moisture content is excluded, because the sample employed is dried after treatment.

Key: $T^1$1 = un detoxified sample

T2 = detoxified by soaking

T3 = detoxified by NH_3

R = replicates

Qualitative test for anti nutrients (Tannins and Saponins)

Reagents for extraction process: Distilled water

n-Hexane

Ethanol

Tannins: Reagent used; $FeCl_3$

Saponins: qualitative test for saponins was determined v ulsion method, using olive oil as reagent

Test for phytin

The finely grounded raw and processed samples were soake illilitres of 2ml HCl as extraction reagents, and allow to stands for 3hrs. The extract er filtered through two

layers of hardened filter paper, the indicator used is ammonium thoicyanate and titrated against $FeCl_3$ until a brownish yellow colour persist for 5min. to confirmed presence of phytin in the seed.