Comparative Determination of Antinutritional Factors in Groundnut Oil and Palm Oil

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Abstract: The research was aimed at determining the degree of antinutritional factors in palm oil and groundnut oil and to know the effect of these factors on the nutritional value of these oils and to know the possible preventive measures. Antinutritional factors are substances found in most food substances which are poisonous to humans and in some ways limit the nutrient availability to the body. The groundnut oil and palm oil were extracted using the soxhlet extraction method. Oxalate and phytate were determined using titration method and aflatoxin was determined using thin layer chromatography. Tannin, trypsin inhibitor, cyanogenic glycosides, hemagglutinin and alkaloids were determined using different procedures. The lethal level of anti-nutritional factors is 50-60 mg/kg for cyanogenic glycosides and phytate and 2.5 g/kg for oxalate and trypsin inhibitor, the lethal doses of these substances were higher compared to the amount that can be possibly found in a food substance at a time from the findings of this research work, groundnut oil contains a higher concentration of the analyzed anti-nutritional factors compared to palm oil.

Key words: Aflatoxin, antinutritional factors, cyanogenic glycosides, groundnut oil, palm oil, phytate

INTRODUCTION

Anti-nutritional factors are those substances found in most food substances which are poisonous to humans or in some ways limit the nutrient availability to the body. Plants evolved these substances to protect themselves and to prevent them from being eaten. However, if the diet is not varied, some of these toxins build up in the body to harmful levels (Norman and Potter, 1987). Some vitamins in food may be destroyed by anti-nutritional substances. Aflatoxin in groundnut has been found to cause severe liver damage if eaten, they are heat stable and can be degraded by strong acid and alkaline treatment (Harold and Pattee, 1985). These anti-nutritional factors must be inactivated or removed, if values of food substances are to be fully maintained.

Palm oil: Palm oil is edible plant oil derived from the palm kernel of the oil palm Elaesis guinensis. It is naturally reddish because of its high amount of β-carotene. Palm oil is obtained from the African oil palm and long recognized in West African people as cooking oil. It was previously the second most widely produced edible oil, after soya bean oil.

Groundnut oil: Groundnut oil is extracted from the seeds of Arachis hypogaea commonly known as groundnut or peanut: it is highly rated over all other edible oils in having no distinct flavour. It retains quite a luscious taste that makes it the preferred oil for chefs all over the world (Clarke et al., 1997).

Aims and objectives:
- To determine the anti-nutritional factors in palm oil and groundnut oil
- To compare the degree of anti-nutrients in palm oil and groundnut oil
- To know the effect of these anti-nutrients on the nutritional value of these oils

Anti-nutritional factors are present in different food substances in varying amounts, depending on the kind of food, mode of its propagation, chemicals used in growing the crop as well as those chemicals used in storage and preservation of the food substances.

Aspergillus flavus is present in groundnut and some other oil seeds. The fungus can only grow when the moisture content of the seeds exceed 9%. This mostly happened due to poor storage conditions which allow pods or kernels to become damaged and contaminated. (Kabagambo et al., 2005).

Aflatoxins are a group of secondary metabolites produced by certain toxigenic strains of Aspergillus flavus and Aspergillus parasiticus of various foods and feed commodities. They are highly toxic and have been implicated epidemiologically as environmental carcinogens in men and animal toxins. Aflatoxins are intensely fluorescent under UV light. There are four major types of aflatoxin namely B1, B2, G1 and G2 (Dutton and Heathcote, 1968). A wide range of agricultural products as well as industrial commodities get contaminated with...
aflatoxin (Wogan et al., 1974). Aflatoxins produce liver lesions and can result to death when the dose is sufficiently high, but lower dose can produce chronic effects like lethargy, hepatitis, etc., (Krough, 1969). Aflatoxin B1 is the most potent hepatocarcinogen (liver cancer promoting compound). Hepatocarcinogens are compounds that cause cancer of the liver. Oxalate: the name oxalate is attributed to its occurrence in plant oxalis (Wood sored). The earliest interest in the toxicity of oxalate arose because of instances of severe or fatal human poisoning following the eating of large quantity of the leaves of certain plants i.e., rhubarb, known to contain relatively large amounts of oxalates, (Osagie, 1998). Phytate: Phytic acid (Inositol hexaphosphoric acid) forms insoluble salts with essential minerals like calcium, iron, magnesium and zinc in food rendering them unavailable for absorption into the blood stream (Bingham, 1978). About half of the phytic acid phosphorus content taken by man is excreted unchanged. The remaining unavailable for utilization, the knowledge of phytate content can be made use of in calculating the quantity of phosphorus available out of the total phosphorus in a diet. Phytic acid and its hydrolysis products are associated with inhibition of calcification in rats (Robert andYudkin, 1999). Tannins: Tannins are made up of complex phenolic polymers which are classified into two structural groups; hydrolysable and condensed tannins inhibit the activities of some enzymes such as trypsin, chymotrypsin, amylase and lipase. Tannins also interfere with dietary iron absorption (Rao and Desothale, 1998) Tannins cause severe growth depression in rats. This effect may be due to reduction in the digestibility of dietary protein and to a lesser extent that of available carbohydrates and lipids (Griffiths, 2000). Trypsin inhibitor: The presence of trypsin inhibitors in the diet leads to the formation of irreversible condition known as enzyme-trypsin inhibitor complex. This causes a drop in intestine trypsin and a decrease in protein digestibility, leading to slower animal growth (Bolhuis, 2003). Cyanogenic glycosides: Cyanogenic glycosides (cyanide) being an effective cytochrome oxidase inhibitor interferes with aerobic respiratory system. Hydrocyanic acid does not occur free, but combines with sugar to form a non-toxic compound known as cyanogenic glycoside (Bolhuis, 2003) respiratory chain at the cytochrome oxidase level is lost during soaking and cooking so that its content in vegetables and fruits poses no danger of toxicity. Hemagglutinins: Hemagglutinins are proteins that have the characteristic ability to agglutinate red blood cells in a fashion similar to antibodies. Like antibodies, they show a remarkable specificity in that they may act in high dilution on one kind of erythrocyte and not at all or only weakly on another (Jaffe, 2003) Plant hemagglutinins are referred to as phytohemagglutinins (PHA). Groundnut oil composition: Groundnut oil obtained from groundnut consists of more than thirteen vitamins including A, B, C and E. it is rich in essential minerals such as calcium, iron, zinc, boron. It consist of saturated fatty acid such as palmitic acid, stearic acid, arachidic acid and lignoceric acid and unsaturated fatty acids such as oleic acid, linoleic acid. Groundnut oil is characterized by low iodine and saponification number. The relative linoleic acid in groundnut oil is a major factor affecting variation in stability of the oil (Kabagambo et al., 2005) Groundnut oil in the diet help matured women in reducing pains associated with menstrual bleeding (Russel, 1980). Palm oil composition: Palm oil is the largest natural source of tocotrienol part of Vitamin E. palm oil contain vitamin E which serve as an anti-oxidant protecting readily oxidizable lipophilic molecules in the cell vitamin K which is a cofactor of post translation gamma carboxylation of N-terminal glutamic acid residue of blood clotting factors II, VII, IX and X when palm oil is present in the diet, it helps in maintaining smooth, healthy and youthful skin (Enig et al., 1983). MATERIALS AND METHODS This research/benchwork was carried out between March to September 2010 in the Food Technology Department of College of Science and Technology of Kaduna Polytechnic, Kaduna -Nigeria. Sampling: The two samples used in the analysis were Groundnut oil (Sample A) and palm oil (Sample B) which were extracted from the raw materials of groundnut and palm kernel respectively. Sample collection: The palm kernel and groundnut used as raw materials were obtained from mallam Auwal Agricultural farm at Rigasa in Igabi Local Government Area of Kaduna. Extraction of groundnut oil: 40 g of the grinded groundnut using pestle and mortar was weighed and rapped twice in a filter paper to prevent leakage of the material. It was then placed in the thimble of the soxhlet chamber. The soxhlet chamber was filled with 250 mL of diethyllether and the set up was heated at 60°C for 4 h to extract the oil. After the extraction, the trace of
diethyether in the oil sample was evaporated using the dryer. The extracted oil was weighed and its percentage calculated.

**Extraction of palm oil:** Twenty gram of the raw material palm kernel bark was peeled and grinded using mortar and pestle and was weighed. The material was wrapped twice in a filter paper to prevent leakage of the material. It was then placed in the thimble of the soxhlet chamber. The soxhlet chamber (the flask) was filled with 250 mL of diethyl ether and the sample heated at 60°C for 4 h to extract the oil. After the extraction, the traces of diethyl ether in the oil sample were evaporated using the dryer. The extracted oil was weighed and its percentage calculated (Clarke et al., 1997).

**ANTI-NUTRITIONAL FACTOR DETERMINATION**

**Oxalate determination:** The oxalate content of the samples was determined using titration method. 2 g of each sample A and B were placed in a 250 mL volumetric flask suspended in 190 mL distilled water. 10ml 6M HCl solution was added to each of the samples and the suspension digested at 100°C for 1h. The samples were then cooled and made up to 250 mL mark of the flask. The samples were filtered and duplicate portion of 125 mL of the filtrate were measured into beaker and four drops of methyl red indicator was added, followed by the addition of concentrated NH₄OH solution (drop wise) until the solution changed from pink to yellow colour. Each portion was then heated to 90°C, cooled and filtered to remove the precipitate containing ferrous ion. Each of the filtrate was again heated to 90°C and 10 mL of 5% CaCl₂ solution was added to each of the samples with stirring consistently. After cooling, the samples were left overnight. The solutions were then centrifuged at 2500 rpm for 5 min. The supernatant was decanted and the precipitates completely dissolved in 10 mL 20% H₂SO₄. The total filtrate resulting from digestion of 2 g of each of the samples were made up to 200 mL. Aliquots of 125 mL of the filtrate was heated until near boiling and then titrated against 0.05 M standardized KMnO₄ solution to a pink colour which persisted for 30 sec. The oxalate contents of each sample was calculated (Munro and Bassiro, 2000).

**Phytate determination:** the phytate of each of the samples was determined through phytic acid determination using the procedure described by Lucas and Markaka (1975). This entails the weighing of 2 g of each sample into 250 mL conical flask. 100 mL of 2% conc HCl was used to soak the samples in the conical flask for 3 h and then filtered through a double layer filter paper. 2.5 mL of saturated Na₂CO₃ solution. The mixture was stirred for 30 minutes at room temperature and centrifuged at 1500 rpm for 5 min. The absorbance of the mixture was taken at 410 nm using 10 mL of the dennis reagent was measured in each flask followed by the supernatants were filtered and the filtrates used for the assay. Two mL of the standard trypsin solution was added to 10 mL of the substrate of each sample. The absorbance of the mixture was taken at 410 nm using 10 mL of the same substrate as blank (Prokopet and Unlenbruck, 2002). The mixture was shaken and allowed to stand for 4h before filtration. The filtrate was evaporated to one quarter of the original volume. Concentrated NH₄OH was added to each of the samples dropwise to precipitate the alkaloid. The precipitate were filtered off with weighed filter paper and

**Cyanogenic glycoside determination:** The method used was alkaline picrate method of Onwuka, (2005). 5 g of each sample A and B were added 50 mL distilled water in a conical flask and allowed to stand overnight. To 1 mL of the sample filtrate in a corked test tube 4 mL of alkaline picrate was added and incubated in a water bath for 5 min. The absorbance of the samples were taken at 490 nm and that of a blank containing 1 mL distilled water and 4 mL alkaline picrate solution before the preparation of cyanide standard curve but there was no colour change in any of the corked test tube containing he sample A and B which is the indication of absence of cyanide in the sample i.e., colour changed from yellow to reddish brown after incubation for 5 min in a water bath (Railes, 1992).

**Tannins determination:** One gram of each sample A and B were dissolved in 10 mL distilled water and agitated, left to stand for 30 min. at room temperature. Each sample was centrifuged and the extract recovered 2.5 mL of the supernatant were dispersed into 50 mL volumetric flask. Similarly, 2.5 mL of standard tannic acid solution was dispersed into a separate 50 mL flask. A 1.0 mL folin dennis reagent was measured in each flask followed by 2.5 mL of saturated Na₂CO₃ solution. The mixture was diluted to 50 mL in the flask and incubated for 90 min at room temperature. The absorbance of each sample was measured at 250 nm with the reagent blank at zero. The % tannin was calculated (Jaffe, 2003).

**Trypsin inhibitor determination:** One gram of each sample A and B were dispersed in 50 mL of 0.5 m NaCl solution. The mixture was stirred for 30 minutes at room temperature and centrifuged at 1500 rpm for 5 min. The supernatants were filtered and the filtrates used for the assay. Two mL of the standard trypsin solution was added to 10 mL of the substrate of each sample. The absorbance of the mixture was taken at 410 nm using 10 mL of the same substrate as blank (Prokopet and Unlenbruck, 2002).
Table 1: Concentration of the anti-nutritional factors in groundnut oil and palm oil

<table>
<thead>
<tr>
<th>Anti-nutritional Factor</th>
<th>Groundnut oil</th>
<th>Palm oil</th>
<th>Lethal dose</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxalate</td>
<td>418 mg/100 g</td>
<td>495 mg/100 g</td>
<td>2-5 g/kg</td>
</tr>
<tr>
<td>Phytate</td>
<td>418 mg/100 g</td>
<td>0.337 mg/100 g</td>
<td>50-60 mg/kg</td>
</tr>
<tr>
<td>Tannin</td>
<td>0.37%</td>
<td>0.13%</td>
<td>30 mg/kg</td>
</tr>
<tr>
<td>Cyanogenic Glycoside</td>
<td>nil</td>
<td>nil</td>
<td>50-60 g/kg</td>
</tr>
<tr>
<td>Alkaloid</td>
<td>0.2%</td>
<td>0.16%</td>
<td>20 mg/100 mg</td>
</tr>
<tr>
<td>Aflatoxin</td>
<td>Present</td>
<td>Absent</td>
<td>-</td>
</tr>
<tr>
<td>Hemagglutinin</td>
<td>200 Hg</td>
<td>100 Hm/g</td>
<td>50 mg/kg</td>
</tr>
<tr>
<td>Trypsin Inhibitor</td>
<td>80.8 Tul/mg</td>
<td>54.7 Tul/mg</td>
<td>2.50 g/kg</td>
</tr>
</tbody>
</table>

washed with 1% NH₄OH solution. The precipitate in each case were dried at 60°C for 30 min and reweighed (Griffiths, 2000).

Hemaglutinin determination: Two gram of each of the sample were added 20 mL of 0.9% NaCl and suspension shaken vigorously for 1 min. The supernatants were left to stand for 1 h the samples were then centrifuged at 2000 rpm for 10 min and the suspension filtered. The supernatants in each were collected and used as crude agglutinating extract.

Preparation of the red blood cells: 3.5 mL of fresh whole blood was collected from the animal using a 5 mL syringe containing Ethylene-diamine Tetracetic Acid (EDTA) antiocoagulant. The whole blood was centrifuged at 2000 rpm for 10 min to separate the red blood cells from the plasma. One volume of the red blood cell was diluted with 4 volumes of cold 0.9% saline centrifuged at 2000 rpm for 10 min and supernatant discarded. The sediment cell was washed with saline three times until the supernatant was colourless. The washed red blood cells were introduced to phosphate buffer saline (about 4 mL of cells per 100 mL of phosphate buffer) to 10 parts of this suspension was added 2% trypsin solution and the mixture incubated at 37°C for 1h. The trypsinized red blood cells were then washed 4-5 times with 0.9% saline to remove traces of trypsin. 1.2-2 mL of parked cells was suspended in 100 mL 0.9% saline. Three wells in a microtitre plate were used each for sample A and B agglutinin extracts and one blank to each of the wells was added 0.3 mL of 0.9% saline with the exception of those containing the 5 samples. After serial dilution, 0.3 mL of 2% suspension of trypsinized red blood cell in saline was added to each well. The contents were mixed and left for 2-4 h at room temperature. The hemagglutinin patterns in the various wells were read (8 rows of 12 wells) (Boyel, 2001).

Aflatoxin determination: Thin layer chromatography technique was used to analyze the presence of aflatoxin. The absorbent used was silica gel with Acetone: Chloroform solvent system. The silica gel absorbent which acted as the stationary phase was spread on a glass slide. It was allowed to dry and activated at a temperature of 120°C. The slide was marked and the samples were spotted on the slide containing the stationary and the mobile phase. The components were visualized using fluorescent glass dyes and the retention factor noted (Enig et al., 1983).

CONCLUSION

From the findings of this research work, Groundnut oil contains higher concentration of the analyzed anti-nutritional factors compared to palm oil (Table 1). Aflatoxin being a carcinogenic anti-nutrient is present in groundnuts and available readily in the oil when the groundnut bark is not removed before extraction. It is safer and healthier to consume palm oil compared to groundnut oil.

RECOMMENDATION

Based on the discoveries from this research work, the following were the recommendations we would like to make:

1. Adequate and conscious growing, production and processing of groundnut should be practiced as it is more likely to be contaminated by many anti-nutritional factors.
2. Varying diet practices may decrease the risk of exposure to antinutritional factors.
3. Palm oil is healthier and safer for consumption and it is recommended for cooking, frying and food processing.
4. We recommend further research on benefits of palm oil and it is nutritive advantages over groundnut oil.

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REFERENCES


