NUTRITIONAL QUALITY AND PHYSICO-CHEMICAL PROPERTIES OF THE SEED AND OIL OF CHINESE FAN PALM

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CERTIFICATION

This is to certify that this project on "nutritional quality and physico-chemical properties of the seed and oil of Chinese fan palm" is the original work approved and carried out by Ezegbe, Clement Chinedum Registration No: 20094698248 under the supervision of Dr (Mrs.) J.N. Nwosu in the Department of Food Science and Technology, School of Engineering and Engineering Technology, Federal University of Technology, Owerri.

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DEDICATION

This research work is dedicated to God, the Almighty and giver of life for giving me life to get to the end of this research work.

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ABSTRACT

The oil of the raw seeds of *Livistona chinensis* (Chinese fan palm*)* was extracted and its physico-chemical properties evaluated. The proximate composition and the anti-nutrients present in the pulp, raw seed, blanched (4, 6, and 8 minutes), cooked (20, 40 and 60 minutes) and the roasted (110^0C for 5, 10 and 15 minutes) seeds were determined. An acute toxicity study was also carried out on the seed during which albino rats were differently fed the raw and also the cooked and roasted samples at 25:75, 50:50, 75:25 and 100: 0 sample-to-commercial feed (Emii feed) ratios respectively for 14 days. The results obtained showed that the oil had a specific gravity (27^0C) of 0.8638g/cm^3, smoke point of 140 – 142^0C, flash point of 239 – 243^0C, iodine value of 35.307mg iodine/g oil, peroxide value of 13.98 MeqO_2/kg oil, saponification value of 40.125mg KOH/g oil, free fatty acids value of 22.031% and acid value of 43.828mgKOH/g oil. The raw seed had a very high crude fibre content (38.21%) and low carbohydrate content (17.82%). Nine (9) anti-nutrients (Tannin: 0.49%, Phytates: 1.20%, Oxalates: 0.18%, Saponins: 5.50%, Hydrogen cyanide: 1.46mg/kg, Alkaloids: 6.00%, total phenols: 0.61%, Flavonoids: 4.00% and oligosaccharides: 27.50%) were found to be present in the raw seed. During processing there was a general reduction trend of the anti-nutrients at different rates but total elimination of saponins, alkaloids and flavonoids was achieved at 40 minutes cooking while oxalates were totally eliminated at 60 minutes cooking. Haematological studies showed a selective effect on some albino rat blood parameters (packed cell volume: 39.00%, haemoglobin: 13.00g/dl, platelets: 864.21 x 10^9/L for albino rats fed 50:50 cooked sample-to-commercial feed mix) and also its ability to cause hyposplenism, thromboembolism and thrombocytosis with possible vascular accidents at higher ratios.

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CHAPTER ONE

1.0 INTRODUCTION.

Livistona chinensis commonly known as Chinese Fan palm or Chinese Fountain palm is of the family, Arecaceae (palm family) and belongs to the genus *Livistona* (Naoto *et al*., 2000). It Is native to southern Japan, Taiwan and several Islands in the southern China sea. It is a medium-sized, slow-growing, single-trunked palm tree that reaches about 15.2m tall in its natural habitat but often seen at much shorter heights of 3 – 8m (Forest, 2003). The leaf sheaths are fibrous, fluffy and brown-coloured somewhat like nests of birds. It has oval to round olive-like fruits that change from green to blue-black when ripe (Wagner *et al.,* 1999).

In most developing tropical countries, the food situation is worsening owing to increasing population, shortage of fertile land, high prices of available staples and restrictions on the importation of food (Nwosu, 2011). This has resulted in a high incidence of hunger and malnutrition, a situation in which children and women, especially pregnant and lactating women are most vulnerable (Potter and Hotchkiss, 1995; Nwosu, 2011). Prediction of future rates of population increase and food production emphasize the seriousness of this problem (FAO, 1990). Okaka *et al.* (1992) and Nwosu (2011) noted that there is

no single solution to the problem of food shortages and crisis. In essence, all information on new sources of food will be of value in the food security struggle.

As recommended by Okaka *et al.* (1992) and noted by Nwosu (2011) that although measures are being taken to boost food production by conventional agriculture, a lot of interest is currently being focused on the possibilities of exploiting the vast number of less familiar food plant resources. Many of such plants have been identified but lack of data on their chemical composition has limited the prospects for their broad utilization (Viano *et al.,* 1995). Most reports on some lesser-known and unconventional crops indicate that they could be good sources of nutrients and many have the potentials of broadening the present narrow food base for humans (Nwosu, 2011).

Livistona chinensis (Chinese Fan Palm) tree resembles that of *Cocos nucifera* (coconut), the cross-sectional profile of the seed also resembles that of coconut and both belong to the same family, Arecaceae (Genini *et al.,* 2009).

It is mainly planted for ornamental reasons (Juliana *et al.,* 2003). The seeds have been noted to be astringent, contain phenolic compounds and used traditionally by the Chinese as an anti-cancer agent (Juliana *et al.,* 2003; Gurpreet and Roman, 2008; Singh and Kaur, 2008; Tao *et al.,* 2009). But in areas where this plant is found in Nigeria,

their seeds are left to waste after maturity. Also its sparing distribution, astringent nature, high phenolic compounds composition, lack of knowledge and documentation of its chemical composition has restricted its use to traditional medication rather than food.

Application of different processing methods to *Livistona chinensis* (Chinese Fan palm) seed, its seed oil properties' determination, proximate composition determination, and its toxicological evaluation will give some useful information, which may increase the utilization of Chinese Fan palm seeds and enhance its potential in food formulations. It is envisaged that a more suitable process for the reduction or elimination of any detected anti-nutritional factors may be found for the production of safer Chinese Fan Palm seed products.

1.1 PROBLEM STATEMENT.

Despite the importance of the palm family*, Arecaceae,* and particularly *Livistona chinensis* which has been used traditionally by the Chinese as an anti-cancer agent (Singh and Kaur, 2008), little has been systematically documented about its utilization as food; the proximate composition, oil extract and effect of processing on the anti-nutrients it contains.

Furthermore, they are mainly planted for ornamental reasons (Corlett, 2005) and the fruits are not utilized as food but rather left to waste after maturity in many places they are found in Nigeria.

The fruit *of Livistona chinensis* has also been noted by many researchers (Gurpreet and Raman, 2008; Juliana *et al.,* 2003; Singh and Kaur, 2008; Fabiana *et al.,* 2006) to be astringent and contain phenolic compounds which could be part of the reason why it is not utilized as food. This research will seek to find answers to some of the problems of its utilization through appropriate processing.

1.2 OBJECTIVES OF THE RESEARCH WORK.

The objectives of this research are;

i. To determine the proximate composition of raw, blanched, cooked and roasted *Livistona chinensis* seed.
ii. To extract oil from *Livistona chinensis* seed and to evaluate the physico-chemical properties of the oil.
iii. To determine the anti-nutrients present in *Livistona chinensis* seed.
iv. To evaluate the effect of blanching, cooking and roasting on the anti-nutrients present in *Livistona chinensis* seed.
v. To evaluate the toxicological effect of *Livistona chinensis* seed on albino rats.

1.3 JUSTIFICATION.

This study will give an insight on the proximate composition, nature of oil, effect of blanching, cooking and roasting on the anti-nutrients in the seed of *Livistona chinensis* as well as its toxicological effects.

Through this way, some possible level of utilization of *Livistona chinensis* seeds will be achieved. This in essence will also be a step forward in the food security struggle in Nigeria through non-conventional food sources.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 BOTANY/DESCRIPTION.

Livistona chinensis commonly known as Chinese Fan Palm or Chinese fountain palm is of the family, Arecaceae (palm family) and belongs to the genus *Livistona* (Naoto *et al.,* 2000). This genus, *Livistona,* is made up of 30 species of which *Livistona chinensis* is one of them (Forest *et al.,* 2003). It is native to southern Japan, Central China, Ryukyu Islands and Taiwan (Forest *et al*., 2003)

It is a medium-sized, slow-growing, single trunked palm tree that reaches about 15.2 metres tall in its natural habitat (Forest *et al*., 2003). However, it is often seen at much shorter heights of 3.0 – 7.6 metres and a frond spread of about 2.4 – 4.0 metres. Its trunk is upright and roundish, up to 46cm in diameter, coloured dark brown and turns grey as it ages (Forest *et al.,* 2003).

It is top-heavy with dense evergreen fronds whose long yellowish-green leaf stalks are sparingly covered with sharp spines towards the base. The bright green fan-shaped leaves are large, up to 1.5cm across and deeply divide with long tampering segments, about 75 in number, which droop gracefully to present a lovely fountain-like palm (Dahot and Mala, 1997; Forest *et al.,* 2003).

The leaf sheaths are fibrous, fluffy, and brown-coloured, somewhat like nests of birds. Hidden with the upper crown of matured trees, 1.8cm inflorescence will emerge, bearing inconspicuous yellow-cream flowers. These will be followed by oval to round olive-like fruits that change from green to blue-black when ripe (Dahot and Mala, 1997; Forest *et al.,* 2003). The diagram of the tree and fruits of Chinese fan palm are shown in figures 1 and 2 respectively.

It is an easy to grow palm that is hardy and robust, and needs little maintenance, prefers full sun and bright locations, drought resistant, adaptable to various soil conditions including clay, loamy and sandy but preferably well drained soil. It survives in tropical and temperate climates, though growth may be slower in temperate areas (Forest *et al.,* 2003).

2.2 DISPERSAL, PROPAGATION AND POLLINATION.

There are several modes of dispersal available to palms species. The three main methods include gravity, humans and animals. *Livistona chinensis* is primarily spread over long distances by humans using the plants in landscaping (Forest *et al*., 2003). From there, plants have been observed spreading primarily by gravity. It may be possible that plants are also spread in water, as seedlings are often observed germinating

Figure 1: *Livistona chinensis* (Chinese fan palm) tree.

Figure 2: *Livistona chinensis* (Chinese fan palm) fruit on its stalk

along ditches. (Forest *et al.,* 2003).

The seeds germinate readily within one to four months of sowing and there is only a single seed inside each fruit (Kulkarni and Mulani, 2004; Forest *et al*., 2003).

In general, as noted by Forest *et al*., (2003), and Kulkarni and Mulani (2004), palms are pollinated by wind and a variety of insects and animals such as beetles, bees, flies, ants and bats.

2.3 DISTRIBUTION.

Livistona chinensis is native to southern Japan, Central China, Ryukyu Islands and Taiwan (Forest *et al.,* 2003; Naoto *et al.*, 2000).

It is one of the hardiest, as well as one of the most widely planted species within the genus. It is cultivated in the United States, pacific islands, including Hawaii and in tropical areas worldwide. It thrives in subtropical and temperate regions withstand harsh weather conditions and cold hardy withstanding cold temperatures down to – 9^0C (Forest *et al.,* 2003).

2.4 COMPOSITION AND PROCESSING.

Generally, ripe fruit of palms is a rich source of lipid but the yield of lipid varies from species to species (Juliana *et al*., 2003 Singh and Kaur, 2008). Mainly it consist of 15 – 64% lipid with low melting point

triacylglycerol and primarily it is composed of oleic and palmatic acid (Dahot and Mala, 1997).

Dahot and Mala (1997) recorded 80.22% moisture, 1.49% ash, 1.92% crude fat, 0.10% crude protein, 16.27% total carbohydrate and11.42% crude fibre for fresh sample of *Livistona chinensis* pulp.

The mineral composition (mg/total ash weight) of *Livistona chinensis* pulp as recorded by Dahot and Mala (1997) include 10.0668mg Sodium, 39.5150mg Potassium, 2.458mg Calcium, 11.920mg Phosphorus, 0.514mg Zinc, 0.223mg Iron and 0.233mg Manganese.

2.4.1 Composition and processing of other related plants

2.4.1.1 Oil palm (*Elaeis guineensis*).

Oil of two kinds is obtained from the oil palm. An individual palm fruit is built up rather like a miniature coconut, with a thick, fibrous layer of pulp on the outside (the pericarp) and a hard kernel (Ihekoronye and Ngoddy, 1985).

The composition of palm oil is rather unique when compared to that of the other major fats and oils. Most of the seed oils, such as soybean, rapeseed, and sun flower seed oils, are composed primarily of unsaturated fatty acids, whilst the palm nut oils, coconut and palm kernel, contain predominantly saturated fats (Ihekoronye and Ngoddy,

1985). Palm oil, however, is composed of approximately 50% saturated fats (primarily palmitic acid) and 40% unsaturated fats (primarily linoleic and oleic acids). Thus, palm oil will tend to be semi-liquid at ambient temperatures (such as in Nigeria), separating on standing into a deep-red liquid fraction and a yellow solid fraction of about equal volumes (Ihekoronye and Ngoddy, 1985). The distinctive colour of the oil is due to fat soluble carotenoids, which are responsible for the high vitamin A content of palm oil (Iheokoronye and Ngoddy, 1985).

Palm kernels yield an oil having a preponderance of saturated fatty acids, primarily lauric acid . Palm kernel oil is very similar to coconut oil and in this respect, the two are virtually interchangeable (Ihekoronye and Ngoddy, 1985).

Palm oil production processes include: fruit reception, steam and power generation, sterilization, mechanical stripping, fruit digestion, oil extraction and oil clarification (Ihekoronye and Ngoddy, 1985). When the fruits are received, power is generated from steam and is used to sterilize the bunch. Stripping is then done to remove the fruits from the bunch. After stripping follows digestion. The digester is a cylinderical vessel with steam jacket. It is open at the top, while it is connected with a chute at the bottom direct to the press. The function of fruit digestion is to break up the pulp physically and raise the temperature of the digested mash usually to about 90^0C to facilitate easy extraction of oil in the press

and also to mash the pericarp so that the oil in the cells is easily released. After digestion follows oil extraction and the ultimate objective of oil extraction is to extract all the oil in the pericarp. This may be achieved in many ways depending on the design of the mill by using various types of presses, e.g. hydraulic press, single screw press or double screw press. The oil is finally clarified to get it rid of its impurities like water, sludge, dirt, fiber particles, etc (Ihekoronye and Ngoddy, 1985).

2.4.1.1.1 Palm wine as a valued product from oil palm.

Palm wine also called palm toddy or simple toddy is the fermented sap of certain varieties of palm trees including Oil palm (*Elaeis guineense*),raphia palm (*Raphia hookeri* or *R. vinifera*), palmyra, date palm (*Phoenix Sylvestris*), Jaggery Palm (*Caryota urens*) (OYeku *et al*., 2009).

It is an important alcoholic beverage in West Africa where it is consumed by more than ten million people (Oyeku *et al*., 2009). It is a highly perishable liquid consisting mainly of water, sugar, vitamins, aroma and flavour components in very small amounts (*Oyeku et al.,* 2009).

The sap is collected by a tapper. Typically the sap is collected from the cut flower of the tree (Wikipedia, 2012). A container, often a guord or bottle is fastened to the flower stump to collect the sap. The initial white

liquid that is collected tends to be very sweet and is not alcohol (Wikipedia, 2012). In some areas, the entire palm tree is felled and the crown exposed to collect the sap. When this method is used, a fire is lit at the root end of the tree to quicken collection of sap.

Palm sap begins to ferment immediately after collection due to natural yeast left in the air which is often spurred by residual yeast left in the collecting container (Wikipedia, 2012). Within two hours, fermentation yields an aromatic wine of up to 4% alcohol content, mildly intoxicating and sweet (Wikipedia, 2012). The wine may be allowed to ferment longer, up to a day to yield a stronger, more sour and acidic taste, which some people prefer. Longer fermentation produces vinegar instead of stronger wine (Wikipedia, 2012).

Palm wine may be distilled to create a stronger drink, which goes by different names depending on the region. In Nigeria, distilled palm wine is called “Ogogoro”, in southern Ghana it is called “Akpeteshi” or “burukutu” while in Togo, it is called “Sodabe” (Wikipedia, 2012).

Palm wine plays an important role in many ceremonies in parts of Nigeria such as Igbo Land. It also has a strong cultural significance among Africans especially central and West African (Wikipedia, 2012). It is used at important traditional ceremonies like marriages, worship rites and other festivals. Although other types of wine are available, palm wine

is irreplaceable for its well known health benefits as well as its cultural significance among Africans.

Palm wine is often infused with medicinal herbs to remedy a wide variety of physical complaints (Wikipedia, 2012).

In Indian State of Kerala, toddy is used in leavening (as a substitute for yeast) a local form of hopper called the Vellai Appam; a staple among the Nasrani Christians (Wikipedia, 2012). Toddy is mixed with rice dough and left over night to aid in fermentation and expansion of the dough causing the dough to rise overnight (Wikipedia, 2012).

In some areas like India, a little lime is added to the palm sap to prevent fermentation, some people evaporate it to leave an unrefined sugar called “Jaggery” while others refrigerate it to take it any time they want (Wikipedia, 2012)

2.4.1.2 Coconut (*Cocos nucifera*).

Coconut trees (*cocos nucifera*) are grown throughout the tropics (Ihekoronye and Ngoddy, 1985). The oil is extracted from the dried copra, which is the white flesh inside the nut. Copra contains about 65% oil which is extracted by crushing and pressing machines (Ihekoronye and Ngoddy, 1985). The oil is a solid below 24^0C and more than 90% of it is glycerol esters of saturated fatty acids. Ihekoronye and Ngoddy (1985) reported that Lauric acid is the main constituent with smaller

amounts of myristic and palmitic acids. The unsaturated oleic and linoleic acids make up no more than 9% of the oil. Because of this high concentration of saturated fatty acids, coconut oil does not go rancid as readily as palm oil (Ihekoronye and Ngoddy, 1985).

The coconut odour is removed by passing steam through the oil and the copra cake left after extraction of oil has a high protein content and is used as animal feed (Ihekoronye and Ngoddy, 1985). Dessicated coconut is prepared from the copra which is shredded and dried in hot air so that it contains less than 2% water. Removal of the oil and grinding of the residue gives coconut flour, but this does not keep well (Ihekoronye and Ngoddy, 1985).

2.4.1.3 Raffia palm.

The most widely accepted and cherished natural traditional alcoholic beverage, especially in the southern parts of Nigeria is the juice of the raffia palm (Ihekoronye and Ngoddy, 1985). Ihekoronye and Ngoddy (1985) explained that when the inflorescence of the palm tree is tapped by tappers, a milky juice, containing initially well over 13% sucrose is collected in a calabash which is hung at the base of the incision. Soon after leaving the tree, yeast spores, especially those of *Saccharomyces cerevisiae* infect the juice and soon start to ferment the fermentable sugar.

Within hours, ethanol has been produced, with the liberation of large amounts of carbon(iv)oxide. This could cause most vessels to explode if they are corked. Within three days, oxidative yeasts and species of *Acetobacter*, oxidize the ethanol into vinegar (Ihekoronye and Ngoddy, 1985). Palm wine is regarded as spoiled when the total acid content measured as a percentage has gone beyond 0.60%.

By heat inactivation of the palm wine yeasts, followed by preservation with low levels of sulphur dioxide and bottling, the Federal Institute of Industrial Research, Oshodi, set the pace for preserved bottled palm wine technology in Nigeria (Ihekoronye and Ngoddy, 1985).

Palm wine has been used for the production of crude alcohol or gin through crude methods locally. However, by using a simple distillation apparatus, a potable alcohol can be produced from fermenting palm wine (Ihekoronye and Ngoddy, 1985). Starting with palm wine or molasses with at least 12% sugar content, the palm wine is allowed to ferment overnight after pitching with yeast, following racking or centrifugation, it is Distilled, discarding the methanol fraction which is collected at 75^0C (Ihekoronye and Ngoddy, 1985).This is not potable. A primary distillation between 75^0C – 85^0C yields a product with 40% v/v alcohol. This can be redistilled to obtain 80% v/v alcohol which can be blended with water, essences and sugar syrup to give gin or brandy (Ihekoronye and Ngoddy, 1985).

2.4.1.4 Date palms (*Phoenix spp.).*

Some other category of palms, like date palms (*phoenix spp*.) are very rich in sugar ranging from 65% to 80% on dry weight basis mostly of inverted forms; glucose and fructose (Erskine *et al*., 2010). Fresh varieties have higher content of inverted sugar while water content is between 7% (dried) and 79% (Fresh) depending on variety (Erskine *et al.,* 2010).

2.5 IMPORTANCE/ UTILIZATION.

Palms represent the third most important plant family with respect to human use (Haynes and Mclaughlin, 2000). Numerous edible products are obtained from palms, including the familiar date palm fruits, coconut palm nuts, and various palm oils (Haynes and Mclaughlin, 2000).. Some less well-known edible palm products include palm “cabbage” or “heart-of-palm”, immature inflorescences, and sap from mature florescences of Tad, Bhirly-mad, Shindhi, etc ((Haynes and Mclaughlin, 2000).

Palms are planted for a varied number of reasons and purposes ranging from food uses, medicinal purposes, ornamental reasons, economic interest, etc (Kulkarni and Mulani, 2004).

2.5.1 Familiar palms for food uses.

Coconut (*Cocos nucifera*), Arecanut (*Areca catechu*), date palm (*Phoenix dactylifera*) and Oil palm (*Elaeis guineensis*) are cultivated for commercial exploitation of their fruits and seeds (Kulkarni and Mulani, 2004).

Palm oil from oil palm is used locally for cooking, the kernel is a source of food, the trees are tapped for palm wine and the fronds are used for building material (Akinyeye *et al*., 2011). Oil palm leaves are used for making brooms, the bark of the frond is peeled and woven into baskets (Akinyeye *et al.,* 2011). The combination of palm oil with caustic soap is used in the manufacture of soap. Palm oil is also a very important component in the manufacture of margarine (Akinyeye *et al.,* 2011; Ihekoronye and Ngoddy, 1985). Palm kernel oil is also obtained from palm kernel which is used in soap and cosmetics and other industrial purposes. Palm kernel cake or meal is primarily and extensively used in animal (Livestock) feed (Akinyeye *et al.*, 2011).

Raffia palm is tapped of its juice and used as an alcoholic beverage (Ihekoronye and Ngoddy, 1985).Date palms are eaten fresh and/or dried as snacks and are used as sweetening component of baked foods as they have very high sugar content (65% to 80%) on dry weight basis (Erskine *et al., 2010).*

Oil is also obtained from Coconut (*Cocos nucifera*) called coconut oil otherwise known as copra oil (Ihekoronye and Ngoddy, 1985). Coconut candies are also produced from coconut.

2.5.2 Less-familiar palms for food uses.

The less familiar palms like Tad (*Borassus flabellifer*), Bhirly-mad (*Caryota urens*) and Shindhi (*Phoenix sylvestris*) palms are extracted for their sugary sap that exudes from their injured stem or flowering axis yielding jaggery or a favourite alcoholic beverage on fermentation (Kulkarni and Mulani, 2004).

The central soft part of the stem of *Corypha umbraculifera*, *C. urens, C. obtusa, Phoenix rupicola, P. acaulis,* and *P. humilis* is a rich source of starch (Haynes and Mclaughin, 2000; Kulkarni and Mulani, 2004).

The stem apices of *C. urens, C. obtusa, Wallichia disticha* and *B. condapanna* are chewed raw or cooked as a delicacy by Indians (Kulkarni and Mulani, 2004).

2.5.3 Palms for ornamental purposes.

Many other palms are cultivated for ornamental purposes. These include Royal (*Oreodoxa spp*.) (Kulkarni and Mulani, 2004), Chinese fan palm (*Livistona chinensis*), *Phoenix canariensis*, *P. dactylifera, P.*

reclinata, Washingtonia filifera, W. robusta, Chamaerops humilis, Trachycarpus fortunei, etc (Armengol *et al.,* 2005). They are used to beautify parks, gardens, avenues, hotels, streets, etc (Kulkarni and Mulani, 2004; Armengol *et al.,* 2005).

2.5.3.1 Chinese fan palm (*Livistona chinensis*) uses.

Chinese Fan Palm (*Livistona chinensis*) more particularly is planted for ornamental reasons and have been used by the Chinese as an anti-cancer agent and posess anti-angiogenic, anti-proliferative, and anti-bacterial activities (Gurpreet and Raman, 2008; Juliana *et al*., 2003; Singh and Kaur, 2008; Dahot and Mala, 1997).

2.6 INTRINSIC TOXICANTS/ ANTI-NUTRIENTS.

Intrinsic toxicants/anti-nutrients are toxic principles that are components of foods by synthesis, or degradation and as such are referred to as naturally occurring food toxicants (Okaka *et al.,* 2002). Probably, all intrinsic toxic substances function as anti-metabolites (Okaka *et al.,* 2002).

Some of the intrinsic toxic principles found in foods include trypsin inhibitor, hemagglutinins, goitrogens, anti-vitamins (oxalic and phytic acid) and other specific anti-nutrient factors (Okaka *et al.,* 2002), tannins,

saponins, alkaloids, polyphenoks, oligosaccharides, steroids and cyanogenic glucosides (Nwosu, 2011).

2.6.1 Cyanogenic glucosides.

The knowledge of the cyanogenic glycosides content of food is vital because cyanide being an effective cytochrome oxidase inhibitor interferes with aerobic respiratory system (Onwuka, 2005) causing gasping, staggering and convulsion (Nwosu, 2011). Hydrocyanic acid does not occur free, but combine with sugars to form a non-toxic compound known as cyanogenic glycoside (Onwuka, 2005). Onwuka (2005) reported that the lethal level for an adult man is 50 – 60mg/kg body weight. Cyanide inhibits the cytochrome oxidase and hydrophenol oxidase enzymes through combination with their copper and iron ions respectively

2.6.2 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 (Onwuka, 2005). About half the phytic acid phosphorus content taken by man is excreted unchanged, thus remaining unavailable for utilization (Onwuka, 2005). Phytic acid and its hydrolysis products are associated with inhibition of calcification in rats (Onwuka, 2005).

2.6.3 Oxalates.

Although oxalates have long been known to occur in nearly all forms of living matter, it is of interest to note that certain families and species contain relatively large amount of this substance, mainly as the soluble sodium or potassium salts or the insoluble calcium salts (Onwuka, 2005). The earliest interest in the toxicity of oxalates arose because of severe or fatal human poisoning following the eating of larger quantities of the leaves of certain plants (i.e. rhubarb) known to contain relatively large amounts of oxalates (Onwuka, 2005). Some of the anti-nutritional and off-flavour problems (bitter and astringent taste and scratches in the mouth and throat) associated with cocoyam are caused by calcium oxalate (Onwuka, 2005).

Oxalates, like phytates, bind minerals like calcium and magnesium and interfere with their metabolism (Soetan and Oyewole, 2009). They also cause muscular weakness and paralysis. Oxalates also cause gastrointestinal tract irritation, blockage of the renal tubules by calcium oxalate crystals, development of urinary calculi, hypocalcaemia and necrotic lesions in the kidney (Soetan and Oyewole, 2009).

2.6.4 Alkaloids.

Alkaloids are basic natural products occurring primarily in plants (Osagie, 1998). They are generally found in the form of salts with organic acids and about 10 – 20% of all higher plants probably contain alkaloids (Osagie, 1998). Common sources of alkaloids include *Mucuna pruriens*, *Physostigma venenosum*, cocoa bean, *Theobromea cocoa,* which contains theobromine steroid glycoalkaloid e.g. solanine and chaconine, which are presented in potato and potato products (Onwuka, 2005).

Alkaloids cause gastrointestinal and neurological disorders (Soetan and Oyewole, 2009). Solanine and chaconine (glycoalkaloids present in potato and *Solanum spp*.) are haemolytically active and toxic to fungi and humans (Soetan and Oyewole, 2009). Some of the toxicological manifestations of potato glycoalkaloids occur at doses in excess of 20mg/100g sample.

2.6.5 Tannins.

Tannins are made up of complex phenolic polymers which are classified into two structural groups; hydrolysable tannins and condensed tannins (Onwuka, 2005). Condensed tannins are far more common in non-grain starch staples and usually exist in the plant tissues as leuco-anthocyanins or proanthocyanidins (Onwuka, 2005). The condensed tannins have been identified in cassava tubers, in the green pulp of many

banana varieties and in yam species (Onwuka, 2005). Tannins can act as anti-nutritional factor in two ways. Firstly, they can provoke an astringent reaction in the mouth and make the food unpalatable. Secondly, they can complex with and thus precipitate proteins in the gut, reducing digestibility or inhibiting digestive enzymes (Soetan and Oyewole, 2009). Tannins inhibit the activities of enzymes such as trypsin, chymotrypsin, amylase and lipase, and also interfere with dietary iron absorption (Onwuka, 2005).

2.6.6 Hemagglutinins.

Hemagglutinins are proteins that have the characteristic ability to agglutinate red blood cells (RBC) in a fashion similar to antibodies (Onwuka, 2005). Moreover, like anti-bodies, they show a remarkable specificity in that they may act in high dilution on one kind of erythrocytes and not at all or only weekly on another (Onwuka, 2005). Hemagglutinins, most frequently have been found mostly in plants and associated with leguminous seeds (Okaka *et al.,* 1992). They are otherwise referred to as lectins.

Hemagglutinins from different seeds cause the clumping of red blood cells from some species of animals but not those of others (Okaka *et al*., 1992). Okaka *et al.,* (1992) also pointed out that soyabean

hemagglutinin, for example, cause the agglutination of rabbit red blood cell but not those of sheep and calves.

2.6.7 Gossypol.

The toxicity of cottonseed meal is usually associated with the presence of gossypol. This compound, first purified as early as 1886, and recognized as the toxic principle in cotton seed (Okaka *et al.,* 1992). Gossypol is a biphenyl compound containing a number of hydroxyl and formyl substituted groups. It is found in most *Gossypium spp.* Gossypol appears to be synthesized in the roots of the cotton plant from where it is translocated to the aerial parts of the plant, where it occurs as innumerable black specks (Okaka *et al*., 1992).

Apparently, gossypol exists in the bound form and only a small fraction is freed during processing. It is the consensus that it is this free gossypol that is toxic to non-ruminants, since chemically bound gossypol is said to be innocuous (Okaka *et al.,* 1992). Gossypol can combine with glucose to produce a compound more toxic than free gossypol (Okaka *et al.,* 1992).

Guinea pigs, weanling pigs and dogs show depressed growth rate and decreased utilization of food, which eventually lead to death when poisoned with gossypol (Okaka *et al.,* 1992). While in rats and mouse, intestinal hemorrhage is a common symptom.

2.6.8 Oligosaccharides (stachyose and raffinose).

Oligosaccharides (stachyose and raffinose) are otherwise referred to as flatulence inducing factors (Okaka *et al.,* 1992). Food items such as melon seed and cowpeas when consumed in appreciable quantities induce abnormal gas distension and belching. The oligosaccharides in these commodities which include raffinose and stachyose are thought to be the major contributory actors in flatulence (Okaka *et al.,* 1992). Okaka *et al.,* (1992) stated that humans do not possess the enzyme – D – galactosidase and these oligosaccharides escape digestive hydrolysis, but are instead fermented in the lower intestine aided by micro-organisms. The fermentative breakdown of these oligosaccharides lead to the production of hydrogen, carbon(iv)oxide and methane, depending on the intestinal micro-flora present.

2.6.9 Saponins.

The anti-nutritional effects of saponins include increased permeability of small intestinal mucosa cells thereby inhibiting nutrient transport, growth depressing action and reduction of protein digestibility, probably by the formation of sparingly digestibly saponin-protein complexes (Jimoh *et al.,* 2011)

2.6.10 Trypsin (protease) inhibitors.

The trypsin (protease) inhibitors are globular proteins found in most leguminous seeds (Okaka *et al.,* 2002). They are characteristically able to attenuate the activity of trypsin but have no effect on pepsin and chymotrypsin (Okaka *et al.,* 2002). The effect of prolonged trypsin inhibitor activity in man is usually seen as an induced enlargement of the pancrease with a concomitant change in the proteolytic action of the exocrine pancreatic secretion (Okaka *et al.,* 2002).

2.7 PROCESSING AND PRESERVATION.

Naturally occurring toxicants are indeed part of the natural food system and unless they are removed or inactivated through processing, they cause acute or chronic toxicity or induce poor nutrients' utilization when consumed (Okaka *et al*., 2002).

Processing and preservation is undertaken to create year round supply of food commodities and to add value to raw produce (Okaka, 1997). By so doing, processing increases the contribution of the food industry to the gross national product (Okaka, 1997).

For many of the foods which contain intrinsic toxic principles, different processing techniques have been developed which make them safe for human consumption (Okaka *et al*., 2002; Potter and Hotchkiss, 1995).

Some of these processes of intrinsic toxicants/anti-nutrients reduction/elimination include blanching, cooking, roasting, malting, dehulling (Nwosu, 2011; Okaka *et al.,* 2002), fermentation and malting (Okaka *et al*., 2002), dehulling and defatting (Jimoh *et al*., 2002), extrusion (Alonso *et al*., 2000), autoclaving and germination (Ugwu and Oranye, 2006), soaking (Olajide *et al*., 2011), crushing, roasting and oven drying, (Montagnac *et al.,* 2009).

Most foods are preserved before and after drying through sun drying and dehydration, smoking, curing and fermentation, freezing and refrigeration, use of chemical additions, and packaging (Ihekoronye and Ngoddy, 1985).

2.7.1 Effect of different processing methods on anti-nutrients of foods .

Martin-Cabrejas *et al.* (2008) assessed the effects of soaking, cooking and industrial dehydration treatments on anti-nutrients and also on protein digestibility of flours from chickpea, lentil and beans. A general decline of phytic acid was observed during dehydration. The study showed that beans had the highest levels of enzyme inhibitors and lectins. However processing such as cooking and dehydration significantly reduced their levels further to negligible concentrations.

Jimoh *et al*. (2011) identified trypsin inhibitor, lectin, tannin, phytin, saponin and oxalate in *Sesamum indicum* (sesame) seed. They observed that cooking and toasting reduced anti-nutrients in sesame seed at lower cooking and toasting time while trypsin inhibitor and lectin were eliminated at higher cooking time.

Alonso *et al.* (1999) studied the comparative effects of extrusion cooking and conventional processing methods (soaking, dehulling and germination) on protein content and reduction of anti-nutrients (phytic acid, condensed tannins, polyphenols, trysin, chymotrypsin, α- amylase inhibitors and haemagglutinating activity) levels in *Vicia faba* (faba beans) and *Phaseolus vulgaris* (kidney beans). In the study, kidney beans showed highest levels of condensed tannins, chymotrypsin, α-amylase inhibitory and hemagglutinating activity. Dehulling significantly increased protein content and greatly reduced condensed tannin and polyphenol levels in both legumes – while extrusion was the best method to destroy trypsin, chymotrypsin, α- amylase inhibitors and haemagglutinating activity.

Nwosu (2011) identified eleven anti-nutritional components (trysin inhibitors, tannins, phytates, oxalates, saponins, hydrogen cyanide, alkaloids, stachyose, raffinose, phenols and steroids) in ‘oze’ (*Bosqueia angolensis*), and all decreased with increased period of heating and malting though not at the same rate. All the anti-nutrients were

eliminated at 20 minutes cooking except for tannins and phytates which had 27% and 22% respectively of their raw material values retained. At 60 minutes cooking, all were eliminated while at 45 minutes roasting (150^0C) all were also eliminated except tannins and phytates which had the values 0.62mg/100g and 2.72mg/100g of the sample.

Ugwu and Oranye (2006) studied the effects of fermentation, boiling, autoclaving and germination on the toxic components of African breadfruit (*Treculia africana*). The African bread fruit was found to contain hydrogen cyanide (26.45mg/kg), tannin (184.10mg/kg) stachyose (1.8%) and raffinose (1.01%) and the different processing treatments reduced them to varying degrees. Autoclaving was found to be best in the elimination of hemagglutinin, stachyose and raffinose while fermentation was effective in the reduction of hydrogen cyanide.

Baber *et al*. (1988) worked on the effects of heat treatments and germination on trypsin inhibitor activity and polyphenols in jack bean (*Canavalia ensiformis*). They found out that the application of dry heat to the seeds and meal of jack bean was not effective in inactivating the trypsin inhibitor and reducing the polyphenol content. They also established that the germination of jack bean seeds for 40 hours decreased the levels of trypsin inhibitor and polyphenols by 31% and 35% respectively.

Olajide *et al*. (2011) investigated the effect of processing on energy values, nutrient and anti-nutrient components of wild cocoyam (*Colocasia esculenta* L.) corm and found out that soaking, cooking and fermentation reduced the array of anti-nutritional factors (tannins, phytate, oxalate, saponin and hydrogen cyanide) identified in the wild cocoyam.

2.8 OIL EXTRACTION.

There are only a few basic production methods to obtain fats and oils from animals, marine and vegetable sources. These include rendering, pressure expelling and solvent extraction. Pressure expelling and solvent extraction are mainly used for oil seeds (Potter and Hotchkiss, 1995).

It is common in large scale operations to remove the oil from cracked seeds at low temperatures with a non-toxic fat solvent such as hexane (Potter and Hotchkiss, 1995). Other solvents which could be used for oil extraction include petroleum ether, chloroform, and methanol (Abdalbasit *et al*., 2009).

2.9 OIL PROPERTIES' DETERMINATION / CHARACTERIZATION .

Potter and Hotchkiss (1995) stated that generally, fats and oils are tested to gain information related to performance in specific food

applications, to measure degree of deterioration (such as oxidation or rancidity) as well as stability of the fat/oil against such change, to check fat properties against purchase specifications, and to identify fats and oils against possible misrepresentation or adulteration. Lee *et al.* (1998) also noted the same view points as mentioned above.

Some of these tests include free fatty acids, acid value, peroxide value, saponification value, iodine value, melting point, smoke point, flash point, solid fat index, specific gravity, fire point etc (Potter and Hotchkiss, 1995).

The degree of unsaturation of the fatty acids in a fat or oil can be quantitatively expressed by the iodine value of the fat or oil, the degree of oxidation that has taken place in a fat or oil can be expressed in terms of peroxide value, the measure of free fatty acids is expressed by the Acid Value, while the average molecular weight of the fatty acids in a fat is indicated by saponification value (Potter and Hotchkiss, 1995).

These are but a few of the chemical tests that have been applied to fats and oils. Much that they reveal can be learned today more quickly by instrumental analytical methods such as gas chromatography and infrared absorption analysis (Potter and Hotchkiss, 1995; Lee *et al.,* 1998) and many of the classical chemical tests have been largely replaced by these newer methods (Potter and Hotchkiss, 1995).

For the physical measurements, the melting point is the temperature at which a chilled cloudy fat within a capillary tube loses its cloudiness from melting of its solidified crystals or the temperature at which chilled fat ion a capillary tube softens just enough to slide within the tube while the temperature at which a melted fat go to its crystalline state by observing the point at which cloudiness of the fat is complete is tagged the solidifying point (Potter and Hotchkiss, 1995). Solid fat index is a measure of solidity of fats and is related to the percentage of the fat that is crystalline at specific temperatures. The response of fats and oils to frying temperatures can be indicated by such measurements as smoke point, flash point and fire point, which correspond to the temperatures at which these occurrences begin (Potter and Hotchkiss, 1995).

2.10 HAEMATOLOGICAL PARAMETERS IN ACUTE TOXICOLOGICAL EVALUATION OF FOODS.

2.10.1 White blood cells (WBC).

White blood cells or leucocytes are cells of the immune system involved in defending the body against both infectious diseases and foreign materials (Hoffbrand *et al.,* 2004). White blood cells are produced in the bone marrow by hematopoietic stem cells (Hoffbrand *et al.,* 2004).

A white blood cell count estimates the total number of white cells in 1 cubic millimeter of blood. It is important in the diagnosis of diseases, especially when accompanied by a differential white blood cell count (Neutrophil, Eosinophil, Basophil, Lymphocyt, Monocyte) (Lewis *et al.,* 2006). The number of white blood cells in the blood is often an indicator of disease or healthy condition (Lewis *et al*., 2006).

A significant increase in white blood cells (WBC) counts indicates toxicity or poisoning while a significant reduction indicates immuno-suppression (Ezeagu, 2005).

2.10.2 Red blood cells (RBC).

The main function of red blood cells is to carry oxygen to the tissues and to return carbon(iv)oxide from the tissues to the lungs (Hoffbrand *et al.,* 2004). In other to achieve this gaseous exchange, they contain the specialized protein haemoglobin (Hoffbrand *et al*., 2004).

Increased red blood ells may be suggestive of one of the following:

- “Relative” (Pseudopolycythaemia) owing to reduced plasma volume (Hoffbrand *et al.,* 2004).
- “Primary” (Polycythaemia vera) as part of the spectrum of myeloproliferative disorders (Hoffbrand *et al*., 2004).
- “Secondary” to chronic hypoxia (e.g. chronic lung disease, congenital heart disease, high affinity haemoglobins) or aberrant erythropoietin production (Hoffbrand *et al.,* 2004).

-Reduced levels of Red blood cells indicates anaemic condition.

2.10.3 Haemoglobin (Hb).

Haemoglobin is the iron-containing protein attached to red blood cells that transports oxygen from the lungs to the rest of the body and picks up carbondioxide at cellular level back to the lungs to be exhaled (Lewis *et al.,* 2006).

A lowered haemoglobin concentration results from impaired haemoglobin synthesis. This may stem from impaired haemoglobin synthesis or failure of haemoglobin (Lewis *et al.,* 2006).

A reduced haemoglobin concentration is a common cause of sideroblastic anaemia and could also be as a result of iron deficiency (Hoffbrand *et al.,* 2004). Haemoglobin synthesis may also be impaired in chronic infections and other inflammatory conditions (Lewis *et al.,* 2006). While high level of haemoglobin suggests a case of thromboembolism (Lewis *et al*., 2006).

2.10.4 Packed cell volume (PCV).

Packed cell volume (PCV) can be used as a simple screening test for anaemia (Lewis *et al.,* 2006) and as a rough guide to the accuracy of haemoglobin measurement (Lewis *et al.,* 2006).

Very low levels of PCV values suggests polycythemia, anaemia and thromboembolism while very high levels of PCV values suggests thromboembolism (Lewis *et al*., 2006).

Packed Cell Volume (PCV) is calculated as a ratio of the Haemoglobin (Hb) of the whole blood to that of the packed cells.

2.10.5 Platelets.

Platelets are produced in the cytoplasm of the large megakaryocytes of the bone marrow, and these cells discharge the platelets into the circulating blood. Sufficient numbers of platelets are necessary for the clotting of blood (Hoffbrand *et al.,* 2004).

High platelets count suggests hyposplenism, myeloproliferative disorders or thrombocytosis (Lewis *et al.,* 2006).

Decreased platelet count is associated with thrombocytopenia, leukemia or cancer in the bone (Hoffbrand *et al.,* 2004).

2.10.6 Mean corpuscular haemoglobin (MCH).

It is of particular importance in conjunction with mean cell volume (MCV) and mean corpuscular haemoglobin concentration (MCHC) in the diagnosis of anemia (Lewis *et al.,* 2006).

MCH is calculated by multiplying haemoglobin by 10 and dividing the value obtained with the red blood cell count.

2.10.7 Mean corpuscular haemoglobin concentration (MCHC)

Mean corpuscular haemoglobin concentration (MCHC) is a measure of the concentration of haemoglobin in a given volume of packed red blood cells (Lewis *et al*., 2006). It is reported as part of a standard complete blood count.

It is calculated by multiplying haemoglobin value with 100 and dividing the obtained value with packed cell volume and is expressed as a percentage.

Mean corpuscular haemoglobin concentration (MCHC) is significantly diminished in microcytic anaemia but is increased in hereditary sherocytosis, sickle cell diseases and homozygous haemoglobin disease (Lewis *et al.,* 2006).

2.10.8 Mean corpuscular volume (MCV).

Mean corpuscular volume (MCV) gives the volume of an individual red bloob cell in cubic microns, and therefore gives information about the cell size (Hoffbrand *et al.,* 2004).

High MCV values are obtained in megaloblastic anaemia when there are macrocytes in the peripheral blood (Lewis *et al.,* 2006).

Very significantly low values are obtained when microcytes are present, for example in iron deficiency anaemia and thalassemia (Lewis *et al*., 2006).

It is calculated by multiplying the value of the packed cell volume by 10 and then dividing it with the value of the red blood cell count.

2.10.9 Differential white blood cell count.

The differential white blood cell count includes lymphocytes, monocytes, basophils, eosinophils, and neutrophils.

2.10.9.1 Neutrophils.

Neutrophils are commonly increased in number during pregnancy and in acute infections, inflammation, intoxication and acute blood loss or destruction (Lewis *et al.,* 2006).

In the absence of any underlying cause, a high neutrophil count with immature myeloid cells suggest chronic granulocytic leukemia (Lewis *et al.,* 2006).

Very decreased neutophils count would be suggestive of overwhelming infection, auto-immune disorders such as irritation, drugs and large granular lymphocyte leukemia (Lewis *et al.,* 2006).

2.10.1.9.2 Lymphocytes.

The lymphocytes are important in the immune responses of the body (Hoffbrand *et al.,* 2004). They are responsible for the production of specific anti-body globulins (immunoglobulins) of different types, known as IgG (Immunoglobulin G), IgM, IgA and IgE (Hoffbrand *et al*., 2004).

Increased number of lymphocytes is a feature of certain infections. It may be especially marked in pertussis, infections mononucleosis, cytomegalovirus infection, brucellosis (Lewis *et al*., 2006). It could also be an indication of physical stress (Hoffbrand *et al*., 2004).

Lymphocytes may be reduced also by stress such as surgery, trauma and infection (Lewis *et al*., 2006).

2.10.9.3 Monocytes.

Monocytes are macrophage cells which have a powerful phagocytic action. They remove bacteria and other harmful or unwanted substances from the blood (Hoffbrand *et al*., 2004).

Increased monocytes may be associated with some protozoal, rickettsial and bacterial infection (Hoffbrand *et al*., 2004; Lewis *et al*., 2006). It could also suggest a myeloid leukaemia.

Monocytes may be reduced by stress such as surgery, trauma and infection (Lewis *et al*., 2006).

2.10.9.4 Eosinophils.

Eosinophil is a little larger than a neutrophil and normally the nucleus has only two lobes (Hoffbrand *et al*., 2004). The cytoplasm contains large red granules, which make the cell easy to recognize (Hoffbrand *et al*., 2004).

Significant increase in eosinophils is typically associated with allergic disorders including drug sensitivity, skin disease and parasitic infections (Lewis *et al*., 2006).

Eosinophils may also be reduced by stress such as surgery, trauma and infection (Lewis *et al*., 2006).

2.10.9.5 Basophils.

These are only occasionally seen in normal peripheral blood. They have many dark cytoplasmic granules which overlie the nucleus and contain heparin and histamine (Hoffbrand *et al*., 2004).

Basophils are present in increased numbers in myeloproliferative disorders and are especially prominent in chronic granulocytic leukaemia.

2.10 TOXICOLOGICAL EVALUATION OF FOODS.

The toxicological nature and safety of novel foods and non-novel foods whose toxicity status are not known or that have toxicity

controversy have been assessed by many researchers. This has been done through acute and sub-chronic toxicity study (Datta *et al*., 2011). In toxicity studies, some of the parameters usually investigated for changes or variations include histopathological parameters (liver, lungs, kidney, spleen, brain, adrenals, gonads, heart etc), serum biochemical parameters (creatinine, uric acid, calcium ions, chloride ions, urea, sodium ions, phosphorus, total cholesterol, low density lipo-protein cholesterol (LDL – C), high density lipo-protein cholesterol (HDL – C), triglyceride, albumin, bilirubin, protein, alkaline phosphate, Y-glutamyl transferase, alanine, aspartate aminotransferases, etc) and haematological parameters (white blood cells, red blood cells, packed corpuscular volume, haemoglobin, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, neutrophils, monocytes, lymphocytes, eosinophils and basophils (Odeyemi *et al*., 2009).

Odeyemi *et al*. (2009) investigated the toxicological effect of the essential oil from *Mentha longifolia L. subsp. capensis* leaves in rats. The oil at 125,250,375 and 500µl/kg of body weight reduced ($p<0.05$) the red blood cell and lymphocytes with no definite pattern on the white blood cells and mean cell volume. The doses significantly increased the neutrophils, monocytes, large unstained cells, liver-body weight ratio, and serum concentrations of cholesterol, triglycerides high-density

lipoprotein-cholesterol, and inorganic phosphate but had no effect on the heart body weight ratio and serum low-density lipoprotein-cholesterol, Na^{+}, Ca^{2+}, Cl^{-}, K^{+}, creatinine and uric acid. In contrast, the oil reduced the serum urea and atherogenic index. The total and conjugated bilirubin together with the total protein and albumin, in the Serum increased only with oil at 125µL/kg of body weight. The serum alkaline phosphatase activity also increased with no significant change in those of Y-glutamyl transferase, alanine and aspartate aminotransferase. The results indicate dose and parameter-specific effect of the essential oil from *M. longifolia* leaves may not predispose to atherosclerosis, it may increase the functional activity of the rat liver at the lowest dose investigated and may not be completely “safe” at the doses investigated.

Ajayi *et al*. (2005) worked on the chemical analysis and preliminary toxicological evaluation of *Garcinia mangostana* seeds and seed oil. They observed that the rats used for the experiments appeared to suffer no toxicological effects when fed with *G. mangostana* seed oil in their diet for 8 weeks. Weekly monitoring of the rats showed good physical appearance and steady weight increase. Histopathological examination of sections of the heart, liver, kidney, spleen and lungs revealed that the kidney of some of the rats had some degree of pathology which included diffuse glomerular and tubular degeneration. No lesion was found in the heart and liver of the rats. They therefore concluded that the seed oil could be useful as an edible oil.

Akinnawo *et al.* (2002) did a toxicological study on the edible larva of *Cirina forda* (westwood) wherein raw and processed larva of *Cirina forda* (westwood) were administered orally to white albino rats and mice. The 14 days investigation showed that the raw extract was toxic to mice, showing signs of irritability and muscular tremor while the processed larva extract did not produce such effects. They registered normal levels of serum enzymes in all the rats which suggested that the larva extracts are not hepatoxic. They finally concluded that the processed larva of *Cirina forda* (Westwood) is neither neurotoxic nor hepatotoxic to mice and rats.

Vendomois *et al.* (2009) investigated the toxicity of three genetically modified corn by comparison of the three. The results of the experiments revealed new side effects for the 3 genetically modified (GM) corn, which were sex and often dose-dependent. The effects were mostly associated with the kidney and liver (the dietary detoxifying organs) although different between the 3 GMOs. They also observed changes in the heart, adrenal glands, spleen and haematopoietic system. They concluded from the experiment that the GM corn indicated signs of hepatorenal toxicity, possibly due to the new pesticides specific to each GM Corn.

The toxicity profile of methanol extract of Aloe Vera (*Aloe barbadensis*) gel was studied in wister rats by Saritha and Anilakumar (2010). A multiple oral administration of the extract at single does of 4, 8,

16g/kg body weight for 14 days did not produce signs of toxicity, behavioural appearances and changes on gross appearance. The sub-acute toxicity was determined by administration of graded doses (1, 2, 4, 8 and 16g/kg body weight orally) of the extract daily for 6 weeks and the effects on body weight, organ weight, histology as well as serum biochemical parameters were estimated. The body weight of dosed and control rats increased throughout the duration of treatment. The data generated demonstrated significantly no difference in serum concentrations of aspartate amino transferase, alanine amino transferase, alkaline phosphate, total protein, albumin, urea, creatinine, total and direct bilirubin. The histopathological findings on the livers, kidneys, small intestines, hearts and brains revealed normal architecture and no obvious pathology was noticed. They therefore concluded that the methanol extract of Aloe vera do not produce significant toxic effect in rats during acute and sub-acute treatments in rats and that it can be utilized for nutraceuticals formulations.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 SOURCE OF RAW MATERIAL.

The fresh fruits of *Livistonia chinensis* was obtained from Amaigbo in Nwangele L.G.A of Imo State, Nigeria.

3.2 EQUIPMENT AND CHEMICALS USED.

All equipments and chemicals used are available at National Root Crops Research Institute (NRCRI), Umudike and Federal University of Technology (FUTO), Owerri, Imo State.

3.3 SAMPLE PREPARATION.

The pulp of the fruit was removed manually with a knife. The separated seed was dried in an oven (Gallenkamp hot box oven) at 60^0C for 3hours. The dried sample was milled and kept in airtight containers. From there, the sample for proximate composition analysis, anti-nutritional properties analysis, rat feeding and oil extraction were taken. The pulp was also analysed for proximate composition and anti-nutritional properties. A completely randomized design and one-way analysis of variance (ANOVA) was used for the experiment.

Plate 1: *Livistona chinensis* (chinese fan palm) fruit.

Plate 2*: Livistona chinensis* (chinese fan palm) seeds

3.4 OIL EXTRACTION

The Chinese fan palm pulp was manually removed from the fruit and the seed was washed to remove dirt. The seed (2kg) was dried in the sun for 5 hours. The dried seed was milled and soaked in N-hexane for 24 hours. The milled and soaked seed was extracted of its oil using the continuous soxhlet extraction technique with an analytical grade N-hexane (boiling point rage 68 – 69^{0}C) for 8 hours and the N-hexane was recovered during the process. The yield of oil from 2kg of the seed was 54ml.

3.5 BLANCHING, COOKING AND ROASTING

Blanching and cooking were done by the procedures described by Nwosu (2011). The fruit was manually removed of its pulp and the seed was taken in its whole form (without milling) for blanching, cooking and roasting treatments. The seed was divided into Nine (9) batches (1, 2, 3, 4, 5, 6, 7, 8 and 9) of 300g each. Batches 1, 2 and 3 were given a hot water (100^{0}C) blanching treatment for 4, 6 and 8 minutes respectively. Batches 4, 5 and 6 were cooked for 20, 40 and 60 minutes respectively. While batches 7, 8 and 9 were roasted at 110^{0}C for 5, 10 and 15 minutes respectively). The samples were left to cool after the treatments. The blanched and cooked samples were dried

(Gallenkamp hot box oven) at 60^0C for 3 hours to a moisture content of 35%. The Nine (9) processed batches were all milled, allowed to cool and stored in airtight containers. Samples were taken from the airtight containers for rat feeding (toxicity study), proximate composition and anti-nutritional factors determination.

3.6 PROXIMATE ANALYSIS.

The proximate analysis was carried out according to the methods outlined by the Association of Official Analytical Chemists (A.O.A.C, 1990)

3.6.1 Moisture content.

Two grams of the dried ground sample was weighed into a crucible and placed in an oven at a controlled temperature of 105^0C. The sample was allowed to dry in the oven to a constant weight.

The percentage moisture content was then expressed as the percentage of the original weight of the sample. The experiment was carried out in triplicates the percentage moisture was thus calculated:

$$\text{Percentage moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times 100$$

Where W_1 = Mass of dried crucible

W_2 = Mass of dry crucible + Sample before drying

W_3 = Mass of dry crucible + Sample after drying

3.6.2 Ash content .

Two grams of the dried sample was measured into a crucible and placed in the muffle furnace at 550^0C until it was burnt to ash. The crucible and content were then allowed to cool in a desiccator and weighed. This was done repeatedly until a constant weight of the ash was obtained.

The percentage ash content was then expressed as percentage of the original weight of the sample on dry basis. The experiment was done in triplicates. Percentage ash content was thus calculated:

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

Where W_1 = Weight of sample analyzed

W_2 = Weight of empty crucible

W_3 = Weight of crucible + Ash

3.6.3 Crude fat content .

Ten grams of the dried ground sample was weighed and wrapped with a clean filter paper and placed into the thimble in a soxhlet extractor. A round bottom flask was cleaned, weighed and 120ml of food grade hexane added. The flask was connected to the sample holder of the

soxhlet extractor and heated slowly on a mantle for 6 hours. Refluxed hexane was recovered and the flask containing the lipid was dried in the moisture extractor in the oven at 60^0C for few minutes to remove any residual solvent. After drying, the flask containing the oil was cooled in a desiccator and reweighed.

By difference, the mass was determined and expressed as the percentage of the fat thus:

$$\text{Percentage (\%) Crude fat} = \frac{\text{Weight of fat}}{\text{Weight of sample}} \times 100$$

3.6.4 Crude fibre content .

Two grams(2g) of the defatted dried sample was transferred into a 100ml flask, followed by addition of 200ml of 1.25% sulphuric acid. The flask was then placed in a digest apparatus on a pre-adjusted hot plate and boiled for 30 minutes with rotation of the flask periodically to prevent solid from adhering to the bottom of the flask. At the end of 30 minutes, the mixture was allowed to stand for one minute, and filtered immediately through the Buchner funnel lined with a muslin cloth. The insoluble matter was washed into the flask for alkali digestion using 0.3M sodium hydroxide. The digest was boiled for 30 minutes and was allowed to cool for one minute and then filtered using a muslin cloth as before. The residue was then washed successively with 0.1MHCl and finally with

boiling water until it was free of acid. It was then washed twice with alcohol and thrice with ether. The residue or insoluble matter was then transferred into a crucible and dried at 105^0C in an oven to a constant weight, cooled and weighed. It was then ashed at 550^0C, cooled and weighed. The difference in weight after ashing was then calculated as the fibre content of the sample and was expressed as a percentage of the original weight. The percentage crude fibre content was this calculated:

$$\% \text{ Crude Fibre} = \frac{W_2 - W_3}{W_1} \times 100$$

Where W_1 = Weight of sample

W_2 = Weight of sample and crucible after drying at 105^0C

W_3 = Weight of sample (as ash) and crucible after ashing

3.6.5 Crude protein content .

0.6g of the dried ground sample was weighed into an already dried kjeldahl flask. A few drops of water was added to the sample to moisten it, using a burette, 3ml of conc. H_2SO_4 acid was added into the flask followed by the addition of 0.5g of $CuSO_4$. The content of the flask was then digested in a fume cupboard with occasional stirring until a clear solution was obtained. The flask was allowed to cool and a small quantity of distilled H_2O added. The digest was then transferred into 100ml

volumetric flask and the initial volume recorded. The mixture was shaken thoroughly to obtain a homogenous solution.

The mixture was now ready for distillation. The distillation apparatus was steamed for 30 minutes as to get rid of traces of alkali left in the flask. With the aid of a pipette, 10ml of the digest was added to the micro distillation apparatus using a funnel. 10ml of 50% NaOH solution was put in the funnel with measuring cylinder, with stopper glass rod in place. A water condenser set was connected with a 100ml conical flask used as a receiver which contained 10ml of 4% boric acid and two (2) drops of mixed indicator (bromocressol green/methyl red). The drop end of the condenser was immersed well into the boric acid. The stopper glass rod was gradually removed to allow the NaOH solution to thoroughly mix with the sample digest solution. The funnel was filled with distilled H_2O and the steam generator was closed at the top and steam passed into the distillation set. NH_3 was liberated and was distilled into 10ml 4% boric acid for 15 minutes. 50ml of the distillate of blue/green colour was collected and the drip end of the condenser was washed with distilled water into the 100ml conical flask containing the distillate. The distillate was then titrated against 0.1N hydrochloric acid till it changed to pink colour.

A reagent blank was run as a control and the protein content was then calculated by multiplying Nitrogen obtained with the factor of 6.25,

expressed on dry basis. The experiment was carried out in triplicates. The formula for % crude protein is given below:

$$\% \text{ Protein} = \% N_2 \times 6.25$$

$$\% N_2 = \left[\frac{100}{W} \times \frac{N \times 14}{1000} \times \frac{V_t}{Va} \right] T - B$$

Where W = Weight of sample

N = Normality of titrant

Vt = Total digest volume

Va = Volume of digest analyzed

T = Sample titre value

B = Blank titre value

3.6.6 Carbohydrate content .

Carbohydrate content was determined by the difference method. This was done by summing up the % moisture, % protein, % fat, % ash and % crude fibre contents and then subtracting their sum from 100. It was also expressed in percentage(%).

3.7 OIL PROPERTY DETERMINATION.

These experiments were carried out in accordance with the methods described by A.O.A.C. (1990).

3.7.1 Free fatty acids.

0.320g of the oil sample was weighed into 250ml conical flask, 25ml of freshly neutralized hot alcohol was added. The mix was boiled on a heating mantle. The content was then titrated with 0.2N NaOH solution using phenolphthalein indicator until the pink colour returned. The volume of the alkali used was recorded and free fatty acid was calculated. The free fatty acids content was done in triplicate. The free fatty acids were thus calculated:

$$\% \text{ FFA (as oleic)} = \frac{\text{ml alkali} \times \text{N of alkali} \times 28.2\text{mg}}{\text{Sample weight}}$$

3.7.2 Acid value.

The acid value was calculated from the free fatty acid (as oleic) values multiplied by 1.99. It was thus calculated:

Acid value= % Free fatty acid (as oleic) x 1.99

3.7.3 Peroxide value.

0.206g of the oil sample was weighed out into a 250ml conical flask 30ml of acetic acid-chloroform solution (with the ratio 3:2 by volume) was added to it. 1.5ml of saturated potassium iodide was then added, the solution was occasionally shaken for 6 minutes. 30ml of distilled water was then added. The solution was titrated with 0.002M sodium

thiosulphate solution using starch indicator until the blue colour disappeared. The peroxide value was done in triplicate and was thus calculated:

$$\text{Peroxide Value (Meq/kg oil)} = \frac{(S - B) \times N \times 1000}{\text{Sample weight}}$$

Where S = Sample titrate value

B = Blank titre value

N = Normality of $Na_2S_2O_3$ solution

3.7.4 Saponification value.

0.755g of oil sample was weighed into a conical flask. 25ml of 0.5KOH was added. A blank experiment was also carried out at the same time with the oil sample. Air condenser were fitted to the two flasks. The two experimental flasks were boiled steadily until saponification was completed as indicated by the absence of an oily matter and the appearance of a clear solution. The flasks were then cooled and the excess KOH was titrated with 0.5N HCl, using phenolphthalein indicator. The saponification value was then calculated. The saponification value was done in triplicate. The saponification value was thus calculated:

$$\text{Saponification value} = \frac{(b - a) \times N \times 56.1}{\text{Weight of sample(g)}}$$

Where b = blank titre value

a = sample titre value

N = Normality of the HCl

56.1 = The molecular weight of KOH

3.7.5 Iodine value.

The oil sample (0.412g) was weighed into 50ml conical flask, 10ml of Wij's solution was added. 5ml of carbon tetrachloride was added, a stopper was fitted on top of the conical flask to avoid evaporation and absorption of CO2 from the air. The sample was kept in the dark for 60 minutes. 10ml of 10% potassium iodine solution was then added. 50ml starch was added to obtain a blue black colour. 0.2N sodium thiosulphate was then used to titrate the sample back to a colourless solution. A blank sample experiment was also carried out along with the oil sample experiment. The iodine value was calculated. The iodine value was done in triplicate. It was thus calculated:

$$\text{Iodine value} = \frac{(b - a) \times 12.69 \times N}{\text{Weight of sample}}$$

Where b = blank titre value

a = sample titre value

N = Normality of sodium thiosulphate solution ($Na_2S_2O_3$)

3.7.6 Smoke point .

30ml of the oil sample was placed into a 50ml conical flask. A thermometer was inserted in a bored rubber cork and was clamped in such a way that the thermometer was suspended in the oil. The whole set up was heated on a heating mantle until smoke appeared. The temperature at which the smoke started appearing was recorded as the smoke point. The smoke point was done in triplicates.

3.7.7 Flash point.

After noting the smoke point (from 3.7.6), the temperature at which the oil started flashing (when flame was applied) without supporting combustion was noted and recorded as the flash point.

3.7.8 Specific gravity.

A 50ml measuring cylinder was dried and weighed, oil sample was poured into it up to the 50ml graduation mark. Marked, weighed and result recorded.

Similarly distilled water was poured up to 50ml graduation mark in another already weighed cylinder re-weighed and the result recorded. The specific gravity was done in triplicate. The specific gravity was this calculated:

$$\text{Specific gravity} = \frac{\text{Weight of Xml of oil}}{\text{Weight of Xml of water}}$$

Where X is the specific volume of sample used.

3.8 ANTI-NUTRITIONAL FACTORS DETERMINATION.

The anti-nutritional factors in ‘Chinese Fan Palm’ seed and pulp were determined as follows:

3.8.1 Determination of tannins.

Tannin content of the sample was determined by Folin Denis Colorimetric method (Kirk and Sawyer, 1998). A measured weight (1g) of the processed sample was mixed with distilled water in the ratio of 1:10 (W/V). The mixture was agitated for 30 minutes at room temperature and filtered to obtain the extract.

A standard tannic acid solution was prepared 2ml of the standard solution and equal volume of distilled water were dispersed into a separate 50ml volumetric flasks to serve as standard and reagent blank respectively. Then 2ml of each of the sample extracts were put in their respective labeled flasks.

The content of each flask was mixed with 35ml distilled water and 1ml of the Folin Denis reagent was added to each. This was followed by 2.5ml of saturated Na_2CO_3 solution. Thereafter each flask was diluted to the 50ml mark with distilled water and incubated for 90 minutes at room temperature. Their absorbance was measured at 710nm in a colorimeter

(Jenway 6051) with the reagent blank at zero. The tannin content was calculated as shown below:

$$\% \text{ Tannin} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{V_t}{Va} \times D$$

Where W = weight of sample

au = absorbance of test sample

as = absorbance of standard tannin solution

C = Concentration of standard tannin solution

Vt = Total volume of extract

Va = Volume of extract analyzed

D = Dilution factor (if any)

3.8.2 Determination of saponins.

This was done by the double solvent extraction gravimetric method (A.O.A.C., 1990). Two grams(2g) of the processed sample was mixed with 100ml of 20% aqueous ethanol solution and incubated for 12hours at a temperature of 55^0C with constant agitation. After that, the mixture was filtered through whatman No 42 grades of filter paper. The residue was re-extracted with 50ml of the ethanol solution for 30 minutes and the extracts weighed together.

The combined extract was reduced to about 40ml by evaporation and then transferred to a separating funnel and equal volume (40ml) of

diethyl ether was added to it. After mixing well, there was partition and the upper layer was discarded while the lower aqueous layer was re-extracted with the ether after which its pH was reduced to 4.5 with dropwise addition of NaOH solution.

Saponin in the extract was taken up in successive extraction with 60ml and 30ml portion of n-butanol. The combine extract (ppt) was washed with 5% of NaCl solution and evaporated with a water bath in a previously weighed evaporation dish. The saponin was then dried in an oven (Gallenkamp Hot box Oven) at 60^0C (to remove any residual solvent), cooled in a desiccator and re-weighed. The saponin content was calculated as shown below:

$$\% \text{ Saponin} = \frac{W_2 - W_1}{W}$$

Where W = Weight of sample used

W_1 = Weight of empty evaporation dish

W_2 = Weight of dish + saponin extract

3.8.3 Determination of alkaloid.

The alkaline precipitation gravimetric method (Inuwa *et al.,* 2011) was used.

A measured weight (1g) of the processed sample was dispersed in 30ml of 10% acetic acid in ethanol solution. The mixture was shaked well

and allowed to stand for 4 hours at room temperature. The mixture was shaken periodically at 30 minutes interval. At the end of this period, the mixture was filtered through whatman No.42 grade of filter paper.

The filterate (extract) was concentrated by evaporation, to a quarter of its original volume. The extract was treated with dropwise addition of concentration NH_3 solution to precipitate the alkaloid. The dilution was done until the NH_3 was in excess.

The alkaloid precipitate was removed by filteration using weighed whatman No.42 filter paper. After washing with 1% NH_4OH solution, the precipitate in the filter paper was dried at 60^0C in an oven (Gallenkamp hot box oven) and weighed after cooling in a desiccator. The alkaloid content was calculated as shown below:

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100$$

Where W_1 = Weight of empty filter paper

W_2 = Weight of filter paper + alkaloid precipitate

3.8.4 Determination of total phenols.

This was determined by the folin-ciocateau spectrophotometeric method (AOAC, 1990). The total phenols was extracted in 0.2g of the sample with 10ml concentrated methanol. The mixture was shaken for 30 minutes at room temperature. The mixture was centrifuged at 500rpm for 15 minutes and the supernatant (extract) was used for the analysis.

1ml portion of the extract from each sample was treated with equal volume of folin-ciocalteau reagent followed by the addition of 2ml of 2% Na_2CO_3 solution. Standard phenol solution was prepared and diluted to a desired concentration.

Iml of the standard solution also treated with the Folin-ciocateau reagent and Na_2CO_3 solution. The intensity of the resulting blue colouration was measured (absorbance) in a colorimeter (Jenway 6051) at 540nm wavelength.

Measurement was made with a reagent blank at zero. The phenol content as calculated using the formula below:

$$\% \text{ Phenol} = \frac{100}{W} \times \frac{au}{as} \times C \times \frac{V_t}{Va} \times D$$

Where W = weight of sample

au = absorbance of test sample

as = absorbance of standard phenol solution

C = Concentration of standard phenol solution

Vt = Total volume of extract

Va = Volume of extract analyzed

D = Dilution factor (if any)

3.8.5 Determination of hydrogen cyanide (HCN)

This was determined by the method used by Balagopalan *et al.* (1998).

1g of the sample was dispersed in 50ml of distilled water in a 25ml conical flask. An alkaline pikrate paper was hung over the sample mixture and the blank in their respective flasks.

The set up were incubated overnight at room temperature and each pikrate paper was eluted (or dipped) into 60ml distilled water. A standard cyanide solution was prepared and diluted to a required concentration. The absorbance of the eluted sample solution and of the standard were measured colourimetrically at 540nm wavelength with the reagent blank at zero.

The cyanide content was determined by the formula shown below:

$$\text{HCN (mg/kg)} = \frac{1000}{W} \times \frac{au}{as} \times C \times D$$

Where W = weight of sample analyzed

au = absorbance of test sample

as = absorbance of standard HCN solution

C = Concentration of standard in mg/dl

D = Dilution factor where applicable

3.8.6 Determination of phytate.

The method described by Nwosu (2011) was used.The phytic acid in the samples was precipitated with excess $FeCl_3$ after extraction of 1g of each sample with 100ml 0.5N HCl. The precipitate was converted to sodium phytate using 2ml of 2% NaOH before digestion with an acid mixture containing equal portions (1ml) of conc. H_2SO_4 and 65% $HClO_4$. The liberated phosphorus was measured colorimetrically (Jenway 6051 Colorimeter) at 520nm after colour development with molybdate solution.

The percentage phytate was thus calculated:

$$\% \text{ Phytate} = \frac{100}{W_t} \times \frac{au \times C \times Vt}{as \quad Va}$$

Where W = weight of sample used

au = absorbance of test sample

as = absorbance of standard phytate solution

C = Concentration of standard phytate solution

Vt = Total volume of extract

Va = Volume of extract analyzed

3.8.7 Trypsin inhibitor determination.

This was done using the spectrophotometric method, described by Nwosu (2011).

A measured weight (10g) of the test sample was dispersed in 50ml of 0.5M NaCl solution and stirred for 30 minutes at room temperature. It was centrifuged and the supernatant filtered through whatman No.42 filter paper. The filtrate was used for the assay.

Standard trypsin was prepared and used to treat the substrate solution (N – benzoyl-DI-arginine-P-anilide; BAPA). The extent of inhibition was used as a standard for measuring the trypsin inhibitory activity of the test sample extract. Into a test tube containing 2ml of extract and 10ml of the substrate (BAPA) 2ml of the standard trypsin solution was added. Also 2ml of the standard trypsin solution was added in another test tube containing only 10ml of the substrate. The latter served as the blank.

The content of the tubes were allowed to stand for 30 minutes and then absorbance of the solution measured at 430nm wavelength with a colorimeter (Jenway 6051). One trypsin activity unit inhibited is given by an increase of 0.01 absorbance unit at 430nm. Trypsin unit inhibited was thus calculated:

$$\text{Trypsin unit inhibited} = \frac{Au \times 0.01 \times F}{As}$$

Where Au = Absorbance of test sample

As = Absorbance of standard (uninhibited) sample

F = Experimental factor given as:

$$\frac{V_f}{Va} \times \frac{I}{W}$$

Where V_f = Total volume of extract

Va = Volume of extract analyzed

W = weight of sample analyzed

3.8.8 Determination of total steroids.

The total steroids was determined colorimetrically with reference to the saponin content (Nwosu, 2011). The saponin crystals were dissolved in a 50ml formaldehyde – Conc. H_2SO_4 mixture (1:1 v/v) and the absorbance was measured at 500nm with a colorimeter (Jenway 6051). The steroid content was calculated as follows:

$$\% \text{ Steroids} = \frac{\text{Absorbance}}{\text{Weight of sample}} \times \frac{100}{1}$$

3.8.9 Oxalate determination.

This was carried out by the procedures described by Nwosu (2011).

one gram (1g) of the sample was weighed into a 100ml beaker, 20ml of 0.30N HCl was added and warmed to (40 – 50^0C) using magnetic hot plate and stirred for one hour. It was extracted three times

with 20ml flask. The combined extract was diluted to 100ml mark of the volumetric flask.

The oxalate was estimated by pipetting 5ml of the extract into a conical flask and made alkaline with 1.0ml of 5N ammonium hydroxide. A little indicator paper was placed in the conical flask to enable know the alkaline regions. It was also made acid to phenolphthalein (3 drops of this indicator added, excess acid decolorizes solution) by dropwise addition of glacial acetic acid. 1.0ml of 5% $CaCl_2$ was then added and the mixture allowed to stand for 3 hours after which it was then centrifuged at 300 rpm for 15 minutes. The supernatants were discarded. 2ml of 3N H_2SO_4 was added to each tube and the precipitate dissolved by warming in a water bath (70 – 80^0C). The content of all the tubes was carefully poured into a clean conical flask and titrated with freshly prepared 0.01N $KMnO_4$ at room temperature until the fist pink colour appeared throughout the solution. It was allowed to stand until the solution became colourless. The solution was then warmed to 70 – 80^0C and titrated until a permanent pink colour that persisted for at least 30 seconds was attained. The percentage (%) oxalate content was thus calculated:

$$\% \text{ Oxalate} = \frac{100 \times 0.00225 \times \text{Total titre volume}}{W}$$

Where W = Weight of sample used

3.8.10 Oligosaccharides determination.

The method of Balagopalan *et al.* (1998) was used. One gram of sample was boiled in 100ml of 2M HCl solution until it was negative to iodine starch test. It was centrifuged and the hydrolysate (supernatant) used for the analysis. 1ml of the hydrlysate was missed with 4mls of anthrone reagent in a test tube and boiled for 10 minutes in a water bath while covering the test tubes. After boiling, the mixture was filtered and diluted with distilled water. Similarly, a standard sugar solution (glucose) was prepared and treated as described above and the absorbances of both the samples and sugar solutions were read with Jenway 6051 Colorimeter against a blank reagent at zero.

The oligosaccharides content was calculated as:

$$\text{Percentage sugar} = \frac{Au \times C \times F}{As}$$

Where Au = Absorbance of test sample

As = Absorbance of standard sugar solution

C = Concentration of sugar solution

F = Experimental factor given by:

$$\frac{V_f \times D \times 100}{Va \qquad W}$$

Where V_f = Total filtrate volume

Va = Volume of aliquot analyzed

D = Dilution factor (where applicable)

W = weight of sample used

3.8.11 Determination of flavonoids

Flavonoid was determined using the method described by Harborne (1973).

A measured weight of the processed sample (1g) was boiled in 100ml of 2M HCl solution under reflux for 40 minutes. It was allowed to cool before being filtered. The filtrate was treated with equal volume of ethyl acetate and the mixture was transferred to a separation funnel. The flavonoid extract (contained in the ethyl acetate portion) was received by filtration using weighed filter paper. The weight was obtained after drying in the oven and cooling in a desiccator. The weight was expressed as a percentage of the weight analysed. It was calculated as shown below:

$$\% \text{ Flavonoid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

W_2 = Weight of filter paper + flavonoid precipitate

W_1 = Weight of filter paper alone.

3.8.12 Hemagglutinin determination.

Hemagglutinin was determined using the method described by Onwuka (2005).

One (1g) of the sample was weighed out and dispersed in a 10ml normal saline solution buffered at pH 6.4 with a 0.01M phosphate buffer solution. The mixture was allowed to stand at room temperature for 30 minutes and was centrifuged afterwards to obtain the extract. 1ml of trypsinized rabbit blood was added to 0.1ml of the extract diluent in a test tube.

A control sample was also prepared containing only the blood cells.

The test tubes containing the sample mixture and the ones containing the control sample were allowed to stand for 4 hours at room temperature. 1ml of normal saline was added to all the test tubes and they were allowed to stand for 10 minutes, after which their absorbances were read in a colorimeter (Jenway 6051) at 620nm. The hemagglutinin units per milligram of the sample was thus calculated:

Hamagglutinin unit/mg = (b – a) x F

Where b = Absorbance of test sample solution

a = Absorbance of the blank control

F = Experimental factor given by:

$$F = \left(\frac{1}{W} \times \frac{V_f}{Va}\right) D$$

Where V_f = Total volume of extract

Va = Volume of extract used in the essay

W = weight of sample used

D= Dilution factor

3.9 ACUTE TOXICITY STUDY.

The method used by the Akinnawo *et al* .(2002) was adopted for the acute toxicity study.

3.9.1 Animals.

Adult (8 weeks) male albino rats (Wistar strain) weighing 80 – 100mg from Emii venterinary farm at 120 Royce Road, Owerri, Imo State were used in this study. The rats were housed in metal cages placed under a well ventilated laboratory with temperature (27±2), Relative humidity (85%) and an alternate 12 hour natural light/12 hour dark cycles. The rats were allowed to acclimatize for a minimum of 5 days in the environment where the experiment was carried out. A commercial rat feed (Emii Finishers feed) and water was provided *ad libitum*.

3.9.2 Treatment.

The albino rats were completely randomized into ten (10) groups (A, B, C, D, E, F, G, H, I and J) consisting of five (5) rats in each group.

Group A (control) was fed 100% of the commercial rat feed (Emii feed) and water while group B was fed 100% of the raw sample (unprocessed sample) and water for 14 days.

Groups C, D, E and F were treated like the control except that they were fed the cooked sample (60 minutes cooked) which was incorporated into the commercial feed (Emii finishers Feed) at 25%, 50%, 75% and 100% levels respectively.

Rats in groups G, H, I and J were also treated like the control except that they were fed the roasted (110^0C, 15 minutes) sample incorporated into the commercial feed (Emii finishers feed) at 25%, 50%, 75%, and 100% levels respectively.

3.9.3 General observations.

All animals were observed twice daily for morbidity and mortality. Any abnormal physical and behavioural changes (skin, fur, eyes, posture, response to handling) were observed. The time of onset, intensity and duration of such symptom if any were recorded.

Individual animal body weights for treatment and control groups were recorded weekly, beginning on the day before the initiation of treatment. Final body weights were recorded a day prior to the final day

of experiment (the 13th day). The amount of feed consumed by each animal was recorded daily.

3.9.4 Haematological analysis.

This was done by the method described by Akinnawo *et al*. (2002). At the end of the experiment, the rats were anaesthetized with choloroform and blood was collected by cardiac puncture.Two (2)ml of blood from each rat was out into sample bottles containing disodium EDTA and used to determine haematological parameters.

The haematological parameters examined are white blood cells (WBC), Red blood cells (RBC), packed Cell Volume (PCV), Haemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Haemoglobin Concentration (MCHC), Neutrophiles, Monocytes, Lymphocytes, Eosinophils and Basophils.

3.9.4.1 Red blood cell count (RBC).

The red blood cell (RBC) count was done using the conventional method of Dacie and Lewis (2001). Blood was diluted to 1:200 with Hayem's fluid which preserved the corpuscles and then counted with an improved Neubauer counting chamber under a light microscope (Mc Arthur Microscopr) using a X40 objective in an area of 1/5 sqmm. Their

characteristic pink-red colour was used for their identification. The number was then calculated thus:

$$\text{Red blood cells} = \frac{\text{Cells counted x blood dilution x chamber depth}}{\text{Area of chamber counted}}$$

3.9.4.2 White blood cell count (WBC).

The counting of total white blood cells was done using a diluting fluid (Turk's fluid) in a ratio of 1:20 and then counted with an improved Neubauer counting chamber under a light microscope (McArthur Microscope) using a X10 objective in an area of 4sqmm. The cells appeared as small black dots. The number was thus calculated:

$$\text{White blood cells} = \frac{\text{Cells counted x blood dilution x chamber depth}}{\text{Area of chamber counted}}$$

3.9.4.3 Haemoglobin estimation.

The conventional method (Sahli's haemoglobinometer) was employed for the estimation of haemoglobin (Hb) content of the blood.

Using the Sahli haemoglobinometer, the colour of the test solution was matched against a coloured glass standard. A graduation test tube was filled to 20ml mark with 10N hydrochloric acid. 0.02ml of blood was added and the content of the test tube was mixed with a glass rod. It was left for 5 minutes (for the haemoglobin to be changed into acid

haematin). More acid was thereafter added and the mixture was stirred until the colour of the test solution matched that of the coloured glass standard. The level of the fluid in the tube was read and the haemoglobin content was expressed as a percentage.

3.9.4.4 Packed cell volume (PCV).

The packed cell volume (PVC) was done using the macrohaematocrit method (Dacie and Lewis, 2001).

The blood sample was added to a bottle containing heparin (0.1mg/ml of blood). The haematocrit tube was filled to 100mm with a capillary pipette and it was centrifuged at 3,000 rpm for 30 minutes. The height of the red blood cells was read and the result was expressed as a percentage.

3.9.4.5 Determination of platelets.

The platelets were determined by diluting the blood in one percent (1%) ammonium oxalate which haemolysed the red blood cells. The platelets were then counted in a definite area using the rulings of an improved Neubauer counting chamber. Their characteristic Mauve-pink colour was used in their identification.

3.9.4.6 Differential white blood cell count.

The differential white blood cell count (Neutrophils, Lymphocytes, Monocytes, Eosinophils and Basophils) was done by making a thin film

of blood on a smooth edged slide. It was allowed to dry on a bench protected from dust, ants, flies, and other insects. The blood film was fixed in a covered staining jar of methyl alcohol for 3 minutes.

Ten(10)ml of May Grunwald Stain (mixture of 5g of May Grunwald powder and 1 litre of methanol) and 10ml of buffered water (pH 6.8) was mixed thoroughly and the smear was covered with the diluted May Grunwald stain for 3 minutes. The stain was tipped off and replaced with diluted Giemsa's stain (5%) for 9 minutes. The stin was washed off with buffered water (pH 6.8) and clean water was dropped on the slide which was allowed to stay for 30 seconds. The water was tipped off and the slide was allowed to dry. It was then examined microscopically (McArthur Microscope) for the identification of Neutrophils (cytoplasm stained pink with small mauve granules), Eosinophils (cytoplasm stained pink with large red granules), Basophils (cytoplasm contained dark mauve-blue granules), Monocytes (cytoplasm stained dull grey-blue) while lymphoctes (cytoplasm stained blue).

3.9.4.7 Mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) determination.

The mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular volume (MCV) were calculated from the values obtained from red blood cells

(RBC), packed cell volume (PCV) and Haemoglobin (Hb) content. They were calculated thus :

Mean Corpuscular Haemoglobin (MCH)

$$= \frac{\text{Haemoglobin content}}{\text{Red Blood Cell count}} \times \frac{10}{1}$$

Mean Corpuscular Haemoglobin Concentration (MCHC)

$$= \frac{\text{Haemoglobin content}}{\text{Packed Cell Volume}} \times \frac{100}{1}$$

$$\text{Mean Corpuscular Volume (MCV)} = \frac{\text{Packed Cell Volume}}{\text{Red Blood Cell count}} \times \frac{10}{1}$$

3.10 DATA ANALYSIS.

The results obtained from the data were subjected to Analysis of Variance (ANOVA) according to Onuh and Igwemma (2000) and SAS (1999).Significant means at $p \leq 0.05$ were separated using Fisher's Least Significant Difference (LSD) test (Onuh and Igwemma, 2000).

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Physicochemical properties of *livistonia chinensis* seed oil.

Some of the physicochemical properties of *L. chinensis* seed oil are shown in Table 1.

The oil content of *Livistona chinensis* seed is 2.86% and is therefore taken not to be an oil seed. *Livistona chinensis* seed oil has a straw colour (yellowish-brown) and is liquid at room temperature (27^0C $\pm$ 2^0C).

The specific gravity at 27^0C was determined to be 0.8638g/cm^3. This value is lower than the values recorded by codex standards (1999) for crude vegetable oils like Arachis oil (0.912 – 0.920g/cm^3), coconut oil (0.908 – 0.921 g/cm^3), cotton seed oil (0.918 – 0.926 g/cm^3), maize oil (0.917 – 0.925 g/cm^3), palm oil (0.891 – 0.899 g/cm^3), palm kernel oil (0.899 – 0.914 g/cm^3), soya bean (0.919 – 0.925 g/cm^3), etc. It is in essence less dense than these oils. Specific gravity is a measure of the heaviness of a substance compared to water (Onwuka, 2005). The oil by implication will readily float in water since its specific gravity (0.8638 g/cm^3) is less than one (1) and it is less dense than water.

Table 1: Physico-chemical properties of *Livistona chinensis* seed oil.

PHYSICOCHEMICAL PARAMETER	VALUE
Oil content (%)	2.86
Colour	Straw Colour(Yellowish-brown)
State (at room temp)	Liquid
Specific gravity (27^0C)(g/cm^3)	0.8638
Smoke point (^{0}C)	140 – 142
Flash point (^{0}C)	239 – 243
Iodine value (mg iodine/g oil)	35.307
Peroxide value (MeqO_2/kg oil)	13.98
Saponification value (mg KOH/g Oil)	40.125
Free fatty acids (FFA) (%)	22.031%
Acid value (mg KOH/g Oil)	43.828

The smoke and flash points of the oil are 140 – 142^{0}C and 239^{0}C – 243^{0}C respectively. The smoke and flash points are measures of the thermal stability of oils. The smoke point (140 – 142^{0}C) and flash point (239 – 243^{0}C) of *L. chinensis* seed oil are lower than the smoke point (221 – 260^{0}C) and flash point (302 – 338^{0}C) of refined corn oil respectively (Potter and Hotchkiss, 1995). In essence, the oil is regarded as being less thermally stable than that of refined corn oil and cannot withstand very high frying and roasting temperatures and very high thermal processing. As such it could be recommended in food for mild frying.

4.2 Chemical properties of *Livistona chinensis* seed oil.

The acid value and free fatty acid (FFA) value of *L. chinensis* seed oil are 43.828mg KOH/g oil and 22.031% respectively. Both values are relatively very high when compared to codex standards (codex standards, 1999) for crude vegetable oils which recommends an acid value of 4.0mgKOH/g oil for cold pressed and virgin oils, 10.0mgKOH/g oil for virgin palm oils and free fatty acid values of 0.0 – 3.0%. The free fatty acids can stimulate off-flavour development in the oil (Oderinde *et al*., 2009). The high free fatty acid value of the oil implies that refining would be required to make them suitable for edible purposes and may be better utilized for industrial purposes.

The peroxide value was determined to be 13.98 Meq O_2/kg oil. This value is lower than the maximum value (15 Meq O_2/kg oil) stipulated for cold pressed or virgin oils (Codex standard, 1999). The peroxide value is a measure of the degree of oxidation (Potter and Hotchkiss,1995) and its value is below the stipulated Codex standard value. The oil is therefore considered to be relatively stable.

The saponification value obtained (40.125mgKOH/g oil) was very low compared to the Codex standard (1999) saponification values for crude vegetable oils like Arachis oil (187 – 196mgKOH/g oil), cotton oil (248 – 265mgKOH/g oil), cotton seed oil (189 – 198mgKOH/g oil), maize oil (187 – 195mgKOH/g oil), palm oil (190 – 209mgKOH/g oil), palm kernel oil (230 – 254mgKOH/g oil), soya bean oil (189 – 195mgKOH/g oil), sun flower seed oil (188 – 194mgKOH/g oil). Saponification value is a measure of both free and combined acids and is inversely proportional to the mean molecular weight of the fatty acids in the glycerides present (Onwuka, 2005). The very low saponification value of the oil indicates that its fatty acids have a high mean molecular weight and it will be suitable for the production of soaps and candles.

The iodine value was determined to be 35.307 mg iodine/g oil. The iodine value is low when compared to the range of many crude vegetable oils recorded by codex standard (1999) like Arachis oil (86 – 107mg iodine/g oil), cotton seed oil (100 – 123mg iodine/g oil), maize oil (103 –

135mg iodine/g oil), mustard seed oil (92 – 125mg iodine/g oil), palm oil (50 – 55mg iodine/g oil), rice bran oil (90 – 115mg iodine/g oil), sesame seed oil (104 – 120mg iodine/g oil), Soya bean oil (124 – 139mg iodine/g oil). While coconut and palm kernel oils have much lower iodine values of 6.3 – 10.6mg iodine/g oil and 14.1 – 21.0mg iodine/g oil respectively than *L. chinensis* (Chinese Fan palm) seed oil. Iodine value is a measure of the level of unsaturation of an oil (Onwuka, 2005) and the low iodine value (35.307 mg iodine/g oil) indicates low level of unsaturation and the presence of saturated fatty acid. This places the oil in the non-drying group and it could be utilized for cooking and may find application as a raw material in industries for the manufacture of vegetable oil-based ice-cream (Oderinde *et al*., 2009).

4.2 Effect of blanching, cooking and roasting on the proximate composition of *Livistona chinensis* seed.

The effect of blanching, cooking and roasting on the proximate composition of *L. chinensis* seed is presented in Table 2.

Results of the moisture content (Table 2) show that the blanching, cooking and roasting processes all had significant effect on the moisture content of *L. chinensis* seed. The dry heat treatment (roasting) significantly reduced the moisture content while the wet heat treatments

Table 2: Effect of blanching, cooking and roasting on the proximate composition of *Livistona chinensis* seed.

Sample	Moisture (%) (Dry weight basis)	Crude protein (%)	Ash (%)	crude fat (%)	crude fibre (%)	Carbohydrate (%)
Raw Seed	35.35^{g} ± 0.05	4.44^{e} ± 0.04	1.31^{b} ± 0.01	2.86^{a} ± 0.01	38.21^{b} ± 0.21	17.83^{e} ± 0.03
4min blanched	35.45^{f} ± 0.40	4.42^{ef} ± 0.07	1.31^{b} ± 0.01	2.86^{a} ± 0.02	38.21^{b} ± 0.10	17.34^{f} ± 0.04
6min blanched	35.86^{e} ± 0.25	4.41^{ef} ± 0.01	1.28^{b} ± 0.02	2.70^{b} ± 0.01	37.14^{d} ± 0.08	17.79^{e} ± 0.08
8min blanched	37.15^{d} ± 0.15	4.40^{ef} ± 0.03	1.27^{b} ± 0.02	2.32^{f} ± 0.00	37.54^{c} ± 0.20	17.32^{f} ± 0.01
20min cooked	39.06^{c} ± 0.06	4.38^{ef} ± 0.08	1.12^{c} ± 0.03	1.91^{g} ± 0.00	37.50^{c} ± 0.00	16.03^{g} ± 0.09
40min cooked	39.83^{b} ± 0.20	4.36^{ef} ± 0.05	1.06^{c} ± 0.02	1.52^{h} ± 0.01	36.02^{f} ± 0.00	17.21^{f} ± 0.05
60min cooked	41.45^{a} ± 0.05	4.32^{f} ± 0.01	0.82^{d} ± 0.01	1.30^{i} ± 0.01	35.92^{f} ± 0.50	16.19^{g} ± 0.05
5min Roasted	30.42^{h} ± 0.22	4.63^{d} ± 0.15	1.34^{b} ± 0.02	2.64^{c} ± 0.02	38.59^{a} ± 0.19	22.38^{d} ± 0.03
10min roasted	27.22^{i} ± 0.01	4.95^{c} ± 0.04	1.35^{b} ± 0.01	2.71^{b} ± 0.00	37.27^{cd} ± 0.10	26.50^{c} ± 0.08
15min roasted	25.44^{j} ± 0.03	5.22^{b} ± 0.02	1.38^{b} ± 0.02	2.51^{d} ± 0.01	37.91^{b} ± 0.00	27.54^{b} ± 0.04
Pulp	11.14^{k} ± 0.02	9.04^{a} ± 0.03	6.92^{a} ± 0.01	2.38^{e} ± 0.01	36.66^{e} ± 0.00	33.69^{a} ± 0.05
LSD	0.36	0.10	0.12	0.02	0.32	0.17

NOTE: Means with different superscripts in the same column are significantly different at $P \leq 0.05$

LSD = Least significant difference.

(blanching and cooking) significantly increased the moisture content of *L. chinensis* seed. The 60 minutes cooking had the most significant increasing effect on the moisture content raising it from its raw seed value of 35.35% to 41.45% while the 15 minutes roasting had the most significant reducing effect on the moisture content reducing it from its raw seed value of 35.35% to 25.44%. The pulp had 11.14% moisture content (dry basis) and no treatment was given to it.

Livistona chinensis raw seed with a crude protein content of 4.44% (Table 2) is much lower than that of *Zea mays* (9 – 10%),*Oryza sativa* (6.8 – 8.0%) and *Triticum valgare* (8 – 10%) (Okaka, 1997).Blanching and cooking had no significant effect on the crude protein content except for 60 minutes cooking while 5,10 and 15 minutes roasting all had significant increasing effect on the crude protein content of *L. chinensis* respectively. This is in agreement with the research carried out by Jimoh *et a*l.(2011) Which indicated a decrease in the crude protein content of sesame seed when cooked for 30minutes and an increase in the crude protein content when roasted for 30minutes. The crude protein levels in the treatments were 4.42%, 4.41% and 4.40% for 4, 6 and 8 minutes blanching respectively,4.38%, 4.36% and 4.32% for 20, 40 and 60 minutes respectively,and 4.63%,4.95% and 5.22% for 5, 10 and 15 minutes roasting respectively. The pulp had a crude protein value of 9.04% and no treatment was given to it. The protein maintained some relative stability and the content did not change significantly during blanching and cooking which could have been as a result of the fact that

L. chinensis seed has a very hard and tough outer coat(shell) very similar to that of palm kernel shell and it was processed in its whole (unmilled) form which may not have allowed for easy heat transfer and leaching during blanching and cooking.

Blanching and roasting had no significant effect on the ash content while cooking had a significant decreasing effect on the ash content, although 20 and 40 minutes cooking had the same effect on the ash content. The raw *L. chinensis* seed had an ash content of 1.31% while the ash content in the treatments were 1.31%, 1.28% and 1.27% for 4, 6 and 8 minutes blanching respectively,1.12%,1.06% and 0.82% for 20,40 and 60 minutes cooking respectively, 4.63%, 4.95% and 5.22% for 5, 10 and 15 minutes roasting respectively. The pulp had an ash content of 6.92% and no treatment was given to it.

The oil content of the raw seed of *Livistona chinensis* is 2.86% (Table 2). The oil content at the different levels of processing as represented in Table 2 are 2.86%, 2.70% and 2.32% for 4, 6 and 8 minutes blanching respectively, 1.91%,1.52% and 1.30% for 20, 40 and 60 minutes cooking respectively, 2.64%, 2.71% and 2.51% for 5,10 and 15 minutes roasting at 110°C respectively while the pulp contains 2.38% oil. This oil content of the raw seed of *L. chinensis* (2.86%) is higher than that of Rice (2%) and wheat (1 – 2%) but is comparable to that of Millet (2 – 4.5%) and is lower than that of maize (3 – 5%) and Guinea Corn

(3%). The oil content of 2.86% for the raw *L. chinensis* seed indicates that it is not an oil seed unlike soya bean that has up to 23.1% fat content (Potter and Hotchkiss, 1995). The oil content decreased generally with increased processing time (Table 2) especially with that of the wet heat treatment (blanching and cooking) which could possibly be as a result of leaching of the oil into the treatment water as pointed out by Okaka *et al*. (2002).

Livistona chinensis seed has a very high crude fibre content of 38.21% as shown in Table 2. There was significant decrease in the crude fibre content during the blanching, cooking and roasting except for 4 minutes blanching and 15 minutes roasting that both had no significant effect on it. Its fibre content of 38.21% (Table 2) is higher than that of many cereals like Guinea corn (2%), maize (2 – 3%), Millet (2%), Rice (0.2%), Wheat (1.5 – 2.5%) (Okaka, 1997) and is also higher than that of many legumes like soya bean (2.2%), Lupin (1.5%), Faba bean (1.4%), Pean bean (1.7%), lima bean (2.1%) and lentil (1.1%) (Potter and Hotchkiss, 1995). This level of crude fibre is an indication that the seed of *L. chinensis* can serve as a good roughage source for animals.

The carbohydrate content of raw *L. chinensis* seed (17.83%) and its values at different stages of processing are shown in Table 2. The

treatments had significant effects on the carbohydrate content except for 6 minutes blanching. The carbohydrate content at the different levels of processing are 17.34%, 17.79% and 17.32% for 4, 6 and 8 minutes blanching, 16.03%, 17.21% and 16.19% for 20, 40 and 60 minutes cooking respectively, 22.38%, 26.50% and 27.54% for 5, 10 and 15 minutes roasting respectively. The pulp had a carbohydrate content of 33.69% and no treatment was given to it. The carbohydrate content (17.83%) of raw *L. chinensis* seed is lower than that of most cereals like Guinea Corn (68 – 80%), maize (65 – 84%), millet (75 – 85%), Rice (80%) and wheat (65 – 75%) (Okaka, 1997). Its value (17.83%) is also lower than that of most legumes like bambara groundnut (65%), broad bean (56.9%) Chick peas (60.9%), Cowpea (61.0%), groundnut (21.0%), Jack bean (61.0%), Lentils (65%), Peas (60%), Soya beans (32%), etc (Okaka, 1997). Its value is nevertheless higher than that of some nuts like walnuts (15.6%), Fiberts (17.7%), Pecans (13.0%) etc (Potter and Hotchkiss, 1995).

4.3 Effect of processing on the anti-nutritional factors in *Livistona chinensis* seed.

The effect of processing on the anti-nutritional factors in Livistona chinensis seed are presented in Table 3 below.

Nine anti-nutritional factors at different levels were found in raw *L. chinensis* seed. There was no trypsin inhibitory activity, hemagglutinin and steroid present in raw *L. chinensis* seed. The anti-nutrients found in the raw seed of *L. chinensis* are tannins, phytates, oxalates, saponins, hydrogen cyanide, alkaloids, total phenols, flavonoids, and oligosaccharides.

The different levels of the anti-nutrients found in the raw seed of *L. chinensis* are as follows: oligosaccharides (27.50%), alkaloids (6.00%), saponins (5.50%), flavonoids (4.00%), Hydrogen cyanide (1.46mg/kg), phytate (1.20%), while total phenols (0.61%), tannins (0.49%) and oxalates (0.18%) were below 1%. The pulp had the same level of tannin (0.49%) with the raw seed. It recorded 0.68%, 0.49mg/kg, 4.00%, 0.50%, 2.00% and 25.00% for phytate, hydrogen cyanide, alkaloids, total phenols, flavonoids and oligosaccharides respectively which were all lower than that found in the raw seed. On the other hand, the levels of oxalate (0.54%) and saponins (6.50%) were higher than that of the raw seed.

Generally, the levels of the anti-nutrients all decreased with increased heat processing (Table 3) except for tannins which remained stable with all the different heat treatments but was just slightly reduced by 40 and 60 minutes cooking from 0.49% to 0.48% and from 0.48% to 0.47% respectively (Table 3). Tannins have been noted for their relative

characteristic heat resistance (Jimoh *et al.*, 2011; Olajide *et al*. ,2011; Nwosu, 2011). The longer persistence of tannins during the heat processing in this study may likely be as a result of the nature of the seed which is very hard and tough, and was in a whole unbroken form during the heat processing.

Phytates, hydrogen cyanide, total phenols and oligosaccharides, all showed some varying levels of stability during the different heat processing (Table 3). It was observed that phytates were only reduced at 60 minutes cooking from the raw seed value of 1.20% to 0.85%, hydrogen cyanide from 1.46mg/kg to 0.49mg/kg, total phenols from 0.61% to 0.33% while oligosaccharides reduced from 27.50% to 7.5% (Table3). At 15 minutes roasting, the phytates, hydrogen cyanide total phenols and oligosaccharides levels reduced to 1.03%, 0.98mg/kg, 0.47% and 20.00% respectively. In essence, there was no total elimination of these five anti-nutrients (tannins, phytates, hydrogen, cyanide, total phenols and oligosaccharides) with all the different heat treatments (blanching, cooking and roasting) at the different levels tested. This as noted earlier, may likely be as a result of the nature and the state of the raw seed which is hard, tough and was processed in its whole seed form.

Table 3: Effect of blanching, cooking and roasting on the anti-nutritional factors in *Livistona chinensis* seed.

Sample	Tannin (%)	Phytate (%)	Oxalate (%)	Saponins (%)	Hydrogen Cyanide (mg/kg)	Alkaloid (%)	Total Phenols (%)	Flavonoids (%)	Oligosacharides (%)	Trypsin inhibitor (T./u/g)	Haemagglutin (Hu/g)	Steroid (%)
Raw Seed	0.49^{a} ± 0.00	1.20^{a} ± 0.02	0.18^{b} ± 0.01	5.50^{b} ± 0.50	1.46^{a} ± 0.49	6.00^{a} ± 1.00	0.61^{a} ± 0.01	4.00^{a} ± 1.00	27.50^{a} ± 2.50	-	-	-
4min blanched	0.49^{a} ± 0.00	1.08^{d} ± 0.00	0.11^{d} ± 0.00	4.00^{d} ± 0.50	0.98^{ab} ± 0.49	6.00^{a} ± 1.00	0.61^{a} ± 0.00	3.00^{ab} ± 0.00	25.00^{b} ± 0.00	-	-	-
6min blanched	0.49^{a} ± 0.00	1.30^{e} ± 0.01	0.09^{e} ± 0.01	3.00^{e} ± 0.00	0.98^{ab} ± 0.49	6.00^{a} ± 0.00	0.53^{c} ± 0.01	3.00^{ab} ± 0.00	25.00^{b} ± 2.50	-	-	-
8min blanched	0.49^{a} ± 0.00	1.00^{c} ± 0.01	0.05^{f} ± 0.00	0.00^{f} ± 0.00	0.98^{ab} ± 0.00	4.00^{c} ± 0.00	0.47^{e} ± 0.01	2.00^{bc} ± 0.00	22.50^{c} ± 0.00	-	-	-
20min cooked	0.49^{a} ± 0.00	0.91^{g} ± 0.00	0.02^{g} ± 0.01	0.00^{f} ± 0.00	0.49^{b} ± 0.00	3.00^{d} ± 0.00	0.42^{f} ± 0.00	1.00^{cd} ± 1.00	17.50^{e} ± 0.00	-	-	-
40min cooked	0.48^{b} ± 0.01	0.88^{h} ± 0.01	0.02^{g} ± 0.00	0.00^{f} ± 0.00	0.49^{b} ± 0.00	0.00^{f} ± 0.00	0.37^{g} ± 0.00	0.00^{d} ± 0.00	15.00^{f} ± 0.00	-	-	-
60min cooked	0.47^{c} ± 0.00	0.85^{i} ± 0.02	0.00^{h} ± 0.00	0.00^{f} ± 0.00	0.49^{b} ± 0.00	0.00^{f} ± 0.00	0.33^{h} ± 0.00	0.00^{d} ± 0.00	7.50^{g} ± 0.00	-	-	-
5min Roasted	0.49^{a} ± 0.00	1.17^{b} ± 0.01	0.16^{c} ± 0.01	5.00^{c} ± 0.50	1.46^{a} ± 0.49	5.00^{b} ± 1.00	0.59^{b} ± 0.02	3.00^{ab} ± 1.00	25.00^{b} ± 2.50	-	-	-
10min roasted	0.49^{a} ± 0.00	1.11^{c} ± 0.01	0.11^{d} ± 0.00	3.00^{e} ± 0.00	1.46^{a} ± 0.49	3.00^{d} ± 0.00	0.52 ± 0.01	2.00^{bc} ± 0.00	22.50^{a} ± 0.00	-	-	-
15min roasted	0.49^{a} ± 0.00	1.03^{e} ± 0.00	0.09^{e} ± 0.01	0.00^{f} ± 0.00	0.98^{ab} ± 0.00	2.00^{e} ± 0.00	0.47^{e} ± 0.02	1.00^{cd} ± 1.00	20.0^{d} ± 0.00	-	-	-
Pulp	0.49^{a} ± 0.00	0.68^{j} ± 0.02	0.54^{a} ± 0.02	6.50^{a} ± 0.00	0.49^{b} ± 0.00	4.00^{c} ± 0.00	0.50^{d} ± 0.01	2.00^{bc} ± 0.00	25.00^{b} ± 0.00	-	-	-
LSD	0.01	0.02	0.02	0.44	0.55	0.88	0.02	1.02	2.21			

Note: means with different superscripts in the same column are significantly different at $p \leq 0.05$, LSD = least significant difference.

Total elimination was achieved for oxalates (at 60 minutes cooking), saponins (at 8 minutes blanching), alkaloids (at 40 minutes cooking) and flavonoids (at 40 minutes cooking). This anti-nutrients' reduction/elimination during heat processing are also in agreement with their reduction/elimination in some other plants during heat processing as established by Ugwu and Oranye (2006), Nwosu (2011), Jimoh *et al*.(2011) and Olajide *et al*. (2011).

Oxalates bind with calcium and magnesium, and interfere with their metabolism, cause muscular weakness and paralysis (Soetan and Oyewole, 2009), Saponins haemolyse red blood cells (Nwosu, 2011), while alkaloids and flavonoids cause gastro- intestinal and neurological disorders (Soetan and Oyewole, 2009). Their total elimination at the different levels of heat treatment (Table 3) would rather make them not to be considered as risk at those levels.

For the anti-nutritional factors which weren't totally eliminated at the highest wet heat treatment of 60 minutes cooking, like the hydrogen cyanides had a value of 0.49mg/kg which is lower than the fatal dose level of 50mg/100g which is equivalent to 500mg/kg (Nwosu, 2011). Cyanide inhibits the cytochrome oxidase through combination with their copper and iron ions respectively (Onwuka, 2005) and also causes gasping, staggering and convulsion (Nwosu, 2011).

At this level of treatment/dose, hydrogen cyanide will likely not be implicated in any toxic activity of whole raw *L. chinensis* seed.

At 60 minutes cooking, the tannins (0.47% which is equivalent to 4,700mg/kg) and total phenols (0.33% which is equivalent to 3,300mg/kg) (Table 3) are very much higher than the lethal dose level of 30mg/kg (Inuwa *et al*., 2011). Tannins are complex phenolic polymers which are capable of inhibiting the activities of trypsin, chymotrypsin, amylase and lipase (Inuwa *et al*., 2011). Tannins can provoke astringent reaction in the mouth, interfere with dietary iron absorption and also cause growth depression (Soetan and Oyewole, 2009). This very high level of tannins and phenolic compounds in *L. chinensis* seed is also in agreement with previous studies on *L. chinensis* seed which established a high level of phenolic compounds in *L. chinensis* seed (Tao *et al*., 2009; Yanna *et al*., 2001; Singh and Kaur, 2008; Huang *et al*., 2007). Tannins and phenols will likely be implicated in any toxic activities of this seed at this level of treatment/dose.

The phytate content was also not totally eliminated and had a value of 0.85% which is equivalent to 8,500mg/kg after 60 minutes cooking. This value is also very much higher than the 50 – 60mg/kg lethal dose level (Inuwa *et al*., 2011). Phytates form insoluble salts with essential minerals like calcium, iron, magnesium and zinc in food, rendering them unavailable for absorption into the blood stream (Onwuka, 2005). It also

causes reduction in protein availability (Nwosu, 2011). In essence, phytates also will likely be implicated in any toxic activities of whole *L. chinensis* seed at this level of processing/dose.

At 60 minutes cooking, the oligosaccharides content (7.50%-which is equivalent to 75,000mg/kg) is also very high when compared to the 5-8% oligosaccharides content range of raw legumes(beans, peas and lentils). Oligosaccharides are the major contributory factors in flatulence (Okaka *et al.,* 1992).

These four anti-nutrients (tannins, phytates, total phenols and oligosaccharides) will most likely also be implicated in any toxic activity of whole *L. chinensis* seed at the 110^0C for 15 minutes roasting done since they even had higher values of 0.49%, 1.03%, 0.47% and 20.00% for tannins, phytates, total phenols and oligosaccharides respectively.

4.4 Feed intake and weight gain.

The albino rats feed intake levels are shown in Table 4 while their weekly weight gain record is shown in Table 5.

From Table 4, groups B, E, F, I and J fed raw, 75:25 and 100:0 cooked and roasted *L. Chinensis* seed sample-to-commercial feed (Emii Finishers Feed) respectively showed a decreased feed consumption. This may be as a result of the non-palatability of the *L. chinensis* seed sample at these relatively high ratios/concentrations.

Groups C, D, G and H feed consumptions were very comparable to that of group A (control group) that was fed the normal commercial feed (Emii Finishers Feed). This may likely be as a result of the lower ratios/concentrations of the *L. chinensis* seed which possibly did not affect the palatability of the feed mix.

Rats from the test group displayed fairly similar body weight gain to those from the normal control group (group A) (table 5).

4.5 General observations on the albino rats.

Albino rats in groups B and I died after 5 days while those in groups E, F and J died after 6, 4 and 11 days respectively (table 4). A very dull behaviour, dim, dull and partially closed eyes with a fur arrangement that revealed some very minute uncovered straight line skin areas were always observed a day prior to the death of the albino rats.

4.6 Effect of cooked (60 minutes) *Livistona chinesis* seed on the haematological parameters of albino rats .

The effect of cooked (60 minutes) *L. chinensis* seed on the haematological parameters of albino rats are presented in table 6.

Assessment of haematological parameters of albino rats can be used to determine the extent of deleterious effect of a foreign compound, including plant extracts, on the blood (Odeyemi *et al.*, 2009). It can also

Table 4: Feed intake (g) of albino rats.

DAY	ALBINO RAT GROUPS									
	A	B	C	D	E	F	G	H	I	J
1	14	8	12	13	7	7	12	13	5	6
2	15	7	14	15	8	7	14	14	8	6
3	13	7	14	15	8	8	14	14	7	5
4	14	7	15	14	8	6	16	16	5	AD
5	15	AD	16	14	10	7	15	13	AD	-
6	16	-	13	14	8	AD	15	16	-	-
7	14	-	14	15	9	-	15	14	-	-
8	15	-	15	16	8	-	13	14	-	-
9	13	-	15	14	10	-	14	16	-	-
10	14	-	15	13	9	-	14	13	-	-
11	14	-	15	14	AD	-	13	13	-	-
12	16	-	14	14	-	-	14	13	-	-
13	15	-	14	14	-	-	16	16	-	-
14	15	-	15	14	-	-	13	14	-	-

NOTE: The values are the means for each albino rat group

AD = ALL DIED , - = No remaining rats

Group A albino rats = Albino rats fed commercial feed -Emii Finishers feed (control rats)

Group B albino rats = Albino rats fed raw sample *(L. chinensis* seed*)*

Group C albino rats = Albino rats fed 25:75 cooked sample-to-commercial feed mix.

Group D albino rats = Albino rats fed 50:50 cooked sample-to-commercial feed mix.

Group E albino rats = Albino rats fed 75:25 cooked sample-to-commercial feed mix

Group F albino rats = Albino rats fed 100:0 cooked sample-to-commercial feed mix

Group G albino rats = Albino rats fed 25:75 roasted sample-to-commercial feed mix.

Group H albino rats = Albino rats fed 50:50 roasted sample-to-commercial feed mix.

Group I albino rats = Albino rats fed 75:25 roasted sample-to-commercial feed mix

Group J albino rats = Albino rats fed 100:0 roasted sample-to-commercial feed mix

Table 5: weekly weight record for the rat groups that survived the 14 days duration of the experiment

WEEK	ALBINO RAT GROUPS				
	A	C	D	G	H
Initial weight (on day 1)	92	83	89	97	100
Week 1	101	93	97	106	105
Week 2	109	98	104	112	109

NOTE: The values are the means for each group

Group A albino rats = Albino rats fed commercial feed -Emii Finishers feed (control rats)

Group C albino rats = Albino rats fed 25:75 cooked sample-to-commercial feed mix.

Group D albino rats = Albino rats fed 50:50 cooked sample-to-commercial feed mix.

Group G albino rats = Albino rats fed 25:75 roasted sample-to-commercial feed mix.

Group H albino rats = Albino rats fed 50:50 roasted sample-to-commercial feed mix.

be used to explain blood-relating functions of a plant extract or its products (Odeyemi *et al.*, 2009; Ajayi *et al*., 2005; Akinnawo *et al*., 2002).

The cooked (60 minutes) sample of *L. chinensis* seed had a significant increasing effect ($p \leq 0.05$) on the packed cell volume (PCV), haemoglobin (Hb) and platelets but did not produce any definite pattern (continuous rise or fall) in white blood cells (WBC), red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), Lymphocytes, Neutrophils, and Eosinophils. It had no significant effect ($p \leq 0.05$) on the mean corpuscular haemoglobin concentration (MCHC), basophils and monocytes (table 6).

The white blood cells showed a significant increase ($p \leq 0.05$) with the increase in sample (*L. chinensis* seed) concentration but did not show a definite rise in pattern while there was no significant difference($p \leq 0.05$) for the monocytes, basophils and eosinophils. It showed no definite pattern of rise or fall of neutrophils and lymphocytes but there was a significant difference between them($p \leq 0.05$). White blood cells (WBC) are important in defending the body against infection (Aboderin and Oyetayo, 2006). The white blood cell count however cannot give specific information but a differential white blood cell count (neutrophils, basophils, monocytes, lymphocytes, eosinophils) narrows down to give specific information about toxicity, poisoning, infections, allergy or immuno-suppression (Aboderin and Oyetayo, 2006).

The primary role of lymphocytes is in humoral antibody formation and cellular immunity (Aboderin and Oyetayo, 2006; Hoffbrand *et al.,* 2004). Neutrophils is mainly responsible for phagocytosis of pathogenic micro-organisms during the first few hours after their entry into tissues (Aboderin and Oyetayo, 2006). Basophils counts increase upon sensitization to an antigen (or allergen), monocytes are responsible for defense of tissues against microbial agents; it increases with bacterial infection and decreases with stress while eosinophils are responsible for allergic reactions and disorders; it increases with allergic conditions and decreases with stress and/or infection (Lewis *et al*., 2006; Aboderin and Oyetayo, 2006; Odeyemi *et al*., 2009).

The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) which are all important in the diagnosis of anaemic conditions all increased significantly($p \leq 0.05$) when compared with the control though they did not show any definite pattern as sample (*L. chinensis* seed) ratio(Concentration) was increased. The red blood cells also did not show a definite pattern (continuous rise or fall) also implying dose independent effect (Odeyemi *et al*., 2009).

The packed cell volume (PCV), platelets and hamemoglobin (Hb) all showed significant increase($p \leq 0.05$) as the sample (cooked *L. chinensis* seed) ratio (concentration) was increased in the sample-to-

Table 6: Effect of cooked (60 minutes) *L. chinensis* seed on the haematological parameters of albino rats.

Haematological parameter	White blood cells (x 10^6/mm^3)	Red blood cells (x 10^6/mm^3)	Packed cell volume (%)	Haemogolobin (g/dL)	Mean corpuscular volume (CUμ)	Mean corpuscular haemoglobin (pg)	Mean corpuscular haemoglobin concentration (%)	Platelets (x 10^9/L)	Monocytes (%)	Lymphocytes (%)	Eosinophils (%)	Neutrophils (%)	Basophils (%)
GROUP A ALBINO RATS	3.60^a	4.90^b	32.00^a	10.80^a	65.30^a	22.04^a	33.75^a	780.12^a	0.00^a	51.00^b	2.00^a	47.00^b	0.00^a
GROUP C ALBINO RATS	4.80^c	4.00^a	38.00^b	12.60^b	95.00^c	31.50^c	33.15^a	830.06^b	0.00^a	68.00^c	0.00^b	32.00^a	0.00^a
GROUP D ALBINO RATS	4.00^b	5.50^c	39.00^c	13.00^c	70.90^b	23.63^b	33.33^a	864.21^c	1.00^a	49.00^a	2.00^a	48.00^c	0.00^a
LSD	0.06	0.06	0.65	0.06	1.47	0.47	0.53	5.15	0.00	0.79	0.00	0.79	0.00

NOTE: Means with different superscripts along the same column are significantly different at $P \leq 0.05$

LSD: Least Significant Difference

Group A albino rats = Albino rats fed commercial feed -Emii Finishers feed (control rats)

Group C albino rats = Albino rats fed 25:75 cooked sample-to-commercial feed mix.

Group D albino rats = Albino rats fed 50:50 cooked sample-to-commercial feed mix.

feed mix that was given to the albino rats. The significant increase in packed cell volume (PCV), platelets and haemoglobin (Hb) is an indication that the rats did not suffer anaemia but rather the sample (cooked 60 min *L. chinensis* seed) enhanced the PCV, Hb and platelets at these ratios (concentration) tested. This enhancement is possibly as a result of the increase in the ash content of the processed *L. chinesis* seed (Table 2) which is an indication of the amount of minerals present including iron(Fe) and copper(Cu) which are important in haemoglobin synthesis.

The significant increase($p \leq 0.05$) in packed cell volume(PCV), haemoglobin (Hb) and platelets with increased concentration of the sample suggests that increased concentration of the sample in the feed mix at those higher concentrations would have also likely increased the values of these parameters (packed cell volume, haemoglobin and platelets). High packed cell volume (PCV) beyond normal limits suggests venous thromboembolism/vascular accidents (heart failure/block, renal failure, ishaemi stroke, possible retinopathy). High haemoglobin levels also suggests thromboembolism while very high platelet counts suggests hyposplenism, myeloproliferative disorders or thrombocytosis (Lewis *et al*., 2006; Hoffbrand *et al*., 2004). Thus it is possible that the deaths recorded may be due to the conditions mentioned.

The deem, dull and partially closed eyes usually observed a day prior to the death of the albino rats may likely be connected to this possible retinopathy mentioned above.

4.7 Effect of roasted (110^0c, 15 minutes) *Livistona chinesis* seed on the haematological parameters of albino rats.

The effect of roasted (110^0C, 15 minutes) *L. chinensis* seed on the haematological parameters of albino rats are presented in table 7.

The roasted (110^0C, 15 minutes) sample of *L. chinensis* seed had a significant increasing effect($p \leq 0.05$) on the white blood cells (WBC), packed cell volume (PCV), haemoglobin (Hb), mean corpuscular volume (MCV), Mean corpuscular haemoglobin (MCH) and platelets. With increase in the ratio (concentration) of the sample (*L. chinensis* seed) of the feed mix, there was no definite pattern (continuous rise or fall) established for the red blood cells (*RBC*), lymphocytes and neutrophils while there was totally no significant effect ($p \leq 0.05$) on the mean corpuscular haemoglobin concentration (MCHC), monocytes, eosinophils and basophils.

It was observed that the roasted (110^0C, 15 minutes) sample of *L. chinensis* exerted a significant increasing effect ($p \leq 0.05$) (definite rising pattern) on the packed cell volume (PCV), haemoglobin (Hb) and platelets just like that of the cooked (60 minutes) sample except that the

Table 7: Effect of roasted (110^{0}C, 15 minutes) *L. chinensis* seed on the haematological parameters of albino rats.

Haematological parameter	White blood cells (x 10^6/mm^3)	Red blood cells (x 10^6/mm^3)	Packed cell volume (%)	Haemogolobin (g/dL)	Mena corpuscular volume (CUμ)	Mean corpuscular haemoglobin (pg)	Mean corpuscular haemoglobin concentration (%)	Platelets (x 10^9/L	Monocytes (%)	Lymphocytes (%)	Eosinophils (%)	Neutrophils (%)	Basophils (%)
GROUP A ALBINO RATS	3.60^a	4.90^a	32.00^a	10.80^a	65.30^a	22.04^a	33.75^a	780.12^a	0.00^a	51.00^b	2.00^a	47.00^b	0.00^a
GROUP G ALBINO RATS	3.90^b	5.30^c	35.00^b	11.80^b	66.03^{ab}	22.26^{ab}	33.71^a	798.17^b	0.00^a	49.00^a	0.00^a	51.00^c	0.00^a
GROUP H ALBINO RATS	4.00^c	5.00^b	38.00^c	12.80^c	76.00^b	25.60^b	33.68^a	806.09^c	1.00^a	59.00^c	0.00^a	40.00^a	0.00^a
LSD	0.06	0.06	0.79	0.01	2.35	0.47	0.51	2.17	0.00	1.56	0.00	1.50	0.00

NOTE: Means with different superscripts along the same column are significantly different at P $\leq$ 0.05

LSD: Least Significant Difference

Group A albino rats = Albino rats fed commercial feed -Emii Finishers feed (control rats)

Group G albino rats = Albino rats fed 25:75 roasted sample-to-commercial feed mix.

Group H albino rats = Albino rats fed 50:50 roasted sample-to-commercial feed mix.

roasted (110^0C, 15 minutes) sample also showed an additional significant effect($p \leq 0.05$) on the white blood cells (WBC), mean corpuscular volume (MCV) and mean corpuscular haemoglobin (MCH). The increase in the haemoglobin content would most likely be connected with the increase in the ash content of roasted (110^0C, 15 minutes) sample as shown in table 2. The ash content of a sample is an indication of the amount of minerals including iron (Fe) and copper (Cu) in the sample which are both particularly important in haemoglobin synthesis.

The white blood cells showed a significant increase($p \leq 0.05$) with increase in the ratio (concentration) of the *L. chinensis* seed in the feed mix but with the examination of the differential white blood cell count no significant effect was observed on the monocytes, eosinophils and basophils while lymphocytes and neutrophils did not show any pattern (continuous rise or fall) though there was a significant effect of the sample on them. The lymphocyte is responsible for immunity, eosinophils are responsible for allergy, neutrophils are responsible for phagocytosis of pathogens, monocytes are responsible for defense against microbial agents while Basophils increase upon sensitization to an antigen (or allergy) (Lewis *et al*; 2006; Aboderin and Oyetaya, 2006). The non-significant effect observed for the monocytes, eosinophils and basiphils, and the indefinite pattern (continuous rise or fall) observed for

the lymphocytes and neutrophils suggests that the significant increase in the white blood cells is not dependent on the increased ratio of roasted *L. chinensis* seed in the feed mix.

The mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) relate to individual red blood cells and are of particular importance in the diagnosis of anaemia (Odeyemi *et al*., 2009). The significant increase($p \leq 0.05$) in the haemoglobin level, mean corpuscular volume (MCV), mean corpuscular haemoglobin and the non-significant effect on the mean corpuscular haemoglobin concentration (MCHC) implies that the sample enhanced the incorporation of haemoglobin(Hb) into the red blood cells (RBC) and by implication, the animal did not suffer anaemia (Odeyemi *et al*., 2009).

There was also a significant increase in the platelets ($p \leq 0.05$). Platelets are the blood cells involved in coagulation (Odeyemi *et al.*, 2009). Coagulation of blood requires that the platelets should be in sufficient size, number and function. The increase in the platelets level may be explained by stimulatory effect on thrombopoietin (Odeyemi *et al*., 2009).

The significant increase($p \leq 0.05$) in the packed cell volume (PCV) and haemoglobin(Hb) which are linked to the total population of red blood cells (Odeyemi *et al*., 2009) and the non-definite pattern(definite

continuous rise or fall) observed in the red blood cell count may imply that the rats were not anaemia and that the osmotic fragility of the red blood cells was not adversely affected (Odeyemi *et al*., 2009). The non-definite pattern (continuous rise or fall) observed for the red blood cells may be an indication that the balance between the rate of production and destruction of the blood corpuscles (erythropoiesis) may not have been altered by the roasted sample (*L. chinensis* seed) at the levels which the test animals survived.

The deaths of the albino rats recorded at higher sample-to-commercial feed mix (75:25 & 100:0) may likely be as a result of possible increases in the packed cell volume(PCV), haemoglobin(Hb), mean corpuscular volume(MCV), mean corpuscular haemoglobin(MCH) and platelets to high levels beyond normal limits. Very high levels of these parameters (PCV, Hb, MCV, MCH and Platelets) have been implicated in the raising of blood to hypercoaguable state (Lewis *et al*., 2006) which probably predisposed the animals to hyposplenism, myeloproliferative disorders, and thromboembolism with possible vascular accidents like heart failure/block, renal failure, Ischaemic stroke and possible retinopathy (eye problems) (Lewis *et al*., 2006), and consequently led to their deaths.

The deem, dull and partially closed eyes usually observed a day prior to the death of the albino rats may likely be connected to this possible retinopathy mentioned above.

It is worthy of note that these effects are very similar to those observed in the albino rats fed the cooked (60 minutes) *Livistona chinensis* seed sample.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATION

5.1 Conclusion.

The findings from the physicochemical properties of the oil from *Livistona chinensis* (Chinese Fan Palm) seed revealed that it has low oil content. The findings also show that the oil has low thermal stability, oxidatively stable, high level of free fatty acids and saturated fatty acids. These suggest that the oil will not be suitable for edible purposes (unless refined) but will rather be better for industrial purposes.

It was discovered that raw *L. chinensis* seed has a relatively low crude protein, fat and carbohydrate content but with a relatively high ash and very high crude fibre content. It is therefore concluded that it can serve as a good source of fibre and roughage for animals. The study also showed that heat processing improves the ash content and crude protein content of *L. chinensis* seed just like many other raw foods that are heat processed before consumption.

Tannins, phytates, total phenols and oligosaccharides were present beyond lethal dose levels after 60 minutes cooking and 15 minutes of roasting of the whole *L. chinensis* seed thus suggesting that the seed is unsafe for human consumption at these levels of treatment.

From the evidences of the toxicity study which showed significant effect on packed cell volume, haemoglobin, mean cell volume, mean cell haemoglobin and platelets, it is concluded that *L. chinensis* seed is capable of causing thromboembolism, thrombocytosis, hyposplenism or myeloproliferative disorders with possible vascular accidents like heart failure, renal failure and retinopathy.

Raw, 60 minutes cooked and 15 minutes roasted *L. chinensis* seed all caused death of albino rats. The death of the albino rats leads to the conclusion that *L. chinensis* seed is unsafe for human consumption at the levels tested.

5.2 Recommendations.

Livistona chinensis seed should not be explored for high level of industrial oil since it contains very low oil content. *L. chinensis* seed is recommended for use as a high source of fibre/roughage in animal feed formulations.

Other methods of reduction of anti-nutrients in foods should be carried out on *L. chinensis* seed to ascertain their own rate and suitability in the reduction of the anti-nutrients in *L.chinensis* seed. The anti-nutrients' reduction/elimination methods should also be carried out on the cracked seed and not as a whole (uncracked) seed to also ascertain

if there is any variation in the anti-nutrients' reduction/elimination rate between the whole (uncracked) and cracked *L. chinensis* seed.

A chronic and histopathological toxicity study should further be carried out to ascertain the effect of *L. chinensis* seed on the different organs of albino rats and *L. chinensis* seed should not be used as food at these levels of treatment.

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APPENDIX 1

ANALYSIS OF VARIANCE FOR ANTI-NUTRIENTS

Class level information

Class	Levels	Values
Treat	11	blan4 blan6 blan8 cook20 cook40 cook60 pulp raw roast15 roast5 roat10

Number of Observations Read	33
Number of Observations Used	33

Dependent Variable: Tannin

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treat	10	0.00125455	0.00012545	13.80	<.0001
Error	22	0.00020000	0.00000909		
Corrected Total	32	0.00145455			

R-Square	Coeff. Var.	Root MSE	Tannin Mean
0.862500	0.618773	0.003015	0.487273

t Tests (LSD) for Tannin

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	9.091E-6
Critical Value of t	2.07387
Least Significant Difference	0.0051

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	0.490000	3	blan4
A	0.490000	3	blan6
A	0.490000	3	blan8
A	0.490000	3	cook20
A	0.490000	3	roast15
A	0.490000	3	roast5
A	0.490000	3	pulp
A	0.490000	3	raw
A	0.490000	3	roast10
B	0.480000	3	cook40
C	0.470000	3	cook60

Class level information

Class	Levels	Values
Treat	11	blan4 blan6 blan8 cook20 cook40 cook60 pulp raw roast15 roast5 roat10

Number of Observations Read	33
Number of Observations Used	33

Dependent Variable: Phytate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treat	10	0.70881818	0.07088182	458.65	<.0001
Error	22	0.00340000	0.00015455		
Corrected Total	32	0.71221818			

R-Square	Coeff. Var.	Root MSE	phytate Mean
0.995226	1.249981	0.012432	0.994545

t Tests (LSD) for phytate

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	0.000155
Critical Value of t	2.07387
Least Significant Difference	0.0211

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	1.20000	3	raw
B	1.17000	3	roast5
C	1.11000	3	roast10
D	1.08000	3	blan4
E	1.03000	3	roast15
E	1.03000	3	blan6
F	1.00000	3	blan8
G	0.91000	3	cook20
H	0.88000	3	cook40
I	0.85000	3	cook60
J	0.68000	3	pulp

Class Level Information

Class	Levels	Values
Treat	11	blan4 blan6 blan8 cook20 cook40 cook60 pulp raw roast15 roast5 roat10

Number of Observations Read	33
Number of Observations Used	33

Dependent Variable: oxalate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treat	10	0.66801818	0.06680182	816.47	<.0001
Error	22	0.00180000	0.00008182		
Corrected Total	32	0.66981818			

R-Square	Coeff. Var.	Root MSE	oxalate Mean
0.997313	7.262682	0.009045	0.124545

t Tests (LSD) for oxalate

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	0.000082
Critical Value of t	2.07387
Least Significant Difference	**0.0153**

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	0.540000	3	pulp
B	0.180000	3	raw
C	0.160000	3	roast5
D	0.110000	3	roast10
D	0.110000	3	blan4
E	0.090000	3	blan6
E	0.090000	3	roast15
F	0.050000	3	blan8
G	0.020000	3	cook20
G	0.020000	3	cook40
H	0.000000	3	cook60

Class Level Information

Class	Levels	Values
Treat	11	blan4 blan6 blan8 cook20 cook40 cook60 pulp raw roast15 roast5 roat10

Number of Observations Read	33
Number of Observations Used	33

Dependent Variable: Saponins

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treat	10	195.6818182	19.5681818	287.00	<.0001
Error	22	1.5000000	0.0681818		
Corrected Total	32	197.1818182			

R-Square	Coeff. Var.	Root MSE	saponins Mean
0.992393	10.63808	0.261116	2.454545

t Tests (LSD) for Saponins

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	0.068182
Critical Value of t	2.07387
Least Significant Difference	**0.4422**

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	6.5000	3	pulp
B	5.5000	3	raw
C	5.0000	3	roast5
D	4.0000	3	blan4
E	3.0000	3	roast10
E	3.0000	3	blan6
F	0.0000	3	cook40
F	0.0000	3	cook20
F	0.0000	3	roast15
F	0.0000	3	cook60
F	0.0000	3	blan8

Class Level Information

Class	Levels	Values
Treat	11	blan4 blan6 blan8 cook20 cook40 cook60 pulp raw roast15 roast5 roat10

Number of Observations Read	33
Number of Observations Used	33

Dependent Variable: HCN

Source	DF	Sum of quares	Mean Square	F Value	Pr > F
Treat	10	4.91096364	0.49109636	4.59	0.0014
Error	22	2.35233333	0.10692424		
Corrected Total	32	7.26329697			

R-Square	Coeff. Var.	Root MSE	HCN Mean
0.676134	35.04631	0.326993	0.933030

t Tests (LSD) for HCN

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	0.106924
Critical Value of t	2.07387
Least Significant Difference	0.5537

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	1.4633	3	roast10
A			
A	1.4633	3	raw
A			
A	1.4633	3	roast5
A			
B A	0.9800	3	roast15
B A			
B A	0.9800	3	blan8
B A			
B A	0.9767	3	blan6
B A			
B A	0.9767	3	blan4
B			
B	0.4900	3	cook20
B			
B	0.4900	3	cook40
B			
B	0.4900	3	cook60
B	0.4900	3	pulp

Class Level Information

Class	Levels	Values
Treat	11	blan4 blan6 blan8 cook20 cook40 cook60 pulp raw roast15 roast5 roat10

Number of Observations Read 33
Number of Observations Used 33

Dependent Variable: Alkaloids

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treat	10	146.1818182	14.6181818	53.60	<.0001
Error	22	6.0000000	0.2727273		
Corrected Total	32	152.1818182			

R-Square	Coeff. Var.	Root MSE	Alkaloids Mean
0.960573	14.72965	0.522233	3.545455

t Tests (LSD) for Alkaloids

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	0.272727
Critical Value of t	2.07387
Least Significant Difference	0.8843

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	6.0000	3	blan4
A	6.0000	3	blan6
A	6.0000	3	raw
B	5.0000	3	roast5
C	4.0000	3	blan8
C	4.0000	3	pulp
D	3.0000	3	roast10
D	3.0000	3	cook20
E	2.0000	3	roast15
F	0.0000	3	cook60
F	0.0000	3	cook40

Class Level Information

Class	Levels	Values
Treat	11	blan4 blan6 blan8 cook20 cook40 cook60 pulp raw roast15 roast5 roat10

Number of Observations Read	33
Number of Observations Used	33

Dependent Variable: T. Phenols

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treat	10	0.26105455	0.02610545	220.89	<.0001
Error	22	0.00260000	0.00011818		
Corrected Total	32	0.26365455			

R-Square	Coeff. Var.	Root MSE	T. Phenols Mean
0.990139	2.206321	0.010871	0.492727

t Tests (LSD) for T_phenols

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	0.000118
Critical Value of t	2.07387
Least Significant Difference	0.0184

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	0.610000	3	blan4
A	0.610000	3	raw
B	0.590000	3	roast5
C	0.530000	3	blan6
C	0.520000	3	roast10
D	0.500000	3	pulp
E	0.470000	3	roast15
E	0.470000	3	blan8
F	0.420000	3	cook20
G	0.370000	3	cook40
H	0.330000	3	cook60

Class Level Information

Class	Levels	Values
Treat	11	blan4 blan6 blan8 cook20 cook40 cook60 pulp raw roast15 roast5 roat10

Number of Observations Read 33
Number of Observations Used 33

Dependent Variable: Flavoniods

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treat	10	50.72727273	5.07272727	13.95	<.0001
Error	22	8.00000000	0.36363636		
Corrected Total	32	58.72727273			

R-Square	Coeff. Var.	Root MSE	Flavoniods Mean
0.863777	31.58690	0.603023	1.909091

t Tests (LSD) for Flavoniods

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	0.363636
Critical Value of t	2.07387
Least Significant Difference	1.0211

Means with the same letter are not significantly different.

t Grouping		Mean	N	Treat
	A	4.0000	3	raw
B	A	3.0000	3	blan4
B	A	3.0000	3	blan6
B	A	3.0000	3	roast5
B				
B	C	2.0000	3	blan8
B	C	2.0000	3	pulp
B	C			
B	C	2.0000	3	roast10
D	C	1.0000	3	cook20
D	C	1.0000	3	roast15
D				
D		0.0000	3	cook60
D		0.0000	3	cook40

Class Level Information

Class	Levels	Values
Treat	11	blan4 blan6 blan8 cook20 cook40 cook60 pulp raw roast15 roast5 roat10

Number of Observations Read	33
Number of Observations Used	33

Dependent Variable: Oligosac.

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treat	10	1026.136364	102.613636	60.20	<.0001
Error	22	37.500000	1.704545		
Corrected Total	32	1063.636364			

R-Square	Coeff. Var.	Root MSE	Oligosac. Mean
0.964744	6.176949	1.305582	21.13636

t Tests (LSD) for Oligosac

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	1.704545
Critical Value of t	2.07387
Least Significant Difference	2.2108

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	27.500	3	raw
B	25.000	3	blan4
B			
B	25.000	3	pulp
B			
B	25.000	3	blan6
B			
B	25.000	3	roast5
C	22.500	3	blan8
C			
C	22.500	3	roast10
D	20.000	3	roast15
E	17.500	3	cook20
F	15.000	3	cook40
G	7.500	3	cook60

APPENDIX 2

MEANS AND STANDARD DEVIATION FOR ANTI-NUTRIENTS

Means and Descriptive Statistics Tannin

Treat	Mean of TANNIN	Std. Dev. of TANNIN
	0.48727	0.006742
blan4	0.49000	0.000000
blan6	0.49000	0.000000
blan8	0.49000	0.000000
cook20	0.49000	0.000000
cook40	0.48000	0.010000
cook60	0.47000	0.000000
pulp	0.49000	0.000000
raw	0.49000	0.000000
roast15	0.49000	0.000000
roast5	0.49000	0.000000
roat10	0.49000	0.000000

Means and Descriptive Statistics Phyatate

Treat	Mean of PHYTATE	Std. Dev. of PHYTATE
	0.99455	0.14919
blan4	1.08000	0.00000
blan6	1.03000	0.01000
blan8	1.00000	0.01000
cook20	0.91000	0.00000
cook40	0.88000	0.01000
cook60	0.85000	0.02000
pulp	0.68000	0.02000
raw	1.20000	0.02000
roast15	1.03000	0.00000
roast5	1.17000	0.01000
roast10	1.11000	0.01000

Means and Descriptive Statistics Oxalate

Treat	Mean of OXALATE	Std. Dev. of OXALATE
	0.12455	0.14468
blan4	0.11000	0.01000
blan6	0.09000	0.00000
blan8	0.05000	0.00000
cook20	0.02000	0.01000
cook40	0.02000	0.00000
cook60	0.00000	0.00000
pulp	0.54000	0.02000
raw	0.18000	0.01000
roast15	0.09000	0.01000
roast5	0.16000	0.01000
roast10	0.11000	0.00000

Means and Descriptive Statistics Saponins

Treat	Mean of SAPONINS	Std. Dev. of SAPONINS
	2.45455	2.48232
blan4	4.00000	0.50000
blan6	3.00000	0.00000
blan8	0.00000	0.00000
cook20	0.00000	0.00000
cook40	0.00000	0.00000
cook60	0.00000	0.00000
pulp	6.50000	0.00000
raw	5.50000	0.50000
roast15	0.00000	0.00000
roast5	5.00000	0.50000
roast10	3.00000	0.00000

Means and Descriptive Statistics HCN

Treat	Mean of HCN	Std. Dev. of HCN
	0.93303	0.47642
blan4	0.97667	0.48501
blan6	0.97667	0.48501
blan8	0.98000	0.00000
cook20	0.49000	0.00000
cook40	0.49000	0.00000
cook60	0.49000	0.00000
pulp	0.49000	0.00000
raw	1.46333	0.48501
roast15	0.98000	0.00000
roast5	1.46333	0.48501
roast10	1.46333	0.48501

Means and Descriptive Statistics

Treat	Mean of ALKALOIDS	Std. Dev. of ALKALOIDS
	3.54545	2.18075
blan4	6.00000	1.00000
blan6	6.00000	0.00000
blan8	4.00000	0.00000
cook20	3.00000	0.00000
cook40	0.00000	0.00000
cook60	0.00000	0.00000
pulp	4.00000	0.00000
raw	6.00000	1.00000
roast15	2.00000	0.00000
roast5	5.00000	1.00000
roast10	3.00000	0.00000

Means and Descriptive Statistics

Treat	Mean of T.PHENOLS	Std. Dev. of T. PHENOLS
	0.49273	0.090770
blan4	0.61000	0.000000
blan6	0.53000	0.010000
blan8	0.47000	0.010000
cook20	0.42000	0.000000
cook40	0.37000	0.000000
cook60	0.33000	0.000000
pulp	0.50000	0.010000
raw	0.61000	0.010000
roast15	0.47000	0.020000
roast5	0.59000	0.020000
roast10	0.52000	0.010000

Means and Descriptive Statistics

Treat	Mean of FLAVONIODS	Std. Dev. of FLAVONIODS
	1.90909	1.35471
blan4	3.00000	0.00000
blan6	3.00000	0.00000
blan8	2.00000	0.00000
cook20	1.00000	1.00000
cook40	0.00000	0.00000
cook60	0.00000	0.00000
pulp	2.00000	0.00000
raw	4.00000	1.00000
roast15	1.00000	1.00000
roast5	3.00000	1.00000
roast10	2.00000	0.00000

Means and Descriptive statistics

Treat	Mean of OLIGOSAC.	Std. Dev. of OLIGOSAC.
	21.1364	5.76530
blan4	25.0000	0.00000
blan6	25.0000	2.50000
blan8	22.5000	0.00000
cook20	17.5000	0.00000
cook40	15.0000	0.00000
cook60	7.5000	0.00000
pulp	25.0000	0.00000
raw	27.5000	2.50000
roast15	20.0000	0.00000
roast5	25.0000	2.50000
roast10	22.5000	0.00000

APPENDIX

Class Level Information

Class	Levels	Values
Treat	11	blan4 blan6 blan8 cook20 cook40 cook60 pulp raw roast15 roast5 roat10

Number of Observations Read	33
Number of Observations Used	33

Dependent Variable: moisture

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treat	10	2319.807273	231.980727	5066.09	<.0001
Error	22	1.007400	0.045791		
Corrected Total	32	2320.814673			

R-Square	Coeff Var	Root MSE	moist4 Mean
0.999566	0.654580	0.213988	32.69091

t Tests (LSD) for moisture

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	0.045791
Critical Value of t	2.07387
Least Significant Difference	0.3623

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	41.4500	3	cook60
B	39.8300	3	cook40
C	39.0600	3	cook20
D	37.1500	3	blan8
E	35.8600	3	blan6
F	35.4500	3	blan4
G	35.3500	3	raw
H	30.4200	3	roast5
I	27.2200	3	roat10
J	25.4400	3	roast15
K	11.1400	3	pulp

Means and Descriptive Statistics

Treat	Mean of MOIST.	Std. Dev. of MOIST.
	32.6909	8.51619
blan4	35.4500	0.40000
blan6	35.8600	0.25000
blan8	37.1500	0.15000
cook20	39.0600	0.06000
cook40	39.8300	0.20000
cook60	41.4500	0.05000
pulp	11.1400	0.04000
raw	35.3500	0.05000
roast15	25.4400	0.40000
roast5	30.4200	0.22000
roat10	27.2200	0.01000

Class Level Information.

Class	Levels	Values
Treat	11	blan4 blan6 blan8 cook20 cook40 cook60 pulp raw roast15 roast5 roat10

Number of Observations Read	33
Number of Observations Used	33

Dependent Variable: protein

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treat	10	57.32127273	5.73212727	1504.85	<.0001
Error	22	0.08380000	0.00380909		
Corrected Total		32	57.40507273		

R-Square	Coeff Var	Root MSE	protein4 Mean
0.998540	1.244083	0.061718	4.960909

t Tests (LSD) for protein4

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	0.003809
Critical Value of t	2.07387
Least Significant Difference	0.1045

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	9.04000	3	pulp
B	5.22000	3	roast15
C	4.95000	3	roat10
D	4.63000	3	roast5
E	4.44000	3	raw
F E	4.42000	3	blan4
F E	4.41000	3	blan6
F E	4.40000	3	blan8
F E	4.38000	3	cook20
F E	4.36000	3	cook40
F	4.32000	3	cook60

Means and Descriptive Statistics

Treat	Mean of PROTEIN	Std. Dev. of PROTEIN
	4.96091	1.33937
blan4	4.42000	0.07000
blan6	4.41000	0.01000
blan8	4.40000	0.03000
cook20	4.38000	0.08000
cook40	4.36000	0.05000
cook60	4.32000	0.01000
pulp	9.04000	0.03000
raw	4.44000	0.04000
roast15	5.22000	0.02000
roast5	4.63000	0.15000
roat10	4.95000	0.04000

Class	Levels	Values
Treat	11	blan4 blan6 blan8 cook20 cook40 cook60 pulp raw roast15 roast5 roat10

Number of Observations Read	33
Number of Observations Used	33

Dependent Variable: ash

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treat	10	89.13235152	8.91323515	1726.15	<.0001
Error	22	0.11360000	0.00516364		
Corrected Total	32	89.24595152			

R-Square	Coeff Var	Root MSE	ash2 Mean
0.998727	4.111180	0.071858	1.747879

t Tests (LSD) for ash

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	0.005164
Critical Value of t	2.07387
Least Significant Difference	0.1217

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	6.92000	3	pulp
B	1.37667	3	roast15
B	1.35333	3	roat10
B	1.34333	3	roast5
B	1.33333	3	blan8
B	1.31333	3	blan4
B	1.31000	3	raw
B	1.27667	3	blan6
C	1.12000	3	cook20
C	1.06000	3	cook40
D	0.82000	3	cook60

Means and Descriptive Statistics

Treat	Mean of ASH2	Std. Dev. of ASH2
	1.74788	1.67001
blan4	1.31333	0.00577
blan6	1.27667	0.01528
blan8	1.27200	0.23094
cook20	1.12000	0.03464
cook40	1.06000	0.01732
cook60	0.82000	0.01732
pulp	6.92000	0.01000
raw	1.31000	0.01000
roast15	1.37667	0.02309
roast5	1.34333	0.01528
roat10	1.35333	0.02082

Class Level Information

Class	Levels	Values
Treat	11	blan4 blan6 blan8 cook20 cook40 cook60 pulp raw roast15 roast5 roat10

Number of Observations Read	33
Number of Observations Used	33

Dependent Variable: Fat

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treat	10	8.60105455	0.86010545	6757.97	<.0001
Error	22	0.00280000	0.00012727		
Corrected Total	32	8.60385455			

R-Square	Coeff. Var.	Root MSE	Fat Mean
0.999675	0.482679	0.011282	2.337273

t Tests (LSD) for Fat

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	0.000127
Critical Value of t	2.07387
Least Significant Difference	0.0191

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	2.860000	3	raw
A			
A	2.860000	3	blan4
B	2.710000	3	roast10
B			
B	2.700000	3	blan6
C	2.640000	3	roast5
D	2.510000	3	roast15
E	2.380000	3	pulp
F	2.320000	3	blan8
G	1.910000	3	cook20
H	1.520000	3	cook40
I	1.300000	3	cook60

Class Level Information.

Class	Levels	Values
Treat	11	blan4 blan6 blan8 cook20 cook40 cook60 pulp raw roast15 roast5 roat10

Number of Observations Read 33
Number of Observations Used 33

Dependent Variable: Fiber

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treat	10	28.18707273	2.81870727	78.18	<.0001
Error	22	0.79320000	0.03605455		
Corrected Total	32	28.98027273			

R-Square	Coeff. Var.	Root MSE	Fiber Mean
0.972630	0.506741	0.189880	37.47091

t Tests (LSD) for Fiber

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	0.036055
Critical Value of t	2.07387
Least Significant Difference	0.3215

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	38.5900	3	roast5
B	38.2100	3	blan4
B	38.2100	3	raw
B	37.9100	3	roast15
C	37.5400	3	blan8
C	37.5000	3	cook20
D C	37.2700	3	roast10
D	37.1400	3	blan6
E	36.6600	3	pulp
F	36.0200	3	cook40
F F	35.9200	3	cook60

Class	Levels	Values
Treat	11	blan4 blan6 blan8 cook20 cook40 cook60 pulp raw roast15 roast5 roat10

Number of Observations Read	33
Number of Observations Used	33

Dependent Variable: carbohydrate

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Treat	10	1036.238830	103.623883	10293.8	<.0001
Error	22	0.221467	0.010067		
Corrected Total	32	1036.460297			

R-Square	Coeff Var	Root MSE	carb4 Mean
0.999786	0.480221	0.100333	20.89303

t Tests (LSD) for carbohydrate

Alpha	0.05
Error Degrees of Freedom	22
Error Mean Square	0.010067
Critical Value of t	2.07387
Least Significant Difference	0.1699

Means with the same letter are not significantly different.

t Grouping	Mean	N	Treat
A	33.69333	3	pulp
B	27.54000	3	roast15
C	26.50000	3	roat10
D	22.38000	3	roast5
E	17.83000	3	raw
E	17.79000	3	blan6
F	17.34000	3	blan4
F	17.32000	3	blan8
F	17.21000	3	cook40
G	16.19000	3	cook60
G	16.03000	3	cook20

Means and Descriptive Statistics

Treat	Mean of CARB.	Std. Dev. of CARB.
	20.8930	5.69117
blan4	17.3400	0.04000
blan6	17.7900	0.08000
blan8	17.3200	0.01000
cook20	16.0300	0.03000
cook40	17.2100	0.05000
cook60	16.1900	0.05000
pulp	33.6933	0.29484
raw	17.8300	0.03000
roast15	27.5400	0.04000
roast5	22.3800	0.03000
roat10	26.5000	0.08000

APPENDIX 4

MEANS AND STANDARD DEVIATION FOR PROXIMATE COMPOSITION

Means and Descriptive Statistics

Treat	Mean of MOISTURE	Std. Dev. of MOISTURE
	32.6909	8.51619
blan4	35.8600	0.40000
blan6	36.6800	0.25000
blan8	37.1500	0.15000
cook20	39.0600	0.06000
cook40	39.8300	0.20000
cook60	41.4500	0.05000
pulp	11.1400	0.04000
raw	35.3500	0.05000
roast15	25.4400	0.40000
roast5	30.4200	0.22000
roat10	27.2200	0.01000

Means and Descriptive Statistics

Treat	Mean of PROTEIN	Std. Dev. of PROTEIN
	4.96091	1.33937
blan4	4.42000	0.07000
blan6	4.41000	0.01000
blan8	4.40000	0.03000
cook20	4.38000	0.08000
cook40	4.36000	0.05000
cook60	4.32000	0.01000
pulp	9.04000	0.03000
raw	4.44000	0.04000
roast15	5.22000	0.02000
roast5	4.63000	0.15000
roat10	4.95000	0.04000

Means and Descriptive Statistics

Treat	Mean of ASH	Std. Dev. of ASH
	2.40818	1.50579
blan4	1.31000	0.00000
blan6	1.59000	0.02000
blan8	1.82000	0.02000
cook20	2.01000	0.00000
cook40	2.49000	0.02000
cook60	2.59000	0.01000
pulp	6.91000	0.01000
raw	1.31000	0.01000
roast15	2.34000	0.02000
roast5	1.91000	0.00000
roast10	2.21000	0.01000

Means and Descriptive Statistics

Treat	Mean of FAT	Std. Dev. of FAT
	2.33727	0.51853
blan4	2.86000	0.02000
blan6	2.70000	0.01000
blan8	2.32000	0.00000
cook20	1.91000	0.00000
cook40	1.52000	0.01000
cook60	1.30000	0.01000
pulp	2.38000	0.01000
raw	2.86000	0.01000
roast15	2.51000	0.01000
roast5	2.64000	0.02000
roast10	2.71000	0.00000

Means and Descriptive Statistics

Treat	Mean of FIBER	Std. Dev. of FIBER
	37.4709	0.95165
blan4	38.2100	0.10000
blan6	37.1400	0.08000
blan8	37.5400	0.20000
cook20	38.7100	0.00000
cook40	36.0200	0.00000
cook60	35.9200	0.50000
pulp	36.6600	0.00000
raw	38.2100	0.21000
roast15	37.9100	0.00000
roast5	38.5900	0.19000
roast10	37.2700	0.10000

Means and Descriptive Statistics

Treat	Mean of CARB.	Std. Dev. of CARB.
	20.8930	5.69117
blan4	17.3400	0.04000
blan6	17.7900	0.08000
blan8	17.3200	0.01000
cook20	16.0300	0.03000
cook40	17.2100	0.05000
cook60	16.1900	0.05000
pulp	33.6933	0.29484
raw	17.8300	0.03000
roast15	27.5400	0.04000
roast5	22.3800	0.03000
roat10	26.5000	0.08000

APPENDIX 5

ANALYSIS OF VARIANCE FOR THE WHITE BLOOD CELLS

OF ALBINO RATS FED (60 MINUTES) COOKED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	3.7	4.9	4
REPLICATE 2	3.6	4.8	4
REPLICATE 3	3.5	4.7	4
TOTAL	10.8	14.4	12
MEAN	**3.6**	**4.8**	**4**
TOTA SQUARED	116.6	207.36	144

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	2.24	1.12	168
ERROR	6	0.04	0.006666667	
TOTAL	8	2.28		

G. total	**=**	**37.2**
GT ²	**=**	**1383.84**
Replicate*treatment	**=**	**9**
Correction factor(CT)	**=**	**153.76**
all sum of squares	**=**	**156.04**
SS total	**=**	**2.28**
TOTAL SQUARED SUM	**=**	**468**
SS TREATMENT	**=**	**2.24**
SS ERROR	**=**	**0.04**

ANALYSIS OF VARIANCE FOR THE RED BLOOD CELLS

OF ALBINO RATS FED (60 MINUTES) COOKED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	4.9	4.1	5.4
REPLICATE 2	4.9	4	5.5
REPLICATE 3	4.9	3.9	5.6
TOTAL	14.7	12	16.5
MEAN	**4.9**	**4**	**5.5**
TOTA SQUARED	216.09	144	272.25

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	3.42	1.71	256.5
ERROR	6	0.04	0.006666667	
TOTAL	8	3.46		

G. total	**=**	**43.2**
GT ²	**=**	**1866.24**
Replicate*treatment	**=**	**9**
Correction factor(CT)	**=**	**207.36**
all sum of squares	**=**	**210.82**
SS total	**=**	**3.46**
TOTAL SQUARED SUM	**=**	**632.34**
SS TREATMENT	**=**	**3.42**
SS ERROR	**=**	**0.04**

ANALYSIS OF VARIANCE FOR THE PACKED CELL VOLUME (PCV)

OF ALBINO RATS FED (60 MINUTES) COOKED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	32	38	38
REPLICATE 2	31	38	39
REPLICATE 3	33	38	40
TOTAL	96	114	117
MEAN	**32**	**38**	**39**
TOTA SQUARED	9216	12996	13689

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	86	43	64.5
ERROR	6	4	0.666666667	
TOTAL	8	90		

G. total	**=**	**327**
GT ²	**=**	**106929**
Replicate*treatment	**=**	**9**
Correction factor(CT)	**=**	**11881**
all sum of squares	**=**	**11971**
SS total	**=**	**90**
TOTAL SQUARED SUM	**=**	**35901**
SS TREATMENT	**=**	**86**
SS ERROR	**=**	**4**

ANALYSIS OF VARIANCE FOR HAEMOGLOBIN(Hb)

OF ALBINO RATS FED (60 MINUTES) COOKED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	10.7	12.6	13.1
REPLICATE 2	10.8	12.6	13
REPLICATE 3	10.9	12.6	12.9
TOTAL	32.4	37.8	39
MEAN	**10.8**	**12.6**	**13**
TOTA SQUARED	1050	1428.84	1521

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	8.24	4.12	618
ERROR	6	0.04	0.006666667	
TOTAL	8	8.28		

G. total	**=**	**109.2**
GT ²	**=**	**11924.64**
Replicate*treatment	**=**	**9**
Correction factor(CT)	**=**	**1324.96**
all sum of squares	**=**	**1333.24**
SS total	**=**	**8.28**
TOTAL SQUARED SUM	**=**	**3999.6**
SS TREATMENT	**=**	**8.24**
SS ERROR	**=**	**0.04**

ANALYSIS OF VARIANCE FOR MEAN CELL VOLUME(MCV)

OF ALBINO RATS FED (60 MINUTES) COOKED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	67.35	92.6	70.37
REPLICATE 2	65.31	95	70.91
REPLICATE 3	63.27	97.44	71.43
TOTAL	195.9	285.04	212.71
MEAN	**65.31**	**95.01333333**	**70.90333333**
TOTA SQUARED	38389	81247.8016	45245.5441

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	1494.865489	747.4327444	217.7185862
ERROR	6	20.59813333	3.433022222	
TOTAL	8	1515.463622		

G. total = 693.68

GT ² = 481191.942

Replicate*treatment = 9

Correction factor(CT) = 53465.7714

all sum of squares = 54981.235

SS total = 1515.46362

TOTAL SQUARED SUM = 164881.911

SS TREATMENT = 1494.86549

SS ERROR = 20.5981333

ANALYSIS OF VARIANCE FOR MEAN CELL HAEMOGLOBIN(MCH)

OF ALBINO RATS FED (60 MINUTES) COOKED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	21.84	30.73	24.26
REPLICATE 2	22.04	31.5	23.64
REPLICATE 3	22.24	32.31	23.04
TOTAL	66.12	94.54	70.94
MEAN	**22.04**	**31.51333333**	**23.64666667**
TOTA SQUARED	4372	8937.8116	5032.4836

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	154.2098667	77.10493333	223.1978386
ERROR	6	2.072733333	0.345455556	
TOTAL	8	156.2826		

G. total	**=**	**231.6**
GT ²	**=**	**53638.56**
Replicate*treatment	**=**	**9**
Correction factor(CT)	**=**	**5959.84**
all sum of squares	**=**	**6116.1226**
SS total	**=**	**156.2826**
TOTAL SQUARED SUM	**=**	**18342.1496**
SS TREATMENT	**=**	**154.209867**
SS ERROR	**=**	**2.07273333**

ANALYSIS OF VARIANCE FOR MEAN CELL HAEMOGLOBIN CONCENTRATION (MCHC)

OF ALBINO RATS FED (60 MINUTES) COOKED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	33.44	33.16	34.47
REPLICATE 2	33.75	33.16	33.33
REPLICATE 3	34.06	33.16	32.25
TOTAL	101.3	99.48	100.05
MEAN	**33.75**	**33.16**	**33.35**
TOTA SQUARED	10252	9896.2704	10010.0025

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	0.5442	0.2721	0.61445239
ERROR	6	2.657	0.442833333	
TOTAL	8	3.2012		

G. total = 300.78

GT ² = 90468.6084

Replicate*treatment = 9

Correction factor(CT) = 10052.0676

all sum of squares = 10055.2688

SS total = 3.2012

TOTAL SQUARED SUM = 30157.8354

SS TREATMENT = 0.5442

SS ERROR = 2.657

ANALYSIS OF VARIANCE FOR PLATELETS COUNT
OF ALBINO RATS FED (60 MINUTES) COOKED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	778.02	820.1	869
REPLICATE 2	780.12	830.06	864.21
REPLICATE 3	782.22	840.02	859.42
TOTAL	2340.36	2490.18	2592.63
MEAN	**780.12**	**830.06**	**864.21**
TOTA SQUARED	5477285	6200996.432	6721730.317

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	10731.3542	5365.6771	127.193254
ERROR	6	253.1114	42.18523333	
TOTAL	8	10984.4656		

G. total	**=**	**7423.17**
GT ²	**=**	**55103452.8**
Replicate*treatment	**=**	**9**
Correction factor(CT)	**=**	**6122605.87**
all sum of squares	**=**	**6133590.34**
SS total	**=**	**10984.4656**
TOTAL SQUARED SUM =		**18400011.7**
SS TREATMENT	**=**	**10731.3542**
SS ERROR	**=**	**253.1114**

ANALYSIS OF VARIANCE FOR NEUTROPHILS

OF ALBINO RATS FED (60 MINUTES) COOKED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	48	33	47
REPLICATE 2	47	32	48
REPLICATE 3	46	31	49
TOTAL	141	96	144
MEAN	**47**	**32**	**48**
TOTA SQUARED	19881	9216	20736

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	482	241	241
ERROR	6	6	1	
TOTAL	8	488		

G. total = 381

GT ² = 145161

Replicate*treatment = 9

Correction factor(CT) = 16129

all sum of squares = 16617

SS total = 488

TOTAL SQUARED SUM = 49833

SS TREATMENT = 482

SS ERROR = 6

ANALYSIS OF VARIANCE FOR THE MONOCYTES

OF ALBINO RATS FED (60 MINUTES) COOKED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	0	0	1
REPLICATE 2	0	0	1
REPLICATE 3	0	0	1
TOTAL	0	0	3
MEAN	**0**	**0**	**1**
TOTA SQUARED	0	0	9

G. total = 3

GT ² = 9

Replicate*treatment = 9

Correction factor(CT) = 1

all sum of squares = 3

SS total = 2

TOTAL SQUARED SUM = 9

SS TREATMENT = 2

SS ERROR = 0

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	2	1	#DIV/0!
ERROR	6	0	0	
TOTAL	8	2		

ANALYSIS OF VARIANCE FOR THE LYMPHOCYTES

OF ALBINO RATS FED (60 MINUTES) COOKED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	50	67	50
REPLICATE 2	52	68	49
REPLICATE 3	51	69	48
TOTAL	153	204	147
MEAN	**51**	**68**	**49**
TOTA SQUARED	23409	41616	21609

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	654	327	327
ERROR	6	6	1	
TOTAL	8	660		

G. total = 504

GT ² = 254016

Replicate*treatment = 9

Correction factor(CT) = 28224

all sum of squares = 28884

SS total = 660

TOTAL SQUARED SUM = 86634

SS TREATMENT = 654

SS ERROR = 6

ANALYSIS OF VARIANCE FOR THE EOSINOPHILS

OF ALBINO RATS FED (60 MINUTES) COOKED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	2	0	2
REPLICATE 2	2	0	2
REPLICATE 3	2	0	2
TOTAL	6	0	6
MEAN	**2**	**0**	**2**
TOTA SQUARED	36	0	36

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	8	4	#DIV/0!
ERROR	6	0	0	
TOTAL	8	8		

G. total = 12

GT ² = 144

Replicate*treatment = 9

Correction factor(CT) = 16

all sum of squares = 24

SS total = 8

TOTAL SQUARED SUM = 72

SS TREATMENT = 8

SS ERROR = 0

APPENDIX 6

ANALYSIS OF VARIANCE FOR WHITE BLOOD CELLS

OF ALBINO RATS FED (110˚C,15MINUTES) ROASTED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	3.7	4	4
REPLICATE 2	3.6	3.9	4
REPLICATE 3	3.5	3.8	4
TOTAL	10.8	11.7	12
MEAN	**3.6**	**3.9**	**4**
TOTA SQUARED	116.6	136.89	144

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	0.26	0.13	19.5
ERROR	6	0.04	0.006666667	
TOTAL	8	0.3		

G. total = 34.5

GT ² = 1190.25

Replicate*treatment = 9

Correction factor(CT) = 132.25

all sum of squares = 132.55

SS total = 0.3

TOTAL SQUARED SUM = 397.53

SS TREATMENT = 0.26

SS ERROR = 0.04

ANALYSIS OF VARIANCE FOR RED BLOOD CELLS

OF ALBINO RATS FED (110˚C,15MINUTES) ROASTED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	4.9	5.4	4.9
REPLICATE 2	4.9	5.3	5
REPLICATE 3	4.9	5.2	5.1
TOTAL	14.7	15.9	15
MEAN	**4.9**	**5.3**	**5**
TOTA SQUARED	216.1	252.81	225

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	0.26	0.13	19.5
ERROR	6	0.04	0.006666667	
TOTAL	8	0.3		

G. total	**=**	**45.6**
GT ²	**=**	**2079.36**
Replicate*treatment	**=**	**9**
Correction factor(CT)	**=**	**231.04**
all sum of squares	**=**	**231.34**
SS total	**=**	**0.3**
TOTAL SQUARED SUM	**=**	**693.9**
SS TREATMENT	**=**	**0.26**
SS ERROR	**=**	**0.04**

ANALYSIS OF VARIANCE FOR PACKED CELL VOLUME (PCV)

OF ALBINO RATS FED (110˚C,15MINUTES) ROASTED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	32	34	39
REPLICATE 2	31	35	38
REPLICATE 3	33	36	37
TOTAL	96	105	114
MEAN	**32**	**35**	**38**
TOTA SQUARED	9216	11025	12996

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	54	27	27
ERROR	6	6	1	
TOTAL	8	60		

G. total = 315

GT ² = 99225

Replicate*treatment = 9

Correction factor(CT) = 11025

all sum of squares = 11085

SS total = 60

TOTAL SQUARED SUM = 33237

SS TREATMENT = 54

SS ERROR = 6

ANALYSIS OF VARIANCE FOR HAEMOGLOBIN (Hb)

OF ALBINO RATS FED (110˚C,15MINUTES) ROASTED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	10.7	11.8	13
REPLICATE 2	10.8	11.8	12.8
REPLICATE 3	10.9	11.8	12.6
TOTAL	32.4	35.4	38.4
MEAN	**10.8**	**11.8**	**12.8**
TOTA SQUARED	1050	1253.16	1474.56

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	6	3	180
ERROR	6	0.1	0.016666667	
TOTAL	8	6.1		

G. total = 106.2

GT ² = 11278.44

Replicate*treatment = 9

Correction factor(CT) = 1253.16

all sum of squares = 1259.26

SS total = 6.1

TOTAL SQUARED SUM = 3777.48

SS TREATMENT = 6

SS ERROR = 0.1

ANALYSIS OF VARIANCE FOR MEAN CELL VOLUME (MCV)

OF ALBINO RATS FED (110˚C,15MINUTES) ROASTED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	67.35	62.96	79.59
REPLICATE 2	65.31	66.04	76
REPLICATE 3	63.27	69.23	72.55
TOTAL	195.9	198.23	228.14
MEAN	**65.31**	**66.07666667**	**76.04666667**
TOTA SQUARED	38389	39295.1329	52047.8596

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	215.2646889	107.6323444	12.23889115
ERROR	6	52.76573333	8.794288889	
TOTAL	8	268.0304222		

G. total = 622.3

GT ² = 387257.29

Replicate*treatment = 9

Correction factor(CT) = 43028.5878

all sum of squares = 43296.6182

SS total = 268.030422

TOTAL SQUARED SUM = 129731.557

SS TREATMENT = 215.264689

SS ERROR = 52.7657333

ANALYSIS OF VARIANCE FOR MEAN CELL HAEMOGLOBIN(MCH)

OF ALBINO RATS FED (110˚C,15MINUTES) ROASTED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	21.84	21.85	26.53
REPLICATE 2	22.04	22.26	25.6
REPLICATE 3	22.24	22.69	24.71
TOTAL	66.12	66.8	76.84
MEAN	**22.04**	**22.26666667**	**25.61333333**
TOTA SQUARED	4372	4462.24	5904.3856

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	24.02026667	12.01013333	34.48985322
ERROR	6	2.089333333	0.348222222	
TOTAL	8	26.1096		

G. total	**=**	**209.76**
GT ²	**=**	**43999.2576**
Replicate*treatment	**=**	**9**
Correction factor(CT)	**=**	**4888.8064**
all sum of squares	**=**	**4914.916**
SS total	**=**	**26.1096**
TOTAL SQUARED SUM	**=**	**14738.48**
SS TREATMENT	**=**	**24.0202667**
SS ERROR	**=**	**2.08933333**

ANALYSIS OF VARIANCE FOR MEAN CELL HAEMOGLOBIN CONC. (MCHC)

OF ALBINO RATS FED (110˚C,15MINUTES) ROASTED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	33.44	34.71	33.33
REPLICATE 2	33.75	33.71	33.68
REPLICATE 3	34.06	32.71	34.03
TOTAL	101.3	101.13	101.04
MEAN	**33.75**	**33.71**	**33.68**
TOTA SQUARED	10252	10227.2769	10209.0816

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	0.0074	0.0037	0.009108813
ERROR	6	2.4372	0.4062	
TOTAL	8	2.4446		

G. total = 303.42

GT ² = 92063.6964

Replicate*treatment = 9

Correction factor(CT) = 10229.2996

all sum of squares = 10231.7442

SS total = 2.4446

TOTAL SQUARED SUM = 30687.921

SS TREATMENT = 0.0074

SS ERROR = 2.4372

ANALYSIS OF VARIANCE FOR PLATELETS COUNT

OF ALBINO RATS FED (110˚C,15MINUTES) ROASTED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	778.02	794	805.31
REPLICATE 2	780.12	798.17	806.09
REPLICATE 3	782.22	802.34	806.87
TOTAL	2340.36	2394.51	2418.27
MEAN	**780.12**	**798.17**	**806.09**
TOTA SQUARED	5477284.93	5733678.14	5848029.793

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	1062.9698	531.4849	71.15782357
ERROR	6	44.8146	7.4691	
TOTAL	8	1107.7844		

G. total = 7153.14

GT ² = 51167411.9

Replicate*treatment = 9

Correction factor(CT) = 5685267.98

all sum of squares = 5686375.77

SS total = 1107.7844

TOTAL SQUARED SUM = 17058992.9

SS TREATMENT = 1062.9698

SS ERROR = 44.8146

ANALYSIS OF VARIANCE FOR THE NEUTROPHILS

OF ALBINO RATS FED (110˚C,15MINUTES) ROASTED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	48	50	42
REPLICATE 2	47	51	40
REPLICATE 3	46	51	46
TOTAL	141	152	128
MEAN	**47**	**50.66666667**	**42.66666667**
TOTA SQUARED	19881	23104	16384

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	96.22222222	48.11111111	13.53125
ERROR	6	21.33333333	3.555555556	
TOTAL	8	117.5555556		

G. total = 421

GT ² = 177241

Replicate*treatment = 9

Correction factor(CT) = 19693.4444

all sum of squares = 19811

SS total = 117.555556

TOTAL SQUARED SUM = 59369

SS TREATMENT = 96.2222222

SS ERROR = 21.3333333

ANALYSIS OF VARIANCE FOR THE MONOCYTES

OF ALBINO RATS FED (110˚C,15MINUTES) ROASTED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	0	0	1
REPLICATE 2	0	0	1
REPLICATE 3	0	0	1
TOTAL	0	0	3
MEAN	**0**	**0**	**1**
TOTA SQUARED	0	0	9

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	2	1	#DIV/0!
ERROR	6	0	0	
TOTAL	8	2		

G. total = 3

GT ² = 9

Replicate*treatment = 9

Correction factor(CT) = 1

all sum of squares = 3

SS total = 2

TOTAL SQUARED SUM = 9

SS TREATMENT = 2

SS ERROR = 0

ANALYSIS OF VARIANCE FOR THE LYMPHOCYTES

OF ALBINO RATS FED (110˚C,15MINUTES) ROASTED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	50	50	58
REPLICATE 2	52	49	59
REPLICATE 3	51	49	53
TOTAL	153	148	170
MEAN	**51**	**49.33333333**	**56.66666667**
TOTA SQUARED	23409	21904	28900

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	88.66666667	44.33333333	11.4
ERROR	6	23.33333333	3.888888889	
TOTAL	8	112		

G. total	**=**	**471**
GT ²	**=**	**221841**
Replicate*treatment	**=**	**9**
Correction factor(CT)	**=**	**24649**
all sum of squares	**=**	**24761**
SS total	**=**	**112**
TOTAL SQUARED SUM	**=**	**74213**
SS TREATMENT	**=**	**88.6666667**
SS ERROR	**=**	**23.3333333**

ANALYSIS OF VARIANCE FOR THE EOSINOPHILS

OF ALBINO RATS FED (110˚C,15MINUTES) ROASTED SAMPLE

SAMPLE	**CONTROL**	**25:75 SMPLE-TO- FEED MIX**	**50:50 SMPLE-TO- FEED MIX**
REPLICATE 1	2	0	0
REPLICATE 2	2	0	0
REPLICATE 3	2	0	0
TOTAL	6	0	0
MEAN	**2**	**0**	**0**
TOTA SQUARED	36	0	0

G. total	=	**6**
GT ²	=	**36**
Replicate*treatment	=	**9**
Correction factor(CT)	=	**4**
all sum of squares	=	**12**
SS total	=	**8**
TOTAL SQUARED SUM	=	**36**
SS TREATMENT	=	**8**
SS ERROR	=	**0**

ANOVA TABLE

SOURCE OF VARIATION	**DF**	**SUM OF SQUARE**	**MEAN SQUARE**	**F - VALUE**
TREATMENT	2	8	4	#DIV/0!
ERROR	6	0	0	
TOTAL	8	8		

APPENDIX 7

LSD for haematological parameters of albino rats fed cooked *Livistona chinensis* seed sample.

WBC: LSD = $\frac{t\alpha}{2}\sqrt{\frac{2EMS}{r}} = \frac{1.943}{2}\sqrt{\frac{2 \text{ X } 0.006666667}{3}}$

$= 0.9715\sqrt{0.004444444667} = 0.064766668 \approx 0.06$

RBC: LSD = $0.9715\sqrt{\frac{2 \text{ X } 0.006666667}{3}}$

$= 0.064766668 \approx 0.06$

PCV: LSD = $0.9715\sqrt{\frac{2 \text{ X } 0.006666667}{3}}$

$= 0.9715 \text{ X } 0.666666666 = 0.64766666 \approx 0.65$

Hb : LSD = $0.9715\sqrt{\frac{2 \text{ X } 0.006666667}{3}}$

$= 0.9715 \text{ X } 0.6666666668 = 0.064766668 \approx 0.06$

MCV: LSD = $0.9715\sqrt{\frac{2 \text{ X } 3.433022222}{3}}$

= 0.9715 X 1.512838881 = 1.469722973 ≈ 1.47

MCH: LSD = $0.9715\sqrt{\frac{2 \text{ X } 0.345455556}{3}}$

= 0.9715 X 0.479899681 = 0.46622254 ≈ 0.47

MCHC: LSD = $0.9715\sqrt{\frac{2 \text{ X } 0.4428}{3}}$

= 0.5278 ≈ 0.53

Platelets: LSD = $0.9715\sqrt{\frac{2 \text{ X } 42.18523333}{3}}$

= 0.9715 X 5.303158388 = 5.152018374 ≈ 5.15

Neutrophils: LSD = $0.9715\sqrt{\frac{2 \text{ X } 1}{3}}$

= 0.9715 X 0.81649658 = 0.793226428 ≈ 0.79

Monocytes = (No significant difference)

LSD = 0.00

Lymphoytes: LSD = $0.9715\sqrt{\frac{2 \text{ X } 1}{3}}$

= 0.9715 X 0.81649658 = 0.793226428 ≈ 0.79

Eosinophils: No significant difference

LSD = 0.00

APPENDIX 8

LSD for haematological parameters of albino rats fed roasted *Livistona chinensis* seed sample.

WBC: LSD = $\frac{t\alpha}{2}\sqrt{\frac{2EMS}{r}} = \frac{1.943}{2}\sqrt{\frac{2 \text{ X } 0.006666667}{3}}$

$= 0.9715\sqrt{0.004444444667} = 0.064766668 \approx 0.06$

RBC: LSD = $0.9715\sqrt{\frac{2 \text{ X } 0.006666667}{3}}$

$= 0.064766668 \approx 0.06$

PCV: LSD = $0.9715\sqrt{\frac{2 \text{ X } 1}{3}}$

$= 0.9715 \text{ X } 0.81649658 = 0.793226428 \approx 0.79$

Hb: LSD = $0.9715\sqrt{\frac{2 \text{ X } 0.016666667}{3}}$

$= 0.9715 \text{ X } 0.011111111 = 0.010794444 \approx 0.01$

MCV: LSD = $0.9715\sqrt{\frac{2 \text{ X } 8.794288889}{3}}$

= 0.9715 X 2.42133419 = 2.3523261667 ≈ 2.35

MCH: LSD = $0.9715\sqrt{2 \text{ X } \frac{0.348222222}{3}}$

= 0.9715 X 0.481817546 = 0.468085746 ≈ 0.47

MCHC: LSD = $0.9715\sqrt{\frac{2 \text{ X } 0.4062}{3}}$

= 0.9715 X 0.5204 = 0.5056 ≈ 0.51

Platelets: LSD = $0.9715\sqrt{\frac{2 \text{ X } 7.4691}{3}}$

= 0.9715 X 2.231456923 = 2.167860401 ≈ 2.17

Neutrophils: LSD = $0.9715\sqrt{\frac{2 \text{ X } 3.555555556}{3}}$

= 0.9715 X 1.539600718 = 1.4957221 ≈ 1.50

Monocytes = (No significant difference)

LSD = 0.00

Lymphoytes: LSD = $0.9715 \sqrt{\frac{2 \text{ X } 3.888888889}{3}}$

= 0.9715 X 1.610152972 = 1.564263612 ≈ 1.56

Eosinophils = No Significant Difference

LSD = 0.00

ABBREVIATIONS USED

LSD = Least significant difference

WBC = White blood cell count

RBC = Red blood cell count

MCV = Mean corpuscular volume

MCH = Mean corpuscular haemoglobin

MCHC = Mean corpuscular haemoglobin concentration

Hb = Haemoglobin

PCV = Packed cell volume

EMS = Error mean square

r = Replicates

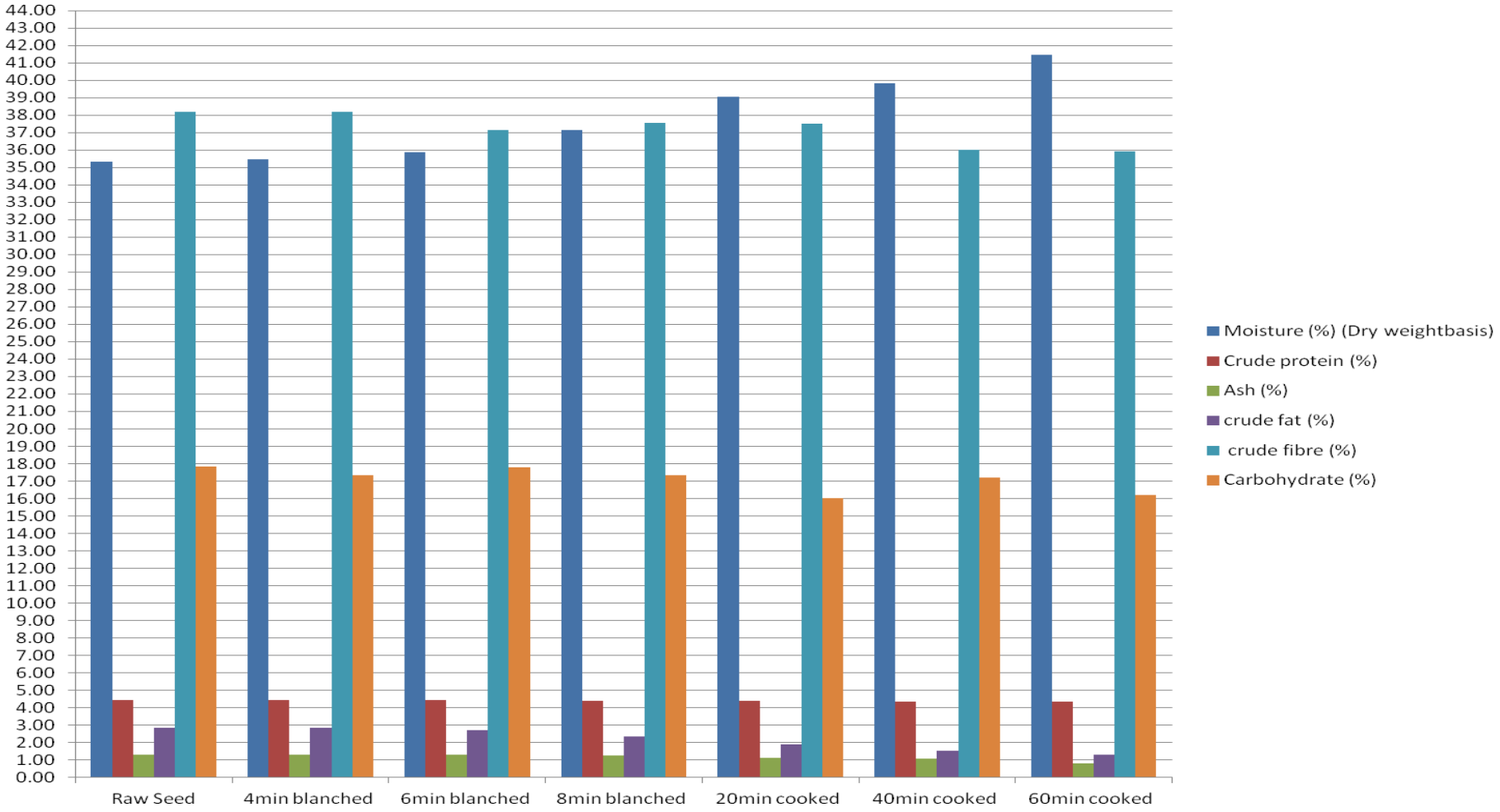

Figure 3: Effect of blanching and cooking on the proximate composition of *Livistona chinensis* seed.

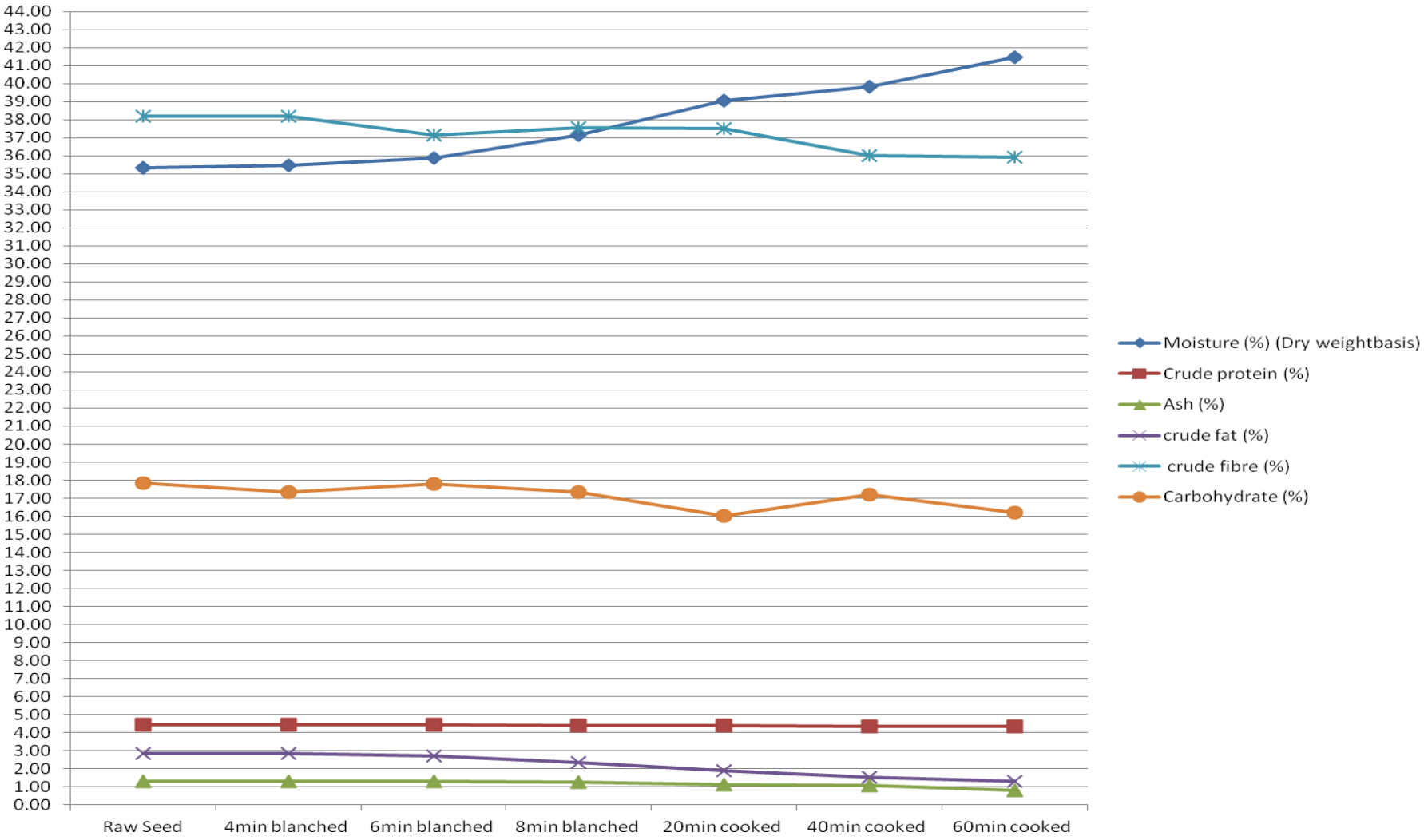

Figure 4: Effect of blanching and cooking on the proximate composition of *Livistona chinensis* seed.

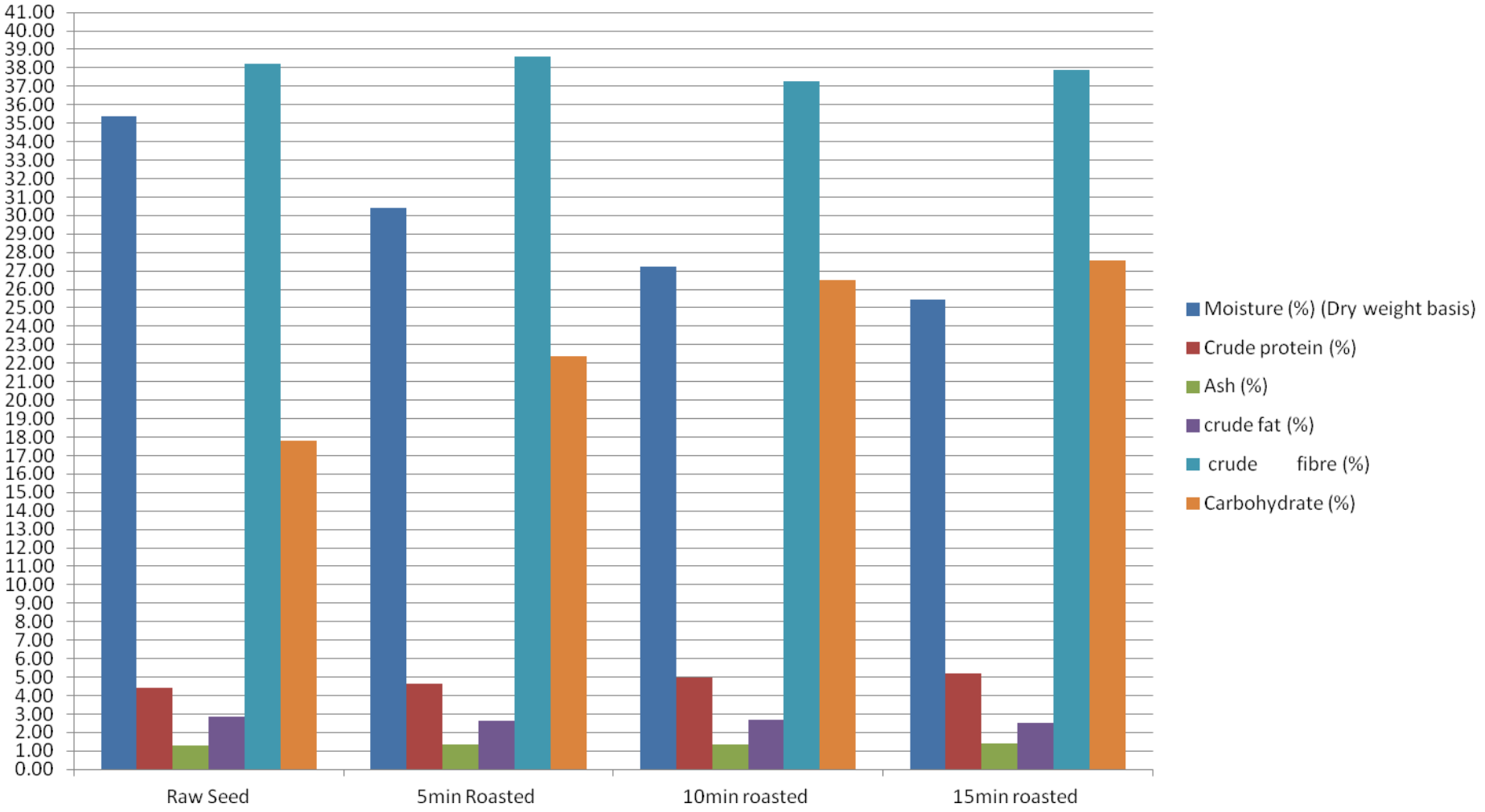

Figure 5: Effect of roasting on the proximate composition of *Livistona chinensis* seed.

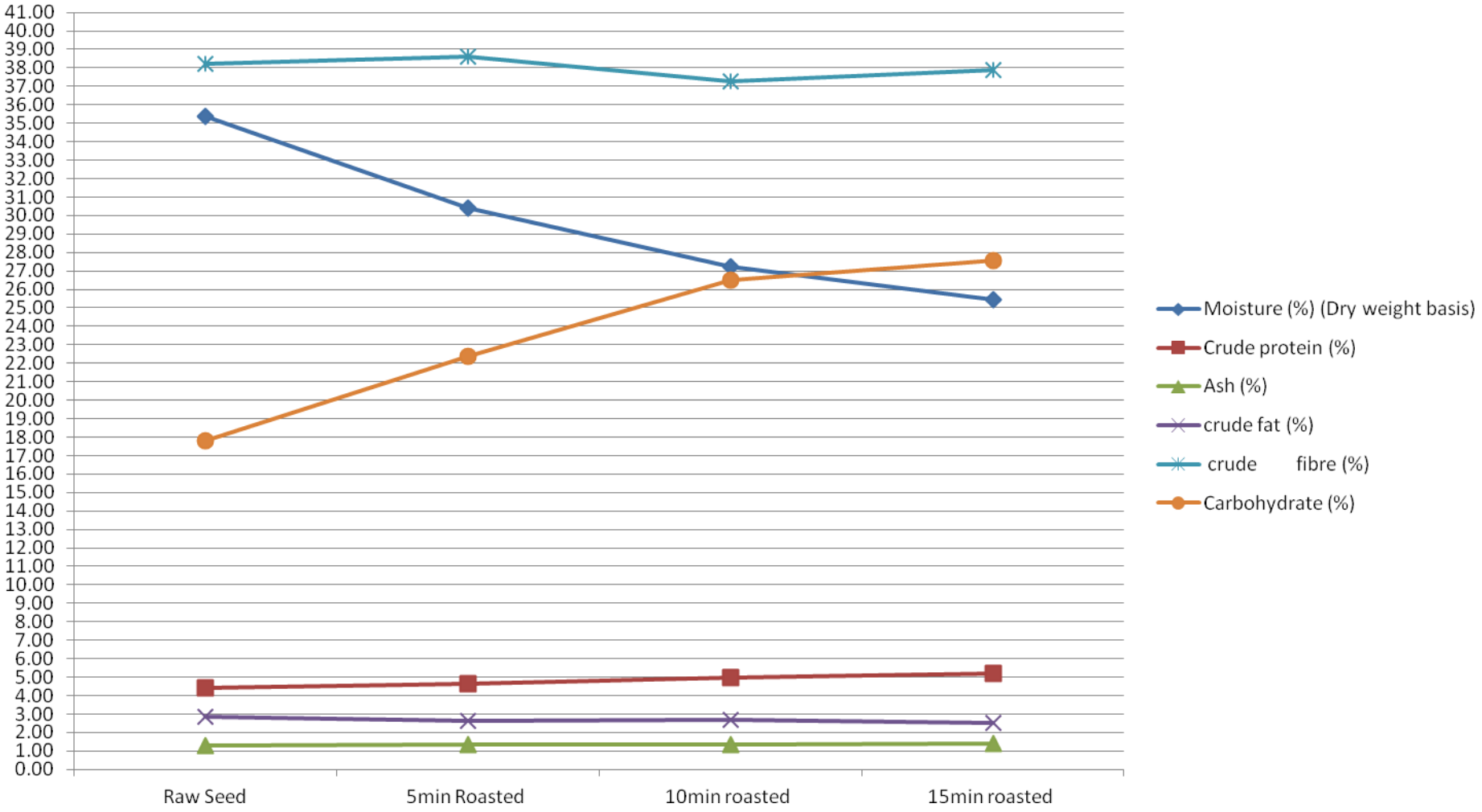

Figure 6: Effect of roasting on the proximate composition of *Livistona chinensis* seed.

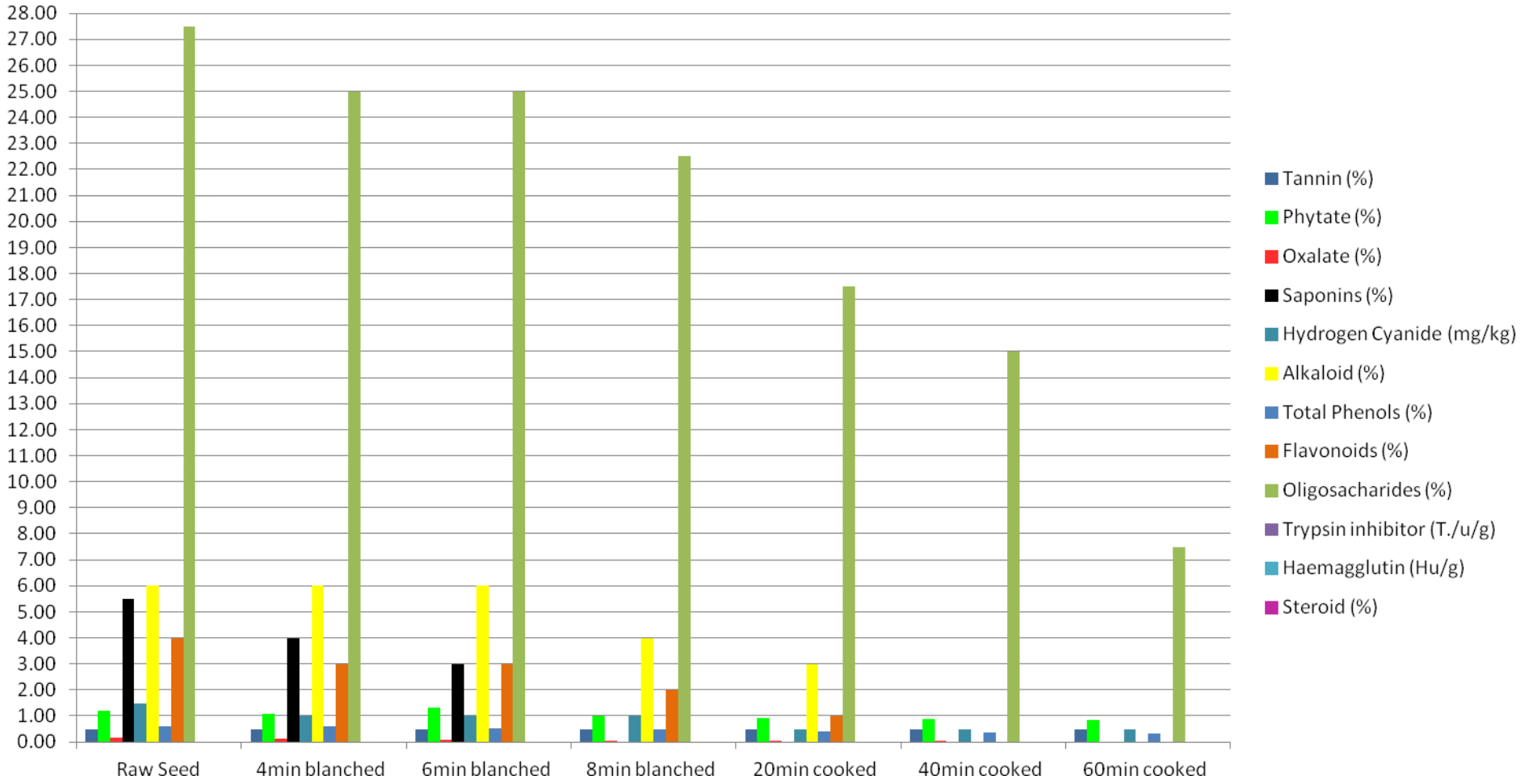

FIGURE 7: Effect of blanching and cooking on the anti-nutritional factors in *Livistona chinensis* seed.

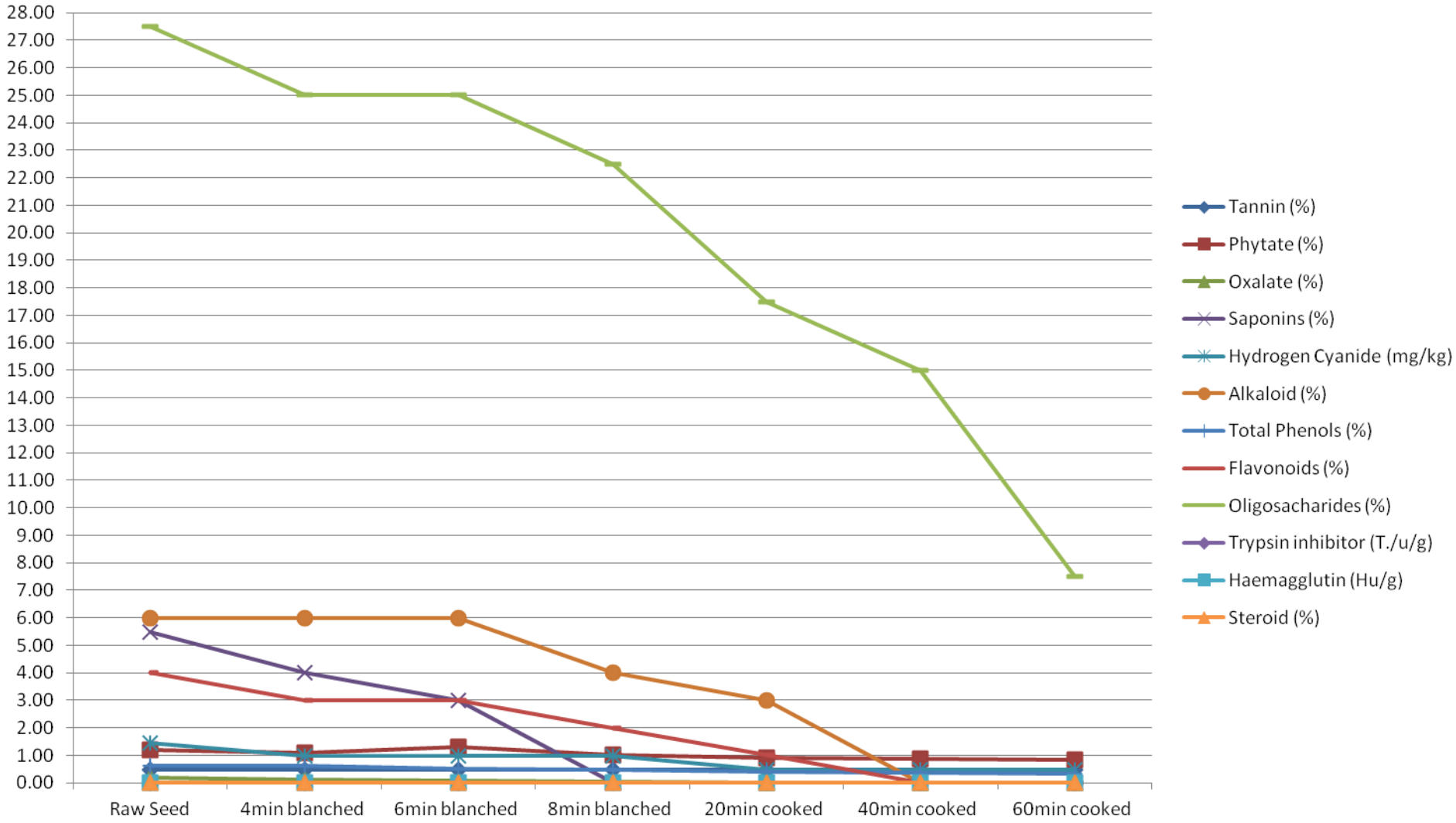

FIGURE 8: Effect of blanching and cooking on the anti-nutritional factors in *Livistona chinensis* seed.

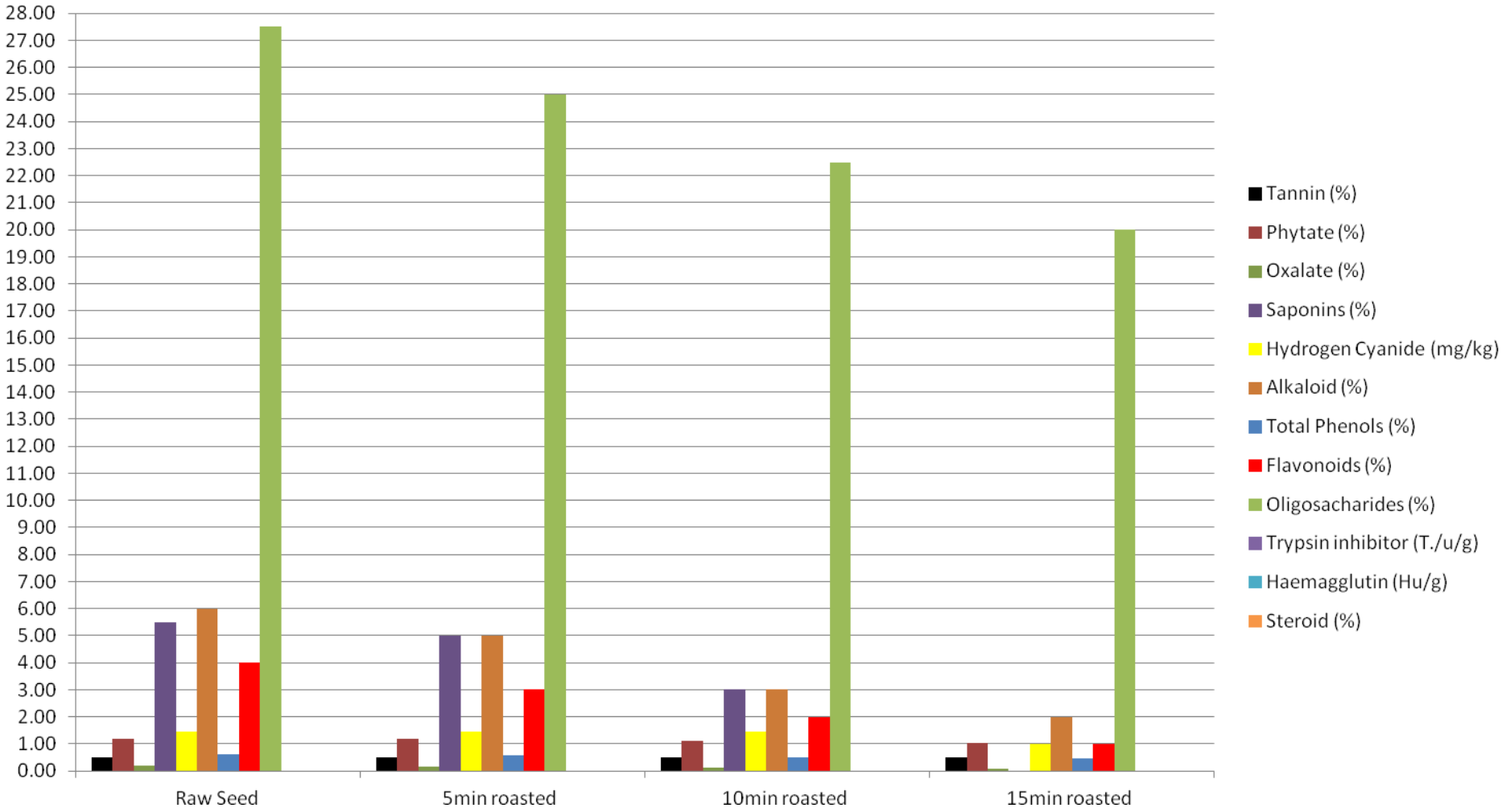

FIGURE 9: Effect of roasting on the anti-nutritional factors in *Livistona chinensis* seed.

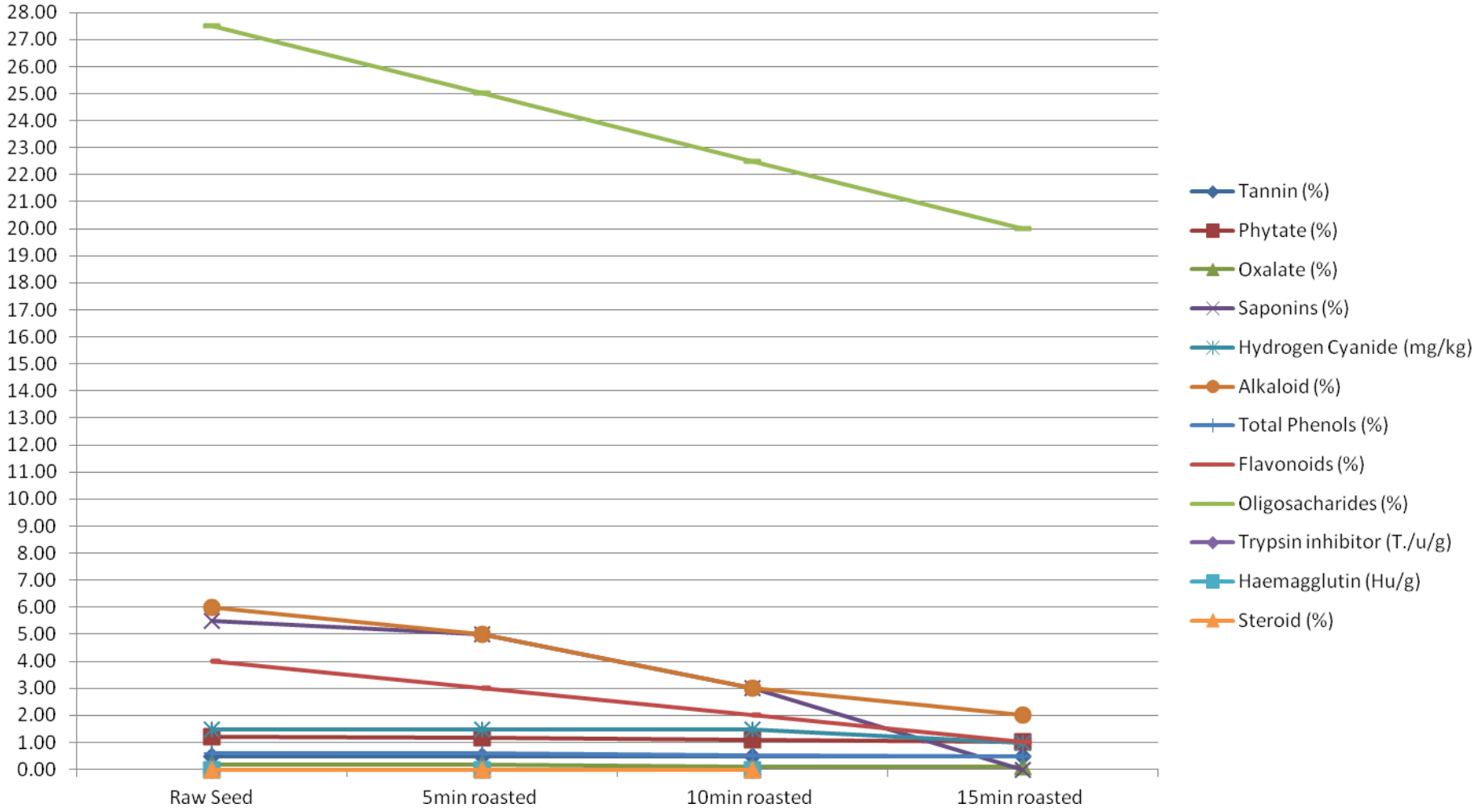

FIGURE 10: Effect of roasting on the anti-nutritional factors in *Livistona chinensis* seed.

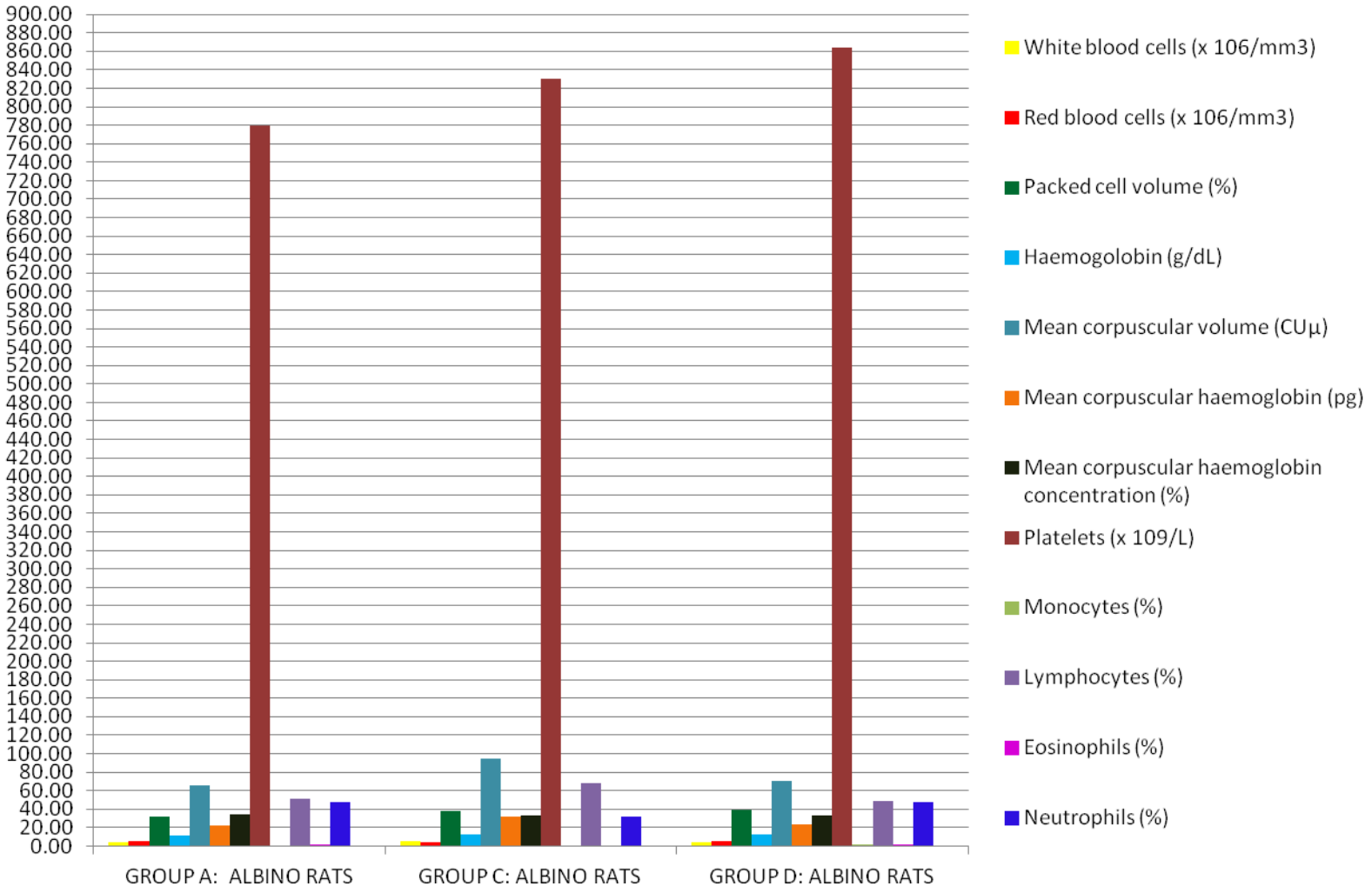

FIGURE 11: Effect of cooked (60 minutes) *L. chinensis* seed on the haematological parameters of albino rats.

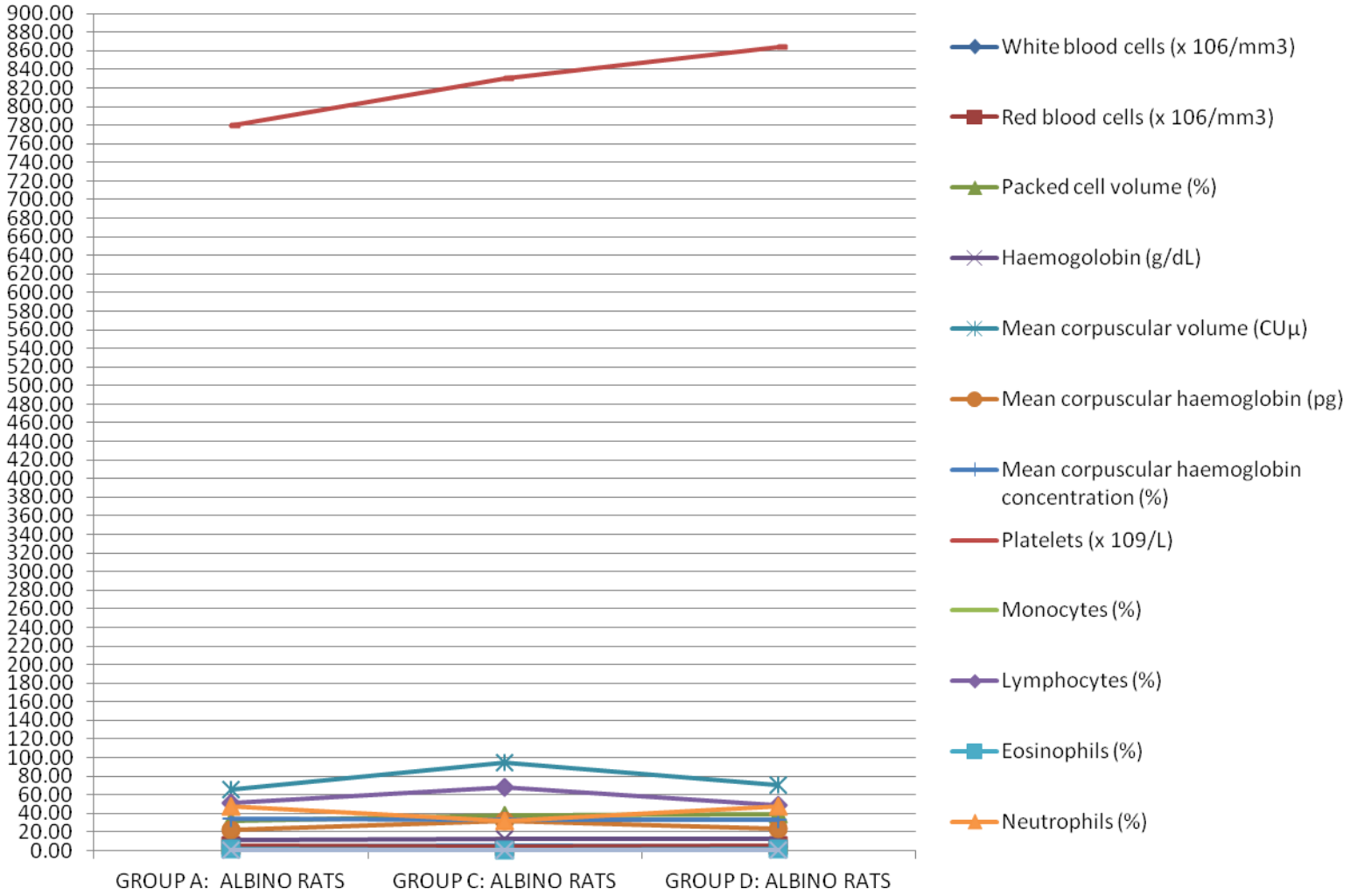

FIGURE 12: Effect of cooked (60 minutes) *L. chinensis* seed on the haematological parameters of albino rats.

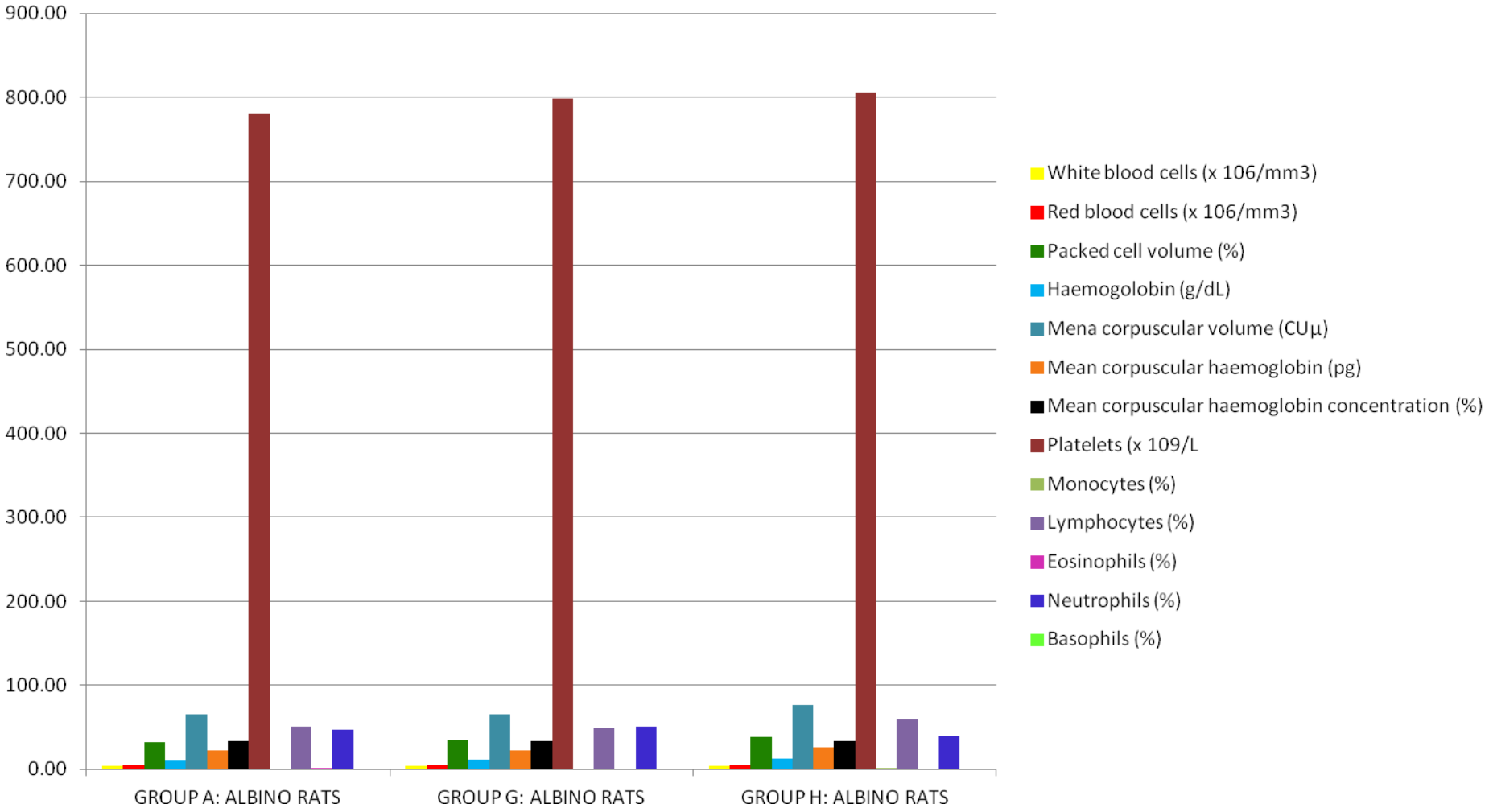

FIGURE 13: Effect of roasted (110^{0}C, 15 minutes) *L. chinensis* seed on the haematological parameters of albino rats.

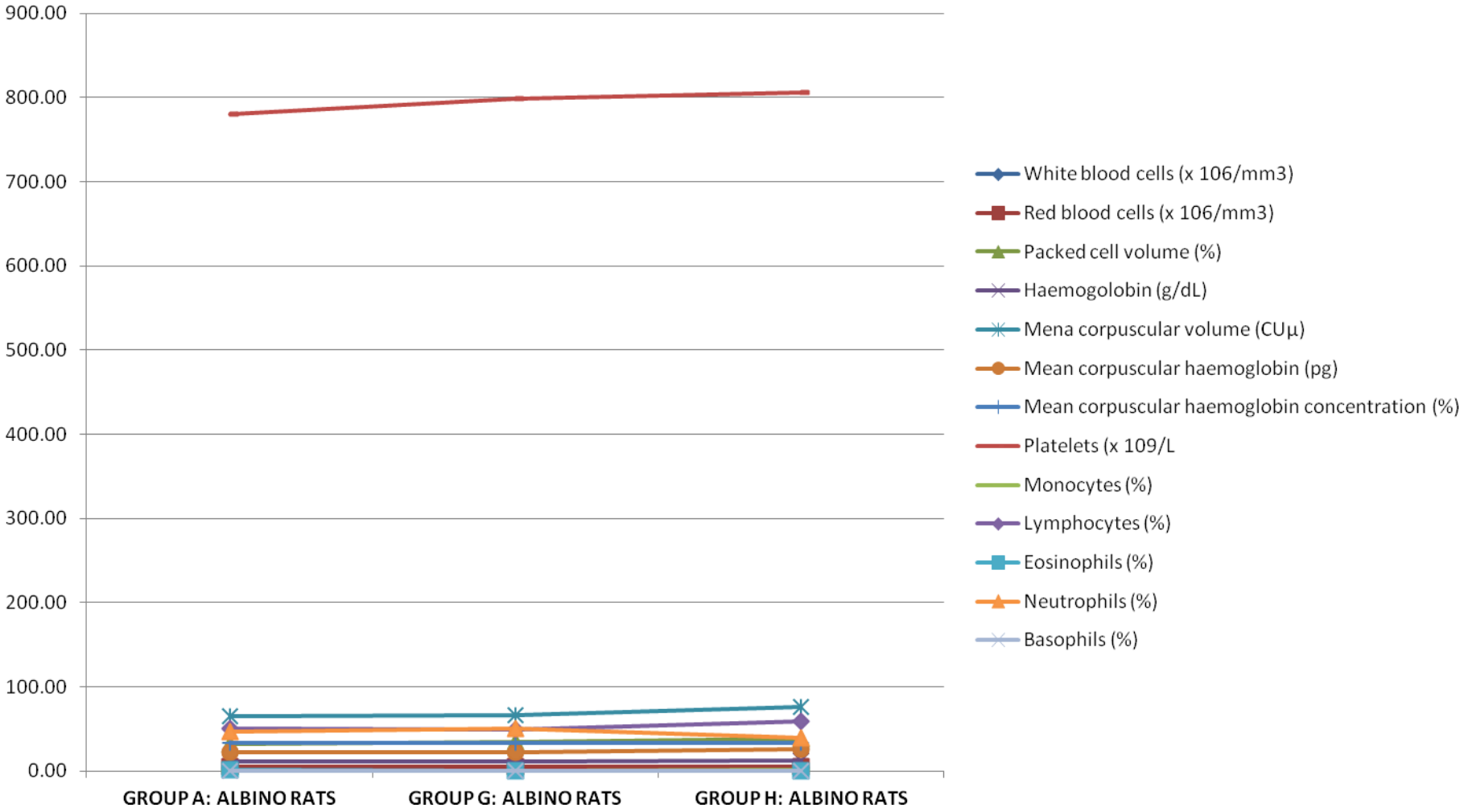

FIGURE 14: Effect of roasted (110^{0}C, 15 minutes) *L. chinensis* seed on the haematological parameters of albino rats.