International Journal of Food Engineering and Technology
2024, Vol. 8, No. 2, pp. 26-33
https://doi.org/10.11648/j.ijfet.20240802.12

Research Article

Toxicological Study on Albino Rat Fed Rich-Fibre Food from Orange (*Citrus sinensis* Linn.) Pomace, Soyameal and Wheat Bran

Abosede Oluwakemi Oduntan[1,*], Oluwafemi Babatunde Oduntan[2], Gibson Lucky Arueya[3]

[1]Product Development Programme, National Horticultural Research Institute, Ibadan, Nigeria
[2]Department of Aquaculture and Fisheries Management, University of Ibadan, Ibadan, Nigeria
[3]Department of Food Technology, University of Ibadan, Ibadan, Nigeria

Abstract

The concentration of phenolics and fibres in the orange pomace suggests that it could serve as an ingredient in novel food. lncrease in consumers demand for functional foods with natural origin like pomace is also on the increase. However, the possible toxicological effect has to be investigated. Orange pomace, wheat bran, and soyameal of different ratios were subjected to extrusion (5Kg per cycle) at fixed cooking temperature (110°C) and screw speed (290 rpm). The extrudates were tested on forty male *Wistar* rats for toxicity for 28 days. Data were analyzed using ANOVA at α0.05. Tested diets on *Wistar* rats resulted in lower weight gain which was at variance with the control diet. White blood cells (5283 – 6400 (x 10^9), creatine (0.63 – 0.70 mg/dL) and glucose (135.3 – 139.7 mg/dL) showed no significant difference between rats fed control and tested diets. Necrosis was not found in the kidney and liver of the rats fed with control and tested diets. The absence of toxic effects on the rat suggested that the food could be consumed for its health-promoting benefit in addition to eliminating environmental pollution by orange pomace. Extrusion of these raw materials yielded products which could contribute significant portion of fibre to human diet without adverse effect especially those that want to control their weight.

Keywords

Orange Pomace, Toxicity, Wistar Rat, Extrusion, Fibre

1. Introduction

Orange pomace is the waste that remains after processing oranges to juice, wine or other products. The wastes from this processing, including peel, seeds and pulp, which make up nearly 50% of unprocessed fruit, are a possible source of valuable byproducts [1]. Orange pulp is rich in fibre. The fibre has superior quality than others because of the occurrence of minerals, sugars, including bioactive substances like polyphenols, flavonoids and carotenes [2].

The phenolic compounds of citrus pomace could undergo enzymatic oxidation at various stages of processing. Dehy-

*Corresponding author: bosetunde12@yahoo.com (Abosede Oluwakemi Oduntan)

Received: 9 August 2024; **Accepted:** 2 September 2024; **Published:** 31 October 2024

dration under suitable conditions enables reduction in water present and water activity in the products. The prevention of oxidative enzymatic reaction and the development of micro-organisms prolong the storability of the products [3].

Several manufacturing plants produce the majority of food remains and many of which are thrown away or utilized at a low industrial and economic level [4]. Indeed, Food industry waste is a promising source of useful substances like dietary fibre, antioxidants, antimicrobial agents, essential fatty acids, and minerals that could be used due to the inherent favourable nutritional, technical and purposeful properties. These food by-products include orange pomace, wheat bran and soyameal and have been identified as excellent origin of dietary fibre [2, 5].

Wheat bran has a dense dietary fibre source with several health gains include decrease in danger of cardiovascular disease and type 2 diabetes, maintaining the normal glucose and cholesterol level [5]. Solta Civelek S noted that inclusion of wheat bran in an amount of 5 to 25% by weight of flour in the formulation of pasta extruded with a single screw extrusion plate improved the sensory sensitivity. [6]

Soyameal is associated with some useful outcome on human welfare such, averting obesity, providing nutrition, reducing blood cholesterol and also plays an important part in the disease prevention [7].

Previous work has involved the use of pomace in the manufacture of several intermediates which include the production of compounds and solvents in flavours and colognes, as components in paints, cosmetics and animal supplements. [8] reported that incorporation of orange pomace up to 10% in cookies preparation increased nutritional value, especially in fibre, physical quality and general acceptance of biscuits. Our previous study [9] that developed and evaluated the extruded food from orange pomace, soyameal and wheat bran came up with the with the 3 best formulations adjudged in sensory out of 13 formulations used in the study, hence this further study to evaluate the possible toxicity of the food to ascertain the safety of the developed food.

2. Materials and Methods

2.1. Sample Preparation

Orange pomace was obtained after juice production from sweet oranges (Agege variety) at the juice processing pilot plant of the National Horticultural Research Institute, Jericho, Ibadan, Oyo State. The pomace was dried at 60 ± 2°C, ground with a laboratory mill (500 µm) and kept in an airtight 250 micron polyethylene until use. Soyameal and Wheat bran was purchased from an Agro processing supplier, Oluyole Estate, Ring Road, Ibadan, individually ground to 500 µm and packed for further use.

2.1.1. Extrusion Cooking

Extrusion cooking was performed according to the modified [10] method. Mixtures of wheat bran, soybean meal and orange pomace with different mixing ratios (Table 1). These were the adjudged best in our previous study [9].

Table 1. *Percentage Formulations of orange pomace, soyameal and wheat bran extruded.*

Sample	Orange pomace	Soyameal	Wheat bran
P2	17	44	39
P3	5	80	15
P4	10	80	10

2.1.2. Animal Requirements

Ethical consent to animals was obtained from ACUREC (UI-ACUREC/App/2016/027), University of Ibadan before study. Wistar albino rat (Rattus norvegicus) of approximate weight of 70- 80 g was used. The animals were obtained from the Department of Veterinary Pathology, University of Ibadan. The venue of the experiment was the Department of Animal Sciences, University of Ibadan. Forty (40) animals were used for the study.

2.2. Animal Treatment and Organ Examination

After one week of acclimatization, the 40 rats were separated into four groups as follows: the first group which was control received basal diet while the other three with different ratio of orange pomace, wheat bran and soyameal (17: 39:44, 5:15:80, 10:10:80)% were fed for 3 weeks. The experimental animals were observed closely for up to 21 days, thereafter biological and hematological tests were carried out.

2.2.1. Nutritional Evaluation on Rats Fed with the Tested Blends

During the experimental period (3 weeks), the consumed diets were recorded every day, and body weights were recorded every week. Nutritional evaluation of various diets was performed by determining body weight gain% (BWG%), feed efficiency ratio (FER), feed conversion ratio (FCR) according to equations described by [11].

2.2.2. Hematological Analysis

About 2 ml of blood samples was taken with heparinized capillary tubes to determine the hematocrit value according to [12]) method. Total red and white blood cells, Hemoglobin strength, mean corpuscular volume, mean corpuscular hemoglobin concentration and mean corpuscular hemoglobin concentration were estimated by the method reported [13].

2.2.3. Biochemical Analysis

About 2 ml of blood was collected and allowed to coagulate in pure dry centrifuge tubes at 3500 rpm for 15 minutes. A section of the pure supernatant serum was taken instantly to determine glucose in accordance to the enzymatic colorimetric method [14]. The actions of alanine aminotransferase (ALT) serum aspartate aminotransferase (AST), and serum alkaline phosphatase (ALP) were estimated [15]. Total cholesterol and total serum lipids were tested following method of [16]. Serum total protein and albumin levels were evaluated by the procedure of [17]. Serum globulin was calculated by [18]. Serum urea and creatinine were determined by the methods reported by [19].

2.2.4. Histopathological Analysis

At the end of the study, cervical dislocation was used to sacrifice the rats for histopathological examination to help identify toxic effects on target organs (liver and kidney) according to methods of [20]. A veterinary pathologist read the result of the Histopathology, biochemical and hematological analysis.

2.3. Method of Euthanasia/Disposition of Animals

At the end of the study, animals certified fit were donated to the Zoological garden of the University of Ibadan.

3. Result and Discussion

3.1. Feed Efficiency/Weight Gain

At the expiration of the experiment, weight gain and food consumed by the rats fed on the experimental diets were lower than the control (Figure 1). There were no significant differences in food intake between control and tested diets, but weight gain was significantly different. Rat fed with food blend of 17% pomace, 44% soyameal and 39% wheat bran had the highest weight gain while those fed with 5 % pomace, 80% soyameal and 15% wheat bran had lowest weight gain. Lower weight gain from the tested food suggests that the food could be considered to make a difference in the onset and progression of morbidity and obesity. This is in line with findings of [21] that fibre is able to reduce body weight gain or decrease weight gain. This has been attributed to various factors such as soluble fibre, which produces glucagon-like peptide (GLP-1) and peptide TY when fermented in the colon. The two intestinal hormones take part in inducing satiety, decrease energy intake and also decrease metabolizable energy.

Rat fed with control diet were able to digest the diet better than the other tested diets which resulted in increased digestion and absorption of nutrients. Soluble dietary fibre in form of neutral detergent fibre was more in the control diet than the tested diets leading to more absorbed nutrients and weight gain. The same trend was observed in the Feed efficiency ratio and conversion ratio (Figure 2 and 3). The result was comparable with the findings of [22] of lowered nutritional parameters as a result of high fibre feed fed to rat. The trend could be due to the distribution of soluble and insoluble fibre fraction; hemicellulose, cellulose and lignin present in the tested and control diets.

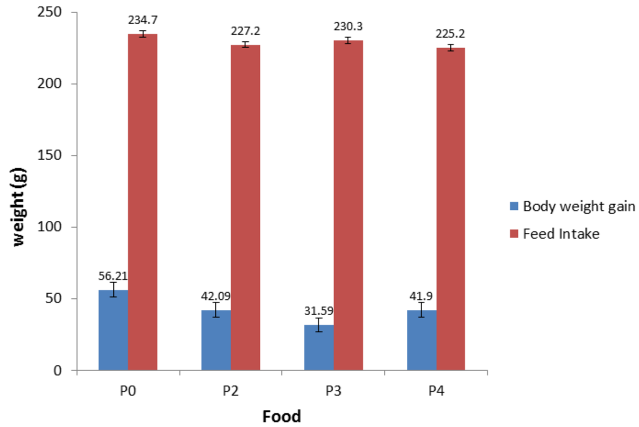


Figure 1. Food intake and Body weight gain by rats fed with control and tested diets.

P0 = control, P2 = 17 % pomace 44% soyameal 39 % wheat bran, P3 = 5 % pomace 80% soyameal 15 % wheat bran, P4 = 10 % pomace 80 % soyameal 10 % wheat bran

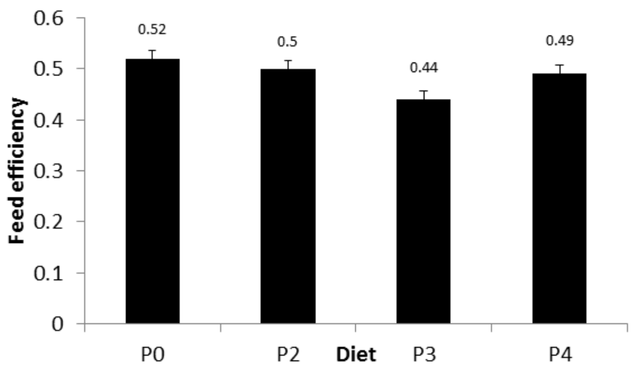


Figure 2. Feed efficiency of control and tested diets.

P0 = control, P2 = 17 % pomace 44% soyameal 39 % wheat bran, P3 = 5 % pomace 80% soyameal 15 % wheat bran, P4 = 10 % pomace 80 % soyameal 10 % wheat bran

3.2. Hematological Parameters

The result of the hematological parameters shows that Packed Cell Volume (PCV) count to be from 35.67 to 37.33% (Table 2) and was within clinical range for rat. The highest PCV was obtained for diet P2 followed by diet P3. No significant difference was observed between the control

(P0) and the tested diets. The PCV known as hematocrit (Ht) or (HCT) or erythrocyte volume fraction (EVF) is the percentage (%) of red blood cells in the blood. According to [23] PCV is involved in the transportation of oxygen and absorbed nutrients. Increased PCV shows better transport and therefore increases the primary and secondary polycythemia.

A higher PCV value for rats fed with the study diet shows that diets are suitable for transporting oxygen and nutrients. The hemoglobin (HGB) value of the albino rat fed the diet P2 (12.63) was the highest, followed by P3, P0 and finally P4. No significant variation (p >0.05) was observed among HGB of rat fed with the food. The result suggests that hemoglobin is sufficient for the albino rats for its physiological function of transporting oxygen to the animal tissues to oxidize red blood cells (RBC). Rats fed control and experimental feed (P0, P2, P3 and P4) were not significantly different. Diet P2 had the highest mean RBC count (6.00 x 1012) while P4 had the least (5.66 x 1012). These values are in the laboratory range for rat [24] and suggested no toxic effect of experimental diet on the rat.

Table 2. Hematological variables of rat fed control and experimental diets.

Diet	PCV (%)	HGB (g/dl)	RBC (x 10^{12})	WBC (x 10^9)	Plt	Lym	Neu	Mo	Eos
P0	36.33^a	12.07^a	5.95^a	5283^a	171667^a	65.33^a	30.33^a	1.67^a	2.67^a
P2	37.33^a	12.63^a	6.00^a	5950^a	191000^a	66.33^a	31.00^a	1.33^a	1.33^a
P3	37.00^a	12.33^a	5.96^a	6400^a	156000^a	70.33^a	25.33^a	2.00^a	2.33^a
P4	35.67^a	11.97^a	5.66^a	6100^a	133333^a	66.67^a	30.67^a	1.33^a	1.33^a

Value are mean of three replicates followed by different letters in the same column are significantly different.
PCV – packed cell volume, HGB – Heamoglobin, RBC –red blood cell, WBC – white blood cell, plt – platelet, lym - Lymphocyte, Neu – Neutrophils, Mo -Monocycte, Eos –Eosinophils.
P0 = control,
P2 = 17 % pomace 44% soyameal 39 % wheat bran,
P3 = 5 % pomace 80% soyameal 15 % wheat bran,
P4 = 10 % pomace 80 % soyameal 10 % wheat bran.

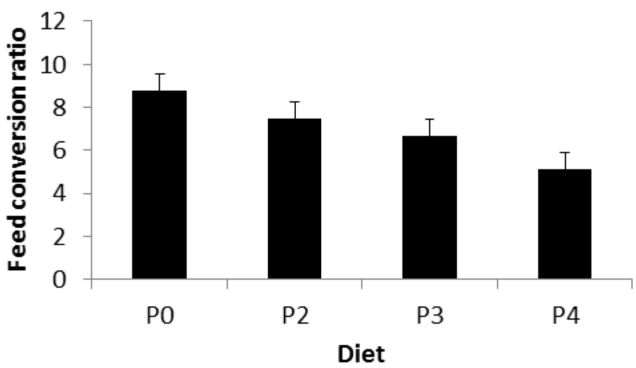


Figure 3. Feed conversion ratio of control and tested diets.

Red blood cells are used for transportation of oxygen and carbon dioxide in the [23] hence, low red blood cell count signifies a decrease in the level of oxygen transported to the tissues and carbon dioxide returned to the lungs [25].

Highest white blood cell (WBC) was recorded for rats fed with diet P3 (6400 x 10 $^{9)}$ which was not significantly different (p > 0.05) from other diets. The main roles of white blood cells are defending the body by phagocytosis against attack by foreign organisms, combating infections, production, transportation and distribution of antibodies in the immune response. The result of the study is within the clinical range suggesting that the albino rats were able to generate antibodies in the course of phagocytosis with high degree of resistance to diseases [25]. It will also encourage their compliance to natural ecology and disease dominant conditions as supported by [26].

Platelet count for rat fed with the diets was between 133333 and 191000, the highest from diet P2 while the lowest was from diet P4. There was no significant variation observed among the control and the test diets (p > 0.05) The difference in the level could be as a result of variation in the type (dose, structure, soluble, insoluble) of dietary fibre in the diets [22]. Table 3 also corroborates the findings showing difference in the Dietary fibre fraction. Platelets are involved in blood clotting, the platelet concentration was within the clinical range, suggesting that the process of clot formation (blood clotting) was quick in the event of an injury in the control diet and on tested diets. Lymphocyte value for the control and test diets were from 65.33 % to 70.33 %. The highest from Diet P3 and lowest from diet P0. No significant variation (p > 0.05) obtained from the diets.

Maximum neutrophils were recorded for the blood samples of rats fed P2 (31%), followed by diet P4 (30.67%) while diet P3 had the least (25.33%). No significant variation ($p > 0.05$) was observed among the diets. The result shows no toxic effect on the blood samples of rat fed the experimental diets.

Table 3. Fibre fraction of control and experimental diets fed to rat.

Diet	%NDF	%ADF	%ADL	%HEMI-CELLULOSE	% CELLULOSE	% LIGNIN
P0	62.19	33.70	15.90	28.49	17.80	15.90
P2	39.67	18.85	2.76	20.82	16.09	2.76
P3	46.28	29.61	5.89	16.67	23.72	5.89
P4	41.63	26.48	3.36	15.15	23.12	3.36

Results are average of three replicates
NDF – Neutral detergent fibre
ADF – Acid detergent fibre
ADL – Acid detergent lignin
P0 = control,
P2 = 17 % pomace 44% soyameal 39 % wheat bran,
P3 = 5 % pomace 80% soyameal 15 % wheat bran,
P4 = 10 % pomace 80 % soyameal 10 % wheat bran.

3.3. Biochemical Parameters

The estimates of biochemical indices of liver and kidney in the study were summarized in Table 4. No significant variation observed ($p > 0.05$) in AST, ALT, ALP, BUN and creatinine of rat fed with control and tested diets. The low value of AST for the tested diets indicated proper functioning of the liver. All serum biochemical parameters were normal except AST which is slightly lower and are in the control range for rats. Reference ranges for AST, ALP, ALT, BUN and creatinine are between 50 and 150, 30 and 130 IU / l, 10 and 40, 12.0 and 25.8 and 0.4 and 2.3 mg / dL, respectively [11]. These suggest that the tested diets had no toxic effect on the liver and kidney of the albino rats. Livers and kidneys are internal organs that have different functions. An important role of these organs is the disposal of waste products and toxic substances. The dysfunction of these organs can lead to biochemical substances entering the bloodstream [23]. The effect of the diet on serum total protein was highest for the rat fed with diet P3 (7.83 g/dl) while lowest was found for diet P4 (Table 5), no significant variation ($p > 0.05$) was found among the control and tested diets. Albumin was between 2.60 and 3.10 for the diets, P3 also had the highest while the lowest was for diet P4. Globulin was highest with diets P2 and P3 (4.73), the lowest for P4 with a value of 4.47. However, Albumin/Globin ratio (A/G) was highest (0.67) for the diet P0 (control) followed by P3 (0.66) with the lowest 0.58 for diet P4. No significant variation ($p> 0.05$) was observed among the diets.

Triglyceride (TG) ranges from 46.30 – 55.0 mg/dL (Table 6), total cholesterol (TC) was from 59.30 to 70.0 mg/dL. Highest value was observed for diet P4 while P0 (control) had the lowest value. High density lipoprotein (HDL), low density lipoprotein (LDL) and glucose (Glu) were highest for diet P4, while lowest value was observed for P0 (HDL and LDL) and P3 for glucose. No significant variation ($p > 0.05$) was observed among the values. This indicated that the diet had no toxic effects on the rats used in this study. It can therefore be concluded that the formulated food is safe for human consumption.

Table 4. Effect of diet on biochemical parameters of rat fed with control and tested diets.

Diet	AST (U/L)	ALT (U/L)	ALP (U/L)	BUN (mg/dL)	CRE (mg/dL)
P0	44.33^a	32.00^a	116.0^a	16.53^a	0.70^a
P2	44.33^a	30.67^a	117.0^a	16.70^{ab}	0.70^a
P3	42.67^a	31.67^a	117.3^a	17.70^a	0.70^a
P4	41.67^a	30.00^a	114.0^a	16.70^{ab}	0.63^a

Averages of three replicates followed by different letters in the same column are significantly different
AST – Aspartate aminotransferase, ALT – Alanine aminotransferase, ALP – Alkaline phosphatase, BUN – Blood urea nitrogen, CRE - Creatinine
P0 = control,
P2 = 17 % pomace 44% soyameal 39 % wheat bran,
P3 = 5 % pomace 80% soyameal 15 % wheat bran,
P4 = 10 % pomace 80 % soyameal 10 % wheat bran

Table 5. *Effect of diet on the serum total protein, albumin, globulin and AG ratio on rat fed with control and tested diet.*

Diet	T.PROT (g/dl)	ALBUL (g/dl)	GLOB	AG.r
P0	7.50^{a}	3.03^{a}	4.53^{a}	0.67^{a}
P2	7.60^{a}	2.87^{a}	4.73^{a}	0.61^{a}
P3	7.83^{a}	3.10^{a}	4.73^{a}	0.66^{a}
P4	7.07^{a}	2.60^{a}	4.47^{a}	0.58^{a}

Averages of three replicates followed by different letters in the same column are significantly
T.PROT – Total protein, ALBUL – Albumin, GLOB – Globulin, AG.r – Albumin globulin ratio
P0 = control, P2 = 17 % pomace 44% soyameal 39 % wheat bran, P3 = 5 % pomace 80% soyameal 15 % wheat bran, P4 = 10 % pomace 80 % soyameal 10 % wheat bran

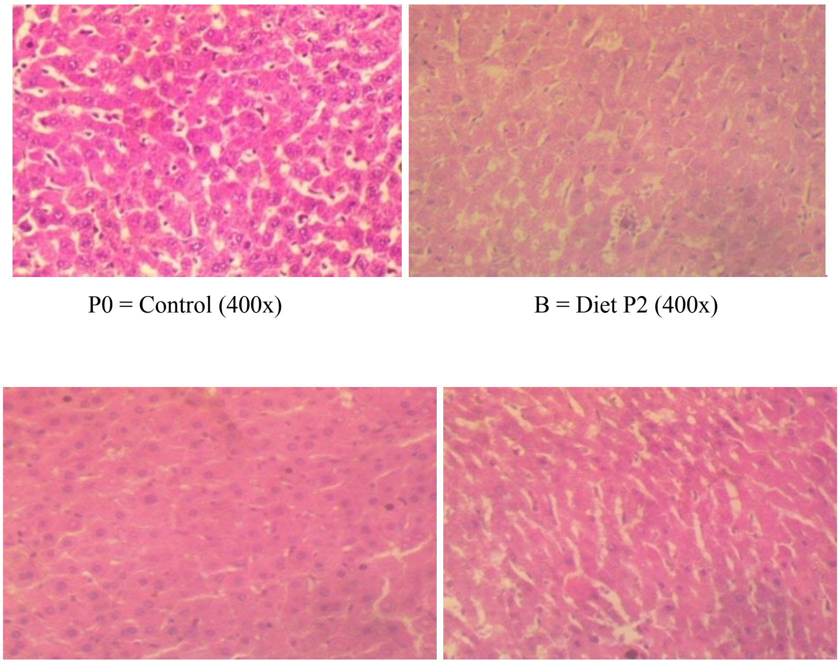
Table 6. *Effect of diet on serum lipids and glucose of rat fed with control and tested diets.*

Diet	TRIG (mg/dL)	CHOL (mg/dL)	HDL (mg/dL)	LDL (mg/dL)	GLU (mg/dL)
P0	46.7a	59.3a	30.0a	20.0a	137.0a
P2	47.0a	64.7a	31.3a	23.9a	135.7a
P3	46.3a	67.7a	35.0a	23.4a	133.3a
P4	55.0a	70.0a	35.0a	24.0a	139.7a

Averages of three replicates followed by different letters in the same column are significantly
TRIG = Triglyceride, CHOL – Total cholesterol, HDL – High density lipoprotein, LDL – Low density lipoprotein, GLU - Glucose
P0 = control,
P2 = 17 % pomace 44% soyameal 39 % wheat bran,
P3 = 5 % pomace 80% soyameal 15 % wheat bran,
P4 = 10 % pomace 80 % soyameal 10 % wheat bran

3.4. Histopathology

On sections of liver tissue in the treated groups there were no visible injuries that could be caused by feed toxicity. (Figure 4). Sections of the kidney of the rat fed on the tested diets show no toxicity effect (Figure 5) on the control and the tested diets. The liver and kidney status of the rats fed on control and tested diets did not differ significantly, which shows no toxicity due to the feed.

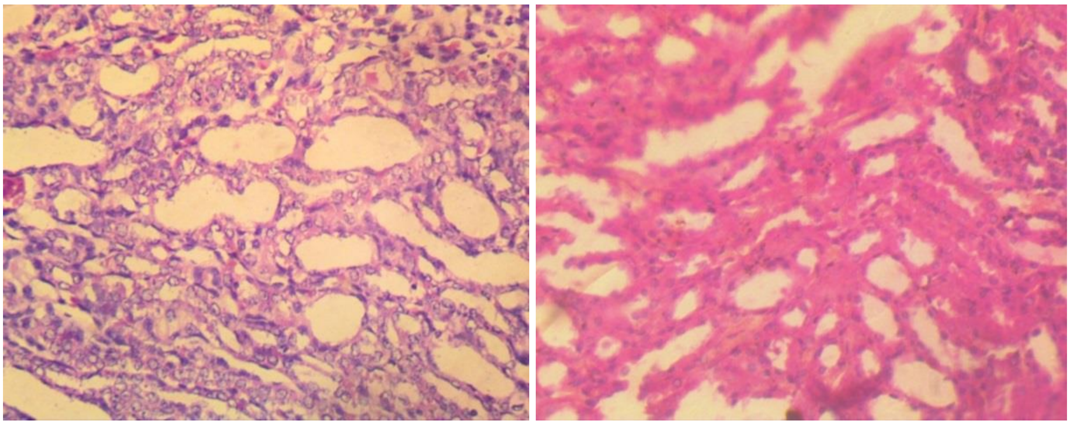


P0 = Control (400x) B = Diet P2 (400x)

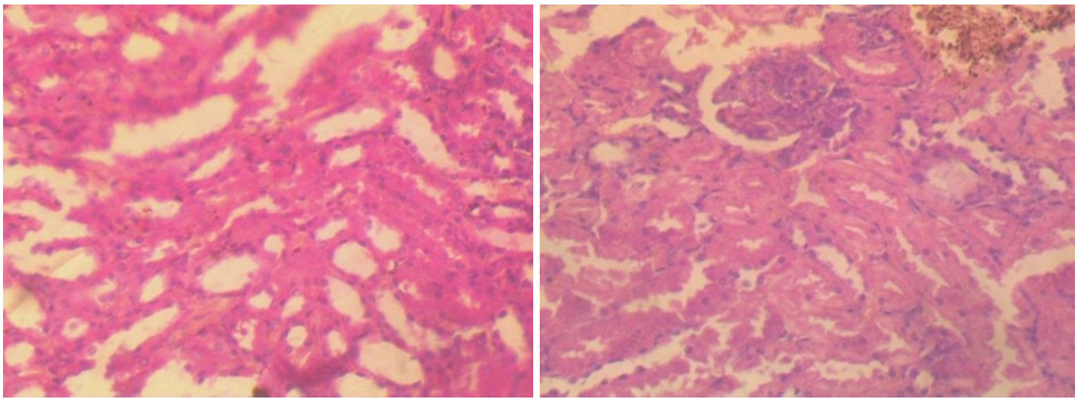
C = Diet P3 (400x) D = Diet P4 (400x)

Figure 4. *Photomicrographs of liver sections of rats fed with the control and tested diets.*

P0 = control, P2 = 17 % pomace 44% soyameal 39 % wheat bran, P3 = 5 % pomace 80% soyameal 15 % wheat bran, P4 = 10 % pomace 80 % soyameal 10 % wheat bran



A= Diet P0 (Control) (x 400) B = Diet P2 (x400)



C = Diet P3 (x400) D = Diet P4 (x400)

***Figure 5.** Photomicrograph of kidney sections of the rat fed with the control and tested diets.*

P1 = control, P2 = 17 % pomace 44% soyameal 39 % wheat bran, P3 = 5 % pomace 80% soyameal 15 % wheat bran, P4 = 10 % pomace 80 % soyameal 10 % wheat bran

4. Conclusion

White blood cells, ceratine and glucose showed no significant difference between rats fed control and tested diets. Necrosis was not found in the kidney and liver of the rats fed with control and tested diets. The absence of toxic effect on the rat suggested that the food could be consumed for its health promoting benefit in addition to eliminating environmental pollution by orange pomace. Extrusion of these raw materials yielded products which could contribute significant portion of fibre to human diet without adverse effect especially those that want to control their weight. The study revealed the possibility of adoption of the ingredients in the production of novel food for food security.

Abbreviations

FER	Feed Efficiency Ratio
FCR	Feed Conversion Ratio
BWG	Body Weight Gain
ALT	Alanine Aminotransferase
AST	Serum Aspartate Aminotransferase
ALP	Serum Alkaline Phosphatase
PCV	Packed Cell Volume
EVF	Erythrocyte Volume Fraction
HGB	Hemoglobin
RBC	Red Blood Cell
WBC	White Blood Cell
TG	Triglycerides
TC	Total Cholesterol
HDL	High Density Lipoprotein
LDL	Low Density Lipoprotein
BUN	Blood Urea Nitrogen
CRE	Creatinine

Conflicts of Interest

The authors declare no conflicts of interest.

References

[1] Gowe, C. 2015. Review on potential use of fruit and vegetables by-products as a valuable source of natural food additives. *Food Science and Quality Management*; *45*: 47-61.

[2] Fernandez-Gines, J. M., Fernadez-Lopez, J., Sayas-Barbera, E., & Perez-Alvarez, J. A. 2003. Effects of storage conditions on quality characteristics of bologna sausages made with citrus fibre. *Journal of Food Science*; 68(2): 710–715.

[3] Mhiri, N., Ioannou, I., & Ghoul, M. Mihoubi boudhrioua N. 2015. Proximate chemical composition of orange peel and variation of phenols and antioxidant activity during convective air drying. *Journal of New Science, Agriculture and Biotechnology, JS-INAT; (9)*: 881-890.

[4] ElMekawy A, Srikanth S, Bajracharya S, Hegab HM, Nigam PS, Singh A, Mohan SV, Pant D. 2015 Jul 1. Food and agricultural wastes as substrates for bioelectrochemical system (BES): the synchronized recovery of sustainable energy and waste treatment. Food Research International.; 73: 213-25.

[5] Topping, D. 2007. Cereal complex carbohydrates and their contribution to human health. *Journal of Cereal Science*, 46, 220– 229.

[6] Solta Civelek S. 2019. *Effects of fiber content and extrusion conditions on quality of pasta*. (Master's thesis, Middle East Technical University).

[7] Mateos-Aparicio, I., Cuenca, A. R., Villanueva –Suarez, M. J., Zapata-Revilla, M. A. 2008. Soybean, a promising health source. *Nutricion Hospitalaria* 23(4): 305-312.

[8] Zaker, M. A., Sawate, A. R., Patil, B. M., Kshirsagar, R. B., Sadawarte, S. K. 2016. Studies on exploration of orange pomace powder on physical, sensorial and nutritional quality of cookies; 7(2): 184-188.

[9] Oduntan, A. O., Arueya, G. L. 2019. Design, formulation, and characterization of a potential 'whole food'using fibre rich orange (Citrus sinensis Lin) pomace as base. Bioactive Carbohydrates and Dietary Fibre; 17: 100172.

[10] Huang, Y. L., & Ma, Y. S. 2016. The effect of extrusion processing on the physiochemical properties of extruded orange pomace. *Food Chemistry*; *192*: 363-369.

[11] El-Sayed, M. E. S. Y., Elsanhoty, R. M., & Ramadan, M. F. 2014. Impact of dietary oils and fats on lipid peroxidation in liver and blood of albino rats. *Asian Pacific Journal of Tropical Biomedicine*; *4*(1): 52-58.

[12] Olayode, O. A., Daniyan, M. O. and Olayiwola, G., 2020. Biochemical, hematological and histopathological evaluation of the toxicity potential of the leaf extract of Stachytarpheta cayennensis in rats. Journal of traditional and complementary medicine, 10(6), pp. 544-554.

[13] Lawal, B., Shittu, O. K., Rotimi, A. A., Olalekan, I. A., Kamooru, A. A., & Ossai, P. C. 2015. Effect of methanol extract of Telfairia occcidentalis on haematological parameters in wister rats. *Journal of Medical Sciencesn*; *15*(5): 246.

[14] Abushofa, F. A., Azab, A. E., & Alkadrawy, S. 2019. Hepatic pathophysiological changes induced by nicotine and/or sodium nitrite injection in male albino rats. East African Scholars *Journal* Med Sci; 2(4): 184-196.

[15] Buncharoen, W., Saenphet, S., Chomdej, S., & Saenphet, K. 2012. Evaluation of biochemical, hematological and histopathological parameters of albino rats treated with Stemona aphylla Craib. extract. *Journal* of Medicinal Plants Research; 6(27): 4429-4435.

[16] Seneviratne, K. N., Kotuwegedara, R. T. and Ekanayake, S. 2011. Serum cholesterol and triglyceride levels of rats fed with consumer selected coconut oil blends. *International Food Research Journal;* 18(4): 1303-1308.

[17] Adegoke, O. A., Bamigbowu, E. O., Braide, A. S., Enyaosa, L. A. 2012. Total Protein and Albumins Concentrations in Albino Rats (*Rattus norvegicus*) Fed Granulated Sugar and Gari. *International Journal of Applied Biological Research*; 4(1-2): 87–94.

[18] Ferreira, E. D. S., Silva, M. A., Demonte, A., & Neves, V. A. 2011. Soy β-conglycinin (7S globulin) reduces plasma and liver cholesterol in rats fed hypercholesterolemic diet. Jo. M. F; 14(1-2): 94-100.

[19] Tandi, J., Yustin, Y., Yanuarty, R. and Handayani, T. W., 2022. Test the effect of miana leaf ethanol extract on ureum and creatinine levels in male white rats. Jurnal Sains dan Kesehatan, 4(SE-1), pp. 23-30.

[20] Dusak, Z. B., Atasoy, N. and Kömüroğlu, A. U., 2022. Protective Effect of Thyme Leaf on Metabolic Changes Induced by Monosodium Glutamate.

[21] Rashad, M. M., and Moharib, S. A. 2003. Effect of type and level of dietary fibre supplements in rats. *Grasas y Aceites*; *54*(3): 277-284.

[22] Isaac, L. J., Abah, G., Akpan, B., & Ekaette, I. U. 2013. Haematological properties of different breeds and sexes of rabbits (p. 24-27). Proceedings of the 18th Annual Conference of Animal Science Association of Nigeria; 6: 7-24.

[23] Sharp PE, La Regina MC, Suckow MA. 1998. The laboratory rat CRC Press. Boca Raton (collection: The Laboratory Animal Pocket Reference Series), Sharp PE, La Regina MC, Suckow MA. The laboratory rat CRC Press. Boca Raton (collection: The Laboratory Animal Pocket Reference Series), 214 pp. 214pp.

[24] Soetan, K. O., Akinrinde, A. S., Ajibade, T. O. 2013. Preliminary studies on the haematological parameters of cockerels fed raw and processed guinea corn (Sorghum bicolor). Proceedings of 38th Annual Conference of Nigerian Society for Animal Production; 49-52.

[25] Iwuji, T. C., and Herbert, U. 2012. Haematological and serum biochemical characteristics of rabbit bucks fed diets containing garcimiola kola seed meal. Proceedings of 37th Annual Conference of Nigerian Society for Animal Production; 87-89.

[26] Atangwho, I. J., Ohaeri, O. C., & Okonkwo, C. O. 2019. Haematological changes in rats exposed to insecticidal oils from the leaves of *Cassia occidentalis and Euphorbia milii. Heliyon*; 5(5).