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Original Research Article

Evaluation of the processing methods of *Dacryodes edulis* var. *edulis* pulp: insights from biochemical changes in Wistar rats

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ABSRTACT

Dacryodes edulis var. *edulis* fruit is a commonly consumed humid fruits, with valuable nutritional and therapeutic importance. However, assessing the effects of these fruits in the biological system are essential; therefore, this study evaluated the outcome of consuming raw and processed *Dacryodes edulis* var. *edulis* supplemented diets on some biochemical parameters of Wistar rats. In this study, Forty nine male and female Wistar rats (110 ± 30g) were used and were distributed into 7 groups of 7 rats each; control (fed normal rat chow) and groups fed rat chow supplemented with 15% or 30% raw, 15% or 30% roasted, 15% or 30% boiled African pear and water and food was allowed *ad libitum*. After 28 days feeding period, alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP), serum proteins, lipid profile, food intake, body weight changes, and haematological profiles, were determined with usual methods. Findings revealed considerably ($p < 0.05$) increased ALT, AST, total protein, albumin, globulin, serum total cholesterol, high density lipoprotein cholesterol and low density lipoprotein cholesterol and red blood cells, as well as the food intake and body weight levels in the experimental animals. The ALP, white blood cells counts, and platelet counts were considerably ($P< 0.05$) reduced in experimental animals, in comparison to the control animals. The study concluded that diet supplementation with 15% and 30% raw *Dacryodes edulis* var. *edulis* pulp can possibly offer anti-anaemic and hepato-protective benefits, with improved immune system.

Keywords: African Pear, Haematology, Liver Enzymes, Raw And Processed, Lipid Profile, Serum Proteins

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Introduction

The role of fruits in dietary abundance, as well as medicinal profusion has long been established, as it is a natural bequest to mankind. *Dacryodes edulis*, commonly called African pear, a native fruit tree in Nigeria and African countries, is a well cherished multipurpose plant, providing both dietary and therapeutic properties.[1] Ethnically, the fruit is named "eben", "ube" and "elemi" by the Ibibios, Igbos and the Yorubas respectively. The fruits are also referred to as "*safou* and "*orumu*" in French and Benin respectively.[2,3] Naturally, *Dacryodes edulis* fruit, when under development, appears pink-like, taking dark-blue to violet colours when fully mature,[4], with a fleshy edible pulp, eaten either raw, boiled, or roasted. It is mostly processed boiled or roasted by softening the eatable portion with boiled water or warm ash. The pulp can be consumed along with processed corn, tapioca, bread or as snack.[5] It's a rich source of health-benefitting nutrients including essential micro and macro-nutrients. [1,5] According to Nwaogu and Oluwamukomi,[6] the pulp of *D. edulis* fruit possess some importance phytochemical/bioactive compounds, which confer medicinal importance on the fruit, and are implicated in the fight against free radicals-related damages.

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The fruits has two distinct species; *Dacryodes edulis* var. *edulis* and *Dacryodes edulis* var. *parvicarpa*, with thick and thin pulp respectively.[7] However, recent studies have shown that var. *edulis* is more nutritious with lesser anti-nutrients levels than the var. *parvicarpa*,[5] thereby prompting an in-depth studies on the effect of their consumption on biochemical parameters. The consumption of processed var. *edulis* has been implicated in peptic ulcer in rat model.[7] Normal food treating techniques including boiling and roasting have been considered to alter dietary profile of food.[8] According to Kim *et al.*[9] the thermal treatment of grains, fruits, and vegetables altered physical and sensory properties, as well as structural conformation of the bioactive compounds of the plant materials. Also, the dietary nutrients and bioactive compounds in *Dacryodes edulis* pulp have been revealed to be affected by processing.[6,10] According to Edem *et al.*[11] consumption of processed food is essential in disease management. Recently, Agiang *et al.*[5] reported that roasting generally increased the concentration of both macro and micro elements of the African pear. The presence of these nutrients, as well as antioxidants in diets have been implicated in disease management.[12] However, the reported significant influence of boiling and roasting on both nutritional and bioactive compounds, as well as the therapeutic properties of *Dacryodes edulis* is a subject of concern. Though, studies have been conducted on the effect of various processing methods on the nutritional profile of *Dacryodes edulis* pulp, there is limited literature on the effect of consuming the traditionally processed *Dacryodes edulis* var. *edulis* pulp on some biochemical parameters. Therefore, the present study evaluated the outcome of consuming raw

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and processed *Dacryodes edulis* var. *edulis* supplemented diets on some biochemical parameters using rat model.

Materials and Methods

Sample collection and identification

The developed fruits of *Dacryodes edulis* var.*edulis* used in the present study, were plucked directly from trees in July 2024 from Ikang Town Farm, Akpabuyo Provinces, Cross River State, Nigeria (GPS: 4° 47'23'' N, 8 ° 31'53" E). The fruits were identified and authenticated by a Botanist (Dr. Michael Eteng) at the University of Calabar, Nigeria, with voucher number UCB/S/0296.

Samples preparation

The *Dacryodes edulis*.var.*edulis* fruits obtained were washed with running tap water and separated into three batches. Batch I (raw), batch II (roasted); hot coals were used for roasting the fruits, and batch III (boiled); the fruits were dipped into a bowl of heated water for 5 minutes. After the processing procedures, the seeds, removed from each pulp were discarded, while the pulps were separately desiccated at 60 ºC, pulverized to fine particles, sieved using a 0.5 mm filter and preserved in a close-fitting flasks.

Animal grouping and study procedures

Forty-nine male and female albino rats, weighing 110 ± 20 g were employed in the present study. The animals were housed in typical metabolic cages in a well-ventilated apartment, with a 7 day acclimatization period prior to the experiments, and allowed free access to the normal Agrofeed livestock pelletized diet and water. At the expiration of the acclimatized period, the study animals were separated into 7 groups, with 7 animals per group and each animal was kept in a separate metabolic cage. **The groups were; I (**control**),** fed regular pelletized diet and water freely**. II:** fed formulated pelletized diet of 15 % and 85 % of raw *Dacryodes edulis*.var.*edulis* pulp and regular rat chow. **III:** fed formulated pelletized diet of 30% and 70 % of the raw sample and regular rat chow. **IV:** fed formulated pelletized diet of 15 % and 85 % of roasted *Dacryodes edulis*.var.*edulis* pulp and regular rat chow. **V:** fed formulated pelletized diet of 30 % and 70 % of roasted *Dacryodes edulis*.var.*edulis* pulp and regular rat chow. **VI:** fed formulated pelletized diet of 15 % and 85 % of boiled *Dacryodes edulis*.var.*edulis* pulp as well as regular rat chow. **VII:** fed formulated pelletized diet 30 % and 70 % of boiled *Dacryodes edulis* var.*edulis* pulp, as well as regular rat chow. The investigational period continued for 28 days, where the animals had free access to the pelletized diet and water and daily food consumption and body weight variations were monitored. Throughout the study, the uses of experimental animals' procedures as stipulated by National Institute of Health procedures (1996) were prudently observed. Also, the University of Calabar Animal Ethical Committee approved the study (146/BCM/3019).

Measurement of food intake and body weight changes

During the study, dietary intake and body weight variations were measured as follows (equations 1 and 2):

Daily dietary intake (g/28days) = diet given to individual animal – unconsumed + leaked diet.....equation 1

Body weight variation (g) = Final weight - initial weight of individual animal…….equation 2

Collection of blood and tissue samples for analyses

At the expiration of the 28 days feeding period, the rats were allowed without food for 24 hours, anaesthetized with ketamine, dismembered and whole blood obtained via cardiac piercing into Ethylene di-amine tetra acetic acid (EDTA) treated sample bottles and plain bottles, which were separately left to coagulate for two hours. Serum, obtained from the coagulated blood, refrigerated at -4 °C, were used for the estimation of the biochemical parameters, while EDTA contained samples were used for the estimation of the haematological parameters.

Determination of Biochemical parameters

Serum ALT and AST activities were measured as described by Reithman and Frankel,[13] using Randox diagnostic kits Ltd-UK, while serum ALP activity was measured in line with the procedure reported by Tietz *et al.*[14] using Randox diagnostic kits Ltd-London. Serum proteins were determined as described by Folin and Ciocalteu[15] using Randox diagnostic kits Ltd-UK. Serum lipid indices were assayed using Agappe Assay Kit (Ames-sera pak-England) with reference to Friedwald's formula.[16] The haematological indices were determined using the automated method (counter machine model: Siemes 1608), in line with Wallace Coulter principle (1956).

Statistical analysis

Data, analyzed using analysis of variance of Statistical Package for Social Sciences software version 20 with Least Significant Difference, expressed as Mean ± SEM, were considered significant at $p<0.05$.

Results and Discussion

As shown in our findings (Table 1), the non-considerable ($p > 0.05$) increase of ALT, as well as AST levels in groups fed diet supplemented 15 % or 30 % raw samples, as well as 15 % roasted sample when compared with the control, suggested non-liver damage. This implies that the raw pulps of the fruit may not have any apparent toxicity on the liver of the experimental rats. However, the reason for the significantly ($p < 0.05$) increase in the ALT and AST activities in the animals fed diet supplemented 30 % roasted, 15 % or 30 % boiled *Dacryodes edulis* var. *edulis* pulp is not clear. Though, it has been reported that antioxidant and bioactive compounds such as flavonoids, which utilizes a membrane-calming action, capable of protecting the liver cells from injury diminished due to heat treatment of *Dacryodes edulis* var. *edulis* pulp, [6] hence, the reduced concentration of these bioactive compounds following heat processing, may have contributed to the increased levels of these enzymes, via the exposure of the liver to toxic substances. The ALP activity in the experimental animals were considerably ($p < 0.05$) decreased.

Table 1: Liver enzyme activities of Wistar rats fed diets supplemented with raw and processed *Dacryodes edulis* var. *edulis* pulp

GROUPS	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	45.09 ± 1.52	10.60 ± 0.63	16.37 ± 0.54
15% Raw Sample	48.09 ± 0.23	10.00 ± 0.09	13.40 ± 0.41*
30% Raw sample	48.07 ± 2.06	10.01 ± 0.31	15.16 ± 0.37[a]
15% Roasted Sample	46.28 ± 1.09	10.86 ± 0.18	14.64 ± 0.18*
30% Roasted sample	52.08 ± 1.39*[,a,b,c]	11.54 ± 0.30[a,b]	14.57 ± 0.27*
15% Boiled Sample	49.31 ± 1.28*	12.60 ± 0.44*[,a,b,c]	14.22 ± 0.79*
30% Boiled sample	50.36 ± 0.81*[,c]	11.60 ± 0.37[a,b]	14.06 ± 0.28*

Values are expressed as Mean ± SEM, n = 7; *significantly different from Control at $p < 0.05$; a = significantly different from 15% Raw sample at $p < 0.05$;b=significantly different from 30% Raw sample at $p < 0.05$;c = significantly different from 15% Roasted sample at $p < 0.05$; ALT Alanine amino transaminase; AST- aspartate amino transaminase; ALP-Alkaline phosphatase.

The results of the serum protein concentration (Table 2) and the liver enzymes activities revealed that processing of the fruit had some positive effect on the liver function, and this agrees with the findings of Uhegbu *et al.*[17] who revealed non-hepato-toxicity of raw *Dacryodes edulis* pulp, suggesting hepato-protective properties of the raw sample of the fruit. The serum total cholesterol, high density lipoprotein cholesterol (HDL- Cholesterol) and low density lipoprotein cholesterol (LDL- Cholesterol) levels of all the experimental animals

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were considerably (P<0.05) higher, with no significant difference in serum triacylglyceride and VLDL-c levels, in comparison with the normal group (Table 3). **Table 2:** Serum protein concentration of male Wistar rats fed diets supplemented with raw and processed *Dacryodes edulis* var. *edulis* pulp

Groups	Total protein (g/dL)	Albumin (g/dL)	Globulin (g/dL)
Control	4.65± 0.13	3.35 ± 0.15	1.30 ± 0.04
15% Raw sample	5.44 ± 0.09*	4.44 ± 0.08*	1.00± 0.02*
30% Raw sample	5.68± 0.07*	4.34 ± 0.11*	1.34 ± 0.10[a]
15% Roasted sample	6.05± 0.07*	4.61 ± 0.07*	1.44 ± 0.12 [a]
30% Roasted sample	6.31±0.01[*,a]	4.64 ± 0.02*	1.66 ± 0.01[*,a,b]
15% Boiled sample	6.07±0.07*	4.52±0.13*	1.54±0.09[a]
30% boiled sample	6.37±0.07[*,a]	4.57±0.12*	1.80±0.15[*,a,b,c]

Values are expressed as Mean ± SEM, n = 7; *significantly different from Control at p < 0.05; a = significantly different from 15% Raw sample at p < 0.05;b=significantly different from 30% Raw sample at p < 0.05;c = significantly different from 15% Roasted sample at p < 0.05.

Table 3: Lipid profile c of male Wistar rats fed diets supplemented with raw and processed *Dacryodes edulis* var. *edulis* pulp

Samples	T- CHOL (mmol/L)	TAG (mmol/L)	HDL (mmol/L)	LDL (mmol/L)	VLDL (mmol/L)
Control	117.95 ±8.20	61.50 ±3.21	23.38 ±0.25*	44.15 ±12.05	12.30 ±0.64
15% Raw Sample	162.78 ±16.85*	63.82 ±0.33	27.32 ±1.73*	86.20 ±16.44*	12.76 ±0.07
30% Raw sample	169.25 ±3.42*	65.12 ±1.78	28.03 ±0.40*	91.11 ±2.05*	13.02 ±0.36
15% Roasted Sample	178.95 ±4.32*	66.36 ±2.98	27.79 ±0.12*	99.32 ±7.82*	13.27 ±0.60
30% Roasted sample	181.09 ±2.66*	69.46 ±5.75	31.98 ±0.07[*,a,b,c]	97.74 ±6.02*	13.89 ±1.15
15% Boiled Sample	151.55 ±1.23[*,b,c,d]	64.47 ±0.48	27.73 ±0.32[*,c]	74.18 ±1.80[*,c,d]	12.89 ±0.10
30% Boiled sample	162.94 ±1.79[*,d]	66.61 ±0.92	28.80 ±0.36[*,c]	83.01 ±1.53*	13.32 ±0.19

Values are expressed as Mean ± SEM, n = 7; * different from Control at p<0.05; a = significantly different from 15% Raw sample at p<0.05; b=significantly different from 30% Raw sample at p<0.05;c = significantly different from 15% Roasted sample at significantlyp<0.05; d = significantly different from 30% Roasted sample at p<0.05; T-CHOL-total cholesterol; TAG-triacylglyceride; HDL-c, high density lipoprotein-cholesterol; LDLc-low density lipoprotein-cholesterol; VLDL-very low-density lipoprotein-cholesterol

This finding supports the earlier study of Ezekwesili and Eneh,[18] who reported non-significant changes in lipid profile of rats fed diet supplemented various percentage of *Dacryodes edulis* var. *edulis* pulp. However, the significant rise in the concentration of HDL suggested *Dacryodes edulis* var. *edulis* pulp as a good source of HDL-c which is beneficial to the body.[19] The HDL-c have been reported to compete with atherosclerosis by eliminating cholesterol from foam cells via its inhibitory effects on LDL-c oxidation[20] However, Ejezie and Ikekpeazu,[21] have suggested total cholesterol reduction as a key player in arresting atherosclerosis . Also, dietary consumption rates (g)/28 days of animals fed 30% raw or 15% roasted *Dacryodes edulis* var. *edulis* pulp were considerably (p< 0.05) higher in comparison to the control group (Table 4).

Table 4: Food intake and body weight changes of male Wistar rats fed diets supplemented with raw and processed *Dacryodes edulis* var. *edulis* pulp

Groups	Initial weight (g)	Final weight (g)	Body weight change (g)	Food intake (g/28days)
Normal Control	73.14±0.94[a]	111.63±2.85[a]	38.49±2.04[a]	393.51±8.08[b]
15% Raw sample	82.30±0.49[b]	111.64±2.85[a]	29.34±2.71[b]	414.39±6.20[b]
30% Raw sample	94.14±0.30[c]	133.45±1.24[b]	39.31±1.13[a]	443.96±4.84[c]
15% Roasted sample	114.33±0.53[d]	143.93±0.75[c]	29.60±0.72[b]	433.97±4.51[c]
30% Roasted sample	103.53±0.84[e]	128.43±0.92[b]	24.91±1.01[b]	405.75±9.64[b]
15% Boiled sample	103.77±0.43[f]	128.61±1.97[b]	24.85±1.99[b]	377.36±7.66[a]
30% Boiled sample	126.48±1.21[g]	145.04±2.42[c]	18.56±1.91[c]	363.91±6.99[a]

Values are expressed as mean ± SEM, n = 7; Means with same superscript along each column were not significantly diifferent (P<0.05)

The cause of the high dietary intake as observed in animals fed diet supplemented 30 % raw *Dacryodes edulis* var. *edulis* pulp could be due to the reported high fatty content of the raw pulp.[5] Edem [22] revealed that high fatty foods increase appetite, with corresponding increase in body weight. The reduction in dietary consumption, observed in animals fed diet supplemented 15 % or 30 % *Dacryodes edulis var edulis* boiled pulp may be linked to increase in moisture, with decreased nutritional value of *Dacryodes edulis var edulis* pulp,[5] capable of increasing palatability. [23] Various traditional food processing methods have been revealed to bring about changes in dietary composition, especially heat sensitive diets and bioactive compounds,[24], capable of influencing their consumption. However, the increase in body weight changes observed in the animals fed *Dacryodes edulis* var. *edulis* pulp is the product of the nutritional endowment of the fruits.[25] Considering the importance of white blood cells (WBCs) in defense mechanism, consuming immune boosting diets, is multifunctional in the fight against diseases. In the present study, the observed non-significant increase in the WBC and lymphocytes levels in almost all the experimental groups, suggest that *Dacryodes edulis* var. *edulis* pulp could provide immunity to the body system, thus supporting its reported therapeutic properties.[26] As observed in our findings, granulocytes volume was considerably decrease in animals fed various portions of the *Dacryodes edulis* var. *edulis* pulp, suggesting a bone disorders like leukemia or aplastic anemia and this was evident in the significant decreases in Mean Corpuscular Haemoglobin (MCH) levels in all the treated groups, which signified a non-macrocytic anaemic condition. The non-significant rise in RBC concentration may have led to the observed non-significant increase in haemoglobin (Hb) and haematocrit (HCT) levels (Table 5). Platelet count is a key screening index for haematological indices and significant reduction of the indices has been implicated in bleeding[27]; hence, the decreases platelet counts observed in the raw samples may indicate possible effects on plasma coagulation.

Table 5: Haematological parameters of Wistar rats fed diet supplemented raw and processed *Dacryodes edulis* var. *edulis* pulp

* Values are expressed as Mean ± SEM, n = 7; *significantly different from Control at p<0.05; a = significantly different from 15% Raw sample at p<0.05; b=significantly different from 30% Raw sample at p<0.05;c = significantly different from 15% Roasted sample at p<0.05; d = significantly different from 30% Roasted sample at p<0.05; WBC= White blood cells, LYM= lymphocytes; GRA= Granulocytes; RBC= Red blood cells; Hb= Haemoglobin; Hct=haematocrit; MCH=Mean corpuscular haemoglobin; PLT= platelet

GROUPS	WBC (10^9cells/µL)	RBC (10^{12}cells/µL)	HB (g/dL)	HCT (L/L)	LYM (%)	GRA (%)	MCH (pg)	PLT (10^9 cells/L)
Control	11.61±2.79	8.15±2.56	16.50±1.79	53.85±15.93	52.40±8.10	40.20±10.70	21.70±4.70	668.00±0.70
15% Raw Sample	11.07±7.11	9.96±0.77	16.80±1.50	60.37±5.43*	55.40±28.80	35.50±28.10	16.80±0.20*	465.50±39.50*
30% Raw sample	11.43±2.33	8.87±1.26	14.77±2.02*,a	56.50±9.64	61.63±16.58	22.87±11.20*	16.70±0.26*	418.00±65.74*
15% Roasted Sample	7.85±1.18*,a,b	10.35±0.38*	16.10±0.55b	59.45±0.41	57.90±10.34	20.43±7.37*,a	15.60±0.06*	553.00±41.77
30% Roasted sample	12.14±3.38c	9.49±0.50	15.73±0.63	55.76±2.67	57.30±8.97	26.93±12.21*	16.57±0.43*	586.00±31.02
15% Boiled Sample	9.10±1.38d	9.56±0.58	15.23±0.92	56.87±3.00	53.60±13.51	24.70±9.87*	15.93±0.03*	533.67±33.46*
30% Boiled sample	8.41±3.73d	9.81±0.85	16.43±1.45b	60.64±5.75*	48.03±4.17	34.73±8.88	16.70±0.12*	582.67±94.34

Conclusion

This finding revealed that the consumption of meal complemented 15 % or 30 % raw *Dacryodes edulis* var. *edulis* pulp could offer hypoglycemic, hypocholesterolemic, antiatherogenic properties and hepato-protective benefits, as well as enhancing immune system. More so, the finding further revealed that consumption of diets supplemented 15 % or 30 % boiled and roasted *Dacryodes edulis* var. *edulis* pulp altered the liver enzymes, lipid profile, serum proteins and haematological parameters, hence, the intake of raw processed *Dacryodes edulis* var. *edulis* pulp should be encouraged. To further enhance our findings, effect of consumption of diet supplemented raw *Dacryodes edulis* var. *edulis* pulp in the management of some disease condition, including obesity should be investigated.

Conflict of interest

The authors declare no conflict of interest

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

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