Effect of Preservation Methods of Oil Palm Sap (*Elaeis guineensis*) on the Reproductive Indices of Male Wistar Rats

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ABSTRACT Thirty male Wistar rats, split into five groups of six rats each, were administered different forms of oil palm tree (*Elaeis guineensis*) sap samples by gavage based on 1.5% of their weekly body weights. Group 1 which served as control received only water, group 2 received pasteurized palm sap (PPS), group 3 received market palm wine (MPW), group 4 received frozen palm sap (FPS), whereas group 5 received fresh palm sap (FrPS). Chemical composition of the sap samples was determined. Normal feed and water were fed *ad libitum*. After 2 months of treatment, each male rat group was allowed 7 days to mate with six female Wistar rats. Thereafter, blood and epididymal samples were collected for testosterone assay and sperm count, respectively, before they were humanely sacrificed and testicular tissues taken for testicular histology. Litter weight and size of the pups produced by the females of each group were determined at birth. The sap samples contained carbohydrate (0.01–11.71%), protein (1.56–1.95%), ash (0.22–0.35%), moisture (92.55–98.24%), and alcohol (0.26–3.50%). PPS-treated rat group had significantly ($P < .05$) decreased sperm count ($42.60 \pm 23.64 \times 10^6$), abnormal increase in testosterone level, and necrosis in the histology of the testes with reduced spermatogenetic activity, compared with other treatment groups. The female rats crossed with male rats fed on FrPS or FPS produced the highest number of pups followed by the control group. This study demonstrated that the intake of FrPS improved fertility in male animals, but its administration for a long period led to necrotic changes in the testes, whereas pasteurization of palm sap, impacted negatively on the reproductive indices of male animals.

KEY WORDS: • fertility • freezing • pasteurization • testosterone • testis

INTRODUCTION

PALM SAP IS THE UNFERMENTED pale yellow exudate from tapped unopened spathe of oil palm tree (*Elaeis guineensis*) that is consumed as a nourishing beverage in some tropical countries. Fermented palm sap on the other hand is usually called palm toddy, or simply palm wine and is widely consumed as a refreshing alcoholic beverage by people in some parts of Africa, Asia, and South America.¹ Proximate analysis carried out on *E. guineensis* saps revealed the following ranges; 85.10–87.20% moisture, 0.05–0.23% crude protein, 0.20–2.25% fat, and 12.375–14.65% carbohydrate.² The freshly tapped sap is often drunk within 1–2 days either as fresh sap or palm wine. It may also be pasteurized and bottled or frozen at industrial level for longer storage or be processed into different products such as caramel, sugar,

spirit,³ or mixed fruit juice.² However, palm wine without any heat treatment is the commonest form of the product in countries like Ghana and Nigeria, where it is served in ceremonies such as traditional festivals, weddings, and funerals.^{4,5} The dregs of palm wine containing yeast sediments and other particles are customarily reserved for the groom in the eastern part of Nigeria, because of the long-standing belief that it boosts male sexual performance. According to Achebe,⁶ the thick dregs of palm wine were supposed to be good for men who were going in to their wives. Palm wine, however, contains alcohol, which is known to affect male reproductive performance.

The increasing prevalence of infertility cases is becoming a major public health challenge in developing countries due to changes in diet and lifestyle.⁷ Numerous studies^{8–10} have indicated that chronic alcohol abuse in males resulted in decreased testosterone production, reduced sperm output, and testis atrophy. In animals, ethanol exposure of adults can increase germ cell apoptosis¹¹ and cause an adverse effect on the secretory function of Sertoli cells.¹² Traditionally, palm wine is given to women after childbirth to

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help stimulate the production of milk as well as to young women in fattening rooms.¹³ It is of great interest to authenticate or refute the traditional reproductive claims. This work was therefore designed to evaluate the effects of consumption of palm sap preserved through various methods on some reproductive indices of male Wistar rats.

MATERIALS AND METHODS

Reagents

Sample procurement and preparation. Fifteen liters of freshly tapped oil palm sap (FrPS) were collected from a palm wine tapper at Ajuona-Nsukka, Enugu State, in a cooler packed with ice blocks, and divided into three lots. Each lot (5 L) was subjected to either heat treatment (70°C for 40 min to obtain pasteurized palm sap [PPS]), freezing (−4°C till frozen [FPS]), or left untreated to serve as fresh palm sap (FrPS) along with 5 L of market palm wine (MPW), and tap water that served as control. The commercial rat feed (Vital Pelletized Growers Feed) used, which was manufactured by Grand Cereals and Oil Mills Ltd. (Jos, Plateau State, Nigeria), contained the following ingredients: cereals/grains, animal protein, vegetable protein, minerals, salts, essential amino acids, antibiotics, antioxidants, and vitamin premix. The nutrient composition as stated in the label included: crude protein, 14.50%; fat, 7.00%; crude fiber, 7.20%; calcium, 0.80%, and metabolizable energy, 2000 kcal/kg.

Experimental animals. Thirty (30) 4 weeks old healthy male Wistar rats weighing between 47.1–72.6 g, were obtained from the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka. The rats were divided into five groups (1, 2, 3, 4, 5) of six rats each. Each study group was split into two subgroups of three rats each, and housed in a stainless steel cage with plastic bottom grid and a wire screen top, in the animal house of the Department of Home Science, Nutrition and Dietetics, University of Nigeria, Nsukka. The room temperature and relative humidity ranges for the rats were 23–24.5°C and 91–96%, respectively, with natural 12-h light–12-h dark cycle. The rats were served 8–10 g of the standard rat chow and at least 5 mL of water per day, *ad libitum*. The animals were acclimatized for 2 weeks before treatment. The group 1 rats were given normal feed and water only; group 2 rats were fed on PPS in addition to the normal feed and water; group 3 rats were given normal feed, water, and MPW; whereas groups 4 and five rats were given frozen palm sap (FPS) and FrPS, respectively, in addition to normal feed and water. The palm sap/wine was administered by gavage based on 1.5% of their weekly body weights. After 2 months, 30 female Wistar rats of the same age and strain were divided into five equal groups (A, B, C, D, and E) and introduced to each male group (1, 2, 3, 4, and 5) for mating. Palm sap/wine was not administered to the female rats. After mating, the females were separated from the males and allowed to produce pups, whereas the males were humanely sacrificed. The pups produced by each group of the mated female Wistar rats were counted, weighed with an electronic

balance (Derive Instrument Company), and recorded. The entire animal study was conducted in accordance with the Ethics and Regulations guiding the use of research animals as approved by the University of Nigeria, Nsukka.

Determination of sperm count, testosterone level, and histology of testes

The sperm count was determined using World Health Organization (WHO)¹⁴ method. Testosterone assay of the male Wistar rats was made by the Microwell Testosterone Enzyme Immunoassay technique using the Quantitative Determination Kit (Syntron Bioresearch, Inc., Carlsbad, CA, USA). The histology of the rat's testes was evaluated by the method of Borch *et al.*¹⁵

Determination of carbohydrate and alcohol contents of the palm products

The phenol-sulfuric acid method described in the Association of Official Analytical Chemists (AOAC)¹⁶ was used to determine the carbohydrate content in palm samples, whereas alcohol content was measured using the specific gravity method.¹⁶

Experimental design and statistical analysis

The experiment was conducted in a completely randomized design. Data generated were analyzed using one way analysis of variance and mean separation was done by Duncan's New Multiple Range Test at 95% confidence interval using the SPSS version 17.0.

RESULTS

The proximate composition of the oil palm sap products is presented in Table 1. There were significant ($P < .05$) variations in the carbohydrate contents of the various samples. The protein content of FrPS was significantly ($P < .05$) higher than the rest of treatment groups, followed by FPS and PPS, with the least being MPW. The ash content of PPS was significantly ($P < .05$) higher than other treatment groups. The moisture content of MPW was significantly ($P < .05$) higher than other treatment groups, with the least being that of PPS.

The alcohol content of the samples is presented in Figure 1, with that of MPW being significantly ($P < .05$) higher than those of FPS, FrPS, and PPS samples.

TABLE 1. PROXIMATE COMPOSITION OF PALM SAP PRODUCTS

Parameters	Pasteurized palm sap	Market palm wine	Frozen palm sap	Fresh palm sap
Carbohydrate (%)	16.38 ± 0.34 ^a	7.01 ± 0.03 ^d	8.43 ± 0.51 ^c	11.79 ± 0.20 ^b
Protein (%)	1.67 ± 0.48 ^b	1.56 ± 0.36 ^c	1.70 ± 0.00 ^b	1.95 ± 0.09 ^a
Ash (%)	0.35 ± 0.42 ^a	0.22 ± 0.00 ^b	0.24 ± 0.00 ^b	0.25 ± 0.42 ^b
Moisture (%)	81.60 ± 0.05 ^c	95.21 ± 0.04 ^a	89.63 ± 0.09 ^b	86.05 ± 0.01 ^{bc}

Values are means ± SD ($n = 3$). Values with different superscript lowercase letters within a row were significantly different ($P < .05$).

FIG. 1. Alcohol content of oil palm sap products.

The effect of palm sap or wine treatment on the sperm count of the rat groups is shown in Table 2. The epididymal sperm count of exposed experimental animals differed significantly ($P < .05$) from the control groups. The rat group fed on PPS, had an epididymal sperm count of $42.603 \pm 9.65 \times 10^6$ compared with that of the control group ($223.04 \pm 55.05 \times 10^6$) that was 423% higher.

There was no significant ($P > .05$) difference between the sperm count of the rat group treated with FPS and the control rat group, whereas significant ($P < .05$) differences exist between them and the rat groups treated with PPS and FrPS. PPS-treated rats had significantly ($P < .05$) low sperm count compared with other treated rat groups.

The testosterone level of the control group (Table 2) was significantly ($P < .05$) higher than the groups that consumed MPW, FrPS, and FPS. However, the level of testosterone detected in the FrPS-treated rat group was not significantly ($P > .05$) different from that of the control rat group.

The number of pups (Table 3) produced by PPS-treated rats decreased significantly ($P < .05$), whereas the males that consumed FrPS and FPS produced the highest number of pups followed by the control. The control (group A) had the highest weight of pups followed by groups B, C, D, and E.

The control group animals showed normal histological texture (Fig. 2A), with the diameter of the seminiferous tubules varying within range. Sertoli cells had many normal sized cytoplasmic processes, while the Leydig cells had normal nuclear size. The spermatozoa with long tail and small distinct head were more visible. The histology of the rat group administered with PPS was clearly altered with marked increase in necrosis of the spermatids (Fig. 2B). The interstitial cells were either not well developed or may have undergone destruction. It appeared that group 2 had lower basement cells with active spermatogenesis compared with rat group 1 that had no treatment. The cytoplasm of the cells was also empty. At the early stages, there was active spermatogenesis that decreased at later stages till spermatids went necrotic.

The histology of rat testis exposed to MPW (Fig. 2C) revealed that uniformity in spermatogonia was totally absent. The cytoplasm was empty and the basement cells were not uniform, whereas spermatids had undergone necrosis. The histology of testes of rats administered FPS (Fig. 2D, E) had more basement cells compared with either group 2 or group 3, but were lower than that of the control group. The lumen cytoplasm was found to be empty. However, there were increased spermatogenic activities at the early stages, but degeneration occurred at later stages. The basement cells lacked uniformity. Figure 2E revealed slight deposit of carbohydrate and blood droplets, and advanced stage of spermatogonia on the basement cells.

The histology of rats administered FrPS (Fig. 2F), compared favorably with that of rat group 1. The lumen contained spermatids that were fully developing, indicating active spermatogenesis in the interstitial cells. The rat group administered FPS (Fig. 2E) showed necrotic spermatids in the basement cells and active spermatogenesis at the early stages, that seemed to be degenerating with prolonged administration of sap, whereas that of groups 2 and 3 treated rats were more pronounced, but could not compare favorably with that of the control rat group. There were also congestion of blood vessels within the testes in groups 2, 3, and 4 rats, though they were more pronounced in groups 2 and 4 treated rats.

DISCUSSION

The significantly high carbohydrate content of the PPS sample, which was twice the level of carbohydrate in the

TABLE 2. EFFECT OF TREATMENT ON THE LEVEL OF TESTOSTERONE AND SPERM COUNT

Histopathology	Rat groups				
	1	2	3	4	5
Sperm count ($\times 10^6/\text{mL}$)	223.04 ± 134.74^a	42.60 ± 23.64^d	174.89 ± 122.60^c	219.18 ± 105.57^a	185.78 ± 142.37^b
Testosterone (ng/dL)	0.83 ± 0.67^b	1.95 ± 0.06^a	0.50 ± 0.05^c	0.56 ± 0.09^c	0.80 ± 0.08^b

Values are mean $\pm$ SD ($n=6$). Values with different superscript lowercase letters within a row are significantly ($P < .05$) different.

Group 1—Male Wister rats treated with normal feed + water (control).

Group 2—Male Wister rats treated with normal feed + water + pasteurized palm sap (PPS).

Group 3—Male Wister rats treated with normal feed + water + market palm wine (MPW).

Group 4—Male Wister rats treated with normal feed + water + frozen palm sap (FPS).

Group 5—Male Wister rats treated with normal feed + water + freshly tapped palm sap (FrPS).

TABLE 3. EFFECT OF TREATMENTS ON THE NUMBER AND WEIGHT OF PUPS PRODUCED BY FEMALE RATS MATED BY MALE RATS ADMINISTERED TREATED PALM SAP/WINE SAMPLES

Parameters	Treatment groups				
	A	B	C	D	E
No. of pups	8.04 ± 6.88 ^b	6.60 ± 0.75 ^c	7.50 ± 0.60 ^b	8.83 ± 0.79 ^a	9.17 ± 0.48 ^a
Weight of pups	5.92 ± 0.07 ^a	5.52 ± 0.09 ^a	5.31 ± 0.09 ^b	5.04 ± 0.64 ^b	5.13 ± 0.05 ^b

Values are means ± SD (*n* = 6). Values with different superscript lowercase letters in the same row were significantly different (*P* < .05).

Group A—Dam mated by male rats (1) treated with normal feed + water (Control).

Group B—Dam mated by male rats (2) treated with normal feed + pasteurized palm sap (PPS).

Group C—Dam mated by male rats (3) treated with normal feed + market palm wine (MPW).

Group D—Dam mated by male rats (4) treated with normal feed + frozen palm sap (FPS).

Group E—Dam mated by male rats (5) treated with normal feed + freshly tapped palm sap (FrPS).

other palm sap/wine samples could be due to moisture evaporation during pasteurization that concentrated the carbohydrate and sugar contents. Carbohydrates have been shown to play roles in male animal's reproductive function and are also used for amino acid production.¹⁷ The MPW may have been adulterated with water, which is a common practice among palm wine marketers/tappers. Freeze-thaw effect may have contributed to the relatively high moisture content of FPS,¹⁸ compared with FrPS.

The significantly higher protein content of FrPS could be due to its lower moisture content compared to the relatively low moisture contents of FPS and MPW. The lower protein content of PPS, which had the lowest moisture content, may

be attributed to loss of volatile nonprotein nitrogen (*e.g.*, NH₃) during the heat treatment. MPW had the lowest protein content probably due to dilution effect resulting from added water. However, the protein contents of the samples were in agreement with Dalibard³ who reported 1.79–2.27% protein content for palm sap used in jaggery production. Chinoy and Mehta¹⁹ reported that protein-rich diet is beneficial to overcome the toxic effects of fluoride on testicular steroidogenesis. According to Glass *et al.*,²⁰ long-time treatment of rat with low protein diets showed significantly smaller prostrate and seminal vesicle weights than the food-restricted control. The high level of ash in PPS sample could be as a result of heat treatment given to the palm sap that led

FIG. 2. Histology of rat groups' testes after 2 months of treatment (×400). (A) Group 1—Male Wister rats treated with normal feed + water. The spermatogonia (G) and seminiferous epithelia (S) are active in spermatogenesis with spermiation occurring in seminiferous lumen (L). (B) Group 2—Male Wister rats treated with normal feed + water + pasteurized palm sap (PPS). (C) Group 3—Male Wister rats treated with normal feed + water + market palm wine (MPW). (D) Group 4—Male Wister rats treated with normal feed + water + frozen palm sap (FPS). (E) Group 4—Male Wister rats treated with normal feed + water + frozen palm sap (FPS) (advanced stage of necrosis). (F) Group 5—Male Wister rats treated with normal feed + water + freshly tapped palm sap (FrPS). N=Invisible Leydig cells in the interstitial spaces. Color images available online at www.liebertpub.com/jmf

to loss of moisture and consequently concentrated other components. The ash content of the samples agrees with 0.40% ash reported by Cunningham and Wehmeyer,²¹ for palm wine from the sap of *Hyphaene coriacea* and 0.23% by Ikegwu² for palm sap from *E. guineensis*.

The slight increase in the alcohol content of FPS on storage compared with that of FrPS, may be attributed to suppression of the activities of the contaminating microorganisms associated with fermentation. It could also be linked to the fermentative activities of contaminating microorganisms during freeze-thawing of FPS. The lower alcohol content of PPS, than those of MPW, FrPS, and FPS, may be due to the evaporation of residual alcohol during pasteurization. The high alcohol content of MPW in this study, is in agreement with the value (3.6%) reported by Cunningham & Wehmeyer²¹ for saps of *H. coriacea* and *Phoenix reclinata*. The significantly high alcohol content in MPW could be attributed to the high fermentative activity of contaminating microorganisms that was driven by the holding ambient temperature (25–28°C) condition. The lowest alcohol content of PPS could be due to heat-induced destruction of fermentative microorganisms and alcohol evaporation.

Alcohol has been shown to reduce the quantity of sperm a man produces as well as its quality,²² by preventing the body from absorbing zinc, which is found in high quantities in sperm.^{23,24} The decreased sperm counts observed in most of the treated rat groups could be attributed to the alcoholic content of the samples. The effect of alcohol on the histology of the testis was clearly demonstrated by the low number of the Leydig cells and necrosis of spermatids (Fig. 2C), thereby supporting the decreased sperm counts observed in most treated groups.

The significantly low sperm count observed in the PPS-treated rat group could be attributed to loss of probiotic effects of palm sap after pasteurization. This was further manifested in the significantly low number of pups produced by dams mated by PPS-treated male rats (Table 3). According to Dolgin²⁵ a team of Massachusetts Institute of Technology researchers studied the effect of probiotic diet on rates of obesity of male rats, and observed in mating experiments that yogurt-eating males inseminated their partners faster and produced more offspring than control mice. The increase in epididymal sperm count of the rat group administered FPS, may result from the antioxidant activity of microbial cells present in the sap in a dormant form. The microorganisms possessed upon consumption antioxidant activity, which protected the sperm cells from reactive oxygen species.^{7,26}

According to Mooradian *et al.*,²⁷ the primary function of Leydig cells is biosynthesis and secretion of testosterone that influences sexual behavior and maintains spermatogenesis. The result revealed higher testosterone levels in rats that were fed palm sap products with low alcohol content, which may also be linked to the level of residual carbohydrates in the samples. The rat group fed on PPS with highest content of carbohydrates, had a significantly higher testosterone level than other groups. This may be due to heat inactivation of microorganisms and partial concentration of

the palm sap during pasteurization, leaving the sap's carbohydrate content (Table 2) almost intact. Anderson *et al.*,²⁸ observed that the testosterone concentrations in seven normal men were consistently higher after 10 days on a high carbohydrate diet (468 ± 34 ng/dL) than during a high protein diet (371 ± 23 ng/dL).

The decrease in the level of testosterone of MPW- and FPS-treated rat groups with highest exposure to alcohol also coincided with their decreased epididymal sperm count. High testosterone level may also have an indirect negative effect in the spermatogenesis.²⁹ Rajalakshmi *et al.*,³⁰ observed that alcohol abuse in men caused impaired testosterone production, shrinkage of testes, reduced sperm counts, abnormal sperm shapes, and altered sperm motility. These observations are similar to those of this study.

There were no significant differences in the level of testosterone detected in the FrPS-treated and control rat groups, which could be as a result of the probiotic effect of *Saccharomyces cerevisiae* in the body. Emmanuella & Emmanuella³¹ observed that heavy alcohol consumption resulted in reduced testosterone levels in the blood, and also impaired the function of the testicular Sertoli cells that play an important role in sperm maturation.

The increased number of pups produced by dams crossed by FrPS- or FPS-treated male rat groups could be attributed to the probiotic effect of palm sap in the testes of the male rats. The significant reduction in the number of pups produced by dams crossed by PPS and MPW fed males agrees with the report of Sreeranjitkumar *et al.*,³² that the number of pups born to dams fed on toddy/ethanol decreased significantly. Efficient utilization of energy due to probiotic yeasts, and presence of alcohol in the palm sap/wine products may have led to the reduction of the pups' weights. This observation agrees with the reports of Sreeranjitkumar *et al.*,³² Breese *et al.*,³³ and Maldaner *et al.*,³⁴ that administration of toddy/ethanol decreased the birth weight of pups.

The significant reduction in the number of pups born by dams crossed to PPS and MPW fed male rats could be due to necrosis observed in the histology of the testes, destruction of Leydig cells, high content of carbohydrate in the PPS administered to the male rats, and reduction in their sperm count.

The histology of the testes showed that the nontreated rat group displayed active spermatogenesis from the top to the lumina. The histology of rat group fed on PPS contained cells that lacked uniformity, showed enlarged Sertoli cells, necrotic spermatids, and absence of granulated cytoplasm signifying lack of rich nutrients. The cells also showed evidence of active spermatogenesis at the early stage, which could be attributed to high intake of carbohydrate-rich palm sap by the rat groups.

The destruction of Leydig cells of the testes of MPW-treated rat group could be as a result of prolonged exposure to alcohol (Fig. 1), and this agrees with the report of Nayanatara *et al.*,³⁵ that alcohol induced oxidative damage or insufficiency of the protective antioxidants in the rat testis.

The Leydig cells within the supporting tissues in the interstitial spaces between the tubules in groups 2, 3, and 4 were not visible due to lipid droplets resulting from

carbohydrate build-up, and blood droplets. Degenerated spermatogonial cell layers that were observed in the treated rat groups may be attributed to decreased testosterone synthesis and disruption of normal androgen status.

In conclusion, it is evident from this study that FrPS improved fertility in male animals, but addiction could lead to necrotic changes in the testes. Heat treatment, which is the proposed method of preservation of palm sap, impacted negatively on the reproductive indices of male animals. These findings suggest the need for detailed chemical evaluation of the oil palm sap products, which is in progress.

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AUTHOR DISCLOSURE STATEMENT

There are no competing interests.

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