



Effect of Locally Prepared Tofu (Curdled Soymilk) on Hematological Status and Serum Antioxidant Enzyme Activity in Albino Rats

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ABSTRACT

Effect of locally prepared Tofu (Curdled soymilk) on Hematological status and Serum antioxidant enzymes activity in Albino rats were evaluated. Tofus were prepared using locally sourced coagulants (lemon extract, alum, steep water, and lemon peel extract). Wister albino rats were used as experimental animal. Total feed consumption and weight gain of the rats were measured. The analysis of packed cell volume (PCV), red blood cells (RBC), hemoglobin (Hb), mean corpuscular volume (MCV), white blood cells (WBC), serum malondialdehyde (MDA), and antioxidant enzymes; Superoxide dismutase (SOD), Catalase (CAT) and Glutathione S-transferase (GST) activities were determined. The result reveals that the total feed consumption and weight gain of rats fed with tofu prepared from alum, lemon extract, and lemon peel extract were not different significantly ($p > 0.05$), compared to rats fed with commercial diet (CD). Hematological parameters of rats fed with various coagulated tofu were significantly ($p < 0.05$) higher compared to the rats fed with commercial diet. Significant ($p > 0.05$) decrease in the levels of MDA of all rats fed with different coagulated tofu were observed compared to rats fed with CD. Similarly, the rats fed with various coagulated tofu had significant higher ($p < 0.05$) serum antioxidant enzyme activities compared to those fed with CD. The results conclude that all tofu curdled with various coagulants could be used in the management of hemolytic anemia, oxidative stress and membrane peroxidation.

Keywords: Albino rats, antioxidant enzymes, coagulants, hematology, peroxidation, tofu

INTRODUCTION

Nutrition is the relationship of foods to human's health. Proper nutrition implies receiving adequate foods and supplements to convey the nutrition required for optimal health (Wei and Shiow, 2001). Food products (cereal crops, fruits, vegetables, juices, and cream) provide a wide variety of reactive oxygen species (ROS)-scavenging antioxidants such as phytochemicals and antioxidant vitamins. It has been proposed that phytochemicals are the major contributors to the antioxidant capacity of plant products (Sun *et al.*, 2002; Liu, 2003). The increase consumption of plant products containing high levels of phytochemicals has been recommended to prevent or reduce oxidative stress in human body (Chu, *et al.*, 2002). Antioxidant supplements or foods containing antioxidants help human body to reduce oxidative damage (Yang *et al.*, 2002). Recently, the antioxidants and health benefits of phytochemicals in food products have become an interesting area of research.

Jackson *et al.* (2002) reports showed that consumption of 25 g of food products such as fruits, vegetables per day could lower the serum cholesterol and prevention of heart diseases.

Tofu is a plant product produced from soybeans. Soybean is among the cheapest, protein, phenolic-rich crop consumed over the world and most commonly cultivated in African countries like Nigeria and South Africa (Rekha and Vijayalakshmi, 2010). Tofu (soybean curd) is produced by curdles of fresh hot soy milk using the coagulant (shokunbi *et al.*, 2011). Traditionally, tofu is produced in the Northern part of Nigeria by curdle of fresh hot soy milk using Alum, Lemon, steep waste water from pap production (Nuwan baka), and

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lemon peel ash extract (Suaka) (Yakubu *et al.*, 2013). It has high protein, polyunsaturated fatty acids, β -carotene, minerals, antioxidant compounds, and essential amino acids (Paganga and Miller, 1999, Poysa and Woodrow, 2002). The low incidence of cancers and aging diseases has been reported in Asian countries that consumed soybean foods (Sarkar and Li, 2003). Several epidemiological studies have shown that the consumption of soy foods has reduced risk for cardiovascular and cancer diseases, and atherosclerosis (Yamamoto *et al.*, 2003, Jyoti *et al.*, 2007, Rekha and Vijayalakshmi, 2010). It was suggested by Anderson and Theron (1990) that soy foods have potent antioxidant properties, which act as a neutralizer of reactive oxygen species (ROS) in the extracellular cells. Oboh (2006) reported that the reduced activities of cholesterol in general, which lead to lower levels of bad cholesterol (Low density lipoprotein) and normal level of good cholesterol (High density lipoprotein) in the body was observed after soy food consumption. Soy foods, fruits, and vegetables have been demonstrated to be rich in phenols and flavonoids (Alfa *et al.*, 2003). It has been postulated that the health benefits of soy foods could be associated with its higher contents of antioxidants, and phenolic compounds (Lee *et al.*, 2003). In developing countries, where animal proteins are beyond the reach of a larger majority of the population, food products are used to manage chronic diseases. Presently, the therapeutic uses of food products owing to the problems of high cost of animal proteins, imported drug formulations and their side effects has been in the sight of researchers. Therefore, this study aims at evaluating the hematological status and antioxidant enzyme activity in albino rats fed with locally prepared tofu.

METHODOLOGY

Source of materials

Soybeans and local coagulants: Alum and *Citrus limonun* (lemon) were procured from a local market. Steep water (waste water from pap production), and lemon peel ash extract were given by a domestically processed pap. All chemicals used were analytical grade and were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Preparation of coagulant solutions

Alum solution (0.2 w/v) was prepared as the coagulant. A fresh weight of lemon fruit and lemon peel ash (20 g), each was soaked, separately, in 50 mL of distilled water for 20 min and ground into slurries using pestle and mortar. The slurries were separately filtered using cheese cloth, and the final volume of each filtrate was made up to 100 mL with distilled H₂O.

The Acidity of coagulants

An aliquot of each filtrate of the prepared coagulants was titrated against 0.1 M NaOH using methyl orange as an indicator. The acidity of each coagulant was determined and expressed as the % anhydrous citric acid (AOAC, 1990). The acidity of each filtrate was adjusted with distilled H₂O to 2% as the final acidity of coagulants (Rekha and Vijayalakshmi, 2010).

Preparation of tofu

Briefly, 150g of cleaned soybean was soaked in 5 dm³ of distilled water at 28 °C for 12 hours. The soaked soybeans were re-washed thoroughly, the outer coat of soybeans was removed, and ground with water (1:8) in a blender. The soybean slurry obtained was then heated slowly at 85°C for 60 min with constant stirring. The hot slurry was filtered using cheese cloth to separate soy milk from the residue (Akara). Then, about 200 cm³ of cold soy milk at 60°C were measured into five separate 500 cm³ labeled (1, 2, 3, and 4) beakers. Into beaker (1), 10 cm³ of the prepared steep water was added, also 10 cm³ of alum, lemon, and lemon peel ash solution were separately added to beakers (2, 3, and 4), respectively. After the addition of respective coagulants, soy milk were stirred for 5 min and kept for 15 min without disturbing for the coagulation to take place. The coagulated soy milk in each beaker was filtered, separately, using cheese cloth and the curdled soy milk (tofu) was pressed with 1 kg weight of cleaned stone for 1 hour. The prepared tofu cake was then kept in a refrigerator (4 °C) for the subsequent analysis.

Animals

Twenty five of three-week old strain albino rats of 50 g, as average weights were used in this study. They were obtained from the animal house of the College of Medicine, University of Ilorin, Kwara State, Nigeria. The

animals were housed in wire mesh cages in the laboratory under ambient temperature and 12 hrs light and dark cycle. They were fed with commercial rat pellets (Neimeth Livestock Feeds Ltd., Ikeja) and water *ad libitum* and allowed to acclimatize for 2 weeks. Animal experiments were conducted in accordance with the internationally accepted principle for laboratory animal use and care (NIEHS, 1985).

Experimental Design

Animals were weighed and randomly divided into five groups of five rats per group (n=5) and treated as follows:

Group I (Control): 20 g/day of basal diet only;

Group II: 20 g/day of steep water tofu.

Group III: 20 g/day of alum tofu only

Group IV: 20 g/day of lemon tofu only

Group V: 20 g/day of lemon peel ash tofu only

The experiment was conducted for 14 days and the mean average weights of the rats were determined at the beginning of the experiment and at every 2 days for two weeks. The weight was determined using weighing scale (OHAUS MODEL Cs 5000, CAPACITY 500 × 2 g). Other parameters measured are total feed consumed and total weight gain in percentage.

Collection of Blood sample

The blood samples collected were analyzed as described by Shettu et al. (2013). On the 15th day, the rats were fasted overnight and the blood was collected using cardiac puncture using ether anesthesia. The bloods collected were divided into two parts; one was used for hematological assessment and the other part was centrifuged, and the supernatants were collected for the assessment of serum malondialdehyde (MDA) and antioxidant enzyme activities.

Determination of hematological parameters

Some blood components (hemoglobin (Hb), red blood cells (RBC), mean corpuscular volume (MCV), packed cell volume (PCV), white blood cells (WBC), were determined using the automated hematologic analyzer SYSMEX KX21, a product of SYSMEX Corporation, Japan (Dacie and Lewis, 2002).

Biochemical assays

The prepared sera from the blood collected were used for the assay of serum malondialdehyde (MDA) content and antioxidant enzyme activities. The protein

concentrations were determined by the method described by Lowry et al. (1951). Bovine serum albumin (BSA) was used as standard. The malondialdehyde (MDA), an indicator of lipid peroxidation was analyzed by the method described by Jain and Levine (1995). Antioxidant enzyme activities; total Superoxide dismutase (SOD) activity (the auto-oxidation and illumination of pyrogallol) was determined as described by Marklund and Marklund (1974). The catalase (CAT) activity (Hydrolysis of hydrogen peroxide H_2O_2) was assayed using the method described by Aebi (1984). The Glutathione S-transferase (GST) activity (measuring the formation of the GSH and 1-chloro-2,4-dinitrobenzene (CDNB) conjugate) was assayed following the method described by Habig et al. (1974).

Statistical analysis

Data were presented as a mean \pm standard deviation. A one-way analysis of variance (ANOVA) by Dunnett's multiple comparison tests was used to determine the significant differences between the means using the GraphPad 5.0 software. Significance different was considered at $p < 0.05$.

RESULTS

Nutrient utilization and performance of albino rats

The results of nutrient utilization and performance indices (total feed intake, and weight gain) of albino rats are presented in Table 1. No significant difference ($p > 0.05$) in total feed intake (TFI), and weight gain (WG) are observed in rats fed with alum tofu (ALT) (375.5 ± 3.20 and $4.33 \pm 1.50\%$), lemon extract tofu (LET) (374.7 ± 3.53 and $4.30 \pm 1.60\%$), and lemon peel extract tofu (LPT) (380.8 ± 2.62 and $4.31 \pm 0.25\%$), respectively, compared to those of the rats fed with commercial diet (CD) (375.8 ± 2.45 and $4.30 \pm 1.50\%$), respectively (Table 1). Rats fed with steep water tofu recorded significantly decrease values of TFI ($362.5 \pm 4.01\%$), and WG (3.60 ± 1.02 compared to those fed with CD with TFI ($375.8 \pm 2.45\%$) and WG ($4.30 \pm 1.50\%$) (Table 1).

Table 1: Nutrient utilization and performance of albino rats fed with different coagulated tofu.

Sample	Total feed intake (%)	Weight gain (%)
CD	375.8±2.45 ^a	4.30±1.50 ^a
ALT	375.4±3.20 ^a	4.33±1.52 ^a
LET	374.7±3.53 ^a	4.30±1.60 ^a
SWT	362.5±4.01 ^b	3.60±1.02 ^b
LPT	380.8±2.62 ^a	4.31±0.25 ^a

Values are presented in means±standard deviations (SD) of the replicates. Means followed by the same superscript(s) in the same column are not significant different ($p>0.05$). CD: Commercial diet, ALT: alum coagulated tofu, LET: lemon coagulated tofu, SWT: steep water coagulated tofu, LPT: lemon peel ash coagulated tofu.

Hematological parameters

The results of hematological parameters of the rats studied are presented in Table 2. The results revealed that the PVC, RBC, Hb, MCV, and WBC levels of rats subjected to

different locally prepared tofu are higher significantly ($p<0.05$), compared to the groups treated with commercial diet (Table 2). Hence, the rats served with steep water coagulated tofu recorded high hematological parameters with PVC (45.1±0.40%), RBC (5.71±0.05 nm²), Hb (13.7±0.20 g/dl), MCV (92±2.80 fl) and WBC (4500±5.42 mm³), followed by the rats treated with lemon extract coagulated tofu and lemon peel coagulated tofu with PVC (42.0±0.38 and 42.2±.37%), RBC (4.97±0.04 and 5.00 nm²), Hb (12.8±0.15 and 12.8±0.12 g/dl), MVC (85±2.72 and 85±2.70 fl) and WBC (3500±5.31 and 3500±5.35 mm³), respectively, while rats fed with alum coagulated tofu had the least hematological values with PVC (38.2±0.37%), RBC (4.81±0.04 nm²), Hb (12.5±0.12 g/dl), MCV (82±2.61 fl) and WBC (3200±5.28 mm³), Table 2.

Table 2: Hematological parameters of albino rats fed with different coagulated tofu

Sample	PVC (%)	RBC ($\times 10^6/\text{nm}^2$)	Hb (g/dl)	MCV (fl)	WBC (mm ³)
CD	35.1±0.35 ^d	4.20±0.02 ^d	11.6±0.12 ^d	80±2.50 ^d	3000±5.25 ^d
ALT	38.2±0.37 ^c	4.81±0.04 ^c	12.5±0.12 ^c	82±2.61 ^c	3200±5.28 ^c
LET	42.0±0.38 ^b	4.97±0.04 ^b	12.8±0.15 ^b	85±2.72 ^b	3500±5.31 ^b
SWT	45.1±0.40 ^a	5.71±0.05 ^a	13.7±0.20 ^a	92±2.80 ^a	4500±5.42 ^a
LPT	42.2±0.37 ^b	5.00±0.04 ^b	12.8±0.12 ^b	85±2.70 ^b	3500±5.35 ^b

Values are presented in means±standard deviations (SD) of the replicates. Means followed by the same superscript(s) in the same column are not significant different ($p>0.05$). CD: Commercial diet, ALT: alum coagulated tofu, LET: lemon coagulated tofu, SWT: steep water coagulated tofu, LPT: lemon peel ash coagulated tofu, PVC: packed cell volume, RBC: red blood cells, Hb: hemoglobin, MCV: mean corpuscular volume, WBC: white blood cells.

Biochemical parameters

The results of biochemical parameters of this study are presented in Table 3. The results showed that there was a significant ($p>0.05$) decreased in the concentration of malondialdehyde (MDA) content in the rats fed with tofu compared to those fed with commercial diet (Table 3). However, recorded values of MDA among the tofu fed rats were significantly different from each other, with the rats fed with alum coagulated tofu

recorded high value of MDA (2.4±0.01mmol/mg/min), followed by lemon extract coagulated tofu and lemon peel ash coagulated tofu with 2.1±0.03 and 2.0±0.01mmol/mg/min, respectively, while steep water coagulated tofu (1.5±0.01 mmol/mg/min) had the least value of MDA content (Table 3).

The rats fed with locally prepared tofu had the higher significant levels of SOD, CAT, and GST compared with those fed with commercial diet, Table 3. Higher values of SOD, CAT, and GST (24.0±1.12, 4.2±0.02 and 100±3.00 mmol/mg/min), respectively, were observed in rats fed with steep water tofu, followed by lemon extract tofu (21.3±1.00, 3.3±0.01 and 94±2.30 mmol/mg/min) and lemon peel ash tofu (20.9±1.00, 3.4±0.01 and 86±2.50 mmol/mg/min), respectively, fed rats (Table 3).

In contrast, the ratio of SOD/CAT in rats fed with locally prepared tofu; alum tofu (6.47±2.00 mmol/mg/min), lemon extract tofu (6.97±2.02 mmol/mg/min), steep water tofu (5.71±1.03 mmol/mg/min), and lemon peel ash tofu (6.10±2.10 mmol/mg/min) were significantly ($p>0.05$) decrease compared to those fed with commercial diet (8.24±1.20 mmol/mg/min), Table 3.

Table 3: The biochemical parameters of albino rats fed with different coagulated tofu

Sample	MDA (mmol/mg/min)	SOD (mmol/mg/min)	CAT (mmol/mg/min)	GST (mmol/mg/min)	SOD/CAT ratio
CD	3.0±0.01 ^a	20.6±1.22 ^d	2.5±0.01 ^d	65±2.00 ^d	8.24±1.20 ^a
ALT	2.4±0.01 ^b	20.7±1.01 ^c	3.0±0.03 ^c	85±3.11 ^b	6.47±2.00 ^c
LET	2.1±0.03 ^c	21.3±1.00 ^b	3.3±0.01 ^b	94±2.30 ^b	6.97±2.02 ^b
SWT	1.5±0.01 ^d	24.0±1.12 ^a	4.2±0.02 ^a	100±3.00 ^a	5.71±1.03 ^d
LPT	2.0±0.01 ^c	20.9±1.00 ^b	3.4±0.01 ^b	86±2.50 ^b	6.10±2.10 ^b

Values are presented in means±standard deviations (SD) of the replicates. Means followed by the same letter(s) in the same column are not significant different. CD: Commercial diet, ALT: alum coagulated tofu, LET: lemon coagulated tofu, SWT: steep water coagulated tofu, LPT: lemon peel ash coagulated tofu, MDA: malondialdehyde, SOD: superoxide dismutase, CAT: catalase, GST: Glutathione S-transferase.

DISCUSSION

The observed insignificant difference in total feed intake and weight gain in rats fed with tofu curdled with alum, lemon extract, and lemon peel extract might be because of good taste and aroma of tofu made from local sources. However, the lower feed intake in rats fed with steep water tofu could be because of the unpleasant odor imparted by waste water to the tofu whereas lower weight gain may be due to low feed intake as stated earlier. These findings are in line with the reports of Yakubu et al. (2013) that rats fed with all tofu curdled with different coagulants experienced lower feed consumption and weight gain than the rats fed with basal diet. The differences in tofu consumption by rats could be as a result of difference in the taste, nutritional quality, and acceptability of the various coagulated tofu (Yakubu et al., 2013).

Furthermore, increased levels of hematological parameters (PVC, RBC, Hb, and WBC) in rats

fed with various tofus produced using different coagulants compared to those fed with commercial diet suggest that all the tofus could have potential effect in managing hemolytic anemia. Even though the mechanism of managing hemolytic anemia cannot be categorically stated, but could be attributed to the present of high antioxidant compounds in soy product, which serve as an extracellular neutralizer of ROS (Anderson and Theron, 1990). Soy food and vegetables have been reported to be riched in many phenols such as flavonoid (Yakubu and Alhassan, 2016). These findings are in line with that of Yakubu (2006) that significant increase ($p<0.05$) of PVC, RBC, and Hb contents were observed in rats fed with tofus curdled with various coagulants. Likewise, the significant increase of white blood cells count observed in rats fed with tofu curdled with different coagulants compared to that of rat fed

with commercial diet could reflect, possibly, immunomodulatory effects of the tofu produced from various coagulants.

This corroborates with the findings of Bashir *et al.* (2015) that soy products have potent immunomodulatory effects, which augmented the production of more WBC. High level of WBC will increase the animal's capability of generating antibodies in the process of phagocytosis and have high degree of resistance to diseases and enhance adaptability to local environmental and disease prevalent conditions (Okunlola *et al.*, 2012). Interestingly, rats fed with steep water coagulated tofu recorded higher levels of WBC compared to the rats fed CD and other tofu, which may reflect the high content of organic compounds, especially, antioxidants (ascorbic acid, phenols and flavonoid) in steep water coagulated tofu than others. Yakubu *et al.* (2013), suggested that high antioxidant activity of waste water coagulated tofu could be resulted from some extraneous substances from waste water.

Peroxidation, which is a measurement of malondialdehyde (MDA) content, was significantly lower in rats treated with tofu produced using different coagulants as compared to the MDA content of rats fed with commercial diet (CD). The decreased in MDA contents in rats fed with tofu may signify that tofu prepared using different coagulants could alleviate the protective mechanisms against oxidative stress. Alleviated defense mechanisms against oxidative stress as stated above could be due the presence of ascorbic acid, phenols, and flavonoid and breakdown of antinutrients, such as tannins during the process of coagulation. Tofu was reported to have high potential compounds that are valuable antioxidants and protecting molecules against peroxidation (Rekha and vijayalakshmi, 2010, Yakubu *et al.*, 2013). Surprisingly, rats served with steep water coagulated tofu recorded lower peroxidation among the other experimented rats, which may reflect the high content of natural antioxidants (ascorbic acid, phenols and flavonoid) in steep water coagulated tofu than others. Yakubu *et al.* (2013) stressed that high protein content, soluble phenol and antioxidant activity in waste water coagulated tofu were resulted from some extraneous substances from waste

water. These results revealed that tofu produced from locally sourced coagulants exhibited good antioxidant activity and could prevent lipid peroxidation.

Elevated activity of the antioxidant enzymes evaluated suggests that tofu produced from locally sourced coagulants could play important role in defense mechanisms against oxidative stress and membrane peroxidation. The activity of antioxidant enzymes (SOD, CAT, and GST) evaluated in rats fed with different tofu produced from locally sourced coagulants increase and protect the rats from peroxidation: as this finding suggests that the defense mechanisms were enhanced. Of the four tested tofu, steep water tofu (SWT) showed the highest levels of antioxidant enzymes than the others. This could reflect the fact that SWT possesses high content of natural organic compounds (ascorbic acid, phenols and flavonoid) than others. Yakubu *et al.* (2013) have suggested that high protein content, soluble phenol and antioxidant activity in waste water coagulated tofu were resulted from some extraneous substances from waste water.

Decreased SOD/CAT ratios in rats subjected to various tofus signified that tofu produced from locally sourced coagulants did not favor the accumulation of H_2O_2 , since O_2 cannot be converted to H_2O_2 and higher H_2O_2 was metabolized to H_2O due to the increased SOD and CAT levels, respectively, as demonstrated earlier. Halliwell (2007), suggests that decrease in the SOD/CAT ratios reduced the formation of H_2O_2 from O_2 and enhanced the degradation of H_2O_2 to H_2O . These observations indicate that various tofu produced using different coagulants are good source of antioxidants and may be used to reduce biological oxidative stress and prevent cellular damage by neutralizing reactive oxygen species (ROS) (Yakubu *et al.*, 2013). Low SOD/CAT ratio observed in rats fed with steep water tofu compared to others could reflect the high content of antioxidant compounds. It was reported that high protein content, soluble phenol and antioxidant activity were experienced in waste water coagulated tofu than others (Yakubu *et al.* 2013).

Conclusion

In conclusion, the present study reveals that tofu prepared from various coagulants could be used in the management of hemolytic anemia. Their consumption should be encouraged in diets as it can be used as a functional food in the management of oxidative stress and membrane peroxidation caused by ROS due to its antioxidant properties.

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