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Biochemical effects of some preservatives on *Glycine max* in adult male albino rats

Chibuzo Carole Nweze *, Semiu O Ajiboso, Happy Abimiku Manasseh, Rahima Yunusa, Wandoo Tseaa, Eneh William Nebechukwu and Adedipe Lateefat Bisola

Department of Biochemistry and Molecular Biology, Faculty of Natural and Applied Sciences, Nasarawa State University, Keffi, Nigeria.

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Abstract

The food insecurity problem in Nigeria is largely due to the inability to preserve food surpluses after harvest. Post-harvest loss of grains account for between 5-25% forcing farmers' use of some pesticides. This study examines the Biochemical effects of some preservatives on *Glycine max* in adult male albino rats. *Glycine max* (soya beans) sample was collected from Keffi Area of Nasarawa state, divided into five groups and preserved with Aluminum Phosphide, Dichlorvos (sniper), Wood-ash and Pepper while the fifth group was kept without preservative. A total of 36 adult male albino rats were obtained from the animal house of the Federal University of Agriculture, Makurdi, and acclimatized for two weeks. They were separated into the group of six (6) each and fed with a dietary intervention for 8 weeks. Group 1 (normal control) was animals administered Feed+ Water, *max* without preservative, Group 2 animals administered Feed+Water+Feed+*Glycine max* preserved with Aluminium Phosphide, Group 3 animals administered Feed+Water+*Glycine max* preserved with Sniper, Group 4 animals administered Feed+ Water+ *Glycine max* preserved with Pepper, Group 5 animals administered Feed+ Water+ *Glycine max* preserved with Wood-ash and Group 6 animals administered Feed+ Water+ *Glycine max.* The liver, kidney functions, lipid profiles and presence of C-reactive proteins were evaluated according to standard methods. The results showed statistically significance at $p \leq 0.05$ with group fed *Glycine max* with preservative having the highest (145.383 ± 3.300^{b}) serum sodium concentration. There were elevated urea, creatinine, AST, ALT, and C-reactive protein concentration amongst the test groups for most of the preservatives. The study concludes that common preservatives used in the preservation of *Glycine max* have significant effects on serum biochemical parameters. Hence, study recommends further research to determine residual content of preservatives after grains preservation.

Keywords: Aluminium phosphide; Dichlorvos, pepper; Wood-ash; *Glycine max*

1 Introduction

Legumes are plants belonging to the family Leguminosae also called Fabaceae that produce seeds within a pod [1] [2]. Common legumes used for human consumption includes soya beans, kidney beans, lima beans, peas, broad beans, lentils, lupins, lotus, sprouts, mung bean, green beans and peanuts and are referred to as grain legumes or food legumes [3]. These seeds are valued worldwide as an inexpensive meat alternative and are considered the second most important food source after cereals [2]. Legumes are very essential especially in terms of nutrition. They contain the essential amino acids that are needed for building proteins, complex carbohydrates, dietary fibre, unsaturated fats, vitamins, and essential minerals for the needed for human diet [4][5].

Soybean, *Glycine max* (L.) Merrill, is a legume belonging to the botanical family *Leguminosae* and subfamily of *Papilionideae* [6]. It grows in warm temperatures with the optimum at 25°C and rainfall of 500–900 mm. Depending on maturity, soybean varieties can be early or late, being harvested within 120–130 days [7]. Globally, a total of 336.7

* Corresponding author: Chibuzo Carole Nweze

million metric tons of soybean were produced in 2017/2018 and the United States produced 119.52 million metric tons [8]. In Africa, a paltry 2.1 million metric tons were produced in 2016 [9].

The major reason behind their cultivation is mainly for beans and useful in providing protein which is used as substitutes in different dairy products. They are rich source of minerals and vitamin B and considered one of the most important sources of livestock feeds. Also, they have been associated with the reduced risk of breast, endometrial and prostate cancer as per data from American Cancer Society [10] [11]. The consumption of soy-food such as tofu was reported to reduce the risk of breast cancer [12].

Despite the usefulness and benefits of *Glycine max*, they have continued to face storage problem resulting in post-harvest losses, spoilage from weevil and other insect attacks as well as economic loses. To avert the trend in post-harvest loss of *Glycine max*, farmers and grain/legumes marketers are relying on chemical preservatives. While this reliance may solve the storage problems and post-harvest loss of crops to pest and weevil, there is also the need to be concerned about their safety to the health of the consumers and the food ecological systems.

The use of pesticides in grains storage becomes more intensive in Nigeria especially across grain markets and in households. The grain farmers and traders' ignorance about pesticide toxicity led to its misuse: abusive application, inadequate usage, among other problems Dichlorvos and Aluminum phosphide are common classes of insecticides referred to as organophosphates used to control households and stored products against insects. It is effective against mushroom flies, aphids, spider mites, caterpillars, thrips, and white flies in greenhouse, outdoor fruits, and vegetable crops [13].

The aim of the study is to investigate the biochemical effect of some preservatives such as Aluminium phosphide, sniper, pepper and wood-ash on *Glycine max* in male adult albino rats.

(Source: alamy.com)

Figure 1 *Glycine max*

2 Material and methods

The samples were identified in the department of Plant science and Biotechnology, Faculty of Science, Nasarawa University Keffi, Nigeria. Glycine max was obtained from Keffi Local Government Area of Nasarawa State, Keffi, Nigeria. *Glycine max* was cleaned and sorted to remove stones and dirt. The chemicals Aluminium phosphide and dichlorvos (sniper) were obtained from a standard agro-allied store in keffi, Nasarawa state. Fresh birds eye pepper was purchased from keffi market and sun-dried. The stem from neem tree was obtained and burnt to ashes. After cooling, wood-ash was sieved to removed dirt and 300 g was weighed and packed in a clean nylon bag. *Glycine max* was divided into five groups weighing 1000 g each. Each group was put in a clean bucket with air-tight lid. The first to the fifth bucket contained *Glycine max* and 4 tablets of Aluminium phosphide, *Glycine max* and sniper, *Glycine max* and pepper, *Glycine max* and wood-ash, *Glycine max* without preservative respectively. The seed was stored for a period of 6 months and keenly observed for any physical change.

Each treatment was milled into powder with a petrol-grinding machine and sealed in cleaned nylon bags and labelled. A total of 36 male adult wistar albino rats weighing between 200-250 g were used for this study. They were purchased from the animal house of University of Jos. They were housed in clean aerated cages, maintained under normal conducive environmental conditions, and left to acclimatize for two weeks prior to experiment. Standard Pellets were used as a basal diet during the experimental period. The control group and the treatment groups were provided with portable water.

The 36 adult male albino rats were randomly divided into six groups each containing 6 albino rats. Group I (normal control) was administered Feed+Water, Group II was administered Feed+Water+*Glycine max* preserved with Aluminium Phosphide, Group III was administered Feed+Water+*Glycine max* preserved with Sniper, Group IV was administered Feed+Water+*Glycine max* preserved with Pepper, Group V was administered Feed+Water+*Glycine max* preserved with Wood-ash and Group VI was administered Feed+Water+*Glycine max* without preservative.

The treated rats were sacrificed after 2 months. Blood serum samples of each rats were collected and labelled as grouped and afterwards taken to the laboratory for assay.

Urea concentration was determined using the method of [14] as described in Randox Kit. Using the principle where urea in serum is hydrolyzed to ammonia in the presence of urease. The ammonia is then measured spectrophotometrically.

To determine kidney function, the serum creatinine concentration was determined using the method of [14] as outlined in Randox kit with the principle where creatinine in an alkaline solution reacts with picric acid to form a coloured complex. The amount of the coloured complex formed is directly proportional to the creatinine concentration.

Sodium ion was determined using the method of [15] and [16] as outlined in Teco Kit. Sodium is precipitated as the triple salt, sodium magnesium uranyl acetate with the excess uranium then being reacted with ferrocyanide, producing a chromophore whose absorbance varies inversely as the concentration of sodium in the test specimen.

Potassium ion concentration was determined using the method of [17] as described in Teco diagnostic kit. The amount of potassium was determined by using Sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension, the turbidity of which was proportional to potassium concentration in the range of 2-7 mEq/L.

Serum Alanine Aminotransferase (ALT) activity was determined using Agappe diagnostic kits P-PCT-1325 [18]. ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine. The colour intensity is measured against the blank at 540 nm.

To determine liver function, serum Alanine Aminotransferase (ALT) activity was determined using Agappe diagnostic kits P-PCT-1325 [18]. ALT is measured by monitoring the concentration of pyruvate hydrazone formed with 2, 4-dinitrophenylhydrazine. The colour intensity is measured against the blank at 540 nm.

Serum AST was determined using Agappe diagnostic kit with Lot number P-PCT-1113 [18]. AST was measured by monitoring the concentration of oxaloacetate hydrazone formed with 2, 4-dinitrophenylhydrazine. The color intensity is measured against the blank at 546 nm.

Cholesterol is measured to assess patients risk to coronary heart diseases (CHD). Cholesterol concentration was determined using the method described by [19]. Cholesterol was determined after enzymatic hydrolysis and oxidation. The indicator quinoneimine is formed from hydrogen peroxidase and 4-aminoantipyrine in the presence of phenol and peroxidase. Low density lipoprotein concentration is determined to diagnose diseases associated with coronary heart diseases. LDL-C can be determined as the difference between total cholesterol and the cholesterol content of the supernatant after precipitation of the LDL fraction by polyvinyl sulphate (PVS) in the presence of polyethyleneglycol monomethyl ether.

High density lipoprotein concentration is determined to diagnose diseases associated with coronary heart diseases. LDL and VLDL (low and very low-density lipoproteins) are precipitated from serum by the action of a polysaccharide in the presence of divalent cations. Then, high density lipoproteins (HDL) present in the supernatant is determined.

Triacylglycerols measurements are used in the diagnosis and treatment of diseases involving coronary heart diseases and peripheral atherosclerosis. The triacylglycerols are determined after enzymatic hydrolysis with lipases. The indicator is a quinonimines formed from hydrogen peroxide, 4-aminophenazone and 4-chlorophenol under the catalytic influence of peroxidase. The C-Reactive Protein test is based on the principle of the latex agglutination [20]. When latex

particles complexes, human anti-CRP are mixed with a patient's serum containing C reactive proteins, a visible agglutination reaction will take place within 2 minutes. This test was carried out according to the method outlined in Welcome Diagnostics kits' directive for the qualitative and quantitative determination of CRP.

2.1 Statistical Analysis

The computer program Statistical Package for Social Sciences (SPSS) version 2.2.0 was used to conduct statistical analysis, which included one-way analysis of variance (ANOVA) and Duncan multiple range test (DMRT). A 95% confidence interval was used to examine the data. At $p \geq 0.05$, significant differences between means were identified, and data were presented as mean SEM.

3 Results and Discussion

Table 1 Effects of Selected Preservatives on Kidney Function Parameters in Blood Serum of Albino Rats Fed with Preserved *Glycine max* Mixed with Vital-Feed

Groups	Na (mmol/L)	K (mmol/L)	Urea (mg/dL)	Creatinine (mmol/L)
Control	128.437±0.716^{a}	7.055±0.659^{a}	5.413±0.390^{ab}	2.000±0.471^{ab}
G. max +AlP	128.323±1.151^{a}	13.085±1.043^{c}	8.607±0.223^{e}	2.625±0.609^{ab}
G. max +Sniper	132.925±1.105^{a}	7.248±0.695^{a}	6.382±0.336^{c}	5.632±0.283^{c}
G. max +Pepper	131.203±0.412^{a}	7.008±0.654^{a}	6.097±0.309^{bc}	1.752±0.327^{ab}
G. max +Wood-ash	129.463±0.757^{a}	9.395±0.447^{b}	7.340±0.272^{d}	2.875±0.420^{b}
G. max without preservative	145.383±3.300^{b}	7.012±0.172^{a}	4.845±0.273^{a}	1.458±0.146^{a}

Note: Glycine max preserved with different preservatives was mixed with vital-feed in all groups, except for the control group. Six albino rats were used in each group and the mean serum mineral and proteins was calculated and reported as mean ± standard errors of means (SEM). Column with different alphabetic superscript were statistically significant at $p \leq 0.05$ confidence level, while column with the same alphabetic superscript were not significant.

The results for the analysis of kidney function showed values that are reported in table 1. The result shows that the levels of Na^{+} was elevated and there was statistical significance when group *G. max* without preservative (145.383±3.300^{b}) was compared against the control group (128.437±0.716^{a}). There were no significant difference when the other groups were compared against control.

The levels of K^{+} did reveal statistically significant difference between the control group (7.055±0.659^{a}), *G. max*+AlP (13.085±1.043^{c}) and *G. max*+wood-ash (9.395±0.447^{b}). The results did not reveal any difference that was statistically significant between the control group and the remaining groups.

The results for urea analysis recorded statistically significant differences between control group compared against all the other groups.

Creatinine levels revealed that the control group (2.000±0.471^{ab}) was statistically different to *G. max*+sniper (5.632±0.283^{c}), *G. max*+wood-ash (2.875±0.420^{b}**)** and *G. max* without preservative (1.458±0.146^{a}**).** The result showed no statistically significant difference between control group and the remaining groups when compared.

The result in table 2 presents the assessment of the changes in the serum levels of liver enzymes of the experimental animals. The results revealed elevated and significantly different levels of serum Aspartate aminotransferase (AST) at $p \leq 0.05$ when control group (15.443±0.770^{a}) is compared to *G. max*+AlP (20.217±0.452^{c}**),** *G. max* +Pepper (17.543±0.436^{b}), *G. max* +Wood-ash (17.635±0.339^{b}) and *G. max* without preservative (15.898±0.735^{ab}). The result did not record any statistically significant difference when control group was compared against group *G. max* +Sniper (15.097±0.770^{a}).

The levels of Alanine aminotransferase (ALT) were also reported in table 2. The control group showed a significantly different level of serum ALT (15.085±0.513^{b}) at $p \leq 0.05$ when compared to the serum levels of ALT in groups *G. max* + AlP (12.683±0.512^{a}**),** *G. max* + Wood-ash (18.372±0.380^{c}**)** and *G. max* without preservative (17.502±0.559^{c}**)**. The serum ALT levels of group *G. max* +Sniper (14.737±0.513^{b}**)** and *G. max* + pepper (14.260±0.594^{b}) did not show any statistically significant difference when compared to the control group.

Table 2 Effects of Selected Preservatives on Liver Function Enzymes of Albino Rats Fed with Preserved *Glycine max* Mixed with Vital-Feed

Groups	AST (IU/L)	ALT (IU/L)
Control	15.443±0.770^{a}	15.085±0.513^{b}
G. max+AlP	20.217±0.452^{c}	12.683±0.512^{a}
G. max +Sniper	15.097±0.770^{a}	14.737±0.513^{b}
G. max +Pepper	17.543±0.436^{b}	14.260±0.594^{b}
G. max +Wood-ash	17.635±0.339^{b}	18.372±0.380^{c}
G. max without preservative	15.898±0.735^{ab}	17.502±0.559^{c}

Note: Glycine max preserved with different preservatives was mixed with vital-feed in all groups, except for the control group. Six albino rats were used in each group and the mean serum AST and ALT were calculated and reported as mean ± standard errors of means (SEM). Column with different alphabetic superscript were statistically significant at $p \leq 0.05$ confidence level, while column with the same alphabetic superscript were not significant

Table 3 Effects of Selected Preservatives on Blood Serum Lipid Profile of Albino Rats Fed with Preserved *Glycine max* Mixed with Vital-Feed

Groups	Cholesterol (mg/dL)	TAG (mg/dL)	LDL-C (mg/dL)	HDL-C (mg/dL)
Control	89.115±0.753^{c}	105.972±1.498^{c}	67.093±0.594^{c}	20.658±1.359^{b}
G. max+AlP	80.447±1.429^{a}	101.392±0.435^{a}	63.345±0.873^{a}	19.880±1.146^{b}
G. max +Sniper	86.713±0.383^{bc}	106.153±0.433^{c}	62.917±0.706^{a}	12.072±0.909^{a}
G. max+Pepper	83.423±0.382^{abc}	104.653±0.479^{bc}	68.537±0.254^{c}	20.795±0.507^{b}
G. max+Wood-ash	82.343±4.165^{ab}	106.648±0.650^{c}	66.632±0.653^{bc}	21.482±1.014^{b}
G. max without preservative	80.667±1.052^{a}	102.712±1.424^{ab}	64.568±1.099^{ab}	22.001±1.885^{b}

Note: Glycine max preserved with different preservatives was mixed with vital-feed in all groups, except for the control group. Six albino rats were used in each group and the mean serum Cholesterol, TAG-C, LDL-C, and HDL-C were calculated and reported as mean ± standard errors of means (SEM). Column with different alphabetic superscript were statistically significant at $p \leq 0.05$ confidence level, while column with the same alphabetic superscript were not significant.

The result in table 3 presents the analysis of the changes in cholesterol level in the serum of experimental animals. The result presented shows statistically significant difference in cholesterol concentration in control group (89.115±0.753^{c}) at $p \leq 0.05$ when compared to all the other groups.

The triglyceride concentration assessment also recorded in table 3 shows statistically significant difference at $p \leq 0.05$ when control group (105.972±1.498^{c}) was compared against *G. max* + AlP (105.972±1.498^{c}**),** *G. max* + pepper (104.653±0.479^{bc}**)** and *G. max* without preservative (102.712±1.424^{ab}**)**. The result did not record statistically significant difference when control group was compared against *G. max* + sniper (106.153±0.433^{c}) and *G. max* + wood-ash (106.648±0.650^{c}**).**

The levels of low-density lipoprotein cholesterol LDL-C reveals that there was no statistically significant difference at $p \leq 0.05$ when control group (67.093±0.594^{c}**)** was compared against group *G. max* + pepper (68.537±0.254^{c}**).** However, there was statistically significant difference when control group was compared against group *G. max* + AlP, *G. max* + sniper, *G. max* + wood-ash and *G. max* without preservative.

The result for high-density lipoprotein cholesterol HDL-C records statistically significant difference at $p \leq 0.05$ when control group (20.658±1.359^{b}**)** was compared against group *G. max* + sniper (12.072±0.909^{a}) alone. It did not record any statistically significant difference when control was compared against all the other groups.

Table 4 Effects of Selected Preservatives on Serum C-Reactive Proteins (CRP) of the Albino Rats Fed with preserved *Glycine max* mixed with Vital-feed

Groups	**CRP (ug/mL)**
Control	62.955 ± 1.835^{b}
G. max+AlP	56.243 ± 1.521^{a}
G. max+Sniper	63.660 ± 1.487^{b}
G. max+Pepper	55.922 ± 1.241^{a}
G. max+Wood-ash	68.068 ± 0.485^{c}
G. max without preservative	72.083 ± 1.293^{d}

Note: soya beans preserved with different preservatives was mixed with vital-feed in all groups, except for the control group. Six albino rats were used in each group and the mean serum C-reactive protein was calculated and reported as mean ± standard errors of means (SEM). Column with different alphabetic superscript were statistically significant at $p \leq 0.05$ confidence level, while column with the same alphabetic superscript were not significant.

Table 4 shows the results for the mean values of the levels of CRP. The table records changes in the values of the CRP in the test groups when compared to the control group. There was a significant decrease in the levels of CRP in groups *G. max* + AlP (56.243 ± 1.521^{a}) and *G. max* + pepper (55.922 ± 1.241^{a}) when respectively compared to the control group (62.955 ± 1.835^{b}). The *G. max* + wood-ash group (68.068 ± 0.485^{c}) and group *G. max* without preservative (72.083 ± 1.293^{d}) recorded significantly higher levels of CRP when compared to the control group (62.955 ± 1.835^{b}). There was no statistically significant difference when the control group was compared against group *G. max* + sniper (63.660 ± 1.487^{b}**).**

This study found no significant difference in the serum Na^{+} concentration of albino rats fed with feeds containing *Glycine max* preserved with Aluminum phosphide, sniper, pepper and ashes and the control group. This finding reveals that the preservatives have no effects on the serum sodium, however, there was a significant difference between the group fed *Glycine max* with no preservatives which was higher in the control and the other groups. The elevated serum Na^{+} concentration found in the unpreserved soya could be linked to high content of sodium in soya beans. The study conducted by [21] found that soya beans contained as high as 2.7 to 3 g of Na^{+} per 100 g which can supported this claim. The potassium level was statistically significantly higher in the group administered *Glycine max* preserved with a luminium phosphide and group administered *Glycine max* preserved with wood-ash as against the control group which implies that the aluminium phosphide and wood-ash preservatives could influence kidney impairment. The serum urea level was statistically significantly higher in the test groups compared to the control group except for the group administered Glycine max without preservative, which was statistically significantly lower than the control group. Elevated urea level can be as a result of the kidney's inability to excrete urea which implies damage to the kidney. This study also found an elevated serum creatinine level in the group administered *Glycine max* preserved with sniper, which was statistically significant. This implies sniper increased serum creatinine that suggests kidney damage. [22] made similar assertion, adding that elevated creatinine can also indicate injury or damages to the kidney or other organs. Also, the serum creatinine concentration was significantly lower in group *G. max* without preservative when compared with the control group. This suggests that *G. max* has nutritional properties that lowers creatinine concentration. These findings indicate that the preservatives increased serum creatinine level which according to [23] and [24] could be a risk factor of cardiovascular diseases or sign of damage to the kidney and/or chronic kidney diseases.

Similarly, this study found a significant increase in the serum aspartate transaminase level amongst all the groups, except for the group fed *Glycine max* preserved with sniper. This finding is like the finding of [25] and indicated there was no damage to the liver or kidney or the sniper had AST hypo-enzymatic properties.

The groups preserved with aluminium phosphide, wood-ash and pepper elevated serum AST at statistically significant level as serum AST was highest in the group fed with *Glycine max* preserved with aluminium phosphide, followed by the group fed *Glycine max* preserved with pepper and wood-ash. Elevated serum AST suggests damage to liver cells, kidney, or its leakage from AST storage organs. [26] made similar finding and pointed to the fact that it indicates liver, kidney or other organ damage/injury as AST is not naturally found in the serum at high concentration. The study found statistically significant elevated serum alanine transferase (ALT) level between the test groups and the control group, except for the group fed with *Glycine max* preserved with sniper and pepper. The group administered *G. max* preserved with AlP was found to lower serum alanine transferase. Lowered serum ALT concentration suggests that the organs

were preserved, or the enzymes were in their bound form. However, [27] stated to the contrary that lowered ALT could mean damage to liver, since the liver is the site for ALT production.

The significantly lowered serum cholesterol concentration found amongst the tests groups in this study suggest atherosclerosis. The similar findings were made for serum triglyceride (TAG), except for group fed with *Glycine ma x* preserved with aluminium phosphide when compared to the control group. However, the study found lowered serum low density lipoprotein cholesterol (LDL-C) in the group fed *Glycine max* preserved with ashes and pepper which had significantly higher serum LDL-cholesterol level when compared with the control group. The group fed *Glycine max* preserved with pepper had the highest serum LDL-cholesterol than the other groups, while the group fed *Glycine max* preserved with sniper had the least level of serum LDL-cholesterol level. Similar findings were made by [28], [29] and [30]. Lowered HDL-cholesterol and high LDL-cholesterol were linked to risk of heart damage or diseases. Hence, the findings of the study suggest that these preservatives could have negative impact on heart health.

C-reactive protein has been classed as an acute inflammation marker. The present study yielded results of the levels of CRP as tabulated in table 4. The consumption of *G. max* has been found to increase the amount of CRP. This study also found statistical significance amongst all the groups including the control group, except the group fed *Glycine max* preserved with aluminium phosphide and pepper. The test groups had significantly higher level of C-reactive proteins concentration in the serum when compared with the control group, which suggest tissue damage or injuries leading to inflammation. Amongst the test groups, the group fed soya beans without preservative 72.083 ± 1.293^{d} had the highest level of C-reactive proteins followed by the group fed soya beans preserved with wood-ash 68.068 ± 0.485^{c}. These observations suggests that soya beans could have properties that can lead to organ damage and elevate the concentration of C-reactive proteins in the serum of the albino rats. The group fed soya beans preserved with aluminium phosphide had the least concentration of C-reactive proteins, which means it lowers the concentration of C-reactive proteins. Elevated level of serum C-reactive proteins observed in this study implies risk of cardiovascular diseases, liver or kidney damage or induced oxidative stress. Similar observations and confirmations were made by [31], [32] and [33].

4 Conclusion

Despite the effectiveness of these pesticides as preservatives in the storage of legumes to prevent pest attack and the benefits associated with the usage of wood ash and pepper. The findings from this study suggest that the selected preservatives used in the experiment showed either significant elevated or lowered biochemical effects on the fed albino rats. According to the findings from the study *G. max* preserved with AlP when consumed showed some level of toxicity in the liver when compared to the control group. Due to the increase in liver enzymes for both the legume without preservatives and the legume preserved with AlP, this effect did reflect on the elevated level of ALT and AST respectively. This suggests that the residual content on the beans may only be harmful to the liver. There was no significant damage to the kidney by the preservatives when compared to the control group. Also, the C-reactive protein from the *G. max* preserved with wood-ash and the *G. max* without preservative showed that there may be severe health implications if the beans are not properly processed before consumption as it increased significantly when compared to the control group. This is to say that, although these preservatives have been useful and often used in small quantity by farmers, they may lead to cellular damage. I recommend the use of airtight containers to preserve soya beans as a way to combat effect of chemical preservatives. Also further study on the residual content of these preservatives on soya beans should be carried out.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of ethical approval

All experimental procedures were made according to Standard Operating Procedure for Institutional Animal Ethics Committee (IAEC).

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