

Full Length Research Paper

Coagulants modulate the hypocholesterolemic effect of tofu (coagulated soymilk)

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The recent increase in soymilk and tofu (coagulated soymilk) consumption especially in western countries is due to the recognition of the health benefits of soy foods. The amount and the type of coagulated biomolecules (such as isoflavones) vary with the type of coagulant, and this will inevitably alter their biological activity. This study sought to assess the effect of some coagulants (calcium chloride, alum and steep water from pap production) commonly used in the production of tofu in Nigeria on the serum cholesterol, high-density lipoproteins (HDL) and low-density lipoproteins (LDL) level in albino rats fed tofu for 14 days. The result of the study revealed that there was no significant difference ($P > 0.05$) in the tofu yield (17.6 – 18.3%), however steep water (12.0 g/kg) had a significantly higher ($P < 0.05$) total phenol content than tofu produced using other coagulants (8.0 – 9.0 g/kg). Furthermore, feeding albino rats with tofu and water *AD LIBITUM* for 14 days caused a significant decrease ($P < 0.05$) in the serum cholesterol and low-density lipoproteins, while there was no significant difference ($P > 0.05$) in the average daily feed intake of the rats. Conversely, there was a significant increase ($P < 0.05$) in the serum high-density lipoproteins when compared with the control. However, rats fed steep water-coagulated tofu had the lowest serum level of cholesterol and LDL followed by those fed CaCl_2 and alum coagulated tofu. Those fed with calcium chloride-coagulated tofu had the highest serum HDL level, closely followed by those fed steep water-coagulated tofu. It was therefore concluded that of all the coagulants, steep water appeared to be the most promising coagulant with regard to the production of tofu with high hypocholesterolemic effect based on the low serum cholesterol, LDL and high HDL.

Key words: Coagulant, soymilk, tofu, phenol, HDL, LDL, cholesterol.

INTRODUCTION

Tofu is one of the most important and popular food products in east and southeastern Asian countries and is gaining an increasing popularity in western countries as well. Tofu is an unfermented soy product which is soft and cheese-like. It is produced by curdling fresh hot soymilk with either salt (CaCl_2 or CaSO_4) or an acid

(glucuno-d-lactone). Traditionally the curdling agent used to make Tofu is calcium sulfate (CaSO_4). The coagulant produces a soy protein gel, which traps water, soy lipids and other constituents in the matrix forming curds. The curds are then pressed into solids cubes (Cao and Chan, 1997; Cao and Chang, 1999).

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The yield and quality of tofu are influenced by soy bean varieties, soybean quality and processing conditions of the coagulants. Coagulation of soymilk is the most important step in the tofu process and the most difficult to master since it relies on the complex interrelationship of the following: soy bean chemistry; soymilk cooking temperature, volume, solid content and pH; coagulant

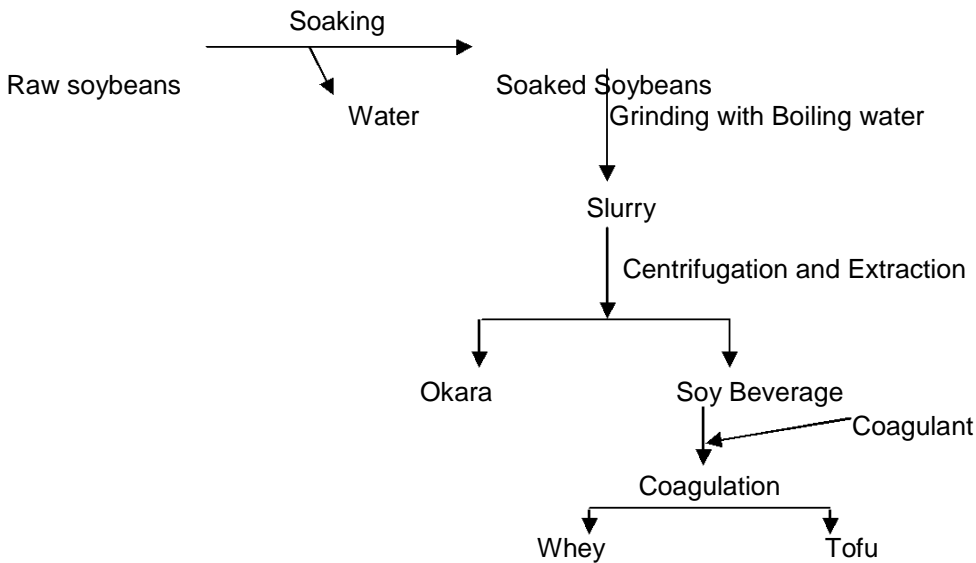


Figure 1. Production of tofu.

type, amount; concentration and the method of adding and mixing; and the coagulation temperature and time (Cao and Chang, 1999). Each coagulant produces tofu with different textural and flavour properties (Poysa and Woodrow, 2001). The texture of tofu should be smooth, firm and coherent but not hard and rubbery. Since tofu is a soy protein gel, the amount of soy protein used to make the soymilk is critical for tofu yield and quality (Poysa and Woodrow, 2001; Jackson et al., 2002).

Isoflavones are a group of naturally occurring heterocyclic phenols found in soybean and its products (Jackson et al., 2002). Isoflavones such as diadzen, genisten, glycitein and their derivatives have been isolated from soybeans and its products. They are also referred to as soy phytoestrogens, and have been credited for performing several health promoting functions (Akio et al., 1997). The lower incidence of certain diseases has been reported in Asian countries where soybean consumption is high with the average intake of isoflavones being about 40-80 mg per day (Jackson et al., 2002). For instance, genistin has been shown to play a protective role in hormonally induced cancers (breast cancer) by acting as an antiestrogen. Tofu also contains these isoflavones but not in quantities as high as those contained in the raw soybean or soy beverage (soy milk). The reduction in isoflavone content of tofu is as a result of the loss during processing. Recent investigation shows that the total recovery of isoflavones in tofu was about 36% based on dry matter (Jackson et al., 2002).

Serum Lipids are fats found in the blood, which are used to determine coronary risk profile, i.e. the serum level of these lipids are indicators of risk for heart disease. Batteries of blood tests are carried out to evaluate serum lipids such as cholesterol, triglycerides, high-density lipoproteins, and low density lipoproteins. Cholesterol is a critical fat and is a structural component

of all membrane and plasma lipoproteins. It is also crucial in the synthesis of steroid hormones, glucocorticoids and bile acids. It is mostly synthesized in the liver although some are also absorbed through the diets especially diets high in saturated fats. Elevated cholesterol has been seen in atherosclerosis, diabetes, hypothyroidism and pregnancy while low levels of these lipids are seen in depression, malnutrition, liver insufficiency and malignancies (Agbedana, 1997). This project therefore sought to assess the effect of various commonly used coagulants in Nigeria on the total phenol content of tofu and the consequential effects on serum cholesterol and lipoproteins (LDL and HDL) of albino rats fed tofu.

MATERIALS AND METHODS

Materials

Soybeans were obtained from the research farm of the Federal University of Technology, Akure, Nigeria. They were stored at room temperature before tofu processing. The calcium salt and alum were industrial grade, while the steep water was collected from a local pap processing industry.

Sample preparation

Soybean (1.0 kg) was soaked in water (6 litres) at 27 - 32°C for 9 h. The soaked beans were drained and ground in the grinder with water. This corresponded to the water to raw bean ratio of 6:1 for extracting solids from soybean into raw milk and brought the total solid content of the soymilk to approximately 11%. The soymilk was subsequently heated to 98°C and maintained for 1 min before delivering to the mixing tank. When cooled to 87°C, soymilk was mixed at 420 rpm with each of the coagulants [CaCl₂.H₂O (20 mM), alum (20 mM) and the steep water (100ml)]. The mixture after mixing was held for 5 s and then filled onto tofu trays and allowed to coagulate for 10 min. The bean curd was pressed and the tofu weight was recorded (Figure 1). The tofu produced was stored in water at 4°C overnight prior to analysis (Cao and Chang, 1999).

Total phenol content

The total phenol content was determined by mixing 0.5 ml aliquot (0.2 g of the sample extracted by 20 ml 70% acetone) with equal volume of water, 0.5 ml Folin-Ciocalteu's reagent and 2.5 ml of sodium carbonate were subsequently added. The absorbance was measured after 40 min at 725 nm (Singleton et al., 1999).

Bioassay

The Bioassay was carried out based on the method reported by Prestamo et al. (2002). Wistar strain albino rats weighing 85-100 g were purchased from Biochemistry Department, University of Ilorin, Nigeria, and acclimatized for 2 weeks during which period they were maintained *ad libidum* on commercial diet. The rats were subsequently divided into four treatment groups. Animals in group 1 were fed the commercial diet (16.0% proteins), while animals in group 2 were fed calcium chloride-coagulated tofu, animals in group 3 were fed alum-coagulated tofu, while animals in group 4 were fed steep water-coagulated tofu *ad libidum*. The experiment lasted two weeks, at the end of which the rats were sacrificed by decapitation after an 18-h fast. The blood was collected and the serum was subsequently prepared. Serum cholesterol, low-density lipoproteins (LDL) and high-density lipoproteins (HDL) were determined by the aid of the Hitachi 705 automated machine.

Analysis of data

The result of the three replicates were pooled and expressed as mean \pm standard error (S.E.). A one way analysis of variance (ANOVA) and the Least Significance Difference (LSD) were carried out. Significance was accepted at $P \leq 0.05$.

Table 1. The yield and total phenol content of tofu.

Coagulant	Yield (%)	Phenol content (g/kg)
Alum	18.3 \pm 0.5 ^a	9.0 \pm 0.6 ^b
Steep water	17.9 \pm 0.3 ^a	12.0 \pm 0.4 ^a
Calcium chloride	17.6 \pm 0.5 ^a	8.0 \pm 0.7 ^b
Value represents mean of triplicate readings		

Values with the same superscript along the same column are not significantly different.

RESULTS AND DISCUSSION

Changes in the serum levels of cholesterol and low-density lipoproteins are considered to be associated with various diseased states; an increase in serum levels of cholesterol and the low-density lipoproteins is associated with hypercholesterolemia and atherosclerosis, respectively. Free radicals have been linked to cardiovascular diseases because of their ability to introduce oxidative damage to biomolecules, for example lipids, DNA and proteins (Halliwell, 1990). Phenols from

various plant materials especially vegetables have attracted a great deal of attention due to their significant antioxidant properties (Sun et al., 2002; Oboh and Akindahunsi, 2004; Oboh, 2005). Soy products have been reported to contain a special class of phenols, isoflavones (Jackson et al., 2002).

The result of the tofu yield is shown in Table 1, the result revealed that there was no significant difference ($P > 0.05$) in the tofu yield by each of the coagulant, however, alum coagulation gave the highest amount of tofu (18.3%), while calcium chloride gave the least yield of tofu. The total phenol content of tofu coagulated with the three different coagulating agents commonly used in Nigeria is also recorded in Table 1.

Phenolic phytochemicals inhibit autoxidation of unsaturated lipids, thus preventing the formation of oxidized low-density lipoprotein (LDL), which is considered to induce cardiovascular disease (Amic et al., 2003). Steep water-coagulated tofu had the highest total phenol content (12.0 g/kg), followed by tofu coagulated by alum (9.0 g/kg). While tofu coagulated with CaCl_2 had the least total phenol content (8.0 g/kg). Amic et al. (2003) has shown that there is a direct relationship between the total phenol content and antioxidant activity, hence steep water-coagulated tofu would have the highest antioxidant activity, followed by alum-coagulated tofu, while CaCl_2 -coagulated tofu would have the least antioxidant activity. The high phenol content in the steep water coagulated tofu could probably be due to the likelihood that some of the phenols present in the steep water might have been transferred into the tofu, as well as the possibility that the steep water coagulated proteins might have trapped more phenols than the other two coagulants. However, there was no significant difference ($P > 0.05$) in the average daily feed intake by the rats fed the various type of tofu, although rats fed calcium chloride coagulated tofu had the highest feed intake (Figure 2), while those fed steep water coagulated tofu had the least feed intake. This lower feed intake by rats fed steep water-coagulated tofu might be due to the odour the steep water might have imparted on the tofu (Oboh and Omotosho, 2005).

Studies carried out by Prestamo et al. (2002) had established the fact that soy and its products effectively lower serum cholesterol and low-density lipoproteins (LDL). The results presented in Figures 3 and 4 agree with the earlier finding by Prestamo et al. (2002) to the extent that there was a significant decrease ($P < 0.05$) in the serum cholesterol and low-density lipoproteins in rats fed tofu coagulated with steep water, calcium chloride and alum when compared with those fed with the commercial diet (control). Tofu may have been able to lower LDL cholesterol by stimulating the hepatic LDL receptor (Agbedana, 1997; Prestamo et al., 2002). The stimulation of the hepatic LDL receptor is related to the

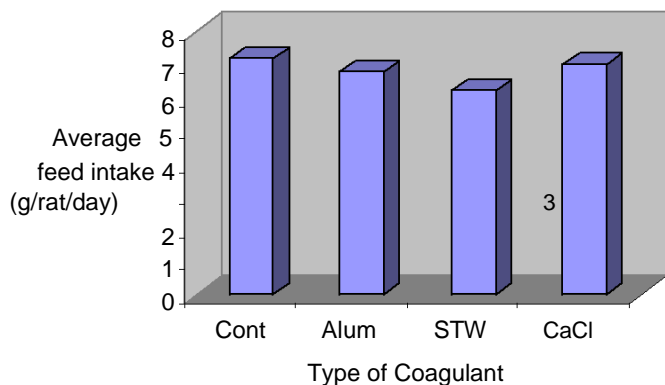


Figure 2. Average daily feed intake by rats fed tofu. Cont: Control; Alum: Alum coagulated tofu. STW: Steep water coagulate tofu. CaCl: Calcium chloride coagulated tofu.

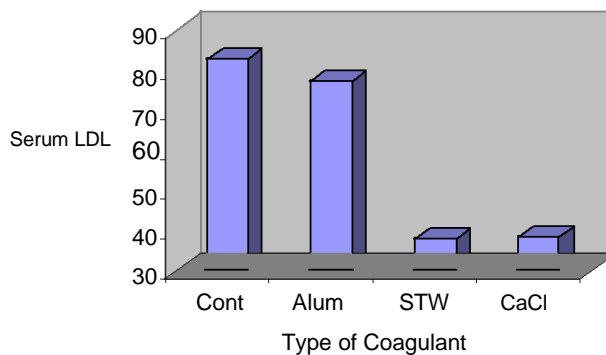


Figure 4. Changes in serum LDL of rats fed tofu. Cont: Control; Alum: Alum coagulated tofu. STW: Steep water coagulate tofu. CaCl: Calcium chloride coagulated tofu.

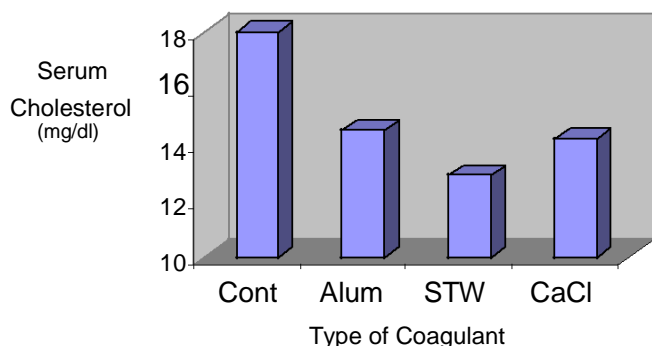


Figure 3. Changes in serum cholesterol of rats fed tofu. Cont: Control; Alum: Alum coagulated tofu. STW: Steep water coagulate tofu. CaCl: Calcium chloride coagulated tofu.

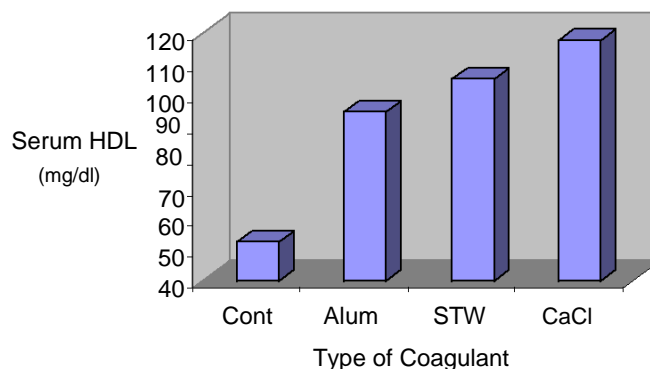


Figure 5. Changes in serum HDL of rats fed tofu. Cont: Control; Alum: Alum coagulated tofu. STW: Steep water coagulate tofu. CaCl: Calcium chloride coagulated tofu.

isoflavones, which resemble the estrogens but contain weak estrogenic activity. Higher levels of estrogens are associated with lower levels of cholesterol. One mechanism proposed for estrogenic effect is also through up-regulation of LDL receptor (Agbedana, 1997; Georgi, 2002).

Although, there was a significant decrease in the serum cholesterol and low-density lipoproteins (LDL) level of rats fed tofu, there was a marked variation in the serum cholesterol and LDL levels of the rats with the type of coagulant used in the production of the tofu. Rats fed with steep water-coagulated tofu had the lowest serum cholesterol and LDL, followed by those fed with calcium chloride-coagulated tofu while those fed alum coagulated-tofu that had the highest serum cholesterol

and LDL levels. This low cholesterol and LDL levels in rats fed steep water-coagulated tofu could be as result of the high total phenol content when compared to alum-and calcium chloride-coagulated tofu that had significantly lower ($P < 0.05$) total phenol contents. This relationship between total phenol content and hypocholesteroleamic effects of the tofu agrees with earlier report by Amic et al. (2003).

The result of the serum levels of high-density lipoproteins (HDL) of rats fed tofu coagulated with steep water, calcium chloride and alum respectively is shown in Figure 5. It revealed that there was a significant increase ($P < 0.05$) in the serum HDL levels of rats fed tofu when compared with those fed the control diet. High serum HDL levels have been proven to protect LDL from

oxidation. High serum levels of HDL are indicative of a healthy metabolic system, if there is no sign of liver disease or intoxication. Thus HDL is sometimes referred to as 'good' cholesterol. Two mechanisms that explain how HDL offers protection against chronic heart disease are: firstly, HDL inhibits cellular uptake of LDL and secondly HDL serves as a carrier that removes cholesterol from the peripheral tissues and transport it back to the liver for catabolism and excretion (Agbedana, 1997; Georgi, 2002).

There was a marked difference in the serum HDL levels of rats fed tofu produced with steep water, alum and calcium chloride respectively. Rats fed calcium chloride-coagulated tofu had the highest serum HDL, followed closely by those fed steep water-coagulated tofu, while those fed alum coagulated-tofu had the least serum levels of HDL. Even though steep water-coagulated tofu showed the greatest effectiveness in lowering serum cholesterol and LDL compared with alum and calcium chloride coagulated tofu, this study also showed that calcium chloride coagulated tofu had the highest serum HDL levels, which is considered as good cholesterol. The basis for the highest serum HDL in rats fed CaCl₂-coagulated tofu despite its low total phenol content when compared to other coagulants is not clear. However, it can be inferred that the total phenols, specifically the isoflavone, may not be responsible alone for the hypocholesterolaemic effect of the tofu. There may have been some other phytochemicals in the tofu, which were more in the CaCl₂-coagulated tofu that may have additive or synergistic effect on the hypocholesterolaemic effect of the total phenol.

In conclusion, steep water appears to be the most promising coagulant with regard to the production of tofu with high hypocholesterolemic effect based on the low serum cholesterol, LDL and high HDL.

REFERENCES

- Agbedana EO (1997). Cholesterol and Your Health, Inaugural Lecture, University of Ibadan, Nigeria. p.50.
- Akio M, Shin-Ichi Y, Hiroshi T (1997). A potent antioxidative and anti-UV-B isoflavonoids transformed microbiologically from soybean components. *Functional Foods for Disease Prevention I*, American Chemical Society, Washington DC 20036 USA. pp. 127 - 138.
- Amic D, Davidovic-Amic D, Beslo D, Trinajstic N (2003). Structure-Radical scavenging activity relationship of flavonoids, *Croatia Chem. Acta*, 2003; 76 (1): 55 – 61.
- Cai TD, Chang KC (1997). Dry tofu characteristics affected by soymilk solid content and coagulation time. *J. Food Qual.* 20: 391 – 402.
- Cai TD, Chang KC (1999). Characteristic of production scale tofu as affected by soymilk coagulation method: Propeller blade size mixing time and coagulant concentrations. *Food Res. Int.* 31: 289-295.
- Georgi E (2002). Soy in the treatment of hypercholesterolemia. <http://www.soysense.com>.
- Halliwell B (1990). How to characterize a biological antioxidant. *Free Raical Res. Commun.* 9: 1- 32
- Jackson CJC, Dini JP, Lavandier C, Rupasinghe HPU, Faulkner H, Poysa V, Buzell D, Dehrandic S (2002). Effect of processing on the content and composition of isoflavones during manufacturing of soy beverage and tofu. *Process Biochem.* 37: 1117-1123.
- Oboh G (2005). Effect of blanching on the antioxidant properties of some tropical green leafy vegetables, *Food Sci. Technology/ LWT* 38(5): 513 – 517.
- Oboh G, Akindahunsi AA (2004). Change in the ascorbic acid, total phenol and antioxidant activity of sun-dried commonly consumed green leafy vegetables in Nigeria. *Nutr. Health.* 18: 29- 36.
- Oboh G, Omotosho OE (2005): Effect of coagulants on the nutritional quality and *In vitro* multienzyme protein digestibility of Tofu. *J. Food Technol.* 3 (2):182 – 187.
- Singleton VL, Orthofer R, Lamuela-Raventos RM (1999). Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagents. *Methods in Enzymol.* 299: 152 – 178.
- Poysa, V and Woodrow, L. (2001) Stability of soybean composition and its effects on soymilk and tofu yield and quality. *Food Res. Int.* 38: 337-345.
- Prestamo G, Lasuncion, MA, Anroyo G (2002). Response of rats to the intake of tofu treated under high pressure. *Innov. Food Sci. Emerging Technol.* 3: 149-155.
- Sun J, Chu Y, Wu X and Liu R (2002). Antioxidant and antiproliferative activities of common fruits. *J. Agric. Food Chem.* 50: 7449 – 7454.