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Coagulants Modulate the Antioxidant Properties and Hypocholesterolemic Effect of Tofu (Curdled Soymilk)

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Abstract: The recent increase in Soymilk and tofu (coagulated soymilk) consumption especially in Western Country is due to the recognition of the health benefits of soy foods; consumption of soybean would prevent heart diseases. In Nigeria Calcium salt, alum and steep water from pap production are usually used as coagulant in tofu production. The effect of those coagulants on the antioxidant properties of tofu and serum cholesterol, High-density Lipoproteins (HDL) and Low-density Lipoproteins (LDL) level of albino rats fed tofu for 14days is been assessed. 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 coagulated tofu had a significantly higher ($p<0.05$) total phenol (12.0 g kg^{-1}) content, reducing power (6.0 OD_{700}) and DPPH free radical scavenging ability (69.1%) than tofu produced using other coagulants. Furthermore, feeding albino rats with tofu and water *ad libitum* for 14days caused a significant decrease ($p<0.05$) in the serum cholesterol and low-density lipoproteins when compared with the control, 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 level followed by those fed $CaCl_2$ and alum coagulated tofu respectively, while those fed with calcium chloride coagulated tofu had the highest serum HDL level and closely followed by those fed steep water coagulated tofu. It was therefore concluded that of all the coagulant, steep water appeared to be the most promising coagulant with regard to the production of tofu with higher antioxidant and hypocholesterolemic effect.

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 state of Asian countries and is gaining an increasing popularity in western countries as well. It was developed some two thousand years ago and has become the world's most popular soy food product. Tofu is an unfermented soy product, also known as soy bean curd and is a soft, cheese-like food produced by curdling fresh hot soymilk with either a 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 Chang, 1997; 1999).

The yield and quality of tofu are influenced by soy bean varieties, soybean quality (growth and storage environment dependent) 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 varieties: soy bean chemistry; soymilk cooking temperature, volume, solid content and pH; coagulant 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, glycetein and their derivatives (glucosidic conjugates which are 9 in numbers) have been isolated from soybeans and products. They are also referred to as soy phytoestrogens and have been credited for performing several health promoting functions. These phytoestrogens have effects on cardiovascular and menopausal health and are noted for cancer prevention. Several investigators have suggested that soy food consumption may contribute to lower rates of certain diseases such as hormone dependent cancers and osteoporosis. Because of resemblances to human estrogen and the observations that Asian population which consume more isoflavones (especially in soy products) compared with women in western countries have less menopausal symptoms, isoflavones are postulated as natural products that may be beneficial to postmenopausal women in cardiovascular health (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 the processing of soybeans into Tofu. Despite the loss in isoflavones, it still contains some amount of isoflavones, which is better than not occurring at all. 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, 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, malignancies etc. (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.

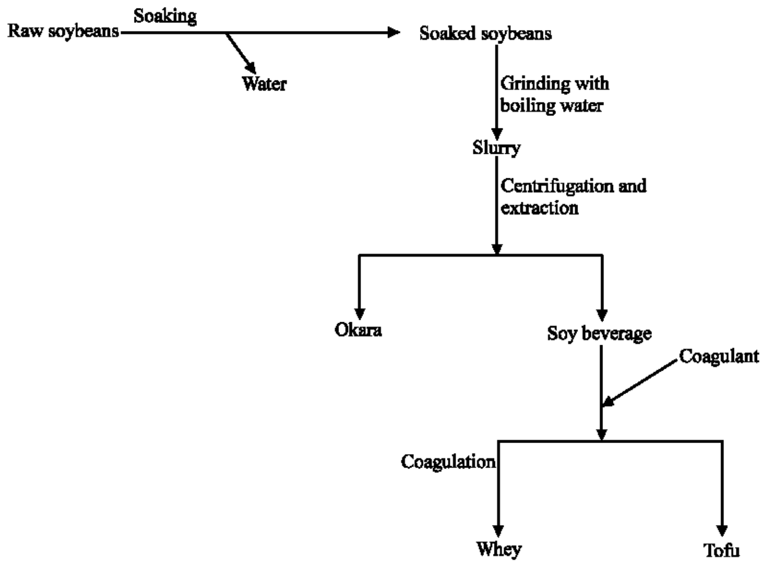

Fig. 1: Production chart of tofu

Methods

Sample preparation

Soybean (1.0 kg) was soaked in water (6 L) 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 respectively with each of the coagulants [$CaCl_2.H_2O$ (20 mM), Alum (20 mM) and the steep water (100 mL]. The mixture after mixing was held for 5 see and then filled onto tofu trays and allowed to coagulate for 10 min. The bean curd was pressed after pressing the tofu weight was recorded (Fig. 1); the tofu produced was stored in water at 4°C overnight prior to analysis (Cao and Chang, 1999).

Sample Analysis

Total Phenol conetnt

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-Cioalteu's reagent and 2.5 mL of Sodium carbonate were subsequently added and the absorbance was measured after 40 min at 725 nm (Singleton *et al.*, 1999).

Reducing Property

The reducing property of the tofu was determined by assessing the ability of the extract to reduce $FeCl_3$ solution. Briefly 2.5 mL aliquot (0.5 g of the extracted by 20 mL 70% acetone) was mixed with 2.5 mL, 200 mM Sodium phosphate buffer (pH 6.6) and 2.5mL of 1% Potassium ferricyanide, the mixture was incubated at 50°C for 20 min, thereafter 2.5 mL, 10% Trichloroacetic acid was added and subsequently centrifuged at 650 rpm for 10 min, 5 mL of the supernatant was mixed with equal volume of water and 1 mL of 0.1% ferric chloride, the absorbance was later measured at 700 nm, a higher absorbance indicates a higher reducing power.

Free Radical Scavenging Ability

The free radical scavenging ability of the tofu against DPPH (1, 1 - diphenyl -2 picrylhydrazyl) free radical was also evaluated (Ursini *et al.*, 1994), briefly, 1mL aliquot (0.5 g of the extracted by 20 mL 70% Acetone) was mixed with 1 mL,0.4 mM methanolic solution containing 1, 1-diphenyl-2 picrylhydrazyl (DPPH) radicals, the mixture was left in the dark for 30 min before measuring the absorbance at 516 nm.

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 and the blood were collected and the serum was subsequently prepared. Serum Cholesterol, Low-density Lipoproteins (LDL) and High-density Lipoproteins (HDL) were determined by the aid of an automated machine called Hitachi 705.

Analysis of Data

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

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 included have been reported to contain a special class of phenols, isoflavones which have antioxidant properties (Jackson *et al.*, 2002).

The result (Table 1) 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 fact that there was no significant difference ($p>0.05$) in the yield indicated that the various coagulants under consideration may not differ substantially in their coagulating ability, however, the slight difference could be as result of extraneous substance introduced by the coagulants. The total phenol content of tofu coagulated with the three different coagulating agents commonly used in Nigeria is shown 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^{-1}),

Table 1: The yield and total phenol content of Tofu.

Coagulant	Yield (%)	Phenol content (g kg^{-1})
Alum	18.3±0.5[a]	9.0±0.6[b]
Steep water	17.9±0.3[a]	12.0±0.4[a]
Calcium chloride	17.6±0.5[a]	8.0±0.7[b]

Value represents mean of triplicate readings, Values with the same superscript along the same column are not significantly different

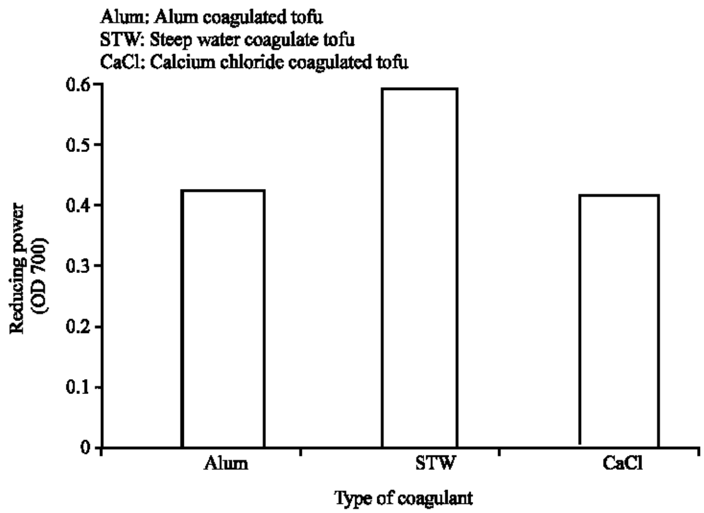

Fig. 2: Reducing power of tofu

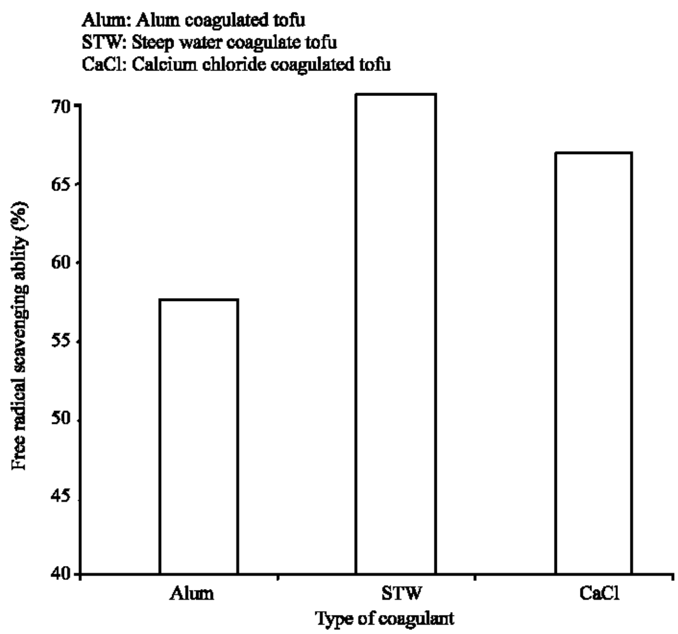

Fig. 3: Free radical scavenging ability of tofu

followed by tofu coagulated by alum (9.0 g kg^{-1}), while tofu coagulated with $CaCl_2$ had the least total phenol content (8.0 g kg^{-1}). Amic *et al.* (2003) has shown that there is a direct relationship between the total phenol content and 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, many reports had establish a correlation between the total phenol content and antioxidant activity of plant food (Sun *et al.*, 2002), this they do by inhibiting oxidation of unsaturated lipids, thus preventing the formation of oxidized Low-density Lipoprotein (LDL), which is considered to induce cardiovascular disease (Amic *et al.*, 2003).

Tofu coagulated using steep water from pap (0.6 OD_{700}) had a significantly ($p<0.05$) higher reducing power than the tofu produced using other coagulants (0.41 OD_{700}), this higher reducing power of steep water coagulated tofu could not be far fetched from the fact that the tofu had the highest total phenol content (Fig. 2). It is what noting that the reducing power of the tofu were higher than that of some commonly consumed and underutilized legumes in Nigeria (Oboh, unpublished data), but lower than that of some consumed green leafy vegetables in Nigeria (Oboh, 2005). Allhorn *et al.* (2005) recently reported that reducing property can be a novel antioxidation defense mechanism, this is possibly through the ability of the antioxidant compound to reduce transition metals. Reduced metals (such as Fe (II) or Cu (I)) rapidly react with lipid hydroperoxides, leading to the formation of reactive lipid radicals and conversion of the reduced metal to its oxidized form (Gogvadze *et al.*, 2003).

The result revealed that steep water coagulated tofu had higher ability to scavenge DPPH free radical than the tofu produced using other coagulants; the reason for this will not be far fetch from the higher total phenol content of the steep water coagulated tofu (Fig. 3). Natural polyphenols exert their beneficial health effects by their antioxidant activity, these compounds are capable of removing free radicals, chelate metal catalysts, activate antioxidant enzymes, reduce α-tocopherol radicals and inhibit oxidases (Amic *et al.*, 2003). The antiradical activity of flavonoids and phenolics is principally based on the redox properties of their hydroxy groups and the structural relationships between different parts of their chemical structure (Rice-Evans *et al.*, 1997). This free radical scavenging ability were within the same range with the free radical scavenging ability of some commonly consumed green leafy vegetables in Nigeria (Oboh and Akindahunsi, 2004; Oboh, 2005) but higher than that of some commonly consumed and underutilized legumes in Nigeria (Oboh, unpublished data).

As shown in Fig. 4, 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 (Fig. 4), 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 Fig. 5 and 6 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 respectively when compared with those fed with the commercial diet (control). Increase in serum cholesterol which is caused by LDL is due essentially to the presence of a mutant allele at the LDL receptor locus, which results in reduced ability to bind and to take up LDL (Agbedana, 1997), leaving LDL and cholesterol in the plasma because LDL is unable to transport cholesterol back to the Liver for biliary excretion or repackaging

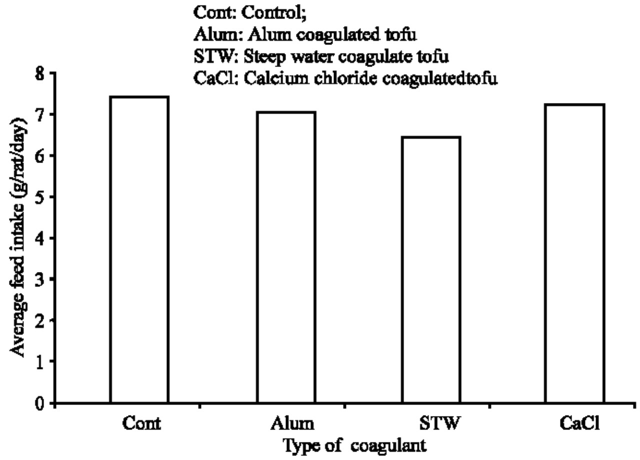

Fig. 4: Average daily feed intake by rats fed tofu

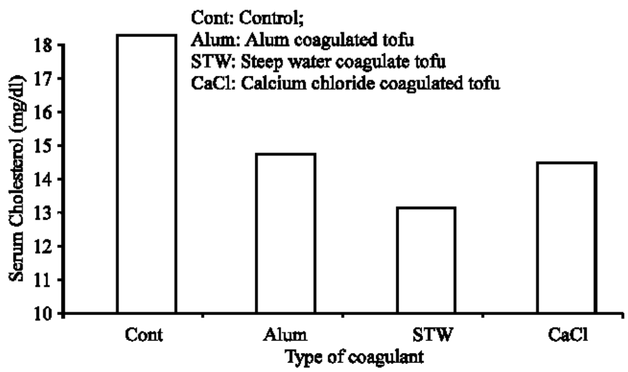

Fig. 5: Changes in serum cholesterol of rats fed tofu

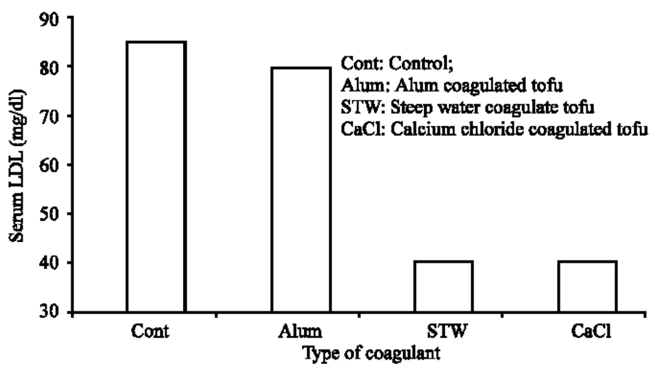

Fig. 6: Changes in serum LDL of rats fed tofu

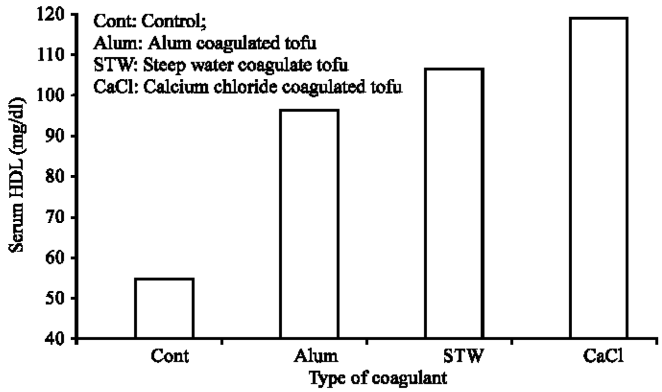

Fig. 7: Changes in serum HDL of rats fed tofu

(Agbedana, 1997), this condition is a characteristic of hypercholesterolemia. However tofu may have 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 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). This study has earlier established the antioxidant property of tofu due to the presence of phenol, which is able to reduce or prevent the oxidation of LDL, which results in cholesterol esters being, deposited in the arteries a condition that characterizes atherosclerosis. Hence the antioxidants, which are present in tofu, are effective in lowering the LDLs present in the serum.

Although, there was a significant decrease in the serum cholesterol and Low-density Lipoproteins (LDL) level of rats fed tofu, however 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), in that there is a direct relationship between the total phenol content and antioxidant activity and the inhibition of autoxidation of unsaturated lipids, thus preventing the formation of oxidized Low-density Lipoprotein (LDL), which is considered to induce cardiovascular disease.

The result 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 (Fig. 7) is 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, that HDL inhibits cellular uptake of LDL and secondly, that 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 $CaCl_2$ coagulated tofu, despite its low total phenol content when compared to others coagulant can not be categorically stated. 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 $CaCl_2$ coagulated tofu that may have additive or synergistic effect on the hypocholesterolaemic of the total phenol.

Conclusions

As shown by this study, steep water coagulated tofu had significantly high total phenols, which could have contributed to the low serum cholesterol and LDL and high serum HDL. This is closely followed by those fed calcium chloride coagulated tofu with low LDL and highest serum HDL, while those fed alum coagulated had the highest serum LDL and lowest serum HDL. Steep water therefore 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.

References

Agbedana, E.O., 1997. Cholesterol and Your Health, Inaugural Lecture, University of Ibadan, Nigeria, pp: 50.

Akio, M., Y. Shin-Ichi and T. Hiroshi, 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.

Allhorn, M., A. Klapyta and B. Akerstrom 2005. Redox properties of the lipocalin alpha1-microglobulin: reduction of cytochrome c, hemoglobin and free iron. Free Radic Biol. Med., 38: 557-67

Amic, D., D. Davidovic-Amic, D. Beslo and N. Trinajstic, 2003. Structure-Radical scavenging activity relationship of flavonoids. Croatia Chem. Acta. 76: 55-61.

Cao, T.D. and K.C. Chang, 1997. Dry tofu characteristics affected by soymilk solid content and coagulation time. J. Food Qual., 20: 391-402.

Cao, T.D. and K.C. Chang, 1999. Characteristic of production scale tofu as affected by soymilk coagulation method: Propeller blade size mixing time and coagulant concentrations. Food Res. Intl., 31: 289-295.

Georgi, E., 2002. Soy in the treatment of hypercholesterolemia http://www.soysense.com.

Gogvadze, V., P.B. Walter, B.N. Ames, 2003. The role of Fe^{2+}-induced lipid peroxidation in the initiation of the mitochondrial permeability transition. Arc. Biochem. Biophys., 414: 255-60.

Halliwell, B., 1990. How to characterize a biological antioxidant. Free Raical Res. Commun., 9: 1-32.

Jackson, C.J.C., J.P. Dini, C. Lavandier, H.P.U. Rupasinghe, H. Faulkner, V. Poysa, D. Buzell and S. Dehrandic, 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. Lebensm. Wissu. Technol., 38: 513-517.

Oboh, G. and A.A. Akindahunsi, 2004. Change in the ascorbic acid, total phenol and antioxidant activity of sun-dried commonly consumed green leafy vegetables in Nigeria. Nutrition and Health, 18: 29-36.

Oboh, G. and O.E. Omotosho, 2005. Effect of coagulants on the nutritional quality and *In vitro* multienzyme protein digestibility of tofu. J. Food Technol., 3: 182-187.

Singleton, V.L., R. Orthofer and R.M. Lamuela-Raventos, 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 L. Woodrow, 2001. Stability of soybean composition and its effects on soymilk and tofu yield and quality. Food Res. Intl., 38: 337-345.

Prestamo, G., M.A. Lasuncion and G. Anroyo, 2002. Response of rats to the intake of tofu treated under high pressure. Innovative Food Sci. Emerging Technol., 3: 149-155.

Rice-Evans, C.A., N.J. Miller and G. Papanga, 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci., 2: 152-159.

Sun, J., Y. Chu, X. Wu and R. Liu, 2002. Antioxidant and antiproliferative activities of common fruits. J. Agric. Food Chem., 50: 7449-7454.