



Influence of Local Coagulants on the Total Antioxidant, Phenolic and Polyphenol Activities of Tofu (Curdled Soy Milk)

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ABSTRACT

Tofu is prepared using locally sourced coagulants which are steep water from pap production (Nuwan kamu), *C. limonum* (lemu baka), and *C. Lemon* peel extract (lemu suaka). The effect of these coagulants on the yield, and total antioxidant, phenolic acid, and polyphenol contents of the tofu were assessed using spectrophotometry method. The results indicated that the tofu yields (31.7-33.8%) were not differ significantly ($p < 0.05$), while coagulants cause a significant increase ($p > 0.05$) in the moisture (52.2-62.1%), fat (18.6-22.1%), protein (55.4-72.1%), ash (1.2-1.6%), energy (5.7-8.0 Cal g^{-1}) contents of coagulated tofu. A significant increase in the antioxidant for the free radical scavenging activity (64.1-82.9%) and antioxidant for reducing activity (0.36-0.49 nm), polyphenol (7.8-13.0 mg/g), and phenolic acid (9.4-14.9 mg/g) contents of coagulated tofu were observed. The results conclude that tofu yield were not significantly difference, while all coagulants elevated the antioxidants, polyphenol and phenolic acid in coagulated tofu.

Keywords: antioxidant, coagulants, coagulated tofu, phenolic acid, polyphenol.

INTRODUCTION

Soybeans are cheap, protein and phenolic-rich crop consumed over the world, most commonly cultivated in Asian countries like Malaysia, Indonesia, Japan, Thailand, and Korea (Rekha and Vijayalakshmi, 2011). Presently, the outbreak of animal diseases (Mad cow disease), shortage and high cost of animal proteins, high demand for healthy (low saturated fats and cholesterol free), and religious halal foods and economically reasons have, globally, increase consumption of soy foods (Wu *et al.*, 2004; Yakubu *et al.*, 2013). Tofu (soybean curd) is a soft cheese-like food produced by curdles of fresh hot soy milk using the coagulant (shokunbi *et al.*, 2011).

Traditionally, in the Northern part of Nigeria, tofu is produced by curdle of fresh hot soy milk using Alum, Lemon, steep waste water from pap production (Nuwan baka), and lemon peel ash extract (Suaka) (Yakubu *et al.*, 2013). It has high protein, polyunsaturated fatty acids, β -carotene, mineral contents, phenolic, 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).

Oboh (2006) have 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. 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. Soy foods, fruits, and vegetables have been demonstrated to be rich in phenolic and flavonoids (Alfa *et al.*, 2003). It has been postulated that the health benefits of soy foods could be associated with its higher content of antioxidant, phenolic and flavonoid compounds (Lee *et al.*, 2003). Phenols are secondary

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metabolites found in plants that are made up of biologically active ingredients. They are grouped into simple phenols and polyphenols, which act to trap down the effects of reactive oxygen species (ROS) by giving out the H⁺ to make the free radicals stable (Marinova *et al.*, 2005). Qader *et al.* (2011) have demonstrated that phenolic compound can form a stable phenoxyl radical, which is an evidence of effective antioxidant property. The biological benefits of phenolic compounds may be due to their ability to act as a reducing agent (hydrogen donors, quenchers of singlet oxygen) and could be able to chelate metal ions (Gulcin *et al.*, 2010). Hence, producing food products with a high source of antioxidants, and phenols could be considered as a way forward for the food manufacturing industries. In Nigeria, due to the lack of tofu manufacturing industries, its consumption, and health benefits have not been acknowledged. Tofu (Waken suya) is locally produced using Alum, Lemon, Steep water, and lemon peel ash extract (Suaka). It is in line with this that the study is undertaken to evaluate the effects of locally used coagulants on the activities of total antioxidants, phenols, and polyphenols in tofu produced in the Northern Nigeria.

METHODOLOGY

Soybeans and local coagulants: Alum, *C. limonum* (lemon) were procured from a local market. Steep water (waste water from pap production), and lemon peel ash extract were obtained by a local pap maker. 1,1-diphenyl-1-picrylhydrazyl (DPPH), Folin-Ciocalteu phenol reagent, 2,6-dichloroindophenol (2,6-DCPIP), rutin, potassium ferricyanide, ferric chloride, aluminium chloride, sodium nitrite, and sodium carbonate were purchased from Sigma Co., St. Louis, Missouri, 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, 2011).

Preparation of tofu

The cleaned soybean (150 g) was soaked in 5 L of distilled H₂O at 28 °C for 12 hours. The soaked soybeans were re-washed thoroughly, the outer coat of soybeans was removed, and ground with H₂O (1:8) in a blender. The soybean slurry obtained was then heated slowly at 85 °C for 60 min with constant stirring. Then, the hot slurry was filtered using cheese cloth to separate soy milk from the residue (Akara). About 200 mL of cold soy milk at 60 °C were measured into five separate 500 ml labeled (1, 2, 3, 4, and 5) beakers. Beaker (1) contained only 200 mL of fresh soy milk, which served as control. Into beaker (2), 10 mL of the prepared alum solution was added, also 10 mL of steep water, lemon, and lemon peel ash extract were separately added to beakers (3, 4, and 5), respectively. After the addition of respective coagulants, soy milk was 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 for 1 hour. The weight of tofu was calculated and expressed as % weight of fresh tofu against the initial amount of soy milk used for its preparation. The prepared tofu cake was then kept in a refrigerator (4 °C) for the subsequent analysis.

Experimental procedure

The % yield of tofu was determined by weighing the amount of tofu (mg) produced per 200 mL of soy milk multiplied by 100. Energy (caloric) values were assessed using Ballistie Bomb calorimeter (Gallemkamp, CBB. 330-0101). The reducing power was analyzed using the method described by Benzie and Strain (1996), 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assay was determined using the method of Wong *et al.*, (2006). The

total polyphenol content was assayed using the method described by Marinova *et al.* (2005). While the total phenolic acid was done by the Folin-Ciocalteu calorimetric reaction as reported by Dewanto *et al.*, (2002).

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 * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

RESULTS

Tofu yield

Tofu yield produced from each of the coagulants was expressed as mg of tofu/200 mL of milk as presented in Figure 1. The result showed that there was not a significant difference ($p > 0.05$) in the tofu yield of each coagulant. However, the amount of tofu yielded by each coagulant was arranged in the following order: steep water > lemon peel ash extract > alum > lemon, with 35.00, 32.40, 32.00, and 31.67 %, respectively.

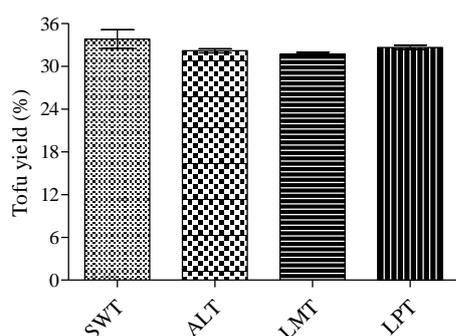


Figure 1: The percentage yield of tofu produced.

SWT: Steep water, ALT: Alum, LMT: Lemon, and LPT: Lemon peel ash extract.

Energy values

The energy values assessed for fresh soy milk and each tofu prepared by different coagulants are presented in Figure 2. Lemon coagulated tofu had higher significant ($p < 0.001$) value of

energy (8 cal g^{-1}) followed by that of steep water (7.8 cal g^{-1}) compared to fresh soy milk (6.2 cal g^{-1}), while that of the lemon peel ash extract (6 cal g^{-1}) was not differed significantly, compared to that of fresh soy milk (6.2 cal g^{-1}). However, a significant decrease in energy value was recorded for the alum coagulated tofu (5.7 cal g^{-1}) compared to that of fresh soy milk with 6.2 cal g^{-1} .

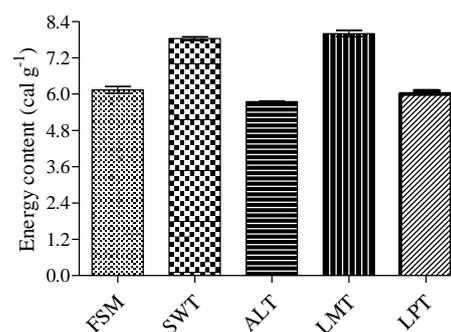


Figure 2: The Energy contents of fresh soy milk and tofu.

FSM: Fresh soy milk, SWT: Steep water coagulated tofu, ALT: Alum coagulated tofu, LMT: Lemon coagulated tofu, and LPT: Lemon peel ash extract coagulated tofu.

Total antioxidant content (DPPH free radical scavenging activity)

The free radical scavenging activities of fresh soy milk and each tofu prepared using different coagulants are presented in Figure 3. The result indicated that steep water, lemon, and lemon peel ash extract coagulated tofu contained the highest significant ($p < 0.001$) value of total antioxidant with 83, 81, and 78.1% compared to that of fresh soy milk (75%), while a significant decreased in total antioxidant was detected in alum coagulated tofu (64.1%) compared to that of the fresh soy milk with 75%.

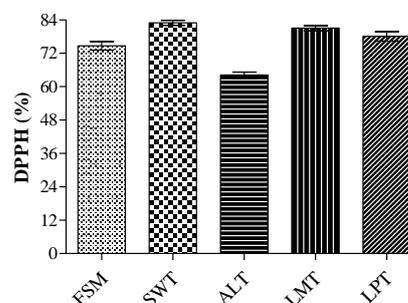


Figure 3: Total antioxidant content measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity.

FSM: Fresh soy milk, SWT: Steep water coagulated tofu, ALT: Alum coagulated tofu, LMT: Lemon coagulated tofu, and LPT: Lemon peel ash extract coagulated tofu.

Total antioxidant content by reducing power

The total reducing ability of fresh soy milk and tofus prepared by different coagulants are shown in Figure 4. The steep water coagulated tofu (0.49 nm) has highest significant content of reducing power compared to that of fresh soy milk (0.42 nm), but alum coagulated tofu, has a significant decrease in reducing the ability (0.36 nm) compared to fresh soy milk (0.42 nm), Figure 4. However, in lemon and lemon peel ash extract, the reducing ability with 0.45 and 0.42 nm, respectively, did not differ significantly, compared to that of fresh soy milk.

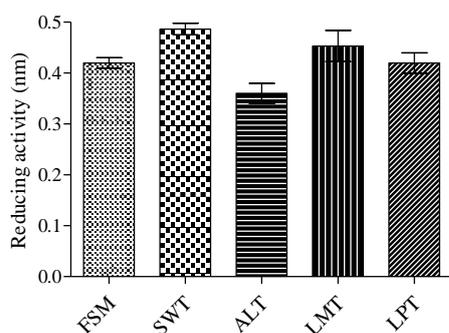


Figure 4: Total antioxidant content measured by reducing the activity.

FSM: Fresh soy milk, SWT: Steep water coagulated tofu, ALT: Alum coagulated tofu, LMT: Lemon coagulated tofu, and LPT: Lemon peel ash extract coagulated tofu.

Total polyphenol content

Figure 5 shows the results of total polyphenol content of fresh soy milk and tofu prepared using different coagulants. The steep water coagulated tofu (13.0 mg/g) had, the highest significant content of polyphenol, followed by that of lemon coagulated tofu 12.1 mg/g, respectively, compared to fresh soy milk (10.1 mg/g). However, significantly lower activity of total polyphenol (7.8 mg/g) was found in alum coagulated tofu compared to fresh soy milk. Similarly, in lemon peel ash extract coagulated tofu, the total polyphenol content (10.6 mg/g) did not differ significantly, from the fresh soy milk with 10.1 mg/g.

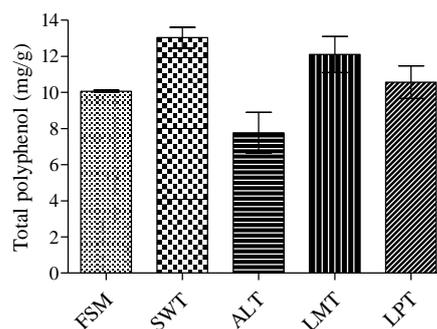


Figure 5: Total polyphenol contents.

FSM: Fresh soy milk, SWT: Steep water coagulated tofu, ALT: Alum coagulated tofu, LMT: Lemon coagulated tofu, and LPT: Lemon peel ash extract coagulated tofu.

Total phenolic acid content

The results of total phenolic acid contents in fresh soy milk and individual tofu prepared using different coagulants are shown in Figure 6. The total phenolic acid content (14.9 mg/g) in steep water coagulated tofu was significantly higher, while, it was significantly lower in alum coagulated tofu (9.4 mg/g) than that of fresh soy milk with mean value of 12.7 mg/g (Figure 6). However, in lemon and lemon peel ash extract coagulated tofu, the phenolic acid contents of 13.7 and 12.5 mg/g, respectively, were not significantly difference from that of fresh soy milk (12.7 mg/g).

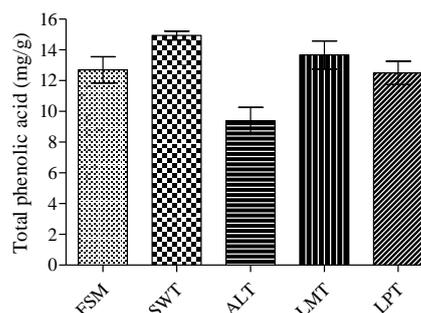


Figure 6: Total phenolic acid contents of fresh soy milk and tofu.

FSM: Fresh soy milk, SWT: Steep water coagulated tofu, ALT: Alum coagulated tofu, LMT: Lemon coagulated tofu, and LPT: Lemon peel ash extract coagulated tofu.

DISCUSSION

The observed insignificant difference ($p > 0.05$) in the yield of tofu prepared from different coagulants may reflect that the coagulation ability by each coagulant used for this study did not differ, substantially and there is no extraneous substances added. Omotosho *et al.*, (2011) have demonstrated that both steep water and alum exhibited almost the same value of the cheese yielded.

The high energy content demonstrated by steep water and lemon coagulated tofu may be probably due to the ability of the protein matrix of the tofu to trap a lot of energy releasing macromolecules. Tofu has been suggested to be very rich in protein and fat that are higher energy producing macromolecules (Prestamo *et al.*, 2002).

The higher total antioxidant (free radical scavenging ability) contents observed in steep water, lemon, and lemon peel ash extract may suggest the presence of natural organic compounds such as ascorbic acid, phenols, and flavonoid and degradation of tannins during the process of coagulation. In addition, the increase total antioxidants in term of reducing ability observed in steep water coagulated tofu may be associated with some extraneous substances (organic chemicals) such as ascorbic acid and phenols from the lemon. 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. These findings correlate with the report of Oboh and Akindahunsi (2004) on some commonly consumed leafy vegetables in Nigeria. These results revealed that tofu produced from locally sourced coagulants exhibited good antioxidant activity and could be a good scavenger of ROS.

Higher total phenolic and polyphenol contents in steep water coagulated tofu and others could be explained probably, that the process of tofu coagulation may affect the tannin content. Food processing methods may induce the breaking down of some of the tannins and other chelating agents thereby increase the total phenol content (Akindahunsi and Oboh, 2004; Tarwadi and Agte, 2003). These observations agreed with the report of Yakubu *et al.*, (2013) on the free phenol contents of coagulated tofu and can also be compared well with the reports of Liu *et al.*,

(2002); Shokunbi *et al.*, (2011) and Nwanna and Oboh (2007). Natural phenolic compounds, which act as a free radical neutralizer may prevent the formation of potent carcinogen (nitrosamines). The nitrosamines are produced through a reaction of nitrite with amines in animals (Ali Al-mamary, 2002). Polyphenols are one of the most plant active compounds with potent antioxidant properties. It can easily react with metallic ions of Fe, Zn, and Cu to form complexes and thereby preventing their absorption (Kumar *et al.*, 2010 and Okafor *et al.*, 2011). It can also reduce the nutrient uptake, but in excess, such metal cations can enhance free radical generation, which can cause the cellular oxidative damage of membranes and DNA (Ara and Nur, 2009).

Conclusion

The consumption of foods (crops, fruits, and vegetables) with potent antioxidant properties, which are known to reduce the process of atherosclerosis, the risk of cancer and degenerative diseases is well documented. In this study, all tofu produced from locally sourced coagulants gave relatively, higher yield, energy value, and antioxidant efficiencies. However, steep water and lemon coagulated tofu are more promising in term of nutritive and antioxidant activities. Finally, more consumption of tofu produced from locally sourced coagulants may have some health benefits and contribute to the wellbeing of individual and society in general.

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