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Effect of Types of Coagulant on the Nutritive value and *In vitro* Multienzyme Protein Digestibility of Tofu

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Abstract: Tofu is popularly consumed in Nigeria because of the various nutritional and medicinal attribute associated with soybean products. In Nigeria Tofu a coagulated product of soymilk is usually produced at household level using various types of coagulants such as Calcium chloride, Alum and steep water (effluent from pap produced from maize). This study therefore sought to assess the effect of the various type of locally used coagulants on the proximate, mineral, energy and antinutrient composition, and *In vitro* multienzyme protein digestibility of Tofu. The result of the study revealed that there was no significant difference ($P>0.05$) in the yield of the Tofu (17.5 - 18.3%), however alum coagulation gave the highest yield, while Calcium chloride gave the lowest yield. The protein (17.6%), fat (6.2%), Mn (0.3), Mg (34.2), energy (6.6cal/g) and *In vitro* multienzyme protein digestibility (75.8 - 77.6%) of steep water coagulated tofu was significantly higher ($P<0.05$) than that of other coagulants. While alum gave Tofu with significantly higher ($P<0.05$) Fe (1.6), Ca (23.5), K (33.9) and Na (21.1) than the tofu produced by other coagulants. The tofu produced by Calcium chloride had the highest Zn (0.6) content but the lowest energy content (5.3cal/g) and *In vitro* multienzyme protein digestibility (61.6 - 63.5%). It could therefore be concluded that of all the locally used coagulants in Nigeria for Tofu production steep water (effluent from pap produced from maize) which is considered to be waste appeared to be the most promising, although it has the least sensory acceptability, however further research will be carried out on how to improve its sensory quality.

Key words: Soybeans, Coagulants, Tofu, Biochemical composition, Digestibility

Introduction

Soybeans have long been a staple of human diet in Asia, especially as soymilk or tofu, which is prepared from soymilk. Soybeans are an inexpensive and serve as high quality protein source. Soymilk and tofu consumption is increasing in Nigeria due to an increase in Asia immigrants, greater acceptance of soy foods by the general population, and increased recognition of the health benefits of soy foods, especially by those who wish to reduce their consumption of animal products (Murphy *et al.*,1997 and Poysa and Woodrow, 2002).

Tofu is one of the most important and popular food products in East and South Eastern Asian countries. Tofu is cholesterol-free, low in saturated fat and high in protein. Western countries have started to take an increasing interest in tofu due to its good nutrition and health benefits to human (Cai and Chang, 1998). In Nigeria, the consumption of tofu and soymilk products is increasing in an alarming rate, due to high cost of animal proteins. The yield and quality of tofu are influenced by soybean varieties, soybean quality and processing conditions (Cai and Chang, 1998). 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 variables: soybean chemistry, soymilk cooking temperature, volume, solid content and pH; coagulant type, amount, concentration and the method of adding and mixing; and coagulation temperature and time (Shurtleff and Aoyagi, 1990; Cai and Chang, 1997; Cai and Chang, 1998). The variation in controlling all these variables greatly affects tofu yield and quality. Increasing coagulation temperature, stirring speed, and coagulant concentration increases tofu hardness but decreased tofu yield (Cai and Chang, 1998)

In Nigeria, tofu is produced at household level using coagulants such as $CaCl_2$. $2H_2O$, alum and steep water (effluent from pap produced from maize). This study therefore sought to investigate the effect of the various type coagulants on the yield and nutritional quality of 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.

Methods

Sample Preparation : Soybean (1.0kg) was soaked in water (6litres) at 27 - 32°C for 9h. The soaked beans were drained and ground in the grinder with tap 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 420rpm respectively with each of the coagulants [$CaCl_2.H_2O$ (20mM), Alum (20mM) 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 10min. The bean curd was pressed after pressing the tofu weight was recorded; the tofu produced was stored in water at 4°C overnight prior to analysis.

Analysis of Products

Sample Analysis: The nutrient composition (ash, fat, moisture, carbohydrate and crude fibre) of the tofu was determined using the standard AOAC (1990) method and the protein content was determined using the micro-Kjeldhal method (N x 6.25). The mineral composition (K, Na, Mg, Fe, Zn and Ca) was determined on aliquots of the solutions of the ash by established flame atomic absorption spectrophotometry procedures using a Perkin-Elmer atomic absorption spectrophotometer (model 372) (Perkin-Elmer, 1982).

Sensory Analysis: The organoleptic properties of the tofu produced using the various kinds of coagulants were carried out using the method of Potter (1968). The products were assessed (aroma, taste, texture, colour and general acceptability) on a 7-point Hendonic scale (7, excellent; 6, very good; 5, good; 4, average; 3, fair; 2, poor; 1, very poor) as described by Potter (1968) and the attribute mean score calculated.

Determination of Energy Values: A 0.1 g sample each of the tofu was ignited electrically in a Ballistic Bomb Calorimeter (Gallemkamp, CBB- 330-010 L) and burned in excess oxygen (25 atm.) in the bomb. The rise in temperature obtained was compared with that of benzoic acid to determine the calorific value of the sample material.

***In vitro* Multienzyme Protein Digestibility** : *In vitro* protein digestibility was carried out using the method of Hsu *et al.*(1977). Sample suspension was prepared by dissolving 1.75 g in 50 ml of distilled water. The suspension was adjusted to pH 8.0 with 0.1 M HCl or 0.1 M NaOH, while stirring in a 37 °C water bath. The multienzyme solution consisting of 1.6 mg trypsin, 3.1 mg chymotrypsin and 1.3 mg peptidase ml^{-1} was maintained in an ice bath and adjusted to pH 8.0 (with 0.1 M HCl or 0.1 M NaOH). 5 ml of the multienzyme solution was added to the sample suspension with constant stirring at 37 °C. The pH of the suspension was recorded 15 min after the addition of the multienzyme solution and the *in vitro* digestibility was calculated using the regression equation of Hsu *et al.* (1977).

$$Y = 210.46 - 18.10X$$

Where Y is *in vitro* digestibility (%), and X is pH of the sample suspension after 15min digestion with the multienzyme solution.

Analysis of Data: The result of the three replicates were pooled and expressed as mean ± standard error (S.E.). A one-way analysis of variance (ANOVA) and the Least Significance Difference (LSD) were carried out (Zar, 1984). Significance was accepted at $P \leq 0.05$.

Results and Discussions

Soybean products are a good source of proteins, carbohydrates, low in fat and rich in mineral contents, they are part of principal meals in eastern countries. The incorporation of soybean food (tofu) into a western diet could be an important means of preventing and treating chronic diseases, such as cancer and cardiovascular studies (Prestamo *et al.*, 2002). Tofu is made by coagulation of soymilk with salt or acid to produced a soy protein gel, which traps water, soy lipids and other constituents in the matrix. Each coagulants produces tofu with different textural and flavour properties (Shurtleff and Aoyagi, 1990). In these studies the effect of $CaCl_2.2H_2O$, alum (a double salt) and steep water (a heterogeneous acidic effluent produced during pap production) on the biochemical composition of tofu and their digestibility is highlighted below.

The result of the tofu yield is shown in Fig. 2, the result revealed that there was no significant difference ($P>0.05$) in the tofu yield by each of the coagulant, however, alum 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. Conversely, there was a significant difference ($P<0.05$) in the protein content of the tofu (13.3 - 17.6%). The protein content of steep water (17.6%) coagulated tofu is significantly ($P<0.05$) higher than that of calcium chloride (15.1%) and alum (13.3%) coagulated tofu. The high protein content of steep water coagulated tofu could possibly be attributed to the likelihood that the protein in the pap effluent might have been transferred into the tofu unlike calcium chloride and alum which are pure salt, without any protein content. Furthermore, it could also be speculated that acidic mediun created by the steep water have created a better coagulating environment for the protein present in the soymilk than the salts.

However the protein content of the tofu produced using all these locally used coagulants had higher protein content than that of the commercially purchased tofu (12%) (Prestamo *et al.*, 2002). Furthermore, the protein content

Table 1: Proximate composition of tofu (% wet weight)

Sample	CH	SW	AM
Protein	15.1±0.3[b]	17.6±0.2[a]	13.3±0.4[c]
Fat	5.2±0.2[b]	6.2±0.2[a]	4.9±0.3[b]
Ash	0.7±0.2[a]	0.5±0.1[a]	0.7±0.0[a]
Carbohydrate	5.1±0.2[b]	3.8±0.1[c]	7.2±0.1[a]
Moisture	73.9±0.3[a]	71.9±0.2[a]	73.9±0.2[a]

Value represents mean of triplicate readings
Values with the same superscript along the same row are not significantly different
CH- Calcium chloride coagulated tofu; SW- Steep water coagulated tofu; AM- Alum coagulated tofu

Table 2: Mineral composition of tofu (ppm wet weight)

Sample	CH	EM	AM
Fe	0.5±0.1[c]	0.9±0.1[b]	1.7±0.2[a]
Zn	0.6±0.0[a]	0.2±0.0[b]	0.3±0.1[b]
Mn	0.2±0.1[a]	0.3±0.1[a]	0.2±0.0[a]
Mg	30.2±0.3[b]	34.2±0.1[a]	29.2±0.1[b]
K	23.1±0.1[a]	26.3±0.1[b]	33.9±0.3[a]
Ca	15.9±0.1[a]	19.0±0.3[a]	23.5±0.1[a]
Na	13.6±0.1[a]	15.4±0.3[b]	21.2±0.2[a]

Value represents mean of triplicate readings
Values with the same superscript along the same row are not significantly different
CH- Calcium chloride coagulated tofu; SW- Steep water coagulated tofu; AM- Alum coagulated tofu

Table 3: Sensory evaluation of tofu

Sample	CH	EM	AM
Taste	6.4±0.1[a]	4.4±0.3[b]	5.2±0.6[ab]
Structure	6.8±0.2[a]	4.8±0.2[b]	6.2±0.4[a]
Colour	6.5±0.3[a]	3.2±0.1[a]	6.7±0.2[a]
Odour	6.2±0.3[a]	2.5±0.1[a]	6.4±0.2[a]
Texture	6.1±0.3[a]	4.8±0.5[b]	5.8±0.3[a]
General Acceptability	6.5±0.2[a]	4.1±0.4[b]	6.3±0.1[a]

Value represents mean of triplicate readings
Values with the same superscript along the same row are not significantly different
CH- Calcium chloride coagulated tofu; SW- Steep water coagulated tofu; AM- Alum coagulated tofu
7-excellent; 6- very good; 5-good; 4-average; 3-fair; 2-poor; 1-very poor

of the tofu was higher than that of some commonly consumed tropical plant foods such as yam (4-10%), cassava products (4-12%) and some commonly consumed green leafy vegetables in Nigeria (Akindahunsi and Oboh, 1998; Akindahunsi and Oboh, 1999; Akindahunsi *et al.*, 1999; Oboh *et al.*,2002 and Oboh and Akindahunsi, 2003). Furthermore, the fat content of steep water coagulated (6.2%) tofu was significantly higher than that of alum (4.9%) and calcium chloride (5.2%) coagulated tofu. Likewise, the higher fat content of steep water could also be attributed to the fact that the coagulated protein in the milk might have trapped some of the fat in the heterogeneous solution of steep water. However, the fat content of the tofu was lower than the value (9%) reported by Prestamo *et al.* (2002) for some commercially purchased tofu. Conversely, the fat content of the tofu produced by the various coagulants were generally high when compared with some commonly consumed plant foods in Nigeria (Akindahunsi and Oboh, 1998; Akindahunsi and Oboh, 1999; Akindahunsi *et al.*,1999; Oboh *et al.*, 2002 and Oboh and Akindahunsi, 2003). However, there was no significant difference ($P>0.05$) in the moisture and ash content of the tofu produced using the various types of tofu.

The result of the mineral content (Fe, Zn, Mn, Mg, K, Ca and Na) of the tofu was generally low when compared with some consumed plant foods in Nigeria. Fe, K, Ca and Na were significantly higher ($P<0.05$) in tofu produced using alum coagulant than the one produced using the other two coagulants, however, steep water coagulated tofu had the highest Mn and Mg content, while calcium chloride coagulated tofu had the highest Zn content (Table 2). The minerals were generally lower than that of some commonly consumed plant foods in Nigeria such as edible wild seeds (Oboh and Ekperigin, 2004), cassava products (Oboh and Akindahunsi, 2003), cultivated and wild yams (Akindahunsi and Oboh, 1998:) and green leafy vegetables (Akindahunsi and Oboh, 1999; Oboh, Ekperigin and Kazeem 2004). The low mineral content in the tofu could be attributed to the possible solubility of some of the salt of those minerals in the whey during tofu production, thereby preventing the trapping of the mineral in the protein matrix of the tofu.

Fig. 3 shows the result of the energy content of the tofu. The energy content of the tofu produced using calcium chloride (5.3cal/g) was significantly lower ($P<0.05$) than the energy content of the tofu coagulated by steep water

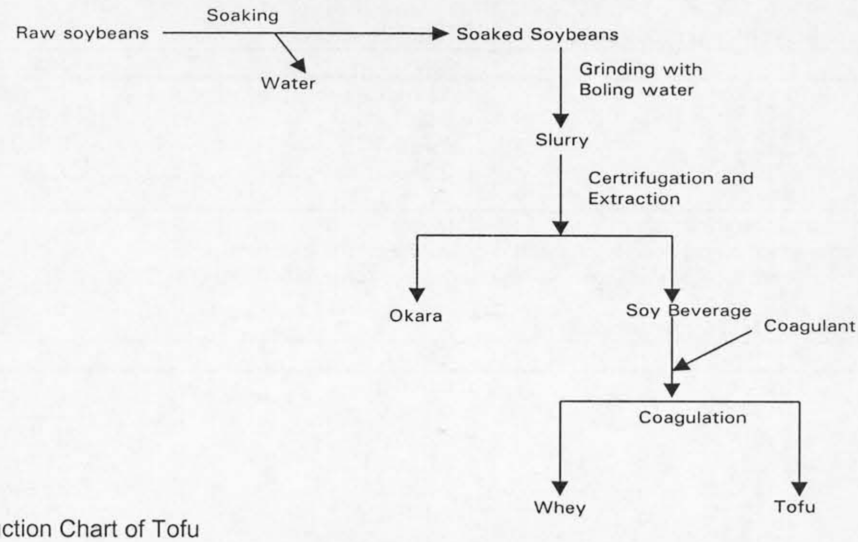

Fig. 1: Production Chart of Tofu

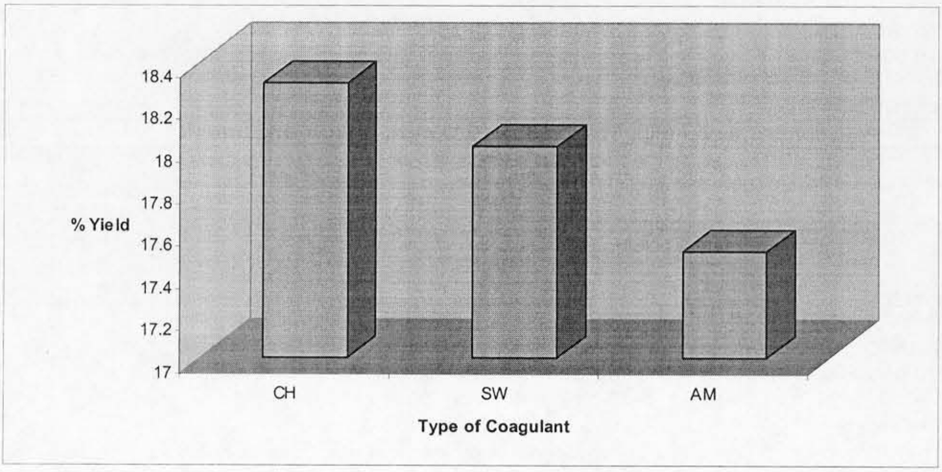

Fig. 2 The Yield of Tofu Produced Using Locally Sourced Coagulants CH- Calcium chloride coagulated tofu; SW- Steep water coagulated tofu; AM- Alum coagulated tofu

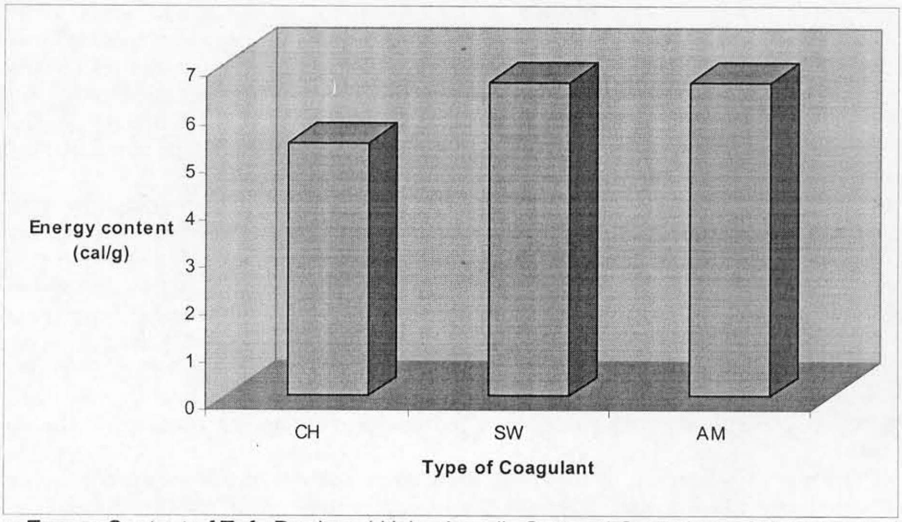

Fig. 3: The Energy Content of Tofu Produced Using Locally Sourced Coagulants CH- Calcium chloride coagulated tofu; SW- Steep water coagulated tofu; AM- Alum coagulated tofu

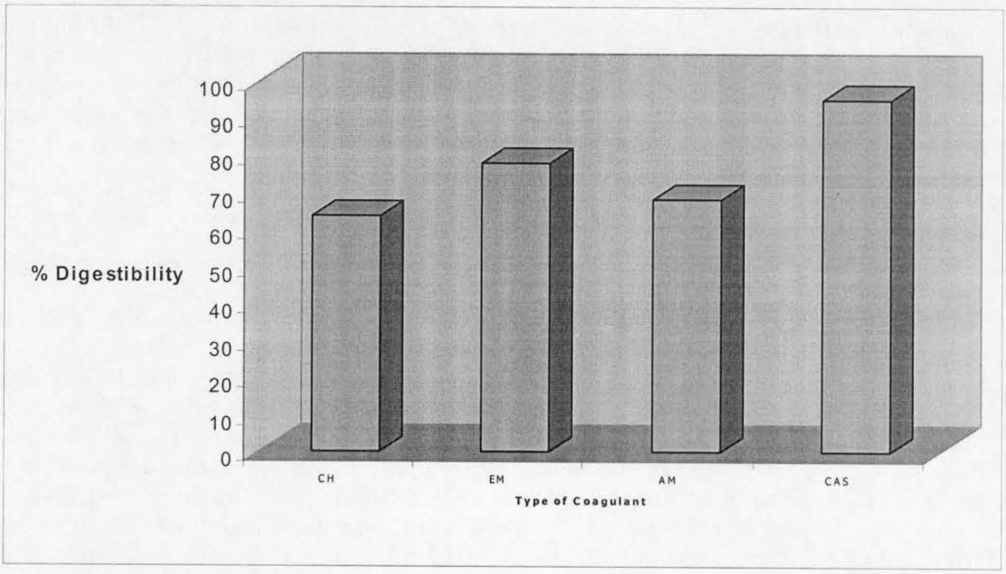

Fig. 4: *In vitro* Multienzyme Protein Digestibility of Tofu CH- Calcium chloride coagulated tofu; SW- Steep water coagulated tofu; AM- Alum coagulated tofu

and alum (6.6 cal/g). However, the energy content of tofu is high when compared to that of cassava products (flour and gari) (3.1 - 3.9 cal/g) (Akindahunsi and Oboh 2003 and Onwueme, 1978), which is considered as one of the main dietary energy source in Nigeria (Oboh and Akindahunsi, 2003 and Oboh *et al.,* 2002). The basis for the high energy content of the tofu could not be categorically stated, however, it could be attributed to the fact that tofu is very rich in protein and fat (Prestamo *et al.*, 2002), which are energy producing macromolecules. Which is an indication that the coagulated protein matrix of the tofu traps a lot of energy giving macromolecules.

The result of the sensory evaluation is shown in table 3. The result revealed that steep water coagulated tofu had a significantly lower ($P<0.05$) general acceptability than alum and calcium chloride coagulated tofu as typified by the taste, structure, texture, odour and colour. This low general acceptability of steep water coagulated tofu compared to alum and calcium chloride coagulated tofu, could be attributed to the fact that the steep water as an heterogeneous mixture with characteristic taste, odour and colour and it might have imparted its taste, odour and colour on the tofu, which actually reduce the acceptability of the tofu produced from the steep water despite its high nutrient content as highlighted earlier. Although the acceptability of the texture and structure was lower than that of the alum and calcium chloride tofu. However, the difference was not significant ($P>0.05$). The tofu produced by alum and Calcium chloride had a very good general acceptability (Potter, 1968), while the general acceptability of steep water coagulated tofu was average (4.5) (Potter, 1968).

In vitro multienzyme protein digestibility of the tofu produced using the various coagulants is in shown in Fig. 4. The result clearly revealed that there was a significant difference in the digestibility of the tofu produced using the various coagulants. Tofu coagulated using steep water (75.8%) had a significantly higher ($P<0.05$) protein digestibility than those produced using either alum (66.9%) or Calcium chloride (61.6%). The percentage digestibility of steep water coagulated tofu was very close to that of maize (76.0%), pigeon pea (77.2%) and African yam bean (77.0%) as reported by Oshodi and Hall (1993), and palmwine yeast fermented cassava flour (79.1%) (Akindahunsi and Oboh, 2003). However, the digestibility of the steep water coagulated tofu was higher than that of fermented and unfermented gari (a fried cassava product popularly consumed in Nigeria) (66.9 - 69.0%), whose value compared well with that of alum (66.9) coagulated tofu. Calcium chloride (61.6%) had a digestibility far below that of some commonly consumed plants food in Nigeria mentioned earlier.

The basis for the wide difference in the digestibility of tofu from the same soybean cannot be categorically stated. However, it could be speculated that the difference in the digestibility could be as a result of the difference in the coagulating ability of each of the coagulant with regard to the different type of proteins, in the presence of the various protease inhibitors such as trypsin inhibitor and chymotrypsin inhibitor (Aletor, 1993). Tannin, trypsin inhibitor and chymotrypsin inhibitors can interact with proteins in the tofu or the digestive enzymes thereby reducing digestibility of the protein in the tofu, and the amount of inhibitors coagulated may have vary from one coagulants to another. However, the reduction in digestibility is more evident in Calcium chloride and alum-coagulated tofu than that of steep water.

In conclusion, tofu produced from a locally sourced coagulant steep water (effluent from pap production) considered to be a waste, appears to be richer in nutritious biochemical macromolecules and have a higher digestibility when compared to Calcium chloride and alum, which is conventionally used. However, the general acceptability of the tofu produced using steep water is low when compared to that of Calcium chloride and alum. Further study is required on how to improve the sensory quality of the tofu produced using this locally source coagulants without compromising is nutritional quality.

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