Physical, Chemical and Sensory Qualities of Roselle Water Extract-coagulated Tofu Compared with Tofu from Two Natural Coagulants

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ABSTRACT
Roselle calyces (Hibiscus sabdariffa) aqueous extracts were used in coagulating soymilk at four different concentrations (2.5%, 5%, 10% and 20%). The physical, chemical and sensory qualities of the tofu preparations were compared with fermented maize liquor and Calotropis procera extract coagulated tofus. pH of roselle extracts was acidic in nature (2.01 – 3.74) which was attributed to the ability to coagulate soy proteins. pH, titratable acidity of the roselle coagulated tofu ranged from 5.32 – 6.26 and 0.16 – 0.43% respectively and the yield and protein content ranged from 87.3 – 95.9 g and 42.6 – 46.3 g/100 g respectively. The yield of roselle coagulated tofu increased with increase in concentration of the roselle extracts. Roselle extract when utilized at 2.5% concentration will yield tofu that is acceptable and comparable to tofu coagulated with fermented maize steep water in terms of appearance, flavour and overall acceptability at p > 0.05. Roselle extract at 5% and 10% also yielded tofus that were acceptable in terms of all of the attributes tested.

Keywords: Hibiscus sabdariffa, aqueous extract, tofu, physical, chemical, sensory.

Introduction
Tofu serves as an inexpensive, highly digestible protein source. It has been eaten for centuries by the Orientals and has played an important role in their nutrition as source of high protein food (Liu, 1997; Evans and Rank, 2000). It is processed from soymilk, a dairy milk substitute using different types of coagulants. Basically, three types of coagulants have been reported to have the ability to coagulate proteins: these are salt, proteinases and acids (Liu, 1997). Soybean curd (tofu) has been processed from different types of coagulants such as salts like CaSO₄, CaCl₂, MgSO₄ and MgCl₂ (Wang and Hessel, 1982). Sodom apple aqueous extract containing plant proteinase has been used in coagulation of cowmilk using the traditional processing. The extract has also been used to coagulate soymilk into tofu (Aworh and Muller, 1987). Acidic coagulants that have been used in tofu processing include vinegar, lemon juice and glucono-d-lactone (Kohayama et al., 1994, Liu, 1997). Various factors such as variety of seeds, processing conditions, seed storage, stability of soybean composition, type of coagulants has been reported to affect qualities of soybean curd (Poysa and Woodrow, 2002; Shi et al., 2002; Bhardwaj et al. 2003; Hou and Chang, 2004; Chang et al., 2002; Prabhakaran et al., 2006). Type of coagulant was reported to affect yield and visco-elastic properties of tofu (Cheng et al., 2005; Sun and Breene, 1991). Temperature of processing and water to bean ratio have also influenced textural qualities of tofu (Obata and Matsuura, 1993; Wang et al., 2003). Based on type of coagulant and processing method, different textural characteristics of tofu such soft,
firm, rubbery, chewy has been described (Shih et al., 1997, Noh et al., 2005). Non-availability and high cost of imported coagulants has led to the search for alternatives for household use in Nigeria. Cheap and locally available natural coagulants for tofu include alum, fermented maize liquor, Calotropis procera leave extract and lime juice (Obatolu, 2008; Omueti and Jaiyeola, 2006).

Roselle is a tropical shrub belonging to the family Malvaceae which produces red, dark red and green form of calyces. The chemical composition of the red calyces revealed that they are good sources of vitamin C, flavonoids, minerals and antioxidants (Babalola et al., 2001; Wong et al., 2002). Roselle aqueous extract is acidic; it is usually processed into a sour tasting refreshing drink called Zobo in Nigeria (Fasoyiro et al., 2005). Based on the acidic nature of the roselle extract, it is worthwhile to study its utilization as an alternative natural coagulant in tofu preparation. The objective of this study is to assess the physical, chemical and sensory qualities of the roselle precipitated tofu in comparison with tofu made from two natural coagulants.

Materials and Methods
Dried dark roselle calyces (Hibiscus sabdariffa) were obtained from Kenaf and Jute Program, Institute of Agricultural Research and Training, Obafemi Awolowo University, Ibadan, Nigeria. Soybean seeds (variety TGX-1448-1E) and maize grains (variety DMR-ESR-Y) were obtained from the seed store of the same Institute. Fresh sodom apple (Calotropis procera) leaves were obtained from a home garden in Bodija, Oyo State, Nigeria.

Preparation of coagulants
Red roselle calyces (300 g) were washed thoroughly with tap water and used in preparation of four concentrations of roselle extract (2.5%, 5%, 10% and 20%). The different concentrations were prepared by soaking roselle calyces 12.5 g in 500 ml of tap water for 10 min (SHSWA), 25 g in 500 ml of tap water for 10 min (SHSWB), 50 g in 500 ml of tapwater for 10 min (SHSWC) and 100 g in 500 ml for 10 min respectively. The extracts were filtered through Whatman paper No.1 and the residue was discarded.

Fermented maize liquor was prepared using the method of Omueti and Jaiyeola (2006) with slight modification. Two hundred grams of the maize grains were soaked in 500 ml of water and allowed to ferment for 72 h. During the time of soaking the water was changed twice. The fermented maize grains were milled in a blender (Nakai Model 462, Japan) with 600 ml of water into slurry. The slurry was sieved using a muslin cloth and the residue was discarded. The filtrate was removed and filtered again through a Whatman paper No. 1 to obtain the coagulant (SCMLF).

One hundred grams of Calotropis procera leaves were washed and blended with 500 ml of tap water. The filtrate used as coagulant was obtained by filtering through Whatman paper No. 1 to obtain the coagulant (SCP).

Preparation of tofu
Soybean seeds (400 g) were sorted to remove extraneous materials and were soaked in 2,500 ml of tap water and stored under refrigerator (4 – 5°C) for 8 h. The soybean seeds were ground in batches with 3000 ml of water with a blender (Nakai Model 462, Japan) to obtain the slurry. Each batch was blended for 5 min at high speed. The slurry was sieved using a muslin cloth to obtain the soymilk and the residue was discarded. The soluble solid content of the soymilk was measured using hand held refractometer ATAGO Model MASTER-α (Cat. No 2351).

The soymilk obtained was boiled at 100°C for 5 min to reduce anti-nutritional factors and later cooled to 40°C. Soymilk (500 ml) at initial temperature of about 35°C was heated on an electric plate and roselle extract was gradually added at 40°C with gentle stirring until soymilk precipitation was completed at 80° – 90°C for 10 min. Complete precipitation is noted by total separation of the curd from the whey. Different amount of roselle
extracts were used in complete precipitation of soymilk (500 ml) as follows: 300 ml of SHSWA, 250 ml of SHSWB, 200 ml of SHSWC and 100 ml of SHSWD respectively. These were used to obtain roselle coagulated tofu SCHWSA, SCHWSB and SCHWSC and SCHWSD respectively. The curds were allowed to stand for 30 min and were later molded in small cheese hoops (50 ml size) lined with one layer muslin cloth to allow for freely draining of water for 4 hours.

The same procedure was repeated in preparing tofu from fermented maize liquor. Soymilk (500 ml) was precipitated with 300 ml of SCFML. Soymilk was coagulated with *Calotropis procera* extract (SCP) using the method of Aworh and Muller (1987).

7. Ten millilitre aliquots were pipetted with 0.1 M NaOH to phenolphthalein end point. The acidity was calculated as g lactic acid/100 g and expressed as percentage.

**Yield**

Yield of soybean curd was expressed as fresh weight of tofu in grams obtained from the 500 ml of soymilk.

**Chemical composition**

Protein content of the soy cheese was determined by Kjeldah method, protein conversion factor was 6.25. Fat content was by Soxhlet extraction. Protein, fat and ash were all determined using AOAC (2000). The analyses were determined as triplicates.

**Sensory evaluation**

Fresh tofu samples were randomly coded and presented to twenty semi-trained panel members who were familiar with tofu. The taste panelists were presented with tofu samples (2 cm x 2 cm x 2 cm) size on a plate. They were asked to assess the curds for appearance, colour, flavour, firmness and overall acceptability. The panelists were presented also with water for mouth rinsing in between tasting of each sample. Attributes tested were rated on nine-point hedonic scale where one represented the lowest point and five represented the mid-point.

**Statistical analysis**

Data obtained were subjected to analysis of variance and means were separated by Duncan multiple range test using statistical analytical systems (2003).

**Results and Discussion**

Table 1 the pH and titratable acidity of the different coagulants. pH of roselle extracts ranged from 2.01 – 3.74, which indicates acidic nature while the titratable acidity of the extracts was within 0.36 – 1.12%. Concentration of acidity in the roselle extracts followed the order SHSWD>SHSWC>SHSWB = SHSWA. While comparing the roselle extracts with fermented
Table 1: pH and titratable acidity of roselle extracts in comparison with two natural coagulants

<table>
<thead>
<tr>
<th>Sample</th>
<th>Concentration (%)</th>
<th>pH</th>
<th>Titratable acidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SHWSA</td>
<td>2.5</td>
<td>3.74±0.01</td>
<td>0.36±0.03</td>
</tr>
<tr>
<td>SHWSB</td>
<td>5</td>
<td>3.02±0.02</td>
<td>0.46±0.02</td>
</tr>
<tr>
<td>SHWSC</td>
<td>10</td>
<td>2.53±0.02</td>
<td>0.74±0.01</td>
</tr>
<tr>
<td>SHWSD</td>
<td>20</td>
<td>2.01±0.02</td>
<td>1.12±0.02</td>
</tr>
<tr>
<td>SCMLF</td>
<td>10</td>
<td>3.45±0.03</td>
<td>1.03±0.01</td>
</tr>
<tr>
<td>SCP</td>
<td>20</td>
<td>6.43±0.02</td>
<td>0.02±0.01</td>
</tr>
</tbody>
</table>

Means with the same superscript within column are not significantly different (p > 0.05)

SHWSA = 2.5% roselle extract, SHWSB = 5% roselle extract, SHWSC = 10% roselle extract, SHWSD = 20% roselle extract, SCMLF fermented maize liquor, SCP = Calotropis procera extract

maize liquor and Calotropis procera extract, the decreasing order of acidity was SHWSD = SCFML>SHWSC>SHWB = SHWSA>SCP. pH values of the fermented maize liquor was acidic while sodom apple aqueous extract was neutral. The natural colours of the roselle extracts were red while CFML was off-white and CF was leaf-green. The coagulation property of the roselle extracts could be attributed to the acidic nature of the extracts. Calotropis procera extract coagulation of soymilk has been reported to be enzymic based (Aworh and Muller, 1987).

Table 2 shows the pH and titratable acidity of different tofus. pH of roselle tofu was within 5.32 – 6.26.

The titratable acidity of the tofu sample coagulated with roselle extract ranged from 0.16% to 0.42%, while in tofu coagulated with fermented maize water was 0.47% and that which was coagulated with Calotropis procera was 0.01%. This indicated that the titratable acidity of tofu is dependent on the coagulant type.

Table 3 shows the yield and chemical analysis of the different tofus. Yield of roselle extract coagulated tofu was within 87.3 – 95.9 g. Yield for SCMLF tofu was 94.2 g and for SCP tofu was 83.2 g. The yield of the roselle coagulated curd increased with increase in concentration of the coagulant. Yield of SCHWSC tofu was similar to that of SCMLF tofu and that of SCP tofu was the lowest. This study shows that the yields of roselle coagulated tofus are generally comparable with tofus prepared from other natural coagulants used in this study.

Protein content of the roselle extract coagulated tofu ranged from 42.6 – 46.3 g/100 g. SCMLF and SCP tofu were not significantly different in protein content. SCHWSA tofu and SCHWSB tofu had higher ash and moisture contents, while SCHWSC tofu and SCHWSD tofu were lower in moisture and ash content.

Table 2: pH and titratable acidity of roselle extract coagulated tofu in comparison with tofus coagulated with two natural coagulants

<table>
<thead>
<tr>
<th>Tofu samples</th>
<th>pH</th>
<th>Titratable acidity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCHWSA tofu</td>
<td>6.26±0.01</td>
<td>0.16±0.02</td>
</tr>
<tr>
<td>SCHWSB tofu</td>
<td>5.88±0.03ab</td>
<td>0.25±0.01c</td>
</tr>
<tr>
<td>SCHWSC tofu</td>
<td>5.89±0.02ab</td>
<td>0.38±0.03d</td>
</tr>
<tr>
<td>SCMLF tofu</td>
<td>6.43±0.01c</td>
<td>0.47±0.01a</td>
</tr>
<tr>
<td>SCP tofu</td>
<td>6.83±0.01a</td>
<td>0.01±0.01e</td>
</tr>
</tbody>
</table>

Means with the same superscript within column are not significantly different (p > 0.05)

SCHWSA = 2.5% roselle extract coagulated tofu, SCHWSB = 5% roselle extract coagulated tofu, SCHWSC = 10% roselle extract coagulated tofu, SCHWSD = 20% roselle extract coagulated tofu, SCMLF = fermented maize liquor coagulated and SCP = Calotropis procera coagulated tofu.
Taste panel evaluation of the tofu for sensory acceptability is shown in Table 4. Among the roselle precipitated tofu, SCHWSA tofu was rated highest for appearance, flavour and overall acceptability, which was not significantly different at $p > 0.05$ from fermented maize steep water coagulated tofu (SCCMLF) in terms of appearance, flavour and overall acceptability. SCHWSA and SCHWSB tofus were not significantly different at $p > 0.05$ for colour and flavour were acceptable in terms of all of the attributes tested. In comparing SCCMFL and SCP tofus with the roselle extract coagulated tofus, SCCMFL tofu had the highest scores for appearance, colour and firmness. SCHWSA and SCHWSB tofus were acceptable in terms of flavour than SCMFL tofu. SCP tofu had the least acceptability among all the samples.

Table 4: Mean sensory attribute of roselle coagulated tofus in comparison with two natural coagulants

<table>
<thead>
<tr>
<th>Sample</th>
<th>Appearance</th>
<th>Colour</th>
<th>Flavour</th>
<th>Firmness</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCHWSA tofu</td>
<td>7.6 ± 0.02$^a$</td>
<td>7.4 ± 0.04$^b$</td>
<td>7.2 ± 0.07$^a$</td>
<td>6.3 ± 0.03$^b$</td>
<td>7.1 ± 0.03$^a$</td>
</tr>
<tr>
<td>SCHWSB tofu</td>
<td>6.6 ± 0.01$^b$</td>
<td>7.3 ± 0.03$^b$</td>
<td>7.5 ± 0.03$^a$</td>
<td>6.2 ± 0.01$^b$</td>
<td>6.9 ± 0.04$^{ab}$</td>
</tr>
<tr>
<td>SCHWSC tofu</td>
<td>6.3 ± 0.06$^b$</td>
<td>6.7 ± 0.05$^c$</td>
<td>5.7 ± 0.02$^b$</td>
<td>6.5 ± 0.02$^b$</td>
<td>6.3 ± 0.03$^c$</td>
</tr>
<tr>
<td>SCHWSD tofu</td>
<td>3.2 ± 0.02$^c$</td>
<td>3.0 ± 0.04$^d$</td>
<td>5.4 ± 0.04$^b$</td>
<td>7.4 ± 0.03$^a$</td>
<td>4.3 ± 0.02$^d$</td>
</tr>
<tr>
<td>CMLF tofu</td>
<td>7.5 ± 0.05$^a$</td>
<td>8.5 ± 0.02$^a$</td>
<td>5.2 ± 0.01$^b$</td>
<td>7.6 ± 0.05$^a$</td>
<td>7.3 ± 0.01$^a$</td>
</tr>
<tr>
<td>SCP tofu</td>
<td>3.4 ± 0.03$^c$</td>
<td>3.6 ± 0.04$^d$</td>
<td>2.0 ± 0.04$^c$</td>
<td>5.5 ± 0.05$^c$</td>
<td>3.7 ± 0.04$^c$</td>
</tr>
</tbody>
</table>

Means with the same superscript within column are not significantly different ($p > 0.05$)

SCHWSA = 2.5% roselle extract coagulated, SCHWSB = 5% roselle extract coagulated, SCHWSC = 10% roselle extract coagulated, SCHWSD = 20% roselle extract coagulated, SCCMLF = fermented maize liquor coagulated and SCP = *Calotropis procera* coagulated tofu

Table 3: Yield and chemical analysis of tofu coagulated with roselle extracts and two natural coagulants (a wet basis, b dry basis)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Yield$^a$ (g)</th>
<th>Protein$^a$ (g/100g)</th>
<th>Fat$^b$ (g/100g)</th>
<th>Ash$^b$ (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCHWSA tofu</td>
<td>87.3 ± 4.8$^c$</td>
<td>43.5 ± 1.4$^a$</td>
<td>12.3 ± 0.7$^b$</td>
<td>5.3 ± 0.02$^b$</td>
</tr>
<tr>
<td>SCHWSB tofu</td>
<td>90.2 ± 4.6$^b$</td>
<td>42.6 ± 2.7$^b$</td>
<td>12.5 ± 0.6$^a$</td>
<td>5.8 ± 0.01$^a$</td>
</tr>
<tr>
<td>SCHWSC tofu</td>
<td>95.5 ± 3.2$^a$</td>
<td>46.3 ± 1.7$^a$</td>
<td>13.1 ± 0.5$^a$</td>
<td>4.3 ± 0.03$^c$</td>
</tr>
<tr>
<td>SCHWSD tofu</td>
<td>95.9 ± 5.2$^a$</td>
<td>45.2 ± 4.2$^a$</td>
<td>12.3 ± 0.3$^a$</td>
<td>4.1 ± 0.02$^c$</td>
</tr>
<tr>
<td>SCCMLF tofu</td>
<td>94.2 ± 3.7$^a$</td>
<td>44.5 ± 1.2$^a$</td>
<td>12.9 ± 0.2$^a$</td>
<td>4.4 ± 0.01$^c$</td>
</tr>
<tr>
<td>SCP tofu</td>
<td>83.2 ± 2.1$^d$</td>
<td>44.4 ± 1.3$^a$</td>
<td>13.4 ± 0.4$^a$</td>
<td>6.1 ± 0.02$^a$</td>
</tr>
</tbody>
</table>

Means with the same superscript within column are not significantly different ($p > 0.05$)

SCHWSA = 2.5% roselle extract coagulated, SCHWSB = 5% roselle extract coagulated, SCHWSC = 10% roselle extract coagulated, SCHWSD = 20% roselle extract coagulated, SCCMLF = fermented maize liquor coagulated and SCP = *Calotropis procera* coagulated tofu
Conclusion
This study shows that roselle aqueous extract could be used in coagulating soymilk into tofu. Coagulating property of roselle extracts is attributed to the acidic nature of the extracts (pH 2.01 – 3.74). Yield of roselle coagulated tofu increased with increase in concentration of the roselle extract from 2.5% to 10%. The yield and protein contents of roselle coagulated tofus were comparable with tofus prepared from other natural coagulants used in the study. Roselle extract when utilized at 2.5% concentration will yield tofu that is acceptable and comparable to tofu coagulated with fermented maize steep water in terms of appearance, flavour and overall acceptability. Roselle extracts will serve as an alternative, cheap and natural coagulant for tofu preparation at household and commercial level.

References