Effect of Calotropis procera on the Proximate Composition and Potential Toxicity of Wagashi (Traditional Cheese) in Benin

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Abstract
The Peulh cheese, locally known as Wagashi, is a popular local milk product in Benin. The most important step in the production of Wagashi is the coagulation of the milk into cheese using fresh leaves and stems of *Calotopis procera*. *Calotropis procera* is a well-known traditional medicinal plant in the world and, particularly utilized in West Africa in the treatment of many infections. Despite the importance associated with this plant, it has been shown that the plant contains toxic substances that can threaten human health. To define the properties of this plant, we embarked on determining the phytochemical composition of *Calotropis procera*, Wagashi and whey, evaluation of the value added by the coagulant by comparing the proximate composition of the milk to the one of the wagashi as well as assess the safety of the Wagashi and the whey. Milk from three different breeds of cows (Girolando, Borgou and Lagunaire) were collected from Benin, and used to prepare the cheese and whey evaluated in this study. Our results revealed that there is significant variation ($p \leq 0.05$) between the gross composition of the milk, Wagashi and whey. The moisture content vary from 84.19% for milk to 62.47% to the cheese, the ash content vary from 0.67% in milk to 1.41% for cheese while the protein content vary from 2.44% in milk to 8.18% for cheese while the lactose vary from 2.02% to 3.0% for cheese; as for the carbohydrate content decrease from 6.12% in the milk to 4% in the cheese for the breed Girolando. The moisture content vary from 81.68% for milk to 57.34% to the cheese, the ash content vary from 0.63% in milk to 1.46% for cheese while the protein content vary from 1.73% in milk to 8.03% for cheese, the fat content vary from 7.5% to 4.52% for cheese while the lactose vary from 2.37% to 2.90% for cheese; as for the carbohydrate content decrease from 5.38% in the milk to 3.77% in the cheese for the breed Borgou. The moisture content vary from 84.19% for milk to 62.74% to the cheese, the ash content vary from 0.67% in milk to 1.41% for cheese while the protein content vary from 2.44% in milk to 8.18% for cheese, the fat content vary from 5.10% to 7.11% for cheese while the lactose vary from 2.02% to 3% for cheese; as for the carbohydrate content decrease from 6.12% in the milk to 4% in the cheese for the breed Lagunaire. However, the variance in composition is dependent on the breed from which the milk was obtained. Besides, the phytochemicals present in *Calotropis procera* such as alkaloids, flavonoids, tannins, cardiac glycosides, phenols, saponins and steroids were not detected in both the cheese and whey. We deduced that the process of Wagashi and/or whey processing denatures the phytochemicals, thus assuring the safety of the products. However, further studies exploiting more sensitive analytical methods are required to confirm these findings
Keywords: Milk, cheese, whey, proximate, *Calotropis procera*, phytochemical analysis

1. Introduction

The supply of healthy and nutritious food is a main element of the food security in Africa. Efforts are thus made to ensure that food and nutrition security is guaranteed. All these approaches are critical in ensuring the food and nutrition security in Africa since the continent is characterised by within and between year food supply fluctuations mainly resulting from relying on rainfed agriculture to produce food. Milk like other agricultural commodities has periods of glut and periods of shortage and this could explain the popularity of cheese making in West Africa coupled with the need to satisfy protein needs in rural poor households. Cheese is a nutritious food being one of the numerous products from processing of milk of cows, goats, sheep, buffalos, camels and yaks. It is produced by coagulation of the milk protein known as casein (Scott, 1986; Akinloye et al., 2014). According to Fox *et al.* (2000), cheese is a product processed from milk by acidification and coagulation. The cheese is produced in a wide range of flavours, textures and forms by coagulation of the milk protein. Cheese production serves as a means of preserving of essential nutrients in milk, and it has been identified as an excellent source of proteins, fat, minerals and vitamins (O'Conner, 1993; Chikpah et al., 2014). Cheese made from milk and *Calotropis procera*, a traditional "Peulh" cheese also called «waragashi» or «wagashi» is one of the base food in West Africa mainly in Benin. In general, its manufacture requires rennet which is the most used enzyme for the coagulation of milk. The latter because of its extremely high cost and limited availability does not favor the manufacture of fresh cheeses in the traditional environment. Then the Fulani found a way to use the leaves of *Calotropis procera* as coagulant for the production of fresh cheese (Abakar, 2012). *Calotropis procera*, a plant of family *asclepiadaceae*, is well known for its medicinal as well as toxic properties. Even the accidental exposure to the latex produces contact dermatitis, keratitis, and toxic iridocyclitis (Raman *et al*., 2005).The toxins in *Calotropis procera* can lead to blindness if its juice is put in to the eyes (Vibha, 2012).Considering the importance of this plant (*Calotropis procera*) in the people's diets and medicinal uses, it is important to know properly about its safety in processed wagashi. Egounléty *et al*., (1994) observed that despite the content of *Calotropis procera* in toxic substances (heterosides cardiot Oxic acid, especially calotropin), no case of food poisoning due to the consumption of cheese has been reported to date. This observation remains to be elucidated by scientific research. The present study was carried out in order to find out the safety of consumption of the traditional cheese Wagashi made with *Calotropis procera*. The objective of this study was to firstly determine proximate in milk, cheese and whey and compare the value in order to show the add value of the *calotropis procera* to the milk into cheese. And finally to do a phytochemical screening to detect the presence of phytochemical especially Cardiac glycosides (which is a toxin) in *Calotropis procera,* cheese and whey.

2. Materials and Methods

2.1 Sampling sites and Breeds used

The milk samples were collected from three different breeds (Girolando, Borgou, Lagunaire) in Benin. The milk samples were collected in 50 ml falcon tubes from 4 cows of each breed. Each milk sample was immediately kept at -20°C (to avoid deterioration) until further analysis. All the samples (Milk, cheese, whey and *Calotropis procera*) were kept at -20ºC in a cool box and transported from Benin to Kenya for further analysis in the food science laboratories at Jomo Kenyatta University of Agriculture and technology.

2.2 Technology of production of cheese

Milk samples collected from different breeds in Benin and *Calotropis procera* leaves were used to produce cheese and whey. The milk was filtrated into the pot on fire for heating. When the temperature of the milk reached 60-70 ºC, the *Calotropis procera* leaves and stem which were previously looted and mixed with some fresh milk were filtrated into the warm milk. The mixture was left on fire and kept at this temperature until coagulation was achieved and the heating was stopped after the separation of curd and whey. The sign of coagulation was observed within the range of 8-12 minutes. The mixture was transferred into a small sieve basket to facilitate whey drainage and characteristic shape. When the cheese was firm enough it was removed from the raffia basket and placed inside a covered plastic container or zip bags for analysis.

2.3 Proximate Analysis

The proximate composition of the milk, whey and cheese samples was determined by a standard method used for moisture (AOAC, 1990), protein (AOAC, 1980), fat (Bligh & Dyer 1959) for cheese and Kirt (1991) for milk, ash (AOAC, 1984), lactose in milk (Lactose scan) lactose scan in cheese (AOOC, 1994) and carbohydrate content (Hedge & Hofreiter, 1962). All the results of proximate analysis were reported on wet basis.

2.4 Phytochemical Analysis

2.4.1 Extraction

About 500g of fresh leaves and stem of *Calotropis procera* were washed using distilled water and dry in the oven at 55ºC for about 72 h. The leaves and the stem were separately grounded to obtain powder. Methanolic extract of the leaves was prepared by soaking approximatively 1g of the dry powdered plant leaves in20 ml Methanol at room temperature for 72 h. The extract was then filtered first through a Whatmann filter paper No. 42 (125 mm) and then through cotton wool. The same procedure was done for the cheese and the whey. The extracts were used for phytochemical screening

2.4.2 Phytochemical Screening

2.4.2.1 Tannins determination:

To 3 ml Methanolic extract was added 3 ml of10% ferric chloride ($FeCl_3$). A formation of blue/ black color was a positive indicator of presence of tannins. (Trease and Evans, 2002).

2.4.2.2 Phenols compounds:

To test for phenols, the ferric Chloride test was used. To 1 ml of the extract, 2 ml of distilled water, 3 drops of 10% aqueous ferric chloride ($FeCl_3$) and 3 drops of potassium ferro-cyanide were added. Formation of blue or green color showed the presence of polyphenols. (Tiwari, *et al* 2011)

2.4.2.3 Flavonoids

This was done according to the method of Harbone (1973). 5ml of dilute ammonia solution was added to a portion of extract followed by addition of concentrated H_2SO_4. A yellow coloration observed indicated the presence of flavonoids.

2.4.2.4 Alkaloids:

This was determined using Mayer's test as describe by Tiwari *et al* (2011). Mayer's reagent (potassium mercuric iodide) was prepared by dissolving a mixture of mercuric chloride (1.36g) and potassium iodide (5g) in water (100ml). To determine the presence of the alkaloids, 1ml of Mayer's reagent was mixed with 1 ml of the samples' extract. Formation of yellowish buff indicated the presence of alkaloids.

2.4.2 5 Cardiac glycosides:

This was determined using Kedde reagent test.5 mL of the sample extract were placed in a test tube, 5mL of kedde's reagent, and 5mL of 2 N sodium hydroxide solution were added. The appearance of purple colour indicated a positive test for cardiac glycosides (Nagy Morsy *et al.,* 2016).

2.4.2.6 Saponins:

The Foam test was used to determine saponins in the samples. To 1 ml of the extracts 5 ml distilled water was added and shaken vigorously. Formation of foam that persisted for over 10 minutes indicated presence of saponins (Sofowora, 1993).

2.4.2.7 Steroids:

This was determined using Liebermann Burchard test. About 5 mL of the solution were evaporated to dryness. The residue was dissolved in 2 mL chloroform and transferred to a small test tube. Acetic anhydride (0.3 mL) was added and mixed gently, then, a drop of concentrated sulphuric acid was added. The appearance of blue-green colour, observed during 60 min, indicated presence of steroids (Rohit., 2015).

2.5 Statistical analysis

The data reported are averages of triplicate observations and were subjected to a one way analysis of variance to determine the significant differences among the samples collected from different breeds and the means were separated using the Duncan test with a significance level of 5%. All data were analyzed using SPSS 16.0 package.

3. Results

3.1 Proximate results

All result of proximate analysis were reported on wet basis

GIROLANDO cattle Breed

The table 1 present the result of proximate analysis of milk, cheese and whey obtained from Girolando cattle breed. The results of proximate composition of milk, whey and cheese produced from the milk using *Calotropis procera* showed that moisture content in the milk, cheese and whey were 89.14, 68.36 and 96.17 % respectively. The highest ash content (0.69%) was obtained in Wagashi while the lowest ash content (0.30%) was obtained in whey. But the highest protein content (9.11%) was obtained in wagashi and the lowest was obtained in whey (0.44%). The result also shows that the highest fat content, carbohydrate content, lactose content respectively 3.02%; 4.61% and 4.26% were obtained in Wagashi.

Table 1: Proximate analysis of milk, cheese, whey from breed Girolando

Parameters	Moisture (%)	ASH (%)	Protein (%)	Fat (%)	CHO (%)	Lactose (%)
Milk	89.14 ± 0.305^{a}	0.69 ± 0.003^{a}	1.32 ± 0.024^{a}	2.15 ± 0.071^{a}	4.38 ± 0.122^{a}	2.53 ± 0.0003^{a}
Cheese	68.36 ± 1.033^{b}	1.58 ± 0.081^{b}	9.11 ± 0.079^{b}	3.02 ± 0.310^{a}	4.61 ± 1.22^{a}	4.26 ± 0.09^{b}
Whey	96.17 ± 0.184^{c}	0.30 ± 0.006^{c}	0.44 ± 0.023^{c}	0.33 ± 0.000^{c}	4.60 ± 1.572^{a}	0.24 ± 0.0001^{c}

Means within the same column with different superscript letters were significantly ($p<0.05$) different. Values are presented as mean± standard deviation, n=3. Each value is mean of triplicate. S.D ()

BORGOU cattle breed

The Table 2 present the result of proximate analysis of milk, cheese and whey obtained from Borgou cattle breed. From the analysis of this table, the whey has the highest moisture content (93.94%) while the lowest moisture content (57.34%) was obtained in Wagashi. The highest ash (1.46%) content was obtained in cheese while the lowest (0.52%) was obtained in whey. As for the highest protein content (8.03%) was obtained in Wagashi while the lowest was obtained in whey. But the highest fat content (7.5%) was obtained in milk and the lowest content (3.77%) was obtained in Wagashi. The highest carbohydrate content 5.38% was in milk while the lowest content (3.77) was in Wagashi. But the highest lactose content (2.90%) was obtained in cheese.

Table 2: Proximate analysis of milk, cheese, whey from breed Borgou

Parameters	Moisture (%)	ASH (%)	Protein (%)	Fat (%)	CHO (%)	Lactose (%)
Milk	81.78 ± 1.735^{a}	0.63 ± 0.002^{a}	1.73 ± 0.057^{a}	7.50 ± 0.000^{a}	5.38 ± 0.244^{a}	2.37 ± 0.0004^{a}
cheese	57.34 ± 0.097^{b}	1.46 ± 0.300^{a}	8.03 ± 0.765^{b}	4.52 ± 0.093^{b}	3.77 ± 0.298^{b}	2.90 ± 0.083^{b}
whey	93.94 ± 0.215^{c}	0.52 ± 0.452^{a}	1.14 ± 0.064^{a}	0.96 ± 0.240^{c}	4.27 ± 0.341^{a}	0.99 ± 0.001^{c}

Means within the same column with different superscript letters were significantly ($p<0.05$) different. Values are presented as mean± standard deviation, n=3. Each value is mean of triplicate. S.D ()

LAGUNAIRE cattle breed

Table 3: proximate analysis of milk, cheese, whey from breed Lagunaire

Parameters	Moisture (%)	ASH (%)	Protein (%)	Fat (%)	CHO (%)	Lactose (%)
Milk	84.19±0.413[a]	0.67±0.035[a]	2.44±0.053[a]	5.10±0.283[a]	6.12±0.560[a]	2.02±0.0003[a]
cheese	62.47±1.214[b]	1.41±0.094[b]	8.18±0.092[b]	7.11±0.459[b]	4.00±0.794[a]	3.00±0.023[b]
whey	93.01±0.085[c]	0.37±0.016[c]	0.70±0.034[c]	0.93±0.300[c]	3.48±0.156[c]	1.36±0.00[c]

Means within the same column with different superscript letters were significantly ($p<0.05$) different. Values are presented as mean± stand standard deviation, n=3. Each value is mean of triplicate. S.D ()

The Table 3 present the result of proximate analysis of milk, cheese and whey obtained from Lagunaire cattle breed.

The highest moisture content (93.01%) was obtained in whey while the lowest content (62.47%) was obtained in Wagashi. The highest ash content (1.41%), protein content (8.18%), fat content (7.11%) carbohydrate content (4.00%) and lactose content (3.00%) were obtained in wagashi while the lowest content (Table 1) were obtained in whey.

3.2 Results for phytochemical analysis

3.2.1 Phytochemical in *Calotropis procera* (stem and leaves)

The Table 4 shows the result of phytochemical screening of *Calotropis procera* (stem and Leaves). From the table tannins and flavonoids presence are just detected (+) in all the plant sample (stem and leaves). However, phenols is more detected (++) in leaves and just detected (+) in stem. However the alkaloids is more intensified (+++) in leaves and stem. The Cardiac glycosides is detected in leaves, more colored (++) in stem. The saponins and steroids are detected (+) in stem and more colored (++) in leaves.

Table 4: present the phytochemical screening results for *Calotropis procera* (stem, leaves) collected from Benin

Phytochemicals	Leaves of plant	Stem of plant
Tannins	+	+
Flavonoids	+	+
Phenols	++	+
Alkaloids	+++	+++
Cardiac glycosides	+	++
Saponins	++	+
Steroids	++	+

- Not detected + detected ++ more colored +++ more intensify

3.2.2 Cheese and whey

The same phytochemical screening analysis was done for the cheese and the whey and any phytochemical was detected.

4. Discussion

4.1 Proximate analysis

The proximate result of milks samples and wagashi and whey obtained from them showed significant difference (table 1, 2 and 3). From that result, we can see that the coagulation of the milk into wagashi using *Calotropis procera* added values to the wagashi by increasing some proximate especially protein, fat ash and carbohydrate. However that increasing or variation depend also on the origin of the milk (breed) used to produce the wagashi. The result of the proximate analysis of the milk Wagashi and whey from breed Girolando (Table 1) shows that the moisture content in milk (89.14%) is significant different from the one (68.36%) in Wagashi and in the whey (96.17%). The same decrease of water content is obtained in milk Wagashi and whey obtained from the breed Borgou (table 3) and breed Lagunaire (table 3). The reduction in moisture content might be due to the heat applied during Wagashi processing (Ogunlade, 2017). The reduction of the moisture content in Wagashi make it to be more preserve for long, ended a high water content could deteriorate the quality of the dairy product by promoting a rapid growth of microorganisms and a reduction in shelf life of the product (Aisso *et al.* 2013). The moisture content (89.14%) obtained in milk from the breed Girolando is the same to the one (89%) obtained by Aisso *et al.* (2013) for the same breed. While the moisture content (81.78%) obtained in milk from the breed Borgou and moisture content 84.19% obtained in milk from breed Lagunaire are similar to the result found by Kora (2005) who found 80.73% and 83.64% for moisture content respectively for breed Borgou and Breed Lagunaire. The moisture content in wagashi obtained (68.36%) from the milk of breed Girolando is slightly higher to the one (65.70%) obtained by Aisso et al. (2013) for the same breed. While the moisture content (table 2 and 3) in Wagashi from milk of breed Borgou and Lagunaire are also slightly lower than that obtained by Kora (2005) who found 65.23% and 65.73% for moisture content in Wagashi respectively for breed Borgou and Breed Lagunaire. These differences may probably due to quantity of coagulant (*Calotropis procera*) used or the technology of production used to process the Wagashi. The moisture value (table 1, 2 and 3) obtained in whey is slightly different than that obtained (94-95%) by Tsakali et al. (2010). The slight variation of moisture content in whey might be related to the fact that the milk used was from a different breed. The highest ash content was obtained in all Wagashi while the lowest was obtained in whey (Table 1, 2 and 3) for all breeds. This can be explained by the fact that the coagulant used (*Calotropis procera*) has an important effect on the milk into wagashi. The ash content is an indicator of the total mineral content in dairy product (Aisso, 2013). Thus, it can be then be conclude that the mineral is higher in Wagashi than milk and in the whey. This shows then the important of the plant *Calotropis procera* as coagulant. The highest protein values also were obtained in wagashi from all breed while the lowest was obtained in whey from all breed (table1, 2 and 3). This variation in high protein content of Wagashi in this study may be attributed to protein content in *Calotropis procera* have migrated into the Wagashi sample . The high protein content of this product shows that its consumption will help eliminate protein deficiencies and also shows the importance of the plant *Calotropis procera* as coagulant. The protein content obtained in Wagashi from all breed are slightly lower than that obtained by Kora (2005) who reported 12.60% and 12.34% respectively for breed Borgou and Lagunaire; and Aisso *et al.* (2013) who found 13,66 for breed Girolando. This variation could be due the technology of production of the Wagashi. Hannon *et al.,* 2006 reported that fat is important as a source of energy to the body; using *Calotropis procera* as coagulant in Wagashi production, it increase the fat content in Wagashi than that obtained in milk (table 1 and 3). However the fat content obtained in Wagashi in this study are lower than that obtained by Kora (2005) who has respectively found 15.05%, 15.22% and 15.62% fat in Wagashi respectively for breed Borgou, Lagunaire and Girolando. The lower fat content obtained in Wagashi (table 1,2 and 3) in this study could be due to the fact that they were frozen. The decrease in lipid level could also be justified by a leaching of the cheese during its passage at room temperature and by the migration of fat to the superficial layer of the Wagashi. Mazou (2011) sated that there is a decrease in lipid levels in the cold-preserved wagashi. The general analysis of the tables(1, 2 and 3) shows that the *Calotropis procera* is an important plant in Wagashi's manufactures. It brings an added value to the milk into Wagashi especially for Protein, Fat, and Ash therefore makes wagashi to be a good source of nutrition.

4.2 Discussion of the phytochemical analysis

- *Calotrpis procera*

The table 4 shows the result of the qualitative analysis of the plant collected from Benin. From the table tannins and flavonoids presence are detected (+) in all the plant sample (stem and leaves), this result is different from what was found by Morsy *et al.* (2016) who showed that the flavoinoids and tannins are not present in the leaves and stem of *Calotropis Procera.* This could be due to the geographical localization of the plant or to the extraction methods. However, phenols were detected (++) in leaves and detected (+) in stem. However the alkaloids were more intensified (+++) in leaves and stem. The Cardiac glycosides were detected in leaves, but more (++) in stem. The saponins and steroids were detected (+) in stem and more (++) in leaves. These results confirm the literature which stated that *Calotropis procera* has biologically active substances such as flavonoids, cardioactive glycosides, alkaloids, tannins and saponins (Shaker *et al*., 2010; Ahmed *et al*., 2005; Elimam *et al*., 2005). Based on the phytochemical results in *Calotropis procera* of the present study, it could be said that the plant extracts contain chemical constituents of pharmacological significance. The presence of these chemical constituents in this plant is an indication that the plant could yield drugs of pharmaceutical significance.

- Wagashi and whey

According to our research no study was done on phytochemical screening of Wagashi, so we discuss our results based on our own result. The absence of phytochemical in cheese and whey could be due to the methodology used or maybe the cheese and the whey do not contain the phytochemical or the cardiac glycosides as they may have been eliminated by the temperature of the coagulation of milk. Past studies reported that this plant (*Calotropis procera*) contains toxic substances but after testing phytochemicals in cheese, this study showed that toxins were not detected in cheese and in whey which might be due to heat reaction during production process. The *Calotropis procera* is plant that is widely used in traditional medicine to cure some diseases such as colds, fever, or as protection and also to manufacture wagashi in Benin by the Fulani people. The absence of phytochemicals especially the cardiac glycosides (toxic component) in cheese and whey could be due to the methodology used for screening or maybe the Wagashi and the whey do not contain the phytochemical due to the fact of the temperature of Wagashi production (≥100ºC), the toxins may be decomposed by the cheese's boiling. This makes the cheese and whey safe for consumption. If so more investigation need to be done.

5. Conclusion

From this study it emerges that *Calotropis procera* has a good coagulation effect therefore it brings an added value to the constituents of milk into Wagashi. The Wagashi be used to instead of fish, chicken in food diet. The whey obtained after the production of Fulani cheese also contains very rich constituents, therefore it can be used for other purposes, such as in animal feed, use in the food industry to obtain other byproducts.

The *Calotropis procera* is a plant rich in biologically active substances such as flavonoids, cardioactive glycosides, alkaloids, tannins and saponins. However those biological substance were not detected in wagashi and in whey. Therefore we deduced that the process of Wagashi and/or whey processing denatures the phytochemicals, thus assuring the safety of the products. However, further studies exploiting more sensitive analytical methods are required to confirm these findings especially on those phytochemical to understand well their behavior with high temperature.

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