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Review Article

Calotropis procera Extract for Wagashi Cheese Making in West Africa: A Review of Applications and Perspectives for Other Plant Coagulants Utilization

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Abstract

Plant extracts are used for centuries in traditional cheese manufacturing worldwide. This paper proposed to review the use of *Calotropis procera* extract in wagashi cheese manufacturing and the opportunities that are studied. After a brief reminder of the socioeconomic importance of the wagashi cheese for West African countries, the review discusses its biochemical composition and microbiological characteristics. The *Calotropis procera* enzymes and their proteolytic activities were described with a special attention paid on their caseinolytic and milk clotting properties. The microstructure and texture of milk-coagulated curds by *Calotropis procera* extracts we presented and discussed from recent studies.

Key words: *Calotropis procera*, plant extracts, wagashi cheese, biochemical composition, proteolytic activities, milk clotting, West Africa

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Milk is a highly demanding food, due to its high contents in water, proteins and lactose. In West Africa, livestock breeder's communities essentially consume fresh milk. Processes such as fermentation to make curd milk, churning to make butter and coagulation to make cheese, are mostly used to process the milk production in order to obtain products of long shelf life. Cheese is an excellent source of protein, fat and minerals such as calcium, magnesium and sodium[1,2]. Coagulation of milk is an important step in cheese making that determines the success of the process and the quality of the product[3].

Calf rennet is the main coagulant use for cheese manufacturing. However, milk clotting enzymes of microbial and plant origin exist. The plant enzymatic extracts are used in the manufacture of many artisanal cheeses around the world (Table 1). In some countries, extracts of *Cynara cardunculus* flowers are used to make traditional cheeses as described in literature[4]. *Gibna bayda*, the mostly consumed cheese in Soudan is traditionally manufactured by coagulating milk with *Solanum dubium* extracts[5]. *Withania coagulans* fruits extract possess the ability to clot milk and are used in India and Iran to make locally soft cheeses[6-9].

Several works took an interest in the milk clotting activity of *Carica papaya* leaves extracts. Since then, many investigations have been carried out on milk clotting properties of various plant species used in traditional cheese manufacturing[16]. Innovative results have been published, showing the main interest accorded by scientists to the milk clotting properties of plant extracts[7,8,21].

In Africa, plant extracts easily replace calf rennet in traditional cheese manufacturing. The use of industrial rennet from animal or microbial origin is limited by supplying, financial constraints and conservation. Consequently, local cheese producers have recourse to traditional methods of calf rennet extraction or use of plant extract for milk coagulation during traditional cheese manufacturing. In addition, the cheese manufacturing in Africa is largely dictated by traditional practices transmitted from generation to generation and the use of rudimentary equipment that did not allow industrial rennet utilization[22]. Therefore, local cheese producers found an alternative in the utilization of plant extracts as coagulant. In West African countries like Nigeria and Benin, the plant extract of *Calotropis procera* is used in the manufacture of the wagashi cheese[23,24]. The plant extracts have the advantage of being available and easy to use on artisanal scale. They do not require special conservation methods or specific abilities instead of industrial rennet and often grow wild near breeding places.

The milk clotting activity of plant extracts is due to the presence of chymosin like proteases that are able to hydrolyze casein micelles in milk, causing by that, their reorganization and the formation of the curd[3]. The milk clotting activity of plant extracts of an enzyme depends on its source, the extraction method employed, the degree of purity and the part of the plant that is used[25,26]. The main milk clotting enzymes isolated from plants are cysteine proteases (ficine from *Ficus carica*, papain from *Carica papaya*, calotropin from *Calotropis procera*, zingibain from *Zingiber officinale*). However, enzymes belonging to the group of serine (cucumisin from *Cucumis melo*, streblin from *Streblus asper*, dubiumin from *Solanum dubium*) and aspartic proteases (cardosins from *Cynara cardunculus*, calotropin from *Calotropis procera*) have also been highlighted in their performance of milk coagulation[27]. The Table 2 presented the characteristics of some milk clotting enzymes from plant origin.

This paper reviews the use of *Calotropis procera* extract in the manufacture of the traditional West African cheese and briefly present last studies on the milk clotting properties of *Carica papaya*[45] leaves and *Balanites aegyptiaca* fruits[21] that are new tracks for milk clotting extracts for traditional cheese making in West Africa.

Table 1: Plant extracts used for cheese making

Plants	Family	Samples	Milk	Cheese	Country	References
Solanum dubium	Solanaceae	Leaves fruits	Cow milk	*Gibna bayda*	Sudan	Kheir *et al.*[5]
Cynara cardunculus	Asteraceae	Flowers	Sheep milk	Torta del Casar	Spain	Sousa and Malcata[10]
				Serra da Estrela	Portugal	Silva and Malcata[11]
Cynara scolymus	Asteraceae	Flowers	Buffalo milk	Tallaga cheese	Egypt	El-Kholy *et al.*[12]
				Jeben	Algeria	Nouani *et al.*[13]
Zingiber officinalis	Zingiberaceae	Rhizome	Cow milk	Jiang zhuang nai	Japan	Hashim *et al.*[14]
			Buffalo milk	Jiangzhinai		Huang *et al.*[15]
Calotropis procera	Asclepiadaceae	Leaves	Cow milk	Warankashi	Nigeria	Aworh and Nakai[16]
		Latex		Wagashi	Benin	Aworh and Muller[17]
Ficus carica	Moraceae	Latex	Goat milk	Cacioricotta	Italy	Faccia *et al.*[18]
Streblus asper	Moraceae	Latex	Cow milk	Dadih	Malaysia	Yazid *et al.*[19]
Withania coagulans	Solanaceae	Fruits	Sheep milk		Iran	Pezeshki *et al.*[20]

Table 2: Characteristics of some milk clotting proteases from plants origins

Plant	Enzyme	Type	Mass weight (KDa)	Optimal pH	Optimal temperature (°C)	References
Cucumis melo	Cucumisin	Serine protease	50	7.1	70	Kaneda and Tominaga[28]
			68.4	9	60-70	Gagaoua *et al.*[29]
				7.1	37	Uchikoba and Kaneda[30]
Cucumis trigonus			67	11	70	Asif-Ullah *et al.*[31]
Streblus asper	Streblin	Serine protease	64	9	65	Tripathy *et al.*[32]
Calotropis procera	Procerain	Cysteine proteases	28.8	7-9	55-60	Dubey and Jagannadham[33]
	Procerain B		25.7	6.5-8.5	40-60	Singh *et al.*[34]
	Proteinase II		25	7	50	Aworh *et al.*[35]
	Proteinase I		30	8	50	
Cynara cardunculus	Cardosin A	Aspartic proteases	31+15	5.1	-	Verissimo *et al.*[36]
	Cardosin B		34+14	5.1	70	Heimgartner *et al.*[37]
Cynara scolymus	Cynarase A	Aspartic proteases	30-15	5	60	Llorente *et al.*[38]
	Cynarase B			5	70	Sidrash *et al.*[39]
Zingiber officinalis	Zingibain	Cysteine protease	36	5.5	60	Hashim *et al.*[40]
			31	5.58-5.22	70	Huang *et al.*[15]
Euphorbia milii	Miliine	Serine protease	-	9	45	Moro *et al.*[41]
Euphorbia nivulia	Nivuliine II	-	43.42	6-6.5	55	Badgujar and Mahajan[42]
Opuntia ficus-indica	-	Cysteine protease	23.2	5.2	70	Teixeira *et al.*[43]
Solanum dubium	Dubiumine	Serine protease	-	10	70	Kheir *et al.*[5]
			66	11	70	Ahmed *et al.*[44]
Carica papaya	Papain	Cysteine protease	23.4	5-9	50	Monti *et al.*[45]
Ficus carica	Ficin	Cysteine protease	23	7	-	Devaraj *et al.*[46]
Ficus religiosa	Religiosin	Cysteine protease	43.4	8-8.5	50	Kumari *et al.*[47]
	Religiosin B	Serine protease	63	8-8.5	-	Kumari *et al.*[48]

Use of *Calotropis procera* extract for traditional wagashi manufacturing

History and socioeconomic importance of wagashi cheese in West Africa: Wagashi is the name given to the traditional West African soft cheese produced in both savannah and forest. It is the West African cheese per excellence considering the importance of its production and the geographical area of its marketing i.e., Benin, Nigeria, Ghana, Togo and all border countries.

The name “wagashi” dates from 17th century, a period during which the Sahelian communities, i.e., Hausa, Songhay and Fulani settled in Northern Benin and adapted themselves and their activities to its weather conditions. The abundance of water and the availability of pasture all year round created the favorable conditions for a great milk production. The traditional commerce of butter and rotten milk practiced by Fulani women did not allow to flow out the overfull of milk. However, the forest populations were not major consumers of milk products because of their eating habits and frequent allergies to lactose. Pastoralists therefore had to find a way to sell their overfull of milk. It is in this context that wagashi was borne. Its manufacture was inspired by a Songhay knowledge as the Songhay of Gao and Tombouctou areas were already manufacturing a traditional soft cheese named “gassi” or “gashi”. The “gassi” cheese was manufactured with goat milk using animal rennet for the milk clotting.

Wagashi was certainly produced to meet the demand of numerous Songhay living beside the Fulani since the fall of the Songhay Empire. With time and practice, the cheese manufacturing was improved and the animal rennet was replaced by *Calotropis procera* extract. Wagashi became that a known and appreciated local cheese[24].

The name wagashi is composed of two parts: “*waa*” which means milk in Songhay language and “*gashi*” which means shuddering[24]. The name’s composition refers to the cheese manufacturing process in which an extract of *Calotropis procera* was added to a heated milk. At the coagulation time and under the effect of heating, the milk gives an effect of shuddering during the formation of the curd. Fulani adapted the name to their vernacular language and call the cheese “*gasire*” in singular and *“gasiije”* in plural.

The trade of wagashi, even as a traditional and local product is very lucrative and its marketing chain is important and well-organized. The cheese is produced in camps by Fulani women and sold in rural markets to sedentary women who collect and resale back the cheese in large urban markets. The cheese’s trade chain is thus in such a way that sale is from rural camps to rural markets, then from rural markets to large urban centers and finally from large urban centers to neighboring countries. Therefore, through wagashi trade, the Fulani in the wagashi production areas became very rich through earning more benefits than Sahelian[24].

Fig. 1(a-c): (a) *Calotropis procera* latex tree, (b) Flowers, fruit and (c) Seeds

Currently, the demand for the cheese is increasing due to urban growth and modernization of its cooking methods. The promoters of Beninese gastronomy are continually making propositions to value the cheese. Thus, wagashi is now valorized in modern restaurants menus that propose new dishes like "Fulani cheese salad" and "Fulani cheese skewers". The official tables also, integrate wagashi as a local specialty.

***Calotropis procera* plant:** *Calotropis procera*, commonly known as sodom apple, dead sea apple, swallow wort or giant milkweed, is a plant from Asclepiadaceae family widespread in West and East Africa, but also in Asia, especially in India, Saudi Arabia and Pakistan[49,50]. The plant is a soft wood shrub, growing in arid and semi-arid areas and does not exceed 6 m high[51]. The leaves are opposite, large, green and covered by a white film-coat when young (Fig. 1a). The inflorescence is dense, multiflowered and appear axillary or terminal. The flowers are pentamerous, white and purple colored (Fig. 1b). The seeds are ovate, brown colored, flattened silky and overcome by a white light fluff (Fig. 1c).

Calotropis procera is a latex plant used in many traditional medicines for its therapeutically properties[51]. The plant extracts possess antimicrobial[52-55], antihelminthic[56-58], antipyretic[50] and anti-inflammatory activities[59,60]. In India, the decoction of the plant is used in traditional medicine to treat dysentery, fever, rheumatism, asthma and as expectorant and purgative[61]. Investigations in the Kingdom of Saudi Arabia reported that the different parts of the plant, including the roots, are commonly used to combat fever, constipation, muscular pain and spasm[51]. In Nigeria, local communities especially in the north, use *Calotropis procera* to treat infectious diseases, indigestion and rheumatism[52,62]. At the opposite of these virtues, the latex of *Calotropis procera* have been reported to induce ocular toxicity[63], burning and throat edema when ingested and inflammation of skin and mucous membranes[56]. Among all the parts of the plant, stems and roots have higher toxicity probably because of their high content in latex[62].

Wagashi cheese

Areas of production and availability: In West Africa, there is traditionally two types of cheeses i.e., the dried one, named "Tchuku", a specialty of nomadic Tuareg and Fulani communities and the fresh one, named "wagashi", manufactured by Fulani and some Yoruba northern communities[17]. In the Tchuku process, coagulation of the milk is obtained by adding an abomasum aqueous extract to whole cow, sheep or goat milk. The Tuareg cheese basin is with Soudan, the only area in Sub Saharan Africa where the use of abomasum extract for cheese making is well mastered by populations[22]. In the manufacture of wagashi, a plant extract of *Calotropis procera* leaves is used as milk coagulant with whole cow milk. The curd obtained is white with a sweet milk taste (Fig. 2a). A stain with sorghum extract gives it a red color reminiscent of that of the meat that it easily replaces in many traditional dishes[64,65] (Fig. 2b).

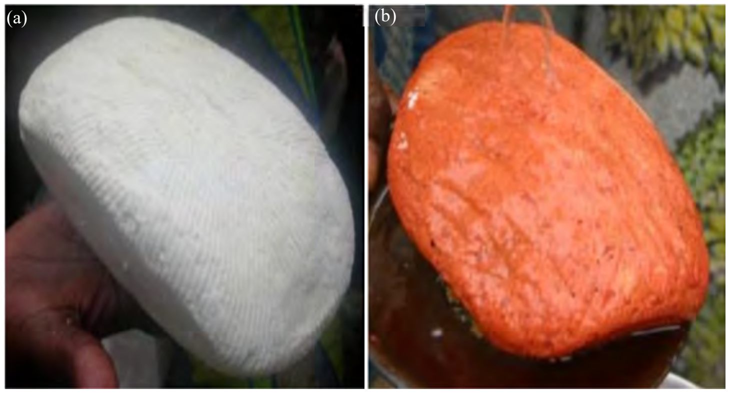

Fig. 2(a-b): (a) Fresh and (b) Colored (B) traditional wagashi
Red color is due to the coloration by the *Sorghum panicum* extract during manufacturing

Fig. 3: Areas of production and availability of Wagashi in West Africa

The traditional West African soft cheese manufactured with *Calotropis procera* extract as coagulant is largely produced in Nigeria, Ghana, Ivory Coast, Togo and the Republic of Benin but is also available in many areas of West Africa, especially in countries that share borders with the cheese's areas of production (Fig. 3). The cheese takes practically the same name in the sub region: « Warankashi » or « Wara » in Nigeria, « Waragashi » or «Woagashi» in the Republic of Benin and « Wagashi» in Ghana[66].

Cheese processing: The West African soft cheese is usually manufactured on artisanal scale by women from Fulani and Yoruba breeders communities[17,22]. The cheese processing is not well standardized and use traditional equipment and empirical methods[67]. The amount of leaves required for clotting a known quantity of milk is not well determined[68]. However, the expertise of traditional manufacturers limits the overdoses of *Calotropis procera* extract during the cheese processing[68]. All the parts of the plant excepted the roots possess the ability to clot milk and are employed for the coagulant extraction.

For traditional Wagashi processing, after milking, fresh whole cow milk is directly heated on wood fire without any standardization. The extraction of the coagulant is made by grinding young leaves of *Calotropis procera* in a little amount of milk or water and filtering. The filtrate obtained is

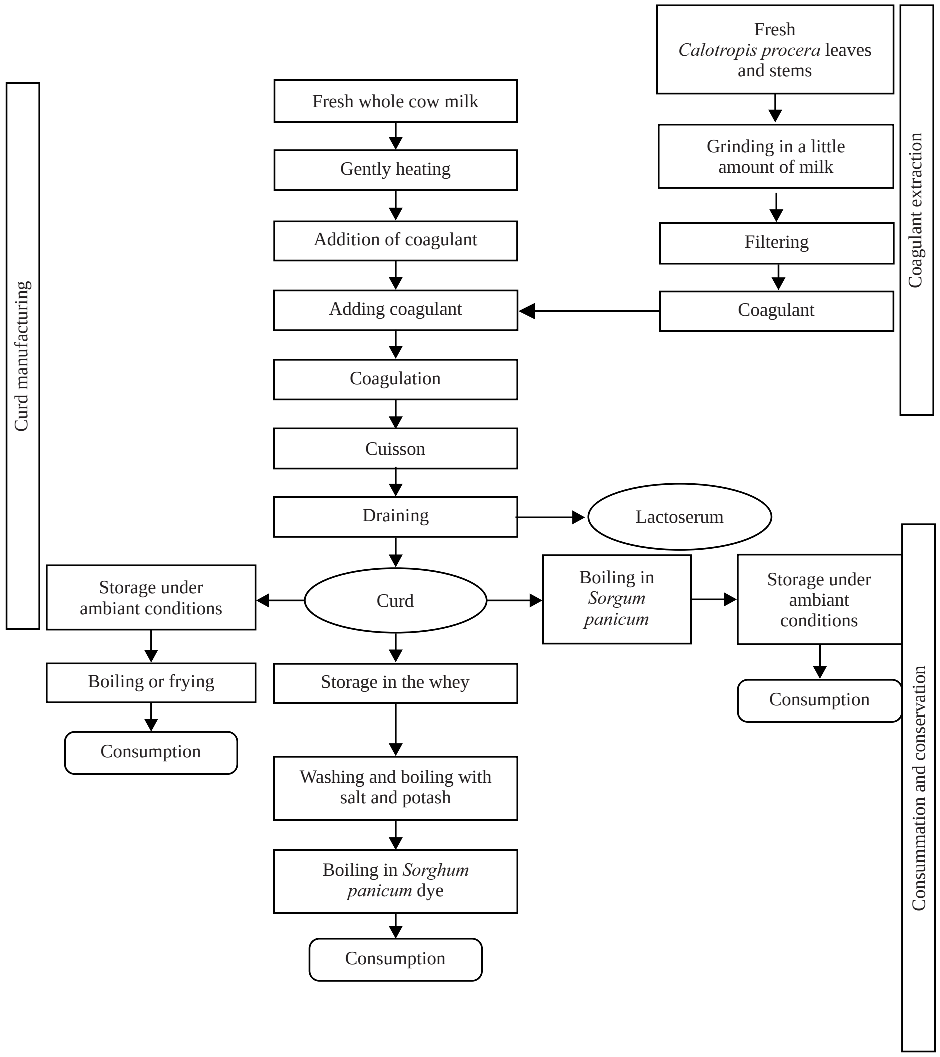

Fig. 4: Wagashi cheese traditional processing

incorporated to a whole cow milk heated on wood fire as described in our previous work. Then the heating continues by increasing fire intensity, until the separation of the curd and the whey. This results in a clear yellowish or greenish whey and crumbly curd with a sweet and creamy taste. The curd obtained, without being pressed, is drained for hours in different types of containers[22,69]. The operation is completed after obtaining a cheese with good physico-chemical and rheological properties. The traditional processing of Wagashi cheese was described in Fig. 4. A yield of about 1 kg of cheese for 5 liters of whole cow milk is classically obtained[70]. The cheese pieces are stored in the whey collected from the cheese making operation. The cheese is sometimes boiled, salted and colored with red sorghum (*Sorghum panicum*) extract that gives to the product a red color comparable to that of meat[64]. The coloring operation provides a protective cheese crust to wagashi and enhances its shelf life for few days.

Consumption and nutritional composition: The wagashi cheese is locally appreciated and consumed as is or incorporated to traditional into traditional food recipes. In rural areas, it is becoming the main supplier of proteins of animal origin, thus replacing meat or fish[71]. Wagashi cheese is consumed fresh, within 2 or 3 days after manufacture without ripening[70,72]. Wagashi easily finds its place in numerous traditional sauces to replace meat or fish or in combination with them. It can also be fried and eaten as fried cake or sandwich filling[73]. Furthermore, the West African soft cheese also finds way in the preparation of celebration and official receptions tables. This increases the local demand of the cheese.

The nutritional evaluation has revealed variable contents in protein, lipid and minerals. These variations can be attributed to the cheese processing method, the quality of the milk used, the storage period and the analytical methods used[68]. As the cheese processing is not standardized, its biochemical and nutritional composition varies a lot. For wagashi samples collected from local markets in Nigeria, the protein and fat contents varies from 20.13 and 4.10% to 37.8 and 39%, respectively[68,74]. Ojedapo *et al.*[75] have proved that salting increases the total solid content of the cheese and extend its shelf life. All the minerals contained in milk are present in the cheese but their concentrations vary also from one sample to another. In spite of these variations, wagashi can be considered as a dietary supplement especially for populations whose supply is poor in protein, minerals and probiotics[70,76]. On the other hand, Mahami *et al.*[77] and Badmos *et al.*[78] reported that *Moringa oleifera* extracts supplementation during wagashi manufacturing enhanced the protein, fat and mineral contents of the cheese from 14.50, 28.15 and 0.87% to 18.50, 34.04 and 0.94%, respectively.

However, as for many other plant proteases, the great inconvenience of *Calotropis procera* extract is its proteolytic activity which leads to a bitter taste in the cheese especially in its fresh state[76,79]. Fortunately, the fermentation that occurs within 24 to 48 hrs after the cheese manufacturing confers a sweet acidic taste that characterizes the cheese and hides the bitter taste when present.

Microbial quality and conservation: Fresh wagashi has a very short shelf life and can't be stored more than 3 days under ambient conditions as traditionally done[67,70-81]. In the traditional conditions of storage, fermentation occurs quickly, probably due to the action of lactic bacteria[80] and grants an acidic taste to the cheese.

Microbial analysis of the cheese as reported by Belewu *et al.*[70] revealed the presence of many fungi in both fresh and boiled-colored wagashi cheeses. Alalade and Adeneye reported a coliform bacterial count of about 475.75 CFU/g in cheese samples collected from local sellers in Nigeria[68]. Other studies showed the presence of pathogenic microorganisms (*E. coli* O157:H7 and *Listeria monocytogenes*) with counts exceeding the international standards in marketed wagashi cheese[82]. Poor hygienic conditions, the warm climate in which the cheese is stored and the vulnerability of dairy products to microbial growth are factors that explain the higher levels of the milk and cheese contamination[83]. At the artisanal scale, frying in vegetable oil or boiling with salt and potash followed by coloration with sorghum (*Sorghum vulgaris*) extract are used to extend the shelf life of the cheese[72]. The products once treated can be stored at ambient temperature for 2 to 3 months without any modification of their organoleptic properties.

Natural and chemical preservatives have been tested in order to reduce the microbial load and extend the shelf life of wagashi. Belewu *et al.*[84] revealed the effect of eucalyptus and lemon grass oil. The lemon grass has proved its positive effect on reducing the microbial growth and thus enhancing the quality and the shelf life of the cheese. Ashayé *et al.*[81] have used *Aframomum danielli* and succeeded in extending the shelf life of the cheese from 3 to 6 days under cold temperature without important modifications in the organoleptic characteristics. Salting, which is traditionally used to extend the shelf life and improve the organoleptic characteristics of wagashi cheese has also proved it's effective on the yield and nutritional composition of the cheese[80]. Alalade and Adeneye[68] studied the short-term frozen conditions and found that this method of preservation reduced the coliform microflora amount in the cheese without any modification to the nutritional value. Freezing can thus extend the shelf life of the cheese and make it safer for consumption.

Texture and microstructure: The microscopic observation by scanning electron microscopy of curds coagulated by the plant extract of *Calotropis procera* have revealed spherical particles of about 200 nm size that is in accordance with the micellar size[85,86] (Fig. 5). In spite of the proteolysis causing the destabilization of the micellar balance and the formation of the curd, casein micelles that had not been destroyed were aggregated together to form a network during the coagulation of milk by the plant extract[87]. The comparison of such curds with those coagulated with chymosin in similar conditions highlights slight differences. The network appeared more compact in the curd obtained with *Calotropis procera* latex compared to that coagulated with chymosin, in which black spaces corresponding to whey pockets were frequently observed, with a more regular gel organization than in the case of *Calotropis procera* extract[85].

The texture profile analysis and crush test performed on curds coagulated by a plant extract of *Calotropis procera* have revealed the values of hardness, cohesiveness and elasticity of 5.6 and 1.2 N, respectively[88]. Ado *et al.*[87] have demonstrated that homogenization of processed milks increases the curds hardness and decreases their adhesiveness.

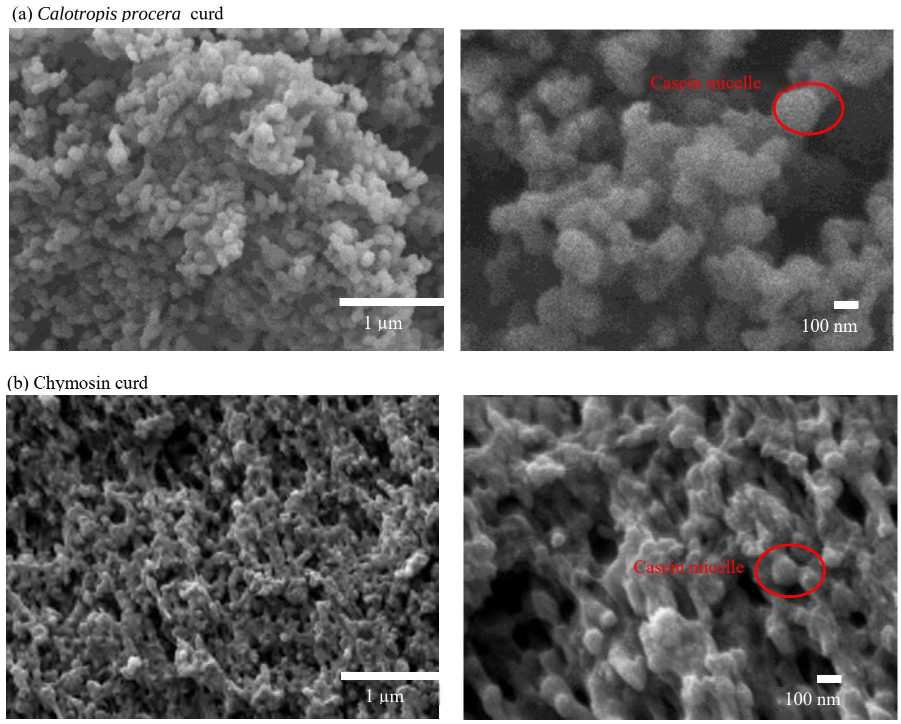

Fig. 5(a-b): Scanning electron micrographs of the milk coagulum obtained with (a) *Calotropis procera* extract and (b) Chymosin curd

Proteases from *Calotropis procera* latex: It has been extracted, purified and characterized two proteases from the latex of *Calotropis procera*. The first one (Procerain), is a cysteine protease (28.8 KDa) with an optimum pH and temperature of 8 and 60, respectively[33]. The second one is cysteine protease (Procerain B) with different characteristics from those of the first one[89]. At the same time, the presence of three novel cysteine proteases of 26.213, 26.133 and 25.086 KDa distinct from procerain and procerain B has been reported (Ramos *et al.*[90]). The milk clotting activity of *Calotropis procera* extract is probably due to the joint action of all these proteases and this explain the great proteolytic activity of the plant extract[85].

Cysteine proteases are characterized by a dyad of cysteine and histidine in their catalytic site[27,91]. In plants, cysteine proteases are involved in protein maturation, degradation and rebuilding in response to various external stimuli such us stress[91-93]. Oseni and Ekperegin[25] demonstrated that cysteine and calcium ions addition enhance the proteolytic activity of the enzyme which is conserved over a wide range of pH and temperature[25,33]. The proteolytic activity of the two characterized proteases is effective on a variety of certain intact and denaturized proteins[35,94]. Rayanatou *et al.*[85] have reported that the mechanism causing milk coagulation by the leaves extract of *Calotropis procera* was a nonspecific hydrolysis of κ casein which probably leads to the destabilization of casein micelles and their rearrangement in gel. Authors have also highlighted the hydrolysis of α_{s1}, α_{s2} and β caseins but assumed that these mechanisms were probably not at the origin of the milk coagulation property of the plant extract and would be due to the presence of a large number of proteases in the extract.

New tracks for traditional cheese making in West Africa: *Carica papaya* leaves: *Carica papaya*, commonly named pawpaw or papaya is an evergreen tree of 3 to 10 m in height, which develops in tropical and equatorial regions. It is essentially cultivated for its fruits but additionally for it's the latex and leaves which are reported to have many nutritional and therapeutic properties[95]. Papaya grows normally as a single stemmed tree. The leaves are green, large and palmately lobed[96]. The flowers appear in the axils of leaves. Papaya fruits are big and oval with a central seed cavity that contains a large quantity of seeds of black color[95]. The flesh of the fruit, initially green change to yellow or orange at the maturity of the fruit[96].

All the parts of the plant are used for treatment of many diseases[97-99]. The leaves, fruits and seeds have shown anti-bacterial activity[100,101]. The latex and leaves of the plant have been reported to possess anti-helminthic activity[102,103] and the dried seeds have shown effectiveness against human intestinal parasitosis[104]. In many traditional medicines, the plant is used in the treatment of infectious diseases such as malaria, dengue and jaundice[105]. The papaya plant, more especially young leaves, exhibit anti-oxidant[106] and anti-tumor[107] activities. Papaya fruits are rich in dietary fiber, provitamin A, proteins and minerals[95]. This rich composition underlies a large nutraceutical and pharmacological properties such as digestive, diuretic, anti-hypertensive and hepatoprotective[97,99].

The latex of *Carica papaya* contains many enzymes but the mostly studied is papain, a proteolytic enzyme widely exploited in the industrial sector[108]. Papain (EC 3.4.22.2) is a cysteine protease of 23.4 KDa, active in a wide range of pH (5-9) and temperature (40-90°C)[109]. Papain exhibit a high reaction speed, about thrice that of *Calotropis procera* extract[17]. In the food industry, papain is essentially used for tenderizing meat and clarifying beer[109].

In Nigeria, *Carica papaya* leaves are sometimes used as *Calotropis procera* surrogate to make traditional wagashi cheese[79]. However, *Calotropis procera* extracts were preferred as they give a white curd with a sweet milk taste instead of *Carica papaya* leaves that slightly colored the curd in green and give them a pronounced vegetable taste[83]. Recently, essays have been conducted to optimize the proteolytic and milk-clotting activity of *Carica papaya* leaves extract[110]. More so, clotting and proteolytic activities are investigated and discussed in relation to their application on the different cheesemaking products[111]. Diouf *et al.*[112] have used different concentrations of fresh *Carica papaya* leaves extracts for clotting raw cow milk. The results obtained have shown that milk clotting times decreased with increasing concentration of *Carica papaya* leaves extracts i.e., the shortest time was observed with the highest concentration but the curd obtained exhibited color (greenish) and taste (bitterness) defaults[112]. Adetunji and Salawu[79] have shown that *Carica papaya* leaves extract can easily replace *Calotropis procera* which is commonly used for traditional cheese making in West Africa. The product, even if slightly less rich in protein and fat than the traditional wagashi, presents an interesting content in minerals such us Zinc and Copper[79]. Akinyolé and Adewumi[113] have compared *Carica papaya* and *Calotropis procera* milk clotting activities using sheep milk and shown that *Carica papaya* leaves extract is more effective than *Calotropis procera*. This suggested that this plant enzyme has more affinity for sheep milk than cow milk, which open an opportunity for production of new traditional products manufacturing.

***Balanites aegyptiaca* fruits:** *Balanites aegyptiaca*, also known as desert palm, wild date or soap berry, is an indigenous tree growing widely in arid and semi-arid regions of African countries[114]. It is a multipurpose tree offering wood, food, medicine and cosmetics to many African populations[21,98,115,116]. *Balanites aegyptiaca* is a multibranched, spiny shrub or tree growing up to 10 m tall[115,116]. The leaves are small and green, alternative and two-foliate[116]. The trunk is covered by green or dark brown barks according to the age of the tree. Fruits are drupes, rather long, green when young but turned to yellow when mature. Flowers are small, greenish white and appear axillary or fascicle[117].

Balanites aegyptiaca tree is well known by West African populations who use all the parts of the plant more for medicine than food. The fruits are sold in local markets and consumed as sweets[115]. In countries like Nigeria and Niger, young leaves and flowers of the plant are cooked and mixed with peanuts past to make a delicious local salad[117]. The kernel produces an edible oil, which is also used by local women as cosmetic to remove dark spots on the facial skin or fight against hair loss.

Next to these nutritional purposes, *Balanites aegyptiaca* is used in many traditional medicines. The plant exhibits anti-microbial activity[118-120] even on antibiotic resistant germs[118,121]. *Balanites aegyptiaca* extracts also show anti-helminthic, larvicidal[122], anti-inflammatory[123], anti-oxidant[120], anti-anaphylactic[98] and anti-carcinogenic[124] activities. These biological evidences account for the traditional use of the plant as laxative, antifungal and antimicrobial agent[124] for skin diseases, as hepatoprotective, purgative and in the treatment of characteristics pathologies such as jaundice, yellow fever, diarrhea, hemorrhoid, syphilis and cough[98,116].

In Cameroon, the fruits extracts of *Balanites aegyptiaca* are traditionally used to thicken a gruel made with flour and fresh cow milk[21]. This encouraged local researchers to conduct further investigations on the milk clotting activity of the fruits. To solve some processing problems, Beka *et al.*[21] provided additional steps for disinfecting fruit samples and pasteurizing milk to reduce the bio burn of the final product, clarifying the extract to improve the coloration of the product, extract concentration to optimize the clotting time and flavoring with *Zingiber officinalis* and *Ocimum gratissimum* to palliate the bitter taste of the cheese. Two proteases with milk clotting activity have been isolated and characterized i.e. a serine and an aspartic protease, that are responsible of the milk clotting activity of the fruits[21].

Aspartic proteases, also known as acidic proteases are characterized by two amino acid aspartic in their catalytic site[91]. *In vitro* experiences show that aspartic proteases in plants are involved in protein synthesis or degradation, thus playing certainly a role in plant senescence, stress response, apoptosis and reproduction[91,93].

Serine proteases contains a reactive serine residue located in their active site that is essential for the function of the enzyme. This active site contains also one Asp and one His amino acid which join the Serine residue to form a "catalytic triad"[92]. Serine proteases achieve quite the same roles in plants as aspartic proteases[93].

CONCLUSION

Calotropis procera leaves extract is the main plant coagulant used to make cheese in West Africa. Cheese making in this part of the word is largely dictated by the availability of milk and traditional empirical practices inherited from generation to generation. In general, African traditional foods and their processing methods have received limited researches. However, investigations conducted on traditional products are essential to provide information to how their production and preservation can be improved and thereby contribute to improve food and nutritional security and reduce poverty in African countries.

SIGNIFICANCE STATEMENT

In West Africa, cheese is becoming more and more part of the dietary habits of a large part of the population. The wagashi cheese replace fish or meat in numerous traditional food recipes. The use of industrial rennet from animal or microbial origin in cheese processing is limited by supplying, financial constraints and conservation. The plant extracts have the advantage of being available and easy to use on artisanal scale. This study reviews the use of *Calotropis procera* extract in the manufacture of the traditional West African cheese and present last studies on the milk clotting properties of other plant leaves and fruits that are new tracks for milk clotting extracts for traditional cheese making in West Africa.

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