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***J. Nat. Prod. Plant Resour.*, 2012, 2 (4):517-523**
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

Carina papaya leaves: a substitute for animal rennet in cheese-making tradition

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ABSTRACT

This present study aims to make a Senegalese traditional fresh cheese by replacing expensive animal rennet, by the leaves of Carica papaya L. It aims to see if these leaves can be used, and to what degree, to coagulate milk and to make cheese. Thus, different proportions of ground papaya leaves (3, 5, 7 and 10 g) were used for coagulation of fresh cow milk. The results showed that indeed, these sheets have a property of coagulating milk. Clotting time of milk in the respective quantities of coagulant used is an average of 25, 20mn35s, 16mn and 14.5 minutes for on liter of milk. The protein levels of cheeses obtained from milk are used on average 28.9 g / l, 23.7 g / l and 10.57%. These values are very low compared to the result of Kora in 2005 which is 33.65% of protein in cheese traditional Fulani in Benin for milk with a 31.55% dry matter. The organoleptic characteristics are also questionable from the slightly bitter taste conferred cheese.

Keywords: coagulant, replacing expensive leaves, substitute, animal rennet cheese.

INTRODUCTION

Milk remains an essential component of the diet of pastoral and agropastoral popolume was estimated at 111 billion liters while in 2008 it rose from 146 million [13]. Despite the high production of milk in Senegal, the producers can not sell their products. Causes related to this situation are the difficulties of market access of local milk (quantity, price, quality), lack of equipment for collecting and processing. All measures aimed at improving the quantity and quality milk production, collection, preservation and processing of local milk are an important part economic and social development of Senegal [16]. It is necessary to establish a controlled system of production, collection and processing more favorable to the promotion of local milk. Therefore, at this situation, this study was done and the results obtained could help to promote local fresh milk, for self-sufficiency and food security, reducing poverty and malnutrition. The development of processing industry is an important issue in Senegal in particular because of the impact of imports on the country's trade balance and income resulting from this activity, both for actors of the transformation for breeders (diversification income). It is also necessary to promote milk and dairy products to support the efforts, support the development of production and ensure a connection between this increase in domestic production and consumption of urban markets. To transform raw milk into cheese, the rennet is essential for the clotting of milk. The latter is limited by cost and availability in rural Senegal. Given this situation, this article reports the results of coagulation of fresh milk in cheese making, the papain extracted from papaya leaves carina.

MATERIALS AND METHODS

Equipment

The raw material used for the manufacture of traditional cheese is fresh cow's milk. Fresh leaves of Carica papaya L., washed, weighed, crushed and filtered after adding a few ml of fresh milk, were used as coagulant. The utensils used include: Mortar and pestle to crush the leaves; sieve to filter milk and crushed leaves; bowls, a ladle, pots and pans. The laboratory equipment includes:

¬ The thermometer, pH meter, balance, water bath, incubator, autoclave etc..
¬ test tube, glass pipettes (5 and 10 ml), graduated beakers, etc..
¬ reagents (alcohol, phenolphthalein, soda Dornic, HCl, culture media etc..).

Methods

Before milk processing multiple sensory tests, physicochemical and microbiological and chemical composition were performed to determine his ability to make cheese.

Thephysico-chemicalparameters :

pH measurement : The pH was measured using a pH meter mark "HANNA".
The Dornic acidity : This test is to determine the levels of lactic acid in 10 ml of milk with the strength of the soda lye Dornic N / 9 in the presence of 3 drops of 1% phenolphthalein (color indicator). Sodium hydroxide solution contained in a burette, until mixture is added to the pink color. The color should persist for at least 10 seconds.
Reading the burette drop is made, multiplied by 10 to convert the result in degrees Dornic (° D) [3].
Alcoholtest
This is a test to determine the suitability of milk for pasteurization. In a test tube, mixed in equal amounts (5 ml) of milk and ethyl alcohol at 68 °. The reaction is immediate. This test is performed on site.
The result is positive if the presence of flocculation: the milk is thermally unstable. It is negative if no flocculation: the milk is stable [3, 10]

Organolepticstests

The assessment of the organoleptic quality (taste, color, odor and appearance of milk) was performed by scoring method [28]

Chemical composition of milk and cheese

Determining the chemical composition of milk and cheese was performed by determining their content, protein, fat, carbohydrates, minerals and solids in milk and cheese by the official methods [22].

Microbiological analyzes of cheese

Microbiological analyzes by the enumeration of faecal Coliform, Salmonella, Staphylococcus aureus and Anaerobic sulphite réduteurs, Total aerobic mesophilic flora and Lactic acid, and yeasts and molds by the standards NF V08 06; NF 08-052, NC 08 to 057; XP V08-061, NF V 08-051, NF-051-V08, V08-NF-059) [1].

Manufacture of cheese

Milk collection

Throughout the period of cheese, milk purchased at point of sale is harvested in two aluminum cans of 10 liters. This milk was transported from the point of sale to the ITA in an air conditioned car.

Before undertaking any purchase, the alcohol test was conducted on site to verify the suitability of milk for processing.

Filtration

At the farm, the milk is filtered automatically after milking and stored in tanks. However, despite the first filtration of the farm, a tight mesh screen was used to filter the milk and the filtrate was collected in a pot.

After filtration, a small amount (50ml) is removed and sent to the chemistry laboratory for chemical analysis.
Preparation of coagulant

She was to pick papaya leaves, wash them, weigh them and then grind them in a clean mortar. The homogenate was then mixed with a small amount of fresh milk. The resulting mixture was filtered through a sieve and then added directly to milk on the fire.

Incorporation of the coagulant
After preheating to heat the milk to 60 ° C for about 5 minutes, the coagulant was added and then heated at a temperature of 95 ° C until the formation of curd whey supernatant. The amount of leaves of Carica papaya L. used varied between 3 and 10 g per kilogram of milk coagulation occurs between 10 to 20 minutes after addition of coagulant and is characterized by the appearance on the surface of the cream oil foam. At this point, it activates the fire for 2-3 minutes to allow the curd formed to cook until the whey becomes light yellow and transparent, whereas the coagulum tends to withdraw into himself. It is divided into small pieces and floats whey. The curd was poured just after cooling in the molds and underwent drip-molding step.

Drip-molding
After coagulation, the curd thus formed is transferred to a ladle in plastic colanders, for the separation of whey. For drainage, we first reduced the whey manually using a sieve, before setting the curd into a mold, which allowed to drain the whey while giving the form of bread more or less circular to the curd. Bread and cheese obtained in each mold, is a very soft hydrated, oozing long hours (24 h). Once manufacturing is complete, the cheeses were stored refrigerated at 4 ° C.

RESULTS

All experimental results: Organoleptic characteristics, frequency of pH of fresh milk, alcohol test, Dornic acidity, ° D frequency milk costs, frequency of variation of pH and Dornic acidity, pH cheese, variation in content protein / curdling time, the chemical properties of milk and cheese, cheese contamination level, are reported in Tables I, II, III, IV, VI, VII, VIII.

Discussion
Organolepticstests
The sensory characteristics of cheeses are a major concern of the streams. The sensory quality of cheese depends on the manufacturing technology and chemical and microbiological characteristics of raw material implementation. The latter in turn depend on many factors of genetic, physiological, food etc.. Such as cows Norman Brune, Montbéliarde or produce milk higher in protein and better cheesemaking than Holstein cows managed under the same conditions [14]:

the gel obtained after addition of rennet is firmer and higher cheese yields. Most of this effect is related firstly to differences in casein content of milk from one race to another and also to changes in the genetic polymorphism of lacto proteins and in particular the frequency of the variant B κ casein. Indeed, it is now well established that variants of this casein, whose frequency varies greatly from one race to another, influence the coagulation of milk [20].

The results of sensory analyzes showed that the cheese produced fresh milk from Zebu Senegalese coagulated with 3 g of papaya leaves (fromage1) is the best due to its taste and color. This cheese has a whitish color and a taste of milk. By cons, other cheeses obtained from coagulated milk but even with 7 and 10 g of coagulant (cheese 3 and 4), gave a better texture and better firmness. The parameter does not smell of cheese and variation is almost identical. Compared to those given the organoleptic quality of cheese depends on the amount of coagulant used. The lower the cheese has more flavor and better color, it is more important over the cheese is firm and he has the best texture. Firm and smooth texture of the cheese could be related to race [14, 20].

This aspect is a smooth texture and enjoyed considerable criterion in the cheese industry [6]. explained by the presence of more whey in milk just curdled and it would contribute to the acidity of the cheese.

Microbiologicaltests
The results obtained showed a very high load in FMAT, fecal coliforms and a low presence of yeasts, molds (<10 CFU / g) and pathogens Staphylococci in different 2.3.2.

Physicochemical tests of raw milk

The Ph
The pH ranged from 5.9 to 6.7 with an average of 6.5 for all 14 samples. 85.72% of these samples have a pH between 6.5 to 6.7. The normal pH of raw milk varies between 6.6 and 6.8 and any pH below 6.5 and above 6.9 is abnormal [5]. The results confirm the suitability of the milk used in cheese making. DOUIK and coll [10] found pH values ranging from 6.6 to 6.91. Sina L. [27] found that pH values vary between 6.69 and 6.79, included in the range of normal pH of fresh milk. Samples with a pH below normal (5.9)

could be explained by the fact that there would be a start of fermentation of milk, because the analyzes were performed at least 3 to 4 h after milking.

The Dornic acidity
57.14% of samples of fresh milk has an acidity ranging from 16.5 to 17 ° D. The Dornic acidity of fresh milk is normally 16 to 18 ° D, with an average of 16.5 ° D. The Dornic acidity of fresh milk used is between 16 and 18 ° D corresponding to the variation limit of the normal acidity of fresh milk cow capable of processing ([7].

Alcohol test
All alcohol tests were negative except two samples only. The negative tests for alcohol, this proves the ability of milk to undergo pasteurization without difficulty (4). This stability may reflect the freshness of milk and its suitability for processing.

pH of fresh cheese
The pH was measured just one hour after coagulation of milk in the four categories of cheese and then 24 hours later. There is no change of pH depending on the amount of papaya leaves used as a coagulant, but observed variation depending on whether the curd is drained at room temperature or refrigerated at 4 ° C.

This variation between t0 and t1 is very remarkable with samples drained at 4 ° C. This variation could be types of cheeses drained at room temperature. It is less important in the drained cheese in the refrigerator. The significant presence of fecal coliforms and FMAT observed could result from contamination of cheese during draining or microbial growth at room temperature. The presence of these smaller organisms in the drained cheese in the refrigerator, could result from inhibition of these germs from the cold. This proves the need to drain the cheeses at low temperature. Cheeses obtained are less contaminated compared to results obtained by Kora in 2005 [18] on the cheese Fulani in Benin (3,7.106 4,2.106 and CFU / g for coliforms and Aerobic mesophilic and 7,36.105 CFU / g for yeasts and molds).

Variation of protein / Time of curdling milk, cheese weight.
The results showed that shredded papaya leaves are actually a property of coagulating milk and the setting time is inversely proportional to the concentration of coagulant used. The greater the amount of the coagulant, the lower curdling time is long. He is averaging 25, 20mn35s, 16mn and 14.5 minutes, respectively with 3, 5, 7 and 10 g of crushed papaya leaves to a liter of milk. Time is slightly longer with the amount of milk and coagulant doubled to 6, 10, 14g of leaves to 2 liter of milk. There is an average of respectively 30, 25.5 20.25 and 17 minutes. The authors DOSSOU J. and coll [9] noted clotting in 20 to 25 minutes with the use of 7 and 12 g of leaves of Calotropis procera in a liter of raw milk. Similar results were found by Egounlety and al. [13] indicate that 5 and 15 grams of leaves of Calotropis procera per kilogram of milk. The results also showed a low protein levels of three cheeses from the cheese of Benin and that obtained by rennet. This low rate of protein could result from proteolytic properties of papain. Indeed, a large number of proteolytic enzymes of animal, vegetable, especially papain, can hydrolyze casein α and β with the release of peptides [23, 25]. If this hydrolysis is too high, it can result in a decrease in the yield of cheese, a soft texture and the appearance of abnormal flavor [19, 20]. The weight of cheese after 24 hours is an average of 195.10 g / l or 1/5 the weight of raw milk. This result is similar to that of Kees [17] who used 5 liters of fresh milk for the manufacture of 1 kg of cheese already drained, with leaves of Calotropis procera.

Chemical characteristics of milk and cheese
The levels of protein, fat content and dry milk samples are used an average of 28.9 g / l, 23.7 g / l and 10.57%. These values are slightly below the range given by Vignola in 2002 [29]. In terms of the average protein content of cheese from the milk (10.56 to 11.55%), it is very small compared to the result of Kora in 2005 which is 33.65% protein, with the Fulani in Benin traditional cheese for milk with a 31.55% dry basis. Alais and Linden in 1993 [2] noted, 19% protein on cottage cheese and skim milk 23% fat in the soft cheese. Diouf noted in 2004 15.2% protein on goat cheese in the Niayes. Although the milk used has an average protein slightly below the normal value of cow's milk, the difference in protein between cheeses obtained with papaya leaves and cheese obtained using rennet is important as this may result from proteolytic properties of papain. This is confirmed by the results Ramet [24] was obtained in 1990 with the coagulating preparations of plant origin (ficines latex of the fig tree3.

CONCLUSION

The papaya leaves have a coagulant property. Cheeses obtained have a low protein, low yield of this protein could result from proteolytic properties of papain. The milk clotting time is inversely proportional to the amount of coagulant incorporated: the greater the amount of coagulant is important the longer the milk coagulation is short, the more it is smaller the coagulation time is long. The lowest amount of coagulant used gave a cheese with the flavor

and color better than those of cheeses obtained with a significant amount of coagulant. However they are better because of their texture and firmness. The use of papaya leaves as a coagulant in cheese making could help alleviate the shortage and unavailability of animal rennet in rural areas. However the following recommendations are made:
o Further research using substances that reduce the high proteolytic effect of papain and correct the bitter taste of cheese;
o extract papain industrially and try it with trace amounts (milliliters);
o experiment with other plants to make effective choice to replace animal rennet coagulant by a cheap plant in traditional cheese making in rural areas.

Table I : Organoleptic characters

cheeses / Parameters	Mean Scores attributed :			
	cheese 1	cheese 2	cheese 3	cheese 4
Color	4	2	2	1
Texture	2	2	3	3
taste	4	2	1	0
smell	4	4	4	4

Cheese 1: 3 g papaya leaf for 1milk liter ;
Gheese 2: 5 g papaya leaf for 1milk liter ;
Cheese 3: 7 g papaya leaf for 1milk liter ;
Cheese 4: 10 g papaya leaf for 1milk liter ;

Table II: Frequency of pH of raw milk

ph	Number of milk samples	simple percentage (%)	cumulative percentage (%)
5.9	2	14 .28	14.28
6.5	6	42.86	57.14
6.7	6	42.86	100

TableIII :Alcoholtest

tests	Results
1	-
2	+
3	-
4	-
5	+
6	-

Table IV: Dornic acidity for raw milk.

Dornic Acidity	Samples Numbers	Variation percentage (%)	Cumulative percentage (%)
16-16,5	3	21.43	21.43
16.5-17	8	57.14	78.57
17-17,5	2	14.3	92.87
17.5-18	1	7.14	100

Table V : frequency of variation for pH and Dornic acidity

pH / °D	5,9	6,5	6,7
17.5 -18	1	0	0
17-17.5	0	2	0
16.5-17	0	3	5
16-16.5	0	0	3

Table VI : Cheese pH

Cheese categories	Cheese				Cheese				Cheese			
	F_3T		F_3F		F_5T		F_5F		F_7T		F_7F	
Temps / Paramètres	t0	t1	t0	t1	t0	t1	t0	t1	t0	t1	t0	t1
pH	6.72	6.60	-	6.68	6.81	6.66	-	6.71	6.82	6.63	-	6.80

Cheese F_3T: cheese with a liter of milk is coagulated with 3 g of papaya leaves and drained at room temperature ; F_3F= drainage at +4 ° C. F_5= 5g of papaya leaf , F_7= 7 g of papaya leaf
t0 : measurement taken just after coagulation;
t1 : measurements taken after 24 h of drainage time.

Table VII: Change in protein content / curdling time

Parameters	Cheese 1	Cheese 2	Cheese 3
Protéines	10.56%	11.53%	11.55%
Temps moyens de caillage	25mn	20mn35s	16mn

Table VIII: Chemical properties of milk and cheese

Parameters	Fresh milk	Cheeses (%)		
	g/100ml	Cheese 1	Cheese 2	Cheese 3
Cendre	0.57	1.41	1.48	1.30
Matières grasses	2.37	14.24	13.10	15.25
Protéine (Nx 6,38)	2.89	10.56	11.53	11.55
Extraits secs	10.57	29.08	28.82	30.77

Table IX : Level of cheese contamination by organisms sought or enumerated.

Micro organisms	Unity	From AF	From AT	From BF	From BT
Yeasts and molds	UFC/g	<10	<10	<10	<10
FMAT	UFC/g	$1.7x\ 10^5$	$1\ .6x\ 10^8$	$1.6x\ 10^3$	$4.3x\ 10^7$
Fecal Coliforms	UFC/g	$3.1\ x\ 10^4$	$1.6\ x\ 10^7$	$9.3\ x\ 10^2$	$1.7\ x\ 10^3$
Pathogens Staphylococcus	UFC/g	0	0	0	0

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