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Dairy curd coagulated by a plant extract of *Calotropis procera*: Role of fat structure on the chemical and textural characteristics

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ABSTRACT

Milk is often subjected to technological treatments which have impacts on the structure of milk constituents and the characteristics of rennet curds. In this paper, the influence of the dairy fat structure on the biochemical and textural characteristics of curds coagulated by an extract of *Calotropis procera* leaves was studied. Standardized milks were reconstituted with the same contents in protein (35 $g \cdot kg^{-1}$) and fat (35 $g \cdot kg^{-1}$) but with different structures of *fat i.e.* homogenized anhydrous milk fat (HAMF), homogenized cream (HC) and non-homogenized cream (NHC). As expected, the size distributions of fat globules in the different milks were different. After their coagulations by the plant extract, the physico-chemical characteristics of the curds and respective wheys were determined. No difference was observed in the coagulation time between the three milks but the whey removed more quickly from HAMF and HC curds than NHC-curd. The biochemical analyses of curds revealed a lower content in dry matter and fat in the NHC-curd compared to HAMF- and HC-curds. Otherwise, the NHC-whey exhibited the highest amount of fat. Observations by confocal microscopy showed that the fat globules were homogenously distributed and well trapped in the protein networks of HAMF- and HC-curds. In the NHC-curd, the fat globules were located in whey pockets, with less connectivity with the protein network. The textural analysis showed that the NHC-curd was more elastic, soft and adhesive than HAMF- and HC-curds. Homogenization significantly reduced the loss of fat during cheese manufacturing and conferred specific textural characteristics to the curds coagulated by an extract of *Calotropis procera*.

1. Introduction

Milk is an interesting food regarding its content in protein, fat, minerals and vitamins. In developing countries, the conservation methods are based on the transformation of milk into adding value products to preserve and store its essential components (Adetunji & Babalobi, 2011). In West Africa, producers that have a direct access essentially consume fresh milk while the rest of the production is transformed into traditional products like fermented milk, fresh or clarified butters, and some cheeses (Oladipo & Jadesimi, 2013). Wagashi is a soft unripened cheese manufactured in West African countries like Benin and Nigeria by women from nomadic breeder's communities (Aworh & Muller, 1987; Aworh & Nakai, 2007). In the traditional manufacture of this cheese, the coagulation of raw whole milk is obtained by addition of an extract of *Calotropis procera* leaves during milk heating on wood fire. After coagulation, the heating is maintained up to obtain a separation of curd and whey. The collected curd is drained, molded, boiled with salt and colored with *sorghum panicum* extract, then sold or consumed. Wagashi cheese is used in numerous traditional food recipes as substitute of meat or fish (Kawo et al., 2009; Mazou, Tchobo, Degnon, Mensah, & Mohamed, 2012; Omotosho, Oboh, & Iwaela, 2011).

Calotropis procera, a plant of the *Asclepiadaceae* family, is a latex tree growing naturally in tropical and subtropical areas of Africa and Asia (Rashmi, Singh, & Arya, 2011). Two proteases of cysteine class (Procerain and Procerain B) with milk clotting properties have been extracted, purified and characterized from the latex of *Calotropis procera*. (Aworh, Kasche, & Apampa, 1994; Kumar, Dubey, & Jagannadham, 2003; Singh, Shukla, Jagannadham, & Dubey, 2010). These proteases are active in wide ranges of pH and temperatures. Ramos et al. (2013)

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highlighted the presence of three novel cysteine proteases of 26.213, 26.133 and 25.086 Da distinct from Procerain and Procerain B. The milk clotting activity of *Calotropis procera* extract is probably due to the joint action of all these proteinases (Ramos et al., 2013). Issa et al. (2017) showed that the proteolysis and coagulation of casein micelles by *Calotropis procera* plant extract was not specific compared to the classical action of chymosin. This can be explained by the proteolytic activity of several enzymes contained in the plant extract.

The processing of wagashi cheese is made by a traditional way using an empirical knowledge transmitted from generation to generation (Adetunji & Babalobi, 2011; Oladipo & Jadesimi, 2013). The losses of raw material are important due to the use of traditional equipment and no standardized procedure. For example, the layer of cream formed at the milk surface during heating is often removed to have a good boiling of the curd. This reduces considerably the fat content of the cheese. Moreover, the significant proteolysis observed with the extract of *Calotropis procera* (Issa et al., 2017) could be the cause of loss of peptides in the whey.

It is admitted that physical treatments of milk such as homogenization modifies fat structures and promotes new interactions especially between fat and proteins (Lopez, Cauty, & Guyomarc'h, 2015). Homogenization reduces the size of fat globules and increases their number. The rupture of native fat globules occurring during homogenization creates a new interface that cannot be entirely covered by the biological membrane. It is described that caseins and whey proteins adhere to this new surface and formed new membrane (Cano-Ruiz & Richter, 1997; Darling & Butcher, 1978; Lopez et al., 2015; Michalski & Januel, 2006; Zamora, Ferragut, Guamis, & Trujillo, 2012). Authors showed that the homogenized fat globules of cream retained a part of their native globule membrane material rich in polar lipids (Henstra & Schmidt, 1970; Keenan, Moon, & Dylewski, 1983).

The size of fat globules and the composition of their surface obtained after homogenization can modulate the interactions with the protein network upon coagulation with consequences on the rheological properties of rennet curds (Lopez & Dufour, 2001; Michalski et al., 2003; Michalski & Januel, 2006). Homogenization is often used to increase the cheese yield and change the textural and organoleptic characteristics of cheeses (Jana & Upadhyay, 1992; Rudan, Barbano, Guo, & Kindstedt, 1998). In cheese processing using chymosin, homogenization has been reported to improve the overall quality of several cheeses like Cheddar, Edam, Roquefort and others (Jana & Upadhyay, 1992). The effect of homogenization on the properties of curds processed with *Calotropis procera* coagulant remains to be elucidated. Increasing knowledge about the biochemical mechanisms involved in the manufacture of wagashi cheese is interesting for food scientists in order to better control the yield and quality of this traditional product. Wagashi cheese is important from a nutritional point of view since it is a source of milk components (proteins, lipids, calcium). Improving the manufacturing efficiency of dairy products in countries where the availability of milk is limited is of primary importance.

The objective of this study was to understand the role of the fat structure on the characteristics of curds manufactured by using an extract of *Calotropis procera*. To answer to this objective, the processed milks were or not homogenized to obtain different structures of fat. After milk coagulation by the plant extract, the biochemical characteristics, texture and microstructure of the resulting curds were determined. A particular attention was given on the yields and recovery rates of dairy constituents in the different obtained curds.

2. Materials and methods

2.1. Materials

2.1.1. Preparation of milks

The milks were prepared from low heat skimmed milk powder (Skim milk powder LH-spray dried, Lactalis Ingredients, Bourgbarré, France), distilled water and 2 sources of dairy *fat i.e.* anhydrous milk fat (99.9% Fat, Corman S. A., Limbourg, Belgium) or cream recovered using a skimmer (Elecrem 3, Fresnes, France) from raw whole milk supplied from a local breeding farm.

The milks were standardized to have the same contents in dry matter (13%; wt:wt), proteins (35 $g{\cdot}kg^{-1}$) and fat (35 $g{\cdot}kg^{-1}$). Three types of milks were reconstituted with differences in the fat structure *i.e.* homogenized anhydrous milk fat (HAMF), homogenized cream (HC) and non-homogenized cream (NHC). From practical point of view, the skimmed milk powder was rehydrated with warmed distilled water at 50–60 °C during 1 h. The anhydrous milk fat was heated at 50 °C and then incorporated into the rehydrated milk before homogenization. The milk was then stored at 4 °C overnight before curd manufacture.

The cream obtained after whole cow milk skimming was preliminary analyzed for protein (noted PC_{cream}) and fat (noted BR_{cream}) contents using a lactoscan (Humeau, La-Chapelle-sur-Erdre, France) and confirming by butyrometric method for fat. Once the fat content was known (about 400 $g{\cdot}kg^{-1}$), the following system of equation with 3 unknowns was solved to determine the values of a, b and c which corresponded respectively to the quantities of water, cream and skimmed milk powder necessary to reconstitute a milk at 35 $g{\cdot}kg^{-1}$ of fat and 35 $g{\cdot}kg^{-1}$ of protein. The amount of protein and fat in water (PC_{water} and BR_{water}) were considered to be 0. The amount of fat and protein in the milk powder were respectively, $BR_{milk\ powder}$ = 15 $g{\cdot}kg^{-1}$ and $PC_{milk\ powder}$ = 350 $g{\cdot}kg^{-1}$. Milks were reconstituted by mixing the appropriate quantities of skimmed milk powder, cream and distilled water preheated at 60 °C. The HC milk was homogenized while the NHC milk was directly cooled and stored at 4 °C before manufacturing.

$$\begin{cases} a + b + c = 12 \\ a{*}BR_{water} + b{*}BR_{cream} + c{*}BR_{milk\ powder} \\ \quad = 12 \times BR_{reconstitued\ milk} \\ a \times PC_{water} + b \times PC_{cream} + c \times PC_{milk\ powder} \\ \quad = 12 \times PC_{reconstitued\ milk} \end{cases}$$

12 = mass of the reconstituted milk(kg)

a = mass of water (kg), b = mass ofcream(kg) and c

= mass of milk powder (kg)

BR = butyric ratio ($g{\cdot}kg^{-1}$), PC = protein content ($g{\cdot}kg^{-1}$)

Homogenization of all milks was carried out with a two-stage homogenizer (Rannie 400Q, Soeborg, Danemark) at 60 °C using first and second stage pressures of 10 and 1 MPa, respectively.

2.1.2. Curd manufacture

The curds manufacture was performed in the same conditions for the three milks on the dairy platform of our laboratory (http://www6.rennes.inra.fr/plateforme_lait). A total volume of 10 L of each milk was used for each fabrication.

For the coagulant preparation, the *Calotropis procera* leaves were picked from the Maradi city in Niger, dried under shade at 25–30 °C during 7 days and then grind to obtain a coarse powder. Before using, the leaves powder was stored at 20 °C in hermetically sealed bottles. The coagulant was prepared by soaking 50 g of dried leaves powder of *Calotropis procera* in 1 L of the processed milk. After 1 h at room manufacturing temperature settled at 30 °C, the mixture was filtered using a polystyrene filter and the filtrate was used as coagulant. The filtrate pH was 6.08 ± 0.06 at 28 ± 1 °C. The remaining 9 L of milk were heated gradually up to 75 °C during 1 h. The pH of milk at coagulation was 6.13 ± 0.04 at 75 ± 1 °C. After coagulation by the *Calotropis procera* extract, gels were left for firming during 20 min, then cutted in cubes of 5 mm × 5 mm × 5 mm size and brewed for 20 min at 30 rpm. Wheys were separated from curds through a polystyrene molding fabric and curds were pressed for 3 h using a mass of 0.01 kg per cm^2.