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Impact of starter culture on curd manufacture – one of the most commonly consumed fermented milk products of Sri Lanka

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Abstract

Curd is a popular type of fermented milk product in Sri Lanka. In commercial curd manufactories, freeze-dried starter cultures are used, whereas small scale curd manufactories make curd in the traditional way using curd from previous days as a starter. Curd can be made from buffalo milk or cow's milk, where buffalo milk is more common. The amount of fat is higher in the buffalo curd compared with the cow curd. However, not much is known about the impact of the processing steps and health benefits of this product.

This study was carried out in March - May 2019 in the middle of the country Sri Lanka at the University of Peradeniya. The aim of the study was to investigate the effect of different starter cultures on the final product of curd made of buffalo milk, with respect to pH, syneresis and sensory aspects. Comparison was done between three starters cultures: Pre-Made Buffalo Curd (PMBC), Pre-made Cow Curd (PMCC) and commercially available Chr Hansen R-704 freeze-dried starter culture (CHR-704).

All curd samples with different starter cultures showed gradual reduction of pH during three days of measurements with only small difference between the products. The highest reduction, 34%, was observed for curd with CHR-704 whereas curd with PMCC showed the lowest pH reduction, 31%. During the three days of observation, the syneresis in the curd decreased. Lowest syneresis, 3.86% was observed in curd with PMBC. Highest syneresis, 3.93% was observed in curd with CHR-704. The sensory aspects of the curd i.e. flavour, texture, odour, colour and overall acceptability were evaluated by 31 members of a sensory panel. Regarding flavor and color, the most preferred curds were curd with CHR-704 and PMCC, respectively. Curd with PMCC was the most preferred one, regarding odour and texture. For the overall acceptability, 36% of evaluators preferred curd with PMCC whereas curd with CHR-704 was least preferred.

Keywords: Curd, buffalo milk, starter culture, lactic acid, Sri Lanka, pH, syneresis, sensory test

Sammanfattning

Curd är en populär, fermenterad mjölkprodukt i Sri Lanka som kan tillverkas med olika typer av starterkultur. Vid kommersiell tillverkning av curd används en starterkultur i frystorkad form. På gårdar gör man curd på traditionellt sätt; man använder curd från dagen innan som starterkultur. Curd kan göras med buffelmjölk och komjölk, vanligast är att man använder buffelmjölk. Fettmängden i buffelmjölk är högre än i komjölk vilket påverkar egenskaper som doft, smak, färg och syneres hos curd.

Studien utfördes i mars - maj 2019 på University of Peradeniya i staden Peradeniya i Sri Lanka. Målet med studien var att undersöka vilken effekt olika starterkulturer har på kvaliteten hos curd gjord av buffelmjölk. En jämförelse mellan starterkulturerna genomfördes, varvid starterkulturer som härstammade från buffel-curd (PMBC), ko-curd (PMCC) och en kommersiell kultur från Christian Hansen (CHR-704) användes. Mätningar av pH, syneres och ett sensoriskt test genomfördes.

Samtliga tre produkterna tillverkade med olika starterkulturer visade en pH-minskning under de tre dygn som mätningar gjordes. Den största minskningen, på 34%, uppvisade curd som tillverkats med CHR-704 medan curd med PMCC visade den minsta pH-förändringen med 31%. Under de tre dygnen minskade syneresen i samtliga curd-produkter. Lägst syneres visade curd med PMBC medan curd med CHR-704 visade den högsta syneresen, 3,93% på dag 3. En sensorisk utvärdering genomfördes där aspekterna smak, textur, doft, färg och övergripande acceptans bedömdes av 31 personer i en testpanel. Angående smak och färg, föredrog de flesta i panelen curd med CHR-704 och PMCC. Curd med PMCC föredrogs avseende på doft och textur. Totalt sett, föredrog 36% av panelens medlemmar curd med PMCC medan curd med CHR-704 var minst populär.

Nyckelord: Curd, buffelmjölk, starterkultur av mjölksyrabakterier, mjölksyra, Sri Lanka, pH, syneres, sensorisk test

Preface

- The study was part of a collaboration between the Department of molecular sciences, Swedish University of agricultural sciences (SLU), and the Department of animal science, University of Peradeniya.

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Abbreviations

β-lg	β-lactoglobulin
CHR-704	Freeze-dried starter culture - Chr Hansen R-704
GMP	Glucomacropeptide
LTTE	Tamil tigers
NSLAB	Nonstarter lactic acid bacteria
PC	Phosphatidylcholine
PMBC	Pre-Made Buffalo Curd
PMCC	Pre-Made Cow Curd
SLS	Sri Lanka Standards

SM	Sphingomyelin
SNF	Solids not fat

Introduction

Sri Lanka is developing country with a population of 21,5 million inhabitants. The island was a British colony from year 1796-1948. Sinhalese and Tamil are two of the largest ethnic groups in Sri Lanka, the ethnic group Sinhalese being the largest. 75 percent of Sri Lanka population are Sinhalese and have an origin from North India. Sinhalese speaks the language Sinhala and has Buddhism as a religion. The minority group of Tamil has an origin from south India and are mainly Hindus and speak the language Tamil (Första klass 2007). From 1983-2009 there was a civil war between the government and *Tamil tigers* (LTTE). Beyond Sri Lanka war history, the country is known to have a great food culture. Food as rice, coconut, curry, buffalo milk and fruit are common in Sri Lanka. Curd, roti and hoppers are examples of Sinhalese courses. Curd is a type of fermented milk product, roti, a coconut bread which is fried in a pan, and hoppers, a type of pancake but with coconut flavour (Sri-Lanka.se 2006).

1.1 Climate of Sri Lanka

The island has a big biodiversity and three climate zones, i.e. dry, wet and intermediate zones (Figure 1). The dry zone has a big variety of agriculture and common for this region is sub-humid-forests, rice growing areas and dairy production. The dry zone has a big variance of different crops due to the small number of mountains and rainfall, comparing with the intermediate and wet zone (Ibrahim 2000). In the dry part the annual rainfall is 900 mm and due the low rainfall, there is a smaller number of big farms in the dry zone (Department of meteorology 2019). Water buffalos (*Bubalus bubalis)* which normally get their feed from grazing alone, are more common in the dry area**.** The domestic fermented product, curd, is mostly made from buffalo milk, and therefore the curd production is high in this area (Samath 2019). The wet and intermediate zones have an annual rainfall up to 2500 mm. Due to this, larger farms with cow (*Bos tarus)* are placed in this area where also coconut cultivation is common (Department of meteorology 2019).

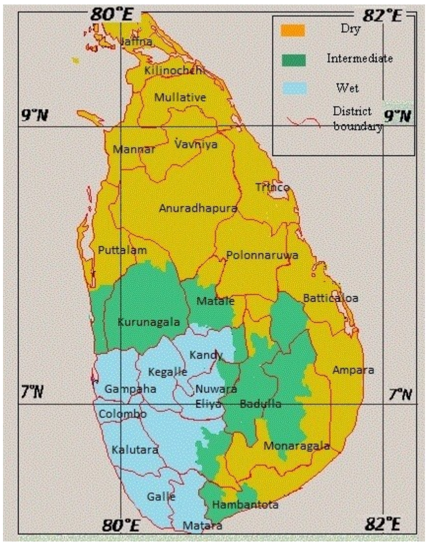

Figure 1. Map of Sri Lanka with the three climate zones. The dry zone; orange, the intermediate zone; green, and the wet zone; blue. Source: Karrunaweera 2014.

In these two zones the number of buffalos is low in contrast to the number of cows. Therefore, curd production is lower in the intermediate and wet zone (Samath 2019).

1.2 Behaviour of water buffalos

The natural environment for buffalos is, because of the sensibility against high and low temperature, shady places, close to water. Buffalos have few sweat glands and dark thick skin which absorb heat. This makes the buffalo sensible to the higher temperatures (Samath 2019). In tropical regions, farmers can make shelters for the buffalos. Low temperature is not good either as the body fat of buffalos does not gives a good insulation. Therefore, buffalos are more common in a tropical climate than a cold climate. Buffalos easily get stressed in an environment which is not adapted for buffalos compare to cows e.g when the temperature is too high or low and when a lot of new people are around them (Ibrahim 2000). Buffalos transforms higher amounts of beta-carotene to vitamin A compare to cows, which makes the colour of buffalo milk very white. Cows transform less amount of beta-carotene to vitamin A; therefore, cow milk is more yellow (Perera 2001). In Sri Lanka there are two breeds of water buffalo called *Nili-Ravi* and *Murrah* (Figure 2). These breeds are often crossed with each other and used for dairy production. *Nili-Ravi* is a cross between *Nili* and *Ravi* which are both domestic buffalo breeds. The colour of *Nili-Ravi* is black with white eye, white mark in the forhead and white mark on the tail. The horns are small and curly. The mean weight value of *Nili - Ravi* is 600 to 700 kg depending on sex (Roy´s Farm 2019a). *Murrah* is also well domesticized and used in dairy production. The colour of *Murrah* is black and the horns are tightly curved and short. The weight of *Murrah* is aproximatly 650 to 750 kg depending on the sex (Roy´s Farm 2019b).

Figure 2. Figure on the left shows a buffalo of the breed *Murra*h. The figure to the right shows a buffalo of the breed *Nili-Ravi. Photo: C. Andersson.*

1.3 Curd

1.3.1 The history of curd

Fermented milk products exist worldwide and are available in different forms and appearing for the first time 10 000-5000 BC (Machado & Mauro 2015). In the Middle East, herdsmen carried abomasum with milk and when intestinal juice from the bags came in contact with the milk, the milk coagulated. Suddenly the herdsman had a fermented milk product. One type of fermented milk product is called curd. When the language Hindi is used, the curd is called Dahi. The year 6000 BC, an ayurvedic script was found and it was about the health benefits of fermented milk, which includes curd (Machado & Mauro 2015). In India, 6000 BC, 700 fermented milk products with health benefits were documented and one of the products was curd. During 6000 BC curd was also spread to nearby south Asia countries e.g. Sri Lanka (Machado & Mauro 2015).

1.3.2 Description of curd

Curd is a type of fermented milk product usually made from buffalo milk instead of cow milk since the higher amount of fat in buffalo milk improves its texture and taste (Sri Lanka Standards Institution Standard 1988). According to (Disanayaka 2019), a good quality curd should have a firm and uniform texture with pleasant aroma and a smooth and glossy surface. The standard for curd made from buffalo milk is final pH 4.5, milk fat 7.5% and milk solids not fat (SNF) 8.5% The standard for curd made from cow milk is pH 4.5, milk fat 5.0% and milk SNF 8.5% (Table 1).

Table 1. *Sri Lanka standard values for cow and buffalo curd. Source: Sri Lanka Standards Institution Standard 1988*

Characteristic	**Cow curd**	**Buffalo curd**
Milk fat, per cent by mass	5.0	7.5
Milk SNF, per cent by mass	8.5	8.5
pH, max	4.5	4.5

In almost all processes of curd, no preservatives are added with the exception of sorbic acid, which sometimes is used. The highest amount of sorbic acid which is legal to add in curd production is 1 000 mg/kg (Sri Lanka Standards Institution Standard 1988). To produce curd, a lactic fermentation of milk is needed~~,~~ using single or a mixed cultures of lactic acid bacteria. (Disanayaka 2019).

1.3.3 Important steps in the process of curd making

When making curd, mesophilic and thermophilic starter cultures containing lactic acid bacteria are added after pasteurising the milk (Sultzer 1961). The optimal temperature for this type of starters is 20-45 °C. According to (Damodaran & Parkin 2017) curd is produced by lowering the pH of milk proteins to their isoelectric point (about pH 4.6) by the fermentation of lactic acid. At this point, the milk proteins aggregate and the milk coagulates. Before the addition of starter cultures, the milk needs to be heat treated (Tauscher

1995). According to (Damodaran & Parkin 2017) the aim with the heat treatment at temperatures of 85-90 °C for 5-20 minutes is to destroy any competing pathogenic and microorganisms. This heat treatment also denatures whey proteins and allows the disulphide bridges and thiol groups to interact with cysteine containing caseins. These reactions modify the micelle surface and contribute to favourable viscous textural properties in the acidified coagulum (Lee & Lucey 2010). At this point syneresis is not present. Syneresis is when whey is expelled from the gel formed mainly by caseins, fat and calcium. Syneresis is in general desired in enzymatic coagulation and unwanted in acidic coagulations (Damodaran & Parkin 2017). Jayarathna (2013) showed that the reduction of pH leads to a stable curd with increased water activity which leads to a decrease of syneresis.

Milk coagulation is formation of a gel, important for a good curd. The casein micelle is a cluster of caseins, e.g. alpha, beta and kappa casein which are kept together by colloidal calcium phosphate clusters (Vaclavik & Christian 2007). The surface of casein micelles consists of negatively charged κ-casein. These negatively charged parts of the protein are called glucomacropeptide (GMP). The negative charge of GMP makes the casein micelles repel each other. When fermentation occurs, lactic acid is produced. The lowering of pH due to increased levels of lactic acid, leads to calcium phosphate particles dissolving and neutralisation of the negatively charged GMP (Walstra 1999). The consequence of this is the aggregation of the casein micelles due to acidic coagulation. The casein micelles form a network together with fat and calcium (Liang 2012). In contrast, in enzymatic coagulation an enzyme, e.g. rennet, is added to the milk. The negatively charged GMP is hydrolysed from the casein micelle and is lost to the whey. On the surface of the casein micelle remains para-κ-casein. The main difference between acidic and enzymatic coagulation is that the gel is stronger, more elastic and the GMP is hydrolysed in the case of the enzymatic one. Acidic coagulation results in weaker but more viscous gels, the GMP is not removed, but neutralised remaining on the micelle surface (Phadungath 2005). Enzymatic coagulation is common in the process of hard cheese, whereas acidic coagulation is used in production of fermented milk products, e.g. yoghurt and curd (Ruettimann & Ladisch 1987).

The fermentation and thus coagulation of the milk takes place in clay pots, plastic containers or glass bottles where (Sri Lanka Standard Institution 1988). The clay pots consist of small pores which have the ability to absorb water from the curd. By that, curd becomes more concentrated with increased flavour of the product (Vidyarathna 2019). With the plastic or glass package a water accumulation occurs. The last part of the process is the labelling of the package. According to the law, following details should be stated: Name of the product with emphases on the source of milk from either cow or buffalo, batch, trademark, net mass in grams, declaration of preservatives added, date of manufacture and the shelf time. The shelf time for curd is approximately one week (Sri Lanka Standards Institution Standard 1988).

1.3.4 Starter cultures

To make curd, addition of a starter culture is needed (Adams et al 2016). At a commercial manufactory, freeze-dried vat cultures are commonly used (Disanayaka 2019). An example of a freeze-dried vat culture is CHR-704, which is made by the Danish company Chr Hansen. CHR-704 are a mesophilic and homofermentative culture, producing lactic acid only (Adams et al 2016). The traditional starter cultures used in the curd manufacture are based on the addition of previously made curd, where the one-day old curd is added into the curd milk of the day. This practice is most common in manufacturing of curd in small scale (Disanayaka 2019). A curd starter culture needs to contain: *Lactococcus lactis* subsp. *lactis, Lactococcus lactis* subsp. *biovar diacetylactis* and *Lactococcus lactis* subsp. *cremoris.* These three bacteria, single or combined, can be also mixed with *Leuconstoc spp, Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus* (Sri Lanka Standards Institution Standard 1988).

1.3.5 Nonstarter lactic acid bacteria

Nonstarter lactic acid bacteria (NSLAB) is a microflora of lactic acid bacteria that can be found naturally in the environment and therefore may affect food products in both positive and negative ways. The negative part of NSLAB will affect the food products negatively e.g inaccurate odour, texture and flavour (Adams et al 2016). However, if the NSLAB contains for example *Streptococcus lactis, Streptococcus diacetylactis* and *Streptococcus cremoris* the effect on the curd is positive (Sri Lanka Standards Institution Standard 1988). NSLAB can also contribute to unique taste and character of a manufactured product.

1.3.6 Sensory test

A sensory test is an evaluation of different sensory properties performed by a test panel. Example of properties include texture, odour, flavour and colour. Comparison of properties is made between products which slightly differ from each other. One simple type of test is when the panel ranks samples without knowing the identity of a sample. A high-ranking number is the most preferred sample and a low-ranking number is the least preferred one. Afterwards an evaluation of the ranks is made in order to get the specific scores of the products (Wijethunge 2015).

1.4 Cow and buffalo milk in comparison

A comparison between cow and water buffalo milk shows that the nutritional value of buffalo milk is higher (Perera 2001). Levels of milk components e.g. lactose, protein (mostly caseins), calcium, magnesium and fat are higher in buffalo milk compared to cow milk (Table 2) (Ahmad et al 2008). The fat globules of buffalo milk are approximately 5 µm, whereas in cow milk the fat globules are smaller, approximately 3.5 µm (Ménard et al 2010). The larger milk fat globules in buffalo milk is probably related to the higher amount of fat, compared to cow's milk (Guo & Hendricks 2010). Cow milk fat globules are fewer and smaller compared to fat globules in buffalo milk. The membranes of both cow and buffalo milk fat globules consist of the same classes of polar lipids, e.g. phosphatidylethanolamine, sphingomyelin (SM) and phosphatidylcholine (PC). The difference between buffalo and cow milk fat globule membrane is the higher percentage of PC and a lower percent of SM in buffalos (Perera 2001). Saturated fatty acids, e.g. palmitic acid, but also trans fatty acids, linolenic acid and conjugated linolenic acid are in a higher amount in buffalo milk than in cow milk (Ménard et al 2010). Buffalo milk has also been reported to have a bigger buffering capacity in comparison to cow (Ahmad et al 2008).

Table 2. *Gross composition of buffalo and cow milk. Source: Perera 2001*

	Buffalo	**Cow**
Fat %	7.45 – 8.95	3.60 – 5.00
Protein %	4.36 – 4.13	3.25 – 3.18
Lactose %	4.83 – 4.78	4.60 – 4.59

Milk of buffalo and cow behave similar with respect to the aggregation of the casein during acid induced coagulation and solubilization of calcium and phosphate (Perera 2001). The composition (Table 2) of the buffalo milk is very important for the manufacture of high-quality curd (Guo & Hendricks 2010) and buffalo milk is due to its unique composition more suitable raw material for curd making than cow milk (Ahmad et al 2008).

1.5 Aim and hypothesis

The general aim of this study was to observe the impact of different starter cultures during the process of curd making.

Hypothesis

- pH decrease will be equal for curd with different starter culture, as all starter cultures in this study produce lactic acid.
- Syneresis will be equal for curd with different starter culture, as the starter cultures should not affect syneresis.
- The most preferred curd with respect to odour, flavour, texture and colour, will be the product based on PMBC starters, as these starters are adapted from buffalo milk.

2 Materials and method

2.1 Study area

The field and experimental study was carried out March - May 2019 in Sri Lanka at the University of Peradeniya in the city Peradeniya, in Kandy region (Figure 3). *Mawalawaththa Livestock Field* Station is a dairy manufactory owned by the University of Peradeniya where the field study was performed. The manufactory provides a big variance of food products, e.g. yogurt, ice cream and curd. The final products are only delivered and available in the milk bars of the University of Peradeniya.

Figure 3. Map of Sri Lanka. The city Peradeniya, where the study was performed is marked in red. Source: Google.se/maps.

2.2 Field and experimental work

2.2.1 Field work

During the field work, there was a plan to visit small-scale and commercial manufactories. The commercial curd manufacturer *Mawalawaththa Livestock field station* is located in Peradeniya, and the small-scale curd manufactory is in south east of Sri Lanka in the district Yala. Questions were prepared for a comparison of the two manufactories (Appendix I). Due the bomb act occurring the 21 April 2019 in the area around Colombo in Sri Lanka, the time and safety was limited and the visit to the curd manufactory in the district Yala was cancelled. Only the visit to *Mawalawaththa Livestock field station* in Peradeniya was fulfilled where the farm manager Chathur Vidyarathna was interviewed (Appendix I).

2.2.2 Description of experimental work

Three curds with different starter cultures were made in order to compare the pH development, syneresis and sensory properties such as flavour, colour, odour, texture and overall acceptability. Curd with CHR-704, PMCC from the manufactory *Kotmale* in Colombo and PMBC from the manufactory *Himalee dairy farms* in Maradankadawala were compared (Figure 4) using buffalo milk. The recipe from *Mawalawaththa Livestock field station* was followed to make own curd (Appendix II). No statistical evaluations were made using data from the experimental work.

Figure 4. Three curds with different starter cultures. From left to right: Chr Hansen-704, Pre-Made Cow Curd and Pre-Made Buffalo Curd. Photo: C. Andersson.

Process of curd making

Firstly, 7.5 litres of buffalo milk of the cross breed *Nili-Ravi* X *Murrah* was poured to a saucepan on a gas stove to be heated at 92 °C for 20 minutes. Secondly, the milk in the saucepan was chilled in a water bath for approximately 10 minutes, until the temperature had decreased to 42 °C. After that, the milk was divided into three fractions. Five tablespoons of each pre-made starter culture were added to the fractions, one starter culture in each fraction. For the CHR-704 0.1 gram was added to the fraction. Stirring occurred in all fractions for 30 seconds. Each fraction inoculated with a specific starter culture was divided into four plastic containers. Totally 12 containers were used with 500 ml of curd milk in each. The containers with the curd milk were incubated at 25 °C for 24 hours for fermentation. After 24 hours, the containers were placed into a refrigerator at a temperature of 4-6 °C until use.

pH measurement

In order to compare the ability of the starters to produce lactic acid, pH was measured in each plastic container in triplicates. This occurred for four days every morning in a row (Appendix III). Mean values and standard deviation of the pH for curds with the same starter cultures were calculated using Excel.

Measurement of syneresis

The levels of syneresis in the curd products were evaluated each morning for each plastic container. This was made for each plastic container for three days in triplicates (Appendix V). The syneresis test was made as described by Jayarathna (2013). In short, 10 grams of curd was taken from each sample. The curd sample was added to a Whatman filter paper number 2 into a Buchner funnel. The Bucher funnel together with an Erlenmeyer flask were connected with a vacuum pump. The curd was filtered for 10 minutes using the vacuum. The whey part was weighed, and the percent of syneresis was calculated (Appendix IV). Mean values and standard deviation for curds with the same starter cultures were calculated using Excel.

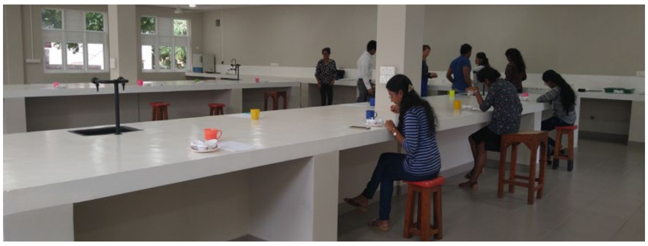

Figure 5. Sensory panel testing curd with three different starters cultures. Flavour, colour, odour, texture and all acceptability are properties which were tested. Photo: C. Andersson.

2.2.3 Sensory test

Day 5, curd made with the three different starters were used for a sensory evaluation (Figure 5). A test panel consisting of 31 persons was assembled at the University of Peradeniya. The three curd samples were labeled with code numbers as follows: CHR-704 - code number 3, PMCC - code number 59 and PMBC - code number 81. Each product was ranked from 1 to 3 for flavour, colour, odour, texture and overall acceptability, where grade 1 was the least preferred and 3 the most preferred one. The results were filled in into the template for the sensory test (Appendix VI) and the curd quality was evaluated (Appendix VII). The rank from the test panel was calculated in mean value and percent, this was called estimated rank. The higher estimated rank the more preferred of the test panel.

3 Result

3.1 pH measurement of the curd

pH was measured from the day of production (day 0) to the third day (day 3). The pH of the milk (day 0), was measured before heating. pH decreased for all samples with time. Using CHR-704 as a starter culture, pH decreased from 6.47 to 4.28 from day zero to day three. Using PMCC, pH decreased from 6.47 to 4.45 from day 0 to day 3. The highest reduction in pH, 34%, took place in curd with CHR-704 whereas curd with PMCC resulted in the lowest pH reduction, 31% (Table 3).

Table 1. *pH values from curd with different starter cultures. Chr- Hansen R-704 = CHR-704, Pre-Made Cow Curd = PMCC, and Pre-Made Buffalo Curd = PMBC. Mean of triplicates and standard deviation is indicated*

Starter culture type	Day 0 Raw milk before processing	Day 1	Day 2	Day 3
CHR-704	6.47 +/- 0	4.33 +/- 0,06	4.28 +/- 0,03	4.28 +/- 0,03
PMCC	6.47 +/- 0	4.55 +/- 0,05	4.52 +/- 0,05	4.45 +/- 0,07
PMBC	6.47 +/- 0	4.42 +/- 0,05	4.38 +/- 0,01	4.37 +/- 0,05

3.2 Syneresis

On day 1, i.e. 24 hours after production of curd, syneresis was measured for the first time. All curds showed a decrease in syneresis from day 1 to day 3. Syneresis in the curd with PMBC was reduced from 4.95 gram on day 1 to 3.86 gram on day 3, a decrease of 19.11%. The curd with PMCC showed the highest amount of syneresis from day 1 to day 3 with 6.17 gram to 3.88 gram, a decrease of 23,94%. The curd with CHR-704 showed a reduction in the amount of syneresis from day 1 to day 3 with 5.10 gram to 3.93 gram, a decrease of 20.04% (Table 4).

Table 4. *Syneresis in curd made with different starter cultures. Chr Hansen R-704 = CHR-704, Pre-Made Cow Curd =PMCC and Pre-Made Buffalo Curd = PMBC. Mean of triplicates of expelled whey in gram and standard deviation is indicated*

Starter culture type	Day 1	Day 2	Day 3
Syneresis in CHR-704	5.10 +/- 0.31	5.86 +/- 0.87	3.93 +/- 0.56
Syneresis in PMCC	6.17 +/- 1.23	5.12+/- 0.12	3.88 +/- 0.41
Syneresis in PMBC	4.95 +/- 0.30	4.99 +/- 0,64	3.86 +/- 0.65

3.3 Sensory test

The most preferred curds with respect to flavour were curds with CHR-704 and PMCC starters, whereas the least preferred product was curd with PMBC starters. With respect to colour and odour, curd with PMCC and CHR-707, respectively, were the most preferred once, whereas curd with PMBC was the least preferred one for both parameters. The most preferred curd with respect to texture was curd with PMCC while curd with CHR-704 was the least preferred one. Overall, curd made with PMCC starter was the most preferred one, while curd with CHR-704 was least appreciated (Figure 6).

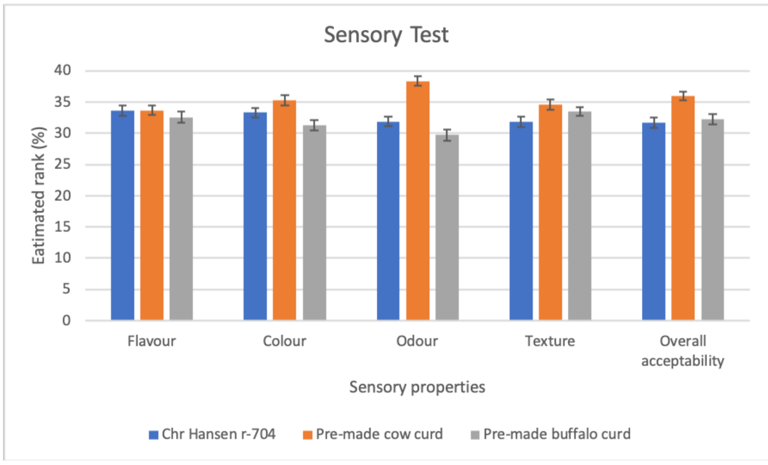

Figure 6. Evaluation of sensory parameters of curds with different starter cultures. Three curds with different starter cultures were tested: Chr Hansen r-704 - blue, Pre-Made Cow Curd - orange and Pre-Made Buffalo Curd - grey. Sensory properties tested: flavour, colour, odour, texture and overall acceptability. 31 replicates were included in the sensory test.

Standard deviation is indicated. Estimated rank (%) is the mean value of the rank percent from the test panel. The higher rank, the higher preferred of the test panel.

4 Discussion

4.1 Experimental work

4.1.1 pH measurements

The hypothesis with respect to pH was that curds with different starter cultures will have the same pH. This assumption was wrong, as the different starter cultures resulted in products which differed in pH at day 3. However, the differences were very small and as no statistical analyses were done, the results must be interpreted carefully. Curd with CHR-704 as a starter showed the highest pH decrease (34%) whereas curd with PMCC showed lowest (31%) from day 0 to day 3 (Table 3). Furthermore, starter CHR-704 reduced the pH more rapidly compared to the pre-made cultures. The curds with pre-made starter cultures showed almost equal changes in pH during the observation period. The pH was however slightly higher compared to the curd with CHR-704. The difference in pH between curd with CHR-704 and the pre-made starter cultures were in the range of 0.09-0.17%, which indicates lower production of lactic acid in curd with the pre-made starters compared to curd with CHR-704. The pH decreased in all curd products, indicating successful fermentation. These observations of how a successful fermentation occur are in agreement with Adams et al (2016) describing fermentation in yogurt.

4.1.2 Syneresis

The hypothesis with respect to syneresis was that curd with different starter cultures will show the same degree of syneresis. The hypothesis was inaccurate. Curd with PMBC showed the lowest syneresis during the time period compared to the curds with the other two starter cultures (Table 4). It is known, that lowering pH of the milk will lead to a lowering of the negative net charge of the milk proteins which makes the protein less able to bind water. Destabilization of milk proteins increase the water activity in the curd (Jayarathna 2013) and high water activity increase syneresis in curd. In this study, differences in syneresis cannot be explained by the differences in pH, as the differences in pH were very small for all curds with different starter culture (Table 3). The observed pH values in the study at day 3 were lower than the isoelectric point for all 3 products (4.6). Jayarathna (2013) showed, studying probiotic vegan yogurt, that syneresis increased closer to the isoelectric point. This finding is in agreement with this study, where the curd with PMCC and pH 4.55 at day 3, i.e. the pH closest to the isoelectric point compared to the other curds in the experiment, also showed the highest syneresis.

4.2 Sensory test

The sensory test was made according to Wijethunge (2015), who developed a method for sensory testing of a feta type cheese. The hypothesis was that curd with PMBC should be the most preferred one. The hypothesis was inaccurate. Curd with PMCC was the most preferred one for all properties. Curd with PMCC had a soft texture, weak sour flavour and odour compared to the curd with PMBC. The test panel preferred the attributes of the curd with PMCC more than of the curd with PMBC. Clear difference in colour was observed between curd produced with different starter cultures. The colour was mostly preferred in the curd with PMCC, which was more yellowish. The colour of this product might be explained by the fact that a small amount of cow milk was added in form of starters. The difference in colour between cow and buffalo milk was reported in Perera (2001) who made a comparison between cow and buffalo milk. Curd with CHR-704 and curd with PMCC, both obtained 34% for the flavour in the sensory test and seem to be similar in popularity. The curd with PMBC was the least preferred product with respect to flavour. For colour and odour, curd with CHR-704 starter was preferred over curd with PMBC. The reason that curd with PMBC obtained a lower score compared to the two other products could be because of its improper texture, containing small cavities in the curd, affecting the mouth feeling negatively. This texture defect could be a result of bad stirring during the curd making. The texture defects were probably not due to a bad fermentation, as all the curd samples obtained approximately the same pH.

4.3 Sources of error

Human error factors can affect the results, for example through mistakes during measurement. The curd with PMBC had small texture defects which probably were due to non-perfect stirring during the process of curd making. Statistical analyses were not performed; therefore, one can not draw conclusions regarding significant differences. To achieve significant results, the number of measurements would need to be increased.

5 Conclusion

The result showed that it is possible to make curds of high quality independent on starter culture. Results suggest that there was no difference in the final pH between the curds with the three different starter cultures. Pre-made starter cultures showed lower syneresis compared to curd with CHR-704. According to the sensory evaluation, the curd containing PMCC starters was the most preferred one.

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Appendix

5.2 Appendix I. Questionnaire for curd manufactory visit

How many employees?

How many cow?

How man breeds of cow?

How many buffalos?

How many breeds of buffalo?

Other animals?

Produce more than curd?

Starter culture for process of curd?

In which form is the starter culture added?

Are additives/ taste enhancers added?

How many litres of buffalo milk needed for 1 500 ml container?

How many litres of cow milk needed for 1 90 ml container?

Do you control pH?

Do you control pathogens?

Is the equipment good?

How is the storage of the curd? How is the curd stored?

How frequently is the curd made?

How is the curd transported to retailer?

Who is customer?

Are you/customers satisfied with the curd?

What do you do with curd of poor quality?

What steps in the process could be developed?

How long is the shelf life of the product?

5.3 Appendix II. Recipe of buffalo curd

	Buffalo curd, 500 ml x 12 cups
Ingredients:	7,5 liters of cattle milk, 0,1 gram of Chr Hanzen R-704 starter culture bacteria, 5 table spoons of premade cattle curd and 5 tablespoons of premade buffalo curd.
Equipment:	Temperature-meter, large saucepan, gas stove, plunder, water bath, small jar, colander, 12 plastic containers
1.	Heat 7,5 liter of milk to 92 degrees in a large saucepan on a gas stove. Heat the milk in approximately 20 minutes. Stir regularly with a plunder to get a homogenization
2.	Put the saucepan with milk in water bath to decrease the temperature from 92 degrees to 42 degrees. This step will approximately take 10 minutes.
3.	Take 1 dl of the heated milk into a jar. Add 0,1 gram of Chr Hansen R-704 starter culture bacteria to the jar of heated milk. Shake gently.
4.	Add the milk and starter culture bacteria to the saucepan with heated milk and stir
5.	Use a colander to filtrate away dirt and unwanted products from the heated milk with starter.
6.	Add 500 ml processed milk with starter culture bacteria to each plastic container.
7.	Put the containers in room temperature for 24 hours. The curd will be set.
8.	Put the curd in a refrigerator with a temperature of 4-6 degrees. After 1 day, you can start to eat.

5.4 Appendix III. Form used to fill in pH

	Day 1- Milk	Day 1	Day 2	Day 2	Day 3	Day 3	Day 4	Day 4

Sample 1a								
Sample 1b								
Sample 1c								
Sample 2a								
Sample 2b								
Sample 2c								
Sample 3a								
Sample 3b								
Sample 3c								

5.5 Appendix IV. Calculation of syneresis in percent

$$\text{Syneresis\%} = \frac{\text{Weight of the liquid}}{Initial\ sample\ weight}\ X\ 100$$

5.6 Appendix V. Form used to fill in syneresis

	Day 1	Day 2	Day 3
Sample 1a			
Sample 1b			
Sample 1c			
Sample 2a			
Sample 2b			

Sample 2c			
Sample 3a			
Sample 3b			
Sample 3c			

5.7 Appendix VI. Ranking card for sensory test

Ranking Card
Sensory evaluation of curd made by using different starter bacteria culture

Date: 2/5-19
Name:
Gender: Male/Female
Age:

Please taste given curd samples and rank by using following attributes and numerical values for given characteristics.

Attribute	Ranks
Least preferred Most preferred	1 2 3

Instructions

- You are kindly requested to taste given curd samples. Taste one curd of each code number.
- Rank each characteristic from 1 to 3.
- Wash your mouth after each and every attempt.

Code no	Flavor	Color	Odor	Texture	Overall acceptability	Remarks
33						
59						
81						

5.8 Appendix VII. Calculation of properties in sensory test in percent

$$Part\ of\ the\ otal$$

$$Percent\ of\ sensory\ properties = \frac{Part\ of\ the\ total}{Total}\ X\ 100$$