A study on the development of starter culture with red chili (Capsicum annuum) fruits for cow’s milk curd

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Abstract
Traditional starter culture for curd is made with a concentration of Lactic Acid Bacteria. Sometimes traditional starter cultures become expensive for the common man to make curd, so to reduce the cost they use a small amount of curd previously made. This causes the quality of the curd to degrade over subsequent generations. Thus, the main purpose of this study was to make curd with sources that are easily available. One of the most easily available sources was Red Chilies (Capsicum annuum) which is widely used in the Indian Cuisine. Curd was successfully developed using the stalks of the red chilies. Another objective of the study was to observe curdling of milk with the addition of sources derived from plants (in this case the fruit). Thus, the two objectives of the study was achieved using the red chili fruits and the reason behind the curdling of milk was also assessed upon.

Keywords: Curd, red chili (Capsicum annuum)

1. Introduction
There are many stories about using plant and other natural sources as dairy fermentation starters. When making gariss, the Sudanese fermented camel’s milk in the absence of a starter from a previous batch, “fermentation is initiated by adding to the container a few seeds of black cumin and one onion bulb,”[1]. Traditionally in India if the starter went bad or if families ran out of starter, they would use the stems of chilli peppers to create a new one[2].

Many other plants have been associated with curdling milk, including stinging nettles (Urtica dioica), fig (Ficus carica), Indian fig (Opuntia ficus-indica), mallow (Malva spp.), creeping Charlie (Glechoma bederacea), lady’s bedstraw (Galium verum), and several different thistles (including Cynara cardunculus, C. humilis, Centaurea calcitrapa, Cirsium arietinum, and Carlina spp.)[3].

Another way that people have used plants in milk fermentation is using the smoke and ash from burning plants, as illustrated in the Kenyan fermented milk- this is prepared by pouring the milk into a gourd or calabash. No artificial starters were used and fermentation and acidification occurred spontaneously after a few days, either from the natural flora of the milk if raw, or from the bacteria found within the vessel. When the milk began to coagulate some whey was removed and the vessel was topped up with fresh milk. The process was repeated and the vessel was shaken regularly for around one week.

The United Nations Food and Agriculture Organization reports, “The practice of smoking the vessels used for the storage of milk is a common feature of the various communities. The treatment has the functions of passing the smoke flavour to the milk or milk product and disinfecting (sterilizing) the vessel.” The FAO lists more than a dozen plants—including grasses, shrubs and hardwoods—used in this way by various communities in Ethiopia, Kenya, and Tanzania[3].

2. Materials and method
2.1 Preparation of Curd
In the initial phase of the study to check whether red chilli stalks aided in the formation of curd, 10 stalks of red chilies were added to 250 ml of full fat cow’s milk (fat-6.00%). It was left for a day covered with cling wrap to maintain conditions under which fermentation due to the stalks can be observed and not due to other organisms present in the surrounding air. 12 hours after the addition of stalks, slight curdling was seen which then increased in consistency in the next 12 hours.
The general process of curd making for this study is stated below:

- 250 ml full fat milk boiled to 100 °C.
- 10 stalks of Red Chilies (*Capsicum annuum*) are added to a glass bowl.
- Boiled milk which was cooled down to 60 °C is added to the glass bowl.
- The bowl is the covered with cling film and kept at room temperature (25 ºC- 35 ºC) to set.

### 2.2 Estimation of ascorbic acid

Once curd formation was seen after the addition of Red Chilli stalks, the ascorbic acid content of the chilli was determined by the 2, 6- Dichlorophenol Indophenol (DCIP) method. Chilies have a high content of ascorbic acid in them. The content of acid in the chilies increases as they ripen over time. For this study, the stalks of red chilies were used. In this method, the ascorbic acid is oxidized by the coloured dye 2, 6-Dichlorophenol Indophenol to dehydro-ascorbic acid and at the same time the dye is reduced to a colourless compound so that the end point of the reaction can be easily determined.

The acid content of the red chilli was determined by measuring 5 grams of stalk and the pulverizing it to a paste with 50 ml of distilled water. This paste was then filtered through a filter paper and the filtrate so obtained was then used to determine the acid content.

### 2.3 Materials/ Reagents required

- Standard Ascorbic Acid solution (20mg/ml)
- 2,6- Dichlorophenol Indophenol dye
- Dilute Glacial Acetic Acid
- Sample (red chilli paste filtrate)

At first the standardization of the dye was done where 1 ml of the dye was mixed with 2 drops of dilute glacial Acetic acid where a pink coloration was seen. This solution was then titrated against standard Ascorbic Acid till the solution turned colourless. The change in colour of the solution determined the end point of the test.

### Table 1: Table depicting the titre value of the dye.

<table>
<thead>
<tr>
<th>Serial No</th>
<th>Initial Volume(ml)</th>
<th>Final Volume(ml)</th>
<th>Mean(ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>0.30</td>
<td>0.30</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0.30</td>
<td>0.30</td>
</tr>
</tbody>
</table>

The strength of the dye was calculated by the following method:

\[ V_1 \times S_1 = V_2 \times S_2 \]

where,
\[ V_1 = 0.30 \text{ ml} \]
\[ V_2 = 1 \text{ ml} \]
\[ S_1 = 20 \text{ mg/ml} \]
\[ S_2 = x \]

Thus, the strength of the dye was 6mg/ml.

Now that the strength of the dye was known, the strength of ascorbic acid in the sample of red chilli stalks was determined. It was done in the same process as the standardization of dye, where 1 ml of the dye was mixed with 2 drops of dilute Glacial Acetic Acid when a pink colour was observed. This solution was titrated against the filtrate derived from the chilli paste till the solution turned colourless thus marking the end of the reaction.

### Table 2: Table depicting the titre value of the sample filtrate

The strength of the sample filtrate was calculated by the following method:

\[ V_1 \times S_1 = V_2 \times S_2 \]

where,
\[ V_1 = 0.30 \text{ ml} \]
\[ V_2 = 1 \text{ ml} \]
\[ S_1 = x \]
\[ S_2 = 6 \text{ mg/ml} \]

Therefore, by applying the given values, the value for ‘x’ was determined as follows,
\[ 0.30 \times x = 1 \times 6 \]
\[ x = 20 \text{ mg/ml} \]

Thus, the strength of the Ascorbic Acid present in the sample filtrate was 20mg/ml.

From the titration it was calculated that 50ml of the filtrate contained 20 mg/ml of Ascorbic Acid. Therefore, the total content of Ascorbic Acid present in the filtrate can also be determined as 1000 mg. Since 5 grams of sample was used, then according to calculations per gram of sample contained 200 mg of Ascorbic Acid. Thus, 10 grams of sample would contain 2000 mg (2gms) of Ascorbic Acid.

To demonstrate effective curdling of milk with help of dehydrated Vitamin C powder, 250 ml of full fat milk was boiled and cooled to 60 °C and Ascorbic Acid content worth 10 grams of sample i.e 2 grams was added to the boiled milk. Upon addition of 2 grams of dehydrated Vitamin C powder (Ascorbic Acid) curd formation was seen.

Thus, it can be noted that Red Chilies (*Capsicum annuum*) contains enough Ascorbic Acid to facilitate the formation of curd.
3. Conclusion
The result of this study reveals that Red Chilli (*Capsicum annuum*) can be used as a source of making started culture for milk fermentation (curdling). The objective of the study i.e., to use a source derived from plant origin to make curd was effectively demonstrated. Ascorbic acid content increases as the chilies ripen over time, thus the red chilies used had enough Ascorbic Acid content in it to curdle milk effectively. In the olden days red chilli was used as a source to curdle milk but the reason behind was unknown. This study has demonstrated the fact that the Ascorbic Acid content of the chilies is enough to cause curdling of milk which can be used as a starter culture for subsequent curd generations.

4. Acknowledgement
The authors take this opportunity to express their gratitude to everyone who helped and encourage them. We would like to thank the faculty and management of the institute for being a constant help to us. Finally, no words of gratitude can express our indebtedness to God and our family, who have always been our guiding spirit, and friends for encouraging us throughout the study.

5. References
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