Extraction, Partial Purification And Characterization Of Milk- Clotting Enzyme From Local Coagulants On The Yield Of Cheese Using Cow Milk

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Research Article

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Abstract

Milk-clotting activity is the most important property of enzymes used in cheese making. The study conducts at the University of Gondar, Institute of biotechnology microbial and cellular biology laboratory. Latex extracted from each plant using an appropriate procedure. Five hundred ml of milk added to each beaker in all extract labels with its replication, heated, each coagulant was added and heated till a separate layer of cheese and whey happened. Then after clotting time, whey product, cheese- fresh weight, cheese dry weight, and the percentage of cheese yield were recorded using appropriate measurement and calculation. All data analyzed using SAS Studio, and analysis of variance showed that highly significant differences have existed among plant extract used for all studied parameters. The highest (59, 74.67, 91.33 gm) amount of cheese- dry weight observes in lemon juice. The highest (11.34, 14.36, and 17.56)% of cheese- yield observe in lemon juice for all extract labels, respectively. The lowest amount (32.67, 25.67, and 17.00) of cheese formation starting time observed from lemon juice, respectively. Therefore, lemon juice, calotropis procera, and Solanum incanum could use in the dairy industry and local community as an alternative source of rennet.

Introduction

Milk coagulation is the primary step for producing cheese and used in cheese making for thousands of years. They seem to the oldest known application of enzymes. Almost all the enzymes used as milk coagulants belong to aspartic proteases, but enzymes from other groups such as cysteine and serine protease also use [1]. The chemical rennin enzyme extracted from the calf stomach of young ruminants uses to coagulate milk and form curds. It is highly essential in the cheese-making process. But its supply had become less available because instead of slaughtering calves to get rennet, they are preferably used to provide meat for human consumption [3]. The shortage of Calf's rennet also highly increased because of religious restrictions and ethnic regulations. These paved the way for the replacement of rennet from more convenient sources [4].

The most important alternative enzyme substitutes fulfilling the requirements of cheese manufacturers include microbial, recombinant, and plant-based enzymes. A plant-derived substitute has a particular value because of its essentially unrestricted availability [5]. Certain plants reported to yield promising rennet activity, such as Withania coagulants; Ficus carica; Pumpkin; seeds of Moringa oleifera; seeds of Ricinis communis; dried Papaya latex, Pineapple, cucumber; Benincasa cerifere, Galium verum; Pinquicule Vulgaris; Drosera rotindifolia; and Ranunculus ligua. However, in Ethiopia, in tradition, they use the plant, but little work reports before [6].

Solanum incanum is one of the most energetic traditional medicinal plants belongs to the *Solanaceae* family. The fruit contains many small and soft edible seeds but is bitter because they have an insignificant amount of nicotinic alkaloids and cyanogenic glycosides [2]. Leaf and fruits use for washing painful areas. Ash of burnt plants is mixed with fat and applied to treat a sore throat, angina, stomach pain, colic, headache, painful menstruation, and liver pain. Even if the wide variation in toxicity makes it

dangerous to transfer specific uses from one region to another, it extensively uses for antibacterial and antitumor activities [7]. Its anti-microbial activities also use for milk clotting activity of cheese production in rural communities and certain dairy farmlands [8].

Calotropis procera is soft-wooded, evergreen, perennial shrubs with broad fleshy leaves that grow wild in the tropics and warm temperate regions of the family Apocynaceae and Asclepiadoideae subfamily (milkweeds). It uses to treat pain, inflammation, digestion, antidysenteric, antirheumatic, diaphoretic, an expectorant, bronchial asthma, and skin conditions [9]. They used roots and Dry latex as an antidote for snake poisoning. They also used it as an abortifacient to treat piles and intestinal worms [10].

Plant extracts used as milk coagulants in cheese making for long times. Cheeses made with vegetable coagulant found mainly in Mediterranean, West African, and southern European countries [1]. However, the excessive proteolytic nature of most vegetable coagulants has limited their use in cheese manufacturing due to lower cheese yield and defects in flavor and texture [11]. Therefore, searching for new potential milk-clotting enzymes from plants is undergoing to make them useful for industry, to go with the increasing global demand for diversified and high-quality cheese production [12].

Several studies performed using plant-derived enzymes for cheese making. Jacob [13] reviewed the importance of milk-clotting enzymes, including animal rennet, microbial coagulants, recombinant coagulants, and plant-derived clotting enzymes. Yegin and Dekker [14] reported the progress of animal, plant, and microbial origin aspartic proteinases with a specific emphasis on structures, functions, catalytic mechanism, inhibition, and engineering.

In Ethiopia, the commercial calf rennet enzyme used to coagulate milk in cheese making is too costly, and the need for calf sacrifice makes it complex to use it at commercial and small-scale dairy production levels in Ethiopia. Very little research performs to use plant-derived protease for cheese making.

These studies have a great significance by solving the problems facing the society in day-to-day life to cheese production, creating awareness for the scientific community in the cheese-making process using natural products, and giving a basic knowledge about the application of calotropis latex, solanum incanum, and lemon juice as it will satisfactorily replace the calf rennet. Therefore, the aim of this study focused on the extraction, purification, and characterization of a milk-clotting enzyme, which was assumed to replace calf rennet produced from animals.

Materials And Methods

The study conducts from Dec to January 2021 at the University of Gondar, Institute of biotechnology, Microbial and cellular biotechnology laboratory using a completely randomized design (CRD). The solanum fruits and calotropis procera latex collected from Gondar town nearby the university, lemon fruits purchased from the local market in Gondar town, and cow milk got through hand milking from Gondar university animal farm at college of veterinary medicine and animal science which have the same feeding.

Preparation of juice and latex

Incisions made on the unripe and ripe fruits of *Solanum incanum* and lemon using a sterile scalpel blade and the green viscous fluid from *Solanum incanum* and lemon juice squeezed out of the fruit, filtered by muslin cloth and Whatmann filter paper No. 1for partial purification, and then the partially purified Juices put into refrigerator till further use. The latexes from calotropis procera got from the succulent bark part, centrifuged at 1500rpm for 15 min at room temperature to separate the aqueous filtrate part from the polymeric gum, the aqueous filtrate part again filtered through muslin cloth and Whatmann filter paper No. 1, and stored in a refrigerator for further use. The experiment comprised three plant with three extract level for each (15,20 and 25 ml), each with three replications.

Preparation of cheese

For each plant and coagulant level used, ten (10) clean Beakers adjust and labeled as lemon juice (M-LJ1, M-LJ2, M-LJ3), solanum juice (M-SI1, M-SI2, M-SI3), and calotropis procera (M-CP1, M-CP2, M-CP3). 500 ml of milk added for all 27 beakers along with its replication and heated with 50 ^{o} C for 15 minutes. Then at each three plant level, 15 ml,20 ml, and 25 ml of each coagulant added, and then the mixture of each extract and milk stirred until boiling point reached and a separate layer of cheese and whey happened, clotting time recorded and the whey separated from cheese by transferring to the same labeled beaker. Then after the amount of whey produced measured through ml, cheese- fresh weight measured using sensitive balance, and cheese dry- weight determined after 72-hour oven drying.

The percentage of cheese yield gained by calculating the ratio of cheese mass got after an oven-dry to the weight of milk used using the following equation.

Where the weight of milk = (ml of milk used) * milk density (1.04 grams).

$$\% \text{ of cheese yield} = \frac{(\text{grams of cheese produced})\ 100)}{\text{Weight of milk used}} \quad [\mathbf{15}]$$

Data analysis

All data analyzed using SAS Studio in SAS University Edition

Results And Discussion

The analysis of variance showed that highly significant differences existed for all studied parameters. The variation between extracts shows highly significant difference during comparison of plants for each studied parameter. But replication didn’t show stastical significance difference for all parameter and treatment. This was due to the experiment was carried out in laboratory condition.

Whey is liquid cloudy water after the precipitation and removal of milk casein [16]. Screening of plants for this parameter is important for whey beverage industry. In this experiment, using (15 ml) of each extract

and (500 ml) of milk, the mean was recorded as (402.22 ml). The highest and the lowest amount were found from calotropis procera (425 ml) and Solanum Inicum (386.67 ml), respectively. When using (20 ml) of each extract, the mean was (420.56 ml) and the highest and lowest amount found from solanum Incanum (440 ml) and lemon juice (400ml), respectively. Whereas, when using (25 ml) of each extract, the mean was (409.11ml), and the highest and lowest amount of whey product gets from solanum Incanum (431.33 ml) lemon juice (372 ml).

Table 1
mean performance of each plant extract for the studied cheese parameter using 15ml of each juice and 500ml of hand milking cow milk.

Extract	Extract (ml)	Milk (ml)	WP (ml)	CHFW (gm)	CHDW (gm)	CHYP (%)	Time (min)
Lemon juice	15	500	395	79.67	59.00	11.34	32.67
Solanum Inicum	15	500	386.67	63.00	43.67	8.40	45.00
Calotropis Procera	15	500	425.00	56.67	42.67	8.21	42.67
Mean			402.22	66.44	48.44	9.32	40.11
F value			2.62	6.41	13.16	13.13	25.20
Pr > F			0.0187	0.0566	0.0174	0.0175	0..0054

Cheese is a widely consumed product by the general population; it is highly-concentrated, rich in proteins and lipids, essential amino acids, and minerals such as calcium and phosphorus [17]. The mean for cheese- fresh- weight, using 15 ml of extract, was (66.44g) and the highest (79.67g) and lowest amount (56.67g) of cheese- fresh weight records from lemon and calotropis procera, respectively. Whereas ,when using 20 ml of extract, mean cheese- fresh- weight was (79.11g) and the highest amount (97g) and lowest amount (70g) of cheese-fresh-weight found in Lemon juice and Solanum Incanum, respectively. When using 25 ml of each extract, the mean for cheese- fresh-weight was (93.33 gm), and the highest (117.67gm) and lowest (76.33 gm) amount observe in lemon juice and Calotropis Procera, respectively.

For cheese- dry- weight, using 15 ml of extract, the mean was (48.44g) and the highest (59gm) and lowest (42.67 g) amount observed in lemon juice and Calotropis Procera, respectively. When using 20 ml of each extract, the mean of this parameter was (59.67 gm), and the highest (74.67 g) and lowest (52 g) amount of cheese-dry-weight observe in Lemon juice and Calotropis Procera, respectively. Whereas when using 25 ml of each extract, the mean for cheese-dry weight was (71.67g) and the highest (91.33 g) and lowest (59.33 g) amount of cheese-dry weight recorded using lemon juice and Calotropis Procera, respectively.

Table 2
mean performance of each plant extract for the studied cheese parameter using 20 ml of each- extract and 500ml of hand milking cow milk.

Extract	Extract (ml)	Milk (ml)	WP (ml)	CHFW (gm)	CHDW (gm)	CHYP (%)	Time
Lemon juice	20	500	400.00	97.00	74.67	14.36	25.67
Solanum Inicum	20	500	440.00	70.00	52.33	10.06	32.33
Calotropis Procera	20	500	421.67	70.33	52.00	10.00	25.67
Mean			420.56	79.11	59.67	11.47	27.89
F value			1.83	5.14	5.35	5.36	400.00
Pr > F			0.0272	0.0785	0.0741	0.0739	< .0001

For cheese yield percentage, the mean proportion of plant extract was (9.32, 11.47, and 13.78) in all used extracts, respectively. The highest (11.34, 14.36, and 17.56, in all extracts, respectively) cheese yield percentage observed in lemon juice, and the lowest (8.21, 10.00, and 11.41, in all extracts, respectively) cheese yield percentage was in Calotropis Procera.

Table 3
mean performance of each plant extract for the studied cheese parameter using 25ml juice of each and 500 ml of hand milking cow milk.

Extract	Extract (ml)	Milk (ml)	WP (ml)	CHFW (gm)	CHDW (gm)	CHYP (%)	Time (min)
Lemon juice	25	500	372.00	117.67	91.33	17.56	17.00
Solanum Inicum	25	500	431.33	86.00	64.33	12.37	27.00
Calotropis Procera	25	500	424.00	76.33	59.33	11.41	20.00
Mean			409.11	93.33	71.67	13.78	21.33
F value			6.46	7.74	6.98	6.97	26.33
Pr > F			0.0559	0.0422	0.0496	0.0497	0.0050

Using 15 ml of each extract, the mean of cheese making efficiency in time was (40.11 min). The highest amount (45 min) from Solanum Inicum and the lowest amount (32.67 min) of cheese formation starting time found from Lemon juice. Using 20 ml of each extract, the mean of cheese formation in time was (27.89 min). The highest amount (32.33 min) was from Solanum Incanum, and the lowest amount (25.67 min) was from lemon juice and Calotropis Procera. Whereas Using 25 ml of each extract, the mean of

cheese forming efficiency in minutes was (21.33 min). The highest (27 minutes) were by Solanum Incanum and the lowest (**17.00 min**) duration of the time observed in Lemon juice.

Conclusion And Recommendation

Milk coagulation is the primary step for producing cheese and used in cheese making for thousands of years. These studies have a great significance by solving the problems facing the society in day-to-day life to cheese production, creating awareness for the scientific community in the cheese-making process using natural products, and giving a basic knowledge about the application of calotropis latex, solanum incanum, and lemon juice as it will satisfactorily replace the calf rennet. The results showed that milk clotting enzymes of plants have cheese-making ability which could be comparable with cheese prepared using rennet. Calotropis procera, lemon juice, and Solanum incanum should use in the dairy industry and could try to solve the societal problems of getting rennet enzyme. The researchers strongly recommend further investigation and attention for Preservation and controlled lab processes, more effective Partial purification methods, more characterization, and the phytochemical analysis and may well done this experiment by increasing the number of replication

Declarations

Ethical Statement

Ethical Statement for Discover Food

Hereby, I **Alemu Tebeje** consciously assure that for the manuscript /Extraction, Partial Purification and Characterization of Milk- Clotting Enzyme from Local Coagulants on the Yield of Cheese Using Cow Milk **/** the following is fulfilled:

This research material has not been previously published, is not currently being considered for publication elsewhere, and is the authors' original work, which properly credits meaningful contributions of co-authors. All sources used are properly cited and copied text indicated by giving proper reference. I agree with the above statements and declare that this submission follows the policies of **Discover Food** as outlined in the Guide for Authors and in the Ethical Statement.

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Alemu Tebeje Tesfaw

Consent for Publication

Not applicable

Data Availability

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request

Competing Interests

The authors declare that they have no competing interests

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Authors' Contributions

All authors read and approved the final manuscript.

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