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Milk Clotting Efficiency of Extracts from different parts of Sudanese *Moringa oleifera*

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Abstract

The limited supply of pure calf rennet enzyme and its high price for cheese industry have necessitated research to come up with an alternative milk coagulant. The aim of the present study was to search for milk clotting substitutes using extracts from different parts of *Moringa oleifera* tree. The most reliable, quick and efficient enzyme extract solution was found to be powder (20 mesh) of *Moringa oleifera* dried samples (flowers, leaves and seeds) suspended in 0.15 M NaCl (4 h at 4^{o}C). The extracted proteins were fractionated by ammonium sulphate at 60% saturation which was used throughout the study. Two experiments were carried out to study the effect of storage period on stability of *Moringa oleifera* extraction. In the first experiment, milk clotting activity of moringa extractions determined at different pH (4.5, 5.5 and 6.5) and different temperature (24, 37and 42^{o}C).According to pH the Highest value of milk clotting activity (3.66±0.07u/ml) obtained by *Moringa oleifera* seeds extract at pH 5.5 at first storage period (week), while the lowest value of milk clotting activity (1.77±0.02u/ml)recorded when *Moringa oleifera* flowers extract used at pH 6.5 at 4thstorage period (week). According to temperature (24, 37and 42^{o}C) the highest value of milk clotting activity (3.80±0.09u/ml) detected when *Moringa oleifera* seeds extract used at 42^{o}C at first storage period (week), while the lowest value of milk clotting activity (1.81±0.02u/ml) obtained by *Moringa oleifera* flowers extract at 24^{o}C at 4thstorage period(week).In the second experiment casinolytic activity of extracts from different parts of *Moringa oleifera* was determined at different storage periods. The highest value of casinolytic activity(2.37±0.00u/ml) recorded when seeds extract used at first storage period, while the lowest value of casinolytic activity (0.77±0.00u/ml) obtained by flowers extract at 4th storage period. It was concluded that *Moringa oleifera* extracts is a suitable substitutes for milk clotting for preparation of white cheese.

Keywords: *Moringa oleifera*, Extraction, Milk clotting activity, casinolytic activity.

Introduction

Milk is a complex biological fluid, secreted by the mammary glands of lactating mammals. The major constituents of milk include water, lactose, fats, protein, minerals, vitamins, and enzymes (Bath et al., 1978). Numerous methods of processing are performed to obtain different forms of milk products such as fermented milk, acidophilus milk, "Koomis", "Kefir", powdered milk, cream milk, butter, ghee and cheese (Fox et al., 2000). Rennet coagulated cheese represent the major milk product (75%), and calf rennet has been and still the most widely used milk-clotting enzyme preparation in cheese making industry (Ahmed et al., 2009). But, the high price of calf rennet and look for enough quantity of natural calf rennet (Ahmed et al., 2010).In addition to the restriction of the use of animal rennet due to religious reasons (e.g., Judaism and Islam), safety reasons (Bovine Spongiform Encephalopathy), and dietary reasons (vegetarianism, or being against genetically engineered foods). All the above reasons have demanded the search for a new enzyme with a high ratio of milk-clotting/ proteolytic activity and low preparation cost to be used as a rennet substitute and/or additive (Tajalsir et al., 2014).

Juices and extracts from fruits and plants have long been used as milk coagulants in many parts of Africa, Asia and the Carrebians. These include the extracts papain from *Carica papaya*, bromelin from pineapple, calotropain from *Calotropis procera*, cyprosin from *Cynara cardunculus* and latex of fig tree which grows abundantly in many parts of Africa. Moreover, cheese made by vegetable coagulants is normally produced on an artisan scale, in a farm house or small scale dairy production (Pontual et al., 2012).

Moringa oleifera (Moringaceae family) is the most widely cultivated species of a Monogeneric family, Moringaceae is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. In Sudan *Moringa oleifera* spread all over the country and used mainly as fences and for traditional water purification "Rawag tree". It also exists in Alcaro area (Khoar Eshkol) and in western desert Altmam area, in the desert area of the Red sea, Kassala, North Kordofan, Darfur, and Blue Nile (Hussein, 2009).

*Moringa oleifera*is rich in calcium, potassium and antioxidants (α and γ- tocopherol), and is used in human diet (Lo Piero et al., 2002). All parts of moringa tree are edible and have long been consumed by humans. *Moringa oleifera* seeds contain high levels of carbohydrates, protein, vitamin C, moderate amounts of B vitamins and dietary minerals (Uchikoba, 1996). Moringa seeds extracted oil - called the ben oil - rich in oleic acid, tocopherols and sterols. It can also withstand oxidative rancidity. The oil can be used in cooking as a substitute for olive oil, as perfumes and also for lubrication (Lalas and Tsaknis, 2002).The leaves of *Moringa oleifera* are rich in minerals like calcium, potassium, zinc, magnesium, iron and copper. Extracts from leaves are used to treat malnutrition. The moringa flowers are great sources of nectar and are used by beekeepers (Gopalakrishnan, et al., 2016).

Despite the wide spread and huge uses of *Moringa oleifera* its uses as a source of milk clotting enzyme is limited (Egito et al., 2007).Casinolytic and milk-clotting activities of extract from *Moringa oleifera* flowers has been used to precipitate milk protein in presence of ammonium sulphate (Pontual et al., 2012). In Sudan, few scientific reports were available in using moringa seeds extracts for milk clotting; however the potential utilization of other parts is still meager.

Materials and Methods

Preparation of *Moringa oleifera* samples

Moringa oleifera seeds, leaves and flowers were obtained from Salah Abdoon farms (Khartoum Bahry, Sudan), August 2016. The samples were carefully cleaned, manually decorticated, then coarsely powdered (60 mesh) using manual grinder and dried at 27± 2^{o}C, relative humidity of70±5%, for 7 days before use.

Figure 1 Preparation of *Moringa oleifera* samples (flowers, leaves and seeds)

Moringa extractions

Fifty grams of the above prepared sample (*M. oleifera* flowers, leaves and seeds) were suspended in 500 ml NaCl (0.15 M) and homogenized using a magnetic stirrer (4 h at 4^{o}C); the extract (clear supernatant) was treated by ammonium sulphate (60% saturation) flowed Green and Hughes (1955) method. The precipitated protein fractions (PP) were collected by centrifugation and kept in a deep freeze at -20^{o}C for two days, then transferred to freeze-dryer at -50^{o}C and at 0.16 Mb pressure for 48 h.

Assessment of Milk clotting time

Ten ml of fresh milk (pH 6.5) were placed in a 40 ml capacity beaker and the contents heated up to 40^{o}C using a constant temperature water bath. Then 2ml of extract solution was added. Curd formation was observed by manually rotating the beaker continuously so as to observe formation of a thin film on the milk surface. The end point was taken instantly when discrete milk particles appeared. A stopwatch was used to record the clotting time in seconds.

Determination of milk-clotting activity

The milk-clotting activity of *Moringa oleifera* extracts were determined following the procedure described by Pontual et al., (2012). The substrate (10% skim separately cow milk) was prepared in distilled water and pH was adjusted at 6.5. The milk (2.0ml) was incubated with 9.0 mg of protein at 37^{o}C, and curd formation was observed. The end point was recorded when the full separation between whey and curd was seen. One milk-clotting unit was defined as the amount of enzyme that clots 2 ml of the substrate (milk) within 180 sec. The milk clotting activity (MCA) of the extract was determined according to the following equation:

$$\text{MCA (U/ml)} = \frac{S \times 100}{CT \times E}$$

Where:
S: volume of milk.
CT: clotting time.
E: volume of enzyme.
100: dilution factor

Assessment of Casinolytic activity

Casinolytic activity was determined using azocasein (Sigma Aldrich, USA) as substrate, according to Azeez, et al., (2007). Three mg of protein and moringa parts was mixed with 300 µl of sodium phosphate (0.1 M)at pH 7.5 containing 0.6% (w/v) azocasein. The mixture was supplemented with 100 µl of Triton (X-100) 0.1% (v/v) and incubated at 37^{o}C for 3 h. The reaction was stopped by adding 200 µl of trichloroacetic acid10%(w/v), and after incubation (4^{o}C, 30 min) the mixture was centrifuged at 9,000 rpm for 10 min. The absorbance at 475 nm of the supernatant was determined. One unit of caseinolytic activity was defined as the amount of enzyme that promoted a 0.01 increase in absorbance, NaCl (0.15 M) used as control.

Stability of *Moringa Oleifera* extract

Powdered *Moringa oleifera* extracts were stored refrigerated (4°C) for month. Samples from each extract and control were tested every week for milk clotting time, casinolytic activity and milk clotting activity at different pH4.5, 5.5 and 6.5 and at different temperatures 24, 37 and 42°C.

Statistical analysis

One way ANOVA and two sample-paired Tests were performing to examine significant differences between normally distributed data. The effect of storage periods on stability of *Moringa oleifera* extracts was also measured by One Way ANOVA. Least Significance Difference (LSD) was used for mean separation between treatments. The level of significance ($P<0.05$) was used in this study.

Results

Milk clotting time

Table (1) demonstrated milk clotting time when moringa extracts were added. At four storage periods (1st, 2nd, 3rd and 4th week), the control sample (rennet) exhibited the faster milk clotting time ranging between 19.00 - 19.33 secwhen compared to that of moringa extracts. At the 2nd and 3rd storage periods *Moringa oleifera* seed extract recorded shorter milk clotting time25.33±0.58 sec and 25.67±0.58 sec respectively, compared to that obtained by extracts from leaves and flowers. At the end of the storage period (the last week) flowers extract recorded milk clotting time of 36.67±0.58 sec longer than other moringa extracts and rennet, the control.

Table (1) Milk clotting time (sec) using extracts from different parts of Moringa.

Storage Period (week)	Milk clotting time (sec)			
	Control	Seeds	Leaves	Flowers
1st	$19.00 \pm 1.00^{a}_{a}$	$25.33 \pm 0.58^{a}_{b}$	$30.33 \pm 0.58^{a}_{c}$	$34.33 \pm 0.58^{a}_{d}$
2nd	$19.33 \pm 0.58^{a}_{a}$	$25.67 \pm 0.58^{a}_{b}$	$30.33 \pm 0.58^{a}_{c}$	$34.33 \pm 0.58^{a}_{d}$
3 rd	$19.33 \pm 1.00^{a}_{a}$	$26.00 \pm 1.00^{b}_{b}$	$30.67 \pm 0.58^{a}_{c}$	$34.33 \pm 1.16^{a}_{d}$
4th	$19.33 \pm 1.16^{a}_{a}$	$27.33 \pm 0.58^{c}_{b}$	$32.33 \pm 0.58^{b}_{c}$	$36.67 \pm 0.58^{b}_{d}$

Values are mean ± SD

Means carrying the same superscript letter in the same column \raw are not significantly different at $p<0.05$ using DMRT

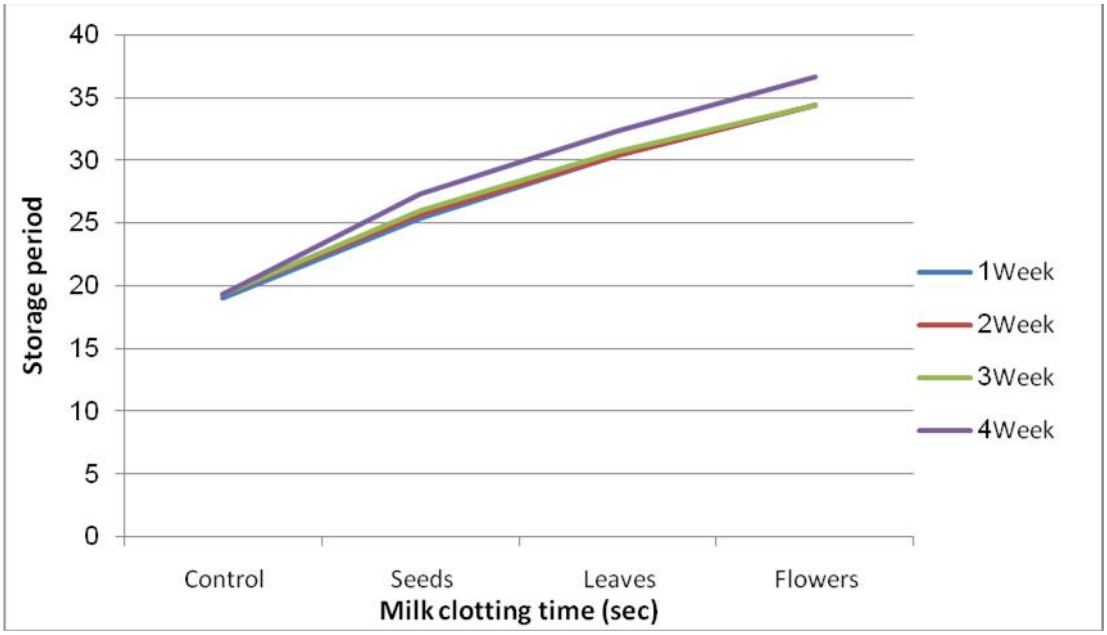


Figure (1) Milk clotting time (sec) using enzymes extracted from Moringa parts

Caseinolytic Activity at Storage Period of enzymes

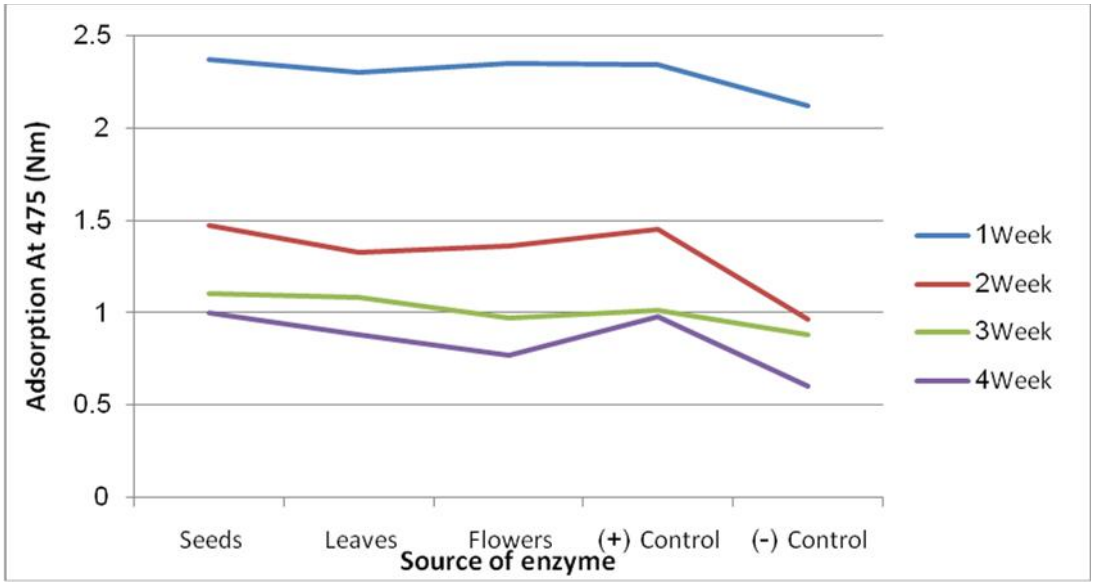
Results in Table (2) showed the caseinolytic activity at different storage periods of extracted moringa parts. One unit of caseinolytic activity on azocasein was defined as the amount of enzyme that give an increase of 0.01 in absorbance. Adsorption (at 475 Nm) of moringa extracts (seeds, leaves and flowers) and control exhibited insignificant difference ($p>0.05$) at the first week of storage period, but significant difference ($p<0.05$) was observed during the other three weeks of storage. Moringa seed extract recorded the highest value (2.37±0.00, 1.47±0.00, 1.10±0.00 and 1.00±0.00) throughout the storage periods. At the fourth week, the flowers extracts showed the lowest adsorption of 0.77±0.00.

Also Table (2) shows that concentration of enzyme (K x B) in the control (+) (rennet enzyme) is higher (39.81±0.00units /ml) than in other extracts (seeds, leaves, and flowers) at all storage periods. Flowers extract had higher concentration of (K x B) (38.00 ±0.00 units /ml) when compared to other moringa extractsat first week of storage, whereas, seeds extract recorded higher concentrations of (K x B) when compared to other moringa extracts at 2nd, 3rd and 4th storage periods.

Table (2) Caseinolytic Activity (unit of azocasein/ml) at Storage Period of Enzymes extracted from Moringa parts.

Parameters	Storage period (weeks)	Source of Moringa Extract				
		Control (+)	Control (-)	Seeds	Leaves	Flowers
Adsorption At 475 Nm	1 st	2.34 ±0.00 $^{b}_{a}$	2.12 ± 0.00 $^{e}_{b}$	2.37±0.00$^{a}_{a}$	2.30±0.00$^{c}_{a}$	2.35±0.00^{d} a
	2 nd	1.45± 0.00 $^{b}_{b}$	0.96 ±0.00 $^{e}_{d}$	1.47 ± 0.00$^{a}_{b}$	1.32± 0.00 $^{c}_{b}$	1.36±0.00$^{d}_{b}$
	3 rd	1.01± 0.00$^{b}_{c}$	0.88±0.00$^{e}_{d}$	1.10±0.00$^{a}_{c}$	1.08± 0.00 $^{c}_{c}$	0.97±0.00 $^{d}_{c}$
	4 th	0.98±0.00 $^{b}_{d}$	0.60±0.00 $^{e}_{d}$	1.00±0.00$^{a}_{d}$	0.88±0.00 $^{c}_{d}$	0.77±0.00$^{d}_{d}$
K x B (units /ml)	1 st	39.81±0.00$^{a}_{a}$	35.96±0.00$^{e}_{a}$	35.20 ±0.00 $^{b}_{a}$	34.68±0.00 $^{d}_{a}$	38.00±0.00$^{c}_{a}$
	2 nd	29.96± 0.00$^{a}_{b}$	23.38±0.00 $^{e}_{c}$	29.61±0.00$^{b}_{b}$	28.93±0.00$^{d}_{b}$	27.03±0.00$^{c}_{b}$
	3 rd	19.48± 0.00$^{a}_{c}$	13.11±0.00$^{e}_{d}$	19.18± 0.00$^{b}_{c}$	18.14±0.00$^{d}_{c}$	17.18± 0.00$_{c}$
	4 th	11.48± 0.00$^{a}_{d}$	7.12±0.00$^{e}_{e}$	11.18± 0.00 $^{b}_{d}$	10.14±0.00$^{d}_{d}$	9.18± 0.00 $^{c}_{e}$

Control (+): rennet enzyme
Control (-): sample blank
K x B: factor of concentration of enzyme
Values are mean ± SD; Means carrying the same superscript letter in the same column/ raw are not significantly different at p (0.05) using DMRT



Figure(2a) Caseinolytic Activity at Storage Period of Enzymes extracted from Moringa parts

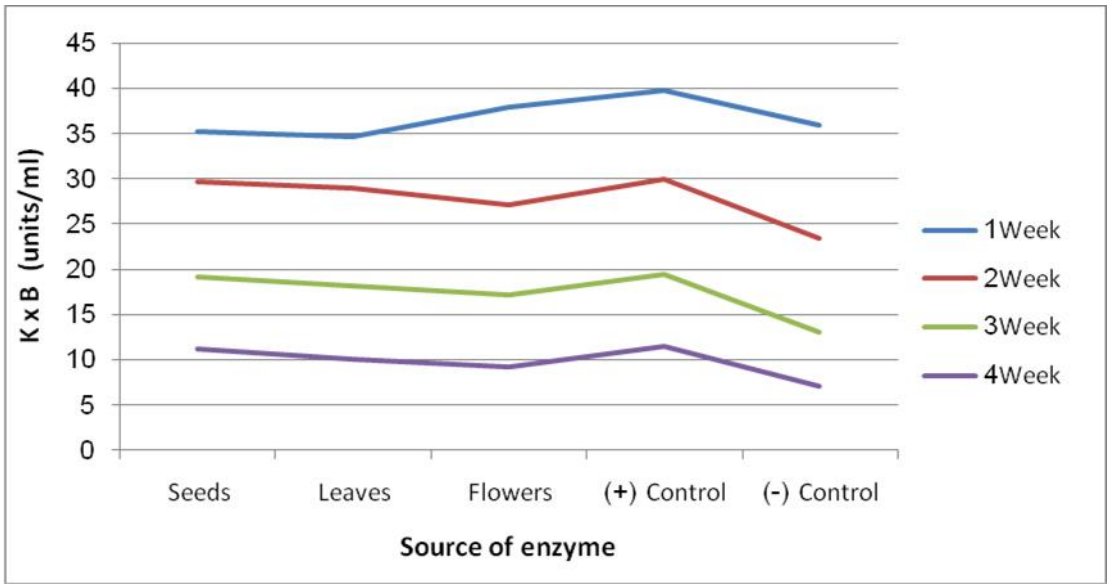


Figure (2b) Caseinolytic Activity at Storage Period of Enzymes extracted from Moringa parts

Stability of *Moringa oleifera* extracts and milk clotting activity

Milk-clotting activity was screened from various parts (seeds, leaves and flowers) of *Moringa oleifera* in order to use the part with the highest milk-clotting activity as a source of enzyme in cheese making. The results showed that milk-clotting activity was found in all parts of moringa with different values on it.

Effect of pH values on milk clotting activity

Table (3) shows the effect of different pH values (4.5, 5.5, and 6.5) on milk clotting activity of extracts from different parts of *Moringa oleifera* at different storage periods. At various pH levels and all storage periods control sample (rennet) recorded significantly higher milk clotting activity than *Moringa oleifera* extracts. Seeds extract resulted in higher milk clotting activity in comparison with other moringa extracts (flowers and leaves) which is significant ($p<0.05$) at pH 4.5 at 3rd and 4th storage periods and at pH 5.5 and 6.5 at all storage periods.

Also Table (3) showed that milk clotting activity is very high at pH (5.5) which is and suitable for white cheese production than other pH levels. In addition, milk clotting activity is significantly different ($p<0.05$) according to extract samples, but there is no significant different ($p>0.05$) due to storage period of one sample.

Table (3) Effect of pH values on milk clotting activity (u/ml)

Parameters	Storage period (weeks)	Source of extract			
		control	Seeds	Leaves	Flowers
M.C.A at pH (4.5)	1 st	$4.22 \pm 0.07^{a}{}_{a}$	$2.75 \pm 0.03^{a}{}_{b}$	$2.43 \pm 0.06^{a}{}_{b}$	$2.20 \pm 0.03^{a}{}_{b}$
	2 nd	$4.22 \pm 0.07^{a}{}_{a}$	$2.75 \pm 0.03^{a}{}_{b}$	$2.43 \pm 0.06^{a}{}_{b}$	$2.20 \pm 0.03^{a}{}_{b}$
	3 rd	$4.17 \pm 0.07^{a}{}_{a}$	$2.73 \pm 0.03^{a}{}_{b}$	$2.39 \pm 0.06^{a}{}_{c}$	$2.19 \pm 0.03^{a}{}_{c}$
	4 th	$4.17 \pm 0.07^{a}{}_{a}$	$2.73 \pm 0.03^{a}{}_{b}$	$2.39 \pm 0.06^{a}{}_{c}$	$2.19 \pm 0.03^{a}{}_{c}$
M.C.A at pH (5.5)	1 st	$4.38 \pm 0.10^{a}{}_{a}$	$3.66 \pm 0.07^{a}{}_{b}$	$3.14 \pm 0.05^{a}{}_{c}$	$2.75 \pm 0.03^{a}{}_{d}$
	2 nd	$4.38 \pm 0.10^{a}{}_{a}$	$3.66 \pm 0.07^{a}{}_{b}$	$3.14 \pm 0.05^{a}{}_{c}$	$2.75 \pm 0.03^{a}{}_{d}$
	3 rd	$4.33 \pm 0.10^{a}{}_{a}$	$3.62 \pm 0.07^{a}{}_{b}$	$3.14 \pm 0.05^{a}{}_{c}$	$2.73 \pm 0.03^{a}{}_{d}$
	4 th	$4.33 \pm 0.10^{a}{}_{a}$	$3.62 \pm 0.07^{a}{}_{b}$	$3.11 \pm 0.05^{a}{}_{c}$	$2.73 \pm 0.03^{a}{}_{d}$
M.C.A at pH (6.5)	1 st	$3.70 \pm 0.00^{a}{}_{a}$	$2.34 \pm 0.03^{a}{}_{b}$	$2.12 \pm 0.02^{a}{}_{c}$	$1.78 \pm 0.02^{a}{}_{d}$
	2 nd	$3.70 \pm 0.00^{a}{}_{a}$	$2.34 \pm 0.03^{a}{}_{b}$	$2.10 \pm 0.02^{a}{}_{c}$	$1.78 \pm 0.02^{a}{}_{d}$
	3 rd	$3.70 \pm 0.00^{a}{}_{a}$	$2.34 \pm 0.03^{a}{}_{b}$	$2.10 \pm 0.02^{a}{}_{c}$	$1.78 \pm 0.02^{a}{}_{d}$
	4th	$3.58 \pm 0.00^{a}{}_{a}$	$2.327 \pm 0.03^{a}{}_{b}$	$2.10 \pm 0.02^{a}{}_{c}$	$1.77 \pm 0.02^{a}{}_{d}$

M.C.A: Milk clotting Activity

Values are mean ± SD; Means carrying the same superscript letter in the same column/ raw are not significantly different at p ‹ 0.05 using DMRT

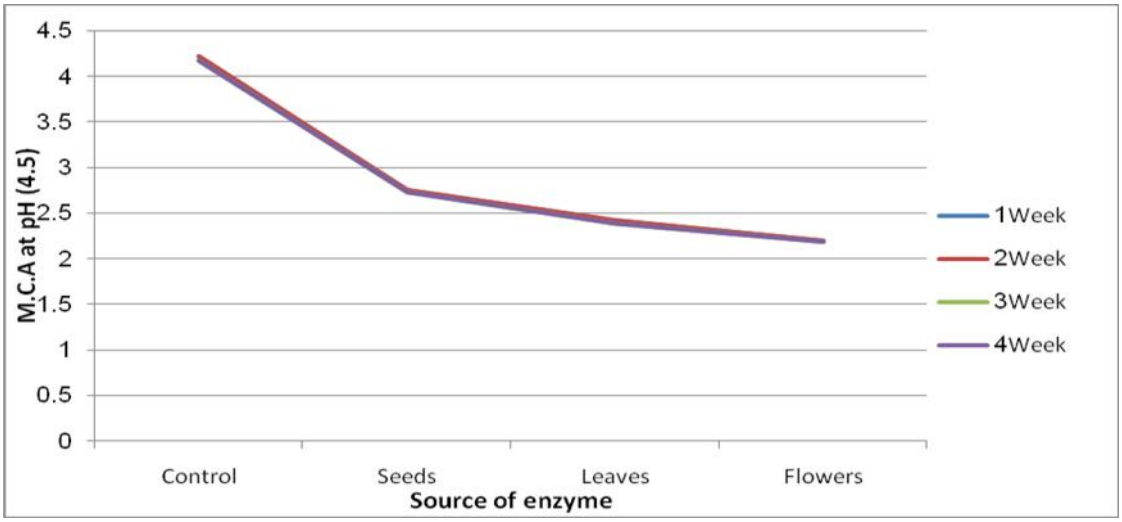


Figure (3a) Effect of pH values on milk clotting activity (u/ml)

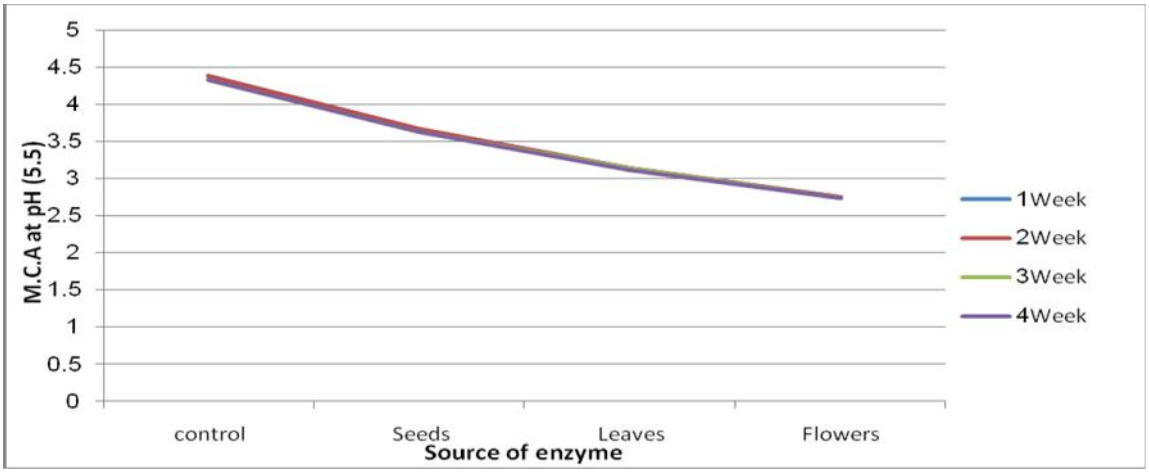


Figure (3b) Effect of pH values on milk clotting activity (u/ml)

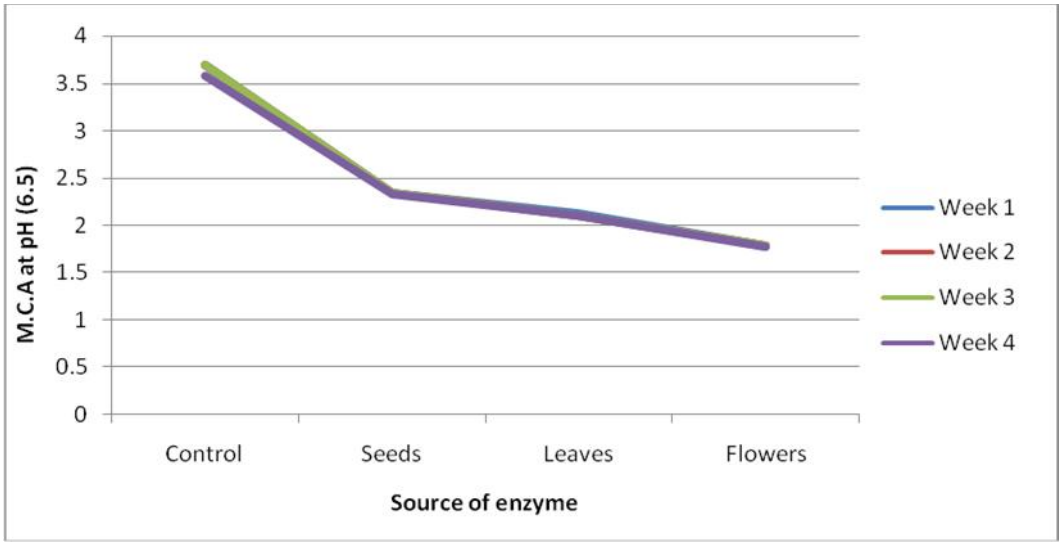


Figure (3c) Effect of pH values on milk clotting activity (u/ml)

Effect of temperature on the extract activity and stability

Milk-clotting activity of the partially purified extract was examined using skim milk as a substrate at different temperatures ranging from 24 to 37 to 42°C. The results showed that (Table 4) milk clotting activity of different kinds of extract increased when temperature increased from 24 to 37 to 42°C. Milk clotting activity of control sample is highest comparing to that of moringa extracts at various levels of temperature at all storage periods. Whereas moringa seeds extract had higher milk clotting activity than those of other moringa extracts at different levels of temperature at all storage periods. The results indicated that milk clotting activity at 42° C is significantly increased and is more suitable than other levels of temperature.

Table (4) Effect of temperature on milk clotting activity (u/ml)

Parameters	Storage period	Storage period			
		control	Seeds	Leaves	Flowers
M.C.A at (24° c)	1 st	$3.66 \pm 0.07^{a}_{a}$	$2.43 \pm 0.06^{a}_{b}$	$2.20 \pm 0.03^{a}_{b}$	$1.84 \pm 0.02^{a}_{c}$
	2 nd	$3.66 \pm 0.07^{a}_{a}$	$2.43 \pm 0.06^{a}_{b}$	$2.20 \pm 0.03^{a\,3}_{b}$	$1.83 \pm 0.02^{ab}_{c}$
	3 rd	$3.62 \pm 0.07^{a}_{a}$	$2.41 \pm 0.06^{a}_{b}$	$2.16 \pm 0.02^{ab}_{b}$	$1.81 \pm 0.02^{ab}_{c}$
	4 th	$3.62 \pm 0.07^{a}_{a}$	$2.41 \pm 0.06^{a}_{b}$	$2.14 \pm 0.02^{b}_{b}$	$1.81 \pm 0.02^{b}_{c}$
M.C.A at (37° c)	1 st	$4.38 \pm 0.10^{a}_{a}$	$3.14 \pm 0.05^{a}_{b}$	$2.75 \pm 0.03^{a}_{c}$	$2.43 \pm 0.06^{a}_{c}$
	2 nd	$4.33 \pm 0.10^{ab}_{a}$	$3.14 \pm 0.05^{a}_{b}$	$2.75 \pm 0.03^{a}_{c}$	$2.43 \pm 0.06^{a}_{c}$
	3 rd	$4.22 \pm 0.09^{ab}_{a}$	$3.06 \pm 0.03^{ab}_{b}$	$2.69 \pm 0.04^{ab}_{c}$	$2.39 \pm 0.06^{a}_{c}$
	4 th	$4.17 \pm 0.09^{b}_{a}$	$3.05 \pm 0.03^{ab}_{b}$	$2.66 \pm 0.04^{b}_{c}$	$2.39 \pm 0.06^{a}_{c}$
M.C.A at (42° c)	1 st	$4.38 \pm 0.10^{a}_{a}$	$3.80 \pm 0.09^{a}_{b}$	$3.14 \pm 0.05^{a}_{c}$	$2.75 \pm 0.04^{a}_{d}$
	2 nd	$4.38 \pm 0.10^{a}_{a}$	$3.75 \pm 0.09^{ab}_{b}$	$3.14 \pm 0.05^{a}_{c}$	$2.75 \pm 0.04^{a}_{d}$
	3 rd	$4.33 \pm 0.10^{a}_{a}$	$3.66 \pm 0.07^{ab}_{b}$	$3.11 \pm 0.05^{a}_{c}$	$2.73 \pm 0.04^{a}_{d}$
	4 th	$4.33 \pm 0.10^{a}_{a}$	$3.62 \pm 0.07^{b}_{b}$	$3.11 \pm 0.05^{a}_{c}$	$2.73 \pm 0.04^{a}_{d}$

M.C.A: Milk clotting Activity

Values are mean ± SD; Means carrying the same superscript letter in the same column/ raw are not significantly different at p ‹ 0.05 using DMRT

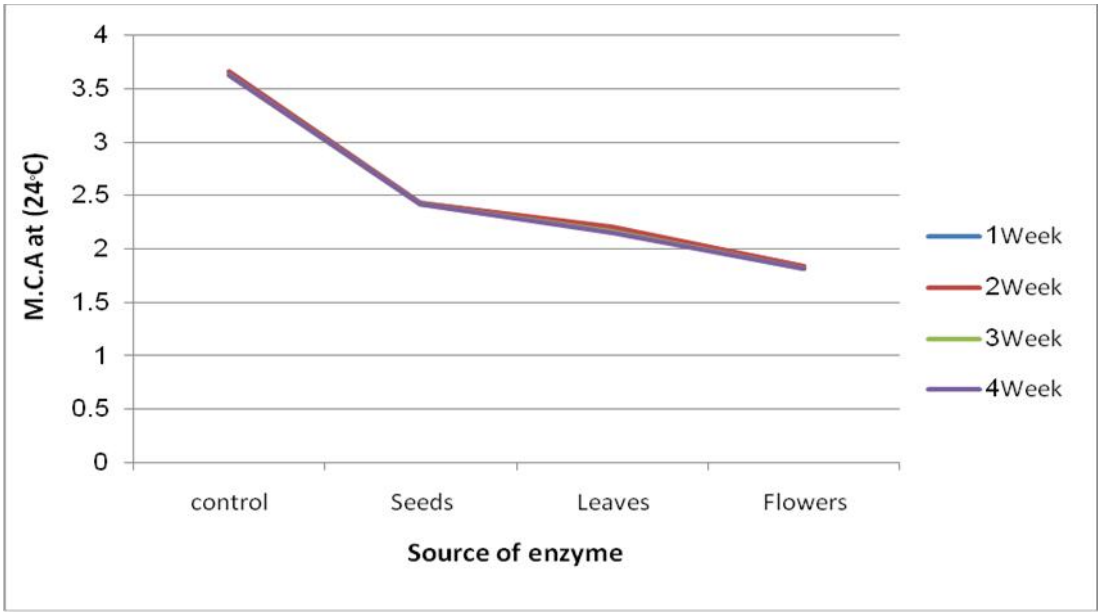


Figure (4a) Effect of temperature on milk clotting activity (u/ml)

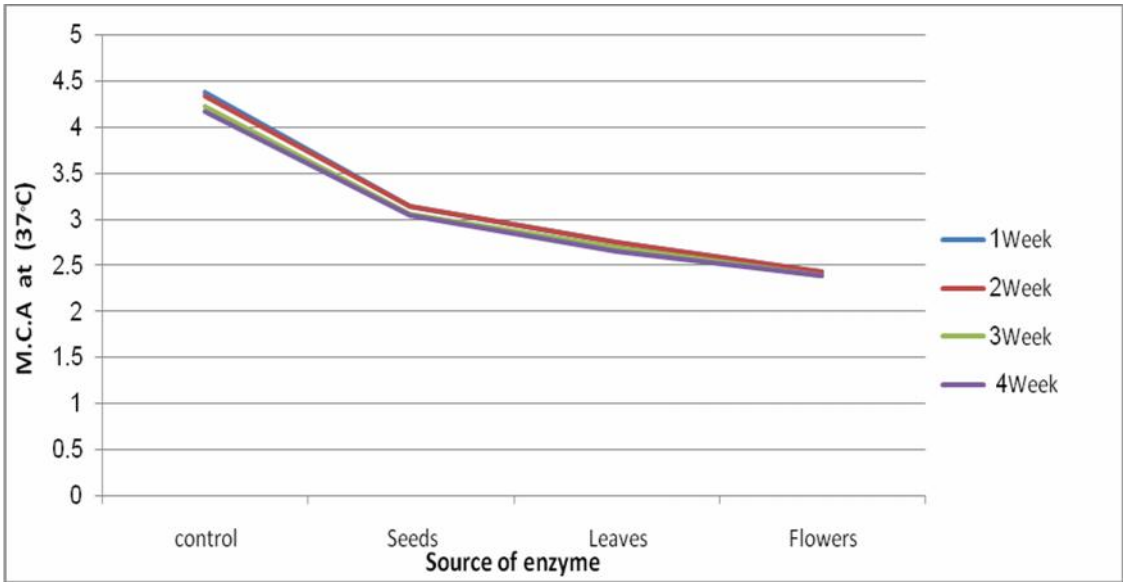


Figure (4b) Effect of temperature on milk clotting activity (u/ml)

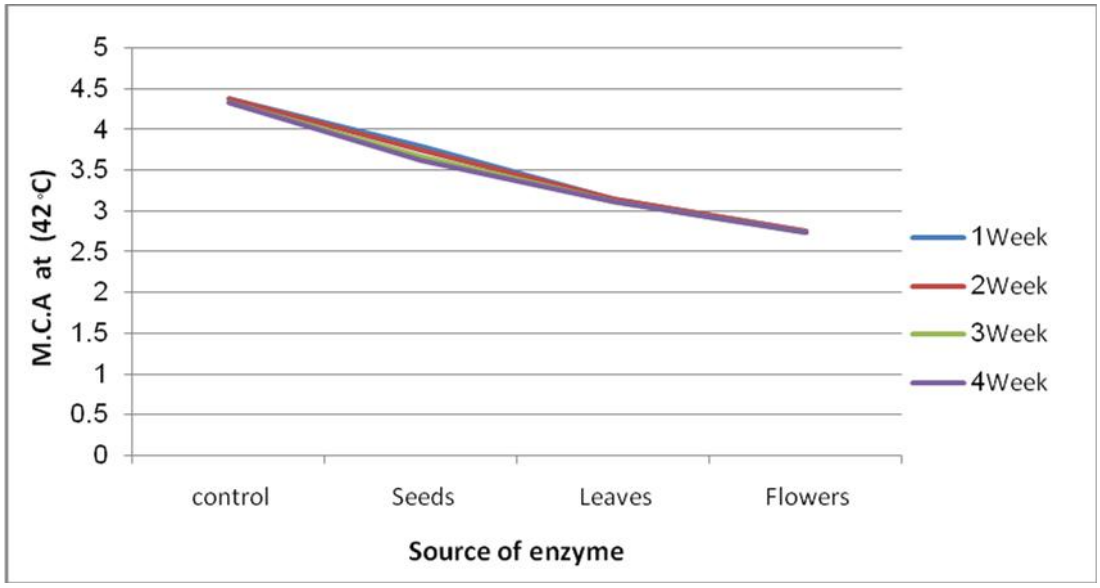


Figure (4c) Effect of temperature on milk clotting activity (u/ml)

Discussion

Tables (1) and (2) exhibited that moringa seeds extract record shorter milk clotting time and higher values of adsorption at 475 Nm which means higher caseinolytic activitythan other moringa extracts (flowers and leaves) at different storage periods (1st,2nd, 3rd and 4th). Also, table (3) showed higher milk clotting activity of moringa seeds extract comparing to other moringa extracts (flowers and leaves) at various pH levels (4.5, 5.5, and 6.5) at all storage periods. These findings are in line with the findings reported by Muñoz et al., (2017)who declared that moringa seeds extract resulted in higher milk clotting activity and higher caseinolytic activity than moringa flowers extract. Also current results are assured by Tajalsir et al., (2014)who stated the higher milk clotting activity of moringa seeds extract against moringa flowers and leaves extracts. Furthermore, Table (3) showed that milk clotting activity is very high at pH (5.5) and which is suitable for white cheese production than other pH levels. Pontual et al., (2012) disagree these results, researchers detected highest milk clotting activity of moringa flowers extract at pH 3.0 and decreased according to increase in pH.

Table (4) showed that milk clotting activity of all extracts is significantly increased at 42° C indicating that it is more suitable temperature for preparation of white cheese. These results are opposite to those observed by Pontual et al., (2012) who mentioned at 30 to 40° C moringa flowers extract didn't show any milk clotting activity and increased at 50° C. This disagreement may be due to different plant source as mentioned by Mazorra-Manzano et al., (2013) who reported that temperature profile of the milk-clotting enzyme from plant extracts depends on several factors such as the plant source, tissue, concentration and type of protease.

Conclusion

It was concluded that *Moringa oleifera* seeds extract can be used as a substitute for rennet extract to produce white cheese because it is less expensive and has high nutritional value than other coagulant to manufacture white cheese.

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