

Phytochemical Screening and Antioxidant Properties of coagulants and Soft cheese Produced
from Goat milk using Different Biocoagulants of Plant Origin

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Abstract

The nutritional importance and easy accessibility of soft cheese has made it indispensable. This study assesses the phytochemical constituents and the effects of antioxidant of biocoagulants used in soft cheese produced from goat milk. Different biocoagulants such as *Calotropis procera*, *Carica papaya*, lemon juice and steep water from cereals (maize, millet, and sorghum) were used. The results of the antioxidant properties revealed that *Carica papaya* had the highest ferric reducing property and displayed better DPPH scavenging activity of 14.94 mg AAE/g and 10.82%, respectively when compared with other biocoagulants also the results of phytochemical screening revealed that cheese coagulated with *Carica papaya* displayed highest DPPH scavenging activity (1.93%) when compared with other cheese samples. Cheese coagulated with lemon juice had the highest phenol content (19.88 mgGAE/100g) and also displayed the highest ferric reducing property (10.31mg AAE/g). Cheese coagulated with steep water from millet had the highest flavonoid content (0.20 mgGAE/100g) and cheese coagulated

with *Calotropis procera* had the highest alkaloid content (13.42 (mgGAE/100 g). Therefore cheese produced from goat milk coagulated with *Carica papaya* or lemon juice should be incorporated into the daily diet because of its high phenolic content which can improve the health status of the consumers. It also possesses some natural antioxidant compounds, which can effectively scavenge free radicals.

Keywords: Phytochemical, antioxidants, soft cheese, goat milk

1. Introduction

Soft cheeses are usually made from raw milk gotten from animal husbandry. They varied widely in nutritional composition, depending on whether they belong to the ripened or unripened varieties as well as the biocoagulants used. Soft cheese (Wara) is an unripened cheese consumed in several parts of **Western** Africa. Goat often referred to as the “poor man’s cow” due to his great contribution to the health and nutrition of the landless and rural people (Dresch, 1988) produces milk differs from cow or human milk in having better digestibility, alkalinity and buffering capacity (Park, 1994). It contains vitamins, minerals, trace elements, electrolytes, enzymes, proteins, and fatty acids that are easily assimilated by the body. Therefore awareness about advantage of consumption of goats milk should be popularized so that production and utilization of goat’s milk could be enhanced (Kumar *et al.*, 2012). Coagulating agents has been greatly used in the coagulation of milk for the production of cheese (Chikpah *et al.*, 2014). Conventionally, soft cheese is prepared by coagulating the fresh milk with the leaf extract of Sodom apple (*Calotropis procera*). Other coagulants such as lemon juice and *Carica papaya* can also be used. Medicinal plants such as *Calotropis procera* and

Carica papaya are the oldest form of healthcare known to mankind and have been used for centuries as remedies for human and animal diseases as they contain phytochemicals of therapeutic value (Ali, 2015). These medicinal plants have many pharmacological effects such as antimicrobial, anti-inflammatory, analgesic, anticancer, anti-angiogenic, immunological, antidiabetic, cardiovascular, gastroprotective, hepatic protective, renal protective, antidiarrheal, antioxidant, anticonvulsant as well as enhancement of wound healing. Rennet is a coagulant of animal origin which served as alternative for the coagulation of raw milk from cow for soft cheese production. The use of rennet is being frowned at due to the high cost, some vegetarians abhor it and also the issue of religion (Judaism and Islam). However, if *Calotropis procera* plant should go into extinction due to unfavorable weather, or there is higher demand because of its wide range of usefulness without adequate replacement, there is going to be a compulsion on cheese makers to source for alternative coagulants. It is therefore necessary to source for other coagulants of plant origin which are cheaper, easily accessible and add value to the final cheese product as well as other animal milk sources that can produce adequate and quality milk for soft cheese production.

2. Materials and Methods

2.1 Sampling

The raw milk sample was collected from Goats at Aba Baba Medinat, a Fulani farm settlement along Afao road, Ado-Ekiti, Nigeria. It was collected aseptically and subsequently transferred to the laboratory for analysis using an ice pack.

2.2 Collection of coagulants

The leaves of *Carica papaya* and *Calotropis procera* were collected from Erifun community around The Federal Polytechnic, Ado-Ekiti, Nigeria. Authentications of the Plants were done at the Department of Plant Science and Biotechnology, Ekiti State University, Ado-Ekiti, Nigeria. The voucher specimens of UHAE 2018/022 for *Carica papaya* and UHAE 2018/023 for *Calotropis procera* have been deposited at the University herbarium. Other biocoagulants like lemon fruits were purchased from Oba market, a local market in Ado Ekiti Metropolis, Nigeria, West Africa. Steep water from maize, sorghum and millet were produced by soaking the well sorted and washed grains in water for 3 days and then milled, the milled grain were later steeped again for 2 days and the effluent was collected and preserved in the refrigerator at 4°C to be used as biocoagulant.

2.3 Production of soft cheese

Raw milk (1000 mL) from goat were heated slowly for approximately 45-50°C for 30-40 minutes. The milk was stirred gently during the heating process with a wooden spoon. 4 mL of the leaf extract of *Calotropis procera*, *Carica papaya*, lemon juice, steep water from different grains were added separately to the warm milk (1000 mL) and the mixture was heated with intermittent stirring to about 95°C and was kept at this temperature until coagulation was achieved and the heating was stopped after the separation of curd and whey. The sign of coagulation was observed within 10-15 minutes from the time that the coagulants were added. It was transferred into a small, previously sterilized, raffia basket to facilitate whey drainage of the cheese and its characteristic shape, when the cheese was firm enough it was removed from

the rafia basket and placed inside a covered plastic container and kept inside the refrigerator (4°C) for analysis.

2.4 Phytochemical screening of soft cheese sample

2.4.1 Quantitative phytochemical screening

A. Determination of total phenolic content

The samples (100 mg) were weighed accurately and dissolved in 100 mL of triple distilled water (TDW). This solution (1 mL) was transferred to a test tube, then 0.5 mL of the Folin Ciocalteu's reagent (2 mol/L) and 1.5 mL of Na₂CO₃ (20%) solution was added, and ultimately, the volume was made up to 8 mL with TDW followed by vigorous shaking and finally allowed to stand for 2 h after which the absorbance was taken at 765 nm. Gallic acid was used as a standard positive control and was plotted at 0.02, 0.04, 0.06, 0.08, and 0.10 mg/100g gallic acid that was prepared in 80% (v/v) methanol. The total phenol content in the sample was calculated from the standard curve and the results expressed as gallic acid equivalent per 100 g dry weight of the (mgGAE/100 g) sample (Singleton *et al.*, 1999).

B. Determination of total Flavonoids

The modified method of Meda *et al.* (2005) which is based on the formation of the flavonoids – aluminium complex which has a maximum absorptivity at 415 nm was employed. 100 µL of the solubilized wara sample in methanol (10 mg/mL) was mixed with 100 µL of (20 %) aluminum trichloride in methanol and a drop of acetic acid, and then diluted with methanol to 5 mL. The absorption at 415 nm was read after 40 min. Blank samples were prepared from 100

mL of solubilized cheese sample and a drop of acetic acid, and then diluted to 5 mL with methanol. The absorption of standard routine solution (0.5 mg/mL) in methanol was measured under the same conditions.

C. Determination of total Alkaloids

This was done by the alkaline precipitation gravimetric method described by Mboso *et al.* (2013). 5 g of the soft cheese sample was weighed into a 250 mL beaker and dispersed in 200 mL of 10% acetic acid solution in ethanol to form a ratio of 1:10 (10%). The mixture was allowed to stand for 4 h at 28°C. It was later filtered via Whatman No. 42 grade of filter paper. The filtrate was concentrated in a water bath (100°C) to one quarter of its original volume by evaporation and treated with drop wise addition of conc. aqueous NH₄OH until the alkaloid was precipitated. The precipitate was collected on a pre- weighed filter paper, washed with 1% ammonia solution. It was then oven-dried at 80°C to a constant weight. The percentage yield of alkaloid was calculated from the weights of precipitate and that of the original sample. All determinations were carried out in triplicates

2.5 Determination of antioxidant capacity

The antioxidants tests were carried out on the samples as listed below:

A. Determination of Ferric Reducing Antioxidant Power Assay (FRAP)

The reducing power of the samples was determined by assessing the ability of the soft cheese sample to reduce FeCl₃ solution according to the method described Oyaizu (1986). A 2.5 mL aliquot of each solubilized cheese samples was mixed with 2.5 mL of 200 mM sodium

phosphate buffer (pH 6.6) and 2.5 mL 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min, and then 2.5 mL 10% trichloroacetic acid was added. This mixture was centrifuged at 650 rpm for 10 min. The supernatant was collected and 5 mL aliquot of each solubilized sample was mixed with an equal volume of water and 1 mL 0.1% ferric chloride. The absorbance was measured at 700nm. The ferric reducing antioxidant property of soft cheese sample was subsequently calculated using ascorbic acid as a standard.

B. Scavenging effect on 1, 1 -diphenyl-2-picrylhydrazyl (DPPH)

The DPPH free radical scavenging ability of the sample was determined using modified methods of Gyamfi *et al.* (1999). Soft cheese samples of different concentrations (1.0 mL) were mixed with 5.0 mL of 0.4 mM DPPH in methanol. The mixture was incubated at room temperature for 30 min in dark. The control contains only DPPH solution in methanol instead of sample while methanol served as the blank. Absorbance was noted at 517 nm by using UV-visible spectrophotometer. The capacity of scavenging free radicals was calculated as:

$$\text{Scavenging activity (\%)} = [A_b - (A_s - A_{bs})] / A_b \times 100$$

Where, A_b is absorbance of blank, A_{bs} is the absorbance of sample + blank, and A_s is the absorbance of sample.

Sample concentration causing 50% inhibition (IC_{50}) was calculated from the graph, plotting the % inhibition against sample concentration.

2.6 Statistical Analysis

Statistical analyses were carried out and data were obtained using SPSS program (Statistical Package for social Sciences version 16). Significant differences between means were calculated by one-way Analysis of Variance (ANOVA) using Duncan's multiple range test (DMRT) in order to evaluate differences among means.

3. Results and Discussions

3.1. Quantitative phytochemical composition of cheese produced from goat milk using different biocoagulants..

The quantitative phytochemical composition of cheese produced from goat milk using different biocoagulants are presented in table 1. Cheese coagulated with lemon juice had the highest phenol content. Cheese coagulated with steep water from millet had the highest flavonoids content and cheese coagulated with *Calotropis procera* had the highest alkaloids content. While, the lowest phenol content (15.39 mgGAE/100 g) was found for the goat milk coagulated with *Calotropis procera*; flavonoid content (0.06 mgGAE/100 g) was observed for goat milk coagulated with lemon juice, and concerning the alkaloids, the lowest content (7.64 mgGAE/100 g) was for the goat milk coagulated with millet steep water.

Table 1. Quantitative phytochemical composition (mgGAE/100 g)* of soft cheese produced from goat milk using different **biocoagulants**

Cheese samples	PHENOLS	FLAVONOIDS	ALKALOIDS
GCPR	15.39± 0.14 ^f	0.16±0.01 ^a	13.42±0.01 ^a
GCP	17.18± 0.16 ^d	0.08 ±0.01 ^c	9.23±0.01 ^e
GLJ	19.88±0.01 ^a	0.06±0.01 ^d	9.78± 0.01 ^d
GSO	17.87±0.02 ^c	0.09 ±0.00 ^c	10.82± 0.00 ^c
GMI	16.35±0.09 ^e	0.20±0.01 ^a	7.64±0.01 ^f
GMA	18.57± 0.02 ^b	0.09± 0.01 ^c	11.83± 0.02 ^b

KEY: **GSO** – goat milk coagulated with steep water from sorghum, **GMA** - goat milk coagulated with steep water from maize, **GMI** - goat milk coagulated with steep water from millet, **GLJ** - goat milk coagulated with lemon juice, **GCPR** - goat milk coagulated with *Calotropis procera*, **GCP** - goat milk coagulated with *Carica papaya*. *Values are means ± standard deviation (n=3). Means with different letters within a column are significantly different. ($P < 0.05$)

3.2 FRAP assay

The FRAP of the biocoagulants and soft cheese produced from goat milk using different biocoagulants is presented in Figures 1 and 2, respectively. The values obtained for FRAP ranged from 1.43 to 14.94mg AAE/g for biocoagulants and from 7.74 to 10.31mg AAE/g for the soft cheese samples.

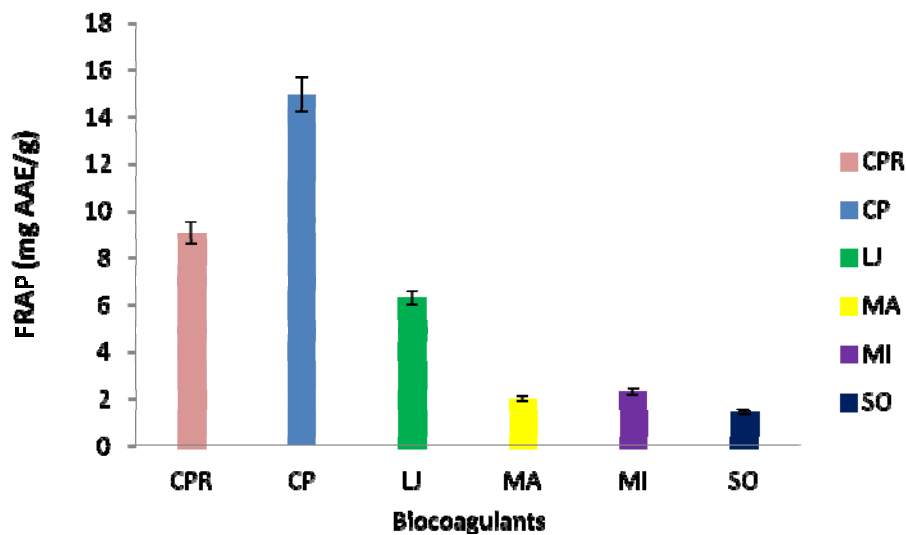


Fig 1: Ferric reducing antioxidant power of biocoagulants

Key: SO- steep water from sorghum, MA- steep water from maize, MI- steep water from millet, LJ- lemon juice, CPR- *Calotropis procera* extract, CP- *Carica papaya* extract.

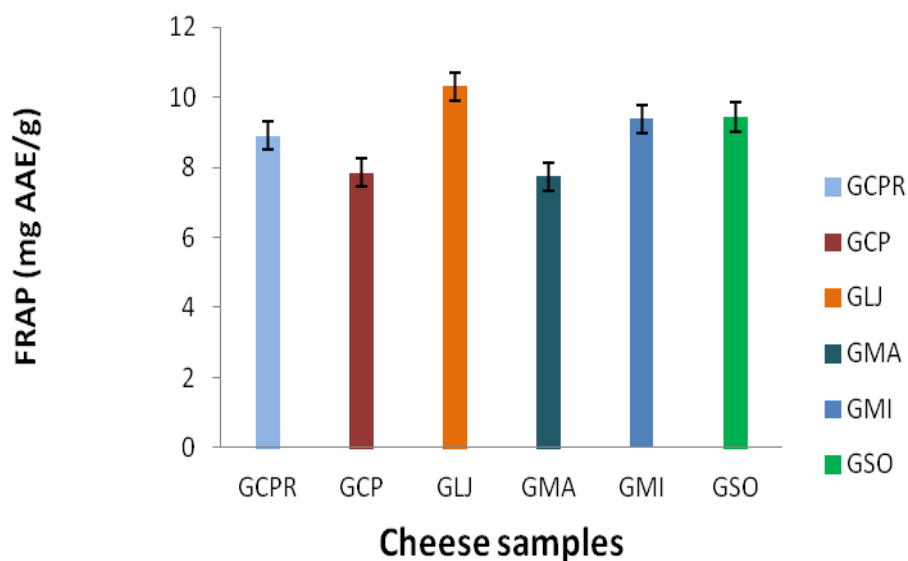


Fig 2: Ferric reducing antioxidant power of local cheese produced from goat

KEY: **GSO** – goat milk coagulated with steep water from sorghum, **GMA** - goat milk coagulated with steep water from maize, **GMI** - goat milk coagulated with steep water from millet, **GLJ** - goat milk coagulated with lemon juice, **GCPR** - goat milk coagulated with *Calotropis procera*, **GCP** - goat milk coagulated with *Carica papaya*. Values are means \pm standard deviation (n=3). Means with different letters within a column are significantly different. ($P < 0.05$)

3.4 DPPH scavenging activity

The scavenging activity of the biocoagulants and soft cheese samples against DPPH free radicals are presented in Figures 3 and 4, respectively. The scavenging activity of biocoagulants against DPPH ranged from 1.37 to 10.82%, while the results of soft cheese from goat milk ranged from 1.56 to 1.93%.

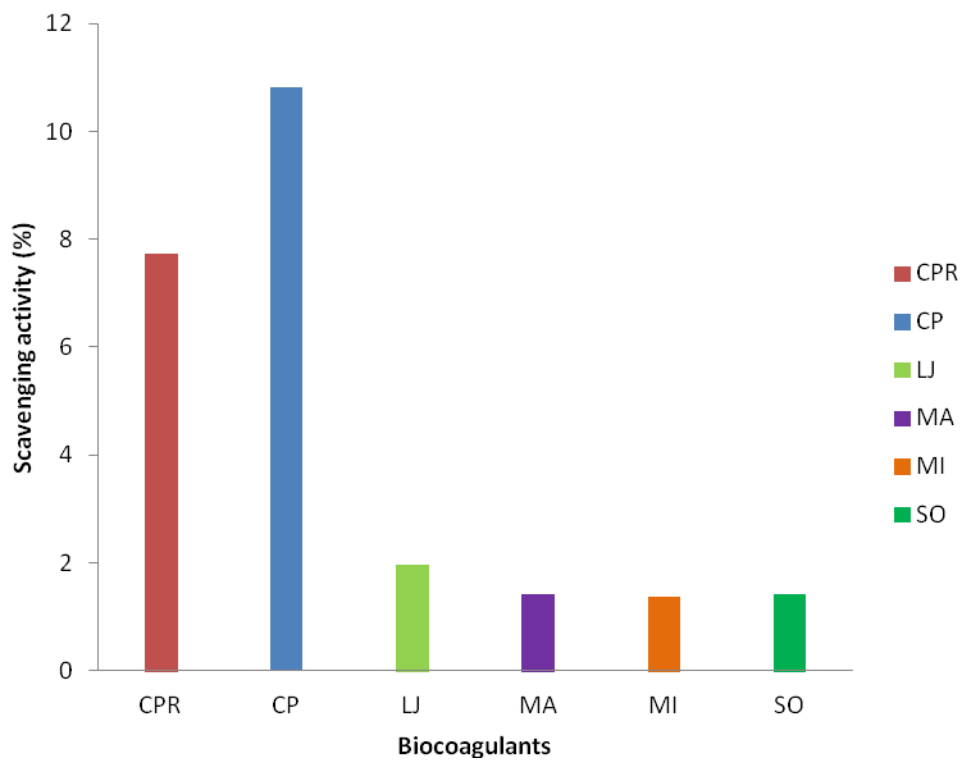


Fig 3: Scavenging activity of biocoagulants used in the production of local cheese on DPPH

free radical

Key: SO- steep water from sorghum, MA- steep water from maize, MI- steep water from millet, LJ- lemon juice, CPR- *Calotropis procera* extract, CP- *Carica papaya* extract.

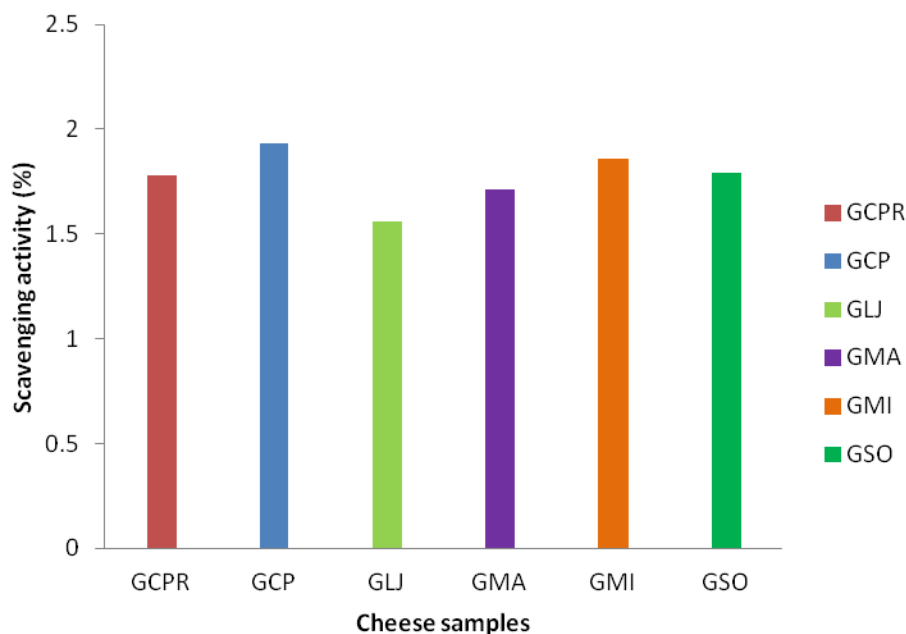


Fig 4: Scavenging activity of local cheese produced from goat milk using different biocoagulants

KEY: **GSO** – goat milk coagulated with steep water from sorghum, **GMA** - goat milk coagulated with steep water from maize, **GMI** - goat milk coagulated with steep water from millet, **GLJ** - goat milk coagulated with lemon juice, **GCPR** - goat milk coagulated with *Calotropis procera*, **GCP** - goat milk coagulated with *Carica papaya*.

The presence of phenols indicates that the cheese may be able to achieve multiple activities such as antioxidant, anticarcinogenic and anti-inflammatory (Asha *et al.*, 2011). Alkaloids are the most significant compounds that play a metabolic role in the living systems and are involved in the protective function in animals. Pure isolated alkaloids and the synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bacterial properties (Stray, 1998). Flavonoids have been used against the cancer causing tumors

and it inhibits the promotion of growth and progression of tumors (Stevens *et al.*, 1992). Phenols and phenolic compounds have been extensively used in disinfection and remains the standard with which other bactericides are compared (Akinyeye *et al.*, 2014). These phytochemicals and phenolic compounds have been shown to contribute immensely to the medicinal and nutritional quality of plant and plant products (Doughari, 2012). It has been reported that intake of these bioactive constituents to an extent has protective and therapeutic effects essential to preventing diseases and maintaining a state of wellbeing (Boyer and Liu, 2004)

The amount of phytochemicals found in the cheese samples was quantitatively determined by standard procedures (Table 1). The presence of the phytochemical constituents, such as alkaloids, flavonoids, phenols in the cheese samples makes it a good source of beneficial bioactive compound that can improve the health status of the consumers. Flavonoids can function as direct antioxidants and free radical scavengers, and have the capacity to modulate enzymatic activities and inhibit cell proliferation (Duthie and Crozier, 2000). High phenolic content recorded in this work is similar to the work of Oboh (2006) who also reported high phenolic content in sheep milk. The highest alkaloids recorded in soft cheese produced from *Calotropis procera* might be due to the fact that *Calotropis procera* contains bioactive compounds such as alkaloids, which might have been introduced into the cheese sample during processing (Ali, 2015), thereby making the final cheese product highly nutritive. The use of biocoagulants such as *Calotropis procera* has been encouraged because it is readily available, cheaper, and safe for consumption as against the use of rennet which is very costly.

Extract from *Carica papaya* (14.94mg AAE/g) and soft cheese produced with lemon juice (10.31mg AAE/g sample) showed the highest ferric reducing property when compared with others ($p < 0.05$). It has been reported that the antioxidant activity of plant material was well correlated with the content of their phenolic compounds (Velioglu *et al.*, 1998, Eze and Airouyuwa, 2014). The ability of the biocoagulants and the soft cheese to scavenge DPPH radicals is presented in Figures 3 and 4, respectively. *Carica papaya* displayed better DPPH scavenging activity (10.82%) when compared with other biocoagulants (1.37%, 1.41%, 1.42%, 1.97%, 7.72%) while soft cheese coagulated with *Carica papaya* displayed better DPPH scavenging activity (1.93%) when compared with other cheese samples (1.56%, 1.71%, 1.78%, 1.79%, 1.86%). Coagulants such as *Carica papaya* have been reported to be a rich antioxidants source due to the presence of phenolic group and carotenoids which can scavenge free radicals (Aravind *et al.*, 2013 and Usman *et al.*, 2012). The results showed that the soft cheese and the biocoagulants possess natural antioxidant compounds, which can effectively scavenge free radicals.

4. Conclusions

The results of phytochemical screening revealed that the soft cheese produced from goat milk using different biocoagulants contain appreciable amount of phenols, alkaloids, and flavonoids. Goat milk coagulated with lemon juice presented the highest phenol content; cheese coagulated with millet steep water had the highest flavonoid content, while *Calotropis procera* coagulated cheese had the highest alkaloid content. The addition of the biocoagulants increased the bioactive content of cheese samples. *Carica papaya* displayed better DPPH

scavenging activity when compared with other samples, and soft cheese produced from goat milk coagulated with *Carica papaya* was able to scavenge free radical better than other samples.

COMPETING INTERESTS:

Authors have declared that no competing interests exist.

ETHICAL APPROVAL:

Not applicable

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