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

Abstract

The nutritional importance and easy accessibility of soft cheese has made it indispensable. This study assesseds 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. Aalso, the results of phytochemical screening revealed that cheese coagulated with *Carica papaya* displayed the highest DPPH scavenging activity (1.93%) when compared with other cheese samples. Cheese coagulated with lemon juice had the highest phenol content (19.88 mg_GAE/100g) and also displayed the highest ferric reducing property (10.31 mg AAE/g). Cheese coagulated with steep water from millet had the highest flavonoid content (0.20 mg_GAE/100g) and cheese coagulated with *Calotropis procera* had the highest alkaloid content (13.42 (mg_GAE/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.

Comment [érdo1]: May be or could be

Keywords: Phytochemical, antioxidants, soft cheese, goat milk

1. Introduction

Soft cheeses are usually made from raw milk ~~gotten~~ obtained from animal husbandry. They vary~~ied~~ widely in nutritional composition, depending on whether they belong to the ripened or unripened varieties, as well as the biocoagulants used. Wara is a type of unripened s~~S~~oft cheese obtained from goats ~~(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 which 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 the advantages of consumption of goat's milk should be popularized so that production and utilization of goat's milk could be enhanced (Kumar *et al*., 2012).

Comment [érdo2]: Add reference to this sentence

-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

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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.

Comment [érdo3]: Reference

Comment [érdo4]: In this work, or in general?

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Comment [érdo7]: These sentences are badly written. Very difficult to understand. Where is th objective of the work?

2. Materials and Methods

2.1 Sampling

The raw milk sample was collected from ~~g~~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.

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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 sources of biocoagulants, such as ~~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 ^{o}C to be used as biocoagulant.

Comment [érdo8]: At WHAT TEMPERATURE THE STEEPING WAS CARRIED?

2.3 Production of soft cheese

Raw milk (1000 mL) from goat ~~were~~ was heated slowly ~~for approximately~~at 45 - 50 ^{o}C~~/~~ ~~for~~ 30 - 40 min~~utes~~. The milk was stirred gently during the heating process ~~with a wooden spoon~~. An aliquot of 4 mL of the leaf extract of *Calotropis procera* ~~and,~~ *Carica papaya*, lemon juice, and 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 ^{o}C and was kept at this temperature until coagulation was achieved. ~~and T~~the heating was stopped after the separation of curd and whey. The sign of coagulation was observed within 10 - 15 min~~utes~~ from the time that the coagulants were added. It was transferred into a small, previously sterilized, rafia basket to facilitate whey drainage of the cheese and allow the formation of its characteristic shape. ~~,~~ ~~W~~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~~refrigerated (4 ^{o}C) ~~for~~ until analysis.

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Comment [érdo9]: How did you prepare this extract

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 Na_2CO_3 (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. The calibration curve was built as ~~and was plotted at~~ 0.02, 0.04, 0.06, 0.08, and 0.10 mg gallic acid/100 g, ~~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 (GAE) per 100 g dry weight (d.b.) 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 cheese sample in methanol (10 mg/mL) was mixed with 100 µL of ~~(20%)~~ aluminum trichloride in methanol (20%) 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

Comment [érdo10]: How?

Comment [érdo11]: Again, Meda et al. did not proposed this methodology. The authors cite this methodology as an adptation made by Arvouet-Grand, Vennat, Pourrat, and Legret (1994).

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 proposed by Harbone (1973) and later described by Mboso *et al*. (2013). ~~5 g of the soft~~Soft cheese sample (5 g) 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 paper 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 NH_4OH 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.

Comment [érdo12]: You should not copy other works. This text is exactly the as of Mboso *et al.* (2013). If you did not modify the methodology, only cite the reference.

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Comment [érdo13]: Harbone JB (**1973**). Phytochemical methods: A guide to Modern technique of plant analysis. Chapman and Hall, London, 279

Comment [érdo14]:

Comment [érdo15]: How the results are supposed to be presented (units)?

Comment [érdo16]: This statement should be clear for all analysis. State it only once at the statistical analysis topic.

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 $FeCl_3$ solution according to the method described Oyaizu (1986), with modifications. A 2.5 mL aliquot of each solubilized cheese samples was mixed with 2.5 mL of

Comment [érdo17]: Please, replace this citation by Benzie and Strain (1996) - The Ferric Reducing Ability of Plasma (FRAP) as a Measure of ‘‘Antioxidant Power’’: The FRAP Assay

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200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min, and then 2.5 mL of 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 of 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.

Comment [érdo18]: How the results are supposed to be presented? What does AAE mean? Make it clear here.

B. DPPH free radical scavenging effect

The DPPH (1, 1 -diphenyl-2-picrylhydrazyl) free radical scavenging ability of the sample was determined using the 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:

Comment [érdo19]: Your methodology is farther different from Gyamfi *et al*. (1999). Which methodology have you followed in order to perform your work?

Comment [érdo20]: I suggest: The DPPH (2,2-difenil-1-picrilhidrazil) free radical-scavenging capacity was estimated using the method of Blois (1958), later modified by Brand-Williams *et al.* (1995). Their methodology are similar to yours. Make sure you state that there are modifications.

Comment [érdo21]: What do you mean by different concentrations of the sample? Are these the extracts? How were they prepared?

Scavenging activity (%) = [Ab - (As-Abs)] / Ab x 100

Where, Ab is absorbance of blank, Abs is the absorbance of sample + blank, and As 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

Comment [érdo22]: State how many times the experiment was carried, and how many replicates did you use to carry analysis. What statistical design have you used? Was it A completely randomized design?

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 mg_GAE/100 g) was found for the goat milk coagulated with *Calotropis procera*; flavonoid content (0.06 mg_GAE/100 g) was observed for goat milk coagulated with lemon juice, and concerning the alkaloids, the lowest content (7.64 mg GAE/100 g) was for the goat milk coagulated with millet steep water.

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

Comment [érdo23]: Is this unit valid for all three analysis (phenols, flavonoids, alkaloids), you should make it clear in the methodology. You only presented the units for phenols.

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 14.94 mg AAE/g for biocoagulants and from 7.74 to 10.31 mg AAE/g for the soft cheese samples.

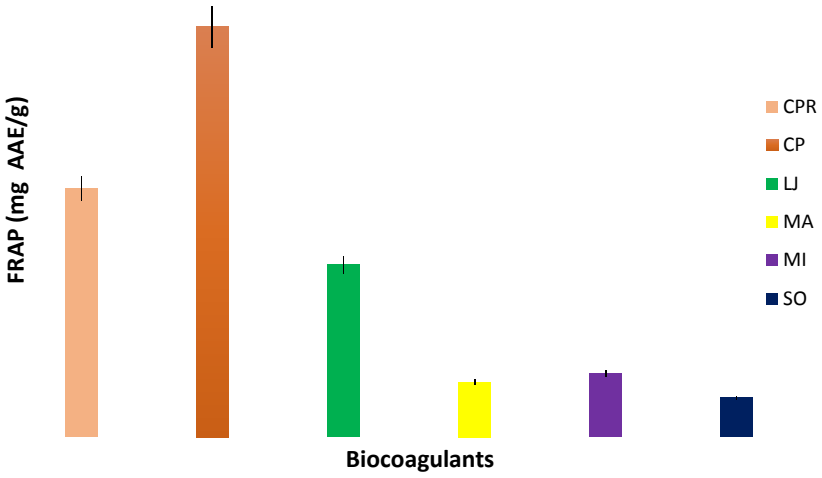

Fig 1: Ferric reducing antioxidant power of biocoagulants (

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

Comment [érdo24]: Again, the lateral legend is not necessary, as the initial are already indicated in the axis of the graph.

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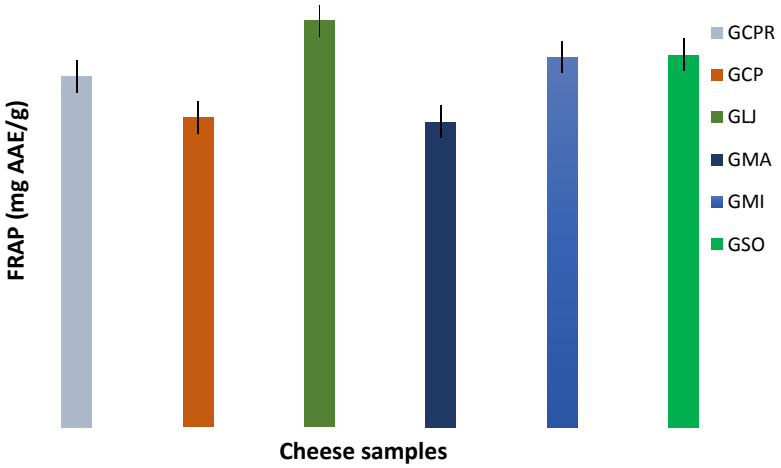

Fig 2: Ferric reducing antioxidant power of local cheese produced from goat milk using different biocoagulants (

~~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*). ~~Values are means ± standard deviation (n=3). Means with different letters within a column are significantly different. (P < 0.05)~~

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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%.

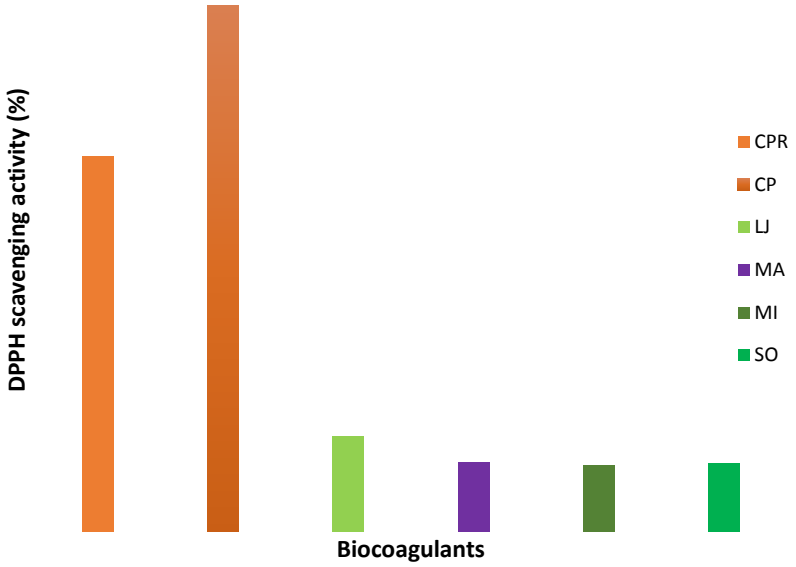

Fig 3: Scavenging activity of biocoagulants used in the production of local cheese on DPPH free radical (

~~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).

Comment [érdo25]: Please, present the standard deviation in the graphic as done for the other figures.

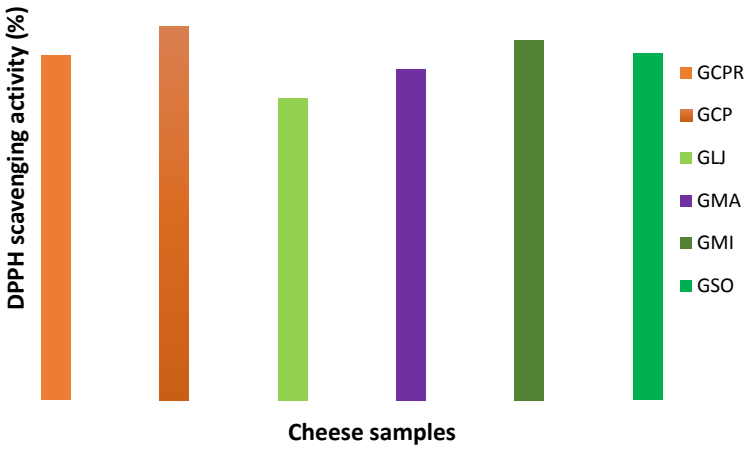

Fig 4: -Scavenging activity of local cheese produced from goat milk using different biocoagulants on DPPH free radical (~~KEY:~~ GSO – goat milk coagulated with steep water from sorghum, GMA - goat milk coagulated with steep water from maize, GMI - goat milk coagulated with steep water from millet, GLJ - goat milk coagulated with lemon juice, GCPR - goat milk coagulated with *Calotropis procera*, GCP - goat milk coagulated with *Carica papaya*).

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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.

Comment [érdo27]: How similar? Present their results.

Extract from *Carica papaya* (14.94_mg AAE/g) and soft cheese produced with lemon juice (10.31_mg 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

ACKNOWLEDGEMENTS:

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