Original Research Article

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

Abstract

This study was carried out to assess the phytochemical constituents and antioxidant properties of coagulants and 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 phytochemical screening revealed that flavonoids, alkaloids, phenols and reducing sugars are present in all the cheese samples while saponins, tannins and cardiac glycosides were absent. However, steroids and glycosides are present only in cheese coagulated with steep water from millet while terpenoids are present only in cheese coagulated with steep water from maize. Cheese coagulated with lemon juice had the highest phenol content (19.88mg/g) while cheese coagulated with steep water from millet and *Calotropis procera* had the highest flavonoids (0.20mg/g) and alkaloids content (13.42mg/g). The result of the antioxidant properties revealed that *Carica papaya* had the highest ferric reducing property and displayed better DPPH scavenging activity (14.94mg GAE/g extract and 10.82% respectively when compared with other coagulants. Cheese coagulated with lemon juice displayed the highest ferric reducing property (10.31mg GAE/g sample) while cheese coagulated with *Carica papaya* displayed better DPPH scavenging activity (1.93%) when compared with other cheese samples. Cheese produced from goat milk coagulated with lemon

juice and *Carica papaya* may be incorporated into the daily diet because of its 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

Wara (soft cheese) is an unripened cheese consumed in several parts of West Africa. Conventionally, it 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 (Ali, 2015) and have been used for centuries as remedies for human and animal diseases as they contain phytochemicals of therapeutic value. They exerted many pharmacological effects such as antimicrobial, anti-inflammatory, analgesic, anticancer, anti-angiogenic, immunological, antidiabetic, cardiovascular, gastroprotective, hepatic protective, renal protective, antidiarrheal, antioxidant, anticonvulsant, enhancement of wound healing effect. The addition of rennet or coagulating agents has been greatly used in the coagulation of milk for the production of cheese (Chikpah *et al*., 2014).

Antioxidant compounds are able to donate electrons to reactive radicals, reducing them into more stable and unreactive species (Gulcin *et al.,* 2003). The reducing ability of a compound generally depends on the presence of reductants (Duh *et al*., 1999) which have been exhibiting antioxidative potential by breaking the free radical chain and donating a hydrogen atom (Gordon, 1990). Goat has been referred as the “poor man‘s cow’’ due to his great

contribution to the health and nutrition of the landless and rural poor (Dresch, 1988). Goat milk differs from cow or human milk in having better digestibility, alkalinity and buffering capacity (Park, 1994). Goat's milk contains vitamins, minerals, trace elements, electrolytes, enzymes, proteins, and fatty acids that are easily assimilated by the body.

Goat's milk has a similarity to human milk that is unmatched in cow milk and also has several medicinal values. 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). However, the different coagulants used which are of plant origin might have impacted their constituents into the soft cheese. It is imperative to carry out phytochemical and antioxidant properties of coagulants and the soft cheese produced from goat milk. Therefore, the objective of this study is to determine the phytochemical and antioxidant properties of coagulants and soft cheese produced from goat milk.

2. Materials and Methods

2.1 Collection of Milk

The raw milk sample was collected from sheep 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.

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. Authetication 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 (effluent from pap produced from maize, sorghum, millet) were produced by steeping the grains in water for 3days after which it was milled and later steeped again for 2 days in the laboratory. The steep water was then collected for use as biocoagulants.

2.3 Production of West African cheese

The milk was stirred gently during the heating process with a wooden spoon. About 4mls of the leaf extract of *Calotropis procera, Carica papaya,* lemon juice, steep water were added to the warm milk and the mixture was heated for the second time with intermittent stirring to about 45-50^{o}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 the range of 10-15 min. It was transferred into a small previously sterilized rafia basket to facilitate whey drainage and characteristic shape, when the cheese was firm enough it was removed from the rafia basket and place inside a covered plastic container for analysis.

2.4 Phytochemical screening of soft cheese sample

Basic Phytochemical analyses were carried out to determine the bioactive compounds present in the sample.

2.4.1 Preparation of samples

Two grams (2g) of cheese sample was carefully weighed into 250mL conical flask and 50mL of distilled water was added to the sample. It was mixed and stoppered with rubber band and then placed in water bath for 2hrs at 37^{o}C, after which it was removed to cool. The content was filtered with the use of Whattman filter paper No 1 and the filtrate was kept for analysis.

2.4.2 Test for Tannins

The dried cheese sample were stirred in distilled water and filtered. Ferric chloride (0.1%) reagent was added to the filtrate. A blue black or blue green precipitate was taken as preliminary evidence for presence of tannin (Trease and Evans, 2004).

2.4.3 Test for Alkaloids

Soft cheese sample (0.5g) was added to 5mL of 10% (v/v) HCl in test tubes and put in a water bath for 2mins, after which the mixture was filtered. The filterate (1mL) was treated with 3 drops of Dragendrof's reagent in order to separate portions. The presence of alkaloids was confirmed by the production of reddish brown colouration (Trease and Evans, 2004).

2.4.4 Test for steroids

Two millimeters (2mL) of acetic anhydride was added to 0.5g of each cheese sample with addition of 2mL of H_2SO_4. A colour change from violet to blue or green indicates the presence of steroids (Trease and Evans, 2004).

2.4.5 Test for Saponins

The ability of saponins to produce frothing in aqueous solution was used as screening test for the saponins. The cheese sample (O.5g) was boiled with distilled water in a water bath and shaken vigorously for stable persistent froth. The frothing was mixed with three drops of olive oil and shaken vigorously for the formation of emulsion (Sofowora, 1993).

2.4.6 Test for Flavonoids

Ten mililitre of ethyl acetate was heated with the sample in a water bath for thirty minutes. The mixture was filtered and 4ml of each filterate was shaken with one mililitre (1mL) of dilute ammonia solution in a conical flask. A yellow colouration indicates the presence of flavonoids (Harborne, 1998).

2.4.7 Test for Cardiac glycosides

A. Legal test: The sample was dissolved in pyridine and few drops of 20% sodium nitro preside together with few drops of 20% sodium hydroxide (NaOH) were added. A colour change from violet to blue to green indicates the presence of glycosides (Trease and Evans, 2004).

B. Lieberman's test: Two mililitre of Acetic Anhydride was used to dissolve 0.2g of the food sample. The mixture was cooled in ice. Sulphuric acid was then carefully added. A colour change from violet to blue to green indicates the presence of a steroidal nucleus i.e aglycone portion of cardiac glycosides (Trease and Evans, 2004).

2.4.8 Terpenoid test

Two mililitre (2mL) of chloroform was used to dissolve 0.2g of the sample. Sulphuric acid was carefully added which form a lower layer. A reddish brown colour at the interface indicates the presence of terpenoid.

2.5 Quantitative phytochemical screening

A. Determination of total phenolic content

The extract of the sample (100mg) was weighed accurately and dissolved in 100 mL of triple distilled water (TDW). This solution (1mL) was transferred to a test tube, then 0.5 mL 2N of the Folin Ciocalteu reagent and 1.5 mL 20% of Na_2CO_3 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 hours after which the absorbance was taken at 765 nm. These data were used to estimate the total phenolic content using a standard calibration curve obtained from various diluted concentrations of garlic acid (Singleton *et al*., 1999).

B. Determination of total Flavonoids

The method is based on the formation of the flavonoids – aluminum complex which has an absorptivity maximum at 415nm. 100µL of the cheese 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 5mL. The absorption at 415 nm was read after 40 minutes. Blank samples were prepared from 100 mL of plant extracts and a drop of acetic acid, and then diluted to 5ml with methanol. The absorption of standard ruotine solution (0.5 mg/mL) in

137 methanol was measured under the same conditions. All determinations were carried out in
138 triplicates (Meda *et al*., 2005).

139 C. Determination of total Alkaloids

140 Soft cheese sample (5g) was weighed into a 250 mL beaker and 200 mL of 10% acetic
141 acid in ethanol was added and covered and allowed to stand for 4 h. This was filtered and the
142 extract was concentrated on a water bath to one-quarter of the original volume. Concentrated
143 ammonium hydroxide was added drop wise to the extract until the precipitation was complete.
144 The whole solution was allowed to settle and the precipitate was collected and washed with
145 dilute ammonium hydroxide and then filtered. The residue is the alkaloid, which was dried and
146 weighed (Harborne, 1998).

147 2.6 Determination of antioxidant activity

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

149 A. Ferric Reducing Antioxidant Power Assay (FRAP)

150 The reducing power of the samples was determined by assessing the ability of the soft
151 cheese sample to reduce $FeCl_3$ solution as described according to the method described by Kong *et*
152 *al*. (2012). A 2.5 mL aliquot of each cheese sample was mixed with 2.5 mL of 200 mM sodium
153 phosphate buffer (pH 6.6) and 2.5 mL 1% potassium ferricyanide. The mixture was incubated at
154 50^{o}C for 20 min, then 2.5 mL 10% trichloroacetic acid was added. This mixture was centrifuged
155 at 650 rpm for 10 min. The supernatants were collected and 5mL aliquot of each sample was
156 mixed with an equal volume of water and 1mL 0.1% ferric chloride. The absorbance was

measured at 700nm. The ferric reducing antioxidant property of soft cheese sample was estimated as mg garlic acid equivalent (GAE)/ g sample in triplicate.

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

The free radical scavenging ability of the sample on DPPH was determined using the methods of Gyamfi *et al*. (1999). Soft cheese samples of different concentrations were mixed with 1.0 ml of 0.4 mM DPPH in methanol (5.0 mL). 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:

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

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

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

2.7 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 multiple range test (DMRT) was used to separate means (Omotosho *et al*., 2011).

3.0 Results

3.1 Qualitative phytochemical properties of biocoagulants used for the production of soft cheese.

Table 1 shows the Qualitative Phytochemical properties of soft cheese produced from goat milk using different biocoagulants and it was observed that flavonoids, alkaloids, phenols and reducing sugar were present in all the cheese samples produced from goat milk coagulated with all the six coagulants while saponins, tannins and cardiac glycosides were absent in all the samples analysed. Terpenoids, steroids and glycosides were present only in cheese samples coagulated with steep water from maize, millet and millet respectively.

Table 1: Qualitative phytochemical screening of soft cheese produced from goat milk using different coagulants.

Samples	Saponins	Flav.	Tann	Alk	Terp.	Ster.	Phenols	Gly.	C.Gly	R.S
GCPr	-	+	-	+	-	-	+	-	-	+
GCP	-	+	-	+	-	-	+	-	-	+
GLJ	-	+	-	+	-	-	+	-	-	+
GSO	-	+	-	+	-	-	+	-	-	+
GMI	-	+	-	+	-	+	+	+	-	+

GMA	-	+	-	+	+	-	+	-	-	+

Keys: **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 steep water from lemon juice, **GCPR -** goat milk coagulated with *Calotropis procera*, **GCP -** goat milk coagulated with *Carica papaya*. Tannins, Alkaloids, Terpenoids, Steroids, Flavonoids, Glycosides, Cardiac glycosides and Reducing sugars

3.2 Quantitative phytochemical properties of biocoagulants used for the production of wara.

Table 2 shows the Quantitative phytochemical screening of cheese produced from goat milk using different biocoagulants. Goat milk coagulated with lemon juice had the highest phenol value of 19.88mg/g, cheese coagulated with steep water from millet had the highest flavonoids value of 0.20mg/g and cheese coagulated with *Calotropis procera* had the highest alkaloids value of 13.42mg/g. while phenol had a lowest value of 15.39mg/g in the goat milk coagulated with *Calotropis procera*, flavonoid had a lowest value of 0.06mg/g in the goat milk coagulated with lemon juice and Alkaloids had a lowest value of 7.64mg/g in the goat milk coagulated with steep water from millet.

Table 2: Quantitative phytochemical screening of soft cheese produced from goat milk using different coagulants

SAMPLES	PHENOLS (mg/g)	FLAVONOIDS(mg/g)	ALKALOIDS (mg/g)

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

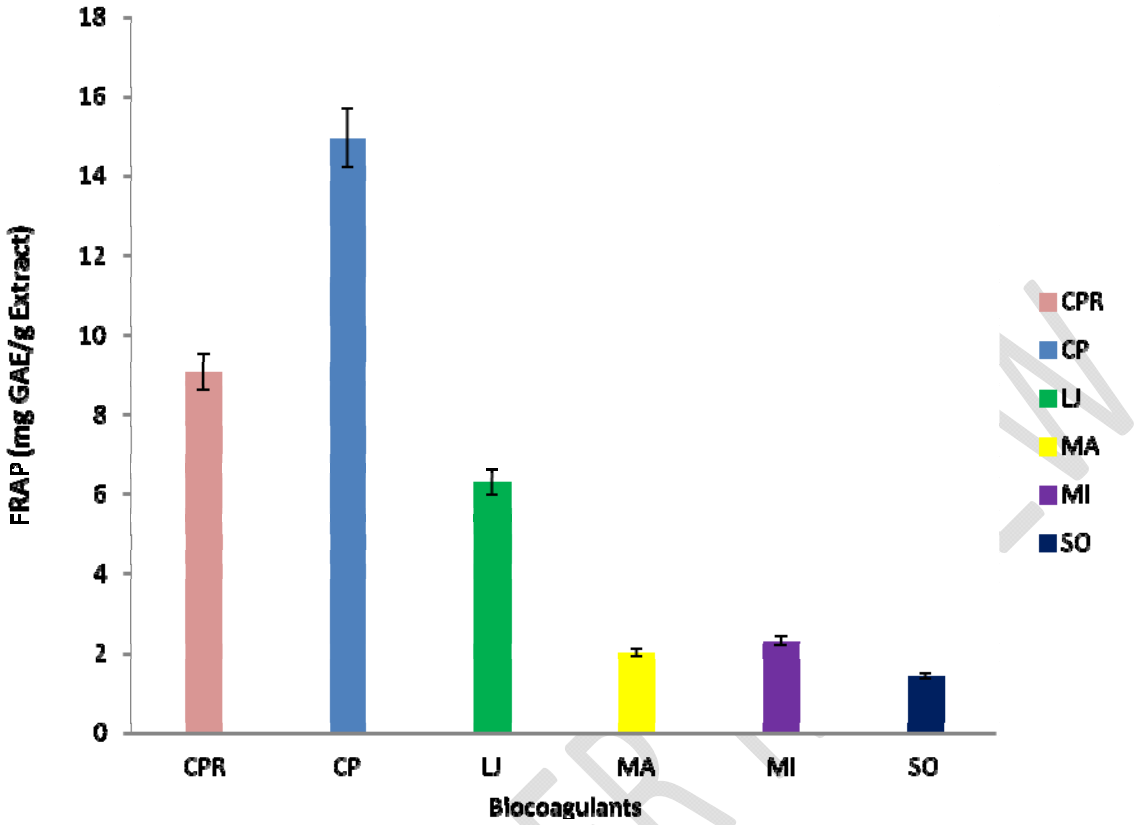
203

204 KEY: G- goat milk, SO- sorghum, MA- maize, MI- millet, LJ- lemon juice, CPR- *Calotropis procera,* CP-
205 *Carica papaya*. Values are means of replicate (n=3), means with different letters within a column
206 are significantly different. ($P<0.05$)

207

208 3.3 FRAP assay of the samples

209 The ferric-reducing antioxidant power (FRAP) of the biocoagulants and soft cheese produced
210 from goat milk using different biocoagulants is presented in Figures 1 and 2. The values
211 obtained for FRAP ranged from 1.43- 14.94mg GAE/g extract for biocoagulants and 7.74-
212 10.31mg Garlic Acid Equivalent/g (mg GAE/g sample) for the soft cheese sapmles respectively.



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214 **Fig 1: Ferric reducing antioxidant power of biocoagulants used in the production of local**

215 **cheese**

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217

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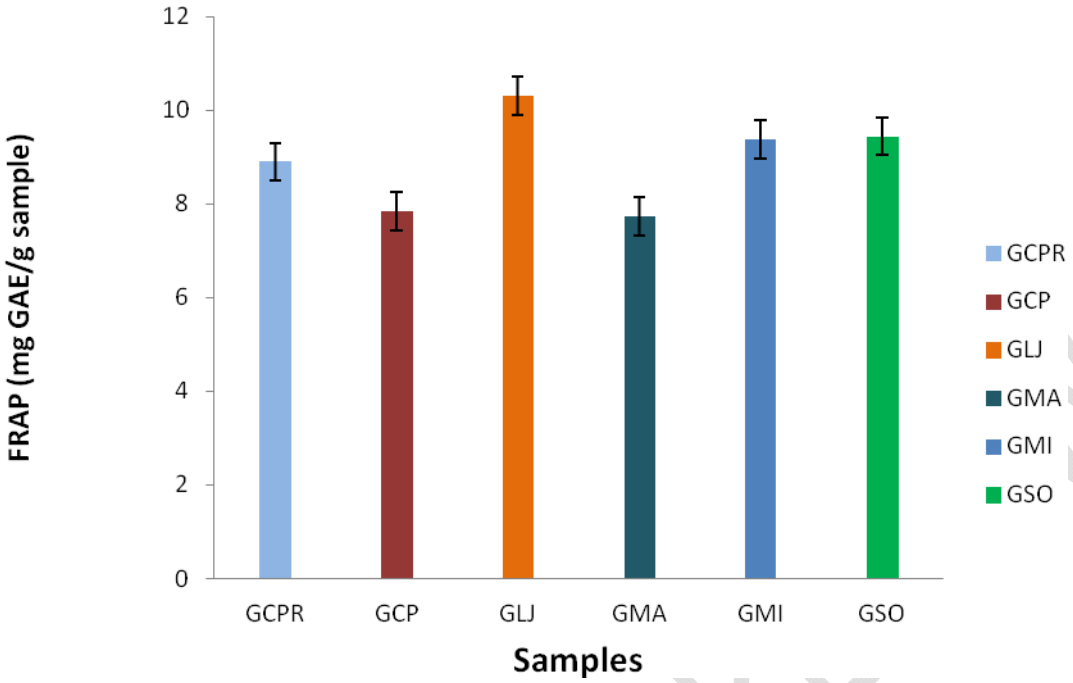

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220 Fig 2: Ferric reducing antioxidant power of local cheese produced from goat milk using

221 different Biocoagulants

222 3.4 DPPH scavenging activity of the samples

223 The scavenging activities of the biocoagulants and soft cheese samples against free radicals are

224 presented in Figures 3 and 4. The scavenging activity of biocoagulants against DPPH ranged

225 from 1.37 to 10.82% while the value of soft cheese from goat milk against free radicals ranged

226 1.56- 1.93%.

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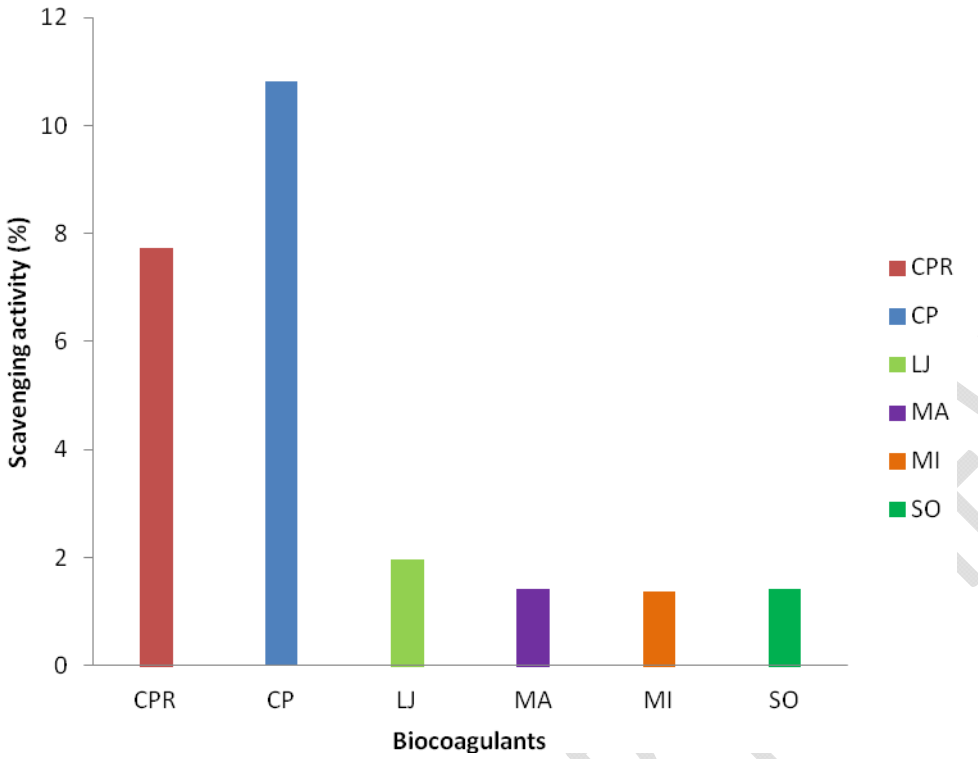

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229 Fig 3: Scavenging activity of biocoagulants used in the production of local cheese on

230 DPPH

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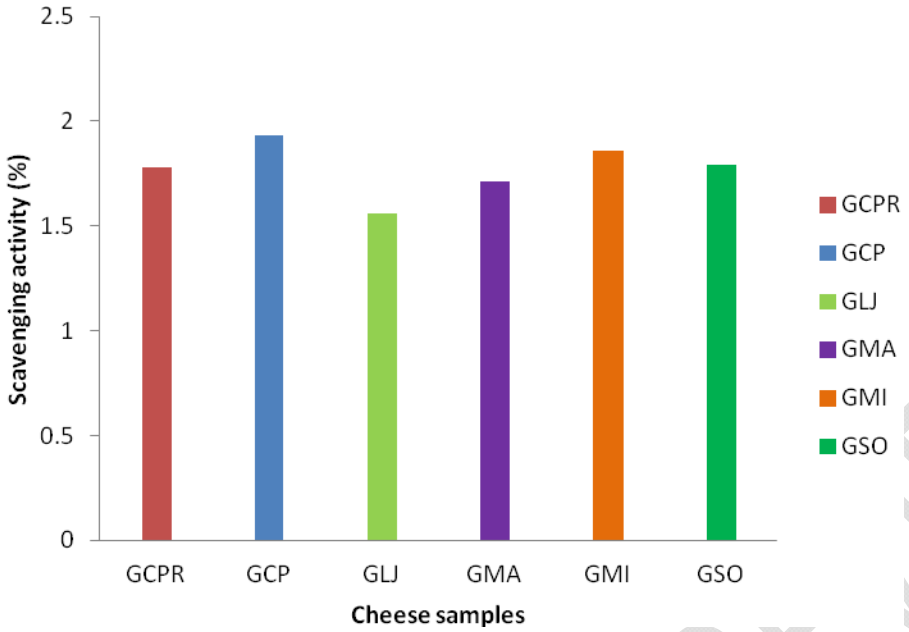

Fig 4: Scavenging activity of local cheese produced from goat milk using different biocoagulants on DPPH

4.0 Discussion

The result of the qualitative phytochemical screening of soft cheese produced from goat milk using different coagulants (Table 1) reveals that phenols, alkaloids, flavonoids and reducing sugar were present in all the cheese samples. However, saponins, tannins and cardiac glycosides were absent in all the samples while, terpenoids, steroids and glycosides were present in cheese coagulated with steep water from maize and millet. The presence of phenols indicates that the cheese may be able to achieve multiple activities like antioxidant, anticarcinogenic, anti-inflammatory e.t.c (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. Steroidal alkaloids are medicinally evolved. Alkaloids are the most efficient

245 therapeutically significant plant substance. Pure isolated alkaloids and the synthetic derivatives
246 are used as basic medicinal agents because of their analgesic, antispasmodic and bacterial
247 properties (Stray, 1998). Flavonoids have been used against the cancer causing tumors and it
248 inhibits the promotion of growth and progression of tumors (Stevens *et al*., 1992). Phenols and
249 phenolic compounds have been extensively used in disinfection and remains the standard with
250 which other bactericides are compared (Akinyeye *et al.,* 2014).

251 The presence of these phytochemical constituents such as Alkaloids, flavonoids, phenols
252 and reducing sugars makes the cheese a good source of beneficial bioactive compound that can
253 improve the health status of the consumers. Flavonoids can function as direct antioxidants and
254 free radical scavengers, and have the capacity to modulate enzymatic activities and inhibit cell
255 proliferation (Duthie and Crozier, 2000). The amount of phytochemicals found in the cheese
256 samples was quantitatively determined by standard procedures (Table 2). The highest phenol
257 content (19.88mg/g) was found in milk coagulated with lemon juice, the highest flavonoids
258 (0.20mg/g) was found in milk coagulated with steep water from millet and the highest alkaloids
259 (13.42mg/g) was found in milk coagulated with *Calotropis procera*. High phenolic content
260 recorded is similar to the work of Oboh (2006) who also reported high phenolic content in
261 sheep milk. The highest alkaloids recorded in soft cheese produced from *Calotropis procera*
262 might be due to the fact that *Calotropis procera* contain bioactive compound such as alkaloids
263 which might have been introduced into the cheese sample during processing (Ali, 2015).

264 The value obtained for FRAP (Figures 1 and 2) ranged from 1.43-14.94 for biocoagulants
265 and 7.74-10.31mg Garlic Acid Equivalent/g sample (mg GAE/g sample) for soft cheese produced

from goat milk. Extract from *Carica papaya* (14.94mg GAE/g extract) and soft cheese produced with lemon juice (10.31mg GAE/g sample) showed the highest ferric reducing property when compared with others at $p<0.005$. 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). The ability of the biocoagulants and the soft cheese samples to scavenge for DPPH radicals is presented in Figures 3 and 4. Soft cheese samples and the biocoagulants used for soft cheese processing displayed concentration dependent DPPH scavenging activity. In this study, the scavenging activity of biocoagulants used for soft cheese processing ranged from 1.37-10.82% while that of soft cheese samples ranged from 1.56-1.93%. *Carica papaya* displayed better DPPH scavenging activity (10.82%) when compared with other coagulants (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 samples (1.56%, 1.71%, 1.78%, 1.79%, 1.86%). Coagulants such as *Carica papaya* have been reported to be a rich source of antioxidants due to the presence of phenolic group and carotenoids in them which can scavenge free radicals (Aravind *et al*., 2013 and Usman *et al*., 2012). The results show that the soft cheese and the biocoagulants possess some natural antioxidant compounds, which can effectively scavenge free radicals.

5.0 Conclusions

The results of qualitative phytochemical screening reveals that the soft cheese produced from goat milk using different biocoagulants contain phenols, alkaloids, flavonoids and reducing sugar. While saponins, tannins, and cardiac glycosides were absent in all the cheese samples.

Goat milk coagulated with lemon juice has the highest phenol content; steep water from millet coagulated cheese has the highest flavonoid content while *Calotropis procera* coagulated cheese has the highest alkaloids content. The addition of the biocoagulants increases the bioactive content of the cheese sample. The results of the antioxidant properties of the biocoagulants revealed that *Carica papaya* displayed better DPPH scavenging activity when compared with other samples and soft cheese produced from goat milk coagulated with *Carica papaya* can scavenge free radical better than other samples.

Conflicts of interest: We declare that we have no conflicts of interest.

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