

**Phytochemical Screening and Antioxidant Properties of Coagulants and Soft  
cheese Produced from Goat milk using Different 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 (19.88 mg/g) while cheese coagulated with steep water from millet and *Calotropis procera* had the highest flavonoids (0.20 mg/g) and alkaloids content (13.42 mg/g). The result of the antioxidant properties revealed that *Carica papaya* had the highest ferric reducing property and displayed better DPPH scavenging activity (14.94 mg 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

**Comment [érdo1]:** Is this unit correct? Is this the result of FRAP? Please review this sentence.

21 juice and *Carica papaya* may be incorporated into the daily diet because of its phenolic content  
22 which can improve the health status of the consumers. It also possesses some natural  
23 antioxidant compounds, which can effectively scavenge free radicals.

24 Keywords: Phytochemical, antioxidants, soft cheese, goat milk

## 25 1. Introduction

26 Wara (soft cheese) is an unripened cheese consumed in several parts of Western Africa.  
27 Conventionally, it is prepared by coagulating the fresh milk with the leaf extract of Sodom apple  
28 (*Calotropis procera*). Other coagulants such as lemon juice and *Carica papaya* can also be used.  
29 Medicinal plants such as *Calotropis procera* and *Carica papaya* are the oldest form of  
30 healthcare known to mankind (Ali, 2015) and have been used for centuries as remedies for  
31 human and animal diseases as they contain phytochemicals of therapeutic value. They exerted  
32 many pharmacological effects such as antimicrobial, anti-inflammatory, analgesic, anticancer,  
33 anti-angiogenic, immunological, antidiabetic, cardiovascular, gastroprotective, hepatic  
34 protective, renal protective, antidiarrheal, antioxidant, anticonvulsant, enhancement of wound  
35 healing effect. The addition of rennet or coagulating agents has been greatly used in the  
36 coagulation of milk for the production of cheese (Chikpah *et al.*, 2014).

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

Comment [érdo2]: Add reference to this sentence.

Comment [érdo3]: This sentence does not conclude the paragraph, neither has a connection to the next one. I suggest moving it to the beginning of the paragraph, and finish the paragraph with the antioxidant subject, in order to connect with the following one.

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

Comment [érdo4]: Again, this subject has no connection to the latter.

46 Goat's milk has a similarity to human milk that is unmatched in cow milk and also has  
47 several medicinal values. Therefore awareness about advantage of consumption of goats milk  
48 should be popularized so that production and utilization of goat's milk could be enhanced  
49 (Kumar *et al.*, 2012). However, the different coagulants used which are of plant origin might  
50 have impacted their constituents into the soft cheese. It is imperative to ~~carry out~~ assess  
51 phytochemical and antioxidant properties of coagulants and the soft cheese produced from  
52 goat milk. Therefore, the objective of this study ~~is~~ was to determine the phytochemical and  
53 antioxidant properties of coagulants and soft cheese produced from goat milk.

Comment [érdo5]: Both sentences are stating the same information. Maintain only the objective to the work in once sentence.

## 54 2. Materials and Methods

### 55 2.1 Collection of Milk

Comment [érdo6]: I suggest: sampling

56 The raw milk sample was collected from sheep<sub>s</sub> at Aba Baba Medinat, a Fulani farm settlement  
57 along Afao road, Ado-Ekiti, Nigeria. It was collected aseptically and subsequently transferred to  
58 the laboratory for analysis.

Comment [érdo7]: Under what conditions? Refrigerated.

### 59 2.2 Collection of coagulants

60 The leaves of *Carica papaya* and *Calotropis procera* were collected from Erifun community  
61 around The Federal Polytechnic, Ado-Ekiti, Nigeria. Authentification of the Plants were done at

62 the Department of Plant Science and Biotechnology, Ekiti State University, Ado-Ekiti, Nigeria.

63 The voucher specimens of UHAE 2018/022 for *Carica papaya* and UHAE 2018/023 for *Calotropis*

64 *procera* have been deposited at the University herbarium. Other biocoagulants like lemon fruits

65 were purchased from Oba market, a local market in Ado Ekiti Metropolis, Nigeria, West Africa.

66 Steep water (effluent from pap produced from maize, sorghum, millet) ~~were was~~ produced by

67 steeping the grains in water for 3 days after which it was milled and later steeped again for 2

68 days in the laboratory. The steep water was then collected ~~for to be used~~ as biocoagulants.

Comment [érdo8]: This sentence is confusing. Please rewrite it.

Comment [érdo9]: Under what conditions this water was kept prior to its use in the experiment?

### 69 2.3 Production of West African cheese

70 The milk was stirred gently during the heating process with a wooden spoon. About 4 mLs of

Comment [érdo10]: Temperature? How long?

71 the leaf extract of *Calotropis procera*, *Carica papaya*, lemon juice, steep water were added to

Comment [érdo11]: All of the coagulants were added together?

72 the warm milk and the mixture was heated for the second time with intermittent stirring to

73 about 45-50°C and was kept at this temperature until coagulation was achieved and the heating

74 was stopped after the separation of curd and whey. The sign of coagulation was observed

75 within ~~the range of~~ 10-15 min. It was transferred into a small, previously sterilized, rafia basket

76 to facilitate whey drainage and characteristic shape, -when the cheese was firm enough it was

Comment [érdo12]: Of what? Cheese?

77 removed from the rafia basket and placed inside a covered plastic container for analysis.

Comment [érdo13]: Refrigerated?

### 78 2.4 Phytochemical screening of soft cheese sample

79 Basic phytochemical analyses were carried out to determine the bioactive compounds

80 present in the sample.

#### 81 2.4.1 Preparation of samples

82 Two grams (2\_g) of cheese sample ~~was-were~~ carefully weighed into 250\_mL conical flask  
83 and 50\_mL of distilled water was added to ~~the sample~~it. It was mixed and stoppered with a  
84 rubber band and then placed in a water bath for 2\_hrs at 37\_°C, after which it was removed to  
85 cool down. The content was filtered with the use of Whattman filter paper No 1 and the filtrate  
86 was kept for analysis.

Comment [érdo14]: Under what conditions?

#### 87 2.4.2 Test for Tannins

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

Comment [érdo15]: I suggest detailing better the methodology as the reference cited is not an easy access one.

Comment [érdo16]: How much of sample and how much of water?

Comment [érdo17]: How much of reagent and how much of extract?

#### 91 2.4.3 Test for Alkaloids

92 Soft cheese sample (0.5\_g) was added to 5\_mL of 10% (v/v) HCl in test tubes and put in a  
93 water bath for 2\_mins, after which the mixture was filtered. The ~~filtrate~~filtrate (1\_mL) was  
94 treated with 3 drops of Dragendrof's reagent in order to separate portions. The presence of  
95 alkaloids was confirmed by the production of reddish brown ~~colouration~~coloration (Trease and  
96 Evans, 2004).

Comment [érdo18]: Which portions?

Comment [érdo19]: Was this color visually identified? Or was it identified through an equipment?

#### 97 2.4.4 Test for steroids

98 ~~Two millimeters~~ (An aliquot of 2\_mL) of acetic anhydride was added to 0.5\_g of each  
99 cheese sample with addition of 2\_mL of H<sub>2</sub>SO<sub>4</sub>. A ~~colour~~color change from violet to blue or  
100 green indicates the presence of steroids (Trease and Evans, 2004).

Comment [érdo20]: Was this color visually identified? Or was it identified through an equipment?

#### 101 2.4.5 Test for Saponins

102 The ability of saponins to produce frothing in aqueous solution was used as screening test for  
103 the ~~saponins~~ substances. The cheese sample (0.5\_g) was boiled with distilled water in a  
104 water bath and shaken vigorously for stable persistent froth. The frothing was mixed with three  
105 drops of olive oil and shaken vigorously for the formation of emulsion (Sofowora, 1993).

Comment [érdo21]: Temperature

Comment [érdo22]: Rpm?

Comment [érdo23]: Was the froth separated from the solution and then mixed? Make your methodologies reproducible.

Comment [érdo24]: How long? RPM?

#### 106 2.4.6 Test for Flavonoids

107 An aliquot of 10 mL ~~Ten millilitre~~ of ethyl acetate was heated with the sample in a water  
108 bath for ~~thirty minutes~~ 30 min. The mixture was filtered and 4mL of each ~~filtrate~~ filtrate was  
109 shaken with ~~one millilitre~~ (1\_mL) of dilute ammonia solution in a conical flask. A yellow  
110 ~~colouration~~ coloration indicates the presence of flavonoids (Harborne, 1998).

Comment [érdo25]: How MUCH? Is this sample the extract?

#### 111 2.4.7 Test for Cardiac Glycosides

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

Comment [érdo26]: ?

115 B. Lieberman's test: ~~Two millilitre~~ 2\_mL of ~~a~~ Acetic ~~a~~ Anhydride was used to dissolve 0.2\_g of the  
116 food sample. The mixture was cooled in ice. Sulphuric acid was then carefully added. A  
117 ~~colour~~ color change from violet to blue to green indicates the presence of a steroidal nucleus  
118 (i.e aglycone portion of cardiac glycosides) (Trease and Evans, 2004).

#### 119 2.4.8 Terpenoid test

120 ~~Two millilitre (2 mL)~~ of chloroform was used to dissolve 0.2 g of the sample. Sulphuric  
121 acid was carefully added which form a lower layer. A reddish brown ~~colour~~color at the interface  
122 indicates the presence of terpenoids.

## 123 2.5 Quantitative phytochemical screening

### 124 A. Determination of total phenolic content

125 The extract of the sample (100 mg) was weighed accurately and dissolved in 100 mL of  
126 triple distilled water (TDW). This solution (1 mL) was transferred to a test tube, then 0.5 mL ~~2N~~  
127 of the Folin Ciocalteu's reagent (2 mol/L) and 1.5 mL 20% of Na<sub>2</sub>CO<sub>3</sub> 20% solution was added,  
128 and ultimately, the volume was made up to 8 mL with TDW followed by vigorous shaking and  
129 finally allowed to stand for 2 h ~~ours~~ after which the absorbance was taken at 765 nm. These  
130 data were used to estimate the total phenolic content using a standard calibration curve  
131 obtained from various diluted concentrations of gallic acid (Singleton *et al.*, 1999).

Comment [érdo27]: What do you mean? Did you prepare another extract? Explain how.

### 132 B. Determination of total Flavonoids

133 The method is based on the formation of the flavonoids – aluminum complex which has  
134 a ~~maximum~~ maximum absorptivity ~~maximum~~ at 415 nm. 100 µL of the cheese sample in methanol (10  
135 mg/mL) was mixed with 100 µL of 20 % aluminum trichloride in methanol and a drop of acetic  
136 acid, and then diluted with methanol to 5 mL. The absorption at 415 nm was read after 40  
137 minutes. Blank samples were prepared from 100 mL of plant extracts and a drop of acetic acid,  
138 and then diluted to 5 mL with methanol. The absorption of standard ~~rutineroutine~~ rutin solution

Comment [érdo29]: What do you mean by cheese sample? Are you sure it is volume (µL)?

Comment [érdo30]: Is it 2 or 20%? If you made modifications to the methodology you should state that.

Comment [érdo31]: Which extract?

139 (0.5 mg/mL) in methanol was measured under the same conditions. All determinations were  
140 carried out in triplicates (Meda *et al.*, 2005).

**Comment [érdo32]:** What about the other assays? Were they carried in triplicates?

#### 141 C. Determination of total Alkaloids

**Comment [érdo33]:** Please, cite the original methodology. Meda *et al.* only reproduced the methodology of other authors. Further, this methodology is adapted, you should state this, once it is not as the original one.

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

**Comment [érdo34]:** Temperature?

**Comment [érdo35]:** How?

**Comment [érdo36]:** Which concentration? Which volume was used to washing?

**Comment [érdo37]:** Temperature? Time?

#### 149 2.6 Determination of Antioxidant activity Capacity

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

##### 151 A. Ferric Reducing Antioxidant Power Assay (FRAP)

**Comment [érdo38]:** This assay is not properly described. please revise the methodology and cite proper references.

152 The reducing power of the samples was determined by assessing the ability of the soft  
153 cheese sample to reduce FeCl<sub>3</sub> solution as described, according to the method described by  
154 Kong *et al.* (2012). A 2.5 mL aliquot of each cheese sample was mixed with 2.5 mL of 200 mM  
155 sodium phosphate buffer (pH 6.6) and 2.5 mL 1% potassium ferricyanide. The mixture was  
156 incubated at 50 °C for 20 min, then 2.5 mL 10% trichloroacetic acid was added. This mixture  
157 was centrifuged at 650 rpm for 10 min. The supernatants were was collected and 5 mL aliquot  
158 of each sample was mixed with an equal volume of water and 1 mL 0.1% ferric chloride. The

**Formatted:** Font: 12 pt

**Comment [érdo39]:** Please cite the original reference. The authors only modified this methodology. And yours is different from the modification carried by Kong *et al.*



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

Comment [érdo40]: I don't believe this is correct.

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

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

Comment [érdo41]: Your methodology does not match the authors cited. Please cite proper references, and state the modifications carried if it is the case.

Comment [érdo42]: How much?

Comment [érdo43]: ?

168 Scavenging activity (%) =  $[Ab - (As - Abs)] / Ab \times 100$

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

171 Sample concentration causing 50% inhibition (IC<sub>50</sub>) was calculated from the graph, plotting the  
172 % inhibition against sample concentration.

## 173 2.7 Statistical Analysis

174 Statistical analyses were carried out and data were obtained using SPSS program  
175 (Statistical Package for social Sciences version 16). Significant differences between means were  
176 calculated by one-way Analysis of Variance (ANOVA) using Duncan's multiple range test (DMRT)  
177 in order to evaluate differences among means was used to separate means (Omotosho *et al.*,  
178 2011).

179 **3.0 Results**

180 3.1 Qualitative phytochemical properties of biocoagulants used for the production of soft  
 181 cheese.

Formatted: Justified

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

Comment [érdo44]: Rewrite the sentence in order not to repeat the term (millet)

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

| Samples | Saponins | Flavonoids | Tannins | Alkaloids | Terpenoids | Steroids | Phenols | Glycosides | Cardiac Glycosides | Reducing sugars |
|---------|----------|------------|---------|-----------|------------|----------|---------|------------|--------------------|-----------------|
| GCPRe   | -        | +          | -       | +         | -          | -        | +       | -          | -                  | +               |
| GCP     | -        | +          | -       | +         | -          | -        | +       | -          | -                  | +               |
| GLJ     | -        | +          | -       | +         | -          | -        | +       | -          | -                  | +               |
| GSO     | -        | +          | -       | +         | -          | -        | +       | -          | -                  | +               |
| GMI     | -        | +          | -       | +         | -          | +        | +       | +          | -                  | +               |
| GMA     | -        | +          | -       | +         | +          | -        | +       | -          | -                  | +               |

Formatted: Font: 8 pt  
 Formatted: Centered, Line spacing: single  
 Formatted: Font: 8 pt  
 Formatted: Font: 8 pt  
 Formatted: Font: 8 pt  
 Formatted: Font: 8 pt  
 Formatted: Centered, Line spacing: single  
 Formatted: Font: 8 pt  
 Formatted: Centered, Line spacing: single  
 Formatted: Font: 8 pt  
 Formatted: Centered, Line spacing: single  
 Formatted: Font: 8 pt  
 Formatted: Centered, Line spacing: single  
 Formatted: Font: 8 pt  
 Formatted: Centered, Line spacing: single  
 Formatted: Font: 8 pt  
 Formatted: Centered, Line spacing: single

190 Keys: **GSO** – goat milk coagulated with steep water from sorghum, **GMA** - goat milk coagulated  
 191 with steep water from maize, **GMI** - goat milk coagulated with steep water from millet, **GLJ** -  
 192 goat milk coagulated with steep water from lemon juice, **GCPR** - goat milk coagulated with  
 193 *Calotropis procera*, **GCP** - goat milk coagulated with *Carica papaya*. ~~Tannins, Alkaloids,~~  
 194 ~~Terpenoids, Steroids, Flavonoids, Glycosides, Cardiac glycosides and Reducing sugars~~

195 3.2 Quantitative phytochemical properties of biocoagulants used for the production of wara.

196 Table 2 shows the qQuantitative phytochemical screening of cheese produced from goat milk  
 197 using different biocoagulants. Goat milk coagulated with lemon juice had the highest phenol  
 198 ~~value of 19.88mg/g content.~~; Cheese coagulated with steep water from millet had the highest  
 199 flavonoids ~~value content of 0.20mg/g~~ and cheese coagulated with *Calotropis procera* had the  
 200 highest alkaloids ~~content value of 13.42mg/g~~. While, the lowest phenol ~~had a lowest value~~  
 201 ~~of content (15.39 mg/g) was found for 15.39mg/g in~~ the goat milk coagulated with *Calotropis*  
 202 *procera*; the lowest flavonoid ~~content (had a lowest value of 0.06 mg/g) was observed in for~~  
 203 the goat milk coagulated with lemon juice, and Alkaloids ~~had a lowest value of concerning the~~  
 204 ~~alkaloids, the lowest content (7.64 mg/g) was 7.64mg/g in for~~ the goat milk coagulated with  
 205 millet steep water ~~from millet~~.

206 **Table 2. Quantitative phytochemical screening of soft cheese produced from goat milk using**  
 207 **different biocoagulants**

| <u>S</u> AMPLES <u>B</u> iocoagula | Phenols (mg/g) | Flavonoids (mg/g) | Alkaloids (mg/g) |
|------------------------------------|----------------|-------------------|------------------|
| G CPR                              | 15.39± 0.14f   | 0.16±0.01a        | 13.42±0.01a      |
| G CP                               | 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      |
| G SO                               | 17.87±0.02cc   | 0.09 ±0.00c       | 10.82± 0.00c     |

**Comment [érdo45]:** SPECIFY THE ACID EQUIVALENT, for exemple: mg GAE/ g Where, GAE stands for gallic acid equivalent. The same should be done for flavonoids and alkaloids

|     |              |             |              |
|-----|--------------|-------------|--------------|
| 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 |

208

209 KEY: G- goat milk, SO- sorghum, MA- maize, MI- millet, LJ- lemon juice, CPR- *Calotropis procera*, CP-  
 210 *Carica papaya*. Values are means ± standar deviation of replicate (n=3); M means with different  
 211 letters within a column are significantly different. (*P* < 0.05)

Formatted: Font: Italic

212

### 213 3.3 FRAP assay ~~of the samples~~

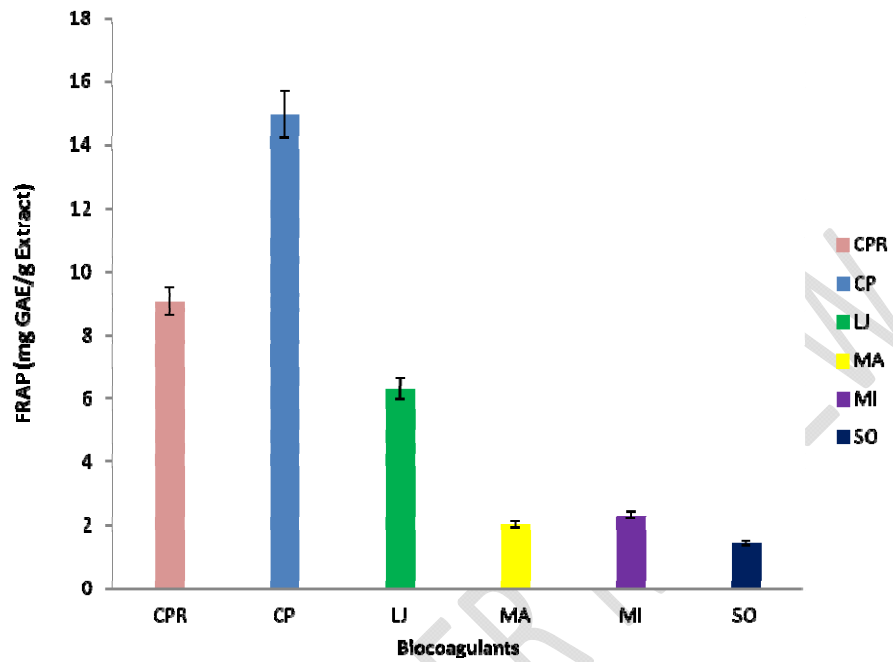
214 The ~~ferric reducing antioxidant power (FRAP)~~ of the biocoagulants and soft cheese produced  
 215 from goat milk using different

216 biocoagulants is presented in Figures 1 and 2, respectively. The values ~~—~~ obtained for FRAP  
 217 ~~anged~~ from 1.43 to

218 14.94 mg GAE/g ~~extract~~ for biocoagulants, and from 7.74 to ~~—~~ 10.31 mg ~~Garlic Acid~~  
 219 ~~Equivalent~~ GAE/g (mg GAE/g sample) for the soft cheese

Comment [érdo46]: Please, make sure this is correct.

220 ~~samp~~les respectively.



221

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

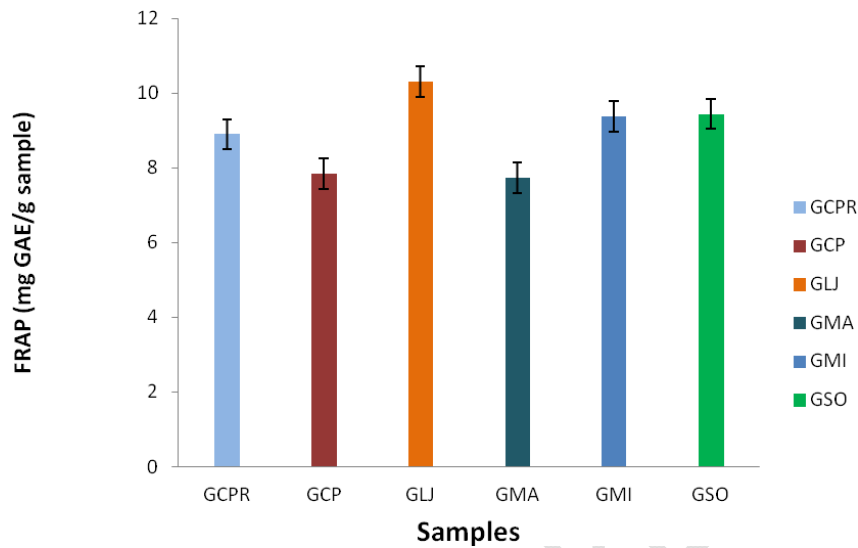
223 **cheese**

224

225

226

**Comment [érdo47]:** Please, remove the lateral legend, once it is repetitive. Explain each of the initials.



227

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

229 different ~~b~~Biocoagulants

230 3.4 DPPH scavenging activity ~~of the samples~~

231 The scavenging ~~activities-activity~~ of the biocoagulants and soft cheese samples against DPPH

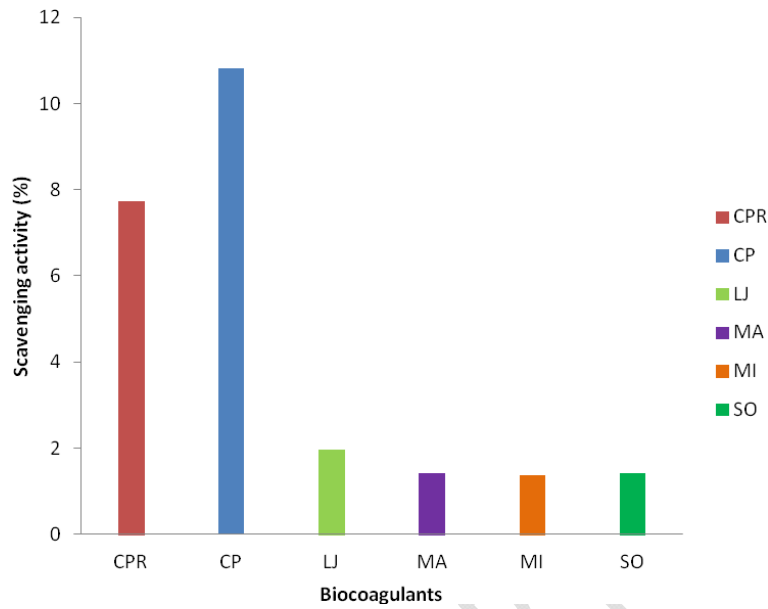
232 free radicals are presented in Figures 3 and 4, respectively. The scavenging activity of

233 biocoagulants against DPPH ranged from 1.37 to 10.82%, while the results for value of-soft

234 cheese from goat milk ~~against free radicals~~ ranged from 1.56 to- 1.93%.

235

Comment [érdo48]: Please, remove the lateral legend, once it is repetitive. Explain each of the initials.



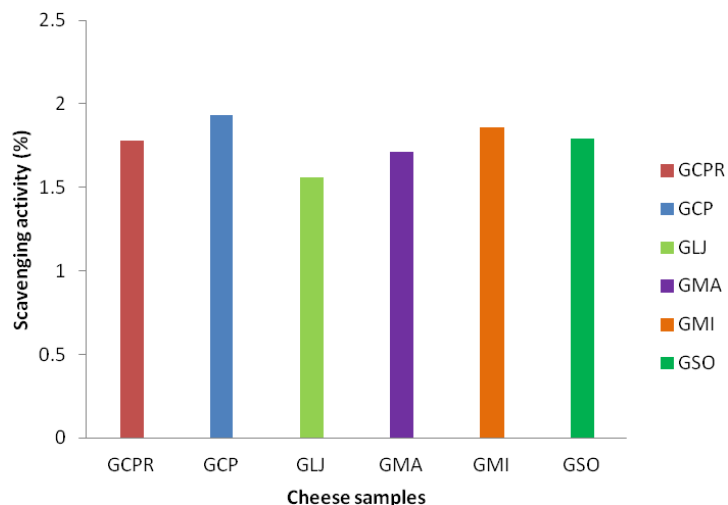
236

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

238 DPPH free radical

239

**Comment [érdo49]:** Please, remove the lateral legend, once it is repetitive. Explain each of the initials.



240

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

242 biocoagulants on DPPH free radical

#### 243 4.0 Discussion

244 The result of the qualitative phytochemical screening of soft cheese produced from goat milk

245 using different coagulants (Table 1) revealeds that phenols, alkaloids, flavonoids and reducing

246 sugar were present in all the cheese samples. However, saponins, tannins and cardiac

247 glycosides were absent in all the samples while, terpenoids, steroids and glycosides were

248 present in cheese coagulated with steep water from maize and millet. The presence of phenols

249 indicates that the cheese may be able to achieve multiple activities, such as ~~like~~ antioxidant,

250 anticarcinogenic, anti-inflammatory, e-t-c. (Asha *et al.*, 2011). Alkaloids are the most significant

251 compounds that play a metabolic role in the living systems and are involved in the protective

252 function in animals. Steroidal alkaloids are medicinally evolved. Alkaloids are the most efficient

Comment [érdo50]: Please, remove the lateral legend, once it is repetitive. Explain each of the initials.

Comment [érdo51]: These are results already presented in the previous topic. Do not repeat.



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

Comment [érdo52]: Add more recent references

Comment [érdo53]: Too many sentences, which are repetitive. Reformulate in order to be more objective.

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

Comment [érdo54]: Again, you are repeating what you have already said in the results section.

273 The value obtained for FRAP (Figures 1 and 2) ranged from 1.43-14.94 for biocoagulants  
274 and 7.74-10.31 mg Garlic Acid Equivalent GAE/g sample (mg GAE/g sample) for soft cheese  
275 produced from goat milk. Extract from *Carica papaya* (14.94 mg GAE/g extract) and soft cheese  
276 produced with lemon juice (10.31 mg GAE/g sample) showed the highest ferric reducing  
277 property when compared with others (at  $p < 0.005$ ). It has been reported that the antioxidant  
278 activity of plant material was well correlated with the content of their phenolic compounds  
279 (Velioglu *et al.*, 1998). The ability of the biocoagulants and the soft cheese samples to scavenge  
280 for DPPH radicals is presented in Figures 3 and 4, respectively. Soft cheese samples and the  
281 biocoagulants used for soft cheese processing displayed concentration dependent DPPH  
282 scavenging activity. In this study, the scavenging activity of biocoagulants used for soft cheese  
283 processing ranged from 1.37-10.82% while that of soft cheese samples ranged from 1.56-1.93%.  
284 *Carica papaya* displayed better DPPH scavenging activity (10.82%) when compared with other  
285 coagulants (1.37%, 1.41%, 1.42%, 1.97%, 7.72%) while soft cheese coagulated with *Carica*  
286 *papaya* displayed better DPPH scavenging activity (1.93%) when compared with other samples  
287 (1.56%, 1.71%, 1.78%, 1.79%, 1.86%). Coagulants such as *Carica papaya* have been reported to  
288 be a rich source of antioxidant sources due to the presence of phenolic group and carotenoids  
289 in them which can scavenge free radicals (Aravind *et al.*, 2013 and Usman *et al.*, 2012). The  
290 results showed that the soft cheese and the biocoagulants possess some natural antioxidant  
291 compounds, which can effectively scavenge free radicals.

Comment [érdo55]: Repetitive, as stated above.

Comment [érdo56]: I suggest adding other references

Comment [érdo57]: Extracts?

Comment [érdo58]: Reformulate this sentence.

Comment [érdo59]: These are results, and not discussion.

Comment [érdo60]: These numbers refer to which coagulants?

Comment [érdo61]: These numbers refer to which coagulants?

## 292 5.9 Conclusions

293 | The results of qualitative phytochemical screening revealeds that the soft cheese  
294 | produced from goat milk using different biocoagulants contain phenols, alkaloids, flavonoids  
295 | and reducing sugar. While saponins, tannins, and cardiac glycosides were absent in all the  
296 | cheese samples. Goat milk coagulated with lemon juice has-presented the highest phenol  
297 | content; ~~cheese coagulated with millet steep water from millet coagulated cheese~~ hads the  
298 | highest flavonoid content, while *Calotropis procera* coagulated cheese has-had the highest  
299 | alkaloids content. The addition of the biocoagulants increaseds the bioactive content of the  
300 | cheese samples. ~~The results of the antioxidant properties of the biocoagulants revealed that~~  
301 | *Carica papaya* displayed better DPPH scavenging activity when compared with other samples,  
302 | and soft cheese extract produced from goat milk coagulated with *Carica papaya* can-was able  
303 | to scavenge free radical better than other samples.

Comment [érdo62]: Extracts?

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

## 305 | References

- 306 | Akinyeye, A. J., Solanke E. O. and Adebiji I. O. (2014). Phytochemical and antimicrobial  
307 | evaluation of leaf and seed of *Moringa olifera* extract. *International Journal of*  
308 | *Research in Medicine and Health Sciences*, 4 (6), 2307-2083.
- 309 | Ali, E. A. (2015). The constituents and Pharmacological properties of *Calotropis procera*-An  
310 | Overview. *International Journal of Pharmacy Review and Research*, 5 (3), 259-275.
- 311 | Aravind. G., Debjit. Bhowmik, Duraivel, S., Harish, G. (2013). Traditional and Medicinal Uses  
312 | of *Carica papaya*. *Journal of Medicinal Plants Studies*, 1 (1), 7-15

313 Asha, K., Rasika, C. T., Nirmala, R. D. and Jyoti, P. S. (2011). Analytical Biology Research, 2(1),  
314 176-180.

315 Chikpah, S. K., Teye, M., Annor, J. A. F. and Teye, G.A. (2014). Potentials of Sodom apple  
316 (*Calotropis procera*) extract as a coagulant to substitute alum in soy cheese  
317 production in Ghana. *Elixir International Journal of Food Science*.

318 Dresch, J. (1988). A plea for the goat. *Production Pastorale-et-Societe OAE*, 1982. 10:81-83.

319 Duh, P. D, Tu, Y. Y and Yen, G. C. (1999). Antioxidant activity of water extract of Harng Jyur  
320 (*Chrysanthemum moifolium* Ramat). *Lebensm-Technology*, 32:269-277.

321 Duthie, G. and Crozier, A. (2000). "Plant-derived phenolic antioxidants", *Current Opinion* in  
322 *Lipidology*. 11: 43-47.

323 Gordon, M. H. (1990). The mechanism of the antioxidant action *in vitro*. In: *Food antioxidants*,  
324 Hudson B.J.F (Ed.). London, Elsevier. 1-18.

325 Gulcin, I., Oktay, M., Kirecci, E., Kufrevioglu, O. I. (2003). Screening of antioxidant and  
326 antimicrobial activities of anise (*Pimpinella anisum* L.) seed extracts. *Food*  
327 *Chemistry*, 8:371-382.

328 Gyamfi, M. A, Yonamine, M and Aniya, Y. (1999). Free-radical scavenging action of  
329 medicinal herbs from Ghana *Thonningia sanguinea* on experimentally- induced liver  
330 injuries. *General Pharmacology*, 32: 661-667.

- 331 | Harborne, J.B. (1998). Method of extraction and Isolation In: *Phytochemical methods*.  
332 | Chapman and Hall, London. 60-66
- 333 Kong, K.W., Mat-Junit, S., Aminudin, N., Ismail, A. and Abdul-Aziz, A. (2012). Antioxidant  
334 | activities and polyphenolics from the shoots of *Barringtonia racemosa* (L.) Spreng in a  
335 | polar to apolar medium system. *Food Chemistry*, 134(1), 324-332. [https://](https://doi.org/10.1016/j.foodchem.2012.02.150)  
336 | [doi.org/10.1016/j.foodchem.2012.02.150](https://doi.org/10.1016/j.foodchem.2012.02.150)
- 337 Kumar, S., Kumar, B., Kumar, R., Kumar, S., Khatkar, S. and Kanawjia, S. K. (2012).  
338 | Nutritional Features of Goat Milk. A Review. *Indian Journal of Dairy Science*, 65(4).
- 339 | Meda, A., Lamien, C. E., Romito, M., Millogo, J. and Nacoulma, O.G. (2005). Determination  
340 | of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as  
341 | their radical scavenging activity. *Food Chemistry*. 91:571–577.
- 342 Oboh, G. (2006). Coagulants Modulate the Antioxidant property and Hypocholesterolemic  
343 | Effect of Tofu (Curdled Soymilk). *Asian Journal of Biochemistry*, (1), 57-66.
- 344 Omotosho, O. E., Oboh, G. and Iweala, E. E. J. (2011), "Comparative effects of local  
345 | coagulants on the nutritive value, in vitro digestibility and sensory properties of wara'  
346 | cheese". *International Journal of Dairy Science*, 6 (1), 58 - 65.
- 347 Park, Y. (1994). "Hypo-allergenic and therapeutic significance of goat milk," *Small*  
348 | *Ruminant Research*. 14(2), 151–159.

349 Singleton, V. L., Orthofer, R., Lamuela-Raventos, R. M. (1999). Analysis of total phenols and  
350 other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent.  
351 *Methods Enzymology*, 299:152-178.

352 Sofowora, E. A. (1993). Medicinal Plants in Africa. Spectrum books Limited, Ibadan,  
353 Nigeria. 2:118-246

354 Stevens, J. F, Hart, H. T., Hendriks, H. and Malingre, T. M. (1992). Alkaloids of some  
355 European and macaronesian diode and semepervivodeae (Crassulaceae).  
356 *Phytochemistry*, 31:3917-3924.

357 Stray, F. (1998). The natural guide to medicinal herbs and plants. Tiger Books International,  
358 London, pp: 12-16.

359 Trease, G. E. and Evans, W. C. (2004). Pharmacognosis Macmillian publishers, Bailvera Tindall,  
360 London. 13: 309-707

361 Usman, R., Khan, A., Gul, S., Rauf, A. and Muhammad, N. (2012). Evaluation of In vitro Anti-  
362 Oxidant properties of Selected Medicinal Plants, *Middle-East Journal of Medicinal*  
363 *Plants Research*, 1(2), 28-31.

364 Velioglu, Y. S., Mazza, G., Gao, L. and Oomah, B. D. (1998). Antioxidant Activity and Total  
365 Phenolics in Selected Fruits, Vegetables, and Grain Products. *Journal of Agricultural*  
366 *Food Chemistry*, 46: 4113-4117

367