



**Original Paper**

<http://ajol.info/index.php/ijbcs>

<http://indexmedicus.afro.who.int>

## Comparative analysis of the antioxidant capacity of milk from different breeds of cow in Nigeria

Abduljalil Muhammad MODE<sup>1</sup>, Umar Faruk MAGAJI<sup>2</sup>, Nnamdi Abiola NZONIWU<sup>3</sup>, Haruna Hassan WAMAKKO<sup>4</sup>, Ibrahim AHMAD<sup>5</sup> and Shamsudeen Umar DANDARE<sup>6\*</sup>

<sup>1</sup>Central Advanced Science Laboratory Complex, Usmanu Danfodiyo University, Sokoto, Nigeria.

<sup>2</sup>Department of Biochemistry and Molecular Biology, Faculty of Science, Federal University Birnin Kebbi, Nigeria.

<sup>3</sup>Abu Dhabi Vocational Education and Training Institute (ADVETI), Mohammed Bin Zayed City, Abu Dhabi, UAE.

<sup>4</sup>Directorate of Animal Health and Fisheries Development, Sokoto, Nigeria.

<sup>5</sup>Directorate of Animal Health and Livestock Development, Gusau, Zamfara, Nigeria.

<sup>6</sup>Department of Biochemistry, Faculty of Life and Chemical Sciences, Usmanu Danfodiyo University, Sokoto, Nigeria.

\*Corresponding author; E-mail: [umar.shamsuddeen@udusok.edu.ng](mailto:umar.shamsuddeen@udusok.edu.ng)

Received: 19-10-2022

Accepted: 29-12-2022

Published: 28-02-2023

### ABSTRACT

Milk is an essential component of human and animal diets, and cattle are a major source of milk for human consumption. Environmental, nutritional, genetic, and physiological factors can affect animal milk composition. This study was aimed at evaluating the antioxidant properties of milk samples from four cattle breeds, namely: White Fulani (WF), Sokoto Gudali (SG), Red Bororo (RB), and the exotic Holstein Friesian (HF) in Sokoto, Nigeria. Fatty acid, amino acid profile, antioxidant vitamins, antioxidant minerals, and antioxidant enzymes activities of the samples were assessed using standard methods. The results of the analysis indicated that milk of SG had the highest values for moisture (85.57%), Vit C (79.24mg/dl), DPPH scavenging activity (80.04%), and FRAP (1083.33mgTE/100g of FW). Milk of RB had the highest ash (4.52%), protein (3.63%), copper (0.19 ppm), zinc (0.16 ppm), iron (3.47 ppm), and antioxidant amino acid composition. The milk of HF had the highest lipid (5.89%), oleic acid (1.17 mg/ml), total polyphenol content (TPC) (0.26 mgTE/100g of FW) and selenium (103.10 ppm) levels. WF had the highest carbohydrate (15.07%), palmitic acid (98.39 mg/ml), stearic acid (35.46 mg/ml), Vit A (55.38 mg/dl), Vit E (52.18 mg/dl) content, catalase (0.02 U/L) and superoxide dismutase (3.00 U/mL) activities. These results show that milk from the WF has better antioxidants and therefore antioxidant capacities than the other breeds. Overall, the study indicates that cow milk is a good source of antioxidants, whose levels vary between the different breeds of cattle. These nutritional differences can be explored during milk sourcing based on nutritional requirements.

© 2022 International Formulae Group. All rights reserved.

**Keywords:** Antioxidant, Cattle, Enzymes, Milk, Minerals, Vitamins.

### INTRODUCTION

Antioxidants are natural or synthetic compounds that protect cells from damage caused by free radicals produced during cell

metabolism and animals depend on an endogenous natural system of antioxidants to protect them from oxidative stress (Wang et al., 2006). In situations where natural

endogenous antioxidants cannot prevent damage caused by oxidative stress, natural or synthetic exogenous antioxidants are used as supplements to salvage the situation. Consumers avoid synthetic antioxidants because they have been reported to be toxic and carcinogenic, making natural antioxidant-rich foods such as broccoli, blueberries, spinach, potatoes, beans, strawberries, and milk safer options (Wang *et al.*, 2006).

Milk is an essential component of the human and animal diet and crucial to the global food industry. It contains a balance of high-quality essential nutrients such as proteins, lipids, vitamins, calcium, phosphorus, and potassium (Pecka-Kielb, 2018). In addition, milk contains natural non-enzymatic and enzymatic antioxidants such as vitamins A, C, and E, carotenoids, uric acids, ascorbic acids, retinol,  $\alpha$ -tocopherol, catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) are all examples of antioxidants found in milk (Cacho and Lawrence, 2017).

Cattle are the primary source of milk, producing about 81% of the global milk supply (FAO, 2020). Globally, more than 6 billion individuals consume milk derived from cows (Fructuoso *et al.*, 2021). In the absence of or inadequate human breast milk, cow milk processed into different dairy formulas is commonly used as a weaning substitute for infants. Nigeria has a large cattle population of 15.3 million, which provides more than 90% of the total milk output in the country (Tibi and Aphunu, 2010). The popular local cattle breeds include White Fulani (WF), Red Bororo (RB), Sokoto Gudali (SG), Wadara, Adamawa Gudali (AG), Muturu, Azawak, Keket, Kuri and Ndama. Foreign breeds such as Holstein Friesian (HF), Brown Swiss and Jersey make up only about 1% of the total cattle population (CSIRO, 2020). Although, Toko *et al.*, (2011) reported that indigenous cows have low milk but are bred and considered a major source of milk, because

WF, RB, and SG, are 1st, 2nd, and 3rd highest cattle populations in Nigeria, respectively (Kubkomawa, 2017).

Previous studies (Dandare *et al.*, 2014) have shown that cow milk has antioxidants and several other nutritional properties. However, environmental, nutritional, genetic and physiological factors affect animal milk composition (Pietrzak-Fiecko, 2018). There is a dearth of knowledge in the comparative study of milk's antioxidants and its nutritional properties from different local cow breeds in Nigeria. Hence, this study was aimed at comparing the antioxidant properties of milk from three local (WF, RB, SG) breeds and an exotic (HF) breed of cattle in Nigeria by evaluating fatty acid and amino acid profiles, as well as carrying out quantitative analysis of antioxidant vitamins, antioxidant minerals, and antioxidant enzymes in milk from each of these cattle breeds.

## MATERIALS AND METHODS

### Study Location

Milk samples from four (4) different breeds of cows (WF, SG, RB, and HF), were collected. One (1) sample was collected from each breed making a total of four (4) samples. The milk of the HF breed was sampled as a positive control for this study. The milk samples were collected from Sidi Akibu Dairy Farms – a semi-intensive farm with about 500 cows, located at plot 21/26, Kano Road, opposite Sultan Abubakar III Mosque Sokoto State, Nigeria (Figure 1). The animals were fed with uniform and nutritious feed such as wheat bran, beans bran, dried and ground bean leaves, rice husk, cotton seeds, and clean water. Furthermore, the animals were kept in optimal environmental conditions and under constant supervision and monitoring by qualified personnel and veterinary doctors.

### Sample collection

Milk was collected from each cow via random selection from the same breed group.

Fresh early morning milk samples were collected in sterile glass containers, appropriately labelled and immediately transported to the laboratory in an ice-packed container. All samples were then stored in a refrigerator at 4°C until use. The samples were collected from April to June 2021.

### **Criteria for sampling**

The followings were the criteria for sampling in this study:

- Pure breeds devoid of any crossing were selected for the study.
- Cows aged between 2 and 6 years and that were not under any physical stress.
- Healthy or non-diseased cows were selected.
- Cows that were vaccinated for the last 3 months.
- Cows that are on antioxidants supplementation or antibiotics.

### **Antioxidant mineral elements and heavy metals determination**

The entire analysis was carried out in a fume cupboard. Briefly, 0.5mL of each sample was taken into a 50 mL conical flask, followed by the addition of 5 mL of Nitric acid (HNO<sub>3</sub>). The mixture was gently heated for 20 minutes until the yellow fumes disappeared. It was then allowed to cool for 30 minutes, after which 2.5 mL of perchloric acid (HClO<sub>4</sub>) was added and heated until it became colourless. Finally, the heated samples were diluted by adding 20 mL of distilled water, filtered into plastic bottles, and stored for analysis using AAS spectrophotometry as described by Bhatti *et al.*, (2006). Antioxidant mineral elements (copper, phosphorus, zinc, iron, and selenium) and heavy metals (lead, chromium, manganese, and cadmium) were quantified using Atomic Absorption Spectroscopy (Model: AA-6300, GBC Scientific Equipment, USA).

### **Amino acids profile determination**

Each milk sample was dried at 70°C to constant weight, defatted, hydrolysed, evaporated in a rotary evaporator, and loaded into the Applied Biosystems PTH Amino acid analyser. The samples were defatted using a chloroform/methanol mixture of ratio 2:1. A 4 g of the sample was introduced into an extraction thimble and extracted for 15 hours in the Soxhlet extraction apparatus (AOAC, 2006). Amino acids were determined using an amino acid analyser (120A PTH, Applied Biosystems Inc, USA) using AOAC (2006) method. The amino acid analyser automatically analyses phenylthiohydantoin (PTH) amino acids derived from the Edman degradation of proteins and peptides.

### **Fatty acids profiles determination**

Liquid-liquid extraction (LLE) was used to obtain lipid fractions from the milk samples as described by Zhang and Hu (2013). A 3 mL aliquot sample was treated with 6 mL of isopropanol and ultrasonicated for 10 minutes at 25°C. Then, 5 mL of hexane (99%) was added, vortexed for 1 minute and centrifuged at 2000 rpm for 5 minutes at 4°C, followed by the removal of the hexane phase. The extraction procedure was repeated twice, while the hexane fractions obtained in the two steps were combined. After solvent removal under vacuum, the lipid extract was dried and reconstituted in 5 mL and 10 mL of chloroform to determine oleic acid and linoleic acid, respectively. Furthermore, 15 mL of n-hexane and 10 mL of ethanol were used for palmitic acid and stearic acid determination, respectively.

Fatty acids such as oleic acid, linoleic acid, palmitic acid and stearic acid were determined using Cary Series UV-Vis spectrophotometer (Agilent Technologies coupled to Z230hp CPU).

### **Proximate analysis**

Using the recommended methods of the Association of Official Analytical Chemists (AOAC) (AOAC, 2000), proximate analysis consisting of percentages of moisture, ash, lactose, crude lipid, and crude protein was evaluated. For estimation of ash content, a dry platinum dish was used, and 2 g of the sample was added into the crucible and weighed. The crucible containing the sample was then put into the muffle furnace and heated at 550°C until fully ashed. The crucible was then cooled in the desiccator and the weight was noted. Similarly, for moisture content, 2 g of the samples were put into the moisture can and each were introduced into an empty crucible and weighed. The crucible was then transferred into the hot air-drying oven set at 105°C for two hours and was quickly transferred into the desiccator to cool. The total crude protein content was determined by the Kjeldahl method.

For the determination of the lipid content of the milk sample, 2 g of each sample was weighed and then put into a labeled porous thimble. A piece of cotton wool was used to cover the porous thimble. Then, 200 ml of petroleum ether was added into the dried 250 ml extraction flask and weighed W1. The thimbles were then placed into the extractor and the apparatus was then assembled. The extraction was done for about 5 to 6 hours. The thimbles were then carefully removed and the petroleum ether was collected on the top of the container (tube) for re-use. When the extraction flask was almost free of petroleum ether, it was removed from the water bath. It was then dried in an oven at 150-110°C for an hour. It was then cooled in a desiccator and weighed as W2 after which the amount of lipid extracted was obtained by the difference between the weight of the flask before and after extraction.

### **Antioxidant vitamins**

Vitamin A was determined using the Bassey et al. (1946) method, whereas Baker and Frank's (1968) method was employed for vitamins C and E estimation. For vitamin A determination, 0.5 g of the sample was dissolved in 10 ml of distilled water and allowed to stand for 1 hour, then filtered using filter paper. The test tube contents were shaken thoroughly and centrifuged for 10 minutes at 2000 rpm. Thereafter, 1 mL of supernatant was measured at 450 nm against a reagent blank. The portion containing petroleum ether solution of each test tube was pipetted into a cuvette and the absorbance was read at 450 nm. For vitamin C, 0.5 g of sample was dissolved in 10 ml of distilled water and incubated for 30 minutes at room temperature, and then filtered using Whatman filter paper, and centrifuged at 2000 rpm for 10 minutes and absorbance was measured at 700 nm. Similarly, for the determination of vitamin E, 0.5g of the sample was dissolved in 10 ml of distilled water, and incubated at room temperature for 30 minutes, then filtered using Whatman filter paper. Thereafter, 0.5 ml of the sample was pipetted and transferred into a clean test tube, and then 0.5 ml of ethanol was also added and shaken vigorously for 1 minute. Also, 3 mL of xylene was also added and shaken vigorously for another 1 minute. The tube was centrifuged at 2000 rpm for 10 minutes, mixed and incubated for 3 minutes and absorbance was spectrophotometrically measured at 539 nm.

### **Antioxidant enzymes [catalase (CAT) and Superoxide dismutase (SOD)]**

Catalase (CAT) and superoxide dismutase (SOD) activities were determined as described by Aebi (1984) and Velikova et al. (2000), respectively. For analysis of catalase activity, 2 mL of sample and 1 ml of hydrogen peroxide solution were added into the sample test tubes while 2 mL of the blank solution and 1 mL of hydrogen peroxide were

added into the blank test tube. The change in the absorbance of the test sample against the blank was measured at 240 nm and recorded every 15 seconds using UV-Visible Spectrophotometer. The catalytic concentration of catalase was calculated (Aebi 1984). For SOD, 0.1 mL, 0.83 mL and 0.5 mL of buffer (pH 7.0), distilled water and sample respectively were pipetted into the sample test tube while 0.15 mL and 0.83 mL of the buffer and distilled water respectively were pipetted and added into the blank test tube. The mixture was incubated at room temperature for 30 minutes and the absorbance was read at 560 nm.

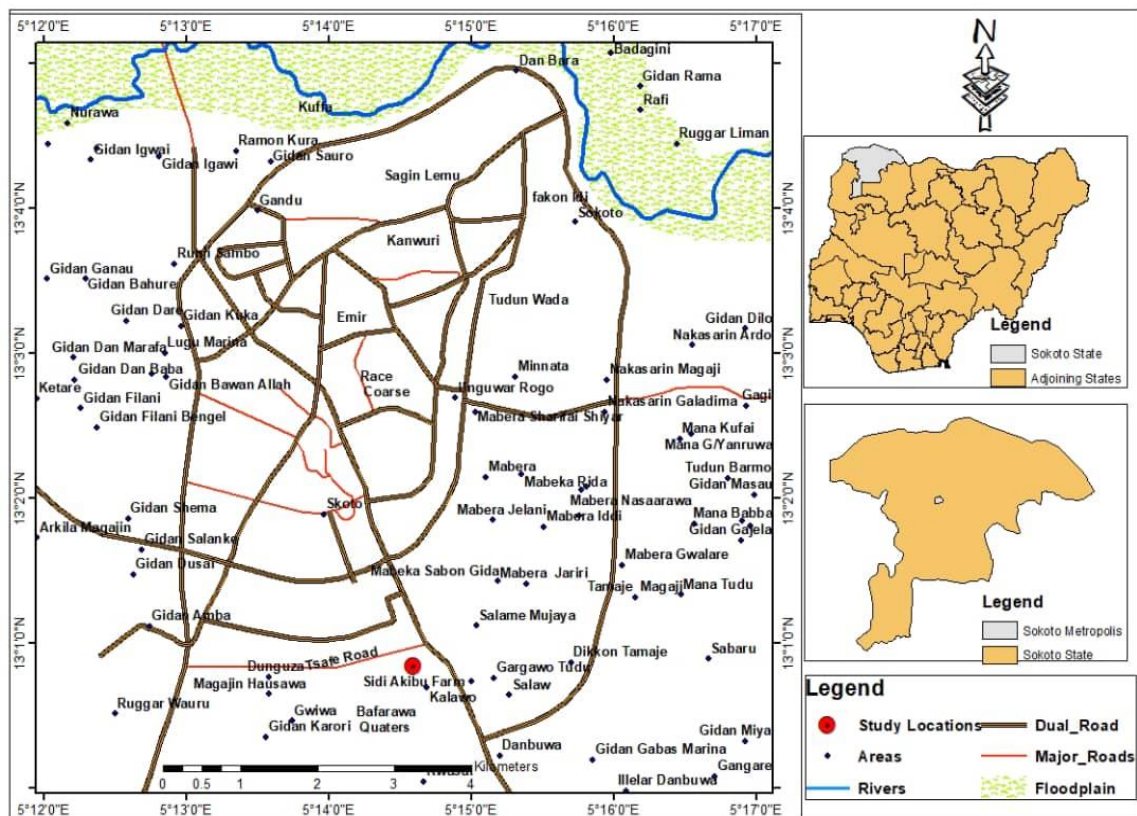
**Antioxidant activity [2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging system, ferric reducing antioxidant power (FRAP) and total phenolic content (TPC)]**

The method of Alyaqoubi et al., (2014), was used to evaluate the antioxidant activity of the milk samples through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) scavenging system, ferric reducing antioxidant power (FRAP) and total phenolic content (TPC). For determination of DPPH activity, forty milligrams were dissolved in 100 mL methanol to prepare the stock solution and stored at - 20°C until use. The absorbance of  $1.0 \pm 0.01$  unit was obtained by mixing 350 mL of the stock solution with 350 mL methanol by using a spectrophotometer (Epoch, Biotek, USA) at 517 nm wavelength. About 100  $\mu$ L fresh milk with 1 mL methanolic DPPH solution were prepared and kept for two hours in the dark for scavenging reaction. The percentage of DPPH scavenging activity was calculated as follows: DPPH scavenging activity (%) = [(A blank - A

sample) / A blank]  $\times$  100. Where A is the absorbance. Similarly, FRAP reagent was prepared fresh using 300 mM acetate buffer, pH 3.6 (3.1 g sodium acetate trihydrate, plus 16 mL glacial acid made up to 1:1 with distilled water); 10 mM TPTZ (2,4,6-tris (2-pyridyl)-s-triazine), in 40 mM HCl; and 20mM FeCl<sub>3</sub>•6H<sub>2</sub>O in the ratio of 10:1:1 to give the working reagent. Then, 100  $\mu$ L from extracted fresh cow milk was added to 1 mL FRAP reagent and the absorbance was monitored at 595 nm wavelength with the spectrophotometer after 30 minutes. A calibration curve of Trolox (as the antioxidant standard) was set up to estimate the activity capacity of samples. The result was expressed as mg of Trolox equivalents per 100 g of fresh samples (mg TE/100 g of FW). For TPC determination, about 100  $\mu$ L of extracted fresh cow milk was added with 0.4 mL distilled water and 0.5 mL diluted Folin-Ciocalteu reagent. The samples with the reagent were left for 5 minutes and then 1 mL 7.5% sodium carbonate (w/v) was added. The absorbance was monitored spectrophotometrically at 765 nm after 2 hours. Calibration curve of gallic acid was set up to evaluate the activity capacity of the samples. The result was expressed as mg of gallic acid equivalents per 100 g of fresh sample (mg GA/100g of FW).

**Statistical analysis**

Data expressed as mean  $\pm$  standard deviation (SD) were analysed using GraphPad Prism Software, version 6.01 (San Diego, USA). Statistical significance was evaluated using one-way variance analysis (One-way ANOVA) followed by Turkey's multiple comparison post hoc test.  $p < 0.05$  was considered statistically significant.



**Figure 1:** Map showing the location of the study area. The red dot indicates the study location (Sidi Akibu Dairy Farms). The map was created using the ArcGIS 10.5 software.

## RESULTS

### Proximate Analysis

Figure 2 shows the mean value (%) of the proximate analysis of milk samples obtained from four (4) different cow breeds. It shows the percentage composition of lactose, ash, moisture, lipids, proteins, and carbohydrates in milk samples of these breeds. The highest percentage composition (12.70%) of lactose was observed in milk samples of the SG breed (Figure 2). The milk sample of HF had the lowest lactose composition ( $4.955 \pm 0.008\%$ ) when compared to the other 3 breeds.

The ash contents, which reflect the mineral composition of the milk samples, had variation within the studied breeds. The difference ranged from 0.35-4.52%. The milk sample from RB had significantly higher ash content (4.52%) when compared to HF,

whereas SG and WF had comparatively lower ash content than the HF.

In this study, lipid content was observed to be significantly higher in the milk of HF (5.89%) when compared to the other three breeds. The lowest percentage lipid was seen in SG (2.24%), while WF and RB had similar lipid compositions (4.171% and 4.233%, respectively).

Carbohydrate content ranged from 4.045-15.068%. No statistical difference was observed between carbohydrate content of HF and WF, while RB and SG milk samples had statistically lower carbohydrate contents than the HF (as shown in Figure 2).

The protein content of the milk samples ranged from 0.106-3.636%, as seen in Figure 2. All three breeds had significantly higher protein content as compared to the HF breed. The RB breed had the highest protein

content (3.633%), while the lowest protein content (0.106%) was observed in the milk sample of the HF breed.

As seen in Figure 2, no significant difference in moisture content was observed between the milk sample of HF and WF, while RB and SG had higher moisture content than the HF breeds. The moisture content ranged between 77.73 to 85.57%. The HF had the lowest moisture (i.e., 77.73%), while SG had the highest moisture content (85.57%).

### Mineral elements and heavy metals

The concentration of mineral elements and heavy metals in milk samples of four (4) cow breeds are presented in Table 1. This study showed that the selenium (Se) content ranges from 33.79-103.10 ppm, with HF having a significantly higher Se level than the other three cow breeds (i.e., RB, SG and WF). The zinc composition of the milk samples ranges from 0.02-0.16 ppm. The RB breed contained a significantly higher level of zinc compared to HF. The levels of zinc based on increasing order is as follows: HF (0.02 ppm) < WF (0.138 ppm) < SG (0.141 ppm) < RB (0.16 ppm). The iron content of milk samples ranges from 1.16-3.47 ppm. The milk of the RB breed had significantly higher iron levels (3.47 ppm) compared to the milk of other cow breeds. The copper content of milk samples analysed in the present study ranges from 0.049-0.191 ppm. RB had a significantly higher Cu content of 0.191 ppm than the WF and HF breeds. The level of manganese (Mn) found in the milk of the RB breed was significantly higher than that of HF and SG. Furthermore, this study observed no significant difference in the level of phosphorus (P) and chromium (Cr) in entire milk samples analysed. The Phosphorus levels in the milk of all four breeds were within the range of 2.49-2.53 ppm. The level of chromium ranges from 0.018 ppm to 0.024 ppm. Moreover, lead (Pb) was not detected in all four breeds analysed. Similarly, Cadmium (Cd) detected was within the ranges of 0.007-0.056 ppm.

### Vitamins

Vitamin A concentration of the analysed samples ranges from 13.85 to 55.38 mg/dl. White Fulani breed had significantly highest vitamin A level (55.38 mg/dl) while HF breed had significantly lowest vitamin A level (13.85 mg/dl) when compared to the other three breeds (Figure 3). The vitamin A levels in RB, SG and HF were 24 mg/dl, 16.91 mg/dl and 13.85 mg/dl, respectively. The concentration of vitamin C in milk samples is depicted in Figure 3. The vitamin C concentration ranges from 51.136 mg/dl to 79.924 mg/dl, with HF having a significantly lowest value (51.14 mg/dl) than the other breeds. The vitamin C in RB and HF are 59.09 mg/dl and 51.14 mg/dl, respectively. There is a significant disparity in the vitamin E contents of the cow milk analysed in the present study (Figure 3). The values range from 7.24-52.179 mg/dl. The SG (18.02 mg/dl) and WF (52.179 mg/dl) had significantly highest vitamin E composition than other cow breeds including HF (12.72mg/dl) control, while the vitamin E content of the RB breed (7.42 mg/dl) was lower than that of the HF breed.

### Antioxidant enzymes and antioxidant activity

The catalase concentration observed in the present study ranged from 0.009 to 0.015 U/L (Table 2). The WF breed had the highest catalase activity corresponding to 0.015 U/L. This was closely followed by RB (0.01476 U/L), then SG (0.0136 U/L). The lowest activity was exhibited by HF (0.0093 U/L). The activity of Superoxide dismutase (SOD) was detected in all milk samples except in the SG breed. The result indicated that WF had significantly higher SOD activity (3.00 U/mol) when compared to RB (0.165 U/mol) and the HF (0.119 U/mol) breed.

In this study, no statistical difference was observed between the DPPH values of all breeds. SG had the highest DPPH value (80.04%), followed by HF (77.53%), RB (77.46%) and the WF (77.12%) (Table 2). Similarly, the FRAP assay showed no significant difference among the breeds except

for SG (Table 2). The FRAP value for SG milk (1083.33mgTE/100g of FW) was statistically higher than that of the other breeds. FRAP value of the breeds in a decreasing order are as follows: SG (1083.33 mgTE/100g of FW) > RB (716.67 mgTE/100g of FW) > HF (516.67 mgTE/100g of FW) > WF (433.33 mgTE/100g of FW). The milk sample of HF had a significantly higher level of TPC when compared to that of the other three breeds (Table 2). This is in the order of HF (0.257 mgTE/100g of FW) > RB (0.253 mgTE/100g of FW) > WF (0.015 mgTE/100g of FW) > SG (0.014 mgTE/100g of FW).

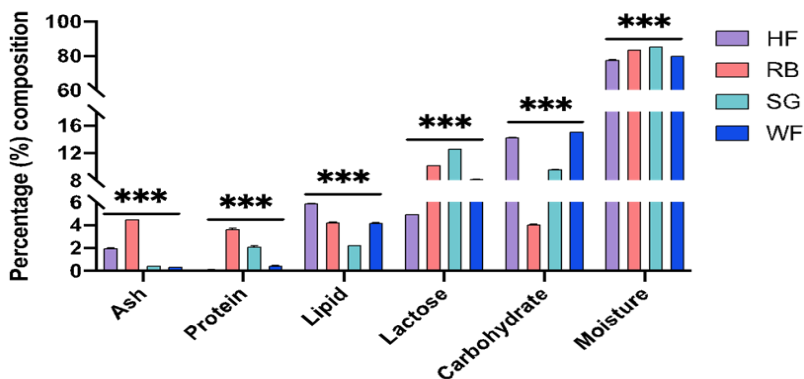
### Fatty acids profile

In this study, four (4) primary fatty acids, namely: stearic acid (C18:0), palmitic acid (C16:0), oleic acid (C18:1) and linoleic acid (C18:3), were evaluated. The outcome indicated that the milk samples contained higher amounts of palmitic acid and stearic acid than oleic and linoleic acid (Figure 4). The HF breed had significantly higher palmitic acid (98.39 mg/ml) composition as compared to both RB and SG, but with lower levels of stearic acid (21.20 mg/mL) than the other three breeds. The RB breed contained significantly higher levels of oleic acid than HF. Contrarily; the HF breed had a significantly higher level of linoleic acid when compared to both SG and WF.

### Amino acids profile

Amino acid profiles of HF, RB, SG and WF, are presented in Figure 5. The result showed that glutamic acid level was higher than other amino acids in all the milk samples of 4 breeds. This present finding indicates no significant difference ( $P < 0.005$ ) in the amino acid profile of the four breeds, except for histidine and leucine. Significantly higher levels of histidine were observed in the WF breed compared with SG breeds. Whereas leucine was significantly higher in the milk of the RB breed. The result of this present study also indicated that milk of RB breed contained the highest composition of essential amino acids such as lysine (7.27 g/100g protein), valine (6.58 g/100g protein), and tryptophan (1.49 g/100g protein).

In general, milk of the WF breed possesses the highest level of eight (8) amino acids (alanine, arginine, aspartic acid, glutamic acid, histidine, methionine, proline, and serine) so also the milk of RB breed had a high level of eight (8) amino acids namely: cysteine, glycine, leucine, lysine, threonine, tryptophan, tyrosine, and valine. Based on this finding, the milk of the RB breed contained a high level of 4 of these antioxidant amino acids (cysteine, lysine, tryptophan and tyrosine). In contrast to milk of WF breed with only 3 of these amino acids (arginine, histidine and methionine).



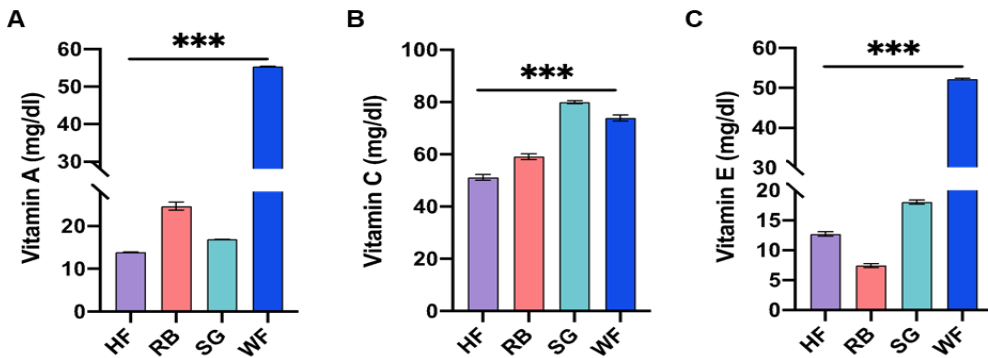
**Figure 2:** Proximate analysis of milk samples from different breeds of cows. Results are expressed as mean percentage  $\pm$  SD of triplicate measurements. Breeds of cow: Holstein Friesian =HF; Red Bororo =RB; Sokoto Gudali =SG; White Fulani =WF. Statistical significance: \*\*\*  $p < 0.001$  vs HF as positive control (One-way ANOVA with Dunnett posttest).



**Table 1:** Concentration of Mineral Elements and Heavy Metal in Milk Samples of Different Cow Breeds.

Minerals/metals	Concentration (ppm)			
	HF	RB	SG	WF
Selenium (Se)	103.103 ± 1.099 <sup>a</sup>	47.427 ± 1.283 <sup>b</sup>	45.033 ± 1.099 <sup>b</sup>	33.791 ± 2.894 <sup>c</sup>
Iron (Fe)	2.951 ± 0.048 <sup>a</sup>	3.474 ± 0.060 <sup>b</sup>	2.102 ± 0.033 <sup>c</sup>	1.157 ± 0.132 <sup>d</sup>
Copper (Cu)	0.049 ± 0.046 <sup>a</sup>	0.191 ± 0.032 <sup>b</sup>	0.142 ± 0.015 <sup>a,b</sup>	0.057 ± 0.062 <sup>a</sup>
Zinc (Zn)	0.024 ± 0.015 <sup>a</sup>	0.161 ± 0.043 <sup>b</sup>	0.141 ± 0.039 <sup>b</sup>	0.138 ± 0.051 <sup>b</sup>
Phosphorous (P)	2.514 ± 0.109 <sup>a</sup>	2.493 ± 0.135 <sup>a</sup>	2.530 ± 0.175 <sup>a</sup>	2.511 ± 0.040 <sup>a</sup>
Lead (Pb)	ND	ND	ND	ND
Chromium (Cr)	0.018 ± 0.020 <sup>a</sup>	ND	0.029 ± 0.024 <sup>a</sup>	ND
Manganese (Mn)	0.167 ± 0.017 <sup>a</sup>	0.364 ± 0.028 <sup>b</sup>	0.223 ± 0.014 <sup>a,c</sup>	0.284 ± 0.076 <sup>b,c</sup>
Cadmium (Cd)	0.056 ± 0.074 <sup>a</sup>	0.007 ± 0.001 <sup>a</sup>	0.014 ± 0.003 <sup>a</sup>	0.013 ± 0.002 <sup>a</sup>

Shows the mean ± S.D. of triplicate measurements. Values with different superscripts along the rows are significantly different ( $p < 0.05$ ), while values with the same superscript in a row are not significant ( $p > 0.05$ ) (Two-way ANOVA with Bonferroni posttest). Breeds of cow: Holstein Friesian, HF; Red Bororo, RB; Sokoto Gudali, SG; White Fulani, WF.

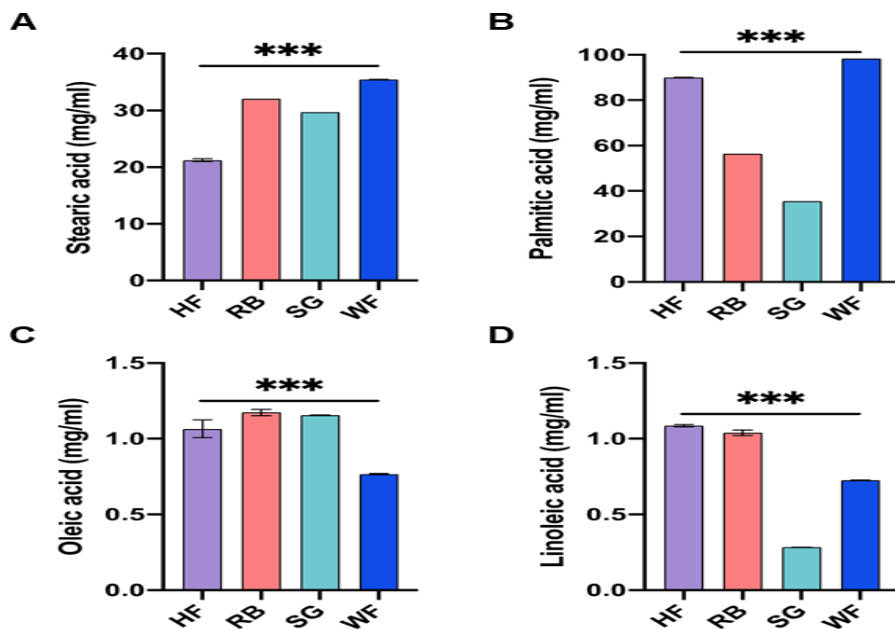


**Figure 3:** Concentration of antioxidant vitamins from different breeds of cows. (A) Vitamin A, (B) Vitamin C, and (C) Vitamin E. Results are expressed as mean ± SD of triplicate measurements. Breeds of cow: Holstein Friesian, HF; Red Bororo, RB; Sokoto Gudali, SG; White Fulani, WF. Statistical significance: \*\*\*  $p < 0.001$  vs HF as positive control (One-way ANOVA with Dunnett posttest).

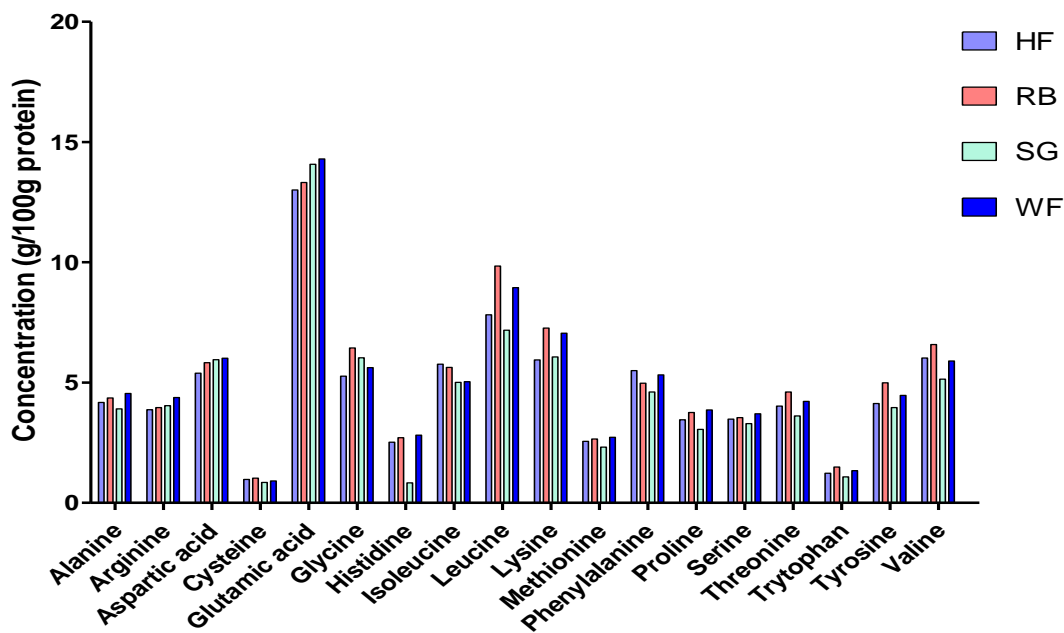
**Table 2:** Antioxidant Enzyme and Antioxidant Activity of Milk Samples from Different Cow Breeds.

Parameters	Activity/Concentration			
	HF	RB	SG	WF
Catalase (U/L)	0.009 ± 0.001 <sup>a</sup>	0.015 ± 0.001 <sup>b</sup>	0.014 ± 0.001 <sup>b</sup>	0.015 ± 0.002 <sup>b</sup>
SOD (U/mL)	0.119 ± 0.007 <sup>a</sup>	0.166 ± 0.005 <sup>b</sup>	ND	3.003 ± 0.003 <sup>c</sup>
DPPH (%)	77.530 ± 1.941 <sup>a</sup>	77.467 ± 0.821 <sup>a</sup>	80.037 ± 0.699 <sup>a</sup>	77.123 ± 3.014 <sup>a</sup>
FRAP (mgTE/100g FW)	516.667 ± 28.868 <sup>a</sup>	716.667 ± 125.831 <sup>a</sup>	1083.333 ± 225.462 <sup>b</sup>	433.333 ± 7.735 <sup>a</sup>
TPC (mgGA/100g FW)	0.257 ± 0.001 <sup>a</sup>	0.253 ± 0.001 <sup>b</sup>	0.158 ± 0.001 <sup>c</sup>	0.157 ± 0.001 <sup>c</sup>

Shows the mean ± S.D. of triplicate measurements. Values with different superscripts along the rows are significantly different ( $p < 0.05$ ), while values with the same superscript in a row are not significant ( $p > 0.05$ ) (Two-way ANOVA with Bonferroni posttest). Breeds of cow: HF = Holstein Friesian, RB = Red Bororo, SG = Sokoto Gudali, WF = White Fulani.



**Figure 4:** Fatty Acid concentration of milk samples from different breeds of cow. (A) Stearic acid, (B) Palmitic acid, (C) Oleic acid and (D) Linoleic acid. Results are expressed as mean  $\pm$  SD of triplicate measurements. Breeds of cow: HF = Holstein Friesian, RB = Red Bororo, SG = Sokoto Gudali, WF = White Fulani. Statistical significance: \*\*\*  $p < 0.001$  vs HF as positive control (One-way ANOVA with Dunnett posttest).



**Figure 5:** Amino Acid concentration (g/100g protein) of milk samples. Bars represent actual concentrations of amino acids from individual measurements. Breeds of cow: HF = Holstein Friesian, RB = Red Bororo, SG = Sokoto Gudali, WF = White Fulani.

## DISCUSSION

### Proximate Analysis

The lactose values obtained in the present study are higher than the result obtained by Dandare et al. (2014) for all the breeds except that of HF, which is slightly lower. Poulsen et al. (2012) revealed that typical cow milk comprises at least 4.9% lactose. The ash contents of the milk samples analysed had variations within the studied breeds. These outcomes were higher than the findings reported by Dandare et al. (2014) (0.73-0.97%) but lower than the values reported by Ajai et al. (2012). The variation in the ash composition may be due to genetic context, as Poulsen et al. (2012) reported that milk from different cow breeds possesses different composition profiles because of genetic background. The difference in ash content observed in the present study can be attributed to the lactation and nutritional status of the cows, as well as the seasons during which the experiments were conducted (Gaman and Sherrigton, 1998). Ash is an important inorganic residue as leftovers following the removal of water or organic substance by heating in the presence of an oxidising agent, thereby providing a quantity of the entire aggregate of minerals present in a product sample (Omola et al., 2019).

The fat content range for RB, WF and HF (4.23-5.89%) was a bit lower than what was previously reported by Dandare et al. (2014) (i.e., 5.96-6.80%). Furthermore, Adesina (2012) reported broad differences in milk fat composition among different breeds of cow. This variation in the fat content may be due to genetic differences or the physiological status of the cow breed (Frank, 1988). The milk of the WF breed had higher carbohydrate content of 15.07%, while RB had the lowest value of 4.05%. These outcomes are lower than the mean carbohydrate content of 9.10 to 22.27% in raw cow milk reported by Ajai et al. (2012). The range of protein content obtained falls within the permissible limit of 3% approved FAO (2004). This range is similar to the value (3.34±0.72%) reported by Adesina (2012) but lower than the range (30.38 to 42.72%) reported by Ajai et al. (2012).

The moisture contents obtained in this study are within FAO (2004) standards. The outcome conforms to the findings of Omola et al. (2019), who reported a range of between 77.12-88.00%. On the contrary, the moisture content observed in the present study is not in agreement with the report of Dandare et al. (2014) (81.33-83%). The microbial stability of milk depends on its moisture content (Enache et al., 2017). Enache et al. (2017) also reported that high moisture content is directly proportional to higher water activity which accelerates microbial growth, thereby reducing the shelf life of the milk sample. On the other hand, high moisture decreases lipid oxidation (Vu et al., 2020). Based on the assertion of Enache et al. (2017) and Vu et al. (2020), milk from SG (with the highest moisture content) would have the lowest shelf life, while that from HF (with the lowest moisture content) will have the highest shelf life. Furthermore, the present findings suggest that milk from SG may have lower lipid oxidation but might have a comparatively low shelf life due to potentially higher microbial activities.

### Mineral elements and heavy metals

Minerals are naturally occurring inorganic micronutrients essential for the normal biological activity of organisms. These antioxidant minerals serve as co-factors to numerous metalloenzymes such as glutathione peroxidase (e.g., Se), catalase (e.g., Fe), and superoxide dismutase (e.g., Cu, Zn, and Mn) that are critical in protecting cellular components from oxidative damage (Mcdowell et al., 2007). This study showed that the selenium (Se) content in the milk sample analysed ranges from 33.79-103.10 ppm. The milk of HF had significantly highest Se level compared to the milk from other three breeds. Selenium is a mineral element specifically related to antioxidant function (Mcdowell et al., 2007). At least 35 antioxidant Se proteins (such as selenoprotein P, glutathione peroxidases and thioredoxin reductases) detoxify harmful ROS and facilitate gene expression of other cytoprotective antioxidants (Nakamura, 2005).

The values of zinc obtained in this study are lower than the ranges previously reported by Ogabiela et al. (2011) and Omola et al. (2019). Zinc is an essential mineral that serves as a cofactor of more than 300 enzymes and 2000 transcription factors involved in gene regulation (Marreiro et al., 2017). Marreiro et al. (2017) also reported that zinc is a structural component of superoxide dismutase found in the cytoplasm of cells. This critical enzyme significantly modulates the function and activity of the antioxidant defence system. Zinc deficiency has been reported to induce a mutant-like conformation in superoxide dismutase, thereby inducing chronic endoplasmic reticulum stress (Marreiro et al., 2017).

The difference in iron content of the breeds in the present study conformed to studies of Adesina (2012) and Abbaya et al. (2020), who separately reported similar differences across different cow breeds. Iron is an essential co-factor for enzymes, including catalase - the antioxidant enzyme that converts hydrogen peroxide to water- and heme proteins involved in oxygen transport (Mcdowell et al., 2007; Adesina, 2012). CAT prevents cellular damage posed by free radicals. Iron (Fe) is critical element in the food of pregnant women, nursing mothers, infants, convulsing patients and elderly in order to prevent anaemia and other related diseases (Adjatin et al., 2013).

Copper is an essential micronutrient and an integral part of antioxidant enzymes (such as glutathione peroxidase and superoxide dismutase) that helps prevent oxidative stress and maintain antioxidant status and also plays an essential physiological role in foetal growth and early postnatal development (Omola et al., 2019).

The milk of RB breed has significantly highest level of manganese (Mn) than that of HF and SG which contradicts the findings of Ajai et al. (2012), who reported no significant difference in Mn concentration of milk samples from different cow breeds. Contrary to the report of Dandare et al. (2014), where they observed significant differences in the phosphorus levels among the animal breeds, no significant difference in

the phosphorus (P) and chromium (Cr) levels in milk samples were observed in this study. The levels of chromium were lower than the ranges of 0.78-1.58 ppm earlier reported by Omola et al. (2019). Chromium (Cr) is also involved in the breakdown and absorption of carbohydrates, fats, and proteins. Moreover, lead (Pb) was not detected in all four breeds analysed. This agrees with other studies conducted by Ogabiela et al. (2011) and Omola et al. (2019). Similarly, the level of cadmium (Cd) detected in the entire milk sample were within the ranges of 0.007-0.056 ppm, which is similar to the value of 0.03 g/L reported by Omola et al. (2019) but lowers than 1.63 mg/l obtained by Ogabiela et al. (2011).

#### **Antioxidant vitamins and enzyme activities**

Studies have shown that beta carotene acts as an oxygen radical scavenger, while vitamin A aids antioxidant defence systems against oxidative stress (Ma et al., 2005). The vitamin A content in the cow milk as observed can help to alleviate the symptoms of vitamin A deficiency as well as to contribute to daily vitamin A required by the body as reported by Cosmas et al. (2010). Vitamin C is the most important extracellular antioxidant that protects the biomembrane against lipid peroxidation (Mcdowell et al., 2007). Vitamin C also exerts its antioxidant action by acting as an enzyme co-factor, particularly for dioxygenases (iron-dependent enzymes) which are involved in numerous activities such as glucose metabolism, collagen synthesis, angiogenesis, iron homeostasis, cell survival and many other vital functions (Du et al., 2012). Vitamin E plays a vital role as a membrane-bound antioxidant, thereby trapping lipid peroxy free radicals produced from unsaturated fatty acids during oxidative stress (Mcdowell et al., 2007). Thus, Mcdowell et al. (2007) suggested that vitamin E is the first line of defence against peroxidation of phospholipids.

#### **Antioxidant enzymes and antioxidant activity**

Catalase is a heme-containing antioxidant enzyme that converts hydrogen

peroxide to oxygen and water. The catalase concentration observed in the present study was lower than the average catalase activity in cow milk (i.e., 0.86 units and 0.56-1.80 units, respectively) reported by Prajapati et al. (2017). Fox and Kelly (2006) suggested that catalase activity in milk is a good indicator of the udder health status of cows. SOD and CAT in milk originate from secretory cell membranes, somatic cells, plod plasma and/or fat globule membranes (Fox and Kelly, 2006). The enzymes' levels in milk correlate with inter and intra-species variations. More so, physiological status, feeding, stage of lactation, age, breed, and infection can affect the level/activity of these enzymes in milk samples (Fox, 2003; Fox and Kelly, 2006). Thus, these factors may be responsible for the variation of the enzymes' activities in the milk analysed in this present study. However, the low level of these activities is also a strong indication that the subjects are not diseased (Sharma et al., 2011).

To evaluate the antioxidant activity of the milk samples, 2,2-diphenyl-1-picrylhydrazine-hydrate (DPPH), ferric reducing antioxidant power (FRAP) and total phenolic content (TPC) methods were used. This is because antioxidants have different mechanisms of action, and no single standard quantitative technique can account for all the antioxidant activities (Karadag et al., 2009), therefore, different assay techniques will likely give different antioxidant outcomes/values (Sethi et al., 2020). DPPH is a stable free radical used to measure antioxidants' radical scavenging activity (Otohinoyi et al., 2014). It is one of the best generally used assays for the determination of the antioxidant capacity of milk and beverages (Khan et al., 2017). The higher the DPPH value, the greater the antioxidant function of the food sample. Cow milk can improve lipid metabolism, the oxidative status of the animals, and the performance of lactating cows by improving rumen metabolism (Alyaqoubi et al., 2014). The level and significance of antioxidant activity in cow milk are subject to the animal feed/diet quality (Stobiecka et al., 2022).

### **Fatty acids profile**

Cow milk is reported to contain an average of 60-70% saturated fatty acids, with palmitic acid (C16:0) accounting for about 30% by weight, while stearic acid (C18:0) account for 12% by weight (Mansson, 2008). Likewise, 25% of the fatty acids are mono-unsaturated, with oleic acid (C18:1) constituting about 23.8% by weight. The 2.3% of the total fatty acids in cow milk are poly-unsaturated fatty acids, in which linoleic acid (C18:2) accounts for 1.6% by weight (Mansson, 2008). Wang et al., (2007) reported that stearic acid could efficiently protect cortical neurons against oxidative stress by enhancing the activity of antioxidant enzymes such as SOD, glutathione peroxidase (GPx), and CAT. Fatty acid profile of the cow milk examined herein might have varied due to diverse factors that include animal origin (breed and selection), stage of lactation, lactation number, age of cow, seasonal and regional effect, rumen fermentation and most notably feed related factor (fibre, energy intake, dietary fats) which is reported to be responsible for 95% of the variation (Pietrzak-Fiecko et al., 2009).

### **Amino acids profile**

The level of glutamic acid ranged from 13.01 to 14.3 g/100g protein which concurs with the findings of Landi et al. (2021), which indicated that glutamic acid is the most abundant free amino acid in raw cow milk (approximately 43% of the total). Similarly, Sabahelkhier and Murwan (2012) reported higher levels of glutamic acid in cow milk than any other amino acids, thus suggesting that glutamic acid was the major non-essential amino acid in cow milk. This study observed that the milk of the WF breed had the highest concentration of glutamic acid and aspartic acid which conforms with a study by Abbaya et al. (2022), where WF cattle milk had the highest level of glutamic acid (11.77 g/100g). WF breed has the highest cattle population in Nigeria (Kubkomawa, 2017) and is the foremost milk producer in the country (Adesina, 2012). The milk of WF breed may be excellent for antioxidative response and as an immune booster due to substantial high

level of glutamic and aspartic acid, since glutamate, glutamine and aspartate are indicated to play a critical role in regulating gene expression, cell signalling, antioxidant responses and immunity (Wu, 2009). The findings of this study contradict the report of Adesina (2012), who observed low levels of glutamic acid ( $14.97 \pm 1.62$  g/100g protein) and aspartic acid ( $8.57 \pm 0.27$  g/100g protein) in the milk of WF breed when compared to RB and Muturu breeds. Similar to the findings of Abbaya et al. (2022), the result of this study also showed that milk of RB breed contained the highest composition of essential amino acids such as lysine, valine, and tryptophan.

The milk of the RB breed also had the highest amount of cysteine; thus the milk of the RB breed can be an excellent antioxidant booster since cysteine (sulphur-containing amino acid) plays a critical role in the intracellular biosynthesis of glutathione. The high level of valine, leucine, glycine, and threonine observed in RB conforms to the findings of Adesina (2012). Valine and leucine are branched-chain amino acids critical for body tissue maintenance, growth and repair, and prevention of several catabolic actions during exercise (Rafiq et al., 2016). Rafiq et al. (2016) reported that threonine is one of the most limiting amino acids in protein sources and is essential for metabolism and protein synthesis.

Both the milk of the WF and RB breed possesses the highest level of eight (8) amino acids each compared to the other 2 breeds (HF and SG). Xu et al. (2017) suggested that out of the 20 amino acids, seven (7) amino acids (namely: tryptophan, methionine, histidine, lysine, cysteine, arginine and tyrosine) are called antioxidant amino acids due to their strong total antioxidative activity, while the remaining 13 amino acids had low or almost no antioxidant activity. Based on this finding, the milk of the RB breed contained a high level of 4 of these antioxidant amino acids (cysteine, lysine, tryptophan and tyrosine). In contrast to milk of WF breed with only 3 of these amino acids (arginine, histidine and methionine). Thus, the RB's milk and that of the WF breed are good sources of antioxidant amino acids. The amino acid composition in

the milk of the four breeds is in the order: WF > RB > HF > SG. This finding is contrary to the report of Adesina (2012), who observed that the milk of the RB breed contained the highest total amino acids, followed by the Muturu breed and then the WF breed. Moreover, the findings agree with the report of Rafiq et al. (2016), and Abbaya et al. (2022). Additionally, variations in diet or feeding system, milking lactation, stage of lactation, animal health status and milking method can affect milk quality (Adesina, 2012; Rafiq et al., 2016). Cow milk is sufficient in amino acids, which play a distinctive role in human metabolism, provide substrate for protein synthesis and gluconeogenesis, suppressing protein catabolism and triggering muscle protein synthesis (Abbaya et al., (2022). Therefore, cow milk can play a significant role in building a healthy society while serving as a tool for rural development through the improvement of income and rural economic status (Rafiq et al., 2016).

## Conclusion

The antioxidant capacity of milk depends on the antioxidant components such as vitamins (A, E and C), minerals (such as phosphorus, selenium, and copper), as well as amino acids or proteins. The findings from this study indicated that milk of different cow breeds contained a significant amount of these antioxidant components, thereby serving as a great source of antioxidants. The milk of the RB breed contained the highest amino acid/protein and antioxidant mineral levels, while WF contained the highest antioxidant vitamin content. Similarly, the milk of WF possesses the highest activities of antioxidant enzymes (CAT and SOD) as well as ferric reducing antioxidant power (FRAP) followed by that of RB breed. Furthermore, the WF breed had a significantly higher level of stearic acid, which is a strong enhancer of antioxidant enzymes (SOD, GPx and CAT). This suggests that WF is a better source of antioxidants, but a mixture of WF and RB milk could offer a better nutritional value for the human diet. The difference in the nutritional composition of the milk samples is

most likely due to the difference in the genetic makeup of the different cattle breeds rather than environmental or nutritional factors since the animals were bred in an optimal environment and fed with uniform feed. There is a need to further study and understand the genes and genetic disparities responsible for the variation in milk quality among cow breeds with the aim of improving milk production and its quality.

### COMPETING INTERESTS

The authors have no competing interests to declare.

### AUTHORS' CONTRIBUTIONS

Conceptualisation: SUD, AAM, and UFM. Survey and project administration: AAM. Data processing, formal analysis, and software: SUD, AAM, UFM and NAN. Supervision, validation, and visualization: SUD, AAM, and UFM. Drafting – revision and editing: AAM, UFM, NAN, HHA, IA and SUD.

### ACKNOWLEDGEMENTS

The authors wish to thank Dr Andrew Onu and Rilwanu Ibrahim Yeldu a Senior Lecturer and Senior laboratory technologist respectively, at the Department of Biochemistry Usmanu Danfodiyo University Sokoto, Nigeria for their technical support. We also want to thank Mahmood Sidi (Sidi Akibu Dairy farm) and Sidi Ahmad Shehu for their help with milk samples.

### REFERENCES

Abbaya HY, Adedibu II, Kabir M, Iyiola-Tunji AO. 2020. Milk yield, composition and their correlated relationships in some selected indigenous breeds of cattle in late wet season of Adamawa state, Nigeria. *Nig. J. Anim. Prod.*, **47**(1): 47. 1-11.

Abbaya HY, Yohanna H, Jechoniah MB, Dauda A, Adamu J, Dubagari NG. 2022. Breed effect on milk amino acids profile of selected indigenous breeds of cattle in Nigeria. *Fudma Journal of Sciences*, **6**(2): 96-102. DOI:

<https://doi.org/10.33003/fjs-2022-0602-886>

Adjatin A, Dansi A, Badoussi ME, Sanoussi F, Dansi M, Azokpota P, Ahissou H, Akouegninou A, Koffi A, Sanni A. 2013. Proximate, mineral and vitamin C composition of vegetable Gbolo [*Crassocephalum rubens* (Juss. ex Jacq.) S. Moore and *C. crepidioides* (Benth.) S. Moore] in Benin. *International Journal of Biological and Chemical Sciences*. **7**(1): 319-331. DOI: 10.4314/ijbcs.v7i1i.27.

Adesina K. 2012. Effect of Breed on the Composition of Cow Milk under Traditional Management Practices in Ado-Ekiti, Nigeria. *J. Appl. Sci. Environ. Manage.* **16**(1): 55 – 59.

Aebi H. 1984. Catalase *in vitro*. *Methods in Enzymology* (2nd Edn), Packer L (Ed.). Academic Press; 121–126.

Ajai AI, Ochigbo SS, Ndamitso MM, Ola OJ. 2012. Proximate and Mineral Composition of Different Raw Cow's Milk in Minna. *European Journal of Applied Engineering and Scientific Research*, **1**(1): 23-29.

Alyaqoubi S, Abdullah A, Addai ZR. 2014. Antioxidant activity of goat's milk from three different locations in Malaysia. AIP Publishing, 1-5. DOI: 10.1063/1.4895195

Amal H, Hamza. 2017. *Vitamin C: An Antioxidant Agent*. Publishing Process Manager, edi Lipovic; 1-29.

AOAC. 2000. *Official Methods of Analysis* (17th Edition). The Association of Official Analytical Chemists, Gaithersburg, MD, USA. Methods 925.10, 65.17, 974.24, 992.16.

AOAC (Association of Official Analytical Chemicals). 2006. *Official Method of Analysis of the AOAC* (18th Edition), Horwitz W (Ed.). AOAC: Washington D. C.

Baker H, Frank O. 1968. Determination of serum tocopherol. In *Clinical Biochemistry* (6th edition). Eimememenn Medical Books: London; 902-903.

Bassey OA, Lowry OH, Brock MJ, Lopez JA. 1946. The determination of vitamin A

- and carotene in small quantities of blood serum. *J. Biochem.*, **234**(166): 177-188.
- Bhatti M, Peter SJ, John E. 2006. Determination of trace element using Unicam 969 Atomic absorption Spectrophotometer. *Ann. Inter. Med.*, **205**(1): 96-105.
- Cacho NT, Lawrence RM. 2017. Innate Immunity and Breast Milk. *Frontiers in Immunology*, **8**: 584. DOI: <https://doi.org/10.3389/fimmu.2017.00584>
- Commonwealth Scientific and Industrial Research Organization (CSIRO). 2020. Dairy production in Nigeria. Retrieved Dec 27, 2021, from: CSIRO- Dairy production in Nigeria.
- Cosmas U, Ujowundu F, Ezim O, Agha N, Alisi C, Nwaoguikpe R. 2010. Evaluation of the chemical composition of *Dacryodes edulis* (G. Don) seeds. *International Journal of Biological and Chemical Sciences*, **4**(4): 1225-1233. DOI: 10.4314/ijbcs.v4i4.63058.
- Dandare SU, Ezeonwumelu IJ, Abubakar MG. 2014. Comparative analysis of nutrient composition of milk from different breeds of cows. *European Journal of Applied Engineering and Scientific Research*, **3**(2): 33-36.
- Du J, Cullen JJ, Buettner GR. 2012. Ascorbic acid: chemistry, biology and the treatment of cancer. *Biochimica et Biophysica Acta*, **1826**(2): 443-457. DOI: <https://doi.org/10.1016/j.bbcan.2012.06.003>
- Enache E, Podolak R, Kataoka A, Harris LJ. 2017. Persistence of *Salmonella* and other bacterial pathogens in low-moisture foods. In *Control of Salmonella and other Bacterial Pathogens in low Moisture Foods*, Podolak R, Black DG (eds). John Wiley & Sons, Ltd: Chichester, UK; 67-86.
- Food and Agriculture Organization of the United Nations (FAO). 2020. Milk Facts. Retrieved Dec 27, 2021, from: Milk Facts (fao.org).
- Fox P, Kelly A. 2006. Indigenous enzymes in milk: Overview and historical aspects—Part 1. *International Dairy Journal*, **16**(6): 500-516. DOI: <https://doi.org/10.1016/j.idairyj.2005.09.013>.
- Fox P. 2003. Indigenous Enzymes in Milk. Advanced Dairy Chemistry—1 Proteins. Berlin, Germany: Springer. Elena Franco-Robles and Joel Ramírez-Emiliano, IntechOpen. DOI: 10.5772/intechopen.88212. Available from: <https://www.intechopen.com/chapters/68503>
- Frank O. 1988. Rural Dairy Technology Experience in Ethiopia. ILCA Manual.4: 1- 10.
- Fructuoso I, Romao B, Han H, Raposo A, Ariza-Montes A, Araya-Castillo L, Zandonadi RP. 2021. An overview on nutritional aspects of plant-based beverages used as substitutes for cow's milk. *Nutrients*, **13**(8): 2650. DOI: <https://doi.org/10.3390/nu13082650>
- Gaman PM, Sherrington KB. 1998. *The Science of Food: An introduction to Food Science Nutrition and Microbiology* (2<sup>nd</sup> edition). Pergamon Press: Oxford; 196-215.
- Karadag A, Ozelcik B, Saner S. 2009. Review of methods to determine antioxidant capacities. *Food Analytical Methods*, **2**(1): 41-60. DOI: <http://dx.doi.org/10.1007/s12161-008-9067-7>.
- Khan IT, Nadeem M, Imran M, Ayaz M, Ajmal M, Ellahi MY, Khaliq A. 2017. Antioxidant capacity and fatty acids characterization of heat-treated cow and buffalo milk. *Lipids in Health and Disease*, **16**(1): 163. <https://doi.org/10.1186/s12944-017-0553-z>
- Kubkomawa HI. 2017. Indigenous Breeds of Cattle, their Productivity, Economic and Cultural Values in Sub-Saharan Africa: A Review. *International Journal of Research Studies in Agricultural Sciences (IJRSAS)*, **3**(1): 27-43. DOI: <http://dx.doi.org/10.20431/2454-6224.0301004>



- Landi N, Ragucci S, Di Maro A. 2021. Amino Acid Composition of Milk from Cow, Sheep and Goat Raised in Ailano and Valle Agricola, Two Localities of 'Alto Casertano' (Campania Region). *Foods (Basel, Switzerland)*, **10**(10): 2431. DOI: <https://doi.org/10.3390/foods10102431>.
- Ma X, Yang Z, Yang W, Song M. 2005. Effect of different vitamin A levels in diets on antioxidant ability of beef cat\_tle (in Chinese). *Chin J Anim Nutr*, **17**: 31–35.
- Mansson HL. 2008. Fatty acids in bovine milk fat. *Food & nutrition research*, **52**. DOI: <https://doi.org/10.3402/fnr.v52i0.1821>
- Marreiro, DD, Cruz, KJ, Morais, JB, Beserra, JB, Severo, JS, & de Oliveira, AR. 2017. Zinc and Oxidative Stress: Current Mechanisms. *Antioxidants (Basel, Switzerland)*, **6**(2): 24. DOI: <https://doi.org/10.3390/antiox6020024>
- Mcdowell L, Wilkinson N, Madison R, Felix T. 2007. Vitamins and minerals functioning as antioxidants with supplementation considerations.
- Nakamura H. 2005. Thioredoxin and its related molecules: update 2005. *Antioxidants & Redox Signaling*, **7**(5-6): 823–828. DOI: <https://doi.org/10.1089/ars.2005.7.823>
- Ogabiela EE, Udiba UU, Adesina OB, Hammuel C, Ade-Ajayi FA, Yebpella GG, Mmereole UJ, Abdullahi M. 2011. Assessment of metal levels in fresh milk from cows grazed around Challawa Industrial Estate of Kano, Nigeria. *Journal of Basic and Applied Science Resources*, **1**(7): 533-538.
- Omola EM, Kawo, AH, Ahmadu Bukar. 2019. Evaluation of nutritional quality and mineral composition of fermented milk (nono) sold in Kano, north-western Nigeria. *Bayero Journal of Pure and Applied Sciences*, **12**: 81-88. DOI: 10.4314/bajopas.v12i2.11.
- Otohinoyi D, Ekpo O, Ibraheem O. 2014. Effect of ambient temperature storage on 2,2-diphenyl-1-picrylhydrazyl (DPPH) as a free radical for the evaluation of antioxidant activity. *International Journal of Biological and Chemical Sciences*. **8**(3): 1262-1268. DOI: 10.4314/ijbcs.v8i3.39.
- Pecka-Kiełb E, Czerniawska-Piątkowska E, Kowalewska-Luczak I, Vasil M. 2018. Polymorphism in ovine ANXA9 gene and the physio-chemical properties and the fraction of protein in milk. *Journal of the Science of Food and Agriculture*, **98**(14): 5396–5400. DOI: <https://doi.org/10.1002/jsfa.9081>
- Pietrzak-Fiecko R, Tomczyński R, Świstowska A, Borejszo Z, Kokoszko E, Smoczyńska K. 2009. Effect of mare's breed on the fatty acid composition of milk fat. *Czech Journal of Animal Science*, **54**(9): 403-440. DOI: 10.17221/1683-CJAS.
- Pietrzak-Fiecko R. 2018. Relationship Between the Content of Chlorinated Hydrocarbons and Fatty Acid Composition of Milk Fat. *Journal of veterinary research*, **62**(1): 71–78. DOI: <https://doi.org/10.1515/jvetres-2018-0010>
- Poulsen NA, Gustavsson F, Glantz M, Paulsson M, Larsen LB, Larsen MK. 2012. The influence of feed and herd on fatty acid composition in 3 dairy breeds (Danish Holstein, Danish Jersey, and Swedish Red). *Journal of dairy science*, **95**(11): 6362–6371. DOI: <https://doi.org/10.3168/jds.2012-5820>
- Prajapati DB, Kapadiya DB, Jain AK, Mehta BM, Darji VB, Aparnathi KD. 2017. Comparison of Surti goat milk with cow and buffalo milk for physicochemical characteristics, selected processing-related parameters and activity of selected enzymes. *Veterinary World*, **10**(5): 477–484. DOI: <https://doi.org/10.14202/vetworld.2017.477-484>
- Rafiq S, Huma N, Pasha I. 2016. Chemical Composition, Nitrogen Fractions and Amino Acids Profile of Milk from Different Animal Species. *Asian-Australian Journal of Animal Science*, **29**(7): 1022–1028. DOI: 10.5713/ajas.15.0452.
- Sabahelkhier G, Murwan A. 2012. Amino Acid Composition of Human and

- Animal's Milk (Camel, Cow, Sheep and Goat). *ARPN Journal of Science and Technology*, **2**(2): 32-34.
- Sethi S, Joshi A, Arora B, Bhowmik A, Sharma RR, Kumar P. 2020. Significance of FRAP, DPPH, and CUPRAC assays for antioxidant activity determination in apple fruit extracts. *European Food Research and Technology*, **246**(3): 591–598. DOI: <https://doi.org/10.1007/s00217-020-03432-z>
- Shankar AH, Prasad AS. 1998. Zinc and immune function: the biological basis of altered resistance to infection. *The American Journal of Clinical Nutrition*, **68**(2): 447S-463S. DOI: <https://doi.org/10.1093/ajcn/68.2.447S>.
- Sharma N, Singh NK, Singh OP, Pandey V, Verma PK. 2011. Oxidative stress and antioxidant status during transition period in dairy cows. *Asian-Australasian Journal of Animal Sciences*, **24**(4): 479-484. DOI:10.5713/ajas.2011.10220
- Stobiecka M, Król J, Brodziak A. 2022. Antioxidant Activity of Milk and Dairy Products. *Animals: an open access journal from MDPI*, **12**(3): 245. DOI: <https://doi.org/10.3390/ani12030245>
- Tibi KN, Aphunu A. 2010. Analysis of Cattle Market in Delta State: The Supply Determinants. *African Journal of General Agriculture*, **6**(4): 199-203.
- Toko RC, Dahouda M, Gbaguidi F, Soumanou S. 2011. Diet supplement effect based on cottonseed meal and Vitellaria paradoxa leaves on Borgou cows performance in rainy season. *International Journal of Biological and Chemical Sciences*. **4**(6): 2427-2432: DOI: 10.4314/ijbcs.v4i6.64950.
- Velikova V, Yordanov I, Edreva A. 2000. Oxidative stress and some antioxidant systems in acid rain-treated bean plants: Protective role of exogenous polyamines. *Plant Science*, **151**(1): 59–66. DOI: 10.1016/S0168-9452(99)00197-1
- Vu TP, He L, McClements DJ, Decker EA. 2020. Effects of water activity, sugars, and proteins on lipid oxidative stability of low moisture model crackers. *Food Research International*, **130**: 108844. DOI: <https://doi.org/10.1016/j.foodres.2019.108844>
- Wang Y, Yu R, Chou C. 2006. Antioxidative activities of soymilk fermented with lactic acid bacteria and bifidobacteria. *Food Microbiol.*, **23**: 128–135. DOI: 10.1016/j.fm.2005.01.020.
- Wang ZJ, Liang CL, Li GM, Yu CY, Yin M. 2007. Stearic acid protects primary cultured cortical neurons against oxidative stress. *Acta pharmacologica Sinica*, **28**(3): 315–326. DOI: <https://doi.org/10.1111/j.1745-7254.2007.00512.x>
- Wu G. 2009. Amino acids: metabolism, functions, and nutrition. *Amino Acids*, **37**: 1–17. DOI: <https://doi.org/10.1007/s00726-009-0269-0>
- Xu N, Chen G, Liu H. 2017. Antioxidative Categorization of Twenty Amino Acids Based on Experimental Evaluation. *Molecules*, **22**(12): 2066. DOI: 10.3390/molecules22122066.
- Zhang J, Hu B. 2013. Liquid-Liquid Extraction (LLE). Separation and Purification Technologies in Biorefineries. Wiley Online Library; 61–78. DOI: 10.1002/9781118493441.ch3
- Zhao Y, Tan Y, Dai J, Li B, Guo L, Cui J, Wang G, Shi X, Zhang X, Mellen N, Li W, Cai L. 2011. Exacerbation of diabetes-induced testicular apoptosis by zinc deficiency is most likely associated with oxidative stress, p38 MAPK activation, and p53 activation in mice. *Toxicology Letters*, **200**(1–2): 100–106. DOI: <https://doi.org/10.1016/j.toxlet.2010.11.001>