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Antibacterial activity of papain hydrolysates of isoelectrically-isolated casein and thermoprecipitated alpha-lactalbumin from bovine and caprine milk on diarrheagenic bacteria

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Abstract: The study compared antibacterial potential of hydrolysates of casein and alpha-lactalbumin from cow and goat milk on diarrhea-causing *Escherichia coli* and *Staphylococcus aureus*. Milk samples were aseptically obtained from lactating cows and goats. The samples were skimmed; casein was isolated using acetic acid and alpha-lactalbumin by filtrate thermoprecipitation at 75°C. 50% of each isolate was reconstituted in a buffer and hydrolyzed with papain at 55°C for 2 hours. The hydrolysates were heated to 75°C to inactivate papain, cooled and their antibacterial activity determined by disc diffusion method. Results showed alpha-lactalbumins had higher degrees of hydrolysis and antibacterial activity than caseins; goat alpha-lactalbumin had the highest antibacterial activity with mean inhibition zones of 19.60mm and 19.50mm on *E. coli* and *S. aureus*. Cow alpha-lactalbumin inhibited *E. coli* more than *S. aureus* with inhibition zones of 16.80mm and 12.50mm. Cow and goat milk casein hydrolysates inhibited *E. coli* with mean inhibition zones of 8.00mm and 10.90mm and inhibited *S. aureus* with inhibition zones of 4.13mm and 1.90mm respectively. The research showed that the milk hydrolysates can be a source of antibiotics for diarrhea treatment. Research should be done to establish the peptide fractions associated with the observed bioactivity.

Keywords: Assay, diarrhea, isolate, hydrolysis, proteins, inhibition zone.

1. Introduction

Human life menacing infections such as tuberculosis, influenza, pneumonia, dysentery, diarrhea, cholera and typhoid has left intolerable death toll worldwide. Diarrhea, particularly is a grave justification of infantile deaths. According to WHO records, close to 5 billion medical incidences of diarrheic infections are annually reported globally, wherein infants get diarrheagenic infections up

to thrice a year [1]. Gross diarrhea-related mortality, approximated at 1.26 million in 2013 from 2.58 million in 1990, was the penultimate most common justification of infantile deaths in the 2012 global medical data [1] . Diarrhea is a medical state wherein an individual experience more than two loose excrements diurnally which is medically deemed aberrant. Diarrhea may be acute, persistent, chronic, osmotic, secretory, exudative or inflammatory. Diarrhea can be surpassed through maintenance of good sanitation, taking boiled or treated water and vaccinal inoculation against rotavirus. Use of oral rehydration salts (ORS), Zinc tablets and antibiotics such as nitazoxanide can treat diarrhea [1]. Albeit protozoic and viral causes, serious diarrhea is often caused by bacteria namely; *Campylobacter*, *Clostridium difficile*, diarrheagenic *Escherichia coli, Bacillus cereus, Clostridium perfringens, Staphylococcus aureus* and *Salmonella enterica.* Regrettably, the otherworldly rate at which these causative microbes have presented sturdy resistance to synthetic drugs is a prognosticatory signification that the world is soon getting thrown into a post-antibiotic era hence the need to search for novel antibiotics with new modes of action.

Milk, a translucent white heterogeneous mixture of lacteal secretion by mammary glands of lactating mammals is bound with biomolecules (chiefly water, proteins, carbohydrates, vitamins, minerals and lipids) [2] indispensable for sustenance of neonate life through its various biological, chemical, physiological and functional activities [3]. Water, the chief composition of milk, is the milieu for other polar milk components. Two distinct phases of milk globular proteins can be appreciated; casein complexes and a soluble serum fraction of whey proteins which are representatively 80% [4]and 20% of the total bovine milk proteins respectively [5] . These often fold into compact, virtually spheroidal units unimpededly dispersible in water. The casein fraction, disorganized structurally, is hesitant to thermodenaturation. The calcium-casein phosphoprotein assemblage bestows the characteristic white and opaque appearance of milk when clumped in clusters as micelles. The amino acid sequence in casein micelles possess hydrophobic and hydrophilic regions, hence the assemblage is a multidispersed surfactant system of spheroidal aggregates with quadradic subunits differing significantly in molecular weight, isoelectric point (pI) and phosphate groups. The quadrature is strictly only electrophoretically differentiable as alpha s1 (αs1), alpha s2 (αs2), beta (β) and kappa (κ) caseins in order of their decreasing degree of motion at pH 7.0.1.[6]. Similarly, neither beta nor alpha casein is singly soluble in milk, nor in fusion, though inclusion of kappa casein to one or both, leads to a soluble complexation due to micelle formation [7]. Addition of an acid to casein causes colloidal calcium hydroxyphosphate in the casein micelle to solubilize as exemplified in natural milk souring. Whey proteins, the filtrate after casein isolation from skimmed milk, is a heat labile family of globular milk proteins composed predominantly of beta lactoglobulin (β-lg), alpha-lactalbumin (α-la), serum albumin (SA), immunoglobulins (IGs), folate-binding protein, lactoferrin (LF), lactoperoxidase, transferrin, ferritin, proteose peptone, calmodulin (calcium binding protein) and prolactin [8]. Alpha lactalbumin, more frequently called lactalbumin (LALBA), is the second most predominant whey protein after beta lactoglobin biosynthesized from a code transcribed from the LALBA gene with translation in primates upregulated by elevated prolactin levels that successively

upregulates lactose synthesis. It constitutes the regulatory subunit of the β-1,4-galactosyltransferase allosteric effector thus enhances lactose synthesis via carryover of galactose moieties to glucose [9]. Serum albumin is not biosynthesized in the mammary glands but rather carried over from the mother's blood to milk. Immunoglobulins, the protein complexes fused by the B lymphocytes, are functionally immunologic [9]. The amino acid profile of casein and whey protein fractions vary significantly between mammals and this will dictate their ease of proteolysis [10]. The carbohydrate profile of milk is dominated by lactose(4-O-(β-D-galactopyranosyl)-D-glucopyranose) which is fully miscible in water. Lactose is a disaccharide composite of two renown monosaccharides (glucose and galactose) responsible for the saccharine taste and colligative attributes of milk such as osmotic pressure, boiling point elevation and freezing point depression. Milk also contain vitamins: A, B_1, B_2, B_3, B_{12}, B_5, B_6, C and D. The minerals bound in milk include calcium, potassium, sodium, phosphorus, iodine, magnesium, zinc, potassium, sodium, chloride, iron, selenium, copper and fluoride [5]. Milk is an emulsion of fat globules in spatial containment of an aqueous fluid wherein every globule is beset by phospholipid-proteinaceous membrane emulsifiers, inhibiting globular patch up into noticeable fat grains and which protect the fats from lipases in the milk serum. Milk fat, secreted in the mammary epithelial cells as fat globules, houses fat-miscible vitamins: D, A, K and E, phospholipids, tri-, di- and monoacylglycerols, cerebrosides, gangliosides, sterols, their esters and derivatives, carotenoids, tocopherol and free fatty acids. Other insignificant milk constituents include enzymes (lipoprotein lipase, lactoperoxidase, xanthine oxidase, alkaline phosphatase) and pigments [**Error! Reference source not found.**].

Milk is inherently antimicrobial purposely to furnish neonate protection [11]. In the mammary glands, milk is incorporated with immense immunity factors, including immunoglobulins fetched from the mammalian mother's blood [12]. Lactoferrin and enzymes (lysozyme and lactoperoxidase) are antimicrobials empirically reported in raw milk [11]. The former antibacterially secludes iron while the latter interjects microbial cell walls by enhancing their porosity [12]. Lahov and Regelson [13] obtained isracidin from bovine αS1-casein segment 1-23 proteolyzed by chymosin and reported it is antibacterial against *lactobacilli,* Gram-positive and Gram-negative bacteria with most Gram-positive bacteria inhibited by aliquots between 0.1mg/mL to 1mg/mL. Murphy and Meullenet [14] in another investigation reported that iscradin was defiant against infectious *Listeria monocytogenes* and *S. aureus*. Otani and Suzuki [15] isolated isracidin from chymosin-mediated neutral pH digestion of αs1-casein. The biopeptide reportedly exhibited antifungal action on *Candida albicans,* inhibited *Lactobacilli, Staphylococcus species (sp), Sarcina sp, Bacillus subtilis, Diplococcus sp, pneumoniae sp* and *Streptococcus pyogenes* with minimum inhibitory concentration (MIC) between 0.1 to 1mg/mL. Birkemo *et al* [16] also reported that isracidin is antibacterial *in vivo* against *E. coli* DPC6053 with a MIC of 0.2 mg/mL. Furthermore, casecidin 15 and 17 on the C-terminal of bovine β-casein from acidified colostrum reportedly had a MIC of 0.4 mg/mL against the test *E. coli* DPC6053 [13]. Hayes *et al* [17] identified fragments of αs1-casein, caseicin A, B and C from bovine milk αS1-casein soured using *Lactobacillus acidophilus* DPC6026 that manifested bacteriostatic potential against *Enterobacter*

sakazakii. Caseicin A, B and C suppressed the growth of *E. coli* DPC5063 with MIC of 52µg/ml, 0.22µg/ml and 1.48mg/ml respectively stated [17]. Recio and Visser [18] proteolyzed successively bovine αS1-casein and αS2-casein with proteases: pepsin, trypsin, alpha-and beta-chymotrypsin. The hydrolysates suppressed the growth of a list of Gram bacteria. Zucht *et al* [19] isolated and identified a cationic biopeptide from acidified bovine milk. The segment 165-203, called casocidin-I, was proven antibacterial against *Staphylococcus carnosus* and *E. coli* [19]. Minervini *et al* [20] isolated casecidin 15 and 17 from acidified colostrum of bovine milk beta-casein that suppressed the growth of a spectrum of Gram bacteria including potentially pathogenic strains of daily increasing clinical importance such as *E. coli* (with MICs of 0.4mg/mL), *Enterococcus faecium*, *Bacillus megaterium* and *Yersinia enterocolitica*. The 26-amino acid peptide housed a copious content of non-polar residues, that hampered further proteolysis and thus had limited antibacterial potential [20].

Thoma-Worringer *et al* [21] isolated caseinomacropeptide following proteolysis of bovine milk κ-casein and tested its bacteriostatic potential on *Streptococcus mutans*, *Streptococcus sanguinis* and *Streptococcus sobrinus.* The biopeptide repressed the adherency of *S. mutans*, *S. sanguis* and *S. sobrinus* to the mouth and controlled the congruity of dental microbial flora. An antibacterial pentapeptide, kappa-casecidin from a tryptic digest of bovine milk kappa-casein suppressed the growth of *S. aureus*, *E. coli* and *Salmonella typhimurium* as reported by Tidona *et al* [22]. Malkoski *et al* [23] isolated glycomacropeptide (GMP), a hirsute stretch on casein micelle chymosin proteolyzed from κappa-casein in cheese processing. The GMP segment (polar residues 106–169), comprising roughly 15-20% of the gross whey protein content inhibited *Streptococcus mutans*, *Porphyromonas gingivitis* and *E. coli* effectively [24]. Rutherford-Markwick and Moughan [25] isolated GMP and tested it on *Vibrio cholerae* and *E. coli* enterotoxins which were bound by GMP, a mimicry of enterotoxin binding carbohydrate structures reported in literature for cell receptors. Lopez *et al* [26] isolated six biopeptides from a peptic digest of kappa-casein and reported they had remarkable antibacterial potential *in vivo* against *Listeria innocua* and *Salmonella carnosus.* da Costa *et al* [27] hydrolyzed Ethawah breed goat milk αS2-casein and tested the digests against *Listeria monocytogenes, Bacillus cereus, Salmonella typhi* and *Shigella flexneri.* The digests inhibited the bacteria with optimal concentrations of 5mg/ml (milligram per milliliter). McCann *et al* [28] isolated isracidin from caprine milk αs1-casein that demonstrated antibacterial potential against *E. coli*.

The search for novel antibacterial agents paid due attention to bovine milk with little attention to other mammalian milk proteins. This study reported the digestibility of bovine and caprine milk casein and alpha lactalbumin by papain and their antibacterial potential on two diarrheagenic bacteria*: E. coli* and *S. aureus.*

2. Materials and Methods

2.1 Reagents and apparatus

The chemicals used in this investigation were of high analytical purity. The assortment of volumetric glassware used in the experiment was presterilized in an autoclave at 121°C for 15 minutes and oven dried prior to analysis. Mettler PM200 digital analytical balance (Marshall scientific, Hampton, New Hampshire, USA) was used for all weighings. Hanna 211 digital microprocessor-based bench top pH/mV/°C meter (Hanna instruments, Italy) precalibrated using pH 4.01, pH 7.01 and pH 10 buffers was used for all pH measurements.

2.2 Sampling procedure, sample size and sample preparation

3 Litres of fresh cow milk sample was aseptically collected in triplicate in sterilized sample containers from Kyambogo University Farm, Kyambogo University, Kampala-Uganda from healthy lactating cows (*Bos taurus)* milked under clean sanitary conditions on Monday, 15th May 2017. Goat milk (3 litres) was aseptically obtained in triplicate from lactating Saanen goats (*Capra aegagrus hircus*) milked under clean sanitary conditions on the same date from Bloom for Saan Farm, Mukono, Uganda. The pooled milk samples at 4℃ were taken to Uganda Industrial Research Institute (UIRI), Department of Biotechnology and Product development for skimming, isolation of casein and alpha-lactalbumin. Papain (papaya proteinase I) used for proteolysis was obtained from the same Department.

2.3 Preparation of samples

Skimmed milk (plasma phase of milk) was prepared by low speed centrifugation (using NIX-521, Tomy Kogyo Co. Ltd, Tagara, Nerima-Ku, Tokyo, Japan) of pooled whole milk samples at 5000 rotations per minute (rpm) for 15 minutes. Exactly 150ml of skimmed milk samples in 250ml beakers were warmed on a water bath to bring the temperature to 40°C. 1M acetic acid solution was added to the warmed solutions drop by drop while constantly stirring until a pH of 4.6 (isoelectric point) was attained. The beakers were kept on a serological water bath maintained at 40°C until no observable precipitation occurred and subsequently filtered through a cheese cloth. The residues were labelled as casein isolates from cow (CMC) and goat (GMC) milk respectively. The filtrates obtained were heated to 75°C for 5 minutes to produce precipitates that were filtered from the hot solutions through Whatmann No.1 filter papers (Sigma-Aldrich, US) to produce cow milk alpha-lactalbumin (CMAL) and goat milk alpha lactalbumin (GMAL) isolates.

An aliquot (50% of total solid) of each non-fat milk protein isolate was reconstituted in pH 6.5 phosphate buffer for optimal proteolysis [29]. The isolates were heated in a water bath for 5 minutes until complete dissolution. The resultant solutions were then hydrolyzed with papain in an optimized enzyme to substrate ratio of 1:100 w/v at 55°C in a water bath for 2 hours. The pH of the solutions was measured at 1-hour intervals. After 2 hours, the hydrolysates were heated to 75°C for 10 minutes to inactivate papain. The hydrolysates were cooled to room temperature and centrifuged in a refrigerated centrifuge at 10,000 rpm for 30 minutes. The supernatants were subsequently dispensed in sterile sample bottles and finally transferred to a refrigerator at -4°C awaiting antibacterial assay.

2.4 Determination of degree of hydrolysis

The degree of hydrolysis (DH) of the hydrolysates after 2-hour proteolysis was obtained as percentage of soluble milk protein in 10% (w/v) trichloroacetic acid (TCA) vis-a-vis the total protein content of the sample according to Hoyle and Merritt [30]. Pipetted 500µL of the hydrolysates were vortexed with equivalent volumes of 20% (w/v) TCA and allowed to stand for half an hour followed by low speed centrifugation at 3000rpm for 20 minutes, and the soluble protein content of the supernatants was obtained using a modified analytical procedure previously employed elsewhere by Hartree [31]. Total protein content (TPC) of the hydrolysates were quantified using Kjeldahl method and the DH was computed as the numerical quotient of the solubilized protein in TCA to the total protein content in miligrams expressed as a percent [29].

2.5 Bacterial cultures

The diarrheagenic bacteria used in this investigation were obtained and identified from Department of Food Processing Technology Laboratory, Kyambogo University-Kampala, Uganda.

2.51 Preparation of bacterial media

Molecular biology grade bacterial media used were prepared following standard guidelines according to their respective manufacturers. Exactly 12.0g of Nutrient agar powder (Stratech, U.K) was weighed and dissolved in 500ml of distilled water in a 500ml beaker. It was mixed thoroughly using a sterilized stirrer and dissolved by heating with frequent agitation until complete dissolution. The solution was dispensed into a 500ml bottle.

13.0g of Nutrient broth powder (DM1SOD, Merseyside, U.K) was weighed and swirled in 500ml of distilled water. It was then mixed thoroughly using a stirrer and distributed into a 500ml bottle.

Exactly 3.8g of peptone water powder (Madrid, Spain) was accurately weighed and dissolved in 250ml of distilled water in a beaker. The solution was dispensed into a 500ml bottle. All the media prepared were then sterilized in an autoclave at 121°C (15psi) for 15 minutes.

2.52 Antibacterial activity assay

Cultures of diarrheagenic *E. coli (E. coli* 057:H7*)* and *S. aureus* previously in a refrigerator at -4°C were used for preparation of working cultures. The agar disc diffusion technique previously described by Bauer *et al* [32] with modifications was employed. Briefly, 0.5 McFarland standard was prepared by the method of Koneman *et al* [33] and the turbidity adjusted to 1.5×10^8 CFU/mL. Four (4) 90mm sterile petri dishes were uniformly filled three-quarter with liquid nutrient agar. Two (2) antibacterial discs were soaked in each of the hydrolysates and allowed to stand for 1 hour with intermittent shaking. A loopful of the bacterial cultures were seeded onto nutrient agar in labelled petri dishes. The antibacterial discs soaked in the hydrolysates were carefully removed from the hydrolysates using sterile forceps and placed in the petri dishes. Tetracycline discs (30μg disc content) (Hi Media Lab. Pvt. Ltd, Mumbai, India) were placed in the opposite side of the discs soaked in the hydrolysates. The petri dishes were inverted and wrapped in Aluminium foils (Hotpack, Kampala) and labelled.

They were then transferred to a thermostatically regulated bacteriological incubator (DESCO, India) where they were incubated at 37°C for 24 hours. The petri dishes were observed for zones of inhibition which were measured and recorded in millimeters.

3. Results

3.1 pH and DH progression

The pH changes and the extent of hydrolysis were recorded in Table 1.

Table 1 Changes in pH and DH during the 2-hour proteolysis

*Parameter	Proteolysis time (hr)	Hydrolysate			
		CMC	GMC	CMAL	GMAL
pH	1	6.46±0.01	6.47±0.01	6.48±0.01	6.48±0.01
	2	6.43±0.01	6.44±0.02	6.45±0.03	6.47±0.01
DH (%)	2	5.91±0.01	7.68±0.01	10.93±0.01	15.3±0.03

* Values are presented as mean±standard error (S.E) of analysis done in triplicate.

3.2 Antibacterial assay and statistical analysis

The experiment was done in triplicate and replicated. The observed zones of complete inhibition (ZOI) of the hydrolysates were measured and recorded (Table 2). One-Way Analysis of Variance was done followed by Tukey's Honest Significant Difference (HSD) test to determine the significant differences between the antibacterial potential of the hydrolysates ($p<0.05$) using Minitab Statistical software (Minitab Inc., USA).

Table 2 Zones of inhibition of the hydrolysates on *E. coli* and *S. aureus*

Bacteria	**Papain hydrolysate**	**Zone of complete inhibition in mm**			
		1	**2**	**3**	**Mean±S.E**
E. coli [1]	Cow milk casein	8.0	7.9	8.1	8.00±0.06 [a]
	Goat milk casein	11.0	10.8	10.9	10.90±0.06 [b]
	Cow milk alpha-lactalbumin	12.0	13.0	12.5	12.50±0.29 [c]
	Goat milk alpha-lactalbumin	19.0	20.0	19.5	19.5±0.29 [d]
S. aureus [2]	Cow milk casein	4.0	4.1	4.3	4.13±0.09 [e]
	Goat milk casein	2.0	1.8	1.9	1.9±0.06 [f]
	Cow milk alpha-lactalbumin	17.0	16.5	17.0	16.8±0.17 [g]
	Goat milk alpha-lactalbumin	19.0	20.0	20.0	19.6±0.33 [h]

[1] the positive control disc had a mean ZOI of 19.80±0.01m, [2] the positive control disc had a mean ZOI of 9.90±0.03mm, [*] Means within the same column that have alphabetical letters (a-h) are statistically different as determined by Tukey's HSD test. Statistical means are presented as Mean±S.E.

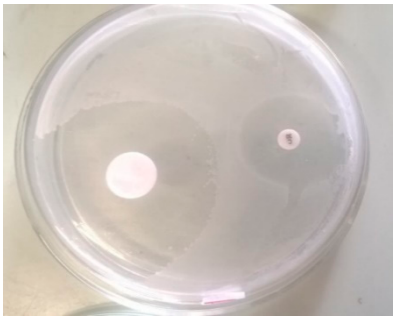

Figure 1 Zone of inhibition of cow milk alpha-lactalbumin hydrolysate *on E. coli*

Figure 3 Zone of inhibition of goat milk alpha-lactalbumin hydrolysate on *E. coli*

Figure 2 Zone of inhibition of goat milk alpha-lactalbumin hydrolysates on *S. aureus*

4. Discussion of results

4.1 pH and DH progression

The initial media pH wherein proteolysis progresses influences the reaction rate. A gradual decrease in pH was observed in this investigation (**Table 2**). Changes in pH is known to alter the structural and functional configuration of proteinaceous substrates, ultimately influencing enzyme-substrate interactions. The least pH (6.46±0.01) was registered in the milieu containing cow milk casein while

the highest pH of 6.48±0.01 was observed in alpha-lactalbumin hydrolysates after one hour of hydrolysis. After 2 hours, the cow milk casein media registered the least pH (6.43±0.01) followed by goat milk casein (4.46±0.01), cow milk alpha-lactalbumin (6.45±0.01) and finally goat milk alpha-lactalbumin (6.47±0.01). The gradual pH decrease could be due to protons cleaved from amino acids during proteolysis [29]. An identical pH decrement involving proteolysis of ovine and camel caseins have been reported by other researchers [29, 34, 35].

The degree of hydrolysis (DH) is an analytical measure of the soluble biopeptides enzymatically released during the proteolysis. The highest DH after 2 hours of continuous proteolysis (15.3±0.03%) was attained with goat milk alpha-lactalbumin and the least DH (5.91±0.01%) was obtained in cow milk casein. The lower DH in cow milk casein could be due to the lower pH observed in it, which could supposedly have potentiated denaturation of the functional proteins of the papain enzyme thus loss of catalytic activity. Another close possibility could be the distortion of the ionic charges of the substrate by the lowered pH, reducing the chances of interaction between the enzyme and the substrate. It is also possible that the enzyme papain is highly specific, rendering it incapable of further proteolyzing the residual bonds within the previously generated biopeptides [29]. This study employed finite proteolysis to preserve the amino acid integrity and structural configuration of the biopeptides produced so as to enhance the expression of their antibacterial potential.

4.2 Antibacterial activity assay

Statistical analysis showed that there is no significant difference between the antimicrobial activities of cow and goat milk casein on *E. coli* although there is a significant difference between the hydrolysates in inhibition of *S. aureus*. Goat milk casein hydrolysate showed a higher antibacterial activity on *E. coli* than cow milk casein hydrolysate. Goat milk casein contains A2-beta casein as the major casein, traces of alpha-s1-casein [36] and prominent levels of alpha-s2-casein while cow milk contain traces of A2-beta casein, much A1-beta casein and a high concentration of the allergenic alpha-s1-casein [37]. This genetic polymorphism causes goat milk casein to be easily digestible as the A2-beta casein variant of beta casein in goat milk forms casein micelles that are larger in size, less solvated, contributing to the formation of a softer curd and gentler proteolysis. Therefore, goat milk casein is more easily digestible than cow milk casein [38-40] and due to a higher degree of hydrolysis (7.68±0.01%), goat milk casein contained a higher concentration of antibacterial peptides than the cow milk casein, thus exhibited a higher antibacterial activity on *E. coli* with a mean zone of inhibition of 10.9mm. Goat milk casein showed a lower antibacterial effect on *S. aureus* than cow milk casein; this could be because of the difference in the cell wall properties of *S. aureus.* It is known that most antibacterial peptides are positively charged, thus electrostatically bind to the oppositely (negatively) charged components on bacterial cell walls, potentiating cell destruction [41-43]. This could be the reason why *E. coli* was highly inhibited by the goat milk casein while the Gram-Positive *S. aureus* was comparatively less inhibited. This result is concordant with the literature of Park [44] that caprine milk casein derived biopeptides are more antibacterial on Gram negative bacteria.

Alpha-lactalbumin hydrolysates showed a higher antibacterial activity than the cow and goat milk casein hydrolysates. This is because casein is resistant to enzymatic proteolysis and requires extensive hydrolysis using unpromisingly long incubation periods [45]. Goat milk alpha-lactalbumin showed a higher inhibitory effect on *E. coli* and *S. aureus* than cow milk alpha-lactalbumin because goat milk alpha-lactalbumin is more easily digestible than cow milk alpha-lactalbumin. Therefore, due to a high degree of hydrolysis (15.3±0.01%), the papain hydrolysate of goat milk alpha-lactalbumin contained a higher concentration of antibacterial peptides than the cow milk alpha-lactalbumin, hence exhibiting a higher antibacterial activity.

5. Conclusions and recommendations

The study revealed that papain hydrolysis of bovine and caprine milk casein and alpha-lactalbumin enhanced their antimicrobial activity against *E. coli* and *S. aureus.* The hydrolysates could be an alternative source of better bioactive, effective and ethnofriendly antidiarrheal drug candidates for treatment of diarrhea caused by diarrheagenic *E. coli* and *S. aureus.*

Alpha-lactalbumin hydrolysates have a higher antibacterial activity than the corresponding casein hydrolysates; goat milk alpha-lactalbumin have the highest antibacterial activity of all the hydrolysates tested against diarrheagenic *E. coli* and *S. aureus*.

Further research to establish the fractions of associated with the observed antibacterial activity should be done. The minimum inhibitory concentration and minimum bactericidal concentration of the papain hydrolysates on *E. coli* and *S. aureus* should be determined.

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