Benha Veterinary Medical Journal 40 (2021) 129-133

Official Journal Issued by Faculty of Veterinary Medicine

Benha Veterinary Medical Journal

Journal homepage: https://bvmj.journals.ekb.eg/

Since 1990

Original Paper

Antibacterial activity of pepsin hydrolyzed goat whey protein and its application in pasteurized milk.

Wafaa S. Nassar, Ekbal A. Ibrahim[1], Hend A. Elbarbary, Hamdi A. Mohamed

Department of Food Hygiene& Control, Faculty of Veterinary medicine, Benha University, Egypt.

ARTICLE INFO

Keywords

Goat whey protein
Pepsin enzyme
Antibacterial
Pasteurized milk

Received 18/07/2022
Accepted 30/08/2022
Available On-Line 09/10/2022

ABSTRACT

In light of current trends favoring healthy foods, there is growing interest in goat milk and its derivatives. Goat milk is more nutritious than cow milk, especially in terms of fat and protein. Goat milk whey protein has gotten a lot of attention as a result of its diverse biological activities, particularly with enzymatic hydrolysis. So, the aim of current study was to measure antibacterial activity of goat`s whey protein hydrolysates and probability of its application in pasteurized milk. Goat whey protein was separated from whole goat`s milk, then hydrolyzed using pepsin enzyme at 2:100 E/S (Enzyme/Substrate) ratio at different times (30, 60, 120 and 240 min), Antimicrobial activity of the produced hydrolysates was tested in -vitro against *E.coli* and *S.aureus*, then tested in-vivo in food model as pasteurized milk. Results revealed that degree of hydrolysis (D.H) is significantly higher after 240 min of hydrolysis, at concentration 10 mg mL^{-1}. Goat milk whey protein hydrolysates 120 (GMWH 120) completely inhibited both *E.coli* and *S. aureus*. Application of hydrolysates in pasteurized cow`s milk reduced bacterial population about 4 log and 2.5 log in case of *E.coli* and *S. aureus*, respectively. So, the present study highlights the great potential effect of GMWH to be used as a safe bio preservative in the dairy industry.

1. INTRODUCTION

Milk and dairy products are nutrient-dense, supplying high-quality proteins, micronutrients, vitamins, and energy. As a result, the food industry is very concerned about dairy sector safety and quality, as they are more susceptible to pathogenic and spoilage bacteria, posing serious health risks as well as economic losses to consumers. So many efforts have been made in the dairy industry to combat microbial growth by using natural antimicrobial agents (Osman et al., 2016; Luz et al., 2020).

Goat's milk has a high concentration of all milk components while being low in lactose. According to studies, goat milk has smaller fat particles, is less allergenic, is easier to digest and absorb and has higher bioactivities than cow's milk, making it a better simple replacement for baby food yield (Kostić et al., 2021). Milk protein is an essential nutritional component in goat milk and is a reasonable guideline for assessment the nutritional quality of dairy products. Goat milk contains 70% casein, 25% whey protein, and 5% milk fat globule membrane protein (Zhu et al., 2018; Chen et al., 2019).

Goat whey features a wide range of intriguing contents, including proteins, which are available in the form of a protein stream and have the capacity to perform a variety of therapeutic functions as well as unique and interesting properties. So a great interest focused on goat whey protein, Enzymatic hydrolysis of whey protein by commercial enzymes (pepsin and trypsin) released a peptides with a great antimicrobial activities in addition, antioxidant and antihypertensive effects (El-Zahar et al., 2004; Corrêa et al., 2014).

Throughout the last few years, there has been a sharp rise in interest in developing natural antimicrobial agents as proper way to control either bacterial or mold growth, from this point, many researches have been applied on whey (Ribes et al., 2018).

Whey is the primary liquid waste from the cheese industry, accounting for roughly 90% of milk volume; it incorporates lactose (3.8-4%), protein (0.8-1%) and minerals (0.7-0.8%) in crude form. Whey protein contains immunoglobulin, lactoferrin, lactoperoxidase, glycomacropeptide, serum albumin, α-lactalbumin, and β-lactglobulin), and enzymatic hydrolysis of whey protein released bioactive components with biologically important properties such as antibacterial, antioxidant and antihypertensive effects (Corrêa et al., 2014; De Gobba et al., 2014). For goat whey, characterized by higher α-lactalbumin reached to 21.4% and serum albumin/lactoferrin (12.8%) than other species, while being lower in β-lactglobulin (54.2%) and immunoglobulin (11.5%) than other species (Rafiq et al., 2016).

As a result, the study goal was enhancement and keeping quality of pasteurized milk in following steps: I) Extraction of whey protein. II) Evaluation the antimicrobial activity of its hydrolysates obtained by enzymatic hydrolysis. III) Incorporation of whey protein hydrolysate in pasteurized milk to check its antibacterial efficacy.

* Corresponding author: wafaa_nassar1991@yahoo.com

2. MATERIAL AND METHODS

2.1. Materials:

Whole Caprin`s milk was collected from Faculty of Agriculture, Benha University, Egypt.

S. aureus and *E. coli* were obtained microbiology department, faculty of veterinary medicine, Benha University.

Pepsin enzyme 1:2500 (>2500 unit's mg^{-1} protein) was obtained from Sigma (EC.3.4.23.1). All the chemicals (trichloroacetic acid, vertical electrophoresis kites, hydrochloric acid, and sodium hydroxide) had excellent quality.

2.2. Separation of goat whey:

Raw whole caprine milk (3.8% protein) was heated to 37 °C, then centrifuged at 2500 ×g, 30 min at 10 °C for removal of fat. Whey protein was separated from casein by addition of 10% acetic acid to reach pH 4.6 (isoelectric point of casein), followed by centrifugation at 5000 ×g, 30 min at 4 °C). Finally, whey was collected, dialyzed for 48 h, lyophilized, and stored at -20 °C until use (Salami et al., 2010).

2.3. Hydrolysis of goat whey protein:

Hydrolysis was carried out using pepsin enzyme. Whey protein was dissolved in buffer solution adjusted at pH 3 at concentration of 5% (w/v). The degree of hydrolysis was determined at various intervals (30, 60, 120 and 240 min). The peptic hydrolysis was terminated by heat inactivation of the enzyme at 95 °C for 10 min. Hydrolysates were subjected to centrifugation at 3000 ×g for 10 min, then the supernatant was collected and lyophilized, then stored at -20 °C (Abdel-Hamid et al., 2017).

2.4. Degree of hydrolysis (D.H):

According to Hoyle & Merritt (1994) D.H was calculated, The hydrolyzed mixture was mixed with 20% trichloroacetic acid (TCA) (1:1) and incubated at 4 °C for 30 min before being centrifuged at 3000 g for 10 min at 4 °C. Bradford technique was used to assess the protein of the supernatant, which was expressed as mg of protein. The degree of hydrolysis was determined by dividing the solubilized protein content in 10% g(w/v) TCA (mg) by the total protein (mg).

2.5. Antibacterial activity:

The antimicrobial activity of goat whey, goat whey hydrolysates was monitored against indicator pathogens *S. aureus* ATCC 25923 and *E. coli* ATCC 25922 as described earlier Pellegrini et al., (1999). Goat whey and it`s hydrolysates were dissolved in sterile distilled water, filtered with a 0.45 µm membrane filter. Single colonies of bacteria were transferred to sterile Muller Hinton broth (MHB) and grown overnight at 37 °C under constant agitation (200 rpm). The overnight cultures were diluted 1:50 in fresh growth medium and incubated with agitation at 37 °C to mid-logarithmic phase (optical density at 600nm reaching 0.4), the bacterial cultures were further diluted in fresh MHB (1:500) to obtain final inoculum concentration ranging from 5.3-5.9 log10 cfu mL^{-1}. Finally equal volume of bacteria and test protein to give 10, 5, and 2.5 mg mL^{-1} sample concentration, and subsequently mixed with 100 µL of MHB. The mixtures were kept at 37 °C for 2 hr, then ten-fold serially diluted in PBS before plating on Muller Hinton agar. Colony forming units were counted after incubation for 24-48 hr at 37 °C. All assays were performed in triplicates.

2.6. In-vivo antibacterial activity in food model (pasteurized milk):

Raw skimmed cow milk was subjected to laboratory pasteurization (63 °C/ 30 min LTLT (low temperature long time model). The milk was then immediately transferred into sterilised lock bottles (10 mL each) and subcultured with either *S. aureus* or *E. coli* test organisms to achieve a final concentration of 5 log cfu mL^{-1}. The desired concentration of pepsin hydrolyzed goat whey protein (120 min hydrolysates) was then added (2.5, 5, and 10 mg mL^{-1}). Control mixtures were created without the use of hydrolysates. For 7 days, the samples were kept at 4 °C. Following that, the mixtures were diluted in TSB (Elbarbary et al., 2018).

2.6.1. Enumeration of E.coli viable count

E.coli were counted on tryptone-bile-glucuronic medium (TBX) and incubated for 24 hr at 44 °C (ISO, 2001).

2.6.2. Enumeration of S. aureus

Baired parker agar is the preferred medium for counting *S. aureus* through using surface plating technique (ISO, 2003).

2.7. Statistical analysis:

The data were described as mean ± standard errors (n = 3). For the statistical methods, Graph Pad Prism 8.0 was used. A one-way ANOVA test was used to analyze the data (Feldman et al., 2003).

3. RESULTS

3.1. Degree of hydrolysis (D.H):

The D.H increased gradually after 30 minutes, significantly ($p < 0.05$)increased after 60 minutes (21.1 %), and then non-significantly decreased at 120 minutes of hydrolysis until reaching the highest D.H (27 %) after 240 minutes of hydrolysis (Figure 1). The degree of hydrolysis can also be seen on SDS-PAGE (Figure 2), where all whey protein bands vanished except for β-lactoglobulin (18 kDa).

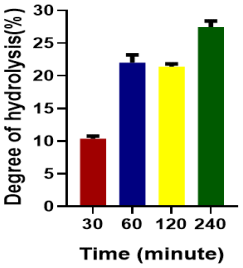

Figure 1 Degree of hydrolysis (DH) of goat whey protein by using pepsin.
30: goat milk whey after 30 min of hydrolysis
60: goat milk whey after 60 min of hydrolysis
120: goat milk whey after 120 min of hydrolysis
240: goat milk whey after 240 min of hydrolysis

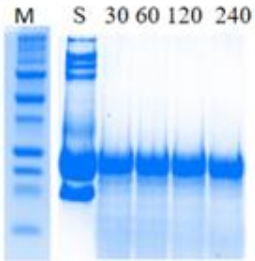

Figure 2 Electrophoretic patterns of goat whey proteins (before and after enzymatic hydrolysis on 12% polyacrylamide gels of reducing sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). M = molecular weight marker S= whey protein before hydrolysis.
30: goat milk whey after 30 min of hydrolysis
60: goat milk whey after 60 min of hydrolysis
120: goat milk whey after 120 min of hydrolysis
240: goat milk whey after 240 min of hydrolysis

3.2. Antibacterial activities of goat whey protein hydrolysates:

Goat milk whey (GMW) and time-dependent goat whey hydrolysates Goat milk whey hydrolysates (GMWH),

GMWH30, GMW60, GMW120 and GMW 240 were examined for antimicrobial effect against *Escherichia coli* and *S. aureus* at different concentrations (10, 5, and 2.5 mg mL^{-1}) (Table 1, 2).

Table 1 Comparison the Antibacterial activity between GMW, GMWH30, GMWH60, GMWH120 and GMWH240 against *Escherichia coli* (log_{10}cfu mL^{-1}) The experiment was performed using liquid broth method where test bacteria incubated with protein samples in different concentrations (0, 2.5,5,120 mg mL^{-1}) for 2h then plated for 24 h in presence of control. The experiment repeated 3 times.

Group	GMW	GMWH30	GMWH60	GMWH120	GMWH240	Control positive
Conc						
0 mg mL^{-1}						6.10 ± 0.1
2.5 mg mL^{-1}	5.8 ± 0.07	5.06 ± 0.12	3.82± 0.04	ND	1.3 ± 0.15	
5 mg mL^{-1}	5.52 ± 0.09	4.9 ± 0.06	2.1 ± 0.06	ND	ND	
10 mg mL^{-1}	5.10± 0.087	4.71± 0.14	ND	ND	ND	

Values indicated are the mean of three replicates (mean± SEM). GMW: goat milk whey GMWH30: goat milk whey after 30 min of hydrolysis. GMWH60: goat milk whey after 30 min of hydrolysis after 60 min of hydrolysis. GMWH 120: goat milk whey after 30 min of hydrolysis after 120 min of hydrolysis. GMWH240: goat milk whey after 30 min of hydrolysis after 240 min of hydrolysis. Means within rows and columns considered significant when ($p < 0.05$)

Table 2 Comparison the Antibacterial activity between GMW, GMWH30, GMWH60, GMWH120 and GMWH240 against *Staphylococcus aureus* (log_{10}cfu mL^{-1}). The experiment was performed using liquid broth method where test bacteria incubated with protein samples in different concentrations (0, 2.5,5,120 mg mL^{-1}) for 2h then plated for 24 h in presence of control. The experiment was repeated 3 times.

Group						
Conc	GMW	GMWH 30	GMWH 60	GMWH 120	GMWH 240	Control positive
0 mg mL−1						5.30 ± 0.17
2.5 mg mL−1	4.92 ± 0.04	3.87 ± 0.05	3.4 ± 0.08	1.1 ± 0.2	3.3 ± 0.08	
5 mg mL−1	4.7 ± 0.07	3.74 ± 0.07	3.1 ± 0.14	ND	ND	
10 mg mL−1	4.2 ± 0.12	3.55 ± 0.08	2.8 ± 0.1	ND	ND	

3.3. Antibacterial activities of goat whey hydrolysates in pasteurized milk:

The true impact of GMW120 on the viability of *E. coli* and *S. aureus* in chilled milk form zero hr till 168 hr was explored at various concentrations (10, 5, and 2.5 mg mL^{-1}) were examined where it achieved the best result against *E. coli* reached to 90% (Table 3, 4).

Table 3 Comparison the Antibacterial activity of GMWH 2.5, GMWH 5 and GMWH 10 against *E. coli* (log_{10}cfu mL^{-1}) in pasteurized milk at 4 ◦C for 168 hr.

Group				
Times	GMWH 2.5	GMWH 5	GMWH 10	Control
zero	5.04± 0.11	5.04± 0.0.07	5.04± 0.13	5.04± 0.11
24 hr	4.3± 0.05	4.2±0.09	3.10± 0.11	4.54± 0.01
72 hr	3.92±0.12	3.8±0.07	3.01±0.04	4.04± 0.04
120 hr	3.8±0.04	3.01±0.08	2.1±0.11	4.34± 0.17
168 hr	2.73±0.08	2.52±0.11	0.51±0.12	4.50± 0.11

Values indicated are the mean of three replicates (mean± SEM). GMWH 2.5: goat milk whey hydrolysates at concentration 2.5 mg mL^{-1}
GMWH 5: goat milk whey hydrolysates at concentration 5 mg mL^{-1} GMWH 10: goat milk whey hydrolysates at concentration 10 mg mL^{-1}
Means within rows and columns considered significant when ($p < 0.05$)

Table 4 Comparison the Antibacterial activity of GMWH 2.5, GMWH 5 and GMWH 10 against *S. aureus* (log_{10}cfu mL^{-1}) in pasteurized milk at 4 ^{O}C for 168 hr.

Group				
Times	GMWH 2.5	GMWH 5	GMWH 10	Control
zero	4.99± 0.05	4.98± 0.05	4.99± 0.12	4.98± 0.04
24 hr	4.3± 0.04	4.11±0.12	3.5± 0.01	4.54± 0.03
72 hr	3.92±0.09	3.72±0.07	3.11±0.13	4.04± 0.05
120 hr	3.6±0.09	3.21±0.05	2.6±0.06	4.64± 0.07
168 hr	2.93±0.06	2.72±0.04	2.01±0.02	4.90± 0.09

4. DISCUSSION

Goat whey protein was hydrolyzed with a pepsin enzyme (2:100 E/S) ratio to produce various hydrolysates. The degree of hydrolysis was calculated using the Bradford assay at various times (0, 30, 60, 120 and240 min) (Figure, 1).

This D.H results is nearly identical to that reported by Osman et al., (2021), who found that the D.H of bovine whey protein by pepsin at a concentration of 1:200 (E/S) ratio was 8%, 12%, 20%, and 25% after 1, 2, 3, and 4 hours. Nearly identical D.H values were recorded for of goat whey hydrolysates after 4 hrs. at 50 ^{O}C, but with a 1:200 Enzyme/Substrate ratio (Osman et al., 2016) . However, it is slightly lower than pepsin hydrolysates from buffalo milk protein (33 %) (Abdel-Hamid et al., 2017). β-lactoglobulin (18 kDa), which is highly resistant to pepsin enzyme degradation due to the intermolecular stabilizing effect (Figure,2) (Le Maux et al., 2014).

For antimicrobial activity of hydrolysates, GMW had only a log inhibitory effect against both *E.coli* and *S. aureus* at 10 mg mL^{-1} (Table 1, 2). Some of the proteinaceous components of GMW that may be responsible for its antibacterial activity include lysozyme, lactoperoxidase, lactoferrin, and immunoglobulin (Haque and Chand, 2008). Pepsin hydrolysis of goat whey protein significantly improved its antibacterial activity due to the emission of antibacterial bioactive compounds during hydrolysis. Many researches have shown that after digestion, whey protein released latent compounds with higher activity than the parent proteins (Wang et al., 2020).

For example, Salami et al. (2010) discovered that activity of whey protein improved by enzyme hydrolysis. Peptic and tryptic digestion enhanced antibacterial effect of β-Lactoglobulin (Pihlanto-Leppälä et al., 1999), According to Tomita et al. (1991) peptic digestion of bovine lactoferrin managed to produce low molecular weight peptides capable of completely inhibiting *E. coli* with a MIC of 0.25 mg mL^{-1}, whereas higher concentrations (2 mg mL^{-1}) of crude form were requested to make the same effect.

Antibacterial activity of goat whey protein was discovered to be time dependent, with activity increasing after 30 minutes of hydrolysis and peaking after 120 minutes. If more potent peptides are released during hydrolysis, this could be reversed. After 60 minutes, 120 minutes, and 240 minutes of hydrolysis at 10 mg mL^{-1} , goat whey protein hydrolysates inhibited *E.coli* completely (100%), while GMWH 120 can inhibited it completely (100%) at 2.5,5 and 10 mg mL^{-1} , respectively (Table, 1).

Concerning *S. aureus*, at concentrations of 10, 5 mg mL^{-1} , GMWH completely inhibited it (100%) with a 4 log_{10} reduction at concentration 2.5 mg mL^{-1} (Table, 2). This finding is similar to that of (Ibrahim et al., 2005), who found that after 120 minutes of hydrolysis by pepsin enzyme, lysozyme hydrolysates inhibited both *E.coli* and *S. aureus* by 5 log and 6 log, respectively. As a result, goat whey protein hydrolysates after 120 min of hydrolysis were found to be the best antimicrobial agent to be applied pasteurized milk.

Pepsin digest of goat whey exerted strong antibacterial activity in TSB. In control samples (no hydrolysate), the *E. coli* population decreased slightly (0.5 log) at 168 hr in milk kept at 4 ◦C (Table, 3). At 2.5 mg mL^{-1}, *E. coli* count was markedly inhibited (1.8 log) at 168 hr. while, whereas at 10 and 5 mg mL^{-1}, microbial cells were decreased by about 4 and 2 log, respectively after 168 h , this is higher than that reported by (Elbarbary et al., 2018).

The hydrolysates had a noticeable impact on growth of *S. aureus*, however at 2.5 mg mL^{-1} during 168 hr of storage (Table, 4). While the bacterial population was significantly decreased in both hydrolysates at a concentration of 10 and 5 mg mL^{-1} reached to 2.8 log and 2.2 log, respectively during 168 hr of incubation (Table, 4). The majority of projects assessed the activity of purified protein hydrolysates against a diverse range of pathogens in simple nutrient media (Almaas et al., 2011). Current research, on the other hand, proved that goat whey hydrolysates significantly inhibit the growth of bacteria in chilled milk.

5. CONCLUSION

The current work highlights on the importance of goat`s whey protein as natural agent, it revealed that goat milk whey (GMW) exerted antibacterial action reached to nearly one log reduction with inhibition percentage 21% and 17% respectively at 10 mg mL^{-1}. This activity increased by hydrolysis reached to 100% inhibition of *E.coli and S.aureus* at concentration 2.5 and 5 mg $mL^{-1,}$ respectively after 120 minute of hydrolysis in-vitro, The in-vivo application of hydrolysates in pasteurized milk, illustrated that it can inhibit bacterial growth to about 4 log in case of *E.coli* with inhibition percentage reached to 90% at 10 mg mL^{-1} during 168 hr of incubation. So it supported incorporation of goat whey protein in dairy sector as strong antimicrobial agent.

6. REFERENCES

1. Abdel-Hamid, M., Otte, J., De Gobba, C., Osman, A. and Hamad, E., 2017. Angiotensin I-converting enzyme inhibitory activity and antioxidant capacity of bioactive peptides derived from enzymatic hydrolysis of buffalo milk proteins. International Dairy Journal, 66, 91–98. https://doi.org/10.1016/j.idairyj.2016.11.006**.**
2. Almaas, H., Eriksen, E., Sekse, C., Comi, I., Flengsrud, R., Holm, H., Jensen, E., Jacobsen, M., Langsrud, T. and Vegarud, G.E., 2011. Antibacterial peptides derived from caprine whey proteins, by digestion with human gastrointestinal juice. British Journal of Nutrition, 106(6), 896–905. https://doi.org/10.1017/S0007114511001085**.**
3. Chen, D., Li, X., Zhao, X., Qin, Y., Wang, J. and Wang, C., 2019. Comparative proteomics of goat milk during heated processing. Food Chemistry, 275(September), 504–514. https://doi.org/10.1016/j.foodchem.2018.09.129**.**
4. Corrêa, A.P.F., Daroit, D.J., Fontoura, R., Meira, S.M.M., Segalin, J. and Brandelli, A., 2014. Hydrolysates of sheep cheese whey as a source of bioactive peptides with antioxidant and angiotensin-converting enzyme inhibitory activities. Peptides, 61,48–55. https://doi.org/10.1016/j.peptides.2014.09.001**.**
5. De Gobba, C., Espejo-Carpio, F.J., Skibsted, L.H. and Otte, J., 2014. Antioxidant peptides from goat milk protein fractions hydrolysed by two commercial proteases. International Dairy Journal,39(1),.28–40.https://doi.org/10.1016/j.idairyj.2014.03.015**.**
6. Elbarbary, H.A., Ejima, A. and Sato, K., 2018. Generation of antibacterial peptides from crude cheese whey using pepsin and rennet enzymes at various pH conditions. Journal of the Science of Food and Agriculture,99(2),555–563. https://doi.org/10.1002/jsfa.9214**.**
7. El-Zahar, K., Sitohy, M., Choiset, Y., Métro, F., Haertlé, T. and Chobert, J.M., 2004. Antimicrobial activity of ovine whey protein and their peptic hydrolysates. Milchwissenschaft, 59(11–12), 653–656**.**
8. Feldman, D.; Hoffman, R. and Simpson, J. 2003. The solution for data analysis and presentation graphics. 2 nd Ed. Abacus Landcripts, Inc., Barkeley, CA, USA.
9. Haque, E. and Chand, R., 2008. Antihypertensive and antimicrobial bioactive peptides from milk proteins. European Food Research and Technology, 227(1),7–15. https://doi.org/10.1007/s00217-007-0689-6**.**
10. Hoyle, N.T. and Merrltt, J.H., 1994. Quality of Fish Protein Hydrolysates from Herring (Clupea harengus). Journal of Food Science, 59(1),76–79. https://doi.org/10.1111/j.1365-2621.1994.tb06901.x**.**
11. Ibrahim, H.R., Inazaki, D., Abdou, A., Aoki, T. and Kim, M., 2005. Processing of lysozyme at distinct loops by pepsin: A novel action for generating multiple antimicrobial peptide motifs in the newborn stomach. Biochimica et Biophysica Acta (BBA) General Subjects,1726(1),102-111. https://doi.org/10.1016/j.bbagen.2005.07.008
12. International Organization for Standardization (ISO) 2001. International Organization for Standardization No.16649-2. Microbiology of food and animal feeding stuffs — Horizontal method for the enumeration of glucuronidase-positive Escherichia coli - Part 2: Colony-count technique at 44 °C using 5-bromo-4-chloro-3-indolyl-D-glucuronide**.**
13. International Organization for Standardization (ISO) 2003. International Organization for Standardization. No. 6888- 1:1999, A1:2003. Microbiology of food and animal feeding stuffs-Horizontal method for the enumeration of coagulasepositive staphylococci (Staphylococcus aureus and other species)-Part 1: Technique using Baird-Parker agar medium (includes amendment A1:2003)**.**
14. Kostić, A.Ž., Milinčić, D.D., Stanisavljević, N.S., Gašić, U.M., Lević, S., Kojić, M.O., Lj. Tešić, Ž.,

Nedović, V., Barać, M.B. and Pešić, M.B., 2021. Polyphenol bioaccessibility and antioxidant properties of in vitro digested spray-dried thermally-treated skimmed goat milk enriched with pollen. Food Chemistry, 351, 129310. https://doi.org/10.1016/j.foodchem.2021.129310.

15. Le Maux, S., Bouhallab, S., Giblin, L., Brodkorb, A. and Croguennec, T., 2014. Bovine β-lactoglobulin/fatty acid complexes: binding, structural, and biological properties. Dairy Science & Technology, 94(5), 409–426. https://doi.org/10.1007/s13594-014-0160-y.
16. Luz, C., Izzo, L., Ritieni, A., Mañes, J. and Meca, G., 2020. Antifungal and antimycotoxigenic activity of hydrolyzed goat whey on Penicillium spp: An application as biopreservation agent in pita bread. LWT, 118, 108717. https://doi.org/10.1016/j.lwt.2019.108717.
17. Osman, A., Goda, H.A., Abdel-Hamid, M., Badran, S.M. and Otte, J., 2016. Antibacterial peptides generated by Alcalase hydrolysis of goat whey. LWT - Food Science and Technology, 65,480–486. https://doi.org/10.1016/j.lwt.2015.08.043.
18. Osman, A., Enan, G., Al-Mohammadi, A.-R., Abdel-Shafi, S., Abdel-Hameid, S., Sitohy, M.Z. and El-Gazzar, N., 2021. Antibacterial Peptides Produced by Alcalase fromCowpeaSeedProteins.Antibiotics,10(7),870.https://doi.org/10.3390/antibiotics10070870.
19. Pellegrini, A., Thomas, U., Bramaz, N., Hunziker, P. and Von Fellenberg, R., 1999. Isolation and identification of three bactericidal domains in the bovine α-lactalbumin molecule. Biochimica et Biophysica Acta - General Subjects. https://doi.org/10.1016/S0304-4165(98)00165-2.
20. Pihlanto-Leppälä, A., Marnila, P., Hubert, L., Rokka, T., Korhonen, H.J.T. and Karp, M., 1999. The effect of α-lactalbumin and β-lactoglobulin hydrolysates on the metabolic activity of Escherichia coli JM103. Journal of Applied Microbiology, 87(4), 540–545. https://doi.org/10.1046/j.1365-2672.1999.00849.x.
21. Rafiq, S., Huma, N., Pasha, I., Sameen, A., Mukhtar, O. and Khan, M.I., 2016. Chemical composition, nitrogen fractions and amino acids profile of milk from different animal species. Asian-Australias Journal of Animal Sciences, 29,1022–1028.
22. Ribes, S., Fuentes, A., Talens, P. and Barat, J.M., 2018. Prevention of fungal spoilage in food products using natural compounds: A review. Critical Reviews in Food Science and Nutrition, 58(12),.2002–2016. https://doi.org/10.1080/10408398.2017.1295017.
23. Salami, M., Moosavi-Movahedi, A.A., Ehsani, M.R., Yousefi, R., Haertlé, T., Chobert, J.M., Razavi, S.H., Henrich, R., Balalaie, S., Ebadi, S.A., Pourtakdoost, S. and Niasari-Naslaji, A., 2010. Improvement of the antimicrobial and antioxidant activities of camel and bovine whey proteins by limited proteolysis. Journal of Agricultural and Food Chemistry, 58(6),3297–3302. https://doi.org/10.1021/jf9033283.
24. Tomita, M., Bellamy, W., Takase, M., Yamauchi, K., Wakabayashi, H. and Kawase, K., 1991. Potent Antibacterial Peptides Generated by Pepsin Digestion of Bovine Lactoferrin.Journal of Dairy Science,74(12), 4137–4142. https://doi.org/10.3168/jds.S0022-0302(91)78608-6.
25. Wang, R., Han, Z., Ji, R., Xiao, Y., Si, R., Guo, F., He, J., Hai, L., Ming, L. and Yi, L., 2020. Antibacterial Activity of Trypsin-Hydrolyzed Camel and Cow Whey and Their Fractions. Animals, 10(2), 337. https://doi.org/10.3390/ani10020337
26. Zhu, Y., Wang, J. and Wang, C., 2018. Research on the preparation, uniformity and stability of mixed standard substance for rapid detection of goat milk composition. Animal Science Journal, 89(5),794–801. https://doi.org/10.1111/asj.12985.