REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 15 June 2022; Accepted: 25 June 2022; Publication: 31 August 2022

Effect Antimicrobial Activity of Chitosan against *S.Aureus* in Locally Bovine Soft Cheese in Babylon Prevalence

Amer Jebur Obayes Al-Isawi [*1], Mohamed Ibrahem Rahma [2] and Sumod Abdul Kadhem Salman[3], Moaed E. Al-Gazally[4]

[1,2] Department of Public Health, College of Veterinary Medicine, AL-Qasim Green University, Babylon, Iraq
[3] Department of microbiology, College of Veterinary Medicine, AL-Qasim Green University, Babylon, Iraq
[4]Collage of Medicine, University of Al-Ameed, Karbala, Iraq
***Corresponding author: dr.amer_42_vet@vet.uoqasim.edu**

Abstract
This work focused on antimicrobial activity of chitosan , the result showed a pronounced antimicrobial activity against *S. aureus* with different inhibition zones. The highest activity was found against *S.aureus* by using 1% concentration with inhibition zones 18mm. *S.aureus* was completely inhibited at 1st day when the cheese was preserved in 4^0C with 1% of chitosan while in 0.5 % chitosan, the count of *S.aureus* was reduced until complete inhibition of the growth at 5rd day. So, chitosan is expected to show good potential application for the inactivation of microorganisms in food and use as natural food preservation material.

Keywords: Dairy animals, *Staphylococcus aureus***,** chitosan**,** soft cheese

Introduction

Milk still up in the air by parts of cleanliness and organization. Milk is an extremely valuable food that go about as a reasonable development mode for an extensive variety of microorganism (1). The nature of milk and dairy items is affected by a few trademark like handling conditions ,greenery of crude milk and after heat treatment defilement. The tainting of cheddar might make from a few sources like starter culture, bundling material, cheddar compartment , cutting blade and creation room air (2). Also storage coolers considered another source of cheese contamination (3) .Many forms of control method are effective to minimize microbial contamination of cheese and to inhibit the growth of pathogen (4).

Three tubes for each treatment were filled with 10 ml of the same fresh cheese solution (10 gm of white cheese were added to 90 ml of sodium citrate) and mixed with 1 ml of 0.5% and 1% chitosan. After mixing, these tubes were stored at 4^0C at 2,3,5-days intervals, one tube from each treatment were taken for viable bacterial count by spread plate method at three days. Staphylococci are often found on the skin and mucous membranes of worms, but they are also found in various environments, such as soil, water, and are considered mastitis in lactating animals (5). S. aureus is important foodborne pathogens due to the production of enterotoxins in food matrices (6). Contamination of S. aureus cheese can be caused by its presence in raw milk, which is especially important in countries with high production of dairy products such as cheese (7). Other sources that give high levels of this genus in milk are the poor sanitation(8). Cheese is a dynamic microbial ecosystem have large variety of microorganisms including bacteria, yeasts, and molds that contribute to the organoleptic quality of cheese, while other microorganisms may lead to cheese spoilage . The presence of this pathogens after pasteurization can be attributed to inefficiency of the thermal process (9).

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 15 June 2022; Accepted: 25 June 2022; Publication: 31 August 2022

Chitosan is a particle with a characteristic natural beginning. It is a polysaccharide gotten from chitin and is fundamentally made out of (β)- 1,4 d-glucosamine connected to N-Acetyl-d-glucosamine buildups (10,11). Chitosan has extraordinary properties like biocompatibility and biodegradability demonstrating to be a minimal expense viable option with numerous applications in food handling (12,13) It is recognized to have antibacterial movement against Gram-positive microorganisms like S. aureus and Gram-negative like E.coli and Salmonella typhimurium (14,15,16). The chitosan use in the dairy business is extremely encouraging on the grounds that it permits to productively handle milk sugar and protein unrefined components, without huge energy costs.This study aimed to elucidating the antimicrobial properties of chitosan and to investigate about using it as food preservation material.

MATERIALS AND METHODS

Samples collection:

A total of 15 white cheese samples were collected from Al-Qasim market using sterile containers. The samples were collected from May 2021 to July 2021 ,the investigation was done following collection. The laboratory tests were accomplished in the laboratories of Collage of veterinary medicine in Al-Qasim Green University .

Isolation and identification of *S. aureus*:

10 gm of white cheese were added to 90 ml of sodium citrate in a sterilized plastic sacs and mixing well by hands . Three 10-fold dilutions were made and then loopful from the last tube were streaked onto 5% sheep blood agar incubated at 37°C for 24 hours. The presumptive colonies of *S. aureus* were further cultured onto mannitol salt agar (MSA). These isolates were conserved for further bacterial identification:

Morphological characteristics:

The bacterial smear was prepared from the isolated culture on clean microscopic glass slide and stained with Gram's stain. The stained smear was tested under microscope.

Biochemical examination:

Biochemical tests were performed to confirm *S. aureus* using Oxidase test, catalase, hemolysis (blood agar),coagulase ,urease, Voges-proskauer, Methyle red test.

Preparation of Chitosan Solution

To prepare 0.5% and 1% chitosan solution respectively,0.5 gm and 1 gm of chitosan was mixed with 100 ml of 1% of glacial acetic acid and stirred until dissolved it.

Efficiency of chitosan in cheese preservation

Three tubes for each treatment were filled with 10 ml of the same fresh cheese solution (10 gm of white cheese were added to 90 ml of sodium citrate) and mixed with 1 ml of 0.5% and 1% chitosan. After mixing, these tubes were stored at 4^0C at 2,3,5-days intervals, one tube from each treatment were taken for viable bacterial count by spread plate method at three days.

Results and discussion:

In our study, all the isolates were identified conferring to microscopic morphological, cultural and biochemical tests according to Brooks *et al.*, (2010) as shown in table (1).

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 15 June 2022; Accepted: 25 June 2022; Publication: 31 August 2022

Table (1) Biochemical test of *S. aureus* isolated from white cheese

Tests	**S. aureus**
Gram stain	+ve
Shape of bacteria	Cocci grape-like clusters
Mannitol fermentation	+ve
Oxidase	-ve
Catalase	+ve
Hemolysis (Blood agar)	+ve
Coagulase	+ve
Urease	+ve
Voges-proskauer	+ve
Methyle red	+ve

Staphylococci normally found on the skin and mucous layers of worm-blooded creatures yet they additionally can be tracked down various ecological like soil , water and it is viewed as the causative specialist of mastitis in lactating creatures (5). Several studies showed that *S.aureus* are isolated from cheese at concentration 85% and 11.1% (18,19). The preparation of the cheese with polluted conditions could lead to the spread of the *S. aureus* in cheese and it can form a high risk for public health.

Antimicrobial activity of chitosan

The antimicrobial activity of chitosan was evaluated against *S.aureus* with two concentration (0.5 % and 1%) , the result showed a pronounced antimicrobial activity against the tested bacteria with different inhibition zones as mentioned in table (2).

The highest activity was found against *S.aureus* by using 1% concentration of chitosan (Fig.1) .

Table (2):- Antimicrobial activity of different concentration of chitosan against *S.aureus*

Chitosan concentration (%)	Inhibition zone (mm)
0.5	15 ± 0.09
1	18 ± 0.10

Chitosan and its derivatives have been given effective for Gram-negative bacteria and Gram-positive bacteria (20).Though the mechanism of antibacterial activity has been understood, little evidence has been provided to exhibit the association between the surface characteristics of cell wall and antibacterial activity of chitosan .

Three tubes for each treatment were filled with 10 ml of the same fresh cheese solution (10 gm of white cheese were added to 90 ml of sodium citrate) and mixed with 1 ml of 0.5% and 1% chitosan. After mixing, these tubes were stored at 4^0C at 2,3,5-days intervals, one

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 15 June 2022; Accepted: 25 June 2022; Publication: 31 August 2022

tube from each treatment were taken for viable bacterial count by spread plate method at three days (21).

Another researchers thought that this antimicrobial activity is directly associated with the absorption of the polysaccharide to the bacterial cell and this absorption will cause alterations in the structure of cell wall, consequently, in the cell membrane permeability (22).

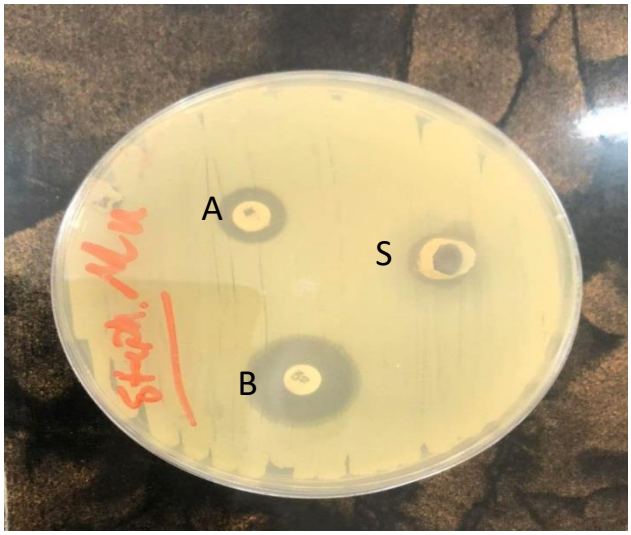

Figure (1):Antimicrobial activity of two concentration of chitosan against *S.aureus* . A=0.5%chitosan , B =1% chitosan, S=normal saline

Efficiency of chitosan in cheese preservation

As shown in table (3) , 1% chitosan after 1st day was completely inhibited the growth in cheese .While in 0.5 % chitosan, *S.aureus* reduced the count of *S.aureus* until complete inhibition of the growth at 5rd day.

Table (3): The effect of chitosan on viable count of *S.aureus* in cheese

Chitosan Conc.(%)	1st	3st	5st
0.5	24×10^6	17×10^6	No growth
1	No growth	-	-

This result was similar to another studies recorded by, Fernandes et al. (2008) and Shanmugama et al. (2016) which found the antibaceterial effects of chitosan against *S. aureus* and *E. coli* but at 0.1% chitosan (23) . Antimicrobial activity of chitosan may be return to its ability to interference with the metabolism by binding to the bacterial surfaces or by blocking the DNA and RNA transcription by binding to the DNA after penetration into cell. The strong antibacterial effect of chitosan , suggested that it had great potential as a natural preservative for raw milk at 4C.

Conclusion

This study revealed the presence of antimicrobial activity of chitosan against *S.aureus.* Chitosan would have advantages as new antimicrobial agents due to their higher activity

REDVET - Revista electrónica de Veterinaria - ISSN 1695-7504
Vol 23, No. 3 (2022)
http://www.veterinaria.org
Article Received: 10 April 2022; Revised: 15 June 2022; Accepted: 25 June 2022; Publication: 31 August 2022

.Additional work is wanted to better know the mode of action of chitosan as antimicrobial agents.

References

[1]. Rajagopal, M., Werner, B. G and Hotchkiss, J. H. Low pressure Co2 storage of raw milk: Microbiological effects. J. Dairy Sci. ,2005, 98: 3130-3138.

[2]. Temelli,S., Anar,S.,Sen, C. and Akyuva, P. Determination of microbiological contamination sources during Turkish white cheese production. Food Control ,2006, 17: 856-861.

[3]. Brito, J.R.F., Santos, E.M.P., Areuri , E.F., Lange, C.C., Brito, M.A. V.P., Souza, G.N., et al. Retail survey of Brazilian milk and Mina's freseal cheese and a contaminated dairy plant to establish prevalence, relatedness and source of Listeria monocytogenes isolates. ,2008, Applied and Environmental Microbiology, 74,4954-4961.

[4]. Mohammad Y.Alfaifi,J.Alkabli ,Reda F.M.Elshaarawy .Suppressing of milk-borne pathogenic using new water-soluble chitosan-azidopropanoic acid conjugate: Targeting milk-preservation quality improvement. International Journal of Biological Macromolecules.Volume 164, 1 December 2020, Pages 1519-1526

[5]. Bartolomeoli, I.; Maifreni, M.; Frigo, F.; Urli, G. and Marino, M. 2009. Occurrence and characterization of Staphylococcus aureus isolated from raw milk for cheese making. Int. J. Dairy Technol. 62, pp:366-371.

[6]. Duquenne M, Fleurot I, Aigle M, Darrigo C, Borezée-Durant E, Derzelle S, et al. 2010. Tool for quantificationof staphylococcal enterotoxin gene expression in cheese. Appl Environ Microbiol. 76, pp:1367-74.

[7]. Holeckova, B.; Holoda, E.; Fotta, M.; Kalinacova, V.;Gondol, J.; Grolmus, J. 2002. Occurrence of enterotoxigenic Staphylococcus aureus in food. Ann Agric Environ Med .9, pp: 179–182.

[8]. Aileen Tan, S. H., Sim, Y. K., Norlaila, M. Z., Nooraini, I., Masthurah, A., Nur Aqilah, M. D., . . . Noraisyah, A. B. (2021). Causes of fish kills in penang, malaysia in year 2019, in conjunction to typhoon lekima. Journal of Survey in Fisheries Sciences, 7(2), 231-247. doi:10.18331/SFS2021.7.2.20

[9]. Ateba, C. N.; Mbew, M.; Moneoang, M. S. and Bezuidenhout, C.C. 2010. Antibiotic resistant Staphylococcus aureus isolated from milk in the Mafikeng area, North West province, South Africa. S. Afr. J. Sci. 106, pp:1-6.
9- Rall, V.L.M.; Vieira, F. B., Rall, R.; Vieitis, R.L.; Fernandes, A.; Candeias, J.M.G.; Cardoso, K.F.G. and Araujo, J. P. 2008. PCR detection of staphylococcal enterotoxin genes in Staphylococcus aureus strains isolated from raw and pasteurized milk. Vet. Microbiol. 132, pp:408-413.

[10]. Liaqat, F.; Eltem, R. Chitooligosaccharides and their biological activities: A comprehensive review. *Carbohydr. Polym.* 2018, *184*, 243–259

[11]. Youssef, A.M.; Abou-Yousef, H.; El-Sayed, S.M.; Kamel, S. Mechanical and antibacterial properties of novel high performance chitosan/nanocomposite films. *Int. J. Biol. Macromol.* 2015, *76*, 25–32.

[12]. Najafabadi, S.A.A.; Mohammadi, A.; Kharazi, A.Z. Polyurethane nanocomposite impregnated with chitosan-modified graphene oxide as a potential antibacterial wound dressing. *Mater. Sci. Eng. C* 2020, 110899.

[13]. Xie, M.; Huang, K.; Yang, F.; Wang, R.; Han, L.; Yu, H.; Ye, Z.; Wu, F. Chitosan nanocomposite films based on halloysite nanotubes modification for potential biomedical applications. *Int. J. Biol. Macromol.* 2020, *151*, 1116–1125.

[14]. Khan, F.; Manivasagan, P.; Lee, J.W.; Pham, D.T.N.; Oh, J.; Kim, Y.M. Fucoidan-stabilized gold nanoparticle-mediated biofilm inhibition, attenuation of virulence and motility properties in *Pseudomonas aeruginosa* PAO1. *Mar. Drugs* 2019, *17*, 208.

[15]. Paomephan, P.; Assavanig, A.; Chaturongakul, S.; Cady, N.C.; Bergkvist, M.; Niamsiri, N. Insight into the antibacterial property of chitosan nanoparticles against Escherichia coli and Salmonella Typhimurium and their application as vegetable wash disinfectant. *Food Control* 2018, *86*, 294–301.

[16]. Huang, J.; Liu, Y.; Yang, L.; Zhou, F. Synthesis of sulfonated chitosan and its antibiofilm formation activity against E. coli and S. aureus. *Int. J. Biol. Macromol.* 2019, *129*, 980–988.

[17]. Brooks ,G.F.; Carroll, K.C.; Butel, J.S.; Morse ,S.A. and Mietzner, T.A. (2010) Jawetz, Melnick.

[18]. Survey in Fisheries Sciences, 8(3), 33-47. doi:10.18331/SFS2022.8.3.3

[19]. Alley, M. L., Royal, K. D., & Lewbart, G. A. (2021). Survey of pet stores regarding medical advice provided for pet fish and the potential impact on welfare. Journal of Survey in Fisheries Sciences, 7(2), 63-70. doi:10.18331/SFS2021.7.2.5

[20]. Hussien, M.F.; Amin, Manal M. and Sadek O.A. (2013): Comparison Between The Microbiological Quality Of Kareish Cheese Manufactured From Raw And Pasteurized Skimmed Milk Sold In Assiut City Markets. Assiut Vet. Med. J. 59 (138).

[21]. *Rahimi, E. (2013):* Enterotoxigenicity of Staphylococcus aureus isolated from traditional and commercial dairy products marketed in Iran. Brazilian Journal of Microbiology. 44(2): 393-399

[22]. Goy RC, Morais ST, Assis OB. Evaluation of the antimicrobial activity of chitosan and its quaternized derivative on E-coli and *S.Aureus* growth. *Rev Bras Farmacognosia Brazilian J Pharmacogn.* 2016;26:122–7

[23]. Avadi MR, Sadeghi AM, Tahzibi A, Bayati K, Pouladzadeh M, Zohuriaan-Mehr MJ, et al. Diethylmethyl chitosan as an antimicrobial agent: Synthesis, characterization and antibacterial effects. Eur Polym J. 2004;40:1355–61

[24]. Zheng LY, Zhu JA. Study on antimicrobial activity of chitosan with different molecular weights. *Carbohydr Polym.* 2003;54:27–530.

[25]. Fernandes, J.C.; Tavaria, F.K.; Soares, J.C.; Ramos, O.S.; Joao Monteiro, M.; Pintado, M.E. and Xavier Malcata F. (2008): Antimicrobial effects of chitosans and chitooligosaccharides, upon Staphylococcus aureus and Escherichia coli, in food model systems. Food Microbiol. 25(7): 922-8.