

The Preventative Effects of Nicin and Preservation Temperature on the Growth and Activity of *Clostridium perfringens* in Lactic Cheese

Aida Agheli, Ali Mohammadi Sani*

Department of Food Science and Technology, Quchan Branch, Islamic Azad University, Quchan, Iran

Received: October 29, 2014

Accepted: December 31, 2014

ABSTRACT

One of the problems in lactic cheese is causing gas defect together with producing unpleasant odor, during which defecting microorganisms release gases such as carbon dioxide and produce unpleasant-smelling compounds like indole and skatole by breaking down the interior nutrients of the cheese. The aim of this research was to investigate the effect of Nicin and preservation temperature on the growth and activity of *Clostridium perfringens* in lactic cheese. Since the spore of sporulated bacteria survives in pasteurization, a particular method rather than pasteurization should be applied for taming these types of bacteria in dairy products. In this study, the effect of different concentrations of Nicin and preservation temperature was investigated on the growth and survival of bacterial spore of *clostridium perferingens* in lactic cheese. Nicin in concentrations of 20, 25 and 30 ppm was added into consumable yoghurt with acidic pH and into raw milk and the samples were kept at 6 and 25°C for 60 days. Counting the bacterial spore was carried out on days 0, 1, 8, 15, 30, 45 and 60. Based on the results of this study, adding Nicin to the yoghurt in concentration of 30 ppm caused the bacterium grow less during the cheese preservation period; moreover, all samples which were kept in 6°C including the contaminated one, remained intact. According to the results of this study, keeping lactic cheese in inappropriate temperature makes the spore, to change into vegetative form, which causes following problems and therefore concentration of 30 ppm of Nicin can be regarded as a natural preservative. It goes without saying that with an increase in the number of spore in the raw milk sample, the applied dose of Nicin with change.

KEYWORDS: Spore, Nicin, Lactic cheese, *Clostridium perferingens*

INTRODUCTION

Robinson (1990) claimed that cheese is one of the most important dairy product and like milk it is a nutritious product for the human. Cheese is much more perceivable than milk; however, anyway it is exposed to spoilage causes same problems for cheese production industry (21).

Jay (1986) stated that speculated bacteria are capable of tolerating high temperatures. This kind of bacterium tolerates the pasteurization temperature; remain in milk and cause splurge cheese and other derivative dairy products.

A kind of spoilage in cheese which is caused by sporulated bacteria is gas defect. This type of spoilage causes gas release and changes in wrapping form and results in less supply and selling (19). Not only gas defect causes changes in wrapping form but also sometimes this spoilage is so server that makes the cheese shred into pieces and causes unpleasant odor and it reduce the product supply (16). Anaerobic microorganisms which produce Butyric acid can cause gas and unpleasant odor in come kinds of cheeses. Late blowing which appears by these organisms in cheese, not only makes the product rotten, but also it causes financial loss. According to the result of the study about dairy farm of NIRD research institute in Reading, England, *Cl. perferingens*, *Cl. butyricum*, *Cl. tyrobutyricum* and *Cl. beijerinckii* were isolated from milk samples under scrutiny (7).

The growth of *clostridium*s in cheese, in addition to the possibility of causing swelling and crack and also acid flavor, also causes bigger and than usual holes and unpleasant odor (5and9). The consistent of *clostridium*s are found in bedding and defecation and alimentary canal of humans and farm animals and it can enter milk by defecation, soil, form animal food and especially silo (24).

Nowadays, the health of foodstuff is very important due to its huge production and long distribution chains.

The centimeters don't show any interest toward using chemical preservatives or serer thermal processes. They prefer healthy feedstuffs which have longer preservation time and high quality (12). Nicin is a natural preservative which is produced by subspecies of *lactococcus lactis* and it is

* Corresponding Author: Ali Mohammadi Sani, Department of Food Science and Technology, Quchan Branch, Islamic Azad University, Quchan, Iran

only bacteriocin which is used in foodstuffs confirmed by FDA/FAO as a GRAS harmless additive Daeschel (1989) stated that Nicin antibiotic has a taming effect on gram-positive bacteria especially *clostridium*s and it can be applied to reduce their growth. In 1965 Heinmann, attributed 10 ideal features to Nicin, these characteristics indicate that Nicin antibiotic is one of the best among 650 different preservatives which cause reduction in the growth of bacteria spores (4).

Hirtch (1954) applied Nicin to the prevention of gas defect resulted from *clostridium*s in Switzerland cheese. The same process was carried out latter by other researchers such as Daeschel (1989) and also by Prescott (1969) (15).

People have consumed Nicin for a long time without experiencing any pathogenic effect, because *lactococci* which produce Nicin exist in milk and cheese (6). According to the results obtained by researchers, Nicin lacks any particular toxic effect (LD^{50} like other ordinary salts, approximately 7 grams per each Kg of body weight) and is an innocuous substance in foodstuff (26) which is deactivated quickly by digestive enzymes (13).

Also unlike antibiotics, there is no evidence indicating any increase in resistance to Nicin like antibiotics (25). This peptide synthesis has 3.5KDa molecular weight, 34 amino acids, positive charge and antimicrobial activity on gram-positive bacteria such as *bacilluses*, *micrococcus*, *staphylococcus aureus*, *listeria monocytogenes* and *clostridium*s.

But it has little antimicrobial activity on gram-negative bacteria, yeasts and molds (18). Nowadays, Nicin is used for protecting and increasing preservation time of pasteurized cheeses, dairy desserts, canned food, Salted meat and sea food. Since heating milk has adverse effect on the quality of produced cheese, Nicin can be used as a healthy preservative in cheeses (26).

The aim of this study is to determine the effect of different densities of Nicin (20-25-30 ppm) on sporulated bacterium existing in cheese mad from pasteurized milk, during the preservation period and also the effect of the preservation temperature on the growth of aforementioned bacterium in lactic cheese.

MATERIALS AND METHODS

Preparing Nicin Solution

For producing Nicin solution, 1 gram Nicin (Pure Nicin was Purchased from Sigma Aldric Inc. in England) was dissolved in 100 ml Hydrochloric Acid %2 normal (pH 1.0) to reach the density of 10^4 IU/ml (the International unit of Nicin) ($40 \text{ IV}=\text{Hg}$), then it been became sterile by 0/45 micrometer filter and was used for different dilutions of distilled water.

Preparation of Bacterial suspensions and producing lactic cheese in laboratory scale

The bacterium under study was *clostridium perferingens* (PTCC 1765) which was supplied in a lyophilized way from Iran's Microbial collocation. The preparation of the bacterium for inoculation was carried out by transferring the bacterium from sterile micro tubes to RCMA medium (Merck, Germany) and keeping it for 24 hours in anaerobic jar in 37°C .

Again, another culture was supplied from the first 24 hour culture in another RCM medium in 37°C for 24 hours and the amount of bacteria per each ml was identified by McFarland Method. Finally 10^6 bacteria in volume of 10 ml were added to the raw milk. Inoculation to the raw milk was carried out up to several levels until the number of spores in the first raw milk finally reached 3000 CFU/ml. The applied milk was supplied from Astan-e-Ghods-e-Razavi industrial farmland (the first spore 27 CFU/ml) and the production lactic cheese was accomplished by pasteurizing the milk up to 80°C for 2 minutes. For making flocculation in predating this cheese, the yoghurt with pH 3.80 was used which was prepared 48 hours earlier by Astan-e-Ghods-e-Razavi milk. The identified doses of Nicin were added once to the yoghurt and once to the raw milk and the cheeses, after the productions, process and the addition of salt water (3%), were wrapped up (in polystyrene containers) and were carried to refrigerating room with 6°C temperature. The first cheese sample as the first evidence was produced by the earlier raw milk (before contamination) and the second sample was produced as the second evidence by the contaminated raw milk without additive (Nicin). The samples 3, 4 and 5 contained yoghurt and doses of Nicin (20-25-30 ppm) and the samples 6, 7 and 8 contained raw milk and doses of Nicin. The samples after being kept in refrigerating room (cultivation period) in 6°C were transferred to 25°C and the counting of the spore was carried out by heat shock (from bout samples (6°C and 25°C) on days 0-1-8-15-30-45-60, In a way that particular dilutions of the sample were cultivated after giving heat shock by poured plate method on BA (Blood Agar), EYA (Egg Yolk Agar), RCMA (Mack, Germany) mediums and after incubation in anaerobe condition, the number of colonics in one gram firm the first sample was counted up (28).

Then different kinds of bacteria grown on the mediums were identified by biochemical experiments.

RESULTS

The comparison of different densities of Nicin and pH conditions was carried out based on the number of existing spore in 8 produced cheese groups by the statistical method of variance and SPSS software pack was applied to accomplish it.

Chart No.1

Counting the number of spores during cheeses preservation period for the sample of the first evidence (the earlier raw milk).

It can be observed that the spor growth has taken place with higher speed but because of the limited number spores, the cheeses remained healthy during 60 days.

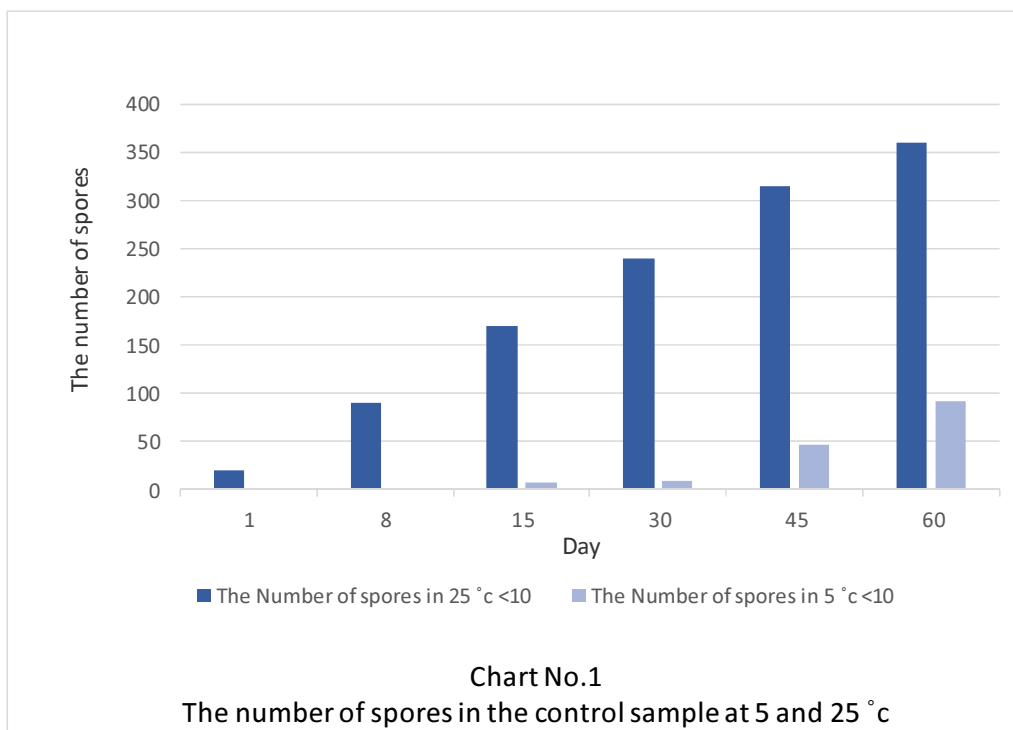


Chart No.2

Counting the number of spores during cheese preservation period for the sample of the second evidence. (the contaminated milk without Nicin). It can be observed that the spore growth has taken place with much lower speed and also no problem was seen in any of the samples but 100% of which have been in 25°C had unpleasant odor and swelling and disintegration of tissues.

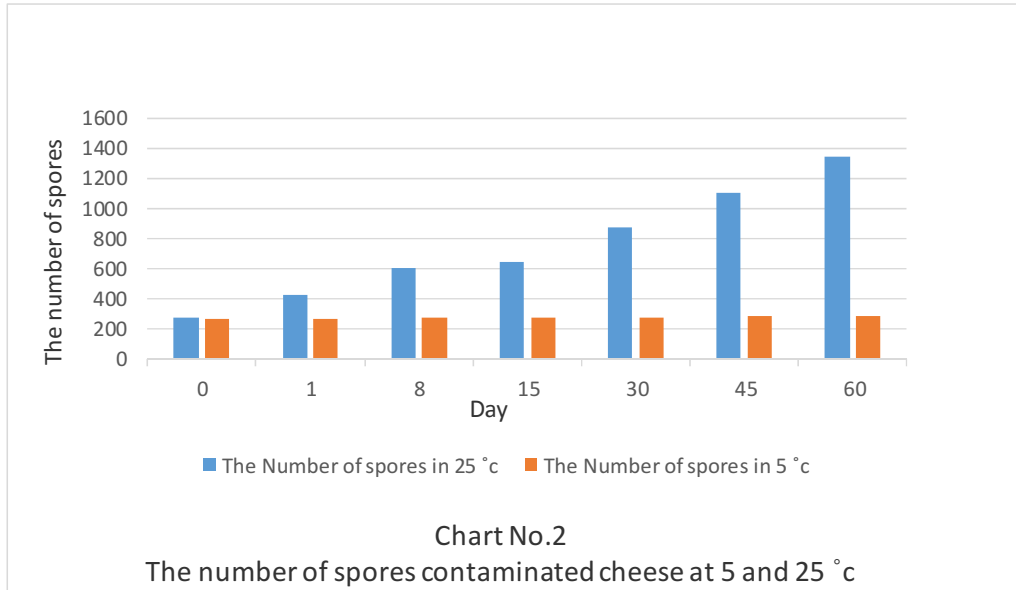


Chart No.3

Counting the number of spores was carried out in cheeses which Nicin had been added to the yoghurt and in 30 ppm dose none of the cheeses had problems in 25°C, but in doses 20 and 25 ppm of Nicin, some of the cheeses which were kept in 25°C had some problems.

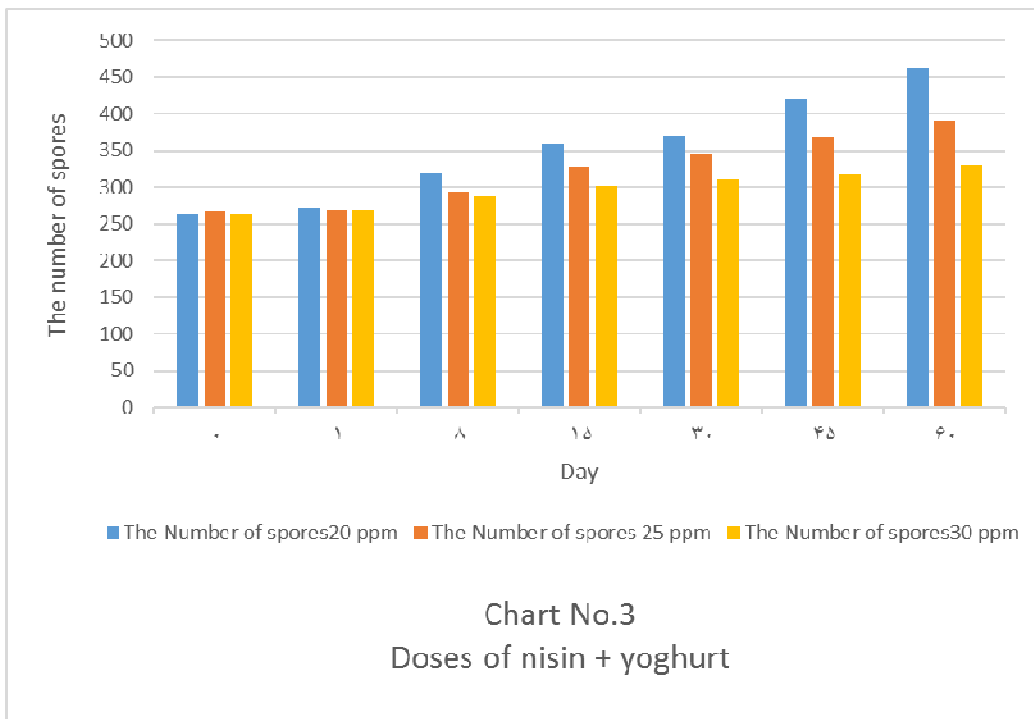
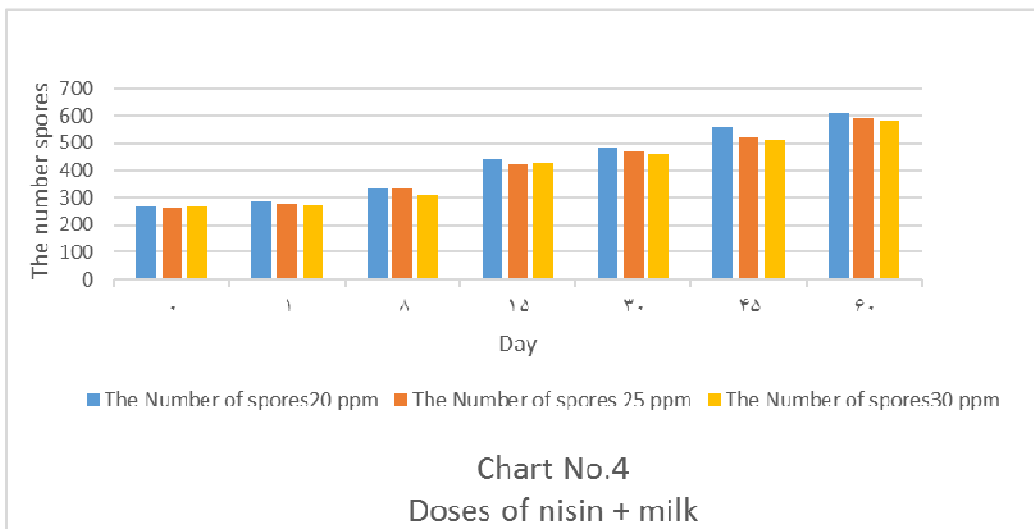


Chart No.4

Counting the number of spores in cheeses which raw milk Nicin has been added to there raw indicates that approximately 80% of samples with the highest applied dose of Nicin, had problems in 25°C.



DISCUSSION

Jay (1986) mentioned that the spore of sporulated bacteria are not capable of tolerating pasteurization temperature and don't vanish by HTST method (temperature of 71°C for 15 seconds) (19). During this study, these kinds of bacteria were more separated from pasteurized milk. Also, by experiment it became apparent that some of these bacteria are capable of fermenting lactose and releasing gas and breaking down protein. Sporulated bacteria were also separated from gas-defected cheeses.

Jay (1986), Frazier (1998) and Fox (1993) mentioned that the antibiotic of Nisin causes prevention of gram-positive bacteria especially sporulated bacteria from growing (19,9,11).

During this study, the truth of the fact was also verified by conducting AntibioGram test and it was indicated that sporulated bacteria which were separated from pasteurized milk were sensitive to this antibiotic. For there are many more researches have been conducted in the field of antibacterial effects of Nisin on experimental models and foodstuff.

Beuchat and his colleagues (1997) investigated the effect of Nisin on the way of growth and secretion of antrotoxin which causes diarrhea in BHT broth and according to the results, Nisin in density of 1 µg in each ml prevented the growth and secretion of antrotoxin by *Bacillus cereus* vegetative cells. In another research Nisin in density of 2.5 µg in ml was effective in the control of *Mesophilic clostridium spores* and thermophile *Bacillus stearothermophilus spore* before thermal process (14, 27, 23).

The preservation period of caramel cream pudding increased from less than 6 days to more than 35 days by adding 3.75 µg Nisin. Flavoured milks like chocolate milk may contain several sporulated organisms due to their additives. In these kinds of foodstuff, Nisin is used as a natural additive. Nisin affects cytoplasmic membrane. Nisin causes in cytoplasmic membrane and deactivates protonic activating force. Therefore, the absorption of amino acid stops and tiny metabolites of ions or soluted substances inside the cytoplasm like amino acids and nucleotides exit from the cells (1).

The amount of Nisin remains unchanged in refrigerator temperature however, by an increase in temperature and the preservation time, the break down of Nisin also takes place with higher speed (5, 19).

Researches indicate that the activeness of Nisin reduces in alkaline and high pH and probably samples in which Nisin has been added to raw milk due to lack of Nisin activity, the cheese had problems and also the applied yoghurt pH has been appropriate for Nisin activity. Adding Nisin together with salt water on the cheese surface is not appropriate, because in addition to its relatively alkaline pH, Nisin is not completely absorbed in cheese interior tissues. Moreover, Bell and his colleague (1985) investigated the simultaneous effect of sodium chloride and Nisin on preventing the growth of *B. licheniformis* and indicated that salt ruins the spore-killing effect of Nisin by stopping the absorption of Nisin in the cell.

Conclusion

The result of this study revealed that complete observation of cold chain without applying any preservative can prevent lactic cheese from swelling and unpleasant odor, because any temperature

change causes the spores, change into vegetative form and finally causes secretion of proteolytic and saccharolytic enzymes furthermore, adding Nicin due to the number of early spores of the milk, can applied as a natural preservative.

REFERENCES

- [1] Abee, T., Krockel, L. and Hill, C. (1995). Bacteriocins: modes of action and potentials in food preservation and control of food poisoning. *International Journal of Food Microbiology*, 28:169-185.
- [2] Bell R. G. & Karen M. De –lacy (1985) The effect of nisin – sodium chloride interactions on the outgrowth of *Bacillus licheniformis* spores.
- [3] Daeschel, M.A 1989 ; Antimicrobial substances from lactic acid bacteria for use as food preservatives. *Food Technol.* 43:148-155
- [4] Defigueiredo, M.P., Splittstoesser, D.F. 1976:Food microbiology: public Health and spoilage aspects. The Avi publishing Company , Inc., Westport p.p.356-386.
- [5] Delves-Broughton, J. (1990). Nisin and its uses as a food preservative. *Food Technology*, 44: 100, 102, 104, 106, 108, 111-112 and 117 .
- [6] Department of food science, School of Veterinary Medicine, University of Urmia, IRAN,
- [7] FDA. (1988). Nisin preparation: affirmation of GRAS status as a direct human food ingredient. *Federal Register*, 53: 11247-11251 .
- [8] Fox,P.F.1993; Cheese. 2nd ed. Chapman & Hall, London. PP:471-462.
- [9] Fox.P.F.1987; Cheese : Chemistry, Physics and microbiology, vol 2 Elsevier Applide., Essex. p.p.85-86.
- [10] Frazier, W.C.2 and D.C. Westoff. 1988; Food microbiology. Mc Graw. Hill Book Co. New York. PP:325-369.
- [11] Gould, G.W. (1992). Ecosystem approaches to food preservation. *Journal of applied bacteriology symposium supplement*, 73: 58S-68S.
- [12] Heinemann, B. and Williams, R. (1966). The inactivation of nisin by pancreatin. *Journal of Dairy Science*, 49:312-313.
- [13] Heinemann, B., Voris, L. and Stumbo, C.R. (1965). Use of nisin in processing food products. *Food Technology*, 19: 592-596.
- [14] Hirsch, A. E.Grinsted, H.R. Chapman , & A.T.R. Mattick.1951; A note on the inhibition of an anaerobic sporeformer in swiss type cheese by a nisin producing streptococcus. *J . Dairy Res.*18:205207
- [15] Hirsch, A, M. McClintock, and G. Mocquot. 1952; Observations on the influence of inhibitory substances produced by the lactobacilli of Gruyere on the development of anaerobic spore formers. *J. Dairy Res.* 19:176-185.
- [16] Hurst. A, (1978) nisin : Its Preservative effect and Function in the growth cycle of the producer organism. The Societyfor Applied Bacteriology Symposium Series No:7.Streptococci ed by F.A.Skinner and L.Baulsnel
- [17] Hurst, A. (1983). Nisin and other inhibitory substances from lactic acid bacteria. In:Antimicrobials in Foods, Branen, A. L. and Davidson, P . M., (Eds.), Marcel Dekker, New York, pp. 327-351.
- [18] Jay, J. M.1986; Modern food microbiology. Van Nostrand Rwinhold Co, New York. pp:268-286.
- [19] Leverentz, B., Conway, W.S., Camp, M.J., Janisiewicz, W.J., Abuladze, T., Yang, M., Saftner, R. and Sulakvelidze, A. (2003). Biocontrol of *Listeria monocytogenes* on Fresh-Cut Produce by Treatment with Lytic Bacteriophages and a Bacteriocin. *Applied and Environmental Microbiology*, 69:4519-4526.

- [20] Rayman, M. K., B. Aris and A. Hurst.(1981)Nisin : a possible alternative or adjunct to nitrite in the preservation of meats. *Applide and Environmental microbiology* . 41(2), 375-380.
- [21] Robinson , R. K. 1990; *Dairy microbiology* . Elsevier Science Publishers Ltd, London. Pp:203-286.
- [22] Scott v. n. and S. L. Taylor(1981)Effect of nisin on the outgrowth of clostridium botulinum spores. *J. of food science*. 46, 117-120.
- [23] Shehata, A.E., Khalafalla, S.M., Magdoub, M.N.I. and Hofi, A.A. (1976). The use of nisin in the production of sterilized milk drinks. *Egyptian Journal of Dairy Science*, 484: 37-42.
- [24] Stewart, D.B.1978: The incidence of bacterial spores in Northern Ireland milk supplies. 20 th International Dairy congress, Paris p.p:91-82.
- [25] Szybalski, W . (1953). Cross resistance of *Micrococcus pyogenes* var. *Aureus* to thirty-four antimicrobial drugs. *Antibiotics and Chemotherapy*, 3:1095-1103.
- [26] Thomas L.V. and Delves-Broughton, J. (2005). Nisin, In: Michael Davidson, P., John N. Sofos, J.N. and Branen , A.L., *Antimicrobial in foods*, Third edition, Published by CRC Press, pp.237-274.
- [27] Trämér, J. (1964). The inhibitory action of nisin on *B. stearothermophilus*. In: *The Action, Use and Natural Occurrence of Microbial Inhibitors in Foods*, Molin, N., (Ed.), Almquist and Wikesell, Stockholm, pp.25-33.
- [28] Varon,E.J. & L.R.Peterson.1994; *Bailey & Scott s diagnostic microbiology*. Mosby, USA.