Available online at www.jpsscientificpublications.com
Volume – 1; Issue - 4; Year – 2015; Page: 320 – 326

Indo – Asian Journal of Multidisciplinary Research (IAJMR)
ISSN: 2454-1370

EFFECT OF ORGANIC ACIDS AND CHEMICAL PRESERVATIVES ON THE INHIBITION OF GROWTH OF DAIRY PRODUCT SPOILAGE BACTERIAL STRAINS

J. Bhuvaneswari* and D. Kanchana,
Department of Microbiology, Annamalai University, Annamalai Nagar – 608 002, Tamil Nadu, India.

Abstract

Milk is the common universal product with more commercial demand, regardless of the source, adding important protein content (casein) to the various age peoples for their diet. The microbiological characteristics and quality of the milk and milk products are very important to the consumer. In the present study, the effects of different concentrations of organic acids (Acetic acid, Lactic acid and Sorbic acid) and chemical preservatives (Potassium sorbate, Sodium nitrate and Sodium propionate) on the growth of the dairy product spoilage bacterial strains were studied. The diameter of the inhibition zone of all the bacterial strains increased with increase in concentration of organic acids from 1000 to 5000 µg ml^{-1}. Highest zone of inhibition was recorded against *Listeria monocytogenes*, *Staphylococcus aureus*, *Yersinia enterocolitica* and *Pseudomonas aeruginosa*. Lowest zone of inhibition was recorded in *Campylobacter jejuni*.

Key words: Dairy products, Chemical preservatives, Organic acids, Bacteria and Spoilage.

1. Introduction

Preservation of foods stuffs has been necessary for the survival of human beings. The food preservation techniques used in early days relied without any understanding of the microbiology on inactivation of the food spoilage causing bacteria and fungi. Usually preservation of food stuffs from microorganisms was carried out by drying, salting, heating, chemical preservatives or fermentation. Various food preservation methods are used in preservation and combining various lightly preservation procedures to inhibit growth of food spoilage causing microorganisms. Microbial food spoilage was characterized by the change in food products that renders it unacceptable to the consumer from a sensory point of view. Spoilage of food stuffs may be caused by physical damage, chemical changes (oxidation and colour changes) or change in flavours and odours resulting from microbial growth and metabolism in the food products. Microbial growth in food substance is the most common cause of food spoilage and manifest itself as visible growth (slime, colonies), as textural changes (degradation of polymers) or as off-odours and off-flavours (Anonymous, 1985).

Various types of dairy product spoilage causing microorganisms *viz*., *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Listeria monocytogenes*, *Campylobacter jejuni*, *Yersinia enterocolitica* and *Salmonella typhi* has been reported as one of the most common pathogenic microorganisms which are related to food borne diseases, food borne diseases and food borne intoxification in the dairy industry. This microorganism is a short rod that can grow under aerobic and microaerophilic conditions. The optimum temperature for growth of dairy product spoilage causing microorganisms is 35 - 37 °C, but some kind of spoilage causing organisms can

Corresponding author*: **J. Bhuvaneswari
E-mail: jbhuvann@gmail.com
Received: 20.03.2015; *Revised*: 12.04.2015;
Accepted: 12.04.2015.

grow under refrigerated temperature, over a wide range of pH which was ranged from pH 4 to 9 with salt concentration upto 10 %. Although, the dairy industry is most concerned about the presence of *Listeria monocytogenes* in dairy products such as cheese, yoghurt, raw milk and curd (Jageethadevi *et al*., 2012; Savitha Janakiraman *et al*., 2012).

The use of chemical preservatives and organic acids currently employed to control the dairy product spoilage causing microorganisms which are capable of growing in milk products including sulphites, sulphur dioxide, sodium chloride, phosphates, hydrogen peroxide, nitrates, nitrites, sodium diacetate, citric acid, acetic acid, lactic acid, benzoic acid, fumaric acid and therapeutic antibiotics are increasingly being questioned with regard to their impact on human health (Magnuson, 1997; Kennedy *et al*., 2000). These type of queries challenging the food processing and manufacturing industries to focuss on natural means of food preservation as the food service customers demand high quality products that have a relatively long shelf-life, but still prefer the appearance of minimally processed dairy food products (Hugas *et al*., 2002; Ross *et al*., 2002; Saranraj and Geetha, 2012).

2. Materials and Methods

2.1. Effect of organic acids on the inhibition of growth of Dairy product spoilage bacterial strains by Agar well diffusion method

The effects of different concentrations of organic acids on the growth of the dairy product spoilage bacterial strains were studied using Acetic acid, Lactic acid and Sorbic acid. The sterilized Nutrient agar medium was prepared and seeded with standard inoculum of the five bacterial strains, *Listeria monocytogenes* (MPB - 1), *Yersinia enterocolitica* (MPB - 2), *Campylobacter jejuni* (MPB - 3), *Staphylococcus aureus* (MPB - 4) and *Pseudomonas aeruginosa* (MPB - 5) separately and plated. Different concentrations of acetic acid, citric acid, and lactic acid *viz*., 1000, 2000, 3000, 4000 and 5000 µg ml^{-1} were prepared. On the surface of the medium wells made by using cork borer (6 mm diameter) and discharged 100 µl of concentrations into well. The plates were incubated at room temperature for 24 hrs and the diameter of inhibition zone (in mm) around the well was measured. Three replications were maintained in each treatment.

2.2 Effect of Potassium sorbate, Sodium nitrate and Sodium propionate on the inhibition of growth of bacterial strains by Agar well diffusion method

The effect of different concentrations of the three different chemical preservatives (Potassium sorbate, Sodium nitrate and Sodium propionate) on the inhibition of growth of the dairy product spoilage bacterial strains was studied. The sterilized Nutrient agar medium was prepared with pH 7.0 and seeded with the five red meat bacterial strains of *Listeria monocytogenes* (MPB - 1), *Yersinia enterocolitica* (MPB - 2), *Campylobacter jejuni* (MPB - 3), *Staphylococcus aureus* (MPB - 4) and *Pseudomonas aeruginosa* (MPB - 5). Different concentrations of Potassium sorbate, Sodium nitrate and Sodium propionate *viz*., 1000, 2000, 3000, 4000 and 5000 µg ml^{-1} were prepared using sterile water. The well made (6 mm in diameter) by using sterile cork borer and inoculated 100 µl of different concentrations of Potassium sorbate, Sodium nitrate and Sodium propionate were discharged into the well. The plates were incubated at room temperature for 24 hrs and the diameter of inhibition zones (in mm) was measured around the well using antibiotic zone scale.

3. Results and Discussion

Organic acids, short chain fatty acids, hydrogen peroxide, reuterin, diacetyl, secondary metabolite bacteriocins which was mainly extracted from Lactic acid bacterial isolates like *Lactobacillus lactis*, *Lactobacillus acidophilus*, etc., and proteinaceous bacteriocin like inhibitory substances are some of the secondary metabolic products of these bacteria which was suggested by

many researchers to have potential antibacterial, antifungal, antiviral, antioxidant, anticancer and various pharmacological effects (Cleveland *et al.*, 2001; Saranraj and Geetha, 2012).

The effect of acetic acid on the inhibition of growth of five dairy spoilage bacterial strains *viz., Listeria monocytogenes, Yersinia enterocolitica, Campylobacter jejuni, Staphylococcus aureus* and *Pseudomonas aeruginosa* was studied and the results were presented in Table – 1. The diameter of the inhibition zone of all the bacterial strains increased with increase in concentration of acetic acid from 1000 to 5000 µg ml^{-1}. Highest zone of inhibition was recorded against *Listeria monocytogenes* (38.83 mm), *Staphylococcus aureus* (36.70 mm), *Yersinia enterocolitica* (35.67 mm) and *Pseudomonas aeruginosa* (32.53 mm). Lowest zone of inhibition was recorded in *Campylobacter jejuni* (30.06 mm).

The effect of lactic acid on the inhibition of growth of five dairy spoilage bacterial strains was tested and the results were tabulated in Table – 2. The diameter of the inhibition zone of all the bacterial strains increased with increase in concentration of lactic acid from 1000 to 5000 µg ml^{-1}. Highest zone of inhibition was observed against *Listeria monocytogenes* (33.21 mm), *Staphylococcus aureus* (31.92 mm), *Yersinia enterocolitica* (28.65 mm) and *Pseudomonas aeruginosa* (26.58 mm). Lowest zone of inhibition was observed in *Campylobacter jejuni* (25.40 mm).

The effect of sorbic acid on the inhibition of growth of five dairy spoilage bacterial strains was determined and the results were furnished in Table – 3. The diameter of the inhibition zone of all the bacterial strains increased with increase in concentration of sorbic acid from 1000 to 5000 µg ml^{-1}.

Table – 1: Effect of Acetic acid on the inhibition of Dairy product spoilage bacteria by Agar well diffusion method

Acetic acid (µg ml^{-1})	Diameter of zone of inhibition (mm)				
	Listeria monocytogenes	***Yersinia enterocolitica***	***Campylobacter jejuni***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
Control	NZ	NZ	NZ	NZ	NZ
1000	17.36	15.56	13.47	16.13	14.62
2000	22.51	20.42	17.20	21.34	18.59
3000	27.60	25.97	21.42	26.17	23.46
4000	32.66	30.50	25.67	31.43	27.84
5000	38.83	35.67	30.06	36.70	32.53
S.Ed	5.55	5.16	4.32	5.26	4.69
CD (P = 0.05)	11.02	10.33	8.65	10.53	9.38

NZ – No zone of inhibition; *****Values are average of three replications

Table – 2: Effect of Lactic acid on the inhibition of Dairy product spoilage bacteria by Agar well diffusion method

Lactic acid (µg ml^{-1})	Diameter of zone of inhibition (mm)				
	Listeria monocytogenes	***Yersinia enterocolitica***	***Campylobacter jejuni***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
Control	NZ	NZ	NZ	NZ	NZ
1000	15.20	12.20	11.52	14.46	11.37
2000	19.33	15.31	13.39	17.34	13.55
3000	23.47	19.63	16.21	21.71	17.39
4000	27.76	23.54	20.17	25.32	21.66
5000	33.21	28.65	25.40	31.92	26.58
S.Ed	4.72	4.07	3.53	4.46	3.76
CD (P = 0.05)	9.44	8.15	7.07	8.93	7.53

NZ – No zone of inhibition; *****Values are average of three replications

Table – 3: Effect of Sorbic acid on the inhibition of Dairy product spoilage bacteria by Agar well diffusion method

Sorbic acid (µg ml^{-1})	Diameter of zone of inhibition (mm)				
	Listeria monocytogenes	***Yersinia enterocolitica***	***Campylobacter jejuni***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
Control	NZ	NZ	NZ	NZ	NZ
1000	9.46	8.40	7.90	9.36	8.29
2000	12.37	10.56	9.65	11.45	9.86
3000	16.90	13.43	11.34	15.54	12.56
4000	20.35	17.96	14.85	19.50	15.73
5000	26.35	22.82	20.16	24.45	21.61
S.Ed	3.74	3.23	2.77	3.48	2.97
CD (P = 0.05)	7.49	6.47	5.55	6.97	5.95

NZ – No zone of inhibition; *****Values are average of three replications

Table – 4: Effect of Potassium sorbate on the inhibition of Dairy product spoilage bacteria by Agar well diffusion method

Potassium sorbate (µg ml^{-1})	Diameter of zone of inhibition (mm)				
	Listeria monocytogenes	***Yersinia enterocolitica***	***Campylobacter jejuni***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
Control	NZ	NZ	NZ	NZ	NZ
1000	9.46	8.40	8.12	9.11	8.23
2000	11.59	10.25	9.24	11.43	9.86
3000	14.65	12.42	11.40	13.60	11.54
4000	17.59	15.38	13.65	16.86	14.70
5000	24.80	21.50	19.45	23.43	20.17
S.Ed	3.39	2.93	2.63	3.20	2.75
CD (P = 0.05)	6.79	5.87	5.27	6.05	5.51

NZ – No zone of inhibition
*****Values are average of three replications

Table – 5: Effect of Sodium nitrate on the inhibition of Dairy product spoilage bacteria by Agar well diffusion method

Sodium nitrate (µg ml^{-1})	Diameter of zone of inhibition (mm)				
	Listeria monocytogenes	***Yersinia enterocolitica***	***Campylobacter jejuni***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
Control	NZ	NZ	NZ	NZ	NZ
1000	9.21	8.21	7.12	8.45	7.24
2000	11.30	10.17	9.00	11.06	9.26
3000	13.45	12.35	11.26	13.27	11.40
4000	16.50	15.24	13.69	15.30	14.23
5000	22.37	20.17	18.75	20.42	19.80
S.Ed	3.06	2.78	2.58	2.82	2.72
CD (P = 0.05)	6.13	5.57	5.17	5.65	5.45

NZ – No zone of inhibition
*****Values are average of three replications

Table – 6: Effect of Sodium propionate on the inhibition of Dairy product spoilage bacteria by Agar well diffusion method

Sodium propionate (µg ml^{-1})	Diameter of zone of inhibition (mm)				
	Listeria monocytogenes	***Yersinia enterocolitica***	***Campylobacter jejuni***	***Staphylococcus aureus***	***Pseudomonas aeruginosa***
Control	NZ	NZ	NZ	NZ	NZ
1000	9.27	8.35	7.45	8.60	7.86
2000	11.45	10.26	9.34	11.32	9.47
3000	13.70	12.30	11.17	12.65	11.36
4000	16.65	14.69	13.70	15.77	14.30
5000	23.46	20.85	19.56	21.60	20.00
S.Ed	3.19	2.83	2.66	2.95	2.73
CD (P = 0.05)	6.37	5.67	5.33	5.10	5.47

NZ – No zone of inhibition

*****Values are average of three replications

Highest zone of inhibition was noticed against *Listeria monocytogenes* (26.35 mm), *Staphylococcus aureus* (24.45 mm), *Yersinia enterocolitica* (22.82 mm) and *Pseudomonas aeruginosa* (21.61 mm). Lowest zone of inhibition was noticed in *Campylobacter jejuni* (20.16 mm). The effect of potassium sorbate on the inhibition of dairy spoilage bacterial strains was determined and the results were furnished in Table – 4. The diameter of the inhibition zone of all the bacterial strains increased with increase in concentration of potassium sorbate from 1000 to 5000 µg ml^{-1}. Highest zone of inhibition was noticed against *Listeria monocytogenes* (24.80 mm), *Staphylococcus aureus* (23.43 mm), *Yersinia enterocolitica* (21.50 mm) and *Pseudomonas aeruginosa* (20.17 mm). Lowest zone of inhibition was noticed in *Campylobacter jejuni* (19.45 mm).

The effect of sodium nitrate on the inhibition of dairy spoilage bacterial strains was evaluated and the results were given in Table – 5. The diameter of the inhibition zone of all the bacterial strains increased with increase in concentration of sodium nitrate from 1000 to 5000 µg ml^{-1}. Highest zone of inhibition was observed against *Listeria monocytogenes* (22.37 mm), *Staphylococcus aureus* (20.42 mm), *Yersinia enterocolitica* (20.17 mm) and *Pseudomonas ginosa* (19.80 mm). Lowest zone of inhibition was record in *Campylobacter jejuni* (18.75 mm).

The effect of sodium propionate on the inhibition of dairy spoilage bacterial strains *viz., Listeria monocytogenes, Yersinia enterocolitica, Campylobacter jejuni, Staphylococcus aureus* and *Pseudomonas aeruginosa* was studied and the results were showed in Table – 6. The diameter of the inhibition zone of all the bacterial strains increased with increase in concentration of sodium propionate from 1000 to 5000 µg ml^{-1}. Highest zone of inhibition was recorded against *Listeria monocytogenes* (23.46 mm), *Staphylococcus aureus* (21.60 mm), *Yersinia enterocolitica* (20.85 mm) and *Pseudomonas aeruginosa* (20.00 mm). Lowest zone of inhibition was noticed in *Campylobacter jejuni* (19.56 mm).

Organic acids like lactic acid were more effective than malic, citric, propionic, acetic acid in limiting the growth of various dairy product spoilage organisms like *Pseudomonas aeruginosa, Listeria monocytogenes, Campylobacter jejuni, Yersinia enterocolitica* and *Staphylococcus aureus* (Rice and Pederson, 1954). Baumgartner and Hersom (1956) proposed that the activity of various chemical preservative on the effect of microorganisms was primarily dependent based on its concentration and the increase in concentration of chemical preservatives increases the zone of inhibition against dairy product spoilage causing microorganisms. Subba Rao and Johar (1959) also stated that the acetic acid can act as a

chemical preservative which was high toxicity for the microorganisms even at low concentrations and it showed non-toxic effect on humans and commercially availability and the low cost. From the research of Davidson (1983), it was reported that chemical preservative benzoic acid exhibited maximum effective at various pH ranges upto 8.0. Gomashe and Tumane (2006) revealed that the citric acid is highly effective against multiple drug resistant urinary tract infection causing pathogens such as *Escherichia coli* and *Klebsiella* sp. (2000 µg ml^{-1}), *Proteus* sp. (1180 µg ml^{-1}) and *Staphylococcus* sp. (1080 µg ml^{-1}). Citric acid as a chemotherapeutic agent, active against various drug resistant dairy product spoilage causing bacterial isolates like *Pseudomonas aeruginosa, Listeria monocytogenes, Campylobacter jejuni, Yersinia enterocolitica* and *Staphylococcus aureus* has been reported (Nagoba *et al*., 1998).

4. References

1) Anonymous, 1985. Subcommittee on Microbiological Criteria: Committee on Food Protection; Food and Nutrition Board National Research Council, an Evaluation of the Role of Microbiological Criteria for Foods and Food Ingredients. National Academy Press, Washington, DC.
2) Baumgartner, J.G and A.C. Hersom. 1956. An introduction to Microbiology, D. Van Nostrand CO., Princeton, New Jersey.
3) Cleveland, J., T. J. Montwille, I. F. Nes and M. L. Chikindas. 2001. Bacteriocins: Safe natural anti-microbial for food preservation. *International Journal of Food Microbiology*, 71: 1.
4) Darwina, D. Kanchana and P. Saranraj. 2012. Biocontrol efficacy of various preservatives against food borne pathogens in poultry chicken. *Novus International Journal of Biotechnology and Biosciences*, 1 (1): 1 – 13.
5) Davidson, P.M. 1983. Phenolic compounds. In: Antimicrobials in foods, ed. A.L. Branen and P.M. Davidson, pp. 37-73, New York: Marcel Dekker.
6) Gomashe, A. U and P. M. Tumane. 2006. *In vitro* antimicrobial activity of citric acid against multiple drug resistant uropathogens. *Journal of Current Science*, 9(2): 595 - 598.
7) Hugas, M., M. Garriga, and J.M. Monfort. 2002. New mild technologies in meat processing: high pressure as a model technology. *Meat Science*, 62: 359-371.
8) Jageethadevi, A., P. Saranraj and N. Ramya. 2012. Inhibitory effect of chemical preservatives and organic acids on the growth and organic acids on the growth of bacterial pathogens in poultry chicken. *Asian Journal of Biochemical and Pharmaceutical Research*, 1 (2): 1 – 9.
9) Kennedy, M., A. O'Rouke, J. McLay and R. Simmonds. 2000. Use of a ground beef model to assess the effect of the lactoperoxidase system on the growth of *Escherichia coli* 0157:H7, *Listeria monocytogenes* and *Staphylococcus aureus* in red meat. *International Journal of Food Microbiology*, 57: 147-158.
10) Magnuson, B. 1997. Food Additive Petition Submission Requirements. http://extoxnet.orst.edu/faqs/additive/aprorv.htm. 17 February 2004.
11) Nagoba, B. S., R.C. Gandhi, B. J. Wadher, S. R. Deshmukh and S. P. Gandhi. 1998. Citric acid treatment of severe electric burns complicated by antibiotic resistant *Pseudomonas aeruginosa*. *BURNS*, 481-483.
12) Rice, A.C and C.S. Pederson. 1954. Factors affecting growth of *Bacillus coagulans* in canned tomato juice. II. Acidic constituents of tomato juice and specific organic acids. *Food Research*, 19: 124 - 131.
13) Ross, R.P., S. Morgan and C. Hill. 2002. Preservation and fermentation: past, present and future. *International Journal of Food Microbiology*, 79: 3-16.

14) Saranraj, P and M. Geetha. 2012. Microbial spoilage of Bakery products and its control by preservatives. *International Journal of Pharmaceutical and Biological Archives*, 3 (1): 204 - 214.
15) Savitha Janakiraman and Malini Maria. 2012. Detection of heat stable bacteriocin from *Lactobacillus acidophilus* NCIM5426 by liquid chromatography/ mass spectrometery, 3: 2325 - 2332.
16) Subba Rao, M. S and D. S. Johar. 1959. Inhibitory effect of acetic acid and sodium benzoate on the growth of two strains of osmophilic yeast. *Journal of Food Science*, 383 - 386.