Trends in Applied Sciences Research

ISSN 1819-3579

Academic
Journals Inc.

www.academicjournals.com

Trends in Applied Sciences Research 2 (1): 57-60, 2007
ISSN 1819-3579
© 2007 Academic Journals Inc.

Comparison of the Antibacterial Activity of Locally and Laboratory Prepared Cheese Wheys Against Some Common Diarrheic Bacteria

O.B. Olorunfemi, T.T. Adebolu and O.S Oturuhuyi
Department of Microbiology, Federal University of Technology,
P.M.B 704 Akure, Ondo State, Nigeria

Abstract: Cheese whey produced at two different temperatures; ≥100°C that is normally employed by the local producers in Nigeria and laboratory controlled temperature (60±5°C) were tested for growth inhibitory effect on some common bacteria that cause diarrhea in the south west part of the country. Seven bacteria namely *Bacillus cereus*, Enterotoxigenic *E. coli*, (NCTC 10418), *Enterobacter faecium*, *Salmonella typhi*, *Staphylococcus aureus* and *Shigella dysentariae* were used in this investigation using agar diffusion technique. Although both inhibited the growth of all the test organisms, the inhibitory activity of laboratory produced at controlled temperature was superior to that of the locally produced whey. From this investigation therefore, the temperature of production of whey has effect on its antibacterial property.

Key words: Whey, temperature, diarrheic bacteria, growth inhibition

INTRODUCTION

Diarrhea is one of the most important health problems globally and a leading cause of morbidity and mortality in children especially in developing countries (WHO, 1998). This is as a result of absence of potable water, proper sanitary habits, good faecal disposal systems, good hygienic practices by the impoverished citizens and over crowding (Cheeshbrough, 1994).

Although diarrhea is self-limiting, at times it may require antibiotic therapy (Prescott *et al*., 2002). However, because of the growing resistance of microorganisms to conventional antibiotics most of the commonly employed antibiotics are becoming ineffective.

Whey is the solution left during cheese-making process. It contains a variety of factors and compounds which have been reported by researchers such as Philanto- Lappala (1999), Ha and Zemel (2003) and CDRF (2006) to have health promoting effects and prevent diseases. Some of the factors are immunoglobulins, lactoferrin, lactoperoxidase, glycomacropeptide, bovine serum albumin, α-lactalbumin and β-lactoglobulin. In addition to the health promoting effect of whey, Adebolu and Ademulegun (2005) also found that it has growth inhibitory activity against common bacteria that cause diarrhea in South-West Nigeria. So, it could be exploited in treating bacterial diarrhea in the absence of antibiotics.

However because all the above mentioned factors are proteins so they can easily be denatured by heat above 65°C (Alais and Linden, 1997; Gutman, 2005). So majority of them are likely destroyed during cheese-making process by the local people which use excessive heat at temperature≥100°C. This present investigation therefore is designed to know whether the temperature of production of cheese has effect on the antibacterial property present in whey against selected common bacteria that cause diarrhea in South-West Nigeria.

Corresponding Author: O.B. Olorunfemi, Department of Microbiology, Federal University of Technology, P.M.B 704 Akure, Ondo State, Nigeria

MATERIALS AND METHODS

Organisms Used

Bacillus cereus, *Enterobacter faecium*, *E. coli* NCTC 10418, *E. coli* ETEC, *S. typhi*, *S. dysenteriae* and *Staphylococcus aureus* were obtained from the Drug Research Unit of Obafemi Awolowo University, Ile Ife, Federal Institute of Industrial Research Lagos, Nigerian Institute of Medical Research Lagos all in Nigeria. The isolates were maintained throughout the period of study aseptically by subculturing them into a freshly prepared nutrient agar medium.

Location of Study

This investigation was carried out at the department of Microbiology, Federal University of Technology, Akure, Ondo State, Nigeria between September-October, 2006.

Source of Local Cheese Whey Used

Cheese whey was obtained from the Fulani cattle rearers in Akure, Ondo State, Nigeria. The pH of the collected whey (LW) was determined daily using Jenway pH meter before use.

Production of Cheese Whey at Controlled Temperature (60±5°C)

Fresh cow milk collected at the point of milking from Fulani cattle rearers in Akure, Ondo State, Nigeria was transferred to the laboratory immediately for processing into cheese. *Calotropis procera* (Sodom apple) leaf sap (1 mL L^{-1}) was added to the milk and gently agitated. The milk was heated to 60±5°C for 30 mins and skimmed. The resulted curd was separated from the whey with the aid of a properly washed handmade basket. The pH of the prepared CW was determined using Jenway pH meter and monitored for 5 days.

Determination of Antibacterial Activity of Cheese Whey on Diarrheic Bacteria

The test organisms used were aseptically transferred from agar slants to different test tubes containing sterile nutrient broth and incubated at 37°C for 24 h. After incubation, 0.05 mL (containing approx. 1.59×10^6 cfu mL^{-1}) of each of the inoculated broth was aseptically transferred separately to different sterile petri dishes one organism per plate and each plate was overlaid with prepared nutrient agar, swirled to allow even distribution of the organisms within the agar and allowed to gel. With the aid of a sterile cork-borer (6 mm), three wells were made into the gelled agar, into one of the wells, 0.1 mL of LW was introduced, into another well, 0.1 mL of CW was introduced, the third well was filled with sterile distilled water which served as control. The plates were incubated at 37°C for 24 h, the inhibitory zones around the well were noticed and the diameters of these inhibitions were measured. This assay was repeated daily for five consecutive days using each of the whey samples kept at 29±2°C against the test organisms.

RESULTS AND DISCUSSION

The results of the antibacterial activity of LW and CW are presented in Table 1. The two whey samples inhibited the growth of the diarrheal bacteria with varying diameters of clearance. The growth inhibitory effect ranged from 1.0-14.0 and 2.0-21.0 mm, respectively. CW stored for four days gave the highest inhibition on the growth of all the test organisms except ETEC whose growth inhibition reached maximal level with CW stored for 3 days. The same pattern was observed with LW on the growth of the test organisms except for the deviation seen with its effect on ETEC and *B. cereus* whose growth inhibition reached the highest level with LW stored for two days and three days, respectively. The pH value of the whey samples used decreased as the storage period progressed (Table 2). The values decreased from 6.1 to 3.8 for LW and 6.5 to 2.5 for CW by the 5th day.

Table 1: Growth inhibitory effect of locally prepared cheese whey and laboratory prepared cheese whey at controlled temperature (60±5°C) on common diarrheal bacteria

Fermentation days	Whey sample	*E. coli* NCTC 10418	*E. coli* ETEC	*Staph. aureus*	*Shigella dysenteriae*	*Salmonella typhi*	*Bacillus cereus*	*Enterobacter faecium*
1	LW	0.0	0.0	2.0	0.0	0.0	0.0	0.0
	CW	4.5	3.0	3.0	2.0	0.0	0.0	5.0
2	LW	1.0	1.5	5.0	7.0	0.0	5.0	5.0
	CW	6.0	4.0	8.0	7.0	0.0	0.0	9.0
3	LW	0.0	0.0	7.0	4.0	0.0	14.0	3.0
	CW	11.0	10.0	15.0	14.0	6.0	17.0	13.0
4	LW	8.0	0.0	9.0	10.0	6.0	9.0	9.0
	CW	13.0	10.0	18.0	19.0	10.0	21.0	15.0
5	LW	0.0	0.0	4.0	2.0	0.0	0.0	1.0
	CW	0.0	0.0	6.0	4.0	1.0	7.0	3.0

Table 2: The pH values of the locally and laboratory prepared cheese whey samples stored at room temperature (29±1°C)

	pH values	
Days	LW	CW
1	6.10	6.50
2	4.12	4.25
3	4.00	3.95
4	4.20	3.30
5	3.80	2.50

The results in Table1 showed that the locally prepared whey at temperature of ≥ 100°C and the laboratory prepared whey at controlled temperature (60±5°C) exhibited varied inhibitory effects against all the test organisms used. The antimicrobial activities exerted by the whey prepared under controlled temperature (60±5°C) in the laboratory was greater than that of the locally prepared whey as shown by the zones of inhibition mediated by the two on the table. The reason for this may not be unconnected with the bioactive antimicrobial components present in the whey prepared at controlled temperature (60±5°C) which must have been severely affected by the high temperature (≥100°C) being used by local producers of cheese in this area. This finding agrees with the report of Bounous (2000) that temperature above 65°C denatures the fragile vital protein components present in whey during cheese production. So the denaturation of these proteins was likely responsible for the diminished antibacterial effect of the locally produced whey at temperature ≥100°C on the test organisms when compared with the values got with the laboratory prepared whey.

However, since the locally produced whey also had inhibitory effect on the test organisms, it shows that some other components present in whey that are heat stable are also participating in the inhibition process. For example, the low pH which ranged from 6.5 to 2.5 (Table 2). Low pH inhibits growth of microorganisms. Other factors responsible for inhibition might be due to the presence of microflora of milk such as *Lactobacillus* species which have the ability of producing antimicrobial substances such as bacteriocin. According to Gilliland and Speck (1977) and Warny *et al.* (1999), *Lactobacillus* species exhibit growth inhibitory effects on various gram positive and gram negative bacteria through production of organic acids such as lactic and acetic acids, hydrogen peroxide and bacteriocin but the effect is more pronounced on gram positive bacteria than gram negative bacteria. This might explain the reason why the whey used recorded greater zones of inhibition on the growth of gram positive organisms such as *Bacillus cereus* and *S. aureus* than that of almost all the gram negative test organism used in this study.

From this investigation, the temperature used in getting whey from milk during cheese production has effect on the antibacterial activity of whey on the test organism used, it reduced the growth inhibitory effect of the whey on the test organism. So it been suggested that temperature below 65°C should be used in making cheese from raw milk so that the proteins present in whey which have health promoting effects and antibacterial properties will not become denatured.

REFERENCES

Adebolu, T.T. and O.H. Ademulegun, 2005. Effect of cheese whey on diarrhea causing bacteria in southwestern Nigeria. Biosci. Res. Commun., 16: 57-60.

Alais, C. and G. Linden, 1997. Food Biochemistry. Aspan Publisher. Maryland, pp: 301.

Bounous, G., 2000. Whey Protein Concentrate (WPC) and glutathione modulation in cancer treatment. Anticancer Res., 6C: 4785-4792.

CDRF., 2006. California Dairy Research Foundation. http://www.Healthywhey.org

Cheeshbrough, M., 1994. Medical Laboratory Manual for Tropical Countries. Vol. II; Microbiology. ELBS, pp: 479.

Gilliland, S.E. and M.I. Speck, 1977. Antagonistic action of *Lactobacillus acidophilus* towards intestinal and food borne pathogens associated culture. J. Food Prod., 40: 820-823.

Gutman, J., 2005. Glutathione, its Role in Cancer and Anticancer Therapy. American Family Physician. http://www.kudo.ca.

Ha, E. and M.B. Zemel, 2003. Functional property of whey, whey component and essential amino acid; mechanisms underlying health benefit for active people. J. Nutr. Biochem., 14: 251-258.

Philanto-Lappala, O.T., 1999. Antagonistics activity of proteins on microbes. J. Dairy Sci., 54: 2568-2572.

Prescott, L.M., J.P. Harley and D.A. Klein, 2002. Parthogenicity of Microorganisms. In: Microbiology, 5th Edn. The Mc Graw-Hill Company. Inc.,pp: 788-801.

Warny, M., A. Fatima and E.F. Bostrick, 1999. Bovine Immunoglobulins concentrate *C. difficile* retains, *C. difficile* toxin neutralizing activity after passage through the human stomach and small intestine. Gut, 44: 212-217.

World Health Organization, 1998. The state of the World Heath. In: The World Health reports 1998. Lite in the 21st century: A vision for all. WHO Geneva, pp: 57-58.