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CAN HONEY REPLACE REFRIGERATED STORAGE OF MILK?

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ABSTRACT
Milk is a wholesome nutritive product but is as a good medium for the growth of microbes. Bovine milk has various naturally occurring inhibitory substances of which lactoperoxidase (LP) system is very well exploited .The increased demand for milk can compromise on quality and with lack of refrigeration facilities, preservatives can largely aid in maintaining quality with the added advantage of increasing short storage life as in households. The study explored the potential of honey as an antibacterial preservative in raw milk sources and identified its positive preservative efficacy. This natural preservative property of honey as an alternative method for storing raw/pasteurized milk was found very encouraging .The results are tabulated and discussed.

KEYWORDS: Honey, Antibacterial activity, Preservation, Antimicrobial property.

INTRODUCTION

With the rise in the demand of milk and lack of refrigeration facilities in rural setting, particularly there is need to identify alternate to maintain shelf life of milk. The use of honey as a traditional conventional remedy for many medicinal purposes and in the food industry are due to its nutritive, therapeutic and dietetic quality[1]. Since ancient times it was discovered that honey is medicine[2]. Numerous studies on the preservative efficacy of honey have been reviewed. Honey does not do well when diluted and undiluted form is effective natural preservator as a viscous barrier to bacteria and infection. Krushna *et al.* studied the preservative efficiency of honey in milk by using disk diffusion assay and turbidometry assays[3]. The most important chemical compounds of honey which proved to be responsible for antimicrobial activity are osmolarity, acidity, the enzymatic generation of hydrogen peroxide via glucose oxidase and the presence of various non peroxide compounds[4]. Most of the inhibition activity of honey caused by hydrogen peroxide generation is via glucose oxidase[5]. Lactoperoxidase is an enzyme, which is naturally present in fresh raw milk and bovine milk contains about 30mg/l of Lactoperoxidase. The concentration of Lactoperoxidase is fairly constant throughout the lactation. The reactivation of LP system in the raw milk extends the shelf life under tropical conditions for a further 7 to 8 hrs (Codex Alimentarius Commission).

To accomplish the anti bacterial property and the preservative effect of honey with its nutritive value, we used a novel approach to identify the antibacterial preservative efficacy of honey in raw milk samples.

MATERIALS & METHODS

Milk Samples

The study was carried out in 24 milk samples collected from different sources which included 18 unpasteurized raw milk samples, two pasteurized commercial milk samples. Each sample was collected in a sterile container and stored in refrigerated condition until further processing.

Honey

Two different types of honey were used are unprocessed natural honey obtained from hill region and a commercial honey.

Methods

To assess the bacterial spoilage susceptibility of milk and opportunistic microbial load the collected milk samples were microbiologically examined by propagating them in nutrient broth and were streaked on plates of Eosin Methylene Blue (EMB) agar and Baird Parker Agar (BPA). Based on the colonial morphology the pathogens that were commonly present in milk were identified in specific agar media. In order to determine the antibacterial activity and efficacy of honey, wells of 2mm diameter were punched into the plates which showed Specific colonies and 100µl of each natural and commercial honey were added. The zone of inhibition after overnight incubation was observed and measured for analysis.

1 ml of Milk sample was propagated in Nutrient Broth at 37^0C. From the overnight grown samples a loopful was streaked on two plates of EMB and Baird Parker Medium. Plates which showed specific colonies, a well was cut and 100µl of honey (Natural and Commercial) was added and observed for Zone of Inhibition after overnight incubation at 37^0C. After incubation the diameter of the inhibition zones produced around the wells was measured *(Fig 4 & 5)*. Studies were also done to standardize the role of

honey as preservative under different storage conditions. 100µl of honey was added in 1ml of two milk samples and stored in two different storage conditions (37^0C & 4^0C). The preserved milk samples were then assessed and explored microbiologically by propagating them in specific media.

Agar diffusion assay

To assess the antibacterial activity of honey, agar diffusion method was also used[6]. A loopful of grown culture in specific media from milk samples was inoculated in Muller Hinton agar plate and a well measuring 2mm diameter were punched into the agar after overnight incubation at 37 ^{0}C. 100µl of natural and commercial honey was added and plates were incubated at 37^0C overnight. After incubation the diameter of the inhibition zones produced around the wells was measured. Size of inhibition zone signifies *(Fig. 1)* a qualitative measure of antibacterial activity of honey.

RESULTS

Out of 24 milk samples propagated in nutrient broth and streaked on two plates of EMB and Baird Parker Medium 18 showed colonies of *E.coli* and 16 showed colonies of *Staphylococcus sp*. After adding honey in Baird Parker medium 16 and 14 respectively out of 24 samples showed zone of inhibition with natural and commercial honey, whereas in EMB agar 18 and 16 respectively showed zone of inhibition with natural and commercial honey. These plates showed various sizes of zone of inhibition from broad inhibition zone (23mm) *(Fig. 5)* to minimal inhibition zone (11mm) and no zone of inhibition also. Observed zone of inhibition is listed in Table 1. Samples of raw milk stored in the presence of honey at 37^0C & 4^0C were showed no bacterial growth compared to samples stored without honey. No visible growth was noticed in milk stored at 4^0C. Except some unidentified growth in 3 plates of EMB and 4 plates of BPA, all plates showed no growth at 37^0C.

TABLE 1: Diameter of inhibition zone for samples (≈mm)

Sample No.	Zone of inhibition (≈ in mm)				Sample No.	Zone of inhibition (≈ in mm)			
	EMB Agar Plate		BPA Plate			EMB Agar Plate		BPA Plate	
	Natural honey	Commercial Honey	Natural Honey	Commercial honey		Natural honey	Commercial honey	Natural honey	Commercial honey
1	19	18	16	16	13	17	-	17	16
2	15	16	17	14	14	19	18	14	13
3	21	18	14	13	15	2	-	-	-
4	16	-	-	-	16	18	17	-	-
5	-	-	-	-	17	14	15	13	13
6	14	15	12	15	18	-	-	-	-
7	18	17	14	14	19	15	16	15	-
8	19	19	15	14	20	23	18	14	12
9	20	18	14	12	21	-	-	-	-
10	-	-	-	-	22	17	18	14	12
11	-	-	-	-	23	18	16	15	13
12	16	17	14	12	24	20	20	13	11
					Total	339	276	231	200
					Mean	18	17	15	12.5

DISCUSSION

The preservative efficacy of honey for storage of raw milk has been identified in our study. The *E.coli* plates showed high sensitivity to honey (higher zone of inhibition maximum of 23mm) and the most pronounced effect was against natural honey than commercial honey. Although still more study is needed to indicate the potential inhibition property of honey against the growth of both Gram negative and Gram positive opportunistic pathogens in milk. Zahoor *et al* in their study found that honey was more active against Gram negative bacterial strains (*Escherichia coli* and *Klebsiella sp*) than the Gram positive bacterial strains (*Staphylococcus aureus* and *bacillus cereus*) [7].

FSSAI reports that nearly 68.4% of milk across the country is adulterated with chemicals in order to increase the storage life. Most of these chemicals cause various health problems including multisystem failure when it is used for a long time. This can be better avoided by using mechanical refrigeration or by activation of Lactoperoxidase (LP) system in milk. In rural settings the availability of refrigeration facility for the storage of raw milk is constrainable factor. Normal sized refrigerator consumes 4.8 kWh per day (100-300 watts an hour) and the refrigeration power consumption cost will comes around Rs.15 per day. Further it involves huge initial setting up cost. This will increase the cost involved in the milk production. Honey is a natural food product and it is freely available everywhere. The storage of one litre of raw milk approximately requires 5ml of honey and which will cost around Rs 5. The cost of honey can be further decreased by encouraging the women self help group in the production of honey. Apart from these the use of honey will saves electricity also. Considering these points and the findings of our study reveals that honey could be a better indigenous antibacterial agent of choice to preserve raw/pasteurized milk during collection and transportation until it reaches further processing/storage point. Further research could make them promising candidates for storing the milk without refrigeration.

FIGURE 1: Plate showing Zone of inhibition(18mm) in MHA agar

FIGURE 2: Zone of inhibition(18mm) in MHA agar (Zoom in view)

EMB agar plate shows E.*coli* metallic sheen

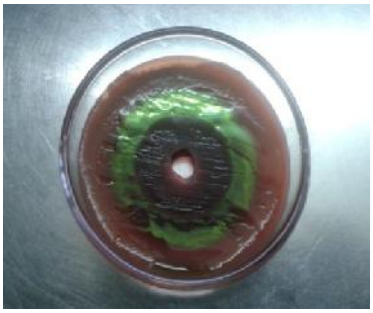

FIGURE 4: Plate showing Zone of inhibition(19mm) in EMB agar

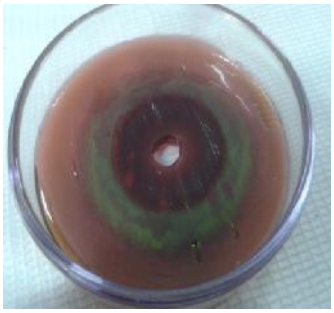

FIGURE 5: Plate showing Zone of inhibition(23mm) in EMB agar

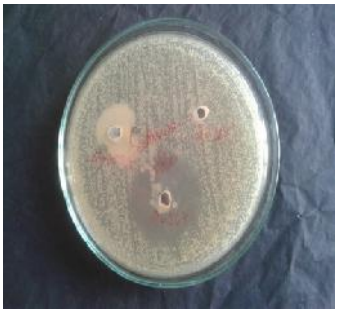

FIGURE 6: Plate showing Zone of inhibition(18mm) in BP agar

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