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Evaluation of microbial contaminants (CFU/g) of certain energy foods at different intervals under controlled 85 laboratory conditions and impact of utilization processes on them

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Abstract

In the present paper, efforts have been made to evaluate microbial contaminants in term of CFU/g of three sealed Bournvita samples (B1, B2, B3) and three sealed Horlicks samples (H1, H2, H3) at interval of one month for 120 days (4 months) under controlled laboratory conditions. Further to find out the effect of consumer's handling on microbial load, these packs after 120 days were distributed to consumer A, B and C respectively and were examined for extended period of 28 days at interval of 7 days. The results revealed that microbial contaminants were present since beginning (when opened) in all the energy food samples. Maximum microbial load in Bournvita sample was recorded in B2, followed by B3 and B1 respectively and in Horlicks, it was maximum in H2 followed by H3 and H1 respectively. In general the microbial contaminants were more in Bournvita than Horlicks. No significant change occurred in their CFU till 120 days but handling process enhanced the contaminants significantly both in their quality and quantity in term of microbial group. Fungi and Actinomycetes were recorded in sampling from the samples of consumers A, B and C. Total CFU enhanced from both energy food and reached in several lakhs. Total Bacterial species isolated and identified were *Staphylococcus aureus, Bacillus cereus, Enterococcus, Escherichia coli*, *and Pseudomonas aeruginosa.* After 2 and half years (Feb 2010) the bacterial count was found 3, 00000 CFU/g of Horlicks and fungal count was 250CFU/g.

Keywords: *Contaminants, Food Sample, Microbial load , Serious effects*

Introduction

Food may be defined as an essential element which is must needed for growth and to keep body system healthy and in proper active state. In the twenty-first century, the demand for food and agricultural products in general reached unprecedented levels. Form and varieties of food has been changed time to time. In the last few decades after independence, a great change in food habits have been recorded in our country due to economic revolution. A significant revolution has been occurred in baby's food also. A number of baby foods for infants are available in the market. Albeit to these, some high energy yielding tinned dry foods are also in common use in society for growing childrens such as Bournvita, Horlicks etc.

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Suitable to human health, safe food is a consumer's basic right. The quality of the baby food is very essential for both in terms of its listed nutrients which should provide requisite elements and energy for growth as well as should be free from any kind of contamination. Assuring safe food is the most difficult task in preparation and distribution units, especially in small and medium-size companies (Baş *et al*., 2005; Ayçiçek *et al.*, 2004; Bermúdez-Millán *et al.*, 2004; Sun and Ockerman, 2004; Walczak and Reuter, 2004; Walker *et al*., 2003; Walker and Jones, 2002; Worsfold, 2001). Albeit in the food processing industry, unwanted and growth of pathogenic microorganism is a key concern. Therefore the production of safe foods is based on the implementation and application of Good Hygienic Practice (GHP) and Good Manufacturing Practice (GMP) (CAC, 1997). Since the infant foods are milk and cereal based and are consumed by most vulnerable group of population, therefore there is

need to exercise great care at every stage of processing and precautions to be taken at consumer level. Several outbreaks regarding energy foods and other infants milk and cereal based food products have been widely reported in the literature. Andreas *et al.* (2004) examined twenty eight milk samples from 18 different countries for Thermophillic bacilli. Of 742 isolates examined, 96.8% were assigned to the same strain of bacilli as previously found in New Zealand powder. Three cases with *Salmonella Tennessee* in infants in Canada and United States have been linked to consumption of contaminated powdered infant formula. Outbreaks of Salmonellosis caused by powdered milk products have been reported in US and else where (Leuschner *et al.* 2004).

The presence of *Enterobacter sakazaki* other than *Salmonella* and other Enterobacteriaceae from powdered infant formula milk and other food products have been widely reported in the literature (Farmer *et al* 1980; Leuschner *et al*., 2004; Iversen *et al*., 2004; Gurtler *et al*., 2005). Infection caused by *Enterobacter sakazaki* are rarely reported. The first outbreak of *Enterobacter sakazaki* from powdered infant formula was reported in 2001 (CDC, 2002; Himelright *et al*., 2002). The bacterium has been implicated most frequently in causing illness in neonates and children from 3 days to 4 years (Iversen and Forysthe, 2003). Therefore, as these foods are directly concerned with human health and may cause serious effects, therefore the main aim of this research paper is to protect the society from unwanted epidemics associated with these energy foods.

Materials and Method

Collection and storage of samples

Bournvita (MFD-07/2007), Horlicks (MFD-07/ 2007), Net weight- 200gms. Test samples/ materials of bournvita of batch number W29T7W2 and horlicks 8903. H were collected randomly from different shops located in Yamuna Nagar, Haryana, India. Samples were stored at room temperature (30 ± 5°C) until required.

Sampling procedure

Three sets of test materials were kept in laboratory and were opened from their purchase date adjoining to their manufacturing date and were sampled under aseptic conditions for quality analysis for a period of 120 days. Samples were evaluated at an interval of 30 days. Precautions were taken to close the pack properly under aseptic conditions after sampling and were kept in dry conditions. After 12 days these samples were given to three consumers (A,B,C) to use as such and samples were taken for evaluation of handling effect on microbial load at interval of 7 days from them for the total period of 28 days. Quantitative enumeration of microorganism was done by serial dilution method and qualitative enumerations of isolated bacteria from the user's samples were examined microscopically and were characterized biochemically.

Test procedure

For the enumeration of microorganism from energy foods, different medias were used each for bacteria, fungi and actinomycetes. Nutrient agar media for bacteria, Czapek-Dox Agar for Fungi and Kenknight's Media for Actinomycetes. All ingredients used for specific medium were weighed properly and mixed in proper volume of distilled water. pH of the media was adjusted and measured by the pH meter and prepared media were sterilized properly and were kept in the laminar flow hood for conducting the experiments. Serial dilution procedures were adopted for isolation of microorganism from selected energy food. Isolated organisms were identified by their morphological, physiological and biochemical characteristics (Cowan and Steel, 1974; Leanor and Carey, 1978).

Results and Discussion

Results obtained during this investigation of microbial load (CFU/g) of test material *i.e.,* Bournvita and Horlicks during different storage period and storage conditions in terms of mode of consumption of consumer A, B and C have been presented in Table 1 below. Results revealed that microbial load in Bournvita sample B1, B2 and B3 had shown only bacterial count and other organism *i.e*. Fungi and Actinomycetes were absent. Maximum bacterial count of 35,000 CFU/g was recorded on zero day in bournvita sample B2 and minimum bacterial load of 32,000 CFU/g was found in Bournvita sample B1. Maximum bacterial load of 34,000 CFU/g was found in sample B2, and minimum 29,000 CFU/g in sample

B1, after four months of storage under clean conditions and fungi and actinomycetes count were not found in any of the sample during four months of storage. In case of Horlicks samples H1, H2 and H3, was found contaminated with heterogenous group of bacterial species however, fungi and actinomycetes were found completely absent even after four months of storage. Maximum bacterial count recorded in sample H2 was found to be 31,000 CFU/g and minimum load of 27,000 CFU/g was found in sample H1. Profound increasing trend of total microbial load (CFU/g) in all three samples of each Bournvita and Horlicks was recorded when these were used by consumers A, B and C. Results showed that till first sampling (7th day) in Bournvita, only bacterial population could be observed constantly. On second sampling *i.e*. on 14 days actinomycetes started to appear and fungi started to appear from 21st day (third sampling). Maximum total microbial load of 9,44,000 CFU/g was recoded on 28th day from the sample given to consumer B followed by consumer A with microbial load of 8,59,000 CFU/g and than consumer C with microbial load of 7,14,000 CFU/g. Similar trend has been recorded in Horlicks. On 7th day (first sampling) only bacterial population was recorded and actinomycetes started to appear on 14th day (second sampling) and Fungi started to appear from 21st day (third sampling). Maximum total microbial load *i.e*. 7,90,000 CFU/g, was recorded on 28th day in consumer B followed by consumer A *i.e.* 7,72,000 CFU/g and consumer C *i.e.* 6,31,000 CFU/g.

Comparative account of total microbial load of both Bournvita and Horlicks on effect of consumer use for extended 28 days period after 120 days has been presented in the Table 2. Data recorded has shown that average value of microbial load was always higher in Bournvita than Horlicks, since 7th day (first sampling) to till 28 days of sampling. Comparative account of microbial load of both Bournvita and Horlicks on storage period of 120 days (four months) has been presented in Table 1. Data recorded showed that the total microbial load in Bournvita is slightly higher than Horlicks under storage conditions for 120 days (four months).The organism identified from different samples of Bournvita (B1, B2 and B3) and Horlicks (H1, H2 and H3) after a storage period of 28 days when given to consumer A, B & C are *Staphylococcus aureus*,*Bacillus cereus*, *Enterococcus*, *Escherichia coli*, *Pseudomonas aeruginosa* (Table 3.)

Table 1: Comparative account of Total Microbial Load (CFU/g) of Bacteria, Fungi and Actinomycetes in Bournvita (B1, B2 and B3) and Horlicks samples (H1, H2 and H3) during a storage period of 120 days.

Storage period (sampling day)	Dilution	Bournvita Total Microbial Load (CFU/g)			Average count	Dilution	Horlicks Total Microbial Load (CFU/g)			Average count
		B1	B2	B3		10^{-3}	H1	H2	H3	
0 day	10^{-3}	32,000	35,000	33,000	33333	10^{-3}	27,000	31,000	29,000	29000
30 days	10^{-3}	30,000	36,000	32,000	32666	10^{-3}	28,000	32,000	30,000	30000
60 days	10^{-3}	30,000	36,000	32,000	32666	10^{-3}	28,000	32,000	31,000	30333
90 days	10^{-3}	30,000	35,000	33,000	32666	10^{-3}	27,000	32,000	31,000	30000
120 days	10^{-3}	29,000	34,000	32,000	31666	10^{-3}	27,000	30,000	31,000	29333

Table 2: Effect of consumers (A, B and C) handling on Microbial Load (CFU/g) of Bacteria, Fungi and Actinomycetes of Bournvita (B1,B2 and B3) and Horlicks (H1,H2 and H3) Samples after 120 days at interval of 07 days interval

S. No	Test Energy Food Under Different Conditions	Sampling Period (days)	Dilution	Effect of Handling on Microbial Load			
				Bacteria	Fungi	Actinomycetes	Total CFU
1.	**Bournvita (Consumers)**						
	B1 (A)	**7**	10^{-3}	2,00,000	--	--	2,00,000
		14	10^{-3}	3,30,000	--	1,10,000	4,40,000
		21	10^{-3}	4,70,000	46,000	1,60,000	6,76,000
		28	10^{-3}	5,70,000	69,000	2,20,000	8,59,000
	B2 (B)	**7**	10^{-3}	1,90,000	--	--	1,90,000
		14	10^{-3}	5,40,000	--	45,000	4,25,000
		21	10^{-3}	6,20,000	12,000	2,70,000	9,02,000
		28	10^{-3}	6,30,000	14,000	3,00,000	9,44,000
	B3 (C)	**7**	10^{-3}	1,70,000	--	--	1,70,000
		14	10^{-3}	3,80,000	--	45,000	4,25,000
		21	10^{-3}	5,30,000	12,000	1,20,000	6,62,000
		28	10^{-3}	5,70,000	14,000	1,30,000	7,14,000
2.	**Horlicks (Consumers)**						
	H1 (A)	**7**	10^{-3}	1,80,000	--	--	1,80,000
		14	10^{-3}	3,90,000	--	1,30,000	5,20,000
		21	10^{-3}	4,50,000	81,000	1,90,000	7,21,000
		28	10^{-3}	4,60,000	82,000	2,30,000	7,72,000
	H2 (B)	**7**	10^{-3}	2,30,000	--	--	-2,30,000
		14	10^{-3}	3,90,000	--	41,000	4,31,000
		21	10^{-3}	5,30,000	71,000	1,10,000	7,11,000
		28	10^{-3}	5,80,000	80,000	1,30,000	7,90,000
	H3 (C)	**7**	10^{-3}	1,40,000	--	--	1,40,000
		14	10^{-3}	2,10,000	--	11,000	2,21,000
		21	10^{-3}	4,50,000	--	45000	4,95,000
		28	10^{-3}	5,10,000	40,000	81,000	6,31,000

Table 3: Bacterial species identified from different samples of Bournvita (B1, B2 and B3) and Horlicks (H1, H2 and H3) after used by consumers (A, B and C)

Consumer	Energy food	Bacteria Identified
A	B1	*Staphylococcus aureus, Bacillus cereus, Enterococcus*
	H1	
B	B2	*Staphylococcus aureus, Bacillus cereus, Enterococcus, Escherichia coli, Pseudomonas aeruginosa*
	H2	
C	B3	*Staphylococcus aureus, Bacillus cereus, Enterococcus*
	H3	

Results obtained from the present investigation revealed that bacteria were present in all the samples of Bournvita and Horlicks while fungi and actinomycetes were completely absent in the samples when container were opened and closed in sterilized conditions under laminar air flow. It is much obvious that microbial load interim of bacterial species was already present in Bournvita and Horlicks since beginning. Although bacterial population could not show any constant pattern of their increase or decrease in CFU/g value but has shown variation in their declined and enhanced value. However, enhanced bacterial count has been recorded in Horlicks sample (Table 1). These findings are in accordance to the other workers who have reported the contaminants in cereal based food as well as raw and dried milk.

Infants food and dried milk harbours the microbial contaminants since their sealed finished product and several reports have been made in this regard. Variation in bacterial count among the different container may be related with manufacturing conditions *i.e.* manpower, industrial cleanliness, and packing container quality. Although opening and closing of sealed pack under strict aseptic conditions could control to minimize the bacterial growth in both energy foods. Speedy enhancement in generic level (fungi and actinomycetes) as well as their quality (in bacterial count) could establish the impact of handling and storing environmental conditions. It is directly related with handling as all consumers (A, B and C) handled routinely as normal and common practice in the society. Besides this, time and temperature may be a major factor for enhancing the bacterial population. Time and temperature, which governs the microbial proliferation in food items changes the color and pH therefore, this can be used for monitoring of food quality (Hariklia *et al.* 2008).

Presence of certain bacterial species *Staphylococcus aureus, E.coli, Enterococcus* and *Bacillus cereus* in both energy foods is a serious problem. Therefore, it is highly needed to improve manufacturing conditions as well as to educate the consumers to follow hygienic conditions in public interest to prevent any outbreak from these energy foods.

Conclusion and Recommendations

On the basis of the results of present investigation, the microbiological quality of energy foods *i.e.* Bournvita and Horlicks are influenced to varying degrees of microbial contamination even in their sealed finished packets. Presence of certain bacterial species such as *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Enterobacter* and *Bacillus* in both energy food is a serious problem and it is directly related with inappropriate production method, production environment handling and packaging material. Moreover high count of harmful microorganism such as *P.aeruginosa*, *S.aureus*, *E.coli* etc may affect the human health and can cause serious health hazards in children and infants. Therefore, there is a necessity of improving the energy food quality and to establish better hygienic conditions in public interest to overcome any serious hazard/ outbreak through such type of energy foods.

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