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Ascertaining shelf life of selected food items stored in household refrigerators for consumer safety

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Study was planned with the objective to undertake microbiological and toxicity analysis to ascertain shelf-life of selected perishable products stored under refrigeration conditions in selected storage materials. This was being done to suggest safe refrigerated food storage practices for health safety. Results indicated that seepage of chromium content from stainless steel containers in stored food items resulted in maximum seepage (996.7%) in milk after two days followed by presence of chromium in *paneer* (569.56%); cooked meat (164.86%) and wheat flour dough (151.04%). Recommended food containers for storage in refrigerator were stainless steel and glass bottles in which food items come packed for minimizing growth of yeast and mould and Total Plate Count of selected food items. However due to heavy seepage of chromium content in stored food items (in stainless steel container) up to 966.7 per cent; it is not advisable to store food items in refrigerator for longer duration.

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INTRODUCTION

Perishable foods are those foods which deteriorate and usually require some sort of refrigerated storage. Refrigeration is employed to control the rates of certain chemical and enzymatic reactions as well as rates of growth and metabolism of food microorganisms (Srivastava and Kumar, 1994). Household refrigerators usually run at 4.4-7.2°C. However, studies have shown that perishable food will deteriorate, even at refrigerator temperature, due to spoilage because of microorganisms, enzymes and oxidation (Jay, 2000).

Principal requirements for effective refrigerated storage are controlled low temperature, air circulation and humidity control. However, aerial oxygen, type of container or wrapping material they are stored in and duration of storage are also important factors that influence type of microbial growth, toxicity and spoilage of food during refrigerated storage. Refrigerated foods are therefore subject to spoilage by moulds and bacteria (Roday, 1999). Moreover, the temperature and climatic conditions prevalent in India, as well as the pH values of most food items are conductive to growth and proliferation of bacteria causing food borne diseases.

There is a wide variety of food containers and packing materials available in the market including glass, metals, paper and the recent entry as food storage containers is that of plastic and stainless steel containers. Styrofoam food containers emit benzene and styrene into our food which can cause vomiting, irritation of the stomach, dizziness, sleepiness, convulsions, rapid heart rate and even death (Potter, 2003). However, there are evidences of seepage of metals or chemicals from materials and containers in which food is cooked and stored even for shorter duration as reported by Bhutani (2005). Therefore, a clear indication of other factors like effect of food container and unhygienic handling of food items (in refrigerator) is seen.

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A lot of work has been done to ascertain health hazards in refrigerated products by way of undertaking microbiological analysis and examining toxicity content; but no study has pinpointed the effect of metals or materials of containers and wrapping material on refrigerated foodstuffs by consumers and housewives. Hence, present study was been planned with the objective to undertake microbiological and toxicity analysis to ascertain shelf-life of selected perishable products stored under refrigeration conditions in selected storage materials.

METHODOLOGY

Study was conducted in 2009. Four most commonly used food items were selected. These were milk, cooked meat, *verka paneer*, and wheat flour dough. These were stored in two types of food containers, one the existing practice and other the experimental one, thus constituting the food sample of 8.

Samples were stored at 3 to 4°C.For microbial analysis, *verka paneer* and wheat flour dough were stored for 2 days; and milk (both raw and boiled) and cooked meat for 5 days. Toxicity analysis was performed to look into seepage of chromium content into stored food.

For microbial study of samples, two types of media (nutrient agar and glucose yeast agar) were prepared. Prescribed procedure was followed for testing *i.e.* by sterilizing, preparing dilution and transferring it into sterilized Petri dishes, pouring warm media in the dishes and placing it in incubator for 24 hours. After 24 hours bacterial count of food samples was taken.

Table A.

Food items stored	Quantity stored	Storage practice	
		Existing practice	For experiment
Verka paneer	250 g	Plastic container	Stainless steel bin
Wheat flour dough	350 g	Plastic container	Stainless steel bin
Cooked meat	250 g	Glass container	Stainless steel bin
Milk (raw & boiled)	500 ml	Aluminum vessel	Stainless steel vessel

Analysis to toxic contents of samples was done by drying of samples in hot air oven at 70°C for 48 hours and doing wet digestion of the samples (Piper, 1950). Nitric acid and perchloric acid of analytic reagent (AR) grade were mixed in the ratio of 5:1. Food samples (0.5 g) were digested with 25 ml of diacid mixture and kept overnight. These were heated at low temperature until 1 ml of clear and colourless liquid was left, which was then transferred with deinonized water into a 25 ml volumetric flask. Double beam atomic absorption spectrophotometer, with automatic background correction was employed. Analysis was carried out using hollow cathode lamp of respective metallic element under standard instrumental operational conditions. For chromium detection air acetylene was used as a fuel.

OBSERVATIONS AND ASSESSMENT

The results obtained from the present investigation have been presented under following heads :

Microbiological analysis of refrigerated food items:

Verka paneer:

Fresh samples of *verka paneer* stored in three types of containers (*verka* packet, stainless steel and plastic bin) were analyzed for possible microbial growth and data is presented in Table 1a and b. The *paneer* samples' TPC was 1.1 x 10^3 cfu/ml in *paneer* stored in its own *verka* packet, 2.0 x 10^3 cfu/ml when kept in stainless steel container and 4.0 x 10^3 cfu/ml during its storage in plastic container. The yeast and mould count in fresh *paneer* sample of *verka* packet was 3.0 x 10^2 cfu/ml 1.0 x 10^3 cfu/ml when *verka paneer* was stored in stainless steel container and 3.0 x 10^3 when it was kept in plastic container. yeast and mould count increased after 2 days of storage of all these samples in refrigerator.

The *verka paneer* sample stored in ordinary plastic containers showed the highest total plate count (5.5 x 10^3 cfu/ml) followed by sample kept in stainless steel container (4.0 x 10^3 cfu/ml) and *paneer* of original *verka* packet (1.2 x 10^3 cfu/ml) after 24 hours in refrigerator. The yeast and mould count of *verka paneer* (when kept) in plastic containers was 3.0 x 10^3 cfu/ml; of the sample which was kept in stainless steel it was 1.5 x 10^3 cfu/ml and the one taken out directly from *verka* packet had yeast and mould growth of 5.0 x 10^2 cfu/ml. The highest rate of growth of yeast and mould was thus recorded in sample of *verka paneer* taken from ordinary plastic containers (3.0 x

Table 1(a). Effect of storage materials on total plate count of *verka paneer* stored in refrigeration temperature (3-4°C)

Storage time (days)	Storage material (TPC CFU*/ml)		
	Verka packet	Stainless steel container	Plastic container
0	1.1 x 10^3	2.0 x 10^3	4.0 x 10^3
2	1.2 x 10^3	4.0 x 10^3	5.5 x 10^3

Incubation temperature: 37°C; Incubation time: 24-36 hours;
* Colony forming unit

Table 1(b). Effect of storage materials on total yeast and mould count of v*erka paneer* stored in refrigeration temperature (3-4°C)

Storage time (days)	Storage material (TPC CFU*/ml)		
	Verka packet	Stainless steel container	Plastic container
0	3.0 x 10^2	1.0 x 10^3	3.0 x 10^3
2	5.0 x 10^2	1.5 x 10^3	3.0 x 10^3

Incubation temperature: 37°C; Incubation time: 24-36 hours;
* Colony forming unit

10^3 cfu/ml) and the lowest rate was recorded from *paneer* kept in original *verka* packing (5.0 x 10^2 cfu/ml).

It may be due to processing and handling of the product. Milk products containing amorphous carbohydrates like lactose from milk can undergo physical changes such as crystallization, clumping, sticking and caking during processing, handling and storage. Such physical changes lead to deterioration of food quality and increase relative humidity (WHO 1996). The ordinary plastic containers showed higher TPC as compared to stainless steel and original packing of *verka paneer* after storage in refrigeration. Similarly it was observed ordinary plastic containers showed highest yeast and mould count and lowest rate was found in original *verka* packing after 2 days in refrigerator.

Wheat flour dough:

Data in Table 2a and b reveal the results of fresh sample of wheat flour dough stored under refrigeration in two types of containers (plastic and stainless steel) for microbial growth *i.e.* the total plate count and yeast and mould count. Total plate count and yeast and mould count of fresh sample of wheat flour dough stored in plastic container was 1.5 x 10^2 cfu/ml and 3.5 x 10^2 cfu/ml. Same sample when stored in stainless steel container resulted in TPC and Y and M of 6.5 x 10^3 cfu/ml and 4.0 x 10^3 cfu/ml, respectively. However, both the kinds of microbial growth increased after 2 days of storage in refrigerator.

The TPC and yeast and mould count in samples of wheat flour dough sample stored in 2 types of containers (steel and plastic) after the end of second day can also be observed from Table 2a and b. TPC and yeast and mould count of sample of wheat flour dough stored in stainless steel containers was 7.1 x 10^3 cfu/ml and 4.5 x 10^3 cfu/ml, respectively at the end of second day and in the dough kept in plastic container TPC and yeast and mould count was 2.0 x 10^3 cfu/ml and 5.0 x 10^2 respectively. So the samples stored in steel container showed lower TPC and yeast and mould count than plastic containers as far as storage under refrigeration conditions is concerned.

Table 2(a). Effect of storage containers on total plate count of wheat flour dough stored in refrigeration temperature (3-4°C)

Storage time (days)	Storage container (TPC CFU*/ml)	
	Plastic container	Stainless steel container
Wheat flour dough		
0	1.5 x 10^2	6.5 x 10^3
2	2.0 x 10^2	7.1 x 10^3

Incubation temperature: 37°C; Incubation time: 24-36 hours; * Colony forming unit

Table 2(b). Effect of storage containers on total yeast and mount count of wheat flour dough stored in refrigeration temperature (3-4°C)

Storage time (days)	Storage container (TPC CFU*/ml)	
	Plastic container	Stainless steel container
Wheat flour dough		
0	3.5 x 10^2	4.0 x 10^3
2	5.0 x 10^2	4.5 x 10^3

Incubation temperature: 37°C; Incubation time: 48-72 hours;
* Colony forming unit

Cooked meat:

Table 3a and b show growth pattern of TPC and Yeast and mould count of meat samples stored in 2 types of containers (*i.e.* stainless steel and glass) in refrigerator. In fresh samples of meat, the total plate count (in both types of containers) was nil on first day. On third day TPC had increased upto 2 x 10^5 cfu/ml in the sample which was stored in glass container and to 1.5 x 10^5 cfu/ml of meat which was stored in stainless steel container. But on 5th day TPC increased to 3 x 10^5 cfu/ml in cooked meat sample which was stored in glass container and found to be 2.5 x 10^5 cfu/ml in the meat samples which were stored in stainless steel container. In fresh samples of meat, the yeast and mould count was also not detected on first day (in samples stored in both types of containers).

Table 3(a). Effect of storage containers on total plate count of cooked meat stored in refrigeration temperature (3-4°C)

Storage time (days)	Storage container (TPC CFU*/ml)	
	Glass container	Stainless steel container
0	ND	ND
3	2.0 x 10^5	1.5 x 10^5
5	3.0 x 10^5	2.5 x 10^5

Incubation temperature: 37°C; Incubation time: 24-36 hours;
* Colony forming unit

Table 3(b). Effect of storage containers on total yeast and mould count of cooked meat stored in refrigeration temperature (3-4°C)

Storage time (days)	Storage container (TPC CFU*/ml)	
	Glass container	Stainless steel container
0	ND	ND
3	1.5 x 10^5	1.0 x 10^5
5	3.2 x 10^5	2.7 x 10^5

Incubation temperature: 37°C; Incubation time: 48-72 hours;
* Colony forming unit

However, on third day yeast and mould count was observed to be 1.5 x 10^5 cfu/ml in case of meat sample which was stored in glass container and it was 1.0 x 10^5 cfu/ml for cooked meat stored in stainless steel container. But on 5th day yeast and mould count was increased to 3.2 x 10^5 cfu/ml of the meat sample which was stored in glass container and it was seen to be 2.7 x 10^5 cfu/ml for meat which was stored in stainless steel container. The meat sample stored in glass container showed highest TPC and yeast and mould count than the one which was stored in steel container.

Milk:

Methylene blue reductive test was used to check level of

Table 4: Effect of storage containers on quality of milk

Storage time (days)	Storage container (TPC CFU*/ml)	
	Glass container	Stainless steel container
Raw milk	Aluminum container	Stainless steel
Reduction time (hrs)	½	½
Quality of milk	Very poor quality	Very poor quality
Duration	Boiled milk (stainless steel container)	
	Reduction time (hrs)	Quality of milk
0	No change in 6 hrs	Good quality
2	No change in 6 hrs	Good quality
3	4	Fair quality
4	2½	Fair quality
5	2	Poor quality
	Boiled milk (stainless steel container)	
0	No change in 6 hrs	Good quality
2	4	Fair quality
3	2	Poor quality
4	1	Poor quality
5	½	Very poor quality

MBRT – Methylene blue reductase test

spoilage in case of samples of boiled milk and raw milk. The results are presented in Table 4 which reveals that the blue dye which was mixed with boiled milk sample (which was kept in aluminum vessel) changed colour after 4 hours. It had become white. Milk sample kept in stainless steel vessel did not show any change during this period. On third day of refrigerated storage of milk; the colour of mixture of boiled milk (kept in aluminum vessel) changed colour in 2 hours only and the one kept in stainless steel vessel took 4 hours to get into same condition. On fourth day of refrigerated storage of milk the colour of mixture of boiled milk (kept in stainless steel vessel) changed colour in 2½ hours only. Results on 5th day were more conspicuous as the mixture made from boiled milk (taken out from aluminum vessel) changed its colour within 30 minutes whereas it took 2 hours to become colourless for the milk sample which was taken from stainless steel vessel.

Methylene blue reductive test showed starling results when raw milk was used for experimentation. Colour of mixture become white within 15 minutes in case of samples drawn both from aluminum and stainless steel vessels. Results indicate that raw milk is not safe to keep in any metallic container even under refrigeration conditions. It should be immediately boiled (upon procuring) and stored for minimum duration. Safest method to store milk pouches is in freezer till its intended use. It should be consumed as fast as possible after boiling.

Toxicity analysis of food stored in refrigerator:

Data in Table 5 indicate that the fresh sample of wheat flour dough had 1.43 mg/100 g chromium content. Minimum

Table 5: Chromium content in selected food items under refrigerated conditions stored in stainless steel container per 100 g

Food items	Chromium content (mg/100 g)		
	Fresh food samples	After 2 days	Per cent increase
Boiled milk	0.03	0.32	966.7
Verka paneer	0.23	1.54	569.56
Cooked meat	0.37	0.98	164.86
Wheat flour dough	1.43	3.59	151.04
Permissible limits	0.05 – 0.20 mg/day		

chromium content (0.03 mg/100 g) was seen in fresh milk sample followed by sample of fresh *verka paneer* which showed 0.23 mg/100 g chromium content. Presence of chromium in the food samples after 2 days increased in all samples. Maximum increase was seen in wheat flour dough sample which showed chromium content of 3.59 mg/100 g followed by seepage in *verka paneer* (1.54 mg/100 g), and cooked meat (0.98 mg/100 g). Minimum rise in chromium content was observed in milk sample after 2 days of refrigerated storage.

However, it was milk sample which indicated maximum percentage increase (966.7%) in chromium content followed by *verka paneer* sample which showed percentage increase of 569.56. These results clearly indicate that the milk and milk products cannot be safely kept in metallic containers for longer duration. Approximately 150 to 165 per cent increase was observed in wheat flour dough and cooked meat samples when these were stored under refrigerated conditions in stainless steel container which clearly indicate that prolonged storage (even at low temperature) is not safe for the food items which have more moisture content.

Permissible limits of presence of chromium in any edible product is 0.05-0.20 mg/day and it was only fresh milk sample which had chromium content less than this limit. However, it was significant to note that it was milk sample stored in stainless steel for only two days which showed almost 1000 per cent increase of the chromium content in it.

Kudesia (1985) also revealed that causes of chromium poisoning have been reported in stainless steel industry and alloy industries. Chromium accumulates in lungs with age. It causes cancer, anuran, nephritis, gastrointestinal ulceration and effects central nervous system. Therefore, it is not advisable to store any of these food items in refrigerator for longer duration.

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