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Microbial Analysis of Commonly Stored Food Items in Household Refrigerators in Selected Containers

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ABSTRACT Nine food items were evaluated organoleptically and for microbial growth under refrigeration conditions. Organoleptic evaluation showed deterioration in appearance, colour, texture, and flavour of wheat flour dough, cooked meat, grapes, *Paneer* (Cottage cheese) and fresh *Pudina (Mentha Sativa)*. Grapes stored in ordinary polythene bag were full of fungus in one day only; followed by significant number of yeast and mould count in cooked *dal* (split red lentil) when stored in plastic bin, *Paneer* in *Verka* packet, dough in plastic bin, *Sharbat* (Sweet drink prepared from rose petals) in plastic bottle and cake in its original wrapper after two days only. Total Plate Count was maximum in dough and *Sharbat* in plastic bin after two days of refrigeration followed by *Paneer*, cake, sauce and cooked *dal* (split red lentil) which was kept in plastic bin. Therefore it is recommended that food grade containers be used to ensure food safety in refrigerator.

INTRODUCTION

Refrigeration is employed to control the rate of certain chemical and enzymatic reactions as well as rate of growth of food microorganisms (Srivastava and Kumar 2002). Food spoilage slows down as molecular motion slows which retards growth of bacteria that causes food to spoil (Whitman et al. 2005). However, studies have shown that perishable food will deteriorate, even at refrigerator temperature, due to spoilage because of microorganisms, enzymes and oxidation (Jay 2000). Type of container or wrapping material they are stored in and duration of storage are also important factors that influence the type of microbial growth, toxicity and spoilage of food during refrigerated storage. Although low temperature retards spoilage but even a sub- freezing temperature of about 7°C does not prevent multiplication of all microorganisms. Refrigerated foods are therefore subjected to spoilage by moulds, yeasts and bacteria (Roday 2002). Moreover, temperature and climatic conditions prevalent in India; are conductive to growth of bacteria causing food borne diseases.

There is a wide variety of food containers and packing materials available in the market including glass, paper, plastic and stainless steel. Some of plastic containers are food grade and some are non food grade. Other like plastic wraps, bags or air tight containers are best choices for storing most of foods in refrigeration (Potter 2003). Packaging also must protect against a variety of assaults including physical damage and contamination from biological vectors including microorganisms. When better quality of containers and original packaging is used for the refrigeration; it can slow down the growth of microorganisms.

Many diseases are caused by microorganisms, such as bacteria, viruses and parasitic infestations arising out of food spoiled due to prolonged storage of perishable and semi perishable food items. Like *Staphylococcus* bacteria which produces a toxic as by-product of growth and multiplication and cause food intoxication (Haghi 2011). Although ample research points out these conditions, none has so far been able to look into effect of containers/wrappers on food deterioration in refrigeration conditions. Hence the present study was been planned with the following objectives:

1. To organoleptically evaluate selected foods stored in selected containers under refrigeration conditions.
2. To undertake microbiological analysis to ascertain shelf-life of selected food products stored under refrigeration conditions in selected storage materials.

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3. To suggest safe refrigerated food storage practices.

MATERIAL AND METHODS

Experiments were conducted in the Microbiology Laboratory in Punjab Agricultural University, Ludhiana. Nine most commonly used and locally available food items (perishable and semi-perishable) were selected. These were grapes, cooked meat, *Paneer* (Cottage cheese-*Verka*), cooked *dal* (split red lentil), wheat flour dough, green leafy herb (*Pudina (Mentha Sativa)*), cake, tomato sauce and *Rooh Afza Sharbat* (Sweet drink prepared from rose petals). These were stored in two types of food containers, one the existing (commonly used) practice and other the recommended one (by the panel of judges), thus constituting the food sample of 18. Already prevalent storage practices were: storing food items using polythene bag, plastic bin, glass jar with plastic lid and glass bottle with plastic lid. The recommended method for storing semi- perishable and perishable food items included use of plastic net bag, stainless steel bin and Tupperware bin. Samples were stored at 3 to 4°C in the pre selected refrigerator shelf. Grapes and *Pudina (Mentha Sativa)* were stored in vegetable basket. *Paneer* (Cottage cheese), wheat flour dough and cake were stored in second shelf of the refrigerator. Cooked *dal* (split red lentil) and cooked meat were stored in first shelf of the refrigerator and sauce and *Rooh Afza Sharbat* (Sweet drink prepared from rose petals) were stored in side door of refrigerator. The duration of storage was pre determined. *Paneer* (Cottage cheese), *Pudina (Mentha Sativa)*, cooked *dal* (split red lentil) and cooked meat, wheat flour dough cooked meat and cake was stored for 2 days, grapes were stored for 3 days whereas sauce and *Sharbat* (Sweet drink prepared from rose petals) was kept for 3 weeks.

For microbial study of samples, two types of media (nutrient agar and glucose yeast agar) were prepared. Prescribed procedure was followed for testing, that is, 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. Organoleptic evaluation of the sample was done by panel of five judges.

RESULTS AND DISCUSSION

1. Organoleptic Evaluation of Stored Food Products

It can be clearly seen in Table 1 that when these selected food items were stored using the existing practice of storage as compared to the recommended method; the mean scores of organoleptic evaluation items stored by using existing practice were found to be lower; in all food items except for processed products (sauce and *Rooh Afza Sharbat* (Sweet drink prepared from rose petals)). These two products indicated the highest score (mean score 4) too which shows that processed food items do not change their physical properties during refrigeration of storage of less than a week. In existing storage practice, when the food samples were kept in glass bottles with plastic lid in refrigerator (like tomato sauce and *Rooh Afza Sharbat* (Sweet drink prepared from rose petals)) the mean score of these food items remained unchanged, that is, 4.00; it means appearance, texture, flavour and colour.

Second rank was obtained by sample of cake which was kept in its original packing (existing storage practice) with mean score of 3.04; followed by 3rd and 4th ranking given by panel of judges to sample of cooked dal (split red lentil) (mean score 2.64) and fresh *Pudina (Mentha Sativa)* (mean score 2.52) when these were stored in plastic bin and polythene bags respectively under existing storage practice in refrigerator for 2 days. 5th and 6th rank were obtained by samples of *Verka Paneer* (Cottage cheese) and grapes which were kept in plastic bin and polythene bag respectively with mean score between 2.18-2.06. Minimum score (showing maximum changes in physical appearance) was observed for dough sample with mean score 1.06 followed by change in cooked meat with mean score of 1.84.

However, there was slight improvement in organoleptic storage of all experimental samples (judged by panel) which were kept in suggested containers in refrigerator. Apart from processed food products (*Rooh Afza Sharbat* (Sweet drink prepared from rose petals) and tomato sauce) which indicated no change in organoleptic properties, minimum change 11.18 per cent was observed in cake samples in its original packing when compared to suggested storage practice

Table 1: Organoleptic evaluation of selected samples kept in refrigerators

Food items stored	*Existing storage practice*	*Mean score*	*Suggested storage practice**	*Mean score*	*Percentage change in scoring*	*Rank*
Grapes	Polythene bag	2.06	Plastic net bag	3.78	83.49	2
Paneer (Cottage cheese) *Verka*	Plastic bin	2.18	Stainless steel bin	3.56	63.30	3
Fresh *Pudina (Mentha Sativa)*	Polythene bag	2.52	Stainless steel bin	3.47	37.69	5
Cooked *dal* (split red lentil)	Plastic bin	2.64	Stainless steel bin	3.42	29.54	6
Wheat flour dough	Plastic bin	1.06	Stainless steel bin	3.38	218.86	1
Cooked meat	Glass bin with plastic lid	1.84	Stainless steel bin	2.96	60.87	4
Tomato sauce	Glass bottle with plastic lid	4.00	Tupper ware	4.00	-	-
Rooh Afza Sharbat (Sweet drink prepared from rose petals)	Glass bottle with plastic lid	4.00	Tupper ware	4.00	-	-
Cake	Cake wrapper in which cake purchased	3.04	Tupper ware bin	3.84	11.18	8
Milk	Aluminum vessel	3.16	Stainless steel bin	3.04	21.51	7

of storing it in Tupperware bin refrigerator. As changes observed was 21.51 per cent only. Approximately 30-40 per cent changes in physical properties was evaluated in cooked dhal and fresh *Pudina (Mentha Sativa)* samples with (mean score 3.42 and 3.47) respect to stainless steel container sample (suggested one). More than 60 per cent improvement in organoleptic assessment was observed in case of cooked mean and *Verka Paneer* (Cottage cheese) sample (stored in stainless steel bin).

There was remarkable improvement (83.49%) in grape sample (kept in net bag) stored in refrigerator appearance, texture, flavour and colour. Top ranking (218-86%) on organoleptic evaluation was obtained by wheat flour dough sample which was kept in stainless steel bin. It may be due to large content of water in dough, grapes, *Paneer* (Cottage cheese), meat etc. which could be responsible for changes in the physical properties of food items during refrigerated storage. Mordi and Olorunda (2003) also revealed similar findings in their study on visual qualities and storage life of fresh tomatoes.

2. Microbiological Analysis of Refrigerated Food Items

Grapes: It can be clearly seen in Table 2 that the total plate count (TPC) and yeast mould count of grapes stored in two types of bags (polythene and net bag) stored at refrigerated temperature (3°C) increased. The TPC was 1.7 x 10^3 cfu/ml in polythene 1.0 x 10^2 cfu/ml in net bag. This may be due to contamination during handling of product and through utensils, equipment and air. It was observed that TPC increased over the time period. Jeya et al. (2005) also reported that increase in TPC could be due to favourable environment factors like temperature, relative humidity, storage conditions and food factors like pH, water activity, moisture content and nutrient present. The growth of microorganisms during storage of samples in refrigerated was also affected by the type of container and packaging material. The sample stored in polythene showed highest TPC (1.7 x 10^3 cfu/ml) followed by net bag (1.0 x 10^3 cfu/ml) after 24 hours in refrigerator. Growth rate of yeast and mould was highest in polythene (fungus is present) and the lowest rate was recorded in the samples of net bag.

Paneer (Cottage Cheese): These samples stored in ordinary plastic containers showed the highest TPC (5.5 x 10^3 cfu/ml) followed by steel (4.0 x 10^3 cfu/ml) and original *Verka* packet (1.2 x 10^3 cfu/ml) after 24 hours in refrigerator. The yeast and mould count of *Paneer* (Cottage cheese) in plastic containers (3.0 x 10^3 cfu/ml), in steel (1.5 x 10^3 cfu/ml) and in *Verka* packet (5.0 x 10^2 cfu/ml). The highest rate of growth of yeast and mould was recorded in sample of ordinary

Table 2: Microbial analysis of selected food samples stored in refrigerator

Foot items stored	*Containers used*	*Refrigeration duration*	*Microbial growth counts*	
			TPC CFU/ml*	*Y and M, CFU*/ml*
Grapes	Polythene	2 days	1.7×10^3	Present
	Net bag		1.0×10^2	Nil
Verka Paneer	*Verka* packet	2 days	1.2×10^3	5.0×10^2
(Cottage cheese)	Stainless steel		*4.0×10^3*	*1.5×10^3*
	Plastic bin		5.5×10^3	3.0×10^3
Fresh *Pudina*	Stainless steel	2 days	4.0×10^3	1.5×10^3
(Mentha Sativa)	Polythene		*ND*	*3.5×10^3*
Cooked *Dal*	Stainless steel	1 day	1.2×10^3	4.0×10^2
*(split red lentil)**	Plastic bin		5.0×10^3	7.0×10^2
Wheat flour dough	Plastic bin	2 days	2.0×10^3	5.0×10^2
	Stainless steel		7.1×10^3	4.5×10^3
Cooked meat*	Stainless steel	1 days	2.8×10^3	1.5×10^3
	Glass jar		4.0×10^3	2.5×10^3
Sauce	Glass bottle	3 weeks	1.0×10^2	Nil
	Plastic		5.0×10^2	2.0×10^2
Sharbat (Sweet drink	Glass jar	3 weeks	2.0×10^2	Nil
prepared from	Plastic		7.0×10^2	5.0×10^2
rose petals)*				
Cake	Cake wrapper in which cake was purchased	2 days	2.2×10^3	5.0×10^2
	Tupper ware		5.0×10^3	3.8×10^3

Incubation time = 48 hours, **Incubation time = 24 hours; ND – Not detected; *Colony forming units; TPC – Total Plate Count, Y and M – Yeast and Mould

plastic containers (3.0×10^3 cfu/ml) and the lowest rate was recorded in original *Verka* packing (5.0×10^2 cfu/ml). It may be due to processing and handling of product. Milk products containing amorphous carbohydrates 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 (Passmore and Eastward 1986). The ordinary plastic containers have higher TPC and steel and original packing after storage in refrigeration. Similarly it was observed ordinary plastic containers shows highest yeast and mould count (3.0×10^3 cfu/ml) and lowest rate was found in original *Verka* packing after 24 hour in refrigerator.

***Pudina (Mentha Sativa)*:** Samples of *Pudina (Mentha Sativa)* stored in 2 types of containers, steel and in simple polythene showed TPC in polythene over crowding and in steel 4.0×10^3 cfu/ml. This may be due to contamination during handling and hardening of product through utensils, equipment and air. The sample stored in ordinary polythene containers highest TPC (over crowding) followed by steel (4.0×10^3 cfu/ml) after 24 hours in refrigerator. The yeast and mould count was 3.5×10^3 cfu/ml for sample stored in ordinary polythene container and the lowest rate was recorded in the samples of steel container (1.5×10^3 cfu/ml). It may possible that ordinary polythene had direct air contact that allowed moisture into samples and favored the growth of microorganisms. These findings are in line with Ashenafi and Busse (1991)'s work.

Wheat Flour Dough and Cooked Dal (Split Red Lentil:) Samples of dough and *dal* (split red lentil) samples stored in 2 types of containers too showed TPC and yeast and mould count in sample of *dal* (split red lentil) in plastic container to be 5.0×10^3 and 7.0×10^2 and in steel containers 1.2×10^3 cfu/ml and 4.0×10^2 cfu/ml, respectively. TPC and yeast and mould count sample of wheat flour dough in plastic containers 7.1×10^3 cfu/ml and 4.5×10^3 cfu/ml and in steel containers, it was 2.0×10^3 cfu/ml and 5.0×10^2, respectively. So samples stored in steel container showed lower TPC and yeast and mould count than plastic containers.

Cooked Meat: Meat samples stored in steel container had TPC 2.8×10^3 cfu/ml and in glass 4.0×10^3 cfu/ml. Yeast and mould count in steel container was 1.5×10^3 cfu/ml and in glass it was 2.5×10^3 cfu/ml. Sample stored in glass container showed highest TPC and yeast and mould count than in steel container.

Sauce and Sharbat (Sweet Drink Prepared From Rose Petals): TPC of sauce sample in plastic was 5.0×10^2 cfu/ml and in plastic container it was 7.0×10^2 cfu/ml. Yeast and mould count of sauce sample stored in glass container was nil and in plastic it was 2.5×10^3 cfu/ml and 2.0×10^2 cfu/ml. This may be due to environment factors such as packaging. It was observed that count was less for both TPC and yeast and mount count because only high salt content, sugar acid content, any preservative, pH, water activity and control growth of microorganisms can grow. Lower growth rate was observed in glass container. Both TPC and yeast and mould were observed after 24 hours.

Cake: TPC of cake in original packing and in Tupperware was 2.2×10^3 cfu/ml and 5.0×10^3 cfu/ml, respectively. This may be due to contamination during handling of product or through utensils, equipment and air. Yeast and mould count found to be in original packing (5.0×10^2 cfu/ml) and in Tupperware (3.8×10^3 cfu/ml). So highest count of yeast and mould was recorded in sample of Tupperware and lowest in original packing (5.0×10^2 cfu/ml).

3. Recommendations for Safe Containers for Use in Refrigeration Storage

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, Total Plate Count and organoleptic properties of selected food items has been given in Table 3.

Table 3: Recommended food containers for storage in refrigerator

Food items to be stored	*Safe storage container recommendation*
Grapes	Plastic net bag
Paneer (Cottage cheese)	Stainless steel bin
Fresh *Puidna*	Stainless steel bin
Cooked dal (split red lentil)	Stainless steel bin
Wheat flour dough	Stainless steel bin
Cooked meat	Stainless steel bin
Sauce	Glass bottle with plastic lid
Sharbat (Sweet drink prepared from rose petals)	Glass bottle with plastic lid
Cake	Cake wrapper in which cake was purchased

CONCLUSION

It can be thus concluded from this study that there is a certain effect of storage containers on the food items stored even under refrigeration conditions. Looking into the multiplication of both yeast and mould, and total plate count, in the selected food items which were stored for a specific duration in the refrigerator, it becomes essential to select the container which inhibits growth of both these microorganisms. Moreover, the organoleptic evaluation of selected samples also can not be overlooked as score improved when recommended containers were used. Therefore, consumers need to follow these recommendations while purchasing new storage bins, and using existing ones for storage of perishable as well as semi perishable food items in household refrigerators.

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