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IDENTIFICATION OF IRRADIATED DRIED FRUITS AND PLANT NUTS BY DIFFERENT ANALYTICAL TECHNIQUES

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ABSTRACT

In order to standardize methods to identify irradiated dried fruits and plant nuts different analytical techniques such as chemiluminescence, spectrophotometry, electrical conductivity and acidity/pH were employed. For this purpose apricot, date, raisin, almond, peanut, pinenut and walnut were irradiated to the dose level of 0.5, 1.0 and 1.5 kGy using gamma radiations from Co-60 source. Chemiluminescence (CL) intensities of the samples were measured using luminol and lucigenin reactions. The CL values obtained for as such, moisture free, and fat free samples were found to be inconsistent and irreproducible. However, the CL values measured using the ashed material (mineral matter) of samples were reproducible, consistent and dose dependent. The electrical conductivity, pH/acidity and the spectrophotometric measurements of the solvent extracted materials showed clear differences between irradiated and unirradiated samples.

Key words: chemiluminescence; spectrophotometry; electrical conductivity; gamma-irradiation.

INTRODUCTION

Identification of irradiated food stuffs is very important to promote international trade and prevent malpractices either in selling unirradiated foods at the price of irradiated ones, or by marketing irradiated foods in countries where laws prohibit such practices. Unfortunately no standard method for this purpose so far has been developed, which can differentiate irradiated and unirradiated foods in relation to the dose absorbed. In about 40 countries more than 50 different kinds of foods are now approved for irradiation treatments. Due to the differences in national regulations concerning food irradiation, it is worthwhile to ensure proper control of irradiated foods in international trade. A number of analytical methods showing promise have been reported (Delincee, 1992; Bogl and Heide, 1985). Preliminary work on the development of detection methods and aquous/non-aquous dosimeters was initiated Sattar *et al.* (1987) and Khan *et al.* (1989). Sattar *et al.* (1995) and Ahmad *et al.* (1995) reported the results of their chemiluminescence and the measurements for detecting irradiated foods. In view of the potentials of food irradiation processing in Pakistan, these studies were conducted for the development of suitable detection methods for dried fruits and plant nuts.

MATERIALS AND METHODS

The samples of dried fruits and plant nuts such as apricot, date, raisin, almond, peanut, pinenut and walnuts were obtained in fresh condition from wholesale market at Peshawar. The samples were packed in clear polyethylene pouches for gamma irradiation treatment (0.5, 1.0 and 1.5 kGy) using Co-60 ISSLEDOVATEL (CIS) at the dose rate of 2.4 kGy/hr with maximum/minimum ratio of 1.07 as determined by Fricke and Hart (1966). Mineral matter of the samples was obtained by dry-ashing in a muffle furnace at 550^{o}C. The chemiluminescence (CL) values of the mineral matter were determined using luminol and lucigenin photosensitizing reactions immediately after irradiation and subsequently during storage of 7-90 days. Spectral analysis and electrical conductivity (EC) were performed by collecting volatiles of test samples in a glass assembly especially designed by this group. For acidity (free and total) and pH measurement, the samples were extracted in distilled water Rayas-Duarte and Rupnow (1994). The data were statistically analysed by measuring the means and coefficient of variation (CV).

RESULTS AND DISCUSSIONS

The chemiluminescence (CL) values of dried fruits and plant nuts as measured on the basis of their mineral matter are presented in Table 1 and 2. The results showed that the CL intensities as a result of luminol and lucigenin photosensitizing reactions increased with increasing radiation doze in all the samples tested as is evident from the means obtained for each dose applied. It was also observed that the CL response of the stored samples was quite consistent up to the entire storage period of 90 days.

Table 1. Chemiluminescence intensities (MV) of mineral matter from dried fruits with respect to irradiation doses and storage time

Sample/ storage days		Luminol reaction Radiation doses (kGy)					Lucigenin reaction Radiation doses (kGy)				
		0	0.5	1.0	1.5	Mean	0	0.5	1.0	1.5	Mean
Apricot	0	0.95	0.98	2.05	2.51	1.62	0.6	1.1	5.3	6.3	3.33
	7	0.99	1.10	2.11	2.61	1.70	0.5	1.0	5.1	6.0	3.15
	15	0.98	1.17	2.13	2.70	1.75	0.5	1.1	5.0	6.0	3.15
	30	1.20	1.80	2.15	2.70	1.96	0.6	1.9	5.0	6.0	3.40
	90	1.80	1.90	2.33	2.81	2.21	0.7	2.5	6.1	6.50	3.95
Date	0	0.90	1.05	1.65	1.96	1.39	0.50	2.50	5.50	7.20	3.90
	7	0.91	1.11	1.72	1.99	1.40	0.45	2.12	5.30	6.91	3.70
	15	0.93	1.19	1.91	2.00	1.50	0.49	2.21	5.20	6.83	3.68
	30	0.99	1.30	4.70	5.00	2.99	0.50	2.20	5.50	6.80	3.75
	90	1.10	1.50	6.50	7.00	4.02	0.80	2.60	5.80	6.70	3.97
Raisin	0	1.7	2.7	3.5	6.6	3.60	7.9	13.0	18.0	20.0	14.72
	7	1.8	2.9	3.5	6.8	3.75	7.5	12.5	17.0	19.5	14.13
	15	1.9	3.0	3.9	6.9	3.90	7.6	12.5	17.8	19.0	14.23
	30	1.8	3.0	3.9	6.5	3.80	7.0	13.0	18.5	20.6	14.77
	90	1.9	3.9	4.5	6.0	4.07	7.0	14.5	19.0	22.0	15.62
Mean		1.32	1.90	3.10	4.27		2.84	5.65	9.60	11.08	

Values are mean of 3 determinations.

Table 2. Chemiluminescence intensities (MV) of mineral matter of dry nuts with respect to irradiation doses and storage time

Sample/ Storage days		Luminol reaction Radiation doses (kGy)					Lucigenin reaction Radiation doses (kGy)				
		0	0.5	1.0	1.5	Mean	0	0.5	1.0	1.5	Mean
Almond	0	1.6	6.1	7.6	8.0	5.83	0.8	1.2	1.5	2.0	1.38
	7	1.8	6.9	7.8	8.1	6.15	0.8	1.1	1.3	2.0	1.30
	15	1.8	6.9	8.1	8.2	6.25	0.7	1.0	1.0	1.8	1.12
	30	1.9	7.0	7.9	8.0	6.20	0.7	1.0	1.0	2.0	1.17
	90	1.9	7.0	7.9	8.5	6.32	0.7	1.2	1.6	2.3	1.31
Peanut	0	0.60	0.81	1.5	4.5	1.85	0.19	1.40	1.90	2.9	1.60
	7	0.75	0.92	1.8	4.8	2.07	0.18	1.20	1.80	2.8	1.50
	15	0.77	0.93	1.9	4.9	2.09	0.17	1.17	1.77	2.7	1.45
	30	0.90	0.95	2.3	5.8	2.48	0.17	1.20	1.80	2.80	1.49
	90	1.00	0.99	2.7	6.0	2.67	0.17	1.50	1.90	2.99	1.62
Pinenut	0	0.85	1.1	2.8	7.3	3.01	0.80	0.95	1.30	2.70	1.44
	7	0.91	1.2	2.9	7.3	3.08	0.78	0.92	1.23	2.69	1.40
	15	0.92	1.2	2.9	7.8	3.21	0.77	0.91	1.20	2.65	1.38
	30	0.99	1.8	2.9	7.8	3.37	0.89	1.00	1.30	2.60	1.44
	90	1.00	2.5	2.9	7.5	3.47	0.90	1.01	2.40	2.30	1.65
Walnut	0	6.5	8.6	35.5	35.5	21.5	0.50	0.40	1.30	2.60	1.20
	7	6.4	8.7	37.0	36.5	22.15	0.47	0.39	1.20	2.50	1.14
	15	6.7	8.9	39.0	39.0	23.40	0.45	0.37	1.10	2.50	1.10
	30	6.5	8.9	39.5	40.9	23.95	0.50	0.45	2.00	2.60	1.40
	90	6.5	9.2	40.5	50.5	26.67	0.60	0.50	2.50	2.70	1.57
Mean		2.51	4.53	12.77	15.34		0.56	0.94	1.55	2.51	

Values are mean of 3 determinations.

Table 3. Effect of gamma irradiation on electrical conductivity (μ-mho) in dried fruits and plant nuts

Sample	Radiation doses (kGy)	
	0	1.5
Apricot	8.0 ± 2.0	17.5 ± 3.5
Date	9.5 ± 2.5	18.0 ± 2.0
Raisin	16.5 ± 3.0	30.0 ± 3.5
Almond	12.0 ± 2.8	21.0 ± 3.0
Peanut	13.0 ± 2.3	26.0 ± 2.0
Pinenut	12.0 ± 2.9	22.0 ± 3.5
Walnut	10.0 ± 2.0	17.0 ± 2.0
Mean	11.57	21.64
CV	23.98	22.42

1 g sample distilled to 40 mL.
Values are average of 3 determinations.

On the basis these observations taken at different intervals, it was found that irradiated food materials can be distinguished from unirradiated foods even after 90 days of storage. Bogl and Heide (1985) found that CL values of spices and herbs were higher in irradiated samples. Dried irradiated vegetables were identified with the help of CL measurements by Delincee (1987). Sattar *et al.* (1987) showed that CL intensities of irradiated pepper and papain were higher than their respective controls. Bogl and Heide (1990) studies 19 different spices, irradiated and subsequently reacted with luminol. They found that the CL intensities elevated with increasing irradiation doses and some samples were identifiable even after 2 months of storage. Recently Khan *et al.* (1993) also reported that there was clear difference of luminescence between irradiated and unirradiated samples.

In addition to CL measurement, electrical conductivity (EC) of the samples-steam distillates were measured using conductivity bridge (Table 3). Clear differences in EC values among treated and untreated samples were detected and the values of irradiated samples were found al-

Table 4. Effect of irradiation on pH, free acid (FA) and total acid (TA) in dried fruits and plant nuts

Samples	Tests	Radiation doses(kGy)					
		0	0.5	1.0	1.5	Mean	CV
	pH	6.3	5.5	4.3	4.0	5.02	21.27
Apricot	F.A	3.5	12.9	18.9	24.7	15.00	60.36
	T.A.	9.3	15.0	19.9	26.5	17.67	41.33
	pH	5.2	4.8	4.4	4.2	4.65	9.53
Date	F.A.	2.7	7.2	11.0	11.5	8.10	50.37
	T.A.	5.7	11.3	16.3	17.4	12.67	42.24
	pH	4.7	3.0	2.0	2.0	9.92	43.54
Raisin	F.A.	5.2	11.0	15.0	19.5	12.70	47.91
	T.A.	7.3	13.5	17.6	19.5	15.0	41.22
	pH	6.5	6.0	5.8	5.5	5.90	7.06
Almond	F.A.	3.5	3.7	4.1	4.7	4.0	13.23
	T.A.	3.8	3.9	4.5	4.9	4.0	12.13
	pH	6.8	5.9	5.5	5.1	5.80	12.48
Peanut	F.A.	3.0	4.2	4.7	5.0	4.20	20.84
	T.A.	5.0	5.3	5.9	6.1	5.60	9.19
	pH	5.8	5.6	5.0	4.8	5.30	8.98
Pinenut	F.A.	4.8	5.0	7.0	8.3	6.27	24.71
	T.A.	4.8	9.5	10.3	12.7	9.27	36.66
	pH	5.7	5.0	4.5	4.0	4.80	15.12
Walnut	F.A.	5.0	5.3	5.7	5.9	5.40	7.36
	T.A.	7.9	9.9	10.3	11.6	9.90	15.44

Values are average of 3 determinations
FA and TA = meq of acid (H^+).

most double than their unirradiated counterparts. Hayashi (1988) identified irradiated potatoes by measuring its EC and reported that applied dose can be estimated even after 6 months of storage. Under laboratory conditions, change in impendence at frequencies of 50 Hz to 100 KHz gave indications about radiation treatment and the radiation dose applied in fish (Ehelermann, 1972).

Free/total acidity and pH of the samples were determined and the data are presented in Table 4. It was observed that both the free and total acidity increased with radiation doses, while the pH value decreased. It revealed that decrease/increase in relation to absorbed doses was of lesser magnitude in case of plant nuts than dried fruits. Rayas-Duarte and Rupnow (1994) indeed reported that the free and total acidity increased with irradiation doses from 2.5-20 KGy.

CONCLUSION

It was concluded from the results obtained that the CL intensity values of mineral matter of test samples gave reproducible and dose dependent values. The EC and pH changes also were found to be somewhat dose dependent.

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MICROBIOLOGICAL DECONTAMINATION OF SPICES BY GAMMA-IRRADIATION ALONE AND BY COMBINATION TECHNIQUES

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ABSTRACT

Results regarding initial fungal counts of spices such as turmeric (*C. lonza*), coriander (*C. sativum*), red chillies (*C. annum*), caraway (*C. cyminum*) and black pepper (*P. nigrum*) revealed that these were heavily infested with fungi ranging from 3.3×10^3 to 2.0×10^6/g which decreased to a range level of 1.0×10^1 - 6.0×10^1/g with 4 kGy of gamma irradiation dose. Complete decontamination of fungi was achieved with a dose of 8-10 kGy. Similarly the bacterial load which initially ranged from 1.4×10^5 to 1.5×10^7/g decreased to negligible level at 8-10 kGy of irradiation dose. Among the combination treatments, radiation (5 kGy) and solar heating (60-70°C in solar dryer for 6 hours) and electric oven heating (70-80°C in an electric oven for 3 hours) were most effective in curtailing the microbial load to a negligible level. This treatment was followed by irradiation alone (500 krad).

Key words: gamma-irradiation; fungi; decontamination; bacterial load; Pakistan.

INTRODUCTION

Dry spices in both whole and ground forms are essential ingredients of not only Pakistani diets but all over the world. They are added to foods for taste, flavouring and seasoning. Pakistan produces several spices and a big potential exists to earn foreign exchange by exporting these to other countries. Post-harvest contamination of spices by microorganisms is wide spread under the hot and humid climate of Pakistan due to which a large part of this important commodity is rendered unusable annually. Hence the country looses a big chunk of foreign earnings. Although only small amounts of spices are used in the diet, the large number of microorganisms which include molds, bacteria and their hear resistant spores can cause serious microbial contamination of the meat, fish, cheese, baked goods and other food to which they are added (Sharma *et al.*, 1984). Spices have been found to contain large bioburden in several studies (Ito *et al.*, 1972; Baxter and Holzapfel, 1982; Shamshad *et al.*, 1985). Moreover, various results indicated that the spores entering the product together with the spices are more difficult to inactivate by subsequent heat treatments (Horwits and Gangarosa, 1976).

Keeping in view these problems, it was considered essential to find safe methods for decontamination of these spices, not only to lessen the spoilage losses but also making them safe for consumption. For this purpose, different chemicals e.g. methyl bromide etc. have been in use, but due to their health injurious residues, their use has been abandoned in many countries. Research into food irradiation has been conducted for over three decades and the efficacy of this technique to a number of food applications is well established (Collins and Lyme, 1976) including the inhibition of the growth and maturation of fresh fruits and vegetables, microbial disinfection of spices and dry condiments, pathogen decontamination of frozen food of animal origin, control of insect infestation of food and reduction of the number of food spoilage microorganisms (Awan, 1995). Above all, an advancement in food irradiation was achieved in 1980 when the Joint FAO/IAEA/WHO expert committee on the wholesomeness of irradiated food (JECFI) recommended the acceptability of food irradiated up to an overall average dose of 10 kGy. This achievement has greatly promoted development of food irradiation very rapidly with wide applications.

The objective of this investigation was to study the efficacy of gamma irradiation alone and in combination with heat treatments for reduction of bio-load in commercially available spices in Peshawar market.

MATERIALS AND METHODS

Samples of turmeric (*Curcuma longa*), black pepper (*Piper nigrum*), caraway (*C. cyminum*), red chilli (*Capsicum annuum*) and coriander (*Coriandrum salivum*) were collected from the local market of Peshawar. Except for turmeric and red chilli which were in ground state, the other three spices were ground individually in a grinder (Retch Muhle-Germany) so that the whole of the material passed through a 14 mesh sieve. The material was mixed thoroughly and samples of each spice were packed in polyethylene packages and then subjected to irradiation at

a dose of 0, 1.5, 3.0, 4.5, 6.0 and 7.5 kGy of gamma rays respectively.

For the study on combination treatments, red dried chillies were bought from the local market and ground to pass through a 14 mesh sieve. Following 7 individual and combination treatments were given to the samples:

1. Control (No treatment)
2. Radiation (5 kGy dose)
3. Heating (70-80°C in a solar dryer for 3 hours)
4. Solar drying (60-70°C in a solar dryer for 6 hours)
5. Radiation + solar drying + heating
6. Radiation + heating
7. Radiation + solar heating

In the combination treatments No. 5, 6, and 7 the doses of the respective treatments were the same as in 1, 2, 3 and 4. After the treatments, the samples were packed in polyethylene pouches and stored for further studies.

Bacterial loads were determined by plate count method using nutrient agar medium (Collins and Lyme, 1976). Culture media were incubated at 28 to 30°C and colony counting was performed after 26-28 hours. Fungal counts of spices were also determined by plate count method using potato dextrose agar (Clark, 1972). Plates were incubated at a temperature of 28°C for 3-5 days. Fungal counting was made by colony counter.

dose from 1.5 to 7.5 kGy. Initially the fungal count ranged from 6.5×10^3 to 1.5×10^5/g which decreased to 0 to 4.0×10^1/g at 4.5 kGy of radiation dose. At the dose of 6.0 kGy all the spices became completely decontaminated from the fungi.

The results regarding the effect of gamma irradiation (same doses of 1.5 to 7.5 kGy) on the decontamination of bacterial count in these spices is given in Table 2. The data revealed that initial bacterial count ranged from 8.6×10^4 to 6.5×10^5, the highest being in red chillies. With the increasing radiation doses the bacterial load in all spices showed a decreasing trend. At the dose of 7.5 kGy the bacterial load dropped to negligible level ranging from 0 to 10/g. Almost similar results were reported by several workers (Farkas, 1973; Himy *et al.*, 1981; Saputra *et al.*, 1981).

The data regarding effect of combined decontaminating techniques on total count of spices is given (Table 3). The data revealed that total fungal count in control sample was 3.0×10^5 which upon storage rose to a level of 6.6×10^6 at the end of 6 months. All the treatments were able to reduce this bioburden but radiation + solar heating + oven heating was found comparatively more effective and helped to maintain this lower bioburden up to 6 months of storage. In efficacy, this combination treatment was followed by treatment on irradiation alone.

Table 1. Effect of irradiation on total fungal count of spices

Irradiation (kGy)	TFC/g of the sample							
	Black-pepper	Corri-ander	Caraway	Turmeric	Red chillies	Mean	SD	CV
0	6.5×10^3	1.5×10^5	9.0×10^3	3.0×10^4	5.6×10^4	5.0×10^4	5.2×10^4	1.0×10^2
1.5	8.0×10^2	6.5×10^4	2.5×10^2	7.3×10^3	3.4×10^3	1.5×10^4	2.4×10^4	1.6×10^2
3.0	1.5×10^1	3.0×10^2	4.6×10^1	4.5×10^1	6.7×10^1	9.4×10^1	1.0×10^2	1.1×10^2
4.5	0	4.0×10^1	0	2.5×10^1	3.0×10^1	1.9×10^1	1.6×10^1	8.6×10^1
6.0	0	0	0	0	0	0	0	0
7.5	0	0	0	0	0	0	0	0
Mean	1.2×10^3	3.6×10^4	1.5×10^3	6.2×10^3	9.9×10^3			
SD	2.4×10^4	5.6×10^4	3.3×10^3	1.0×10^4	2.0×10^4			
CV	2.0×10^2	1.5×10^2	2.2×10^2	1.8×10^2	2.1×10^2			

RESULTS AND DISCUSSION

Results regarding effect of different doses of gamma radiation on fungal counts of black pepper, coriander, caraway, turmeric and red chillies are given in Table 1. Coriander was found to be highly infested with fungi as compared to other spices. It was observed that fungal counts in each case decreased with increasing irradiation

The effect of combined decontaminating techniques on total fungal count of spices is given in Table 4. In this case again, the treatment (radiation + solar heating + heating in air sterilizer) was found comparatively better in eliminating the fungal load from red chillies and maintaining it in safer limits in sealed polyethylene bags. The treatment (radiation alone) ranked 2nd in efficacy.

Table 2. Effect of irradiation on total bacterial count of spices

Irradiation kGy)	Black-pepper	Corri-ander	Caraway	Turmeric	Red chillies	Mean	SD	CV
0	4.7×10^5	2.5×10^5	8.6×10^4	9.5×10^4	6.5×10^5	2.2×10^5	2.2×10^5	98.9
1.5	9.8×10^3	7.0×10^4	1.5×10^4	2.0×10^4	9.7×10^4	4.2×10^4	3.4×10^4	82.2
3.0	4.8×10^3	1.0×10^4	6.2×10^3	3.5×10^3	3.4×10^3	5.5×10^3	2.4×10^3	44.5
4.5	7.9×10^1	3.6×10^2	9.8×10^1	1.0×10^2	8.5×10^1	1.4×10^2	1.0×10^2	74.8
6.0	2.0×10^1	1.9×10^1	0	1.0×10^1	3.0×10^1	1.5×10^1	20.8	46.0
7.5	0	8	0	0	1.0×10^1	3.3×10^1	4.0	2.0
Mean	1.0×10^4	5.5×10^4	1.7×10^4	1.9×10^4	1.2×10^5			
SD	1.6×10^4	9.0×10^4	3.0×10^4	3.4×10^4	2.3×10^5			
CV	163.3	164.6	172.9	174.3	189.7			

Table 3. Effect of combined decontamination techniques on total bacterial count of spices (TBC/g)

	Storage (months)						Mean	SD	CV
	0	1	2	3	4	5			
C	3.0×10^5	8.2×10^5	9.2×10^5	1.0×10^6	2.6×10^6	6.6×10^6	2.0×10^6	2.1×10^6	105.8
R	6.9×10^2	7.2×10^2	1.6×10^3	5.0×10^3	7.4×10^3	1.4×10^4	4.9×10^3	4.7×10^3	98.3
H	7.0×10^4	9.6×10^4	2.5×10^5	2.3×10^5	6.8×10^5	9.0×10^5	3.5×10^5	3.0×10^5	83.5
S	1.0×10^5	3.6×10^5	4.0×10^5	6.2×10^5	1.5×10^5	3.2×10^6	1.0×10^6	1.0×10^6	103.4
R + S + H	2.0×10^2	7.2×10^2	9.0×10^2	2.5×10^3	5.6×10^4	8.5×10^4	3.0×10^3	3.0×10^3	98.3
R + S	4.0×10^2	5.7×10^2	1.0×10^3	3.2×10^3	6.3×10^3	5.4×10^3	3.6×10^3	3.6×10^3	18.5
R + H	4.5×10^2	5.8×10^2	1.6×10^3	3.5×10^3	3.0×10^4	4.2×10^4	2.9×10^3	2.4×10^4	83.9
Mean	6.7×10^2	1.8×10^4	2.2×10^4	3.1×10^4	7.2×10^4	1.6×10^5			
SD	1.0×10^5	2.8×10^5	3.2×10^4	3.5×10^5	9.1×10^5	2.2×10^6			
CV	151.6	157.2	140.0	111.0	125.4	138.1			

C = Control; R = Radiation; H = Heating; S = Solar heating.

Table 4. Effect of combined decontamination techniques on total count of spices (TBC/g)

	Storage (months)						Mean	SD	CV
	0	1	2	3	4	5			
C	2.5×10^3	4.2×10^3	9.5×10^3	2.0×10^4	7.2×10^5	1.6×10^5	4.4×10^4	5.6×10^4	127.0
R	0	0	0	20	50	1.0×10^2	2.8×10^2	36.0	129.5
H	2.8×10^2	2.0×10^2	4.6×10^2	1.6×10^3	1.6×10^4	8.3×10^4	1.6×10^4	3.0×10^4	189.4
S	9.0×10^2	2.0×10^2	6.0×10^3	9.5×10^3	3.4×10^4	9.5×10^4	2.4×10^4	3.3×10^4	135.5
R + S + H	0	0	0	0	25	70	1.5×10^1	25.8	163.5
R + S	0	0	0	30	32	75	2.2×10^1	27.0	120.2
R + H	0	0	0	27	90	2.4×10^2			
Mean	5.4×10^2	9.8×10^2	2.4×10^3	4.8×10^3	1.8×10^4	5.4×10^4			
SD	8.5×10^2	1.5×10^3	3.5×10^3	6.9×10^3	2.5×10^4	5.8×10^4			
CV	164.0	161.2	157.3	159.6	152.2	123.9			

C = Control; R = Radiation; H = Heating; S = Solar heating.

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MICROBIAL LOAD ON CONTACT SURFACES, PERSONNEL AND ENVIRONMENT IN HOSPITAL KITCHEN

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ABSTRACT

Microbiological assessment of the work surfaces, food handlers and general environment of the kitchen of Khyber Teaching Hospital, Peshawar indicated a tremendous load of microorganisms on the contact surfaces. The mean standard plate count (SPC) on vegetable preparation center, ice service area, meat cutting board and wash unit varied from 6857 to 62347. The range of weekly coefficient of variation (CV) of microbes on these areas was 23 to 30. The mean SPC of floor was 1233 and the airborne microbes were 305/12 sq. cm. The mean colonies per 12 sq. cm of milk kettle, serving spoon, meat/vegetable curry serving bucket and serving trolley ranged from 102 to 268. The mean SPC/12 sq. cm of aprons, dusters and shirts of the food handlers and the cloth that covered a pile of tandoori 'roti' varied from 150 to 11920. The mean SPC on the hands of the meat cutter was 18023, bread piler was 8300 and cook was 3623 which were much more than in their hair. The mean SPC on the hair of these personal ranged from 109 to 405. The mean SPC on the hands of the waiters who carried food to the patients in different wards was 14033 in Psychiatry, 1633 in Gynecology, 450 in Surgical, 443 in Medical, 76 in Ophthalmology and 60 in ENT. Whereas the colonies on the hair of workers in these wards were respectively, 182, 279, 330, 220, 379, and 390. All the surfaces likely to be in contact with foods were found to be contaminated with microorganisms above the accepted standards.

Key words: environment; microorganisms; plate count; nosocomial infection; Pakistan.

INTRODUCTION

Sanitation of various areas in a hospital kitchen and the personnel involved in handling food determines the wholesomeness and safety of foods served to the patients. Bacteria grow very rapidly in right environment which is easily available in kitchen uniforms, aprons, dusters and food covers. The hands of the food handlers are also important vehicles in transmitting microorganisms to the food. Diseases transmitted through food frequently originate from an infected food handler (Awan, 1985). Microbiological studies conducted by Zia *et al.* (1977; 1979) on environment in various wards, operation theaters and personnel on duty in a hospital in Peshawar noted that the hospital environment was grossly contaminated with a large number of pathogenic microorganisms and more polluted than the outside air. The microorganisms isolated from various locations were found to be the causative agents of hospital cross-infection and post-operative infection. Since the sanitation of kitchen of the hospital has not been studied in Pakistan, therefore, it became imperative to determine the microbiological statistics of the hospital kitchen and the personnel engaged in food preparation and service.

MATERIALS AND METHODS

Microbiological studies of the work surfaces, personnel engaged in food preparation and service, selected locations of the floor and general environment in the kitchen of Khyber Teaching Hospital, Peshawar were conducted during the months of July to August, 1989. The following quality tests were performed to collect and analyze the samples.

Microbiological tests

1. Open plate method (OPM) was used to determine the total microbial count in the general environment of the hospital kitchen. Three culture plates of 5% Nutrient Agar were routinely exposed at 3 different areas of the kitchen for 30 minutes once a week for a total period of 3 weeks. The plates were incubated at 37°C for 24 hours. The developed colonies were counted on colony counter and the averages were computed.
2. Swab method was used to assess the microbial load (standard plate count) on the contact surfaces and personnel. For this purpose, a 12 sq. cm hole was drilled in a small piece of wood which was sterilized

and placed on the desired surface. Pre-sterilized swabs were used to quash the selected locations through the hole. Subsequently, the swabs were subjected to touch the Nutrient Agar medium in the pre-sterilized petri-dishes which were covered immediately and were taken to the laboratory for analysis as stated for general environment (Blair *et al.*, 1970).

The results were discussed in the light of the accepted colonies not exceeding 20 in the environmental air per cubic foot of a quiet room such as minor operation theater etc. (Topley, 1975). The colonies not exceeding 5000 per sq. ft. for satisfactory cleansing of milk plant (Baumgartner and Hersom, 1956) as suggested by the Ministry of Agriculture and Fisheries (Frem No. 195/TPY) was taken into account for comparison. For convenience of space in collection of samples, the suggested standard was modified to 65 colonies per 12 sq. cm in place of 5000 colonies per sq. ft., which would indicate satisfactory cleansing of the work surfaces and personnel.

In order to estimate the weekly variation in the bacterial population etc, the coefficient of variation (CV) was also measured. Determination of CV (%) is especially appropriate under conditions where there are extreme values or when it is desired to express variation as a percentage of the average around which deviations occur.

RESULTS AND DISCUSSION

The results of standard plate count/12 sq. cm of the contact surfaces and general environment of the hospital kitchen are presented in Table 1. The data indicate highest microbial count (62347 ± 14343) on the washing of raw vegetables and meat etc had been accumulated. It was reported that the area had never been scrubbed off nor it had ever been washed with an effective detergent or treated with a disinfectant. According to Jernigan (1977) dirty damp areas such as space around dish washing, pot and pan wash units, dirty cutting boards, meat blocks, food slicers and garbage cans provide a favourable environment for the bacteria to grow and multiply. De Wit *et al* (1979) studied the cross-contamination in kitchens during the thawing and preparation of frozen broilers which were artificially contaminated with *Escherichia coli* K_{12}. Cross-contamination occurred in mist kitchens. In some kitchens *Escherichia* K_{12} was isolated from the meat cutting boards, sink or dish cloth after rinsing and washing up.

The next highest count of 151461 ± 14250 per 12 sq. cm was detected from the samples collected from the meat cutting board and floor where meat was placed. Neither the floor nor the meat cutting board appeared thoroughly washed. Even the person who was engaged in cutting meat did not look clean in his dress or general appearance. Ahmad *et al.* (1981) reported 1715 isolates of various species of pathogenic bacteria in meat of different parts of animals obtained from butcher shops at Faisalabad. They pointed out that the quality of animals slaughtered needed improvement along with hygienic measures in and around slaughter houses and butcher shops.

The area where ice was placed on bare floor was also found highly contaminated with 19403 ± 2733 SPC per 12 sq. cm, in spite of the fact that the freezing temperature inhibits the growth and multiplication of most bacteria which thrive best between 10°C-48°C (Jernigan, 1977). The causative factors for such a high bioburden might be the adjacent uncovered manhole as well as the environmental pollution.

The vegetable cutting surface (a jute mat) on which the workers sat along with vegetables showed a comparatively lower microbial count of 6857 ± 21 per 12 sq. cm than the other work surfaces (Table 1). The reason for the lower SPC could be that the vegetables are not as attractive a substrate for microbial growth and multiplication as are the protein rich foods or damp dirty places.

Table 1. Standard plate count on the work surfaces and general environment in the kitchen of Khyber Teaching Hospital, Peshawar.

Surfaces	Mean* ± S.D	CV
Wash unit	62347 ± 14343	23
Meat cutting	51461 ± 14250	28
Ice-service	19403 ± 2733	14
Vegetable preparation	6857 ± 207	30
Floor**	1233 ± 406	40
General environment**	305 ± 78	26

*Mean/12 sq. cm and CV (%) of 3 samples collected on 3 different days.

**Means of 3 different areas in the kitchen on 3 different days.

The kitchen floor although looked clean did show a good number of bacterial population of 1233 ± 496 but comparatively with less intensity than all the work surfaces discussed above (Table 1). Procedures, especially the use of dirty mop may cause the bacterial count on the surface to be higher after cleaning than before. Studies by Gunther (1981) have shown that the most effective cleaning results from a combination of mop or brush scrubbing action and wet vacuum pick up. Such a method has never been applied in the cleaning of the kitchen floor under study, as reported by the kitchen supervisor.

The data on microbiological analysis of the general environment of the hospital kitchen (Table 1) revealed a recognizable content of microbes (SPC) such as 305 ± 78 with a weekly CV of 26 in the kitchen air. The open man-

hole, the uncovered overflowing garbage cans, the improperly washed floor, the poorly functioning exhaust system and cross-ventilation non-existing are some of the probable causes of air contamination in the kitchen. Moreover, the door to the washroom was also left open in the kitchen. Some of the toilets were over flooded because of the blockage or poor sewerage. There was no soap on the washroom to wash hands after toileting. The microbial population of foods vary tremendously in numbers from one plant to another. The microbial population in a plant is related to air quality outside the plant and population levels are related to the level of activity of the workers inside (Frazier and Westhoff, 1978). The importance of air hygiene in relation to the control of potential sources of airborne microorganisms and provision of clean environment has also been highlighted in a conference on Environmental Microbiology and Safety (Lach, 1987).

The utensils such as buckets, spoons, milk kettle and trolley used to serve foods to the patients in the hospital kitchen were also subjected to microbiological examination (Table 2). Among the utensils, the colonies per 12 sq. cm of the serving trolley (268 ± 62) and of the serving bucket (131.3+26.8) were higher than those on the serving spoon ((109.7+7.4) and milk kettle (102.3+5.8). The result showed that none of the utensils tested was free of microbes. Beyer and Sinell (1986) also observed in a hospital kitchen that food transport equipment was insufficiently cleaned, unhygienically stored and was contaminated with a range of microorganisms. Zschaler (1981) has also referred to contamination risk of specific equipment used in food industries.

Table 2. Standard plate count on the utensils and linen used by food handlers in the hospital kitchen.

Surfaces	Mean ±	S.D	CV
Serving trolley	268 ±	61.7	22.9
Meat/vegetable curry serving bucket	131 ±	26.8	20.4
Serving spoon	110 ±	7.4	6.7
Milk kettle	102 ±	5.8	5.7
Aprons	150 ±	92.1	61.3
Dusters	1454 ±	429.7	29.5
Shirts	3881 ±	750.4	19.3
'Roti'	11920 ±	1929.4	16.5

*Mean/12 sq. cm and CV (%) of 3 samples collected on 3 different days.

Table 2 further shows the SPC intensity per 12 sq. cm of the linen such as bread cover, aprons, dusters and shirts of the food handlers in the hospital kitchen. The cloth with which pile of 'tandoori roties' was covered, had the highest bacterial count (11920 ± 1969) among the linens. The mean value for SPC of 150 ± 92 on the aprons was the lowest, whereas these colonies 11454 ± 430 and 3880 ± 750 were intermediate on the dusters and shirts, respectively. This wide variation (150-11920) in the examined microbial contamination of the used linen could be attributed to the thick and heavy fabric used for the bread cover which could not be laundered easily except by the washerman. Since that was the only cover they had, they could not spare it for frequent washing and hence the bread cover was not washed for months and consequently got highly contaminated. Whereas the dusters and aprons were made of light weight fabrics and were small in size, therefore, were laundered more frequently. In spite of this convenience even the dusters and aprons were highly polluted with microorganisms as compared with permissible colonies of 65 per 12 sq. cm. It is interesting to note that the aprons were not worn to protect clothings of the workers but were used as pot holders. This function of the aprons might have contributed to their lowest SPC as they were near the fire most of the time.

The importance of clean clothing for food handler has been emphasized in a study, pointing out the tendency of British work forces to wear clothing for up to a week, while their counterparts in the USA usually change every day (Anon., 1987).

Table 3 presents the microbial load per 12 sq. cm of the hands and hair of the food handlers in the hospital kitchen. The heaviest intensity of SPC was observed on the hands (18024 ± 3706) and hair (405 ± 136) of the meat cutter. This was followed by the SPC on the hands (8300 ± 2426) and hair (391 ± 99) of the bread piler. Heinzel (1984) has emphasized on the importance of personal hygiene in meat processing in view of the high incidence of human origin of food poisoning and the biology of the pathogens mainly spread by the food handlers. The next highest load of SPC was detected from waiter who served food to the patients in the ENT ward. The bioburden on the hands of the personnel (waiters) who served food to the patients in the gynecology (1634 ± 870) and Psychiatric (4034 ± 1706) wards was intermediate. The microbial population on the hair (279 ± 63) of the former was intermediate, while that of the latter (182 ± 111) was lowest. It is amazing to note that the bacterial growth on the hands of workers who served food to the patients in the wards of Ophthalmology (77 ± 7) and ENT (60 ± 29) was the lowest but on their hair it was the highest (379 ± 132 and 390 ± 281 respectively). Anyhow, the possibility of the transfer of bacteria from the hair of the host to his hands and subsequently to the foods, is always there.

Table 3. Standard plate count on the hands and hair of personnel working in the Khyber Teaching Hospital kitchen, Peshawar.

Personnel	Hands		Hair	
	Mean* ± SD	CV	Mean* ± SD	CV
Meat Cutter	18024 ± 3705	20.6	405± 135	20.6
Bread Piler	8300 ± 2426	29.2	391± 99	25.2
Cook	3623 ± 1581	43.6	109± 33	29.7
Waiters for food services to patients in various wards:				
Psychiatry	14033 ± 1705	12.3	182± 110	60.6
Gynecology	1633 ± 869	53.2	279± 63	22.6
Surgical	450 ± 102	22.7	330± 44	13.4
Medical	443 ± 216	48.7	222± 49	22.3
Ophthalmology	76 ± 36	61.6	378± 131	34.8
ENT	60 ± 28.9	48.2	390± 281	71.2

*Means per 12 sq. cm of hands and hair of the personnel and CV (%) of 3 samples collected on 3 different days (once a week)

The microbiological assessment of the hands and hair of the personnel engaged on food processing and service in the hospital kitchen on the whole have shown that the microbial load was much higher than the permissible standard of 65 per sq. cm which might be quite hazardous for the patients. Frazier and Westhoff (1978) noted that personnel in food processing plants can contaminate foods during handling and processing and suggested that human beings shed from 10^3 to 10^4 viable organisms per minute. The numbers and types of organisms shed is closely related to the subject's working environment. Faecal coliforms and Enterococci are obtained from the hands of the workers in the catering service. In the present study, 100% of the personnel and contact surfaces examined were positive with microorganisms. Since in the present study the types of microorganisms were not identified, therefore, it is strongly recommended for further investigation. The unhygienic conditions of kitchen of Khyber Teaching Hospital of Peshawar is a matter of great concern.

CONCLUSIONS

The following conclusions were drawn from the microbiological examination of the contact objects and general environment in the kitchen of the Khyber Teaching Hospital:

1. Every object was contaminated with microorganisms with varied degree of intensity.
2. Pronounced variation among the mean bacterial population of different work surfaces, worker's hands, hair, utensils and linen used by the food handlers reflect the varied hygiene measures adopted by the relevant personnel on duty. All were predominantly below the required standards.
3. The broad weekly variation (C.V.) in the assessed mean colonies of all the contact areas and personnel revealed that there was no permanent adoption of good sanitary measures by the food handlers in the hospital kitchen.

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RELATIONSHIP BETWEEN REDUCTION OF LACTOSE AND LYSINE DURING HEAT TREATMENT OF MILK

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ABSTRACT

Rate of degradation of lactose and lysine was dependent upon temperature and period of heating. These changes were significant at higher temperatures (120° to 150°C) even after a short period of heating (2 to 20 minutes). These changes were also significant at lower temperatures (90° to 110°C) but after a longer time (30 to 120 minutes) of heating.

Key words: lactose; lysine; degradation; thermal treatment; microorganisms.

INTRODUCTION

Milk is a perishable commodity and microorganisms spoil it in a short period of time. Hence it is always heated to check the incidence of microbial spoilage so that shelf life of milk is increased. Many physico-chemical changes occur during this heating process (Adachi and Patton, 1961; Geier and Kloslermeyer, 1983; Andrews, 1985). Among these, degradation of lactose and lysine were found to be the most important (Adachi, 1959; Burton, 1984). However, information in the literature on the extent of such chemical changes during heat treatment of milk are inadequate. Therefore, the aim of the present study was to investigate the effect of heating milk on lactose and available lysine.

MATERIALS AND METHODS

Skim milk was heated in sealed stainless tubes (3.5 mL capacity) in a thermostatically controlled oil bath at a temperature range of 90° to 150°C with holding times ranging from 2 to 120 minutes. Temperature of the oil bath was maintained prior to start of heating the milk. The tubes were kept continuously rotating during the heat treatment. After heating for a specified period, tubes were placed in an ice bath immediately to stop the reaction.

Estimation of lactose

Lactose was estimated by high performance liquid chromatography (HPLC) technique. 0.75 per cent raffinose solution was used as internal standard. Bigg's reagent containing 12.5 per cent zinc acetate, 6.25 per cent phosphotungstic acid and 10% v/v glacial acetic acid was used as a precipitating agent. 3g skim milk was diluted to 35mL with water. Diluted skim milk was combined with internal standard and the Bigg's reagent in a 7:2:1 volumetric ratio. The resulting precipitate was removed by centrifugation and the clear supernatant (20μL) was injected onto the HPLC column (Amine HP x 87 PA column). The effluent used was millipore water with flow rate of 0.6 mL/minute. The concentration of lactose in milk samples was estimated by applying the following relation:

$$\text{Unknown mg of lactose} = \frac{C \times PA \times X}{Pa' \times W}$$

where
C = concentration of standard in mg/L
Pa = peak area of unknown
Pa' = peak area of standard
X = dilution factor
W = weight of sample in g.

Estimation of available lysine

Udy-dye method was used for the estimation of available lys ine in heated milk (Udy, 1971). Principle of the method is a quantitative binding of the azo dye acid orange-12 by the basic amino groups of proteins. Lysine concentrations were determined by a different method in which calorimetric measurements at 482 nm were done before and after blocking the lysine with propionic anhydride. Measurements were made in duplicate and averages were computed.

RESULTS AND DISCUSSION

Effect of heating on lactose

Low temperature long time

Three heating temperatures (90°, 100°, 110°C) were applied for heating skim milk to a heating time of

120 min. (Table 1). It is evident from the results that lactose content were reduced more rapidly at 110°C as compared with 90° as well as 100°C. Lactose was reduced to 14.4 per cent after 60 minutes heating at 110°C, while it took 75 minutes and 120 minutes at 100° and 90°C respectively to reach this level.

Effect of heating on available lysine

Low temperature long time

Under these set of conditions the decrease in available lysine ranged from 2.00 to 16.43 per cent (Table 1). After 120 minutes heating at 90°C available lysine was reduced by 10.24 per cent, while this much reduction was observed after 105 and 75 minutes at 100° and 110°C, respectively.

Table 1. Effect of heating time and temperature on important nutrients of skim milk

Heating time (minutes)	Decrease in lactose (%)			Decrease in lysine (%)		
	90*	100*	110*	90*	100*	110*
30	4.57	9.94	11.23	2.00	3.05	4.05
45	5.61	10.81	12.06	4.05	4.05	6.14
60	6.23	11.64	14.14	6.14	7.14	8.19
75	6.65	13.31	15.59	7.14	8.19	10.24
90	8.52	16.21	16.63	8.19	9.24	12.33
105	11.01	16.63	17.46	9.24	11.29	14.38
120	14.13	16.84	19.33	10.24	12.33	16.43

*Heating temperature in °C.

High temperature short time

Results of this study are reported in Table 2. Heating temperatures of 120° and 130°C did not show much effect on lactose degradation even up to 20 minutes. However, when heating temperature was raised to 140°C for only 2 minutes, lactose reduction was as high as 13.72 per cent which is almost equivalent to reduction values obtained when the milk was heated at 90°C for as long as 120 minutes (Table 1). The maximum damage (26.82% degradation) was done to lactose when the milk was heated for 10 minutes at 150°C (Table 2).

High temperature short time

Available lysine was reduced by 9.24 per cent when the milk was heated at 120°C for 20 minutes, while this much reduction occurred after 12 and 10 minutes of heating at 130° and 140°C, respectively. However, when the milk was heated at 150°C, it took 9 minutes to reach this level of reduction (Table 2).

Table 2. Effect of heating time and temperature on important nutrients of skim milk

Heating time (minutes)	Decrease in lactose (%)				Decrease in lysine (%)			
	120*	130*	140*	150*	120*	130*	140*	150*
2	0.83	2.08	13.72	12.68	2.00	2.00	3.05	4.05
4	2.29	3.33	16.84	14.35	3.05	4.05	5.10	6.14
6	-	-	-	19.33	-	-	-	8.91
8	3.95	4.37	18.92	25.16	4.05	6.14	7.14	9.23
10	-	-	-	26.82	-	-	-	11.29
12	4.78	5.20	17.88	-	6.14	8.19	10.24	-
16	5.41	7.29	20.37	-	7.14	10.24	12.38	-
20	6.65	9.36	28.90	-	9.24	12.33	15.95	-

*Heating temperature (°C).

It is evident from these results (Tables 1 and 2) that reduction in lactose was more than in available lysine when milk was heated at temperatures ranging from 90° to 110°C. However, at higher temperatures (120° to 150°C), reduction in lactose was comparatively lesser than the available lysine. It seems that degradation of lactose depends upon time of heating instead of its severity, while lysine degradation was found to be more dependent upon heating temperatures.

Although, it was expected that heat treatment of milk would degrade lactose and limit the availability of lysine (Martinez and Olano, 1980; Burton, 1984), yet from these experiments it has been ascertained how length of heat treatment and heating temperatures affect the magnitude of these changes in skim milk. However, heating effect on these nutrients may be different in case of full-fat milk which is to be investigated in separate set of experiments.

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EFFECT OF STORAGE CONDITIONS ON THE PHYSICO-CHEMICAL CHARACTERISTICS OF MILK CONCENTRATE (KHOA)

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ABSTRACT

Physico-chemical changes in milk concentrate (khoa) during storage were studied. Total acidity and rancidity of the samples increased progressively with increase in storage temperature and time period. However, there was a gradual decrease in moisture content, while the content remained unchanged during storage of khoa samples. Different market khoa samples were also analysed to study the physico-chemical changes. Acidity and rancidity values of the market samples were comparatively higher than those of the fresh and pure khoa samples. Moisture and fat contents of most market khoa samples showed significant variations which indicated that these were kept under adverse conditions.

Key words: khoa; storage; physico-chemical changes; microbiology; fat contents; moisture.

INTRODUCTION

Milk concentrate (khoa) is a popular indigenous milk product. It is prepared by heat desiccation of whole milk in an open pan to a semi-solid consistency (68-70% total solids). It is usually used for direct consumption or as a base material for several products like "burfi", "kalla-kand", "gulabjaman", etc. The food value of khoa is very high as it contains fairly large quantities of protein, fat, lactose and bone forming materials. It is a common practice that khoa is usually made in open pans without maintaining proper sanitary conditions. It has been observed by many workers that the quality of khoa depends on the initial composition of milk and the method of manufacturing (Byron *et al.*, 1987; Adhikari, 1993). Many reports are also available in the literature regarding quality of khoa during storage (Kumar *et al.*, 1975; Scott and Bishop, 1984) but no such information have been collected in Pakistan. Therefore, the present research was undertaken to study the physico-chemical changes and microbial status of commercially produced khoa and effect of storage conditions on its quality.

MATERIALS AND METHODS

Khoa samples were collected from different milk-shops and analysed for various physico-chemical characteristics. Moisture, ash and fat were estimated by AOAC. methods (AOAC, 1970), while estimation of lactose was carried out by using picric acid as described by Perry and Doan (1950). Protein was determined by micro-Kjeldhal procedure (Markham, 1942), whereas total solids and solids not fat (SNF) content were calculated as elaborated by Rai (1980). Acidity was estimated by titration against 0.1 N NaOH solution (Howard and Leonard, 1982) whereas rancidity was determined by titration against alcoholic 0.1 N KOH solution using phenolphthalein as an indicator in both cases (Morris, 1951). Estimation of iron was carried out by using atomic absorption spectrophotometer. The physical quality of khoa was judged by the general methods as described by Nelson and Malcom (1980).

Microbial analysis especially the total viable count and coliform count were carried out by the procedure described by Seeley and Demark (1981). All data were subjected to analysis of variance and standard deviation was calculated by the method of Steel and Torrie (1980).

RESULTS AND DISCUSSION

Chemical composition of fresh and pure khoa prepared from cow and buffalo milk is given in Table 1. along with the chemical composition of the respective milks. It is apparent from these results that the colour of khoa prepared from cow and buffalo milk was pale yellow and white respectively, while the texture was slightly hard and coarse for cow milk. However, the texture of khoa prepared from buffalo milk was soft and smooth. The concentration of the nutrients in was found to be almost four folds than pure milk samples. It is due to significant reduction of water from milk during khoa making process.

Table 1. Chemical composition of milk and khoa

Constituents	Cow		Buffalo	
	Milk	Khoa	Milk	Khoa
Moisture (%)	87.0	30.9	82.0	22.3
Total solids (%)	13.0	69.1	18.0	77.6
Fat (%)	4.0	22.0	7.9	32.1
Protein (%)	4.0	19.1	3.6	17.7
Solids-not-fat (%)	9.0	47.0	10.1	45.5
Ash (%)	0.7	3.7	0.8	3.7
Lactose (%)	5.0	24.2	5.2	23.7
Titrable acidity (%lactic acid)	-	0.3	-	0.3
Rancidity (%)	-	0.027	-	0.029
Iron (ppm)	-	103	-	101
Colour	-	Pale yellow	-	White
Texture	-	Slightly hard and coarse	-	Soft and smooth

Physical analysis

Khoa is commonly used for preparing various types of sweets along with its consumption as such. Though it is a widely used food item, yet unfortunately proper conditions are not maintained during its preparation and storage, which adversely affect its quality. Therefore, a survey was conducted to investigate into the changes in physico-chemical characteristics of khoa during storage at commercial level. Physical changes of market khoa are shown in Table 3. The colour varied from greenish white to slightly brown. The brown colour of the samples can be attributed to charring of carbohydrates due to vigorous heating temperature and low scraping speed during the last stages of khoa preparation. Texture varied greatly among the various samples. There were a few samples which were hard coarse, while others were soft and smooth. The hard coarse texture might be due to the presence of some adulterant in the khoa. The smell/flavour of the samples was nutty, sour and rancid. This might be due to high acidity of milk used. It is also possible that smell in khoa samples was developed due to oxidation of milk lipids during storage. There were a few samples which were sweet in taste, while others were saltish. It

Table 2. Effect of temperature and time period on the quality khoa

Constituents (%)	20°C				30°C				40°C			
	Days				Days				Days			
	0	2	4	6	0	2	4	6	0	2	4	6
Moisture	30.56 ± 1.1	28.65 ± 1.3	27.61 ± 0.9	26.66 ± 1.6	30.56 ± 1.2	28.70 ± 0.7	25.46 ± 1.0	22.77 ± 0.8	30.56 ± 0.4	25.55 ± 1.3	23.39 ± 0.6	21.73 ± 1.2
Fat	21.88 ± 1.2	21.88 ± 1.7	21.88 ± 1.3	21.88 ± 1.2	21.88 ± 1.4	21.88 ± 0.8	21.88 ± 1.1	21.88 ± 1.0	21.88 ± 1.0	21.88 ± 1.2	21.88 ± 0.9	21.88 ± 1.2
Acidity (% lactic acid)	0.29 ± 0.8	0.53 ± 0.6	0.66 ± 1.2	0.79 ± 1.1	-	0.66 ± 0.9	0.81 ± 1.3	0.90 ± 0.9	-	0.73 ± 1.3	0.98 ± 1.0	1.15 ± 1.0
Rancidity (%)	0.027 ± 0.7	0.041 ± 0.3	0.059 ± 0.7	0.060 ± 1.2	-	0.055 ± 0.4	0.067 ± 0.9	0.088 ± 0.5	-	0.064 ± 0.5	0.087 ± 1.1	0.090 ± 1.0

*Average of three replicates along with standard deviation.

The effect of storage temperature and time on the quality of khoa was also studied (Table 2). Total acidity and rancidity increased progressively with increase in storage temperature and time. It was found that acidity and rancidity increased from 0.29 to 0.79 per cent and 0.027 to 0.06 per cent respectively when the samples were kept at 20^0c for 6 days. Maximum total acidity in khoa samples was 1.15 per cent, whereas rancidity was found to be 0.099 per cent at 40^0c after 6 days. Increase in acidity is associated with an increase in the concentration of fatty acids produced due to hydrolysis of milk fat during storage at different temperatures.

may be concluded that the sensory qualities of khoa depend upon the type of milk used and time and storage temperature. Type of adulterant may also alter the sensory properties of the khoa.

Chemical analysis

Table 4 shows the chemical composition of khoa collected from different milk-shops. The moisture content ranged from 28.7 to 43.7 per cent. The variation might be due to type of milk used (cow or buffalo) and degree of concentration. The fat content of market samples ranged from 15.7 to 31.3 per cent, while of laboratory samples

prepared from pure cow or buffalo milk was 22.0 and 32.1 per cent respectively. Therefore, it may be concluded that the samples having high fat content may have greater portion of buffalo milk and those with somewhat low fat content have more proportion of cow milk.

per cent, while the acidity of market samples ranged from 0.65 to 1.50 per cent. Similarly the rancidity values for fresh khoa made from buffalo and cow milk were 0.08 and 0.04 per cent respectively whereas the values for rancidity of the market khoa ranged from 0.075 to 0.175 per cent.

Table 3. Sensory characteristics of market khoa

Khoa*	Colour	Texture	Flavour	Taste
KS_1	Slightly brown	Hard & Coarse	Nutty	Sweet
KS_2	Slightly brown	Hard & Coarse	Nutty	Saltish
KS_3	Slightly brown	Hard & Coarse	Sour	Slightly sweetish
KS_4	Greenish white	Soft & Smooth	Sour	Saltish
KS_5	Greenish white	Soft & Smooth	Rancid	Slightly sweetish
KS_6	Greenish white	Soft & Smooth	Rancid	Saltish
KS_7	Greenish white	Soft & Smooth	Rancid	Saltish
KS_8	Slightly brown	Soft & Smooth	Rancid	Saltish
KS_9	Greenish white	Soft & Smooth	Rancid	Sweetish
KS_{10}	Greenish white	Soft & Smooth	Sour	Salted

*Samples obtained from different localities.

In about 50 per cent samples, protein value was found to be less than the standard value. The value of protein in market samples ranged from 14.2 to 20.3 per cent while the protein value for khoa prepared from cow and buffalo milk was 19.1 and 17.7 per cent respectively. The low values of protein indicate that khoa was prepared from milk in which water was added for adulteration purpose.

The values of total solids and solids not fat in market khoa ranged from 56.3 to 72.9 per cent and 36.9 to 54.0 per cent respectively. However the ash contents varied from 3.2 to 4.5 per cent. The figures for total solids, solids not fat and ash contents of some of these samples are very high which indicate the chances of mixing pond or canal water as adulterant in the milk.

The lactose content of the samples ranged from 14.5 to 24.5 per cent (Table 4) while the lactose content for fresh khoa made from buffalo milk was 23.7 per cent and from cow milk was 24.2 per cent (Table 1). The low values of lactose contents lin market khoa indicate the presence of adulterant like starch. The iron contents of different samples indicated wide variation (from 44 to 96 ppm), whereas the pure milk contains iron only 2-4 ppm. The high iron contents in samples seem to be due to vigorous scraping of stirrer made of iron during the last stages of manufacturing. The variation in the iron contents may be attributed to conditions of pan and speed of stirring.

The values of acidity for fresh khoa made from buffalo milk was 0.3 per cent and from cow milk was 0.5

High acidity values indicate the presence of high concentration of fatty acids produced due to deterioration of milk fat. Almost 60 per cent market khoa was found to be extremely rancid which can be attributed to lipid oxidation during storage for a longer period at elevated temperatures. It is evident from the values of acidity and rancidity that the quality of "Khao" was significantly affected during storage at higher temperatures. On the basis of this data, it may be concluded that proper conditions were not maintained before and after manufacturing of khoa which ultimately adversely affected the quality of khoa.

Microbial analysis

The total viable count was highly variable. The variability in the total count may be attributed to the varying conditions under which the product was produced and marketed. The total viable count of the samples ranged between 2×10^3 to 5×10^4/g. The coliform count was undetectable because the coliform bacteria present in the raw milk were destroyed during boiling used in khoa making. Thus the presence of viable count indicates post preparation contamination due to handling and storage of the finished product.

Table 4. Chemical composition of market khoa

Khoa samples	Moisture	Total solids	Fat	Protein	Solids-not-fat	Ash	Lactose	Acidity (% lactic acid)	Rancidity	Iron (ppm)
KS_1	29.8 ± 1.2	70.2 ± 0.9	28.8 ± 2.6	17.5 ± 2.0	41.4 ± 2.1	4.5 ± 2.6	19.40 ± 0.8	0.65 ± 1.7	0.08 ± 0.6	87 ± 1.2
KS_2	30.7 ± 1.5	69.3 ± 1.1	28.8 ± 2.1	18.3 ± 2.1	40.5 ± 1.8	3.2 ± 1.9	19.57 ± 0.9	1.30 ± 8.9	0.07 ± 0.4	95 ± 1.1
KS_3	27.1 2.2	72.9 1.3	30.5 2.6	15.7 3.1	42.4 0.9	3.4 1.1	23.52 1.2	1.40 1.2	0.13 0.8	96 ± 1.3
KS_4	36.2 ± 2.1	63.8 ± 3.3	18.3 ± 1.6	17.3 ± 2.1	45.5 ± 1.1	4.3 ± 2.1	24.55 ± 1.6	1.50 ± 1.3	0.09 ± 0.8	57 ± 0.9
KS_5	30.3 ± 1.9	69.7 ± 3.1	15.7 ± 1.7	18.3 ± 2.7	54.0 ± 1.6	4.3 ± 2.2	19.23 ± 1.2	0.70 ± 1.4	0.09 ± 0.9	63 ± 0.8
KS_6	29.2 ± 1.0	70.8 ± 2.6	22.3 ± 1.1	20.3 ± 1.6	49.5 ± 1.5	3.3 ± 2.6	21.23 ± 1.4	0.90 ± 2.2	0.14 ± 0.8	44 ± 1.3
KS_7	38.8 ± 3.1	61.2 ± 2.2	27.8 ± 2.0	15.5 ± 1.5	33.4 ± 2.1	4.4 ± 2.2	13.34 ± 1.6	0.80 ± 2.1	0.15 ± 0.9	93 ± 1.9
KS_8	28.7 ± 2.7	71.3 ± 1.9	28.9 ± 3.7	17.6 ± 2.8	42.4 ± 3.2	4.0 ± 2.6	18.50 ± 0.7	0.74 ± 0.7	0.18 ± 0.6	71 ± 1.1
KS_9	31.8 ± 3.0	68.2 ± 1.1	31.3 ± 1.6	15.3 ± 2.7	36.9 ± 3.3	4.2 ± 2.7	18.37 ± 0.7	0.72 ± 0.9	0.16 ± 0.5	72 ± 1.1
KS_{10}	43.7 ± 1.7	56.3 ± 2.0	18.7 ± 1.0	14.2 ± 2.8	37.6 ± 2.0	4.1 ± 2.1	14.55 ± 0.5	1.50 ± 1.3	0.10 ± 0.3	74 ± 0.9
SE	3.4	1.7	3.3	2.8	2.1	1.3	2.6	1.9	0.8	3.7

*Average of five samples from each milk shop in three replicates along with standard deviation.

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APPLIED RESEARCH NOTE

SURF FIELD MASTITIS TEST: AN INEXPENSIVE NEW TOOL FOR EVALUATION OF WHOLESOMENESS OF FRESH MILK

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INTRODUCTION

Mastitis (inflammation or swelling of udder) is recognized worldwide as the most important and costly disease of dairy animals. In addition to causing colossal economic losses to the farmers, the disease is important from consumers' and milk processors' point of view. This is because the milk from the affected animals may harbour the organisms potentially pathogenic for humans (zoonosis) and processing of such milk results in suboptimal output of substandard finished fermented products like yogurt, cheese, etc.

Mastitis and Milk Processing Industry

In countries where the dairy industry is very well developed (e.g. USA, Europe, Australia), dairy product manufacturers are increasingly becoming concerned about the impact of the quality of raw milk on the quality of finished product. Different types of quality premiums (e.g. one based on somatic cell contents in unpasteurized milk) have been introduced as incentives to farmers to improve milk quality.

The primary changes in milk quality resulting from mastitis are due to breakdown of milk protein and/or milk fat (Barbano, 1989). Milk from clinically or subclinically affected mastitic animal has very high increase in the activity of a very important proteolytic enzyme named plasmin (Saeman *et al.*, 1988). There are also proteolytic enzymes that originate from the somatic cells. Plasmin enzyme can cause extensive damage to the milk casein in the udder prior to milk collection from the animal. At refrigeration temperatures, the rate at which plasmin breaks down casein is very much slower. One of the most significant characteristic of plasmin is its heat stability: it takes approximately 1.4 minutes of holding time at 140°C to reduce its activity in whole milk by 90% (Verdi, 1988). Normal milk pasteurization holds milk at 72°C for 15 seconds. Thus, once there is an elevated level of plasmin in milk it can not be inactivated at the milk processing. Deterioration of milk protein as a result of mastitis will have its greatest influence on cheese yield and texture of some cultured products.

In a fashion similar to protein breakdown, milk fat breakdown that results from mastitis is also caused by enzymes called lipases. The predominant type of fat in milk is triglyceride. Lipases attack triglycerides and release free fatty acids. At very low concentrations, free fatty acids produce off-flavours in milk and dairy products that are characterized as rancid off-flavours. Mastitis may also cause changes in the structure of milk fat globule membrane resulting in increased susceptibility of milk fat to attack by native milk lipases.

Types of Mastitis and their Detection

Mastitis occurs both in clinical (overt) and subclinical (hidden) forms. The detection of clinical form of the disease, due to the manifestations of such signs as inflammed udder and visible changes in the milk (clots, discolouration etc.), is not a problem and the disease is quite obvious to the common dairymen. Subclinical mastitis is 3 to 40 times more common than the clinical mastitis and causes greatest overall losses in most dairy herds. The need to detect udder infection at the subclinical phase has long been recognized as imperative to the success of any mastitis control programme. Since there is no gross swelling of the gland or grossly observable abnormality of the milk, this type of mastitis is recognized usually by laboratory examination of milk. Isolation and identification of the mastitogens is important but the necessary laboratory facilities are not readily available in developing countries. As such reliance has to be placed on indirect field tests for the detection of mastitis at subclinical level.

Development of Surf Field Mastitis Test and its Evaluation

At present, an animal-side mastitis detection test viz., California mastitis test (CMT) is used worldwide to detect subclinical mastitis. The reagent for this test (sodium-alkyl-aryl-sulfonate) is not readily available in developing countries. Ideally an on-farm test for subclinical mastitis should have the following desirable attributes:

i. It should be compatible with the technical capabilities of farmers who happen to be mostly illiterate in developing countries;

ii. It should be cheap to conduct on a regular basis;

iii. The reagents and the other materials needed for the test should be readily available in the country;

iv. The test should be sensitive enough to detect all possible cases at the initial (subclinical) phase of infection;

v. The test should be user-friendly and easy to conduct.

With these criteria in mind, we tested the efficacy of a 3 per cent solution of readily available household detergent, viz., Surf* in tandem with the standard California mastitis test reagent** in detecting subclinical mastitis.

In order to determine the reliability of Surf field mastitis test (SFMT) vis-a-vis the standard California mastitis test for the detection of subclinical mastitis, a total of 225 quarter milk samples from buffaloes and cows were collected. Both tests were performed in tandem on each sample. The two tests were in total agreement on 205 (91%) samples. With respect to scoring of the inflammatory reactions, Surf field mastitis test (SFMT) and California mastitis test (CMT) were found discordant only on 20 milk samples. Discrepancy of the test scores occurred when both the tests were at the borderline of negative and trace i.e. when the inflammatory reaction in the udder was subtle. Disregarding the scoring in the interpretation of two tests and recording the test results either positive or negative only brought them in very close (98%) agreement (Muhammad *et al.*, 1994). In another series of experiments (Fazal-ur-Rehman, 1995), four hundred quarter milk samples from apparently mastitis free buffaloes (n = 50) and cows (n = 50) were examined to determine the sensitivity, specificity, accuracy, predictive values and per cent agreement (Kappa value) of SFMT vis-a-viz CMT, modified Whiteside test and direct microscopic somatic cell counts. Microbiological examination of milk was used as the gold standard. All the direct and indirect mastitis tests gave close figure of animal and quarterwise mastitis prevalence. The very high level of agreement between the SFMT and the standard CMT tempted us to recommend the use of the former test as a cheaper alternative of the later for the detection of subclinical mastitis in dairy animals in developing countries.

Test Principle

Infection in the udder of the milch animals causes irritation which results in higher than normal number of leukocytes (commonly referred to as somatic cells) in milk. The active principle in mastitic milk is deoxyribonucleic acid (DNA), released from the nuclei of ruptured somatic cells. The DNA, thus liberated, reacts with the detergent and leads to the formation of gel of varying degree depending upon the number of somatic cells in the milk (Schalm *et al.*, 1971).

Test Procedure & Interpretation

Under conditions prevalent in most developing countries, farmers and buyers of fresh milk for household consumption can prepare the test solution for performing Surf field mastitis test by dissolving 6 teaspoonfuls of household detergent, Surf (Lever Brothers Pakistan) in ½ litre of clean tap water. A plastic paddle with four receptacles for the respective quarters of an animal can be fabricated from locally available plastic or bakelite. When this paddle is not at hand, suitable container such as tea cups or glass may be substituted. The quarter fore-milk samples are taken and approximately equal quantity of 3 per cent Surf solution is added. The mixture is swirled for about one minute and then examined visually for the presence of small floccules and gel. The udder quarters affected with subclinical mastitis will show floccules or gel formation of varying degrees depending upon the severity of the disease. Such milk is unwholesome for human consumption in as much as it contains pathogenic bacteria and their toxins as well as very high number of white blood (pus) cells. Ideally, milk from all four quarters should be tested separately. The test solution is stable for 6 months at room temperature. The solution should be shaken well before use.

Using Surf Field Mastitis Test in Mastitis Control

In order to catch mastitis at an earliest possible (= subclinical) stage, farmers are advised to run the Surf field mastitis test fortnightly on all quarters of all milch animals. In the event of a positive reaction in one or more quarters of an animal, veterinarian should be consulted immediately for professional treatment. Under the setting of a dairy farm, the dichotomous (positive or negative) test results can be used to divide the lactating animals either as affected (SFMT-Positive) or unaffected (SFMT-Negative). Since mastitis in developing countries with no mastitis control programme in vogue, is mostly caused by contagious mastitis pathogens *(Staphylococcus aureus, Streptococcus agalactiae, Corynebacterium* spp.), the non-infected animals should be milked ahead of infected ones. In addition, it is also advised to run the test at the time of purchase of the dairy animals and should an animal test positive to Surf field mastitis test in one or more quarters, farmers should eschew from 'buying' mastitis. At present, the cost estimate of testing 5-10 animals fortnightly with the new test is Pakistani Rs. 100/= (= US $ 2.8) per year.

* Lever Brothers Pakistan.

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Surf Field Mastitis Test

Step-1: Take 5 teaspoonful of household detergent (e.g. Surf) and dissolve in about half litre of water to make 3% SFMT reagent.
Step-2: Arrange for a plastic paddle or clean bowls for the collection of milk from all four quarters.
Step-3: Collect fore-milk separately from all four quarters in the paddle or bowls.
Step-4: Add equal quantity of the 3% SFMT reagent to the collected milk and swirl to mix them.
Step-5: On indication of any sort of flakes or gel formation, seek advice from the veterinary doctor.
Step-6: Adopt appropriate measures as per advice.

Recommendations

Keeping in view the usefulnes and simplicity of Surf field mastitis test for the farming community, dairy industry as well as for common consumers of fresh milk, it is recommend that:

i. This test be made a part of the curriculum of home economics courses. This will help the housewomen to ensure a supply of safer, wholesome milk for the family members. Ensuring the supply of wholesome non-mastitic milk is all the more important for infants.

ii. Personnel involved in dairy industry should emphasize the use of this novice test before the purchase of fresh milk from the farmers. Avoiding the purchase of milk which is abnormal on the basis of this test results would pay rich dividends to milk processors.

iii. Personnel engaged in animal health extension sector should apprise the farmers about the utility of this test in blunting the losses accruing from the costly mastitis problem.

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EXTENSION ARTICLE

THERAPEUTIC VALUE OF YOGHURT

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INTRODUCTION

The nutritional merits of milk are indicated by the fact that it is indispensable for infants, invalids, young and old. It is especially good for those suffering from depression. Milk has a growth stimulating effect on children. The higher biological value of milk protein makes it of special significance in the treatment of ekwashiorkor: a malnutrition problem found among young children. Milk consumption is a keystone of nutrition in all developing countries. The chemical composition of a foodstuff provides a useful indication of its potential nutritive value. The milk from all mammals is nearly similar in composition but varies in concentration of various ingredients. The nutritional value of milk as a whole is greater than just the sum of its known components. Further, milk is the best food to control the body heat as it provides regulatory substances with moderate amount of calories. Since milk is the basic raw material of yoghurt so we conclude that man's diet should include a generous amount of milk and its products thereby minimizing the possibility of malnutrition.

Traditional and recent developments in yoghurt production

Over the last few decades the process of yoghurt manufacturing has become more rational primarily due to various discoveries and developments in such disciplines as microbiology, enzymology, chemistry, biochemistry, physics and engineering, etc. In order to understand the principles of yoghurt making it will be useful to describe separately the various stages as well as the influence of each ingredient used in the production of yoghurt. The essential requirements for manufacturing good quality yoghurt are:

a. good quality milk free from antibiotics, adulteration and contamination.
b. an active well balanced and contaminant free starter culture.
c. correct heat treatment.
d. a clean and well maintained plant.
e. an avoidance of rough handling of the coagulum particularly during cooling.
f. use of high quality ingredients and
g. correct storage of retail product, preferably below 5°C.

The appraisal of product quality has become a vital function of factory operation and the gamut of examination that may be performed under the following headings:

a. assessment of sensory characteristics,
b. evaluation of physical characteristics,
c. analysis of chemical composition and
d. microbiological analysis.

These quality parameters are judged by a range of tests of varying degrees of objectivity and yet all of them can be useful in ensuring that a product is:

a. safe for human consumption and conform to the regulations laid down by the public health authorities.
b. of a high organoleptic standard and
c. capable of achieving a specific shelf-life without spoilage (Tamime and Robinson, 1985).

On the basis of flavour, three main types of yoghurt are natural or plain, fruit and synthetic or flavoured yoghurt. However, different types produced on commercial scale are governed by the followings:

a. legal standards imposed by FAO/WHO and the legal authorities of a country such as full fat or low fat yoghurt etc.
b. method of production which gives rise to set and stirred yoghurt,
c. post-processing or modified yoghurt includes pasteurized yoghurt, frozen yoghurt, dietetic yoghurt, concentrated yoghurt and dried yoghurt having total solids up to 96%.

In 1960 in Switzerland a major development in yoghurt industry took place with the introduction of fruit and sweetened yoghurt. Since that time its popularity has spread to other parts of the world and its consumption has increased significantly. These days the overall nutritive value of yoghurt is well established but special types of yoghurt are often manufactured for dietetic and therapeutic purposes.

Yoghurt and health

Unfortunately, some people, especially children, are allergic to milk due to lack of lactase. Such persons can use yoghurt and other cultured milk products in which bacteria have converted much of the lactose to lactic acid. In fact, yoghurt is one of the oldest fermented milk products known and consumed by large segment of our society either as a part of our diet or as a refreshing beverage. It has been established that plain yoghurt has valuable therapeutic properties and helps curing gastrointestinal disorders. Although the origin of yoghurt is disputed one yet the belief in its beneficial influence on human nutrition and health has existed in many civilizations for a long time.

Yoghurt and similar foods have long occupied a place in the diets of peoples in the Middle East and Central Europe. The western world adopted a totally casual attitude to the product until rumours of its health-giving properties rife. In particular, the views of Metchnikoff (Tamime and Robinson, 1985) who linked longevity among the hill tribes of Bulgaria and their consumption of yoghurt caused a considerable flurry of interest. The role of yoghurt in curtailing the putrefactive bacterial action was explained as follows:

a. The lactic acid bacteria in yoghurt are tolerant to low pH whereas most pathogenic bacteria show optimum growth and metabolism around neutrality. Therefore, the acidic yoghurt passing along the intestine kills the undesirable microflora.
b. The effect of yoghurt was enhanced by the ability of Lactobacillus to become established in the intestine and gradually dominate the resident microflora. This later change ensured the continued absence of putrefactive organisms during the periods of reduced yoghurt availability and hence the vitality of the consumer would be maintained. However, some scientists observed that lactic acid is the only antimicrobial agent of any importance but the overall consensus still tends to the view that yoghurt does possess antibacterial properties, owing to the starter culture that are not solely dependent on low pH (Pulsani *et al.*, 1979).

Dietetic and therapeutic yoghurt

The fact that most strains of *Streptococcus thermophilus* and *Lactobacillus bulgaricus* do not survive in the intestinal tract may be a limiting factor if yoghurt is used for antibiotic therapy and/or any other medical purpose. However, the incorporation of *Lactobacillus acidophilus* and *Bifidobacterium bifidus* into the yoghurt is reported to be of excellent therapeutic value.

Wide range of yoghurts that have been developed for medicinal purposes are reported. It is recommended that low lactose yoghurt is beneficial for lactose-intolerant patients. The addition of different vitamins to yoghurt improves its therapeutic value. Similarly, low calorie yoghurt is attractive to diet conscious consumers; cholesterol free yoghurt is also beneficial for coronary conditions. The "yoghurt tablet" is a specially developed sugarless confectionery product for patients who suffer from diabetes. The last twenty years have seen yoghurts become one of the healthiest and most natural food available. Through production innovation manufacturers have made room for their brands accordingly to attract consumers of all ages (Anonymous, 1993).

Recently, it has been established that the quality of four cultured yoghurt which include two additional organisms namely *L. acidophilus* and *Bifidobacterium bifidus* possess several advantages (Honer, 1992). As it contains less acidity when fully fermented it results in milder and cleaner acid flavour so making it possible the use of chocolate flavour and enhancing delicate vanilla flavour as well. However, on the basis of biological studies it was observed that this type of yoghurt reduces blood serum cholesterol, provides antiviral protection to the development of immune factors and acts as anticarcinogenic defence mechanism.

This yoghurt has been credited by many United States nutritionists as a therapeutic and probiotic food. When ingested on a regular basis therapy begins with less intestinal disturbances. *Bifidus* and *Acidophilus* dominate the unwanted psychrophilic, putrefactive and pathogenic organisms only when their count is not less than 10^8 per mL.

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RECENT PUBLICATIONS

The abstracts are being published in the Pakistan Journal of Food Sciences to keep scientists and professionals abreast with the latest findings. Those interested in particular articles should please write directly to the author whose address appears with the abstract. The researchers are requested to kindly send **REPRINTS** of their recent publications (from 1990 onwards) to the Editor-in-Chief, Pakistan Journal of Food Sciences, C/o Department of Food Technology, University of Agriculture, Faisalabad for publication in the forthcoming issues of the Journal.

FRUITS AND VEGETABLES

MIAN ABDUL MALIK, ABDUL GHAFOOR, MUHAMMAD SALEEM AND MUHAMMAD MUSHTAQ AHMAD. **Preservation of watermelon squash using sodium benzoate and potassium metabisulphite.** Pak J. Agri. Sci., 31 (1): 58-60, 1994.

Food Technology Section, Ayub Agricultural Research Institute, Faisalabad.

Watermelon squash was prepared according to the standard method. It was divided into two equal lots and preserved by sodium benzoate (0.1%) and potassium metabisulphite (0.06%). The squash so prepared was filled in glass bottles (capacity 250 mL) sealed and stored at ambient temperature for 240 days. The physico-chemical and organoleptic evaluation showed that watermelon squash preserved with sodium benzoate was more stable and superior in quality as compared to potassium metabisulphite during storage.

MIAN ABDUL MALIK, MUHAMMAD AMIN JAVED AND MUHAMMAD MUSHTAQ AHMAD. **Development of bael fruit syrup.** Punjab Fruit J., 46 (1-4): 62-68, 1993.
Food Technology Section, Ayub Agricultural Research Institute, Faisalabad.

Bael fruit (*Aegle marmelos*) is sweet, aromatic, and has a cooling effect. It is helpful in chronic diarrhoea, dysentery, intestinal problems and piles Keeping in view these medicinal properties, an attempt has been made to develop bael fruit syrup. Pulp was obtained after removing hard rind and seeds. Four formulations were prepared, maintaining the pulp: water: sugar ratio @ 1: 1:4.5, 1:2:6.8, 1:3:9.0 and 1:4:11.2, respectively. The syrups so prepared were packed in glass bottles and stored at ambient temperature. The physico-chemical and organoleptic evaluation during storage showed that the syrups prepared from all the four formulations were acceptable, but the syrup developed from the first formulation (F_1) was highly acceptable.

MIAN ABDUL MALIK, NOOR MUHAMMAD, MUHAMMAD MUSHTAQ AHMAD AND MUHAMMAD SARWAR. **Shelf life extension of Kinnow fruit at ambient temperatures.** Punjab Fruit J., 46 (1-4): 39-45. 1993.
Food Technology Section, Ayub Agricultural Research Institute, Faisalabad.

Due to its high yield, attractive bright colour, nutrition and appealing taste and flavour, Kinnow (*Citrus reticulata* blanco) fruit is very popular among growers and consumers. In this study, efforts were made to control the post harvest losses and maintain freshness of fruit using different treatments during storage. The study revealed that the fruits coated with 1000 ppm thiabendazole (TBZ) and fruitex wax (T_3) and 1500 ppm of TBZ and fruitex wax (T_4) were more stable as compared with 500 ppm TBZ and fruitex wax coating (T_2) and control (T_1). However, economically Kinnow fruit coated with fruite wax containing 1000 ppm TBZ (T_3) can be stored at ambient temperature for one month.

HEALTH AND NUTRITION

DILSHAD AKHTAR AND ROHEELA ALBERT. **Nutritional status and dietary habits of teen-age college girls in Peshawar in relation to their family income.** Pak. J. Sci., 41-42: 70-79, 1989-1990.
College of Home Economics, University of Peshawar, Peshawar.

The study suggests that the meal practices of the teen-age college girls are not only a reflection of the family but of many other environmental factors as well. The anthropometry status of these girls is neither up to the western standard nor falls in the malnutrition spectrum. The dietary intake by the girls of all the income groups is much below the acceptable level which has resulted in certain nutritional deficiency disorders such as anaemia, cheilosis, glossitis, poor eyesight, bleeding of gums, dental cavities and general weakness. On the whole the nutritional status of the girls of high income group is found better than the girls of other income groups because

their diet contained more food servings as well as better nourishing foods such as meat, poultry and fish than the diet of girls from other income groups.

DILSHAD AKHTAR SAMINA HABIB, NAEEMA BEGUM AND ABDUS SATTAR. **Effect of dietary phytate on bioavailability of iron.** Nutrition Res., 7: 833-842, 1987.
College of Home Economics, University of Peshawar, Peshawar.

Experiments were carried out on iron-chelating capacity of phytic acid in fortified and unfortified diets of whole wheat, white flour and wheat bran using albino rats in a 10-day balance study. The results revealed that gross digestibility was highest in control diet (93.25%), least in the wheat bran diet with added ferrous sulfate (86.70%) and intermediate in other diets. Among the unfortified and fortified diets, the highest amount of phytate was present in the diet having wheat bran (2.69-4.99 mg/g). Phytate concentration of the rat faeces followed the pattern of dietary phytate content of test diets. The data indicated high values for iron (200.00-236.64 mg/g) in fortified diets while in the unfortified diets, the value was the same (16.67 mg/g) except the casein diet, which contained 69.99 mg/g of iron. Iron concentration of the fat faeces ranged 16.25-23.50 mg/g; the higher value being in the excreta of animals given fortified diets. The casein diet had iron 46.75 mg/g. The overall results showed that the phytate absorption was relatively higher in groups fed fortified diets. Iron absorption almost followed the pattern of phytate absorption. Correlation co-efficient analysis showed insignificant relationship between iron and phytate absorption indicating little effect of dietary phytate on the bioavailability of iron.

DILSHAD AKHTAR AND DILAWAIZ SAEED. **Infant feeding and weaning practices among educated mothers in Pakistan.** Pak. J. Sci., 36 (1-4): 41-45, 1984.
Department of Food and Nutrition, College of Home Economics, University of Peshawar, Peshawar.

Infant feeding and weaning practices among 70 Home Economics graduate mothers from all over Pakistan were investigated during December 1982-83. The relevant informations were collected through a Postal (mailed) questionnaire technique.

The findings indicated that majority of the infants (64%) were introduced to mother's milk and "ghutti" right after birth. The rest of the infants were partially breast-fed (7%) bottled-fed (6%) and fed with sugar solution and ghutti (17%), sugar solution (6%) and ghutti only (6%). After three days of birth the majority of the mothers (64%) started partially breast-feeding their babies while only 14.7% of the babies were purely breast-fed and 20% were purely bottle-fed. The reasons offered were, insufficient/ absence of breast milk mother's employment, allergy to mother's milk, cleft palate of the infant (3%) and the inverted nipple of the mother (3%).

The weaning process of majority of the babies was gradual by increasing the number of bottle-feeds and / or by increasing the solids in the diet. A considerable percentage of the mothers also used the old method of hiding themselves from their babies or smearing their breasts with some repellants during the process of weaning their babies from the breast milk. A large number of the babies were fed with weaning foods/food supplements. All of the mothers did not feed the right food supplements at the right time. Majority of the mothers fed their babies with food supplements and cared to keep their babies in optimum health because of the influence of Nutrition Education they received at colleges of Home Economics. While the rest of them did so on the advice of the doctors and relatives. Maximum percentage of the respondents (95.7%) reported their infants in very good health.

The study has shown the impact of Nutrition Education on the infant-feeding and weaning practices of Home Economics Graduate mothers to a large extent. While they also appeared to be under the influence of social customs as well as suggestions of the relatives and medical advice.

MILK AND DAIRY PRODUCTS

DILSHAD AKHTAR, A. SATTAR AND FAZLE RAB. **Boiling and storage effect on seasonal quality of milk.** Sci. Khyber, 6 (1): 21-27, 1993.
College of Home Economics, University of Peshawar, Peshawar

Effect of seasonal variations on the chemical and microbial quality of unboiled and boiled milk stored at ambient and refrigerated temperature was assessed. The unboiled fresh milk had range values of 6,20,000-10,10,000/mL (cv = 75.77) and 70/mL (cv = 52.27) for standard plate count (SPC) and coliforms respectively during the four seasons of the year. These values were the highest in summer, lowest in winter and intermediate in the rest of the seasons. boiling treatment at 100°C for 2.5 minutes completely eliminated coliforms and drastically reduced SPC (3,000-5,000/mL, cv = 20.32). Storage effect at 6 hourly intervals in both the boiled and unboiled milks resulted in increased titrable acidity and SPC with a decrease in total solids. These changes were more pronounced at ambient room (12°C -40°C) than refrigerator (5 ± 1°C) temperature during different seasons. The range values of SPC in boiled milk increased to 9,200-24,100/ml (cv = 38.28) after 6 hours and 22,500-

1,80,000/mL (cv = 73.41) after 12 hours storage at room temperature compared to SPC 4,200-9,500/mL (cv = 22.72) and 8,500-14,800 (cv = 21.91) at the same time intervals in the refrigerator. The microbial propagation was much faster in the unboiled than milk during refrigerated storage. The multiplication rate of SPC in both the boiled and unboiled milk during storage was much higher in summer followed by autumn, spring and winter. Increase in titrable acidity and decrease in total solids in stored milks presented the same pattern as that of other parameters during different seasons.

DILSHAD AKHTAR, ABDUS SATTAR AND FAZLE RAB. **Effect of processing time-temperature on chemical and microbial quality of milk.** Sci. Khyber, 6 (2): 167-172, 1993.
College of Home Economics, University of Peshawar, Peshawar.

Effect of processing time-temperature (60°C for 7.5 minutes, 81°C, 100° for 2.5 minutes) on the chemical and microbial quality of fresh milk was studied. A wide variation was observed in the standard plate count (CV 106; range 85,000-14,20,000), coliforms count (CV 24.55; range 30-68), total solids (CV 13.3; range: 8.05-12.01), titrable acidity (CV 6.67; range 0.16-019), and specific gravity (CV 0.27; range 1.017-1.025), in unprocessed milk during the seasons of spring, summer, autumn and winter. Standard plate count was found to be the highest in fresh milk during summer, lowest during winter and intermediate in spring and autumn seasons while the reverse was true in the case of specific gravity and total solids. Processing of milk increased the specific gravity and total solids while SPC was considerably reduced. coliforms were completely eliminated with processing treatment whereas acidity was little affected. Among the treatments, heat processing at 100°C for 2.5 minutes was found suitable as it reduced the Standard Plate Counts more than others.

DILSHAD AKHTAR, H.S. HASHMI AND ABDUS SATTAR. **Effect of post-boiling cooling and refrigerated storage on microbial and physico-chemical quality of market milk.** J. Pure App. Sci., 9 (2): 17-21. 1990.
College of Home Economics, University of Peshawar, Peshawar.

The results showed wide variation in standard plate count (SPC), coliform counts and total solids (TS) in relation to retail stores and sampling days. The SPC of unboiled fresh milk ranged 265667-1023667 and coliform 30-350. The range values for total solids (TS) and titrable acidity were 8.6-9.9% and 0.17-0.18% respectively. The mean SPC were 5000, 24963 and 20222 in the milk cooled after boiling by running tapwater, water seeping and fan respectively. However, the mean SPC values increased to 1120518, 109678, 306944 and 232821 after 24 hours refrigerated storage at 4°C in fresh milk and that cooled by running tap-water water steeping and fan respectively. Titrable acidity increased wile TS decreased on refrigerated storage. Effect of cooling procedures on sensory ratings was not significant ($P<0.05$).

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