USE OF ARTIFICIAL SWEETENERS IN KINNOW DRINK

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ABSTRACT

Artificial sweeteners aspartame and cyclamate were used in combination with sucrose for the preparation of a Kinnow based drink. The treatments were analyzed for physico-chemical and sensory evaluation fortnightly for two months. The TSS, degree of settling, acidity and reducing sugar increased while Brix:acid ratio and pH decreased with the storage. The maximum increase in TSS (11.35%), acidity (0.57%), and reducing sugar (1.374%) were observed in samples containing 100% sucrose, 50% sucrose and 50% aspartame and 100% aspartame resptively. The maximum decrease in Brix:acid ratio (7.72) and pH (3.468) were found in samples having 100% aspartame and 100% sucrose. Degree of settling was not affected significantly due to treatments. Treatments and storage periods significantly affected the colour, flavour and taste of various samples. For colour there was a declining trend in scores while the scores for flavour and taste showed high degree of acceptability. Control sample and sample with 70% sucrose and 30% aspartame got maximum score for colour while sample with 100% aspartame and sample with 70%sucrose, 15% aspatame and 15% cyclamate showed higher scores for flavour and taste respectively.

INTORDUCTION

In Pakistan, 19.4 thousand hectares area is under citrus cultivation with annual production of two million tonnes (Anon, 1996). Among citrus, Kinnow mandarin var. blanco occupies about 59% of the total citrus area and 64% of total citrus production in Pakistan.

Nutritionally, Kinnow is a rich source of Vitamin C, having 47 mg/100 mL (Haq, 1993), TSS 7.24%, minerals 4.60%, starch 4.26%, crude protein 1.36% and fat 0.235 on dry weight basis (Ghai *et al.*, 1979)

Alongwith other products, Kinnow juice based drinks are appreciated much by consumers. Sugar is a major ingredient being used as a source of sweetness. The excessive intake of sugar results in its accumulation in the form of fat (Gamman and Sharington, 1981). There may be serious diseases like cardiovascular disease, hyperglycemia, diabetes, hyperavtivity and tooth decay only because of excessive use of sucrose and obesity.

Presently, consumers are becoming more conscious about their health risks. With the help of non-nutritive sweeteners, sugar can be replaced and new products can be developed with low calories. Sucrose is always used as a standard to which the sweetness of other sweeteners is compared. The non-nutritive intense sweeteners are used in conbinations with sugar in the soft drinks, ice cream and comfectioneries. In the developed countires such sweeteners are being utilized for certain preparations to reduce the calories. However in developing countries like Pakistan, these sweeteners are not too much introduced and their use is limited. But being economical, helpful to control obesity and diabetes because of providing very few calories, the present study was conducted to provide a guideline for a low sugar content Kinnow drink with an equi-sweet taste.

MATERIALS AND METHODS

In this research juice was extracted by Rose Head Machine. Drinks were prepared by adding 15% juice into measured amount of water to which stabilizer (CMC) @ 0.4%, citric acid @ 0.15% and potassium metabisulphite @ 0.02% was added. The amount of sweeteners was added according to the formulation given in Table 1.The prepared drinks were filled in 250 mL glass bottles and stored at room temperature for two months.

Table-1:- DETAILS OF TREATMENTS

Treatment	Sweetner's Ratio Equsweetness Level		
	Sucrose(%)	Aspartame(%)	Cyclamate(%)
T_1	100	-	-
T_2	70	30	-
T_3	50	50	-
T_4	30	70	-
T_5	-	100	-
T_6	70	-	30
T_7	50	-	50
T_8	30	-	70
T_9	-	-	100
T_{10}	70	15	15
T_{11}	50	25	25
T_{12}	30	35	35

Physico-Chemical Analysis

TSS and acidity were dertermined by the procedure provided in A.O.A.C. (1984). Brix acid ratio was analyzed by the method of Redd *et al.*, 1986. For the determination of pH and sugars the procedures were adopted as given by Ruck (1963).

Sensory Evalution

Sensory evalution was made on 9 point hedonic scale as described by Larmond (1977). Statistical analysis was done as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Total Soluble Solids (TSS)

It is evident from the data that total soluble solids of all the Kinnow drinks showed an increasing trend during storage for 60 days.

Statistical analysis showed highly significant effect of storage period on artificial sweeteners while thir interaction has non significant effect on TSS of drinks.

Maximum increase in TSS (11.35%) was observed in T1 (100% sucrose) while minimum increase (4.28 and 4.38%) was observed in T5 (100% aspartame) and t9 (100% cyclamate) respcetively in samples stored for 60 days (Tables 2-3). This change may be due to degredation of sugars in to glucose and fructose (Polaninwamy and Muthukrishnan, 1974). Ahmed *et al.*, (1986) observed similar results in citrus and mango juices.

Table-2:- EFFECT OF SWEETENERS ON VARIOUS CHARACTERISTICS OF KINNOW SRINKS

Treatments	TSS (%)	PH	Brix-acid ratio	Colour	Flavour
T_1	11.35a	3.58a	20.70a	4.08a	6.20e
T_2	9.29b	3.54b	16.46b	3.92b	6.44de
T_3	7.36d	3.53c	12.63d	3.76bc	7.04c
T_4	6.50f	3.51f	11.52e	3.56ed	7.44b
T_5	4.28g	3.47h	7.72f	3.48d	8.32a
T_6	9.38b	3.51f	16.19b	3.88ab	6.20e
T_7	7.48b	3.49g	12.89cd	3.80bc	6.64d
T_8	6.70e	3.52e	11.93e	3.60cd	6.72d
T_9	4.38g	3.53c	7.87f	3.56cd	6.76b
T_{10}	9.28b	3.51f	15.99b	3.88ab	6.44dc
T_{11}	7.60c	3.52d	13.34c	3.80bc	7.08c
T_{12}	6.52ef	3.54b	11.38e	3.60cd	7.32bc

Means values carrying same letters are not significantly different.

Table-3:- EFFECTO FSTORAGE ON VARIOUS CHARACTERISTICS OF KINNOW DRINK

Days	TSS (%)	pH	Brix-acid ratio	Degree of settling (cm)	Colour	Flavour
0	7.23c	3.58a	15.17a	0.00e	5.30a	6.63d
15	7.41b	3.54b	12.56b	3.00d	4.37b	6.83c
30	7.52b	3.52c	14.78a	4.80a	3.65c	6.95c
45	7.65a	3.50b	14.89a	5.40b	3.18d	7.05b
60	7.75a	3.46c	8.69c	6.00a	2.22e	7.30a

Means values carrying same letters are not significantly different.

Acidity

The results in Table-4 showed that there is a gradual increase in the acidity of all samples throughout the entire storage priod. the increase in acidity of T3 (1.013%) was mazimum while minimum increase was observed in T8 (0.765%) after 60 days storage. Statistical analysis indicates a significant effect of days, where as artficial sweeteners and interaction has non-significant effect on the acitdity of Kinnow drink.

Table-4:- EFFECT OF STORAGE ON ACIDITY (%) OF KINNOW DRINKS

Treatment	Storage period(days)				
	0	15	30	45	60
T_1	0.467a	0.471a	0.490a	0.587a	0.91a
T_2	0.462a	0.504a	0.495a	0.588a	0.980a
T_3	0.491a	0.501a	0.509a	0.611a	1.013a
T_4	0.476a	0.504a	0.508a	0.593a	0.877a
T_5	0.477a	0.490a	0.502a	0.616a	0.770a
T_6	0.471a	0.513a	0.517a	0.621a	0.943a
T_7	0.481a	0.523a	0.526a	0.621a	0.877a
T_8	0.495a	0.499a	0.503a	0.611a	0.765a
T_9	0.467a	0.495a	0.501a	0.597a	0.835a
T_{10}	0.490a	0.509a	0.512a	0.635a	0.919a
T_{11}	0.476a	0.495a	0.499a	0.635a	0.924a
T_{12}	0.481a	0.504a	0.504a	0.621a	0.873a
T_{13}	0.478b	0.501a	0.642b	0.611b	0.890a

Means values for storage carrying same letters in row are not significantly different

The increase in acidity as a function of storage might be due to the dergradation of sugars into carbonyl groups (Goldbirth *et al* 1961). The increase in acidity is also with the results of Kalra and tonton (1984) for guava and mango nectars.

pH

A gradual decrease is found in pH of all the Kinnow drimk samples having different comninations of artificial sweeteners throughout the entire storage period. this decline in pH is due to respcetive increase in acidity. Statistically all the treatment, storage intervals and their interaction bave a highly significant effect on the pH of Kinnow drinks.

Mazimum decrease in pH was in samples of t5 (3.47) while minmum decrease was observed in t1 (3.58) in samples stored for 60 days (Tables 2-3). The decrease in pH may be attributed to the study of Zia (1987) who also observed declines in juice blends of mango, pomegranate and guava.

Brix-Acid Ratio

Brix-acid ratio is an indication of the relative sweeteners or tartness of a product. the higher the Brix in relation to teh acid content, the higher the ratio and sweeter the taste.

Brix acid ratio decreased in all the treatments during storage (Table-2). The mean valus of Brix-acid ratio decreased from 15.17 at zero day to 8.69 after 60 days (Table-3). The mazimum decrease that all the treatments of artificial sweetners, days and their interaction have a highly significant effet on the Brix-acid ratio of Kinnow drinks.

The decrease in Brix-acid ratio might be due to increase in acidity and TSS during storage of Kinnow drinks at room temperature. It was observed that acid increase was more as compared to TSS which resulted in a low Brix-acid ratio which is alos supported by the study undrtaken by Wajid (1996).

Reducing Sugars

There is gradual increase in reducing sugars throughout the entire storage period in all the samples having different treatments of artificial sweetners. the mean values of reducing sugar (Table 5) indicated on increase from 1.097% at zero day to 1.354% at 60 days. After 60 days of storage mazimum value 1.374 was recorde din T5 (100% aspartame).

Table-5:- EFFECT OF STORAGE ON REDUCING SUGAR(%)OF KINNOW DRINKS

Treatment	Storage period(days)				
	0	15	30	45	60
T_1	1.080a	1.167a	1.217a	1.253a	1.352a
T_2	1.087a	1.189a	1.223a	1.246a	1.365a
T_3	1.101a	1.185a	1.223a	1.249a	1.352a
T_4	1.112a	1.173a	1.205a	1.248a	1.342a
T_5	1.087a	1.174a	1.218a	1.264a	1.374a
T_6	1.091a	1.187a	1.208a	1.254a	1.369a
T_7	1.112a	1.178a	1.194	1.255a	1.348a
T_8	1.117a	1.169a	1.192a	1.247a	1.351a
T_9	1.104a	1.177a	1.198a	1.261a	1.346a
T_{10}	1.082a	1.171a	1.206a	1.227a	1.343a
T_{11}	1.082a	1.178a	1.192a	1.245a	1.351a
T_{12}	1.08a	1.187a	1.195a	1.242a	1.359a
T_{13}	1.097e	1.178d	1.206c	1.299b	1.354a

Means valuse of storage carring same liters in a row are not significantly different.

Statistical analysis indicates a highly significant effect of says on the sweeteners. Where as interaction of days and sweeteners has non-significant effect on reducing sugar of kinnow drink.

A constant increase in reducing sugar might be due to prolonged storage time at higher temperatuer, increase catalytic oxidation and hydrolysis of sugars with increase in acidity and decrease in

pH (Tressler and Josylin, 1961).

DEGREE OF SETTLING

Settling is proved to be an unattractive feature in citrus juice products, Statistical results indicated that storage intervals have highly significant effect on degree of settling while treatments and interactions found to be non-significant.

Degree of steeling increased in all treatment with the pasage of time (Table3). After 60 days higher degree of settling (6 cm) occurred in all the samples. this increase may be due to varitions in processing conditions of foods (Rodinson *et al,* 1956).

Sensory Evaluation

A panel of 5 judges evaluated the Kinnow drink for colour, flavour and taste.

Statistical analysis revealed that treatments and storage perios bave a highly significant effect on the colour and flavour of kinnow drinks. While their respective interactions were found to ber non-significant.

The loss in colour showed a declining trend in all the treatments during storage (Tables 2-3). The degradation of colour is mainly due to destruction of colour is mainly due to destruction of unstable pigment, lycopene, which is largely responsible for desirable colour of fresh drimks. There is also a simultaneous loss of Vit.C. caused by browning reactions between amino acidsm ascorbic acid and sugar present (Nelso and Tressler, 1980).

The results of flavour (Tables 2-3) showed an excellent consumer acceptance for T5 (100% aspartame). Treaatments containing a conbination of sucrose and aspartame got better score for flavour as compared to other combinations. Ther findings coincide with the results reported by Pruthi *et al,* (1984) and Hmad and Chaudhry (1993).

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EFFECTS OF STORAGE ON FUNCTIONAL CHARACTERISTICS OF DEHYDRATED BEEF MINCE & PRODUCTS

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ABSTRACT

Nutritional losses incurred during production and subsequent storage of dehydrated beef mince and mince products have been studied. Major nutrients of these food items undergo quantitative changes due to loss of moisture. Storability of the products in two grades of polypropylene (pp) pouches and their consumer acceptability in terms of sensory evaluation have been investigated.

INTRODUCTION

Dried meat products can be useful alternative to fresh beef as these can be stored over long periods of time without refrigeration. Furthermore, transportation becomes easier and cheaper because of reduced bulk (upto 50%). Dehydration of beef would be the cheapest and most effective means for its preservation. At the PCSIR laboratories Complex, Lahore, work is being carried out on these lines. Various additives are being tried and different methods are being tested for producing acceptable dried beef products.

MATERIALS & METHODS

Proximate Analysis: The moisture, crude protein, fat, fibre and ash were determined according to standard AOAC (1984) procedures with minor modifications to address the nature of dried materials. Rancidity was measured by Tarlidgis (1960) method and non-enzymatic browning was estimated by Gooding (1960) method.

Water Activity Measurement: Chuck beef was obtained from a local butcher, and trimmed to above 90% visual lean. This was cut into chunks approx. 2 x 2 x 2 cm and cooked in boiling water until thorough brown. The chunks were then minced, and the meat was put into an oven at 70°C to dry. Samples of meat were taken regularly during drying to determine the changes in moisture content and A_w over the drying period. A_w was measured using a Decagon CX-1 water activity meter at ODNRI Laboratories, U.K. Proximate analyses were carried out according to standard AOAC (1984) methods.

Sensory Evaluation: Small portions of mince (about 5gm) and fully intact meat balls were evaluated under incandescent light (1970 Lux) at room temperature by 10 panelists, all staff members of the Biotechnology and Food Research Center, experienced in such assessments. Samples were presented to the panel in random order at room temperature. Panelists were instructed adequately to rate each sample on 10 points scale (ranging from extremely desirable to unacceptable). Odour was evaluated by sniffing vapours emanating form sample source.

Preparation of Material: Fresh buffalo meat purchased from the local market was trimmed and cut into cubes of about two inches. The cubes were cured in a curing mixture comprising potassium nitrate, 300 ppm and sodium nitrite, 200 ppm dissolved in water.

These chops were left in the curing solution for 2 hr. at room temperature and then cooked in steam for 3-4 minutes.

Perparation of Meat Balls: Meat balls containing the following spices and condiments were prepared: (For 1 Kg. of beef): Salt (20g), chillies (6 g), white cumin (4 g), coriander (10 g), black pepper (4 g), fresh onions (500 g) and wheat flour (50 g).

Curing of Meat Balls: Lean beef chops of about 4-5 cm were passed through the meat grinder having sieve size of 4 mm. The curing mixture (KNO_3, 300 ppm, and $NaNo_2$, 200 ppm of beef) was dissolved in about 50 ml of water. Curing mixture was applied to the minced meat and left at room temperature for two hours. Fresh onions were peeled off and passed through the same mincing machine. The spices, onions and cured minced meat were then thoroughly mixed with wheat flour, by hand.

Cooking and Drying of Meat Balls: Round shaped meat balls (25-30 g) were prepared manually from the meat dough and then cooked under steam pressure of 12 Ib. for ten minutes. The balls were then allowed to cool to room temperature. These were then spread on the "Sarkanda chic" trays for drying in the forced-air natural gas dryer at 65 ± 5°C for 8-9 hours. The dehydrated meat balls were packaged in laminated polypropylene pouches of two types (PP_1 & PP_2)

RESULTS AND DISCUSSION

Buffalo meat (Beeflo) was used for these studies. It tends to have less intramuscular fat hence preferred over other meats for preparation of dehydrated products. To ensure overall acceptable texture, and proper rehydratability, temperature during dehydration was maintained at 65 ± 5°C. Cabinet drying (electrical dryer) of meat balls not only yielded a product of lighter and more attractive colour but also of better rehydratability (Table-1). Probably because better temperature control was possible in this method of dehydration, the resultant products were less dense in structure. Mechanically dried samples of both beef mince and meat balls were more rehydratable (Table-1) whereas sun or solar dried materials were gritty, closer grain and of lesser rehydratability. Because sun or solar dehydration were carried out usually for long duration and under uncertain drying schedules, The solar dryer temperatures at times soared to 70-80°C which caused extensive cross linking of proteins. Beef mince and products dried mechanically (in a natural gas dryer) and packaged in low and high density polypropylene (PP_1, & PP_2) pouches showed slight variation in chemical composition over a storage period of six months (Table-2).

Table 1:- REHYDRATION CAPACITIES OF DRIED BEEF - MINCE AND MEAT BALLS, DRIED BY TOW DIFFERENT METHODS

Product	Method of drying	Dry weight (gm) (+40 ml H^2O)	Time (Hours)	Weight after soaking (gm)	Rehydrat-ion ratio
Beef Mince	Solar dryer	5.00	0.5	12.13	2.4
		5.00	1.0	12.31	2.5
		No further increase in weight with time			
Beef Mince	Cabinet dryer	5.00	0.5	12.13	2.4
		5.00	1.0	12.80	2.6
		5.00	1.0	13.17	2.6
		No further increase in weight with time			
Meat Balls	Solar dryer	6.39	1.0	13.42	2.1
		6.45	1.5	13.34	2.1
		6.93	2.0	15.11	2.2
Meat Balls	Cabinet dryer	6.93	1.5	16.27	2.4
		7.08	2.0	16.29	2.3

1. Solar dried samples were comparatively darker in colour.
2. The values are average of triplicate samples.

Table 2:- EFFECT OF STORAGE ON PROXIMATE ANALYSIS OF DIFFERENT DRIED DEEF MINCE AND MEAT BALLS USING DIFFERENT PACKAGING MATERIALS

	0 Month	3 Months		6 Months	
Proteins %		PP_1	PP_2	PP_1	PP_2
Beef mince	78.22	77.62	78.15	77.83	77.81
Meat balls with onion	66.07	66.21	66.06	65.98	65.82
Fat %					
Beef mince	8.83	8.93	8.72	8.87	8.68
Meat balls with onions	14.19	14.23	14.16	14.17	14.03
Ash %:					
Beef mince	4.04	4.73	5.02	4.54	5.15
Meat balls with onions	6.83	6.92	7.5	6.96	7.36
Fibre %:					
Meat balls with onions	3.18	3.26	3.56	3.13	3.39
Moisture %:					
Beef mince	8.35	8.47	7.69	8.47	8.26
Meat balls with onions	8.92	9.00	8.47	9.25	8.73

It has been concluded by Acker (1963) that enzymes have to be inactivated for proper stability of low moisture foods. Therfore, beef mince, and products to be made thereof, were cured and cooked before dehydration.

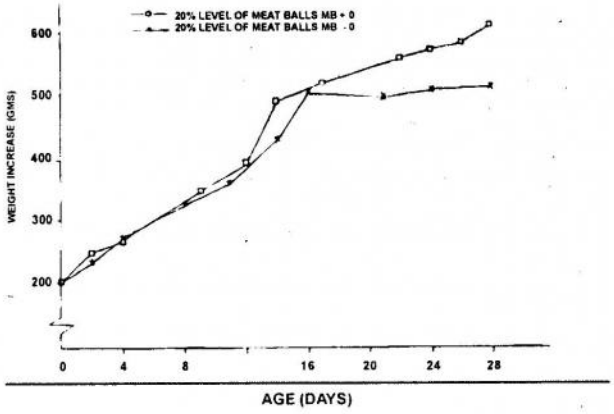
Addition of onions in meat balls increased chewability and over all consumer acceptability (Table-3). Autoclaving destroys most of pungency and also causes hydrolysis of starch to sugars. Dehydration of meat balls containing onions (MBO) created voids for rehydration water. Also capillary spaces were developed in these balls due to differential drying of the constituents. These factors contributed towards better re-hydrat ability. Meat balls containing onions have also shown high F.E. at 20% level (Fig.1)

Table 3 - TASTE PANEL RESULTS OF DRIED BEEF MINCE (NGM) DURING STORAGE STUDY

Parameters	Zero-time without packaging	2 months		4 months		6 months	
		PP_1	PP_2	PP_1	PP_2	PP_1	PP_2
Colour	5.6	6.6	6.6	7.0	6.7	6.3	6.3
Flavour	6.2	6.2	6.6	6.7	6.3	6.0	6.7
Shape/size	6.0	6.2	6.6	6.7	6.3	6.0	6.7
Taste	5.0	6.4	6.4	6.0	6.0	6.7	6.3
Texture	5.2	6.8	6.8	6.7	6.7	6.7	6.3

1 Ten panelists were used. The marks are averages
2 Rating 0-3 means poor, 4-5 means fair, 6-8 means good and 9-10 is excellent

FIG-1 WHEAT FLOUR CONTAINING



TBA values of all fresh and stored products are reported in Table-2. Storage in polypropylene (PP_1 & PP_2) bags has adversely affected all the samples as indicated by high TBA values for 6 months old samples (Table-4). Onions seemed to be playing proxidant role as the initial TBA values of onion containing meat balls were higher than those of other products. In case of meat mince oxidative rancidity can be checked by compressing the mince if long storage is desired. Alternatively, addition of an antioxidant. (BHA or BHT) is the obvious choice.

Table 4 - EFFECT OF STORAGE ON TBA VALUES OF DIFFERENT DRIED BEEF PRODUCTS USING DIFFERENT PACKAGING MATERIALS

Sr. No	Storage Time (Months)	Dried Beef Mince		Meat balls with Onions		Meat Balls without Onions	
		PP_1	PP_2	PP_1	PP_2	PP_1	PP_2
1	0 month	0.231	0.231	0.2418	0.2418	0.1326	0.1326
2	3 month	0.257	0.240	0.2847	0.234	0.234	0.195
3	6 months	0.390	0.312	0.32	0.256	0.315	0.241

It has been shown by Love and Pearson, (1974) and Igene (1979) that cooking of red meat destroys haeme pigment and releases a significant amount of non-haeme iron from bound haeme pigment and provides a source of free iron, which accelerates lipid oxidation in cooked meats. Iron availability in dehydrated mince is very much dependant upon interaction with other components. Lea and Chrysdale (1980) have also reported that heat treatment and additives during processing such as salt, affect iron availability. Availability of moisture (A_w) is one of the most important environment materials. A relationship has been established between moisture content (Fig.2) and A_w of behydrated beef mince. It will belp in defining a limiting moisture content of the dehydrated beef for prolonged storage, virtually ensuring its stability against microbial spoilage and oxidative rancidity. It will also help in ascertaining the degree of drying necessary for accurate cost estimation.

FIG-2 VARIATION IN WATER ACTIVITY WITH MOISTURE CONTENT FOR BEEF DRIED IN AIR AT 70°C

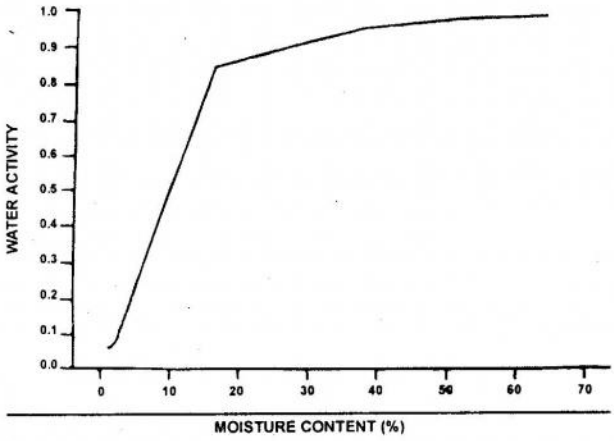


Fig. 2 shows that the A_w of 0.6 is achieved at a moisture content of about 10%. It can be seen that A_w of beef mice during drying remains high at moisture content down to 15-20%. It indicates that slow drying may cause initial spoilage by bacteria. NEB (Non-Enzymatic Browning) estimations showed highest values for meat balls containing onions (Table-5). The highest rate of increase has also been observed in these products. Sugar and starch provided by the

onion shreds seems to be the cause of this phenomenon. Also moisture content of these meat balls were higher in comparison to other products. Water plays a dominant role in determining the rate of browning. It shortens the induction period and accelerates the NEB reaction in such carbonyl containing food systems.

Table 5:- EFFECT OF STORAGE ON NON-ENZYMATIC BROWING OF DIFFERENT DRIED BEEF PRODUCTS USING DIFFERENT PACKAGING MATERIALS

Sr. No.	Storage Time (Months)	NGM		MB + O		MB - O	
		PP1	PP2	PP1	PP2	PP1	PP2
1	0 month	0.148	0.148	0.190	0.190	0.155	0.155
2	3 month	0.175	0.185	0.262	0.270	0.205	0.258
3	6 month	0.162	0.170	0.307	0.312	0.255	0.305

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Acknoovledgement:- Assitence of ONDRI, U.K. in this work is gratefully acknowledged.

USE OF EMULSIFIERS IN THE PRODUCTION OF BISCUITS FROM COMPOSITE FLOUR

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SUMMRY

Previous investigations showed that supplementation of wheat flour with legume flours improved the nutritional status of biscuits. At the same time it also caused some defects in physical characters like increased thickness and reduced spread factor, Lecithin and glyceryl monostearate (GMS) were incorporated at different levels (0.8,1.0 and 1.2 percent) into the dough for the improvement of aforesaid physical characteristics in biscuits. GMS at 0.4 percent performed better than its 0.6 percent level in batter with selected three levels of lecithin. An increase in peroxide value and free fatty acids in all the biscuits was observed during storage. However, all the biscuits remained acceptable even after 90 days of storage at ambient temperature.

INTRODUCTION

Biscuits are an important commodity consumed as snack food by all age groups. Commercially available biscuits are principally manufactured from white flour that lacks fiber, mineral elements and is nutritionally deficient in certain essential amino acids such as lysine, threonine, etc. Biscuits prepared from such flour are nutrituinally poor (Rauf, 1993). One the other hand, whole wheat flour is nutritionally superior to white flour (Awan *et al.* 1991). Composite flours containing wheat and nutritionally rich materials have been prepared in many parts of the world. In this regard numerous substances such as oil seeds, oil seed cakes, fish flour, leaf protein, milk solid, milk whey and edible legumes have been employed (Awan *et al.* 1996). Legumes and pulses exhibit high protein content varying from 20 to 30 percent. Furthermore, quality of protein in legumes is known to be superior to that of cereals, particularly in amino acids like lysine, threonine and tryptophan (Aykroyd and Doughty, 1969).

Previous investigations have shown that biscuits prepared from composite flours containing wheat and legume flours possessed high mutritional values (Ullah, 1990; Awan *et al.* 1995). These have improved protein content, are high in crude fiber and possess a better amino acid profile. However, the use of composite flours causes some physical defects like decreased diameter and increased thickness (Hussain, 1993; Shakoor, 1995). The purpose of present study was to utilize certain emulsifiers to improve the physical qualities of biscuits prepared from composite flour containing whole wheat and gram flours.

MATERIALS AND METHODS

Procurement of raw materials-Wheat (Pb-85) and black gram (Pb-92) were procured from Ayub Agricultural Research Institute, Faisalabad. Emulsifiers, namely lecithin and glyeryl monostearate (GMS), were purchased from the local market.

Preparation of composite flour-Wheat sample was cleaned manually and milled in china mill to obtain whole wheat flour. The cleaned grams were soaked over night to remove seed coat. Dehulled grams were sun dried and ground in china mill to obtain gram flour. Composite flour was prepared by blending 80 percent whole wheat flour and 20 percent gram flour.

Preparation of biscuits-Biscuits were prepared in the Department of Food Technology accord-

ing to the following modified recipe of AACC (1983):
Whole wheat flour = 750g Gram flour = 150g
Hydrogenated Vegetable Oil = 450g
Sugar = 450g Baking powder = 10g
Water = 180mL
Emulsifier According to the respcetive treatment

TREATMENTS. Various doses of emulsifiers (percent proportion by weight) were incorporated into the batter as follows:

Treatments	Lecithin(%)	Glyceryl monostearate(%)
T_0	-	-
T_1	0.8	0.4
T_2	1.0	0.6
T_3	1.2	0.4
T_4	0.8	0.6
T_5	1.0	0.4
T_6	1.2	0.6

Storage Of Biscuts. The biscuits were cooled at room temperature after baking and packed in polyethylene bags. They were stored at ambient temperature for 90 days in laboratory shelf.

Chemical Studies . Whole wheat flour, gram flour and biscuits were analysed according to the methods described in AACC (1983) to dertermine their proximate composition. Peroxide and free fatty acid values of fat present in the biscuits were estimated by the methods of Koniceko (1985). Chemical analyses of the discuits were conducted after 15 days interval upto 90 days storage.

Physical Analysis . The AACC (1983) mehtods were used to evaluate biscuits for diameter (width), thickness and spread factor.

Sensory Evaluation . The biscuits were evaluated for colour, flavour, taste, texture and over all acceptability at an interval of 15 days using nine point hedonic scale (Larmond, 1977).

Statistical Analysis . The data thus collected were analysed using Radomized Complete Block Design (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Chemical Composition of Raw Meterials- Proximate analysis of wheat variety Pb-85 revealed the presence of 10.80 percent moisture, 11.22 percent crude protein, 1.98 percent ether extract, 2.65 percent crude fiber, 1.95 percent mineral content and 71.40 percent nitrogen free extract (Table 1). These results are almost similar as reported by Siddique *et al.* (1996)

Table-1:- PROXIMATE COMPOSITION OF WHOLE WHEAT FLOUR, GRAM FLOUR AND BISCUITS.

Item	Moisture %	Protein %	Fat %	Fiber %	Ash %	NFE %
Whole wheat flou	10.80	11.22	1.98	2.65	1.95	71.39
Gram flour	10.25	20.77	5.43	1.91	2.25	59.39
T_0	2.30	6.56	26.30	1.24	0.99	62.61
T_1	2.43	6.56	27.50	1.24	0.99	61.28
T_2	2.56	6.56	27.86	1.24	0.99	60.79
T_3	2.54	6.56	27.86	1.24	0.99	60.21
T_4	2.47	6.56	27.68	1.24	0.99	61.06
T_5	2.40	6.56	27.66	1.24	0.99	61.06
T_6	2.60	6.56	28.05	1.24	0.99	60.56

Gram flour possessed 10.25 percent moisture, 20.77 percent, crude protein, 5.43 percent ether, 1.91 percent crude fiber, 2.25 percent mineral content and 59.39 percent nitrogen free extract (Table 1). The results of present study are quite close to the observations recorded earlier by lllahi (1978).

Chemical Composition of the Biscuits - The proximate analysis of freshly prepared biscuits and average composition of biscuits over the entire storage period are presented in Tables 2. The results revealed no difference in crude protein, crude fiber and mineral content of all samples as the basic raw material was the same. However, there was a slight difference in moisture, ether extract and NFE content. It was only due the addition of various doses of emulsifiers.

Table-2:- EFFECT OF STORAGE ON CHEMICAL COMPOSITION OF COMPOSTIE FLOUR BISCUITS CONTAINING EMULSIFIERS (MEANS OF TREATMENTS).

Storage period dats	Moisture %	Protein %	Fat %	Fibre %	Minerals %	NFE %	Peroside value	Free fatty acids %
0	2.48a	6.56a	27.56a	1.24a	0.99a	61.16a	1.08a	0.101a
15	3.00b	6.53b	27.39b	1.23b	0.98b	60.87b	1.56b	0.115b
30	3.50c	6.50c	27.24c	1.23c	0.98b	60.56c	2.64e	0.123c
45	3.93d	6.47d	27.11d	1.23b	0.98b	60.28b	2.71d	0.129d
60	4.27e	6.45e	27.03e	1.22c	0.97e	60.06e	3.04e	0.135e
75	4.53f	6.43f	28.98f	1.22c	0.97e	59.87f	3.57f	0.140f
90	4.72g	6.41g	1.21g	1.21d	0.97e	59.73g	4.33g	0.146g

A highly significant increase in moisture was noted in all biscuits during the storage period. The highest increase in moisture was observed in T_6 containing the highest dose of emulsifiers. The lowest increase in moisture was found in T_0 after 90 days of storage. This increase may be attributed to the packaging material, presence of wheat bran which is hygroscopic in nature and to the higher protein content of biscuits. Similar results were reported by Leelavathi and Rao (1993).

Crude Protein . All the biscuits possessed equal amount of protein (6.56 percent) at zero day. A decreasing trend in protein content in all the samples was noted during storage. After 90 days protein content apparently decreased to 6.41 percent in all the biscuits (Table-1). Similar results were reported by Ahmed *et al.* (1997)

Either Eetract . The results revealed a variation in fat content among all the samples that ranged from as high as 28.05 percent in T_6 to as low as 26.30 percent in T_0. A slight decrease in fat content was found in all the samples during entire storage period. Ahmad *et al.* (1997) reported a reduction in fat in biscuits fortified with soy flour after 3 mounts storage.

Crude Fiber . The results suggested that all the biscuits possessed same crude fiber content (1.24 percent) at zero day. A similar descending pattern in crude fiber in all the samples was noted during storage. After 90 days fiber content apparently decreased to 1.21 percent in all the biscuits bue to moisture absorption from atmosphere. Similar reduction in crude fibre content in biscuits was reported by Ahmad *et al.* (1997).

Mineral Content . The results disclosed that mineral content in all the samples were the same (0.99 percent) at zero day. An identical decline in mineral content was observed during storage in all the biscuits. After 90 days mineral content apparently decreased to 0.97 percent due to moisture absorption from the atmosphere. A similar decreasing trend in mineral content of legume fortified biscuits due to moisture absorption was found by Ahmad *et al.* (1997).

Nitrogen Free Ectrect (NFE). The results manifested a slight variation in different samples from 60.56 percent in T_6 to 62.61 percent in T_0. During storage periond NFE content in all the biscuits fel dolwn in a similar fashion. After 90 days storage NFE content was as low as 59.08 percent in T_6 and as high as 61.23 percent in T_0.

Peroxide Valus (PV). The results revealed a variation in PV from 1.00 in T_0 to 1.13 in T_6 at zero day (Table 2). During the entire storage period same increasing pattern of PV was maintained in all the samples. After 90 days, PV in T_6 was the highest. PV is an indicator of incipient rancidity that results from auto-oxidation of fat. It depends on fat content of the sapmle and is accelerated by air, light and water. As moisture was absorbed by all the samples duing storage, hence a rise in PV in the biscuits was noted throughout storage period. The results are in line with the previous findings of Rao *et al.* (1995).

Free Fatty Acids (FFA). The FFA in different biscuits varied from 0.094 in T_0 to 0.106 in T_6 at zero day (Table 2). During storage increase in FFA content was in an identical mode in all the biscuits. After 90 days of storage FFA content was as high as 0.146 and as low as 0.101. FFA are generated from the hydrolysis of fat by the action of water and lipolytic enzymes, Their presence depends on the quality and quantity of fat. Since all the samples absorbed moisture from atmosphere during storage, consequently a rise in FFA content was observed in all the biscuits. Similar increasing trend in FFA was reported by Leelavathi and Rao (1993).

PHYSICAL ANALYSIS OF BISCUITS

Diameter (Width). The width of the biscuits waried from 264.0 mm (T_0) to 282.5 mm (T1). A decreasing trend in width of the biscuits was observed with increasing dose of lecithin from 0.8 to 1.2 percent when applied in combination with 0.4 prcent GMS (Table 3). Almost similar situation prevailed when the same levels of lecithin were employed along with 0.6 percent GMS. On the other hand, addition of 0.4 percent GMS to the dough increased the width of the biscuits, whereas slightly higher level (0.6 percent) decreased it when applied with the same selcted lev-

els of lecithin.

Table-3:- PHYSICAL CHARACTERISTICS OF BISCUITS

Treatment	Diameter(D)(mm)	Thickness(T)(mm)	Sperad factorD/T
T_0	264.00	59.00	44.70
T_1	282.15	56.00	50.40
T_2	270.00	54.00	50.00
T_3	270.00	51.90	52.80
T_4	269.70	54.70	49.30
T_5	276.50	54.40	50.80
T_6	265.30	51.40	51.60

Thickness . The thickness of biscuits varied from 59.0 mm (T_0) to 56.0 mm (T_1). A decreasing trend in the thickness of biscuits was noted with increasing levels of lecithin when employed with 0.4 or 0.6 percent GMS. On the other hand decrease in thickness with 0.6 percent GMS was slightly greater as compared to 0.4 percent when employed with selected doses of lecithin (Table 3).

Spread Factor . A considerable increase in spread factor was ranging from 44.7mm (T_0) to 52.8mm (T_3) due to the incorporation of emulsifiers (Table 3). An increasing trend in spread factor was recorded with higher levels of emulsifiers. Application of 0.4 percent GMS in combination with selected levels of lecithin improved spread factor. However, incorporation of 0.6 percent GMS with the same levels of lecithin reduced the spread factor.

SENSORY EVALUATION

Biscuits were placed for sensory evaluation before a panel of judges after 15 days interval. All the biscuits prepared with emulsifiers proved better than the control (Table 4). During storage, a decline was observed in quality scores for all the sensory attributes i.e. colour flavour, taste, texture and overall acceptability. T_0 and T_6 scored lower than others due to poor sensory characteristics, higher PV and FFA content. Quality scores in other treatments were close to one another. However, T_5 and T_1 proved better than others with respect to overall acceptability. All these biscuits remained acceptable even after 90 days storage.

Table-4:- EFFECT OF STORAGE ON SENSORY CHARACTERISTICS OF COMPOSITE FLOUR BISCUITS CONTAINING EMULSIFIERS (MEANS OF TREATMENTS).

Storage period days	Colour	Taste	Flavour	Texture	Overall acceptability
0	6.64a	6.62a	6.60a	6.57a	6.60a
15	6.57b	6.53ab	6.51b	6.46b	6.55b
30	6.52c	6.39bc	6.38c	6.32c	6.40c
45	6.44d	6.24cd	6.25d	6.17d	6.26d
60	6.35e	6.13de	6.13e	6.06e	6.15e
75	6.24f	6.04e	6.04f	5.97f	6.05f
90	6.17g	5.81f	5.94g	5.88g	5.96b

CONCLUSINONS

Incorporation of emulsifiers positively improed the physical characteristics of the legume flour supplemented biscuits. Addition of emulsifiers increased fat content that in turn raised the PV and FFA content. Results of physical and sensory evaluation proved their acceptabbility even after 90 days of storage. Hence, it may be concluded that GMS at 0.4% can be applied in combination with 0.8, 1.0 and 1.2% lecithin in biscuit munfactured from composite flour for the improvement of physical characteristcs. At the same time antoxidants should be incorporated to avoid increase in PV and FFA content in the biscuits.

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STUDIES ON THE EFFECT OF STORAGE CONDITIONS ON ASCORBIC ACID, ACIDITY AND pH OF TOMATO CONCENTRATE

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ABSTRACT

This paper reports the effect of storage temperatures on ascorbic acid, acidity and pH of tomato concentrate during six months storage. A gradual increase in acidity was observed during storage of tomato concentrate at three different temperatures (-18°C, 6°C and 20°C). On the other hand, ascorbic acid contents were decreased at all these temperatures on storage. pH values were also decreased on storing the tomato concentrate at these three different temperatures. However, these changes were more pronounced at 20°C than at -18°C and 6°C.

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is one of the major vegetables grown in Pakistan. 325.3 tonnes of tomatoes were produced during the preceding year (Agric. Stat. Pakistan, 1997-98). Apart from fresh consumption as a salad, tomato has become virtually part of every dish cooked at home or in the restaurants all over the country. Nutritionly it ranks very high. Its food value is around 20 calories per100 gram. It is an excellent sourec of vitamins A and C (Shah & Zafar, 1975). It also contains considerable amount of food acids such as citric, melic and formic acids alongwith a fair quantities of histidine, lysine and certain minerals (Luh and Woodroof, 1975). Additionally it is also considered as an appetizer due to its chemical composition and refreshing qualities (Cruickshank, 1968).

The major organic acids present in tomatoes are citric acid and melic acid. Citric acid is the most abundant acid and accounts for upto 90 percent of the acid content (Hammer *et al.*, 1945). presently tomatoes are used in fresh state as well as in different processed forms such as ketchup, pickles, sauces, soup, salad, purees, concentrates, pastes, etc. (Cruess, 1958; King *et al.*, 1973).

The principal purpose for the production of concentrated tomatoes had been reported to reduce the weight and bulk (Norman, 1973), while its various products had a considerable demand in the world food tade (Gould, 1974). Quite a few researchers had investigated into the effect of storage temperature on the ascorbic acid (Youssof *et al.*, 1978; Ahmad, 1997) and acidity (Jamil, 1990) of concentrated tomatoes. The aim of the present study was to record the observations regarding the effect of storage temperature on ascorbic acid, acidity and pH of tomato concentrate during a storage period of six months.

MATERIALS AND METHODS

Fully matured tomatoes (Roma cultivar) were procured form local vegetable market. Tomatoes were trated to get plup (Shah and Zafar, 1975), concen treated to 20° Brix in a steam jacketed kettle at 105°C, packed in 250 ml presterilised glass bottles, processed in boiling water for 10 minutes and stored at ambient temperature (18 - 20°C), refrigerated temperature (6°C) and freezer (-18°C). The samples were analyzed just after processing and at 30 days intervals upto storage period of 180 days.

Determination of Ascorbic Acid: Ascorbic acid was determined according to the method of Bajaj and Kaur (1981) by using oxalic acid - EDTA solution. Absorbance was observed at 760 nm by spectrophotometer (Hitachi model 220S). The concentration of ascorbic acid was then calculated by reference to the standard curve.

Titratable Acidity: Titratable acidity was measured by applying AOAC method number 942.15 a (1990), and calculated as percent citric acid (anhydrous) persent in the samples.

pH: pH was determined by using digital pH meter (Knick model number 646) by following AOAC method number 981.12 (1990)

RESULTS AND DISCUSSION

Ascorbic Acid: Results of changes in ascorbic acid are reported in Table-1. There had been gradual decline in ascorbic acid throughout the storage period at all the three storage temperatures. Out of 33 mg/100gm ascorbic acid only 7mg/100gm tomato concentrate was observed after 180 days room temperature (20 ± 2°C) storage, while reduction in this vitamin was faster (45.5%) in concentrate stored at 6°C than samples (36.4%) stored at -18°C.

Table-1:- EFFECT OF STORAGE TIME AND TEMPERATURE ON ASCORBIC ACID, ACIDITY AND pH VALUE OF TOMATO CONCENTRATE.

Storage Time	Ascorbic Acid (mg/100 ml)			Acidity (%)			pH		
Days	20°C	6°C	-18°C	20°C	6°C	-18°C	20°C	6°C	-18°C
0	33	33	33	1.56	1.56	1.56	4.21	4.21	4.21
30	25	32	32	1.58	1.57	1.57	4.20	4.20	4.20
60	20	30	31	1.62	1.59	1.58	4.18	4.19	4.19
90	18	29	31	1.65	1.61	1.59	4.16	4.18	4.19
120	15	29	30	1.71	1.64	1.62	4.13	4.16	4.18
150	12	28	29	1.75	1.66	1.64	4.10	4.15	4.17
180	7	26	27	1.80	1.68	1.65	4.08	4.14	4.17

Statistical analysis of data showed highly significant effect of storage temperature, storage period and their interaction on ascorbic acid. It has been reported that ascorbic is easily destroyed by oxidation aspecially at high temperature storage, as oxidation of this vitamin takes place in the presence of oxygen, which is greatly accelerated by the presence of trances of metals (Clegg and Mortorn, 1968; Norman, 1973). This is also known that oxidation process is accelerated at higher temperture (Moschette *at al.*, 1947). In the present investigations loss was more pronounced at ambient temperature than 6°C and -18°C. These results are in comformity with the observations of Pope and Gould (1974).

Acidity: The data regarding changes in acidity during 180 days storage at various temperatures is reported in Table-1. A gradual increase in acidity at all the three storage temperatures was observed in the tomato concentrate. Maximum increase was recorded in samples stored at ambient temperature followed by 6°C storage conditions, while minimum increase occurred in samples stored at -18°C.

Statistical analysis of the data showed highly significant effect of storage temperature, storage time and their interaction on acidity of the samples. The rise in acidity may be due to increase in the concentration of weakly ionized acids and their salt during storage resulting in increase in the acidity of the samples, this increase is influenced by storage temperature, higher the temperature greater the increase in acidity (Hummel and Okey, 1950). Luh *et al.*, (1958) and Ahmad (1997) have also supported this view point.

pH: Data on pH of tomato concentrate stored at three different temperatures is presented in Table-1. A gradual decline in pH in all the samples at all storage temperatures had been observed. This decrease was maximum in samples stored at higher temperatures, while a minumum change was observed in samples stored at -18°C.

Statistical nalysis of data showed highly significant effect of storage temperature, storage time and their interaction on pH of the concentrate. Phenomenon change in pH is directly related to change in acidity of the samples. This type of observation had already been reported by Shaheen (1995) and Pope and Gould (1974).

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STUDIES ON THE ACTIVITIES OF PEROXIDASE ENZYME DURING THE POST HARVEST RIPENING OF MANGOES *(Mangifera indica var. Chaunsa)* AT LOW TEMPERATUER STORAGE

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ABSTRACT

The results showed that peroxidase (POD) enzyme has highly singnificant effect on the post harvest ripening of mangoes. The activity of this enzyme is temperature dependent and almost negligible change in its concentration was observed during storage at 17°C in the first four experiments. Sooner the mangoes were shifted at 29°C for ripening, the concentration of POD started to increase till the last day of storage, causing metabolic changes in the fruit and ultimate ripening. It also helped improving the colour, flavour, taste, texture and acceptability of the fruit during storage at ripening temperature. Similar trend of its activity has also been observed in the fifth and sixth experimatents during storage.

INTORDUCTION

The mango *(Mangifera indica L.)* is recognixed as one of the popular fruit in the world market due to its excellent flavour, attractive fragrance and therapeutic value (Subramanyam *et al.*, 1975). In the South Asian Region, it is considered to be the King of all indigenous fruits (Singh, 1960). Mango is a good source of minerals such s calcium, sodium, potassium, magnesium, phgosphorus and iron (Anon., 1980; Hubbert and Ledger, 1988). This fruit is very popular and owing to an excellent taste, it is mainly consumed in the fresh state like many other tropical fruits.

Like other fruits number of physico chemical changes occur during storage and ripening of mango. These impotant chemical changes are mainly hydrolysed into simple sugars and are repidly reduced during ripening which is one of the most impotant cri terion of acceptability for the ripened magoes (Selvaraj *et al.*, 1989; Morga *et al.*, 1979). Peel colour during ripening changes gradually from green to orange/yellow (Satyan *et al.*, 1986). The main physiological activities occurring during maturity or ripening of mango are respiration and transpiration, which are responsible for the faster ripening and softening of this fruit (Parikh *et al.*, 1990).

The enzyme specially peroxidase is the vital important biochemical agents responsible to bring the physico-chemical changes in mangoes during post harvest ripening of this fruit. So the studies bave been conducted in order to chalk out the effect of this enzyme on the changes occurring during post harvest ripening of mangoes at low temperature storage.

MATERIALS AND METHODS

Collection of Samples. The green but matured mango fruits of the cultivar "Chaunsa" were randomly picked from different trees of Experimental Garden, Ayub Agricultural Research Institute, Faisalabad. The fresh mangoes were subjected to six different experiments. The details of the each experiment has been shown in Table 1. The mangoes were hydrocooled at 17°C temperature and stored at the same temperature for different days by maintaining the constant relative humidity at 86% in all cooling chambers.

Table-1:- DETAILS OF EXPERIMENTS SHOWING VARIATION IN TEMPERATURE FOR HYDROCOOLING AND STORAGE DAYS FOLLOWED BY RIPENING OF MANGOES AT 29c.

Experement No	Hydrocooling And Storage	
	Temperature (°C)	Days
1	17	7
2	17	14
3	17	21
4	17	28
5	29	-
6	-	-

The objective of hydrocooling process was to extract the field heat from the mangoes qucikly after harvesting which was carried out by using ice water in a large tank. The latex was removed manually from each mango fruit and washed with tap water prior to subjecting the mangoes for hydrocooling treatment. Therafter, the ice water was sprinkled using pump, over the mangoes to cool down and attain the different desired storage temeratures by following the procedure described by Pantastico (1975).

After the storage periods, the mangoes of each experiment were shiftd to a temperature of 29°C for undergoing the ripening process. The mango samples of each treatment were drawn randomly every day during the whole period of storage and ripening, to carry out the analayses and to see through the effect of activities of POD upto maximum acceptability level. The details of the analytical method is described below:

Peroxidase (POD) Avivity. The peroxidase avtivity was estimated according to the method of McLlelan and Robinson (1987) as outlined below:

a. Preparation of buffers

i. Phosphate buffer (for extraction)

The phosphate buffer of pH 7.5 was prepared from sodium dihydrogen orthophosphate and disodium hydrogen orthophosphate.

ii. Acetate buffer

Acetate buffer (100mM, pH 5.6) was prepared from acetic acid and sodium acetate stock solutions and then diluting the resulting solution to 100mL with distilled water.

b. Perparation of mango pulp soluble peroxidase fraction

The mango fruit of each experiment was peeled, destoned and flesh cut into pieces. A flesh sample of 100g was homogenized in 200 mL of ice cold sodium phosphate buffer (10 mM, pH 7.5) for 1 minute using Waring blender. A high speed setting insoluble polyvinyl poly pyrrolidone (1%) was obtained and pulp suspension was filtered through double layer of muslin cloth and the resultant filtrate was centrifuged (19000 x g) for 20 minutes at 4°C. The supernatant was collected and stored at -18°C and designed as the mango pulp soluble peroxidase fraction.

c. Assay of POD

The reaction mixture contained 1.4 mL of 0.1% H_2O_2 in 100 mM sodium acetate buffer (pH 5.6) and 0.2 mL of peroxidase extract. The reaction was initiated by the addition of 1.4 mL of 0.5% aqueous guaiacol solution and initial change in absorbance at 420 nm was measured at 5°C (Fig 1). One unit of peroxidase activity was defined as an increase of 1.0 optical density units at 420 nm/min.

Statistical Analyses

The results of each experiment were subjected to the analysis of variance by applying completely randomized design to ascertain the level of significance of POD, using MSTATC package developed by R.D. Freed based on the methods of Steel and Torrie (1980).

RESULTS AND DISCUSSIONS

The enzymes are the true radicals causing different biochemical changes in all the living things. This study was conducted to see through the effect of POD, the most active enzyme, during the post harvest ripening of mangoes. The drsults of which are discussed below:

Exp. No. 1

In the first experiment, the results in Table 2 indicated a significant variation in the avtivity of POD during storage. The POD activity was found to be significantly low and identical for the first 7 days of storage of mangoes at a temperature of 17°C (Table 3). Whereas, an abrupt and significant increase in its avtivity was observed when the mangoes were shifted at a temperature of 29°C for ripening and this activity was found to be the highest on 27th day, the last day of storage.

Table-2:- F-RATIO SHOWING THE EFFECT OF POD AVTIVITY OF POST HARVEST RIPENING OF MANGOES AT 29°C AFTER LOW TEMPERATURE STORAGE.

Experiment No	df	MS	df	MS	F-Ratio
1	26	0.0008	54	0.0001851	430219**
2	33	0.006	68	0.0001911	31.397**
3	44	0.006	90	0.0001111	59.397**
4	48	0.019	98	0.003265	58.192**
5	15	0.034	32	0.001	34.00**
6	10	0.014	22	0.0002272	61.619**

**Highly Singificant

Exp. No. 2

The statistical results (Table 2) of experiment No.2 have shown almost the similar results and found that the avtivity of POD varied significantly as a function of storage days. The results in Table 3 also indicated that the avtivity of POD was found to be non-singificantly different during first 18 days of storage of mangoes subjected to a temperature of 17°C, but during the storage beyond 18th day, the avtivity of this enzyme increased significantly which was found to be the highest on 34th day of storage i.e. last day of storage.

Exp. No. 3

In the dexperiment No. 3, the results of POD activity showed a significant variation during storage. The mean values in Table-3 showed that the concentration of this enzyme did not vary significantly during the first 16 days in mangoes stored at a temperature of 17° C but when the mangoes were shifted at ripening temperature of 29° C, a drastic change in the activity of this enzyme was noticed. This is also observed that the peroxidase actrivity increased with the advancement of storage days and the maximum activity was recorded on 45th day of storage.

Exp. No. 4

Similarly in experiment No.3, the POD enzyme has shown significant variation in its avtivity and it invreased in an ascending order as a function of storage days and temperature. It was also found that POD activity is temperature dependent and did not change significantly during first 28days of storage at low temperature i.e. 17°C. However, a significant effect was observed in these mangoes after 31st day of storage at a temperature of 29°C. After this period the POD concentration started to increase and the maximum activity was oberved at 49th day of storage.

Exp. No. 5

In the experiment No.5, the avtivity of POD increased significantly as a function of storage days following an ascending order and it was significantly high during last tow days of storage (Table-3).

Exp. No. 6

The last experiment was control in which the activity of POD enzyme was affected significantly between the storage days (Table 2). The result in Thable 3 also indicated the significant increase in POD activity with the advancement of storage period and it was found to be the highest on 11th day of storage i.e. the last day of storage.

Table-3:- MEAN VALUES OF POD AVTIVITY OF DIFFERENT EXPERIMENTS

DAYS	EXP.1	EXP.2	EXP.3	EXP.4	EXP.5	EXP.6
1	0.16m	0.17n	0.18t	0.19o	0.21h	0.17j
2	0.181m	0.17n	0.18t	0.19o	0.21h	0.20ij
3	0.181m	0.17n	0.18t	0.18o	0.25gh	0.22hi
4	0.19klm	0.17n	0.18t	0.19o	0.27efg	0.24fg
5	0.20jklm	0.18mn	0.18t	0.19o	0.28efg	0.26fg
6	0.22jklm	0.18mn	0.18t	0.19o	0.31def	0.28ef
7	0.22ijklm	0.191mn	0.18t	0.19o	0.33cde	0.29de
8	0.25hijkl	0.191mn	0.19st	0.19o	0.34bcd	0.31cd
9	0.26hij	0.20klmn	0.19rst	0.19o	0.36abc	0.34bc
10	0.26hij	0.21klmn	0.19rst	0.19o	0.37abc	0.36b
11	0.26hij	0.22klmn	0.19rst	0.20o	0.38ab	0.40a
12	0.27hij	0.24jklm	0.20qrst	0.20o	0.38ab	
13	0.27hij	0.24jklmn	0.22oqrst	0.20o	0.38ab	
14	0.28ghi	0.24jklmn	0.22pqrst	0.20o	0.39a	
15	0.28ghi	0.26hijklmn	0.22pqrst	0.21no	0.40a	
16	0.28ghi	0.26hijklmn	0.22pqrst	0.21no	0.41a	
17	0.28ghi	0.27hijklmn	0.24opqrs	0.22mno		
18	0.29ghi	0.27hijklmn	0.24opqrs	0.23mno		
19	0.29ghi	0.28ghijkl	0.24opqr	0.251mno		
20	0.31fg	0.28ghijkl	0.25mnop	0.25klmno		
21	0.34fg	0.28ghijkl	0.26mnop	0.26klmno		
22	0.38ef	0.28ghijkl	0.26lmnop	0.26klmno		
23	0.43de	0.29ghijk	0.28klmno	0.26klmno		
24	0.48cd	0.33fghij	0.28klmno	0.27klmno		
25	0.52bc	0.34fghi	0.29jklmno	0.27klmno		
26	0.57ab	0.35fgh	0.29jklmno	0.27klmno		
27	0.62a	0.37efg	0.29jklmno	0.27klmno		
28		0.40ef	0.30ijklmn	0.27klmno		
29		0.42def	0.30ijklmn	0.27klmno		
30		0.47cde	0.31hijklm	0.28klmn		
31		0.50bcd	0.31hijklm	0.28klmno		
32		0.54abc	0.32ghijkl	0.31jklmn		
33		0.60ab	0.32ghijkl	0.32jklm		
34		0.64a	0.32fghijk	0.33ijkl		
35			0.33fghij	0.34ijkl		
36			0.33fghij	0.34ijk		
37			0.34efgi	0.34ijkl		
38			0.35efghi	0.35ijkl		
39			0.36efg	0.36hijk		
40			0.37def	0.40ghij		
41			0.38de	0.43fghi		
42			0.42cd	0.46efgh		
43			0.46c	0.50defg		
44			0.52b	0.53cdef		
45			0.59a	0.56bcde		
46				0.58abcd		
47				0.62abc		
48				0.65ab		
49				0.68a		

Mean values sharing same letters are non significant to each other.

The biochemical changes brought by the POD enzyme in the mango fruit has direct relation with the appearance of the fruit. With the increase in the activity of this enzyme during storage at 29°C, the colour, flavour, taste and texture started to improve and highly acceptable eating quality was observed upto 23,29,39,41,12 and 8 days in the experiments Nos. 1,2,3,4,5 and 6 respectively. After these periods, the overall acceptability of this fruit started to decline with the advanecment of storage days.

Zauberman *et al.*, (1988) reported almost the same results and found an increase in POD activity in mangoes with the advanecment of ripening at room temperature. The results of dirrerent experiments showed that the concentration and the activity of POD remained lower and static at low temperature i.e. 17°C but at higher temperature (29°C) an abrupt invrease in activity was observed.

Mattoo *et al.*, (1968) also observed that with the rise in oxidative activity, the enzymes such as catalase and peroxidase increased considerably during ripening. Similarly Haard (1977) found that peroxidase was the most stable enzyme in plants and contributed to the deteriorative changes regarding colour, flavour, texture and nutritional value after certain period. Gasper *et al.*, (1982) reported that peroxidase where mainly localized in cell walls and vacuoles and had vital role to affect the biochemical changes in mangoes. Therefore, these results are agreeable with reported findings.

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NUTRITIONAL EVALUATION OF BREAD WASTE

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ABSTRACT

Bread waste (BW) was procured from local manufacturing plant and analysed for its chemical constituents. This waste was incorporated in broiler feed at the rate of 5 and 10 % by replacing wheat waste. Results of this investigation indicated that incorporation of BW imporved weight gain and feed efficiency of the broilers.

INTRODUCTION

Rapid changes in the life style of people has lead to changes in the food habits. Fast foods are becomming more popular among the workig class. Consumption of plant manufactured bread has replaced the traditional "Chapati", consequently bread industry has developed tremendously. Due to varying seasonal changes and unhygenic conditions at the general shopping stores, quite a large quantity of bread is returned to production plants as stale bread, which ultimately goes to waste. It has been estimated that around 8% of the total bread produced in wasted.

This waste contains precious nutrients like protein, fat, ash and carbohydrates in reasonable amounts with high biological value that can be utilized to replace grains in rations of all classes of livestocks (Ghol, 1975). According to Dale (1995) dried bakery waste contains crude protein 10.7%, ether extract 12.7%, crude fibre 1.3%, ash 4.4%, calcium 0.14%, phosphrous 0.26%, potassium 0.53% and magnesium 0.26%.

Inspite of good nutritive profile BW is not commonly used for livestock and poultry due to fungal contamination. Fungal contamination can be eliminated by amonia treatment (Shah, 1985) and made it safe for poultry ration. Present study was conducted to determine the nutritive value of BW and to see its positive effects through incorporation into the poultry feed.

MATERIALS AND METHODS

Bread waste was procured from a local plant, dried and analysed for protein, fat, ash, moisture and aflatoxin following A.O.A.C. (1984) methods. Ammonia treatment was given following Shah (1985) methot to remove the aflatoxin from bread waste. Three experimental feeds were formulated, which were desiginated as A, B and C. Feed A served as control, feed B contained 5% and feed C contained 10% bread waste (Table-1).

Table-1:- COMPOSITION OF EXPERIMENTAL FEEDS

Ingrdients	Feeds		
	A (Control)	B (5% Bread Waste)	C (10% Bread Waste)
Maize	25.00	25.00	25.00
Wheat Waste	10.00	5.00	—
Rice Brother	15.00	15.00	15.00
Rice Polshings	8.00	8.00	8.00
Blood Meal	2.00	2.00	2.00
Fish Meal	6.00	6.00	6.00
Corn Ghten Meal	2.00	2.00	2.00
Corn ghuten Meal	4.00	4.00	4.00
Cotton Lead Meal	8.00	8.00	8.00
Rapseed Meal	4.00	4.00	4.00
Soyabean Meal	6.00	6.00	6.00
Lyroun Limestone	1.00	1.00	1.00
Molasses (cane)	3.00	3.00	3.00
Dicalcuin Phosphate	0.50	0.50	0.50
Bread Waste	—	0.50	0.50
Premix			
Total	100.00	100.00	100.00

One hunderd and twenty broiler chicks (Hubbard strain) of day old age were acquired and randomly divided into 3 groups.

Experimental room was white washed, cleaned and disinfected prior to starting the experiment. Experimental room was partitioned into different compartments. Wheat straw was used as bedding material. Fresh and clean water was made available round the clock. Feed was offered *ad bibitum*.

During experimental period following

parameters were observed; weight gain, feed consumption, feed efficiency, dressing percentage and weight of internal organs. Data collected were subjected to analysis of variance (Steel and Torrie, 1980).

RESULTS AND DISCUSSIONS

Results of chemical analysis of BW are presented in Table-2. It contained moisture 26.0 ± 2.0%, protein 10.0 ±1.0%, fat 5.0 ± 0.56%, ash 0.70 ± 0.05% and carbohydrates 58.3 ± 1.80%. Absence of aflatoxin suggested that this material can be used safely for poultry feeding.

Table-2:- CHMICAL COMPOSITION OF BREAD WASTE

Constitutents	Bread Waste
Moisture%	26.0 ± 2.00%
Protein%	10.0 ± 1.00%
Fat%	5.0 ± 0.56
Ash%	0.7 ± 0.05
Carbohydrates%	58.3 ± 1.80
Aflatoxin	Negative

Average weight gained by the chicks fed on different feeds (containing 5% and 10% BW) is presented in Table-3. Results indicated that incorporation of BW improved weight gain, (from 1600 to 1750 gm). This improvement may have been due to the high biological value of BW as reported by Ghol (1975). BW contains yeast protein which is beneficial for poultry growth as has been reported by various research workers who incorporated yeast in poultry feed (Chaudhry *et al.*, 1995; Flores *et al.*, 1993 and Pronczuk *et al.*, 1975).

Table-3:- AVERAGE PERFORMANCE OF VARIOUS FEED GROUPS

Particulars	Feed Groups		
	A	B	C
No of Chicks	40	40	40
Duration of experiment	42	42	42
Weight of (days) the start of experiment/chick (gm)	40	40	40
Weight at the end of experiment/chich (gm)	1600	1700	1750
Total Feed consumption per chick (gm)	3800	3800	3800
Feed Efficiency	2.37	2.23	2.22
Dressing Pereentage	56.00	56.50	55.90
Heart Weight (gm)	10.00	10.50	10.00
Liver Weight	45.00	46.00	45.50
Gzzard Weight (gm)	43.00	44.00	42.50

Ali *et al.*, (1995) also used dried brewer yeast in broiler feed and reported improvement in weight gain. They further reported that incorporatin of BW has no adverse effect on the palatability of feed.

Feed efficiency of different experimental groups indicated that addition of BW improved feed utilization in the conversion of meat. This might be due to the yeast (single cell protein) which is highly digestible. Carbohydrate profile of BW is of high biological values. Dressing percentage data indicated that BW had no detrimental effect on the performance of broiler and no adverse effect was obsrved in the internal organs. It can be concluded that BW can safely be incorporated in the poultry feed replacing wheat waste.

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MILK FOR NUTRITION

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MILK FOR ALL

Nature has endowed milk with the attributes of an ideal food (Tables 1and 2). It is highly nutritious containing all, well almost all, what is essential for development of the growing-ones and maintenance of the grown-ups, both male and female. Milk is in fact a concentrated food designed to produce rapid growth in young mammals and contains more solid material than many of our other common foods. Milk constituents, inclusive of major and minor categories, are easily digestible and are in such assimilable state as can directly meet the needs of human body. The storehouse of supplies in milk include the energy-giving lactose and fat, the body-building proteins, the bone-forming calcium and other minerals, and the health-giving vitamins. Indeed, milk is not just the food for infants and growing children as per the usual perception, it is an important food adjunct for the active adolescents and adults as also for pregnant mothers, invlids, convalescents and the sick.

MAJOR MILK CONSTITUENTS

Water. Milk is mainly water, about 80-87 percent of it. It contains dissolved or suspended within, the milk constituents or total solids as these are usually called, serving as the physiological medium of their active deposit and for their transport wherever needed in the body. Most of it is "free" with only a minor amount bound to lactose, proteins, salts and phospholipids for their maintenance in active state of assimilation and nutrient availability.

Lactose or the Milk Sugar. Except trace amounts of other sugars, lactose for most purposes can be considered as the only carbohydrate source in milk, respectively, accounting for about 5.5% in buffalo, 4.9% in cow and 7% in human milks. Unlike sucrose, the common white sugar which is a compound of fructose and glucose, lactose is made up of a molecule each of glucose and galactose. It is mildly sweet, being only a quarter as sweet as sucrose at its usual concentration in milk. Bacteria ferment lactose to lactic acid, which under controlled conditions is of benefit for the production of yoghurt and buttermilk, but is otherwise the major cause of milk spoilage or sour curdling. Lactose is a direct source of energy for the body. The indirect nutrient role of lactose is through its fermentation to lactic acid by the useful intestinal becteria causing acidity in the digestive tract. This on

Table-1:- AVERAGE COMPOSITION AND CALORIFIC VALUE OF INDIVIDUAL CONSTITUTENTS OF WHOLE MILK OF COW, BUFFALO AND HUMANS. VALUES WITHIN PARANTHESIS ARE THE ENERGY VALUES EXPRESSED IN KILO JOULES

Major Constituents (%)						Calorific Value/ 100 ml Whole milk (kcal)			
Milk Source	Water	Fat	Protein	Lactose	Ash	Fat	Protein	Lactose	Whole Milk
Cow	87.2	3.7	3.5	4.9	0.7	33.3 (137 kJ)	14.0 (59 kJ)	18.4 (78 kJ)	65.7 (274 kJ)
Buffalo	82.7	7.4	3.6	5.5	0.8	66.6 (274 kJ)	14.4 (61 kJ)	20.6 (88 kJ)	101.6 (423 kJ)
Human	87.4	3.7	1.7	7.0	0.2	33.3 (137 kJ)	6.8 (29 kJ)	26.3 (112 kJ)	66.4 (278 kJ)

Table-2 AVERAGE VALUE OF TRACE ELEMENTS IN COW MILK

Ash Minerals (mg/100ml whole milk)		Trace Elements (μg/100ml whole milk)	
Calcium	123	Cadmium	2.6
Magnesium	12	Chromum	1.5
Phosphorus	95	Copper	13
Sodium	58	Iodine	4.3
Potassium	141	Iron	45
Chlorine	119	Manganese	2.2
Sulphur	30	Molybdenum	7.3
Citrate	160	Nickle	2.7
		Zinc	390

the one hand checks the growth of proteolytic bacteria thus inhibiting putrefaction, while on the other causes increased calcium absorption and improves utilization of other minerals.

Milk Fat. This is the most variable constituent in the milk composition not only in respect of the different sources of its origin but also within the same animal. Its percentage may thus range between 6.5-8 in buffalo (even 12.6 in the Chinese buffalo), 3.7-4.6 in cow and 3.6-3.7 in humans. Variability notwithstanding, the typical flavour and taste of milk, its soft body and smooth texture are due to the fat it contains. Even greater dietary significance of fat is its own profile comprising a mixture of 96% energy-giving triglycerides, peculiar to milk. Remainder of the milk fat fraction includes phospholipids, sterols (mainly cholesterol), traces of free fatty acids, and the fat-soluble vitamins A, D, E and K. The presence of essential fatty acids such as linoleic and linolenic acids, from which the body synthesizes hormones, and which if deficient may cause skin disorders in infants, makes consumption of milk fat necessary. Phospholipids have their significance in the blood-clotting process, as structural elements in the cell membrane, in the energy transfer mechanism, and in the nervous tissues. Cholesterol in minor amounts, likewise, is essential for the brain tissues, productin of adrenal hormones and bile acid. Bulk of the fat makes emulsion with water as small globules bounded by an absorption layer made up of a complex between phospholipids and proteins. This so-called globule membrane stabilizes the emulsion and prevents the fat globules from coalescing. These fat globules in the undisturbed milk tend to rise to the surface forming a layer of cream. Higher fat contents, as in buffalo milk, result in larger globules rendering the cream layer thick. Emulsion in the globule breaks on heating, freezing, and low temperature agitation. This property is of industrial importance, for the production of butter fat has been found void of detectable phospholipids and is thus nutritionally different from milk fat.

Milk Proteins. Adequate supply of proteins in the diet is necessary as sources of amino acids which are required for the building and maintenance of tissues. These are also involved in the production of enzymes, some hormones and antibodies. Proteins also help in the regulation of metabolic processes. The building blocks of proteins are the 20 odd amino acids and depending upon what sequence these are linked together determine the kind a particular protein is. The hair protein, keratin, is thus different form the milk protein, casein. Containing all the essential amino acids in fairly large quantities, the milk proteins are of high quality. Compared with carbohydrates and fats, proteins have a very complex molecular structure. The principal protein of milk, casein, makes up 80% of the total, while lactalbumin and lactglobulin, also called the whey or serum proteins, make up the remaining 20%. The protein fraction includes also the milk enzymes. Casein in milk is present as the calcium ceseinate-phosphate complex in the colloidal state. It coagulates in acidic or alcoholic medium, on heating, or when treated with rennet. These attributes are of significance in the commerce of milk and milk products. Protein percentage in cow milk is 3.5, in buffalo milk 3.6 and human milk 1.7. Also contained within the milk serum are a number of free amino acids and peptides, which are of dietary significance in their own right.

Ash or Milk Salts. Included in this fraction are the salts of milk, which though present in samll quantities exert considerable significanec on the nutritive value of milk. The ash in fact represents the minerals contained in milk, which are various salts of potassium, sodium, magnesium, calcium, phosphate, citrate, chloride, sulphate and bicarbonate. Some mineral salts occur as trace solutions, while a part is present in colloidal state. All the milk minerals are essential for nutrition. For example milk is an excellent source of calcium and phosphorus, both of which, together with vitamin D, are involved in bone formation. Ash percentage in cow, buffalo and human milk is , respectively, 0.7, 0.8 and 0.2. Altough the salts comprise less than1% of milk, they play a very important role in the milk and milk product industry since they influence the satbility of milk to heat and alcohol coagulation, the thickening of sweetened condensed milk, the coagulation by rennin in cheese making, and the clumping of the fat globules on homogenization.

MINOR MILK CONSTITUENTS

Vitamins. Though present in minute quantities, vitamins are vital for growth and maintenance of healthy body. Milk contains both water soluble and fat soluble vitamins. Those that are water soluble include vitamins of B complex such as thiamine (B_1). riboflavin (B_2), niacin, pyridoxine (B_6), biotin, folic acid and cyanocobalmine (B_{12}), while to the fat-soluble category belong vitamins A,D,E and K. Deficiency of vitamin A causes cessation of growth, defects in the teeth, disturbances in bone growth, degeneration of sight. Vitamin D is necessary for the absorption of calcium and phosphorus to bulid strong bones and teeteh; the deficiency thus causes bone softening (rickets) in children and osteoporosis in adults. Vitamin E deficiency interferes with the reproductive system leading to sterility; causes degenerative diseases of the nervous system and damage to the liver. Vitamin K is involved in blood clotting and its deficiency thus causes haemorrhagic condition and liver damage. Symptoms of thiamine deficiency include loss of appetite and weight, nervous system disorders, gastro-intestinal disturbances, heart enlargement and general weakness. With riboflavin deficiency are linked itching, lesions and fissures of the skin, eyes, corners of mouth and folds of the nose, Niacin deficiency causes mouth ulcers, intestinal inflammation and mild mental disturbances leading to insanity. Vitamin B_6 is required for the prevention of decreased growth rate, skin diseases, anaemia and convulsions. Folic acid is essential for the normal red blood cell development and thus prevention of anaemia. Biotin deficiency leads to depression, muscle pain, loss of appetite, and skin diseases. Vitamin B_{12} is necessary for growth and formation of red blood cells, therfore, its deficiency results in pernicious anaemia.

Trace Elements. A large number of elements occur in such low quntities that these are reported as parts per million (ppm) or as microgram in a litre of milk. The fact that these are present in trace amounts does not belittle their importance since these are possessed with remarkable physiological and nutritional qualities. Though the list of trace elments in milk is long, cobalt, chromium, copper, iodine, iron, manganese, molybdemum, nickle and zinc are of greater nutritional significance. Cobalt is important in nutrition as it forms the nucleus of vitamin B_{12}. Chromium (within limits; more is toxic) is needed for the production of insulin in the human body and is thus of significance to some diabetics. Copper is associated with a number of enzymes including those related with electron transport chain in the respiratory metabolism and with a copper-containing protein present in red blood cells. Copper deficiency can cause anaemia and other physiological disorders. A deficiency of iodine causes enlargement of the thyroid gland, a condition known as goiter. Iron is important as constituent of haemoglobin and some other respiratory enzymes. Manganese is an integral part of a liver enzyme and it activates a number of other important metabolic enzymes. Molybdenum, nickle and zinc are likewise constituents of a number of enzymes. Zinc deficiency is now being widely recognized to have a rloe in heart ailments, cancer and pregnancy disorders.

Pigments. Milk contains a number of pigments. Carotene and to some extent xanthophyll, the fat soluble pigments, are responsible for the characteristic yellow colour of milk, cream, butter and other milk products. One melecule of β-carotene, additionally, yields two molecules of vitamin A. Cow milk contains more carotene than buffalo milk and is thus more yellow. Riboflavin, besides being a vitamin, is the greenish yellow pigment that gives skim milk and whey their cheracteristic colour. The white or milky appearance of milk is caused by the scattering of reflected light by the fat globules, the colloidal calcium caseinate, and the colloidal calcium phosphate in the milk.

Enzymes. Milk contains a number of enzymes. Their role in nutritive quality of milk is basically indirect. For example, lipase breaks down fats to glycerol and fatty acids, protease acts on proteins to yield amino acids and amides, and alkaline phosphatase converts organic phosphates into phosphoric acid. Enzymes are sensitive to heat. Thus longer the milk is held unheated after milking the more it continues to spoil.

Acidity. Not strictly a constituent by itself but a sum manifestation of its total composition, fresh milk is slightly acid in reaction. Because of many variables, the acidity of fresh milk, having pH of 6.5-6.7, varies between 0.15-0.18% expressed as lactic acid. Acidity values are indicators of the quality of milk, whether it

is going through the spoilage process or it has been adulterated with alkali to unlawfully hide spoilage of bad milk.

MILK ENERGY

Loctose, fat and protein are the energy giving constituents of milk. These on conversion during the respiration process generate metabolic energy. this energy is needed by the body for growth, elimination of wastes by kidney, maintenance of body temperature at 37°C (98.6°F), muscular movements, and maintenance of salt balance. When intake of nutrients is more than the energy needs of the body, the excess is stored as fat or glycogen. Food encergy is expressed as Calories (Cal) or kilocalories (kcal), which is defined as the amount of heat required to raise the temperature of one kilogram of water by 1°C. The international unit of food energy is kilo Joule (kJ), which is about 4.2 times the kilocal value. Since the percentage of constituents in milk is variable no exact energy value can be assigned to it. Nutritional energy of a gram each of fat, protein and lactose are, however, known which respectively are kilocals 9,4 and 3.75 (= kJ 37,17 and 16). Milk energy can be calculated by multiplying these factors with the percentage values of various constituents present in any given milk. Based on this, kcal values have been computed in Table1 based on average compositon of whole milks of cow, buffalo and humans. Evidently buffalo milk is nutritionally the richest, while the energy profile of human and cow milks is comparable.

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CHEMISTRY AND TECHNOLOGY OF DEEP FRYING OILS

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Frying has been a traditional and long used method of cooking. There are two types of frying methods;

a) Shallow frying
b) Deep frying

In shallow frying the quantity of frying oil used is usually small. Shallow frying is done at lower temperatures e.g. frying of egg, shami kabab, pratha etc. Therefore the quality degradation of frying oil during shallow frying is minimum. Moreover, consumers do have preferences for certain types of edible oils for reasons of taste or flavour e.g. butter, peanut oil, mustard oil and coconut oil etc. Therefore, any available oil of preference can be used for the process of shallow frying.

On the contarary, the deep frying process is carried out at elevated temperatures ranging from 160 - 195°C and the food is submerged in the frying oil. The contact time between the food and the frying oil is short. The frying oil is used for the longer periods continously or intermittently. Moreover, deep frying is intended to provide special organoleptic and other desirable properties to the food.

* Act as a heat tranfer medium
* Rapid & deep cooking of food
* Impart colour, flavour, taste and texture to the food
* Become integral & nutritional ingredient of the food
* Improve the shelf-life of the food

In view of the nature of the process, all oils and fats cannot be used as a medium for deep frying. Only those oils/fats can be used which have the physical and chemical properties to withstand the sternness frying conditions or those oils/fats which have been modified or objectively and technically blended to create special functional properties for the purpose of deep frying.

Deep frying is commonly used in the traditional catering restaurants, fast food chains and in industrial frying operations such as potato chips, instant noodle etc.

DEEP FRYING

Deep frying is a complex process. The changes that occur during this process are depicted in fig (I);

* Water vaporization
* Gelatinzation
* Protein denaturation
* Texture changes
* Cooking of food
* Degradation of frying oil
* (Thermal, oxidative & hydrolytic)

STAGES IN FRYING

Stage I - When surface of the food is submerged in hot oil. Immediately the water vaporization starts.

Stage II - Surfacee boiling takes place. Foodstuff starts to form crust on its surface.

Stage III - More internal moisture leaves the food and internal temperature rises to boiling point. Gelatinazation and cooking take place in the

internal part.

Stage IV - After extended frying food gets brown and bubble end-point is reached.

CHEMISTRY OF FRYING

During the deep frying process the oil is exposed continuously and repeatedly to the elevated temperatures in the presence of air, moisture and food. Oxidation, hydrolysis and thermo decomposition of the frying oil take place resulting in degradation of the frying oil. As the reactions proceed, the degradation of the frying oil becomes intense and affects the functional, sensory and nutritional qualities of frying oil as well as that of the food prepared in the degraded frying oil. The complex chemical changes taking place in the deep frying operation are given in fig (II).

The decomposed products of the frying are of two types i.e.

a) Volatile decomplosed products (VDP)

i) Some are retained by the food
ii) Inhaled by the fryer operator
iii) Contribute to the surrounding room odours
iv) Form deposits on the exhaust hood

b) Non Volatile Decomposed Products (NVDP)

NVDP formation is due to the thermal oxidation of unsaturated fatty acids. NVDP are responsible for physical changes in the frying oils such as increases in viscosity, colour, foaming and decrease in smoke point etc. The associated chemical changes increase FFA, carbonyl value, hydroxyl value, polar compounds, polymerised products and decrease in unsaturation (I.V.),.

Table (I) depicts the ups & downs of quality parameters during the frying process.

SELECTION OF FRYING OIL

The selection of frying oil is a technical process and requires balanced approach keeping in view the following factors:

* Trading & inherent qualities of the oil
* Frying life required
* Product appearance
* Eating quality of the fried food
* Specific product requirements
* Ease of handling
* Availability & cost

QUALITY ASPECTS

The quality of dfrying oil is audited for suitablity at three stages;

* Buying
* Before frying
* During frying to determine the discard point

Generally the quality of deep frying oil is adjudged from the fatty acid composition. The oil should have minimum level of polyunsaturation. The fatty acids like linoleic ($C_{18:2}$) and linolenic ($C_{18:3}$) are highly susceptible to oxidation and subsequent deterioration due to the number of double bonds present in the carbon chain of the oil molecule. The deep frying oil with higher contents of $C_{18:2}$ & $C_{18:3}$ will degrade rapidly at the elevated frying temperatures and will form and accumulate concentrations of polar and polymerised compounds. Such compounds are undesirable from the point of view of frying life of oil, food quality and nutritional safety.

In some countries the legislation requires that $C_{18:3}$ level in the frying oil should not exceed 2%. Infact, oils with $C_{18:3}$ contents exceeding 2% cannot be marketed and sold as "frying oils" Similarly, during frying the concentrations of polar and polymerised compounds should not exceed 25% and 10% respectively.

It is also to be ensured that the frying oil

is properly and adequately refined to remove phospholipids, gums, pro-oxidant trace metals and other breakdown compounds from oxidative and hydrolytic processes such as FFA, PV etc. Frying oil should have good clean colour and flavour with smoke point in excess of 210°C and flash point above 316°C. The oil quality must be protected with suitable antioxidants and stored under shade and in an airy place with storage temperature not exceeding 26°C.

Table (II) provides information on quality parameters for frying oil to be controlled and monitored at various stages. While Table (III) & Table (IV) describe the quality of oil before frying and during the frying operation respectively.

OIL DISCARD

After the prolong use the degradation of oil and the food quality reaches a stage where recearch it has to be discarded. There are various ways of judging the discard point of frying oil. Following are some of the imporatnt criteri a to assess the end-life of oil:

* Darkening of colour
* Excessive foaming
* Excessive smoking
* Thickening of oil
* Objectionable smell and off flavours
* Half cooked fried food
* Greasiness of food
* High level of FFA (>0.5%)

STATUS OF PALM OIL AS "FRYING OIL"

The fatty acid composition of Palm oil / Palm olein suggests that these products are ideally suitable for industrial deep frying. The level of undesirable polyunsaturated fatty acids is low and well within the dsesired limits i.e. $C_{18:2}$ level ranges from 6 - 10% and $C_{18:3}$ is less than 0.3%. AOM stability at 100°C is more than 40 hours which could be further enhanced by partial hydrogenation.

Table (V) & Table (VI) provide technical properties and specifications for Palm olein offered for industrial deep frying operations as well as for products like Palm oil, partially hydrogenated Palm oil and Palm olein.

Collaborative study carried out by Palm Oil Research Insitute of Malaysia, Marmara Scientific & Industrial Research Centre and the Yldz Technical University of Turkey comparing commercial samples of mono-unstaurated oils (Palm olein and olive oil) with poly-unsaturately oils (sunflower and soyabean) to deep fry potato chips (French Fries) in large scale laboratory trails. The oils were tested in parallel fryers on five consecutive 8-hour days.

At the end of each frying day, residual oil in each fryer was filtered and the oil level was topped up using fresh oils. The qualities of the starting oils were compared (Table - VII). The performance of the oils was assessed by using the physical and chemical parameters. The results of the trails are summarized in (Table - VIII). Poly-unsaturated oils scored high in some of the simple tests particularly at the starting stage such as colour, PV, FFA, smoke point etc. The mono-unsaturated oils scored high in more sophisticated and subjective tests under the frying conditions such as polar compounds, polymer contents, foam hieght and anisidine value etc. Palm olein attained leading positions in six out of the 12 assessment parameters, olive oil in five tests and soyabean oil in four tests.

Similar study on commercial oils was independently done by the Central Food Control & Consultant Laboratory of Sharjah Municipality using commercially available oils from the market. These oils were subjected to oxidative stability test by placing in the oven at 100°C and monitoring the peroxide value at 2 hours intervals. The results of this stability test are given in Fig (III) which confirm that poly-unsaturated oils like SFO, SBO & CO have reduced stability at elevated temperatures whereas palm oil and palm olein were found to be far more stable.

The above studies support the large scale use of palm olein in commercial practice as frying oil in the catering restaurants and fast food industry of the Middle East Countries, Europe, Far East and the Pacific Countries.

CONCLUSIONS

1. Industrial frying is a complex chemical process which is regulated through the controls on quality of oil & fat to be used, the food to be fried and the fryer's operating conditions.

2. The quality audit for frying oil at the time of manufacture, before use and during use is important and compulsory.

3. Deep frying oils are technical products specially tailored to suite the functional reuirements for the deep frying process.

4. Palm oil / palm olein is technically and commercially suitable oil for deep frying.

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Table (I):- UPS & DOWNS OF DEEP FRYING

PARAMETERS	UPS	DOWNS
Colour	↑	–
FFA	↑	–
PV	–	↓
AV	↑	–
Totax	↑	–
Viscosity	↑	–
Polymer contents	↑	–
Oxidised Fatty Acids	↑	–
Saturation	↑	–
R.I.	↑	–
Foam formation	↑	–
Foam persistence	↑	–
Polar compounds	↑	–
Smoke point	–	↓
Flash point	–	↓
Poly unsaturation	–	↓
IV	–	↓
Antioxidants level	–	↓
AOM	–	↓
Frying life	–	↓
Oil quality	–	↓
Food quality	–	↓

Table (II):- FRYING OILS
Three stages of quality audit:

a) Buying
b) Before Frying
c) Discard poing

Quality Parameters	a	b	c
Appearance	√	√	√
M & I	√	√	–
FFA	√	√	√
PV	√	√	–
I.V.	√	–	√
Taste	√	√	–
Flavour / Odour	√	√	√
Colour	√	√	√
smoke point	√	√	√
Acid value	–	√	√
Induction period	√	√	–
Trace metals	√	√	–
Antioxidants	√	–	–
Additives	√	√	–
Carbonyl value	–	–	√
Polar compounds	–	–	√
Polymerized compounds	–	–	√

Table (VIII):- SUMMARY OF TEST RESULTS
ORDER OF PERFORMANCE (BEST TO WORST LEFT TO RIGHT

Colour	SBO	SFO	POo~OO	-
Free fatty acid	SBO	SFO	POo	OO
Smoke point	SBO	SFO	POo	OO
Unsat: FA reduction	OO	SBO	SFO	POo
Peroxide value	OO=SBO	-	SFO	POo
Induction period	POo	SBO=SFO	-	OO
Totox	OO	POo	SFO=SBO	-
IV reduction	OO=POo	-	SFO	SFO
Anisidine value	OO=POo	-	SFO	SBO
Foam height	POo	OO	SBO	SFO
Polymer content	POo	OO	SBO	SFO
Polar compounds	POo	OO	SBO=SFO	-

Soyabean oil = SBO
Olive oil = OO
Palm = POo
Sunflower oil = SFO

Table (VII):- PROPERTIES AND COMPOSITION OF THE FRYING OILS BEFORE USE

Characteristics	Palm Olein	Olive oil	Sunflower oil	Soyabean oil
Colour				
(1 inch Lovibond cel)				
Red	1.2	1.0	0.7	0.5
Yellow	7.2	8.2	2.0	3.3
Peroxide value	0.7	6.3	1.6	3.3
(PV: meg/kg)				
FFA (%)	0.06	0.43	0.04	0.05
Iodine value	59.6	84.1	128.8	130.4
(IV, Wijs method)				
Induction period (h)	40.0	12.3	8.2	10.0
Smoke point (°C)	220	195	230	229
Polar compounds (%)	9.0	9.1	5.5	7.1
Fatty acid composition				
12:0	0.4	-	-	-
14:0	1.3	-	-	-
16:0	40.3	13.0	6.3	10.7
18:0	3.2	2.6	4.0	3.1
20:0	0.4	-	0.3	-
16:1	0.1	0.5	0.1	-
18:1	43.5	71.2	19.2	18.9
18:2	10.6	12.1	70.0	61.1
18:3	0.2	0.6	0.1	6.1
IV (Wijs method)	59.6	84.1	128.8	130.4
IV (Calculated)	56.4	84.2	138.1	138.0

* TUBITAK Studies

Table (IV):- FRYING OILS & FATS (DURING FRYING)

Quality Parameters	Not Less	Limit	Not More
FFA (%)	-		0.4
Acid Value mg KOH/gm	-		2-2.5
Carbonyl Value	-		50
IV diff (Unused - oil)	-		16
Smoke point (°C)	170		-
Oxidized Fatty Acids (%)	-		0.75-1.0
(PE Insoluble)	-		10
Polar Compounds (Total)	-		
*Viscosity (m Pa - Sec at 50° C)			
Fats	-		37
Oils	-		27
Frying temp (°C)			
Peak Hrs	160		190
Lay off Hrs	90		120
Odour & Taste	-		Objectionable
Others			
Must satisfy	-		Sensory evaluation
Oxifrit Test	-		Below 3 (Scale 1-4)
Fritest (carbonyl compounds)	-		Max 2 (Scale 1-3)
Food oil Sensor	-		Below 4 (Scale 0-6)
(Redox indicator)			
Law forbids frying in equipment without temperature controls			

* DPTG - Dimeric & polymeric triglycerides

FUNCTIONS OF FRYING OIL

* Act as a heat transfer medium
* Rapid & deep cooking of food
* Impart colour, flavour, taste and texture to the food
* Become integral & nutritional ingredient of the food
* Improve the shelf-life of the food

Table (III):- FRYING OILS & FATS (BEFORE USE)

Quality Parameters	Not Less	Limit	Not More
Moisture (%)	-		0.1
FFA (%)	-		0.1
PV (meq/keg)	-		2.0
Acid Value mgKOH/mg	-		-
Solid fat contents (%)	-		50
Linolenic Acid contents (%)	-		2
Meltin point (°C)	38		49
Smoke point (°C)	205		-
Induction period (hr)	40		-
Others			
Odour & flavour	-		Clean & Acceptable
Colour (when melted)	-		Bright & Shiny
Antioxidants	-		BHT, BHA, TBAQ Tocopherols, gallates & polysorbates
Additives	-		0 - 3 ppm
* Dimethyl-Siloxane (Silicon)			
Frying oils must be prepared with GMP			
Frying oils must comply food regulations / standards			
Must be labeled as "frying oil/fat"			
Packing Size (min) 5 kg			

* (Not permitted in some countries such as France, Sweden, Switzerland etc).

Table (VI):- PROPERTIES OF PALM OIL (REFINED) (FOR FRYING PURPOSES)

Characteristics	P.O. Value	H.P.O. Partially	H.P. Olein Partially
Acid value	0.04	0.04	0.04
PV (meg/kg)	0.34	0.3	0.3
M. Pt (°C)	37.3	38-40	30-32
IV	52.4	46-48	50-52
Colour (Lovibond 1"	R 1.1	R 0.8	Y 8.0
	Y 11	Y 8	Y 8.0
Tocols Contents (ppm)	104	-	-
AOM value (hr)	62	100-130	80-110
SFc (%)			
10°C	45.5	62	51
20°C	24.8	40	24
30°C	7.8	16	5
FAC (%)			
C14	1.1	1	1.0-1.5
C16:0	42.3	42-45	39-41
C18:0	5.2	5-7	6
C18:1	39.9	45-48	47-51
C18:2	10.4	3-5	4-6

TYPES OF FRYING

SHALLOW FRYING

Quantity	:	Small
Temperature	:	Low
Contact time	:	Short
Usage time	:	Short
Preference	:	Taste & flavour

DEEP FRYING

Quantity	:	Food submerged in oil
Temperature	:	High (165 - 195°C)
Contact time	:	Short
Usage time	:	Long hours
Preference	:	- Stable oil - Low cost

Table (V):- SPECIFICATIONS FOR PALM OLEIN
(FOR DEEP FRYING INDUSTRY)

M & I	=	0.05% Max
FFA	=	0 1% Max
PV	=	1.0 meg/kg
IV	=	50-60
Rancimat	=	1000 Min at 120°C 40 Hrs at 100°C
Lovibond Colour	=	Red 2.5] Yellow 20] 5 1/2" Cell
Antioxidant (TBHQ)	=	200 ppm
Taste	=	Bland
Smell	=	Odourless
Cu	=	<0.05 ppm
Fe	=	<0.01 ppm
P	=	<4 ppm

Foot Note

* Audit of the oil-supplier must be carried out.
* Audit of the snack food factory is also of vital importance.
* Should be close co-operation between supplier and food processor to control the supply and quality at all stages of production and handling aimed at producing the best quality "Food" products.

Figure (II) CHANGES OCCURING DURING DEEP-FAT-FRYING

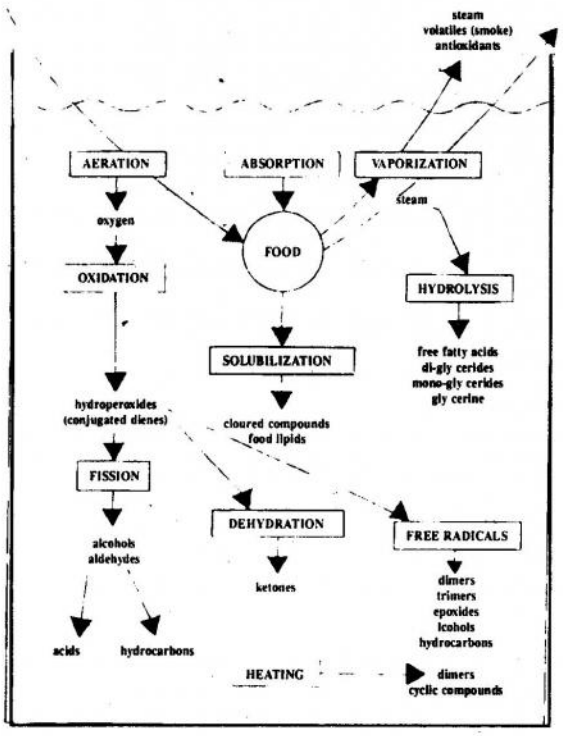
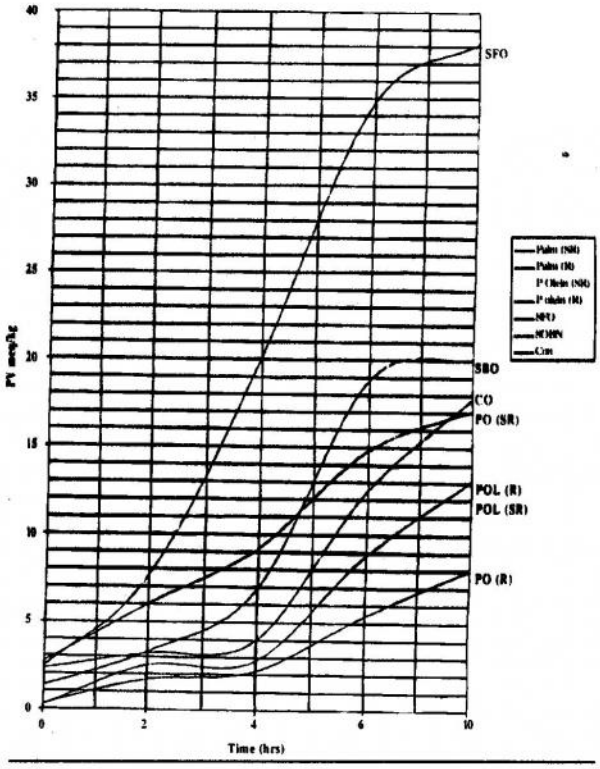
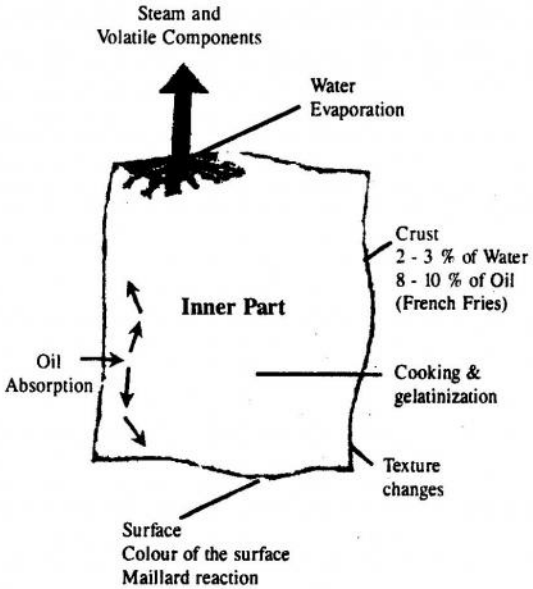


Figure (III) EFFECT OF HEATING ON THE STABILITY OF PALM OIL IN COMPARISION WITH OTHER OILS



Source: Central Food Laboratory Sharjah - UAE

Figure (I) STRUCTURE OF CROSS SECTION OF FRENCH-FRIED POTATO (FORMATION OF CRUST)



CALCIUM HOMEOSTASIS AND THE CALCIUM BINDING PROTEIN CALBINDIN

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INTRODUCTION

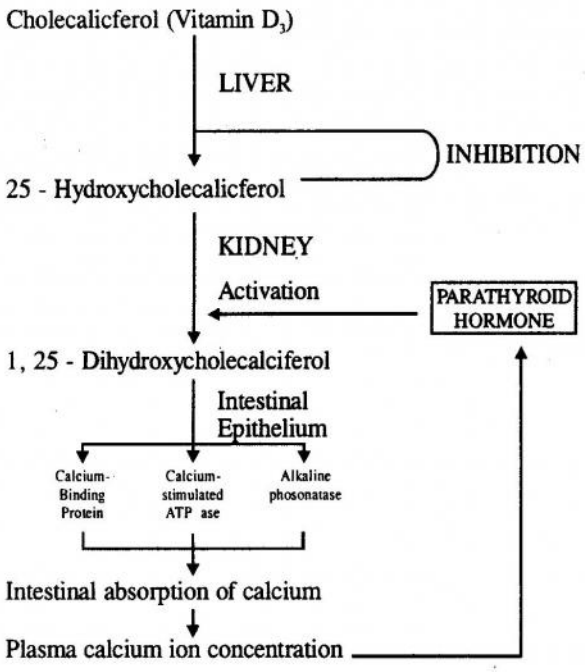
Calcium is absorbed both transcellularly and paracellularly throughout the intestine, both as ionized calcium, and as intact small molecules i.e calcium carbonate and calcium citrate (Karbach, 1992). Transcellular absorption is a saturable process and occurs in the duodenum and jejunum where the pH is more acidic and calcium binding protein, calbindin is present. Absorptive efficiency in this portion of the intestine varies inversely with calcium intake. Less calcium is absorbed with higher intakes and vice versa.

A number of physiological factors affect calcium absorption in the body via altering the calbindin synthesis. For example high vitamin D, calcium and/or phosphorus deficiency, and individual's age (Miller, 1995) are the factors that increase calcium absorption. While factors such as vitamin D deficiency, menopause and old age are responsible for decreased calcium absorption. The presence of oxalates, phytates or pectates also hinder calcium absorption by making insoluble complexes with the calcium. Spinach and wheat bran have low calcium availability due to their high oxalic acid and phytate content (Weaver *et al.*, 1987; Weaver, 1991).

MECHANISM OF CALCIUM ABSORPTION

It is well established that the maintenance of normal level of blood calcium is regulated by vitamin D through its effect on the principal target tissues, *i.e.* intestine, kidney, bone and parathyroid hormone (PTH). In response to low calcium, vitamin D is converted to its active form. The parathyroid gland secretes PTH which converts vitamin D to its active metabolite, calcitriol ($1,25(OH_2)D_3$). Activation of vitamin D occurs in two steps. In the liver it is hydroxylated at C-25 to produce 250H vitamin D_3 by a 25 hydroxylase enzyme. It is the major circulating form of vitamin D, and is further hydroxylated in the kidneys by 1-α-hydroxylase to produce 1-$25(OH)_2D_3$ or calcitriol, the active form of vitamin D_3 (DeLuca and Schloes, 1983). This results in higher concentration of active vitamin D in the blood, which exerts its biological action to increase the absorption of calcium from the intestine by stimulating the synthesis of calcium binding protein, calbindin CaBP-D9K (in mammals) or CaBP-D28K (in birds) (Fig. 1).

Fig. 1 Mechanism of Calcium Absorption



Calbindin is present in all target tissues including intestine, kidney and bones (Balmain *et al.*, 1986). All these tissues contain the 1,25

dihydroxy vitamin D_3 receptor (VDR), which after complexing with vitamin D, interacts with vitamin D response elements (VDRE) in the calbindin gene. This results in the transcription of the gene producing mRNA and the protein calbindin (Darwish and DeLuca, 1992). The concentration of calbindin in chick intestine has been shown to be correlated with the extent or rate of intestinal calcium absorption (Brehier and Thomasset, 1990). It varies inversely with the dietary amount of calcium. Under severe hypocalcemic conditions, there is a massive increase in the amount of calcium binding protein as the animal works very hard to absorb what little calcium is made available from the diet. In contrast, under conditions of high calcium intake, there is down regulation in the amount of calcium binding protein and intestinal absorption of calcium is much less efficient (Norman, 1990).

CALCIUM BINDING PROTEINS-CALBINDINS

Calbindins belong to the calmodulin/ troponin C super family of calcium-binding proteins. Wasserman and Taylor discovered vitamin D dependent calcium binding proteins. Calbindin-D28K was first isolated in 1966 from chick intestine (Wasserman and Taylor, 1966) and calbindin-D9K in rat intestine was isolated in 1967 (Kalffelz *et al.*, 1967). There are now two known classes of vitamin D dependent calbindins, CaBP-D9K and Ca BP-D28K Ca BP-D9K is found in memmals including rat, mouse and humane intestive, placenta, bone and mous kidney. Ca BP-D28K is found in avian inestine, avian as well as mammalian kidney, bone and cerebellum-brain.

There are some similarities in immunological features and biological functions. CaBP-D9K has two and CaBP-D28K has four calcium binding sites binding two verses four moles of calcium per mole of calbindin, respectively, Both calbindins have high calcium binding affinity (ka=10^4 to 10^8 M^{-1}). However, they have no sequence homology (Christakos *et al.*, 1992). Antibodies raised against CaBP-D9K do not recogize avian intestinal or mammalian CaBP-D28K (Thomasset *et al.*, 1982).

INTESTINAL CaBP-D9K and D28K

Most of the calbindin in intestine is found in the duodenum, followed by the upper jejunum and none in the ileum. However, it is found in the cecum, where it is not vitamin D dependent (Mayel-Afshar *et al.*, 1988). Calbindin is a cytosolic protein found in the colunmar epithelial cells of rat (CaBP-D9K) and chick (CaBP-D28K) intestine. It comprises 2% of the soluble protein in the duodenal absorptive cells (Thomasset *et al.*, 1982). It is involved in the transepithelial transportation of calcium by ferrying it from the brush border to the basolateral membrane calcium pump (Kaune, 1996).

A close linear correlation was found between the active calcium trasport across the basolateral membrane and toal content of calcium binding protien in the enterocytes (Roche *et al.*, 1986). However, for the entry into the enterocytes, vitramin D stimulates the induction of calcium binding protein in rat (Perret *et al.*, 1985) and chick (Hall *et al.*, 1988) intestines in the same manner.

Calbindin is not only a calcium transporter across the basolateral membrance, it serves as a cytosolic calcium buffer by trapping and storing the free calcium ions when it is offered in excess concentration at certain times, thus preventing free calcium toxicity (Nemee and Norman, 1991). Age-related decline in CaBP-D9K in the rat duodenum and its reversal with vitamin D supplementation suggests that it is because of decreased circulatory vitamin D and not because of age. Rat colon CaBP-D9K, however, was not affected by age since it is not vitamin D dependent (Mayel-Afshar *et al.*, 1988).

CALBINDIN-D28K IN THE KIDNEY

The kidney is one of the major organ that plays a crucial role in the calcium homeostasis in the body. For the maintenance of a net calcium balance, more than 98 % of the filtered load of calcium is reabsorbed along the nephron via passive as well as active transport. Passive absorption occurs paracellularly.Almost 65% of calcium is reabsorbed in the proximal tubules, with an additional 20% in the thick ascending limb of loop of Henle. Passive absorption occurs in response to electrical or chemical gradients (Matkovic *et al.*, 1994).

Active transport occurs transcellularly in the distal nephron where ~10% calcium is reabsorbed. Active transport occurs against the electrochemical gradient. The distal nephron is the major regulatory site for cacium excretion in the urine. The rate of reabsorption is controlled by hormones, i.e. parathyroid (PTH), calcitonin, and 1,25 $(OH)_2$ vitamin D_3. Calbindin D-28K is localized in distal tubules and collecting ducts (Kumar *et al.*, 1994), and has an importnt role in renal calcium reabsrption. Epitopes for CaBP-D28K were found in the distal tubule starting at gestational day 19 (Johnson *et al.*, 1995) and was immunohistochemically idntified in the developing embryos on 5th day of incubation. Developmental studies show that rat renal mRNA increased between birth and one week of age, the rapid period of nephron differentiation (Verghese *et al.*, 1988).

Renal calbindin-D28K mRNA expression is regulated similarly to intestinal-D9K or avian intestinal-D28K by the calcium concentration and vitamin D. Like brain and intestine, the kidney contains mRNA species that were dependent on vitamin D for their induction (Varghese *et al.*, 1988). Maximum expression was observed at 1-2 mM extracellular in the calbindin -D28K mRNA in chick kidney cells grown in the serum free medium. A striking increase in calbindin mRNA was observed both in the presence of vitamin D (Clemens *et al.*, 1989).

The mouse kidney is unusual because it has both calbindin -D9K and D28K. Both are inducible by 1,25 dihydroxy vitamin D. However, a difference was observed in their time of response to 1,25 vitamin D when a single dose was administered. A peak of CaBP-D28K occurred at 12 hours in vitamin D deficient mice which decrease at 24 hours. While a delayed response was observed in CaBP-D9K mRNA. A peak induction occurred at 24 hours and decreased at 48 hours after 1,25 vitamin D was administered in the same vitamin D deficient mice (Christakos *et al.*, 1992).

There is an age related decline in calbindin-D28K synthesis in rat(Armbretch *et al.*, 1994) However, in another study, this decline was observed to be not because of aging per se but rather due to lowered circulatory vitamin D with aging. The kidney cells from rats of different ages (1 month, 6 months and 12 months) grown in medium not containing 1,25 vitamin D had similar amounts of calbindin regardless of age. On the other hand, the amount of calfindin was increased more in old age (12 months) than in young age (one month) after addition of vitamin D to the growth medium (Chen *et al.*, 1992).

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MICROPROPAGATION OF *ZINGIBER OFFICINALE ROSCOE*, THROUGH AXILLARY MERISTEMS.

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Zngiber officinale ginger is a perennial rhizomatous herb with aromatic tuberous rootstock. Due to its warm pungent taste it is widely used in oriental and continental foods as a spice. More over it is medicinally important as stimulant and carminative (Guenther, 1952).

Natural rate of propagation of giner is restricted due to its vegetaive mode of propagation. Seed ginger is obtained by dividing the root stock into 2 -3 cm pieces each bearing a dormant aspical or axillary bud. Hence number of plants obtained is limited.

Ginger is reported to be cultured *in vitro* for vegetative cloning and production of secondary metabolities. Morphogenetic effect of growth regulators on ginger was reported by Shah and Raju (1976). Micropropagation was studied by Hosoki and Sagawa (1977) & Ilahi and Jabeen (1987). While Sakamura *et al.* (1986) & Ilahi and Jabeen (1992) cultured the ginger *in vitro* for production of essential oils.

The present work was undertaken to prppagate ginger asexially in *in vitro* conditions and explore some means to enhance "Seed Ginger" production.

Explants were obtained by culturing root stocks of *Z. officinale* in polyethlene bags containing damp sand at room termperature in spring season. After two weeks the shoots sprouted. Within next two weeks 5-7 leaves unfolded. The shoot bases consisting of axillary buds ensheated in mother scales provided the explants. Rhizomes were thoroughly washed with detergent and tap water prior to dissection. Explants were re-rinsed with sterilized distilled water. 30 seconds dip in alcohol, then 20 min. soaking in 0.01% mercuric chloride solution *(w/v)* and finally three rinses with sterlized distilled water sufficed the sterilization procedure.

Initially explants were cultured on Murashige and Skoog (MS) medium (1962) with five modification in organic addenda. No. 1-3 media contained Adenine sulphate (Ade), Thiamine HCl and Na_2HPO_4. $2H_2O$ each @ 80, 0.4 and 170 mg/L respectively. To the medium NO. 4 & 5 each, 4 mg/L Ade. and 0.1 mg/L Thiamine HCl were added. Na_2HPO_4. $2H_2O$ was 85 and 170 mg/L in media 4 and 5 respectively. 500 mg/L malt extract was also added to the latter. Medium No. 1 was supplimented with 3.0 mg/L isopentenyladenine (2ip) only. No. 2 medium contained 30 mg/L 2iP and 0.3 mg/L indole acetic acid (IAA); 2.0 mg/L each of Kinetin (Kin.) and IAA were added to medium No.3; No. 4 medium had 1.0 mg/L and 0.1 mg/L Kin. and naphthelene acetic acid (NAA) respectively; while in No. 5 medium 0.1 mg/L Kin. and 0.3 mg/L IAA were present as growth regulator suppliments.

Sucrose and agar were added at a uniform rate *i. e.* 3% and 0.8% respectively. pH of the media was adjusted 5.7 prior to the addition of agar. Media were autoclaved at 15 *p. s. i.* for 15 - 20 min. 5mm pieces from shoot bases and nodal sigments of the rhizomes were cultured on aforementioned compositions of MS medium.

Bud activation was induced in 12 weeks. The proliferated explants were transferred on another MS medium modification after 16 weeks of initial culture. Within 8 week of subculture well developed plantlets regenerated consisting of slendrical shoots with a crown at upper and 2-3 offset aggrigate at the lower end. The plantlets had well developed adventitious root system. Nodal segments were also prominent on the shoot.

Reculture of explants from the regenerated plantlets on the same medium formed well develped plantlets within four weeks. In eight weeks regenerents produced 4 - 8 leaves and elongated adventious roots. Offset induction was successively induced at the bases of root stocks.

Precocious development of otherwise inhibited axillary meristems of shoot tips and nodal segments was promoted in the presence of Kin. supplimented media. Out of five modified MS culture media the bud activation was observed on media with IAA/NAA & Kin. The induction period was prolonged *i. e.* 12 - 16 weeks initially. Subculture on medium containing BAP reduced the proliferation period to 8 week regenerating complete plantlets with grown up stem, adventitious roots and offsets.

Regenerated leafless segmented aerial shoots could be interpreted as thin slendrical variety of rhizomes "the sobole" (without stored food material), morphologically competable with the fleshy rhizomes of ginger growing *in vivo*. The slendriical rhizomes resembled to those of *Convlleria majalis, Cynodon dactylon* and *Carex* growing under natural condition. Its slendrical structure might be due to low intensity illumination which hindered the synthesis of excessive food to be stored in the tuberous form of root stock.

In vitro produced offsets were compareable to those formed dy naturally growing *Phoenix dactylefera* and *Ananas comosus* and serveral other vegetively propagating monocotyledonous plants. Morphogenetically offsets of the rhizomatous species are just like bulbelts, cormlets and tubercles adventively produced vectors for vegetative propagation of bulbous, cormous and tuberous plants. On culturing the various explants from the regenerated plants all axillary meristems whether in shoot tip nodal segments or offset had in variablly undergone direct somatic organogenesis giving rise to independent plants.

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DIETARY FIBER AND COLORECTAL CANCER

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INTRODUCTION

Dietary fiber is defined as the remnants of plant cells resistant to hydrolysis by the alimentary enzymes of man. More recently, dietary fiber is also described as undigested storage polysaccharide present within the contents of the cell as well as undigested polysacchari an dlignin present in the cell wall. In terms of functional properties, dietary fiber is defined as the material in the foods which decreases the transit time, increases the fecal volume and fecal water content.

Dietary fiber may be subdivided into 3 basic components; 1. Cellulose, the major fibular component of the plant cell wall; 2. The non-cellulose polysaccharides, including hemicellulose and pectin substance which is formed in the cell wall matrix and 3. Lignin, a non carbohydrate substance of the plant cell wall. With regard to chemical properties of dietary fiber, it can be grouped in to two major components; 1. Water soluble; and 2. Water insoluble fiber, both of which have a distinctive physiological role in the human body. Water-soluble fiber includes pectin, gum. mucilage and some hemicellulose while cellulose and lignin constitute the non water-soluble substance.

The physiological role of dietary fiber not only depends on the physical properties and chemical composition of the fiber but also the form in which it is ingested. An important property of dietary fiber is that it can absorb water in the colon leading to much softer and increased fecal weight. A determinant in the water holding property of dietary fiber is particle size and it is gernerally agreed that the coarse bran retains more water than fine bran and that fecal bulk is related to the particle size of bran.

As a general rule, the soluble dietary fiber from fruits, vegetable and cereals such as oats and barley exert their effects in the small intertine, whereas the insoluble forms in wheat, exert the strongest effect on large bowel function. All dietary fiber is degraded to a certain extent in the large bowel and provides a little energy to the body. Some starch also escapes digestion in the small intestine and exerts effects in the large bowel, similar to those of the more soluble dietary fiber.Dietary fiber reduces transit time, which is thought to be advantgeous not only in inhibiting the formation of carcinogen by colonic microorganism but also decreasing the risk of contact between the carcinogen and mucosa of the colon. The hypolipidemic effect of dietary fiber is thought to be due to increased excreta of colonic bile acids and thus decreasing the reabsorption of bile acid for cholesterol synthesis. An overview of epidemiological and clinical data suggests the potential role of dietary fiber in certain diseases. These diseases are probably caused by the low intake of dietary fiber and are more prevalent in those areas of the world where refined foods (partially or completely fiber-free foods) are commonly consumed. These diseases are more common in the Western communities than those of the developing world. Eight disease conditions are specifically associated with low dietary fiber intake, which are:

1. Diverticular diseases
2. Appendicitis
3. Varicose Veins
4. Piles (Hemorrhoids)
5. Hiatus Hernia
6. Cancer of the Colon and Rectum
7. Ischemic Heart Disease
8. Gall Stone

The fiber related functions in reducing cancer

incidence among population consuming high fiber diets are thought to result from increased stool velocity and volume. Cancer causing agents can be more readily absorbed in fiber and bence can be eliminated more quickly. It has been suggested that dietary fiber results in an altered type of micro flora in the gut that tends to be more aerobic and bence less likely to produce carcingogenic bile acid metabolites. There are also good body of evidence that dietary fiber imparts by prolonging the chewing time as well as in digestion which tends to reduec the food intake and thus prevent from obesity. Thus it provides safegaurds against obesity related diseases like diabetes, hypercholesteriolemia etc.In the recent years much attention has been focused on the possible protective role of fiber in the prevention of cancer of large bowel (colon and rectum). This interest has stemmed from the observation that the incidence of colonic cancer is lower among black Africans who consume a diet high in unabsorbable fiber, than among Western populations who consume a diet low in fiber. This invoked our interest to search for the recent advances, which have taken place in elucidating the role of fiber in human nutrition.

LINKS WITH OTHER DISEASES

Fiber and Chronic Intestina Diseases

In human, a lack of dietary fiber intake has been implicated to a number of diseases in including constipation, diverticular disease, chronic inflammatory bowel diseases, irritable bowel syndrome and malignant disease of the large bowel (colon).

1. Constipation.

Bran fiber is widely used in the treatment of constipation and undoubtedly has a stool bulking effect. The therapeutic calue of bran in the treatment of consitipation may be due to formation of stool alcohol (such mannitol, dulctiol, xylitol) or the non-metabolisable sugars (such as lactose) since the same bulking effect could readily be achieved and many would find them more portable. Goading (1980) divided constipation into primary and secondary types. Primary constipation includes self and environmental induced constipation varieties, while secondary constipation include idoathic, psychiatric, genetic traumatic, obstetric and pathogenic or tatogenic types. Burkitt (1969) reported that the low fiber content in the Western diet is responsible for many intestinal diseases in the Western part of the world. While the prevalence those diseases in uncommon in population who consume good amount of fiber in the diet.

2. Diverticular Disease

Like constipation, diverticular disease in Western population (Kohler, 1963). The use of fiber in the treatment of diverticular disease was promoted and its value in controlled trials has been extensively documented. However, till to date the treatment of choice has been a low fiber and presumably most clinicians are satisfied that it is a rational and treatment. It has been observed that diverticular disease is a disease of Western civilization and is due to a deficiency of dietary fiber, (Painter & Burkit 1971).

3. Chronic Inflammatory Disease of The Large Bowel

Little is known about the etiology of inflammatory bowel disease. In crohon's disease the clon may have a different microbial etiology than ulcerative colitis. In Corhon's disease low disease low dietary fiber diet has been recommended. (Heaton, *et al.*, 1979).

4. Irritable Bowel Syndrome

Irritable bowel syndrome is disease of unknown actiology in which the symptoms may be diarrhea or constipation like and where no inflammatory or constipation or ulcerous lesions of the large bowel mucous are detectable, THe disease may also be of psychosomatic origin and symptoms are oftem associated with stress. Inevitably a high fiber diet was proposed as the treatment of choice (Manningn, 1977).

FIBER THERAPY AND COLORECTAL CANCER PREVENTION

Burkitt, (1971) reported that diets which are high in fiber content are associated with more rapid intestinal transit and often with greater stool weight. In contrast, communities consuming low residue diets, typified by modern foods of Western Europe and North America have a slower intestinal transit and stools that are firm, small and lower in weight.

Burkitt, (1971) demonstrated that the lack of dietary fiber is related to two broad disease conditions namely colon cancer and constipation according to the physiological function of fiber.

Globber (1974) suggested that diets, which are lacking in dietary fiber cause longer gut transit times and increase the risk of colon cancer.

Fleming (1980) assessed the effects of two sources of dietary fiber on the characteristics of cecal contents directly with swine cannulated to facilitate frequent collections of cecal digesta. The short chain fatty acid (SCFA) concentration increased and the pH decreased in the cecum. It was concluded that SCFA concentration and acidity of the digesta are directly related and that dietary fiber factors influencing caloric health.

Reddy *et al.* (1980) studied the effect of types of supplement fiber on fecal mutagens and bile acids in human volumteers. They concluded that the increased fiber intake in the form of wheat bran or cellulose may reduce the production and / or excretion of mutagens in the stools and decrease the concentration of fecal secondary bile acids in humans.

Caliendo (1981) suggested that the diet related functions in reducing cancer incidence among population consuming high fiber diets may result from increased stool velocity and volume. Cancer causing agents can be more readily absorbed to the fiber and hence can be eliminated more quickly. It has also been suggested that dietary fiber results in an altered type of microflora inthe gut that rends to be more aerobic and hence less likely to produce carcinogenic bile acid.

Cassidy *et al.* (1981) suggested that fibers which bind bile acids may also disrupt the intestinal mucosa and so provide an ideal area for turnover growth which means that fiber increase the risk of tumor growth.

Kritechevski(1984) conducted a study to determine an association between dietary fiber and fecal bile acid. He oberved a positive correlation between fecal bile acids and fiber intake. In addition the concentration of fecal bile acid was positively associated with the incidence of bowel cancer.

Slattery *et al.*, (1988) reported that low fiber diet is related to the development of colon cancer in population. It was observed that crude fiber consistently decreased risk associated with colon cancer in both males and females. Hieghest quantities of fruits and vegetables were also associated with a decreased risk of colon cancer in male and in females compared with lower quartile of intake, whereas high intake of grains were not as protective.

Calver *et al.*, (1988)examined the relationship between colonic thymidine kinase enzyme activity and mucin chemistry and reported the effects of various dietary fibers on chemically induced colon carcinogenesis. Thymidine kinase enzyme specific avtiviy was not significantly different in the fiber free, wheat bran and guar gum groups.

Lupton *et al.*, (1989) studied the potential interactive effects of protein and fiber on cecal and colonic surface areas. This study demonstrates that luminal ammonia concentration is dependent upon both protein level and fiber type and that a fermentable fiber, rather than colonic ammonia concentration actually increases them several fold.

West *et al.*, (1989)studied colon cancer cases and hypothesized that dietary fat increases colon cancer risk while dietary fiber decreases the risk of colon cancer. They further noticed that fat and protein may independently be associated woththe risk of colon cancer risk.

Hoagland (1989) demonstrated the binding of chenodeoxycholate and decanoate to alcohol insoluble residue of carrot, cabbage, broccoli and

onion. Binding of chenodeoxycholate to freeze-dried calcium pectate gel under conditions used for vegetable. Alcohol insoluble residue was observed. They reported that binding of bile acids and fatty acids to vegetable fiber occurs through salt linkage to calcium pectate of the plant cell wall. Such binding may possibly be beneficial to human health by lowering blood cholesterol; levels and by reducing the risk of colon cancer.

Heilbrun *et al.*, (1989) interviewed Japanese and American men for their dietary assessment. They suggested that the reporting of the dietary association with the risk of colon cancer be made with caution. They observed no significant association between dietary variables withthe risk of colon cancer. Also there were no significant association between intake levels of micro nutrients or food groups and risk of rectal cancer.

Alberts *et al.*, (1990) while studying the potential role heat bran fiber as a dietary supplement in the prevention of colorectal cancer used. The researchers obseved that fiber intake decreases the gorwth of rectal adenomatous polyposis in patients with familial polyposis.

Freudenhein *et al.*, (1990) examined the impact of fibers with different solubility characteristics on risk of colon and rectal cancer. They observed that the risk of colon cancer decreased with intake of grain fiber of both females and males. While intake of fruit/ vegetable fiber was effective in decreasing the risk of colon cancer. Insoluble grain fiber was more strongly associated with risk than soluble grain fiber.

Friedman *et al.*, (1990) reproted an association between appendicitis and large bowel cancer in intersociety correlation and hyposthesized prevention of both by a high fiber diet.

Willet *et al.*, (1990)demonstrated that a low intake of fiber from fruits appears to contribute to the risk of colon cancer but this relation was not statistically independent of meat intake.

Sinkeldam *et al.*, (1990) conducted an experiment to examine the effect of dietary fiber (wheat bran) and fat (lard) on genesis of N-methyle-N-nitro-N-nitrosoguamidine- induced colon cancer in rats. The researchers with cereals and vegetables being protective against both cancers, fruit having no effect and starchy roots having a very weak and non significant promoting effect. There is strong current interest in the protective effect of fruit and vegetables against cancers at a number of sites. This sutdy showed that only the current intake of vegetables was protective. Intak early in life seemed to offer no protection. The protective effect of cereals was manifest both early in life as well as for current intake for females breast and colorectal cancer, but only for current period for male colorectal. Caloric restriction, but only early in life, provides protection against all three cancers. In this study fruit was not correlated at all with the risk of either colorectal or breast concers at either time period. Fruit is clearly mor protective against cancers of upper digestive tract and respiratory tract than against the cancers considered here.

Hill (1998) reviewed that nonstarch polysaccharide accounts for only about 25% of the true intake of dietaryfiber and it is better at present to use fibre-rich foods as a measure rather than inaccurate assays of dietary fibre found in epidmiological studies. A re-examination of the epidmiological literature has shown that although the strengh of protection given by dietary fiber may be disputed , there is no doubt about the protection afforded by cereal fiber.

Bespalov *et al.*, (1998) They tested a boilogically active-Fibromed experimentally and clinically. The additive made from wheat bran by the reacon company contains no less then 40% of dietary fibre(cellulose, hemicellulose and lignin). Its effects on multi-organ carcinogenesis induced by N-methy1-N-nitrosouria(MNU) and lipid metabolism was tested in rats. Tumors were induced by combined intramammary injections and intrarectal infusions of the agent. Fibromed was fed (20% by weight) during Post-initiation period. It effectively inhibited the development of mammary and colonic tumors and reduced serum-blood cholesterol, triglycerides and beta-lipopproteins, The influence of Fibromed treatment on stool during early post-operative period

was studied in surgical cases of colorectal cancer. A dose of 60g was administered, starting from days4-5, Fibromed restored intestinal function 36hr earlier than in controls. Fibromed should ber recommended for prevention of breast and colonic tumors, lipid metablism disorders and rehabilitation of patients who underwent surgery for colorectal cancer.

Kritchevsky (1997) reported that dietary fibre could be rrotective against colon cancer. Isolated fibres were tested for anticarcinogenic avtivity in carcinogen-treated rats. Wheat bran was consistently protective. Some human studies suggest that dietary wheat bran may protect against growth of adenomas. The precise mechanism by which fibre exerts its effects is still elusive. Fibre exerts many influences in the digestive tract. It can dilute the tract contents, and studies show that decreased concentration of faecal bile acids is correlated positively with cancer risk. Fibre may enhance energy excretion and caloric intake had been correlated positively with cancer risk. Bacterial degradation of fibre produces, among other compounds, butyric acid which may affect colonic and faecal pH and also shows antiproliferative activity. More data are needed vis-a-vis effects of dietary fibre on human colon cancer. These should include studies of specific fibres as well as of high fibre diets. The mechanism(s) of fibre action, including effects on oncogenesis, steroids metabolites and short chain fatty acids, requires elucidation.

LeMarchan *et al.*,(1997) coducted apopulation-based case-control study among different ethnic groups in Hawii to evalute the role of various types and components of fibre, as well as mirronutrients and foods of plant origion on the risk of colorectal cancer. Interviews were taken from 698 male and 494 female Japanes, Filipino, Hawaiian, and Chinese cases diagnosed during 1987-1991 with adenocarcinoma of the colon or rectum and to 1,192 population controls matched to cases by age, sex and ethnicity. They used conditional logistic regression to estimate odds ratios, adjusted for caloric intake and other covariates.They found a strong, dose-dependent, inverse association in both sexes with fibre intake measured as crude fibre, dietary fibre, or nonstarch polysaccharides. This protective effect of fibre wa Slimited to fibre from vegetable sources, but no clear association with fibre from fruits or cereals. This pattern was consistent between sexes, across segments of the large bowel(right colon, left colon, and rectum), and among most ethnic groups. The effect of vegetable fiber may be independent of the effects of other phytochemicals,since the effect estimates remained unchanged after further adjustment for other nutrients. Intakes of carotenoids, light green vegetables, yellow-orange, broccoli, corn, carrots, banana, garlic, and legumes (including soy products) were inversely associated with risk, even after adjustment for vegetable fiber. The data support a protective role of fiber from vegetables against colorectal cancer, which appears independent of its water solubility property and of the effects of other phytochemicals. The data also indicate that certain vegetables and fruits may be protective against this disease through mechanisms other than their fibr content.report that both dietary fiber and fat effects colon carcinogenesis in a complex interactive manner.

Harris *et al.*, (1991) studied the protective action of some dietary fibers against colon cancer. They prepared two different cell walls from potato tuber and observed the action of adsorption in vitro the hydrophobic mutagen 1,8-dinitropyrene (DNP)using an incubation mixture. They concluded that competition between soluble and insolube fibre components may have major implications for the availablety and distribution of hyprophobic mutagens in the alimentary tract.

Mogrity *et al.*, (1991) evaluated the effects of unsaturated fat and fiber(cellulose) on the growth of human colon cancer explanted to mice. They revealed that a high fat diet stimulated and fiber decreased the growth of human colon concer explanted to mice.

Evan (1998) in a epidiomioligical study and observed that 80-90% of colorectal cancer is caused by dietary and environmantal factors and the prevalence of cancer can be altered in low risk patients by long term alterations in dietary fiber ingestion.

Franceschi *et al.*, (1998) interviewed 1225

subjects with cancer of the colon 728 with cancer of rectum and 4154 controls, hospitalized with acute non-neoplastic diseases, between 1992 and 1996 in six Italian areas. A questionnaire comprising of 79 questions on food items were asked. After allowing for nondietary confounding factors and total energy intake, a sigificant trend towards an increasing risk of colorectal cancer with increasing intake was found for bread and cereal dishes, cakes and desserts and refind sugar. The intake of fish, raw and cooked vegetable and fruit showed an inverse association with risk. Whole meal bread was consumed by only 12.5% of cases and 13.9% of controls and at variance with refined bread, did not show a significant direct association with colorectal cancer risk.In view of these findings current hypothesis on the carcinogenic effects of refind starchy foods and refined sugars should be revised to take into account the digestive physiology of corbohydrates and the possible relationship between insulin and colon cancer.The beneficial influence of most vegetables is confirmed, and a possible difference between refined and wholemeal bread is suggested.

Caygill CP *et al.*, (1998) studied the risk of cancers of the colon and breast with the intake of cereals, starchy roots, vegetables, fruits and total energy, either concurrently with the cancer mortality data, or from 20 years earlier. The patterns of the effects of cereals, roots, vegetables and fruits were very different,

SUMMARY

Dietary fiber which is mainly non-strach polysaccharide is a physiologically important component of the diet. Much of it is degraded in the human gut probably in the colon by the micro flora althrough the extent of breakdown varies with the source of fiber. During breakdown the bacteria obtain energy for cell growth and both gas and volatile fatty acids are produced. Although the clinical signifinance of these fibers up to greater extent is known, the mechanisms are not cleary understood. However, it has been well established that lack of dietary fiber in the diet leads to the development of a variety of diseases in man. These diseases are largely chronic and probably multifactorial in origin. In recent years,there has been an increasing interest in elucidating the role of plant fibers in decreasing the risk of colon cancer and other disease of bowel. Moreover, populations changing from fiber rich to fiber poor diets seem to show increased tendencies to fall prey to these diseases. Jahangir *et al.*, (1986) have summarized in dietary fiber content of various foods which are included in the annexures -1 to 6.

Annexure-I
Dietary Fiber Content of Foods

Bread & Cereal Products	Fiber g/100 gm
Whole wheat bread	8.5
Brown bread	5.1
White germ	4.6
White bread	2.6
Whole Wheat Flour (100%)	7.6
Brown Flour (85%)	7.6
White Flour (for bread baking 72%)	3.0

Annexure-II
Dietary Fiber Content of Foods

Biscuits & Crackers	Fiber g/100gm
Rye crispbread (wafers)	11.7
Plain digestive (gharme wafers)	5.5
Bran wheat	44.0
Oatmeal(raw)	7.0
Pearly bareley(raw)	6.5

Annexure-III
Dietary Fiber Content of Foods

Fresh Vegetables	Fiber g/100gm
Broccoli,(tops raw)	3.6
Cabbage, (red, raw)	3.4
Carrots (raw)	2.9
Carrots (boiled)	3.1
Grourd, butter (raw)	4.0
Spring onion	3.1
Sweet corn	3.7
Sweetcorn (canned)	5.7
Turnip, (raw)	2.8

Annexure-IV
Dietary Fiber Content of Foods

Fresh & Dried Fruits	Fiber g/100 gm
Apple (eating flesh only)	2.0
Apricots (dried, raw)	24.0
Banana (raw)	3.4
Dates (dried, raw)	7.3
Figs (Fresh, raw)	8.7
Figs (dried)	8.7
Gauva (canned whole)	18.5
Peach (dried, raw)	14.3
Pears (eating flesh only)	2.3
Pears (eating with skin and core)	1.7
Prunes (raw with stone)	12.4
Prunes (raw without stone)	16.1

Annexure-V
Dietary Fiber Content of Foods

Legumes (Pulses)	Fiber g/100 gm
Lima bean (raw)	21.0
Lima beans, (boiled)	5.1
Chickpea (raw channa)	15.0
Navy bean (raw)	25.4
Navy Bean (boiled)	7.4
Kidney beans (raw)	25.0
Lentils red (raw)	11.7
Mung bean (raw)	22.0
Mung beans (canned)	3.0
Peanuts(fresh)	8.1
Pea Fresh (raw)	5.3
Pea frozen(boiled)	12.0
Pigeon, pea (raw)	15.0
Soya.flour (full fat)	11.9
Soya flour (low fat)	14.8

Annexure-VI
Dietary Fiber Content of Foods

Nuts	Fiber g/100 gm
Almonds / (Badam)	14.3
barcelona nuts	10.3
Brazil nuts	7.0
Cocunuts (kernal only)	13.6
Walnuts	5.2

RECOMMENDATION

Various recommandations have been made regarding fiber intake that ranges from 15g-30g day. In one of the studies conducted by Nordgard I, *et al.*, 17.9g supplement was used for three months.This fibre therapy increased faecal concentrations of butyrate by 42+/12%, autete by 25+/-6%, propionate by 28+/-9% and total SCFAs by 25+/-% All of these compounds may be very effective against colorectal cancer occurrence.

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