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ANALYTICAL DETECTION METHODS FOR GAMMA-IRRADIATED FRESH APPLES

Abdus Sattar, Anwar Ahmad, Mohammad Jan, and M. Zafar Iqbal*
Nuclear Institute for Food and Agriculture (NIFA), Tarnab, Peshawar,
**Institute of Chemistry, University of Punjab, Lahore.*

ABSTRACT

For distinguishing irradiated from unirradiated apples chemiluminescence (CL) intensities of fresh apples were measured using luminol and lucigenin reactions. The results revealed that the CL values obtained from fresh samples were irreproducible and scattered in case of control and irradiated (0.2, 0.4, 0.6, 0.8, and 1.0 kGy) apples. However, when CL of the charred and ashed (mineral matter) samples was retaken, it was found that there were marked differences in CL intensities of treated and untreated samples, It was observed that the data pertaining reaction of mineral matter with luminol and lucigenin was dose dependent. Electrical conductivity of whole apples and their 20 per cent slurry in distilled water also gave promising results.

INTRODUCTION

Irradiation treatment extends shelf-life and enhances hygienic quality of food materials. An expert committee of IAEA/FAO/WHO has already recommended that foods irradiated up to an overall average dose of 10 kGy are safe for human consumption (Anonymous, 1991). In order to harmonize international marketing of irradiated food materials, there is a need to standardize analytical methods which can help in identifying irradiated food stuffs. World-wide efforts are continued in this regard (Bogl & Heide, 1985; Delincee, 1992). Physico-chemical changes associated with irradiation treatment are nominal and difficult to be detected by simple means. There is a need for suitable sensitive and reliable method to be standardized to facilitate the identification of irradiated food materials. In Pakistan preliminary work on identification of irradiated spices and dried fruits has been conducted (Khan *et al.*, 1993; Ahmad *et al.*, 1995). A commercial food irradiator is likely to be installed at Lahore along with Pakistan Radiation Services (PARAS) and it is, therefore, necessary to develop some routine identification method for irradiated fresh fruits and vegetables to help in implementation of irradiation technology in food preservation and/or for quarantine purposes.

MATERIALS AND METHODS

Fresh red apples were obtained from the fruit market at Peshawar. The apples were irradiated at the dose levels of 0.2, 0.4, 0.6, 0.8, and 1.0 kGy using ISSLEDOVATEL (CIS) gamma source with dose rate of 1.9 kGy/hr. Chemiluminescence (CL) intensities of the fresh apples were taken using luminol and lucigenin photosensitizing reactions with the help of Bio-Orbit 1250 luminometer, the composition of these reagents has already been reported (Sattar *et al.*, 1987). Due to widely dispersed data the method for CL measurements was modified by using charred and ashed (mineral matter) apples instead of fresh samples. Electrical conductivity (μ-mho) of fresh whole apples was taken by puncturing the fruit by stainless steel pins using model ABB/10 sproule electrolytic conductivity cell UK. The EC of the blended (20%) apples in distilled water was also measured. In view of the wide variation in the values the data were analyzed statistically by measuring means and coefficient of variability (CV).

RESULTS AND DISCUSSION

Initial experiments on CL intensities using luminol and lucigenin reaction with fresh apples showed inconsistent results with wide variations. It was, therefore, desired to measure the CL of mineral matter of the apples. The results of CL values of charred and ashed samples are given in Table-1. There was significant difference (more than double) in the CL values of unirradiated and irradiated samples. The data clearly show that on the basis of CL values obtained from the reactions of charred material with both the photosensitizers, irradiated samples can be identified, similar pattern was observed from the results of mineral matter of apples. It was further found that the data of mineral matter were almost dose dependent and quite consistent. The means and CV of the data were

Table-1. Chemiluminescence intensities (mV) of irradiated fresh apples

Material	Sample No.	Radiation Doses (kGy) 0	0.2	0.4	0.6	0.8	1.0
				Luminol reaction			
Apple-charred	1	27.5	33.3	35.3	38.6	40.0	40.0
	2	26.5	31.3	32.5	38.4	40.0	42.0
	3	26.0	32.0	33.4	38.5	40.0	40.7
	Mean	26.67	32.2	33.7	38.5	40.0	40.7
	CV	3.75	3.15	4.23	0.26	0.00	2.84
Apple-ashed	1	3.4	6.52	6.55	6.90	7.0	8.3
	2	3.0	6.42	6.24	7.00	7.8	8.5
	3	3.2	6.50	6.30	7.00	7.8	8.5
	Mean	3.2	6.48	6.36	6.97	7.5	8.4
	CV	6.25	0.82	2.58	0.83	13.27	1.37
				Lucigenin reaction			
Apple-charred	1	13.5	25.8	26.5	25.5	27.5	31.5
	2	18.5	20.8	25.8	24.0	27.0	28.0
	3	17.0	24.0	25.5	27.0	27.0	28.1
	Mean	16.33	23.53	25.93	25.5	27.17	29.2
	CV	15.71	10.76	3.86	5.88	1.06	3.42
Apple-ashed	1	99.8	120.0	100.0	100.0	110.0	100.0
	2	87.7	150.0	120.0	98.0	100.0	120.0
	3	77.0	130.0	110.0	96.0	100.0	130.0
	Mean	88.17	133.33	110.0	98.0	103.3	116.67
	CV	12.94	11.46	0.91	1.02	5.69	13.09

measured and wide differences between irradiated and unirradiated samples were observed. Determination of CV is especially appropriate under conditions where there are extreme values or when it is desired to express variations as a percentage of the average around which the deviations are taken. Although CL intensities of irradiated spices, dried fruits and some other materials have been reported to be much higher than their unirradiated counterparts (Bogl, 1990; Ahmad *et al.*, 1995). The use of the ashed material for CL measurements has been presented for the first time in these and related recent studies. The application of EC technique for detecting irradiated foods has been tried in Pakistan (Khan *et al.*, 1993; Sattar *et al.*, 1995) and abroad (Ehlermann, 1972; Hayashi, 1986). However, it was consistently reported that reproducibility is the factor which limits its usage. Further, it was not possible to determine the absorbed dose of the irradiated materials.

Electrical conductivity values (μ-mho) of the whole fruit as well as its slurry (20%) in distilled water are presented in Table-2. The results revealed that there was increasing trend in the EC values as the radiation dose increased from 0-1.0 kGy in both using whole fruit and slurry. These preliminary experiments on fresh apples indicate that chemiluminescence and electrical conductivity measurements can detect the irradiated samples from unirradiated controls. However, this technique needs to be further improved with regard to accuracy, reproducibility, and measurement of absorbed doses.

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Table-2. Electrical conductivity values of irradiated fresh apples

Irradiation doses (kGy)	EC (μ-mho)	
	Whole fruit	Slurry (20%)
Unirradiated control	95.0	591.0
0.2	100.0	600.0
0.4	170.0	750.0
0.6	240.0	800.0
0.8	270.0	900.0
1.0	250.0	800.0
Mean	187.5	740.17
CV	41.3	16.51

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POST-HARVEST CHANGES IN APPLES DURING STORAGE

Mujeeb-ur-Rahman, Shereen Gul & Wasim A. Farooqi*
PCSIR Laboratories, P.O. Box 387,
Mastung Road, Quetta.
**Post-harvest Expert, UNDP, P.O. Box 234, Quetta.*

ABSTRACT

Studies on the post-harvest changes during storage were carried out and parameters like weight loss, fruit firmness, total soluble solids and organoleptic tests examined in two varieties of apple viz. 'Golden Delicious' and 'Spartan Spur'. On the basis of these results, 'Golden Delicious' was found to have a storage life of 180 days under refrigerated condition, whereas 'Spartan Spur' had 150 days storage life. Physico-chemical changes in 'Spartan Spur' were faster as compared to 'Golden Delicious' during storage.

Key words: storage life; quality; physico-chemical changes

INTRODUCTION

Apple fruit, like others, does not cease its metabolic activities even after harvest. Fruit respires, consumes oxygen (O_2), gives off carbon dioxide (CO_2) and generates heat (Farooqi, 1986). The softening of fruit results due to degenerative changes in cell wall composition (Pentzer and Allen, 1944). The colour of the fruit also changes i.e., from green to yellow, due to decrease in chlorophyll, while yellow pigments of the skin increase. A pigment called anthocyanin gives typical red colour increase in some fruits after harvest. According to Hoffman (1937), apples are some time left in the orchard after picking to promote red colour development. The plum also develops some red or blue colour during storage. Some fruits, including apple produce volatile compounds after harvest, which give typical aroma and flavor of specific variety. Ethylene gas (C_2H_4), considered as ripening hormone, is one of the volatiles found in most fruits. It triggers the ripening process. If more ethylene is produced whole process is accelerated (Pratt and Goeschl, 1969). The skin of some fruits like apple develop wax that give an attractive appearance and help to reduce moisture loss through the process of evapo-transpiration as well as respiration. Wax-coating is also done as a post-harvest treatment to improve external appearance, reduce weight loss and external storage life (Farooqi and Hall, 1973). Some enzymes are synthesized and a small increase in protein is ascribed to this process (Frankel *et al.*, 1968).

Apple is a major commercial fruit of Balochistan. From an area of 32,075 hectares, about 446,151 tonnes of apples are produced in the province, annually. The interest of growers in planting more apple trees is shown from the fact that increase in production during the last 10 years (1984-85 to 1994-95) was 461.9 per cent (Anonymous, 1995). Different cultivars grown in Balochistan are Red Delicious, Golden Delicious, Mashadi, Kashmiri, Amri, Kaja, Spartan Spur, etc. Nutritive value of some of these varieties has been reported by Rahman and Gul (1996).

Due to lack of basic infra-structure, unawareness of post-harvest problems amongst the growers, traders, managers and further lack of technical know-how, about 30-40 per cent of the horticultural produce is wasted from farm gate till it reaches the consumer (Farooqi, 1996). This loss tunes to billions of rupees, annually. This tremendous loss can be minimized if facilities are improved, awareness is created and technology is practically implemented during handling, storage and marketing.

Metabolic activities in fruits are slowed down at cooler environment, therefore refrigeration is considered as one of the principle means of fruit storage. Different kinds and varieties of fruits behave differently at low temperature which determines their shelf-life. Spartan Spur is one of the early varieties introduced recently in Balochistan. Due to its earliness, it gives handsome price to the producer in the market. A trial was carried out to study the shelf-life of Spartan Spur and an old variety, Golden Delicious under refrigerated and non-refrigerated storage conditions. The results are reported in this paper. This information will add to the activities already in progress (Farooqi & Raisani, 1996).

MATERIALS AND METHODS

Two cultivars of apples namely, Golden Delicious (locally known as Shin-Kulu) and Spartan Spur (an imported cultivar) were selected for comparison of shelf-

life in cold storage and at room temperature. Apples of equal size and maturity were harvested from the Deciduous Fruit Development Centre, Quetta. The temperature of cold storage was maintained at 3±1°C, while range of ambient temperature was 25-30°C. The humidity was continuously recorded during cold and room temperature storage using thermograph which was 70-75 per cent. Ten apples were randomly selected, marked for each determination and following data were recorded initially and then at monthly intervals.

Loss in weight

The loss in weight was determined by the formula given as:

$$W_3 = \frac{W_1 - W_2}{W_1} \times 100$$

W_1 = Initial weight
W_2 = Weight after particular storage time
W_3 = Loss in weight

Pressure test

Pressure test was determines by Magness-Taylor tester and expressed in lb cm^{-2}.

Total Soluble Solids

Total soluble solids were determined by Abbe refractometer and expressed as degree Brix (%).

Changes in appearance

This observation was recorded visually using a scale.

Sensory evaluation

Sensory evaluation was carried out by a panel of 10 judges using Hedonic Scale Ratings (Larmond, 1970).

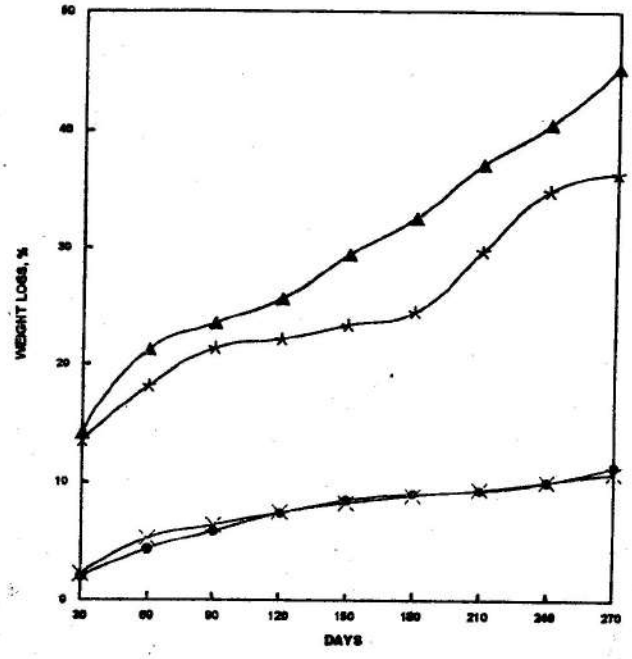
RESULTS

Weight loss

Figure 1 shows the weight loss in apples during storage. There was a progressive decrease in weight with extension of storage period. The loss in weight was minimum in refrigerated storage, while maximum weight loss was recorded at room temperature storage. The loss in weight was significant at Pμ0.01 throughout the storage period in both the cultivars. The reason for less weight loss in refrigerated storage is that the metabolic activities in the fruit slow down at lower temperature.

Pressure test

Data regarding pressure test presented in Figure 2 shows that firmness of the flesh started losing from the day of storage, irrespective of storage condition and cultivar. The decrease in pressure was significant at Pμ0.01 in cold storage and room temperature treatment in both the cultivars. The reason for this decrease in firmness was again dependent upon the rate of decomposition of apple contents.



• Golden delicious C.S. * Golden delicious R.T.
x Spartan spur C.S. Δ Spartan spur R.T.
(C.S. = Gold storage R.T. = Room temperature)

Fig. 1. Weight loss in apples during storage (CVS: Golden delicious and spartan spur)

Total soluble solids

The pattern of increase or decrease in soluble solid contents was alike in both the cultivars i.e., in cold storage upto 150 days (Fig. 3). The increase in soluble solids was progressive in room temperature treatment. The increase or decrease in soluble solids was significant at Pμ0.01 in room temperature treatment, only. In cold storage the total soluble solids increased upto 13.26, 7.49 and at room temperature was 12.35 and 25.52 per cent in Golden Delicious and Spartan Spur, respectively.

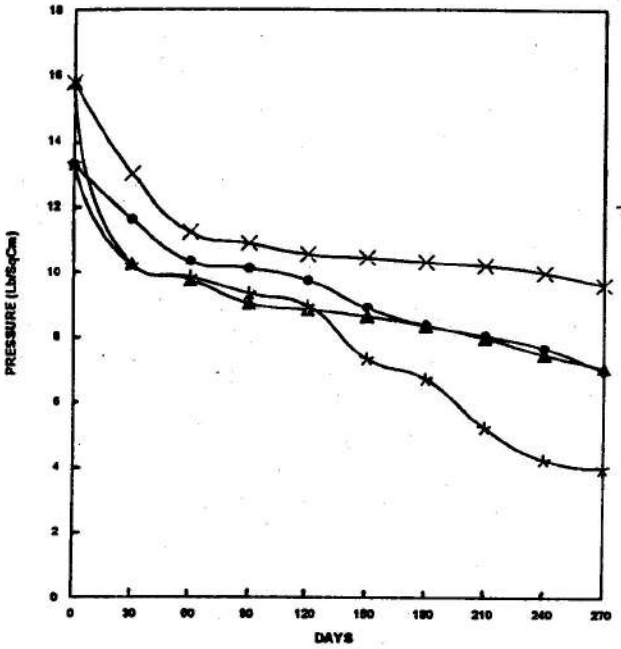
Appearance

Appearance of fruit is an important criterion of consumers acceptability. Table 1 shows that the appearance of Golden Delicious and Spartan Spur was good for upto 180 and 150 days, respectively, in cold storage. The apples of both the cultivars shrivelled at room temperature after 30 days storage, hence reducing the market value of the fruit.

Table 1. External Appearance of Apples (CV. Golden Delicious and Spartan Spur) During Storage.

Duration (days)	Golden Delicious		Spartan Spur	
	Roome temperature	Cold storage	Room temperature	Cold storage
30	SS	G	SS	G
60	SS	G	SS	G
150	SS	G	SS	SS
180	S	SS	S	SS
210	S	SS	S	SS

G = Good
SS = Slightly Shrivelled
S = Shrivelled

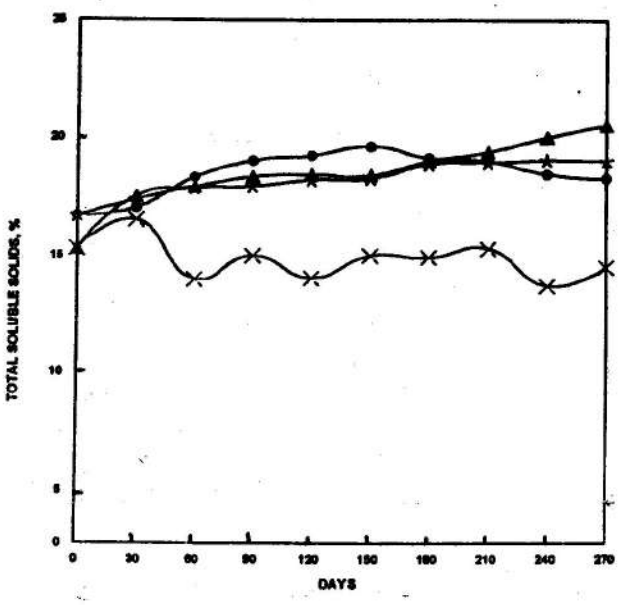


• Golden delicious C.S. * Golden delicious R.T.
x Spartan spur C.S. Δ Spartan spur R.T.
(C.S. = Gold storage R.T. = Room temperature)

Fig. 2. Changes in firmness of apples during storage (CVS: Golden delicious and spartan spur)

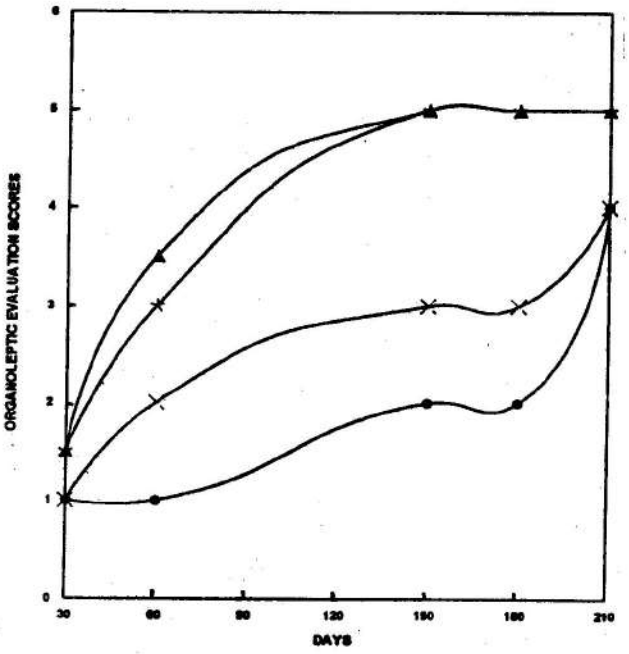
Sensory evaluation

In cold storage treatment the fruit remained acceptable for upto 150 days and average rating was 2. Spartan Spur were marginal and scoring was 3 and also remained acceptable for upto 150 days (Fig. 4).



• Golden delicious C.S. * Golden delicious R.T.
x Spartan spur C.S. Δ Spartan spur R.T.
(C.S. = Gold storage R.T. = Room temperature)

Fig. 3. Changes in total soluble solids in apples during storage (CVS: Golden delicious and spartan spur)



• Golden delicious C.S. * Golden delicious R.T.
x Spartan spur C.S. Δ Spartan spur R.T.
(C.S. = Gold storage R.T. = Room temperature)

Fig. 4. Sensory evaluation of apples during storage (CVS: Golden delicious and spartan spur)

DISCUSSION

Weight loss during storage period depends upon the type of fruit, structure, its composition, storage temperature and humidity. Our results (Fig. 1) are in line with Gac (1955) who has reported 8.3 per cent loss in weight at 4°C and 75 per cent relative humidity in apples in 120 days, whereas, in our experiment the loss in weight was 7.32 and 7.41 per cent.

The firmness of fruit shows its maturity and quality. After picking, several bio-chemical reactions are persistent. The depravity of different substances due to the biochemical reactions influences the firmness of the fruit. Transpiration decreases the weight of the fruit, that is why the firmness decreases faster at room temperature storage than in cold storage. It is obvious from Figure 2 that the firmness of Golden Delicious lasts more than Spartan Spur cultivar. The loss in firmness can also be ascribed to different stages of maturity at harvest and size of fruit.

The increase in total soluble solids (Brix) during the early storage period and decrease in the later period (Fig. 3) is mainly due to respiration of the fruit. In the process of respiration water is produced which becomes a part of water content of the cell and is used for the hydrolysis of starch and other materials. Hence the soluble solids increase but when all the starchy material is converted to sugars, there is gradual decrease in soluble solids which is a net loss of sugar due to respiration.

Loss in weight is mainly due to the loss of water and the appearance of the apples principally depends upon its water content. The apples contain about 84 percent water. According to Smock and Naubert (1950), apples shrivelled after losing 5-6 percent water. The mechanism of loss of water from fruits is the same as the evaporation of the water. The driving force is the vapor pressure of the moisture of the fruits. When it is higher than the vapor pressure of the surroundings, moisture will be lost from the fruit to the atmosphere. The loss of moisture from the fruit is also dependent upon the type of skin of the fruit. If the skin is thick and waxy, water loss will be minimum. In Golden Delicious cultivar the appearance was better than Spartan Spur which may be due to the differences in skin composition.

Conclusion

It is concluded that storage life of Golden Delicious was significantly higher ($P\mu 0.01$) than Spartan Spur apples. Spartan Spur, being an early variety, should be marketed immediately and not stored because many varieties follow this. Contrary to this, Golden Delicious could be marketed immediately fresh, stored and supplied to the market as per need during the off-season.

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EVALUATION OF PROTEIN-ENRICHED BISCUITS USING WHEAT SOY BLENDS

Riaz Ahmad, Javaid Aziz Awan, Salim-ur-Rehman and Abu Saeed Hashmi*
Department of Food Technology, University of Agriculture, Faisalabad.
**Department of Animal Nutrition, University of Agriculture, Faisalabad.*

ABSTRACT

Protein enriched biscuits were prepared from composite flour containing 80 percent wheat and 20 percent soyflours. Physico-chemical and sensory characteristics were studied for 90 days. Biological studies of the selected treatments were also undertaken. Width and spread factor decreased, while thickness increased with increasing protein content in biscuits. Protein, fat, fibre and ash contents of supplemented biscuits increased significantly and NFE decreased. A non-significant change in protein, fat, fibre and ash contents in biscuits was observed during storage. An increase in moisture, PV and FFA was observed during 90 days storage at 25±3/C in polyethylene bags. Sensory characteristics were within acceptable range but score was lower than standard treatment. Biological studies revealed a decrease in digestibility with increase in net protein utilization and biological value.

INTRODUCTION

Soybean is a leguminous crop, rich in protein, fat, fiber and ash. In Pakistan, it is grown for extraction of oil. After oil extraction, its cake is used as cattle and poultry feed. This cake can also be employed as a source of proteins for human beings. Although in Pakistan per capita protein intake of 67.88 grams is 13.1 percent above the recommended dietary allowance of 60 grams, yet children and lactating mothers have high incidence of malnutrition due to uneven income distribution, poverty, unhygienic environment improper delivery of services and ignorance of nutritional practices (Govt. of Pak., 1996).

In order to overcome the protein deficiency, foods preferred by all age groups should be supplemented with some cheap and rich source of protein. Among bakery products, biscuits are popular in general masses, particularly for the school-going-children. Commercially available biscuits are deficient in protein, fiber and ash (Rauf, 1993). They are made from straight grade flour which is deficient in these nutrients. Owing to deficiency of some essential amino acids in wheat flour, there is need for developing proteinaceous flour containing the essential amino acids in proper proportion.

Composite flour technology was developed in various parts of the world, using numerous substances such as oil seeds, oil seed cakes, fish flour, leaf protein, single cell protein, milk solids and edible legumes (Awan *et al.*, 1996). Among legumes, soybean is higher in protein content and is rich source of lysine (6.43 g/16 g of nitrogen) and has higher biological value than wheat (Pyler, 1988). It is also a good source of minerals and vitamins (Pyler, 1988). Moreover, it contains seven out of fourteen phytochemicals that have anti cancerous effects and help to prevent other diseases (Orthoefer and Liu, 1995). Consumption of soy protein significantly decrease serum cholesterol level and low density lipoprotein (LDL) level without reducing high density lipoprotein (HDL) level which protect against heart disease. It also lowers the risk of breast and prostate cancers (Anderson, 1995). Owing to good essential amino acid contents, soyflour, soy protein concentrates and soy protein isolates are used in cereals to complement deficiency of essential amino acids.

The nutritional properties of soy protein products (i.e. absence of cholesterol, reduced fat content and calories) and their technological attributes (i.e. typical soybean flavour, water absorption, emulsifying and anti oxidative capacity) are important advantages for their use in many food applications (Ladd, 1996). As it is rich source of protein, hence wheat flour may be supplemented with soy flour or soy meal for the production of biscuits. This would increase the intake of better quality protein at a cheaper rate for the general masses. It may shorten the protein deficiency gap.

Biscuits were prepared from composite flour containing matri (Ullah, 1990), moth beans (Rehman, 1990, Ahmad, 1993), raw Bengal gram (Hussain, 1993) roasted Bengal gram (Shakoor, 1995) and soybean (Imran, 1996). The results revealed that good quality, crispy and nutritious biscuits could be prepared when flour is fortified at 10 to 20 percent levels with these legumes.

Study on the production of biscuits fortified with a combination of soyflour and peanut butter was done by Ranhotra *et al.* (1980). The authors reported that biscuits contained 15.1 percent protein and sensory evaluation showed that these remained acceptable during 16 weeks of storage. In a similar study, Sirivicha *et al.* (1981) produced cookies using wheat and 6 percent soyflour. These cookies had increased protein and ash contents with enhanced nutritional value. However, soyflour caused noteworthy loss of crispiness and hardness.

MATERIALS AND METHODS

Commercially available straight grade flour was procured from the local market. Soybean was also procured from the local market and subjected to the following treatments:

a) Soybean was ground with the help of laboratory grinder to get raw or untreated full fat soyflour (T_1).
b) Raw soybean was partially defatted with the help of manual press. The meal was grounded to get untreated, partially defatted soyflour (T_2).
c) Raw soybean was soaked overnight in water, boiled for 20-25 minutes. The hulls were removed. Dehulled soybean was dried and ground to get treated full fat soyflour (T_3).
d) Treated dehulled soybean was partially defatted. Soybean meal was ground to get treated partially defatted soyflour (T_4).

Raw materials and composite flours were analysed for proximate composition. Biscuits were prepared from composite flours with some modification in the method described in AACC (1983). Approved Methods of American Association of Cereal Chemists (AACC, 1983) were followed to evaluate width, thickness and spread factor of biscuits.

Biscuits were stored for 90 days and analysed after every 15 days to determine moisture, crude protein, crude fat, crude fibre and ash according to respective methods described in AACC (1983). Peroxide value and free fatty acids were determined by the method of Koniecko (1985).

The stored biscuits were evaluated at regular intervals of 15 days by a panel of 5 judges, for colour, taste, flavour, texture and overall acceptability according to Larmond (1977).

From the results of sensory evaluation (on zero day) the two best treatments were selected for biological evaluation. Biological studies were conducted on these treatments according to Miller and Bender (1955) using weanling Albino rats. Digestibility, net protein utilization and biological value were determined.

The data obtained were analyzed statistically according to Steel and Torrie (1980).

RESULTS AND DISCUSSION

Preliminary studies were undertaken to determine optimum level of soyflour in biscuits. For this purpose composite flour was prepared using 10, 20, 30 and 40% soyflour. Sensory evaluation of the biscuits prepared from these flours reflected that judges did not like the product containing 30% or higher amount of soyflour. Hence 20% level was selected for the present studies.

Biscuits were evaluated for their chemical and sensory characteristics after 15 days interval for 3 months. Physical and biological studies were also undertaken.

Chemical studies

Moisture content in all biscuits was found to increase during storage (Table 1). Biscuits absorbed moisture from the atmosphere because they were not packed air tight. High protein biscuits absorbed more moisture than low protein biscuits. A significant increase in protein content was observed during supplementation with soyflours. Fat, fibre and ash contents also increased significant, whereas NFE decreased during supplementation (Table 1). These results are supported by the findings of Shakoor (1995) and Imran (1996). Non significant changes were observed in protein, fat, fibre, ash and NFE contents in all biscuits during storage. Peroxide value (PV) and free fatty acid (FFA) contents also increased during storage and statistically analysis revealed a significant difference during storage. PV is an indicator of incipient rancidity and it depends upon fat content and storage time. Free fatty acids are freed by hydrolysis from their glyceride combinations through the action of water and lipolytic enzymes. It also depends on quality and quantity of fat. Rao *et al.* (1995) reported an increase in PV and FFA contents of biscuit after 6 months storage in different packaging materials.

Physical studies

Width, thickness and spread factor of different treatments is presented in Table 2. This table revealed a decrease in the width and spread factor with increase in protein content. The results are in accordance with the results of Rehman (1990) and Shakoor (1995).

Table 1. Average Chemical Composition of Biscuits.

	T_0	T_1	T_2	T_3	T_4
Moisture%	6.94	7.56	8.16	7.57	7.85
Protein%	7.30	10.63	10. 95	10.69	11.01
Fat%	28.18	33.05	31.99	33.25	32.03
Fibre%	0.14	0.46	0.50	0.36	0.39
Ash%	0.27	0.65	0.68	0.60	0.62
NFE%	63.93	55.18	55.81	55.06	55.94
PV (Meq/kg)	2.51	3.06	2.86	3.07	2.91
FFA %	0.14	0.15	0.14	0.15	0.14

T_0 = Biscuits prepared from 100 percent wheat flour.
T_1 = Biscuits prepared from composite flour containing 80 percent wheat flour and 20 percent untreated full fat soyflour.
T_2 = Biscuits prepared from composite flour containing 80 percent wheat flour and 20 percent untreated partially defatted soyflour.
T_3 = Biscuits prepared from composite flour containing 80 percent wheat flour and 20 percent treated full fat soyflour.
T_4 = Biscuits prepared from composite flour containing 80 percent wheat flour and 20 percent treated partially defatted soyflour.

Table 2. Physical Evaluation of Biscuits

Treatment	Width (mm)	Thickness (mm)	Spread factor
T_0	271.0	56.7	47.8
T_1	265.6	59.2	44.8
T_2	260.5	61.3	42.5
T_3	262.0	60.5	43.3
T_4	259.1	62.0	41.8

T_0 = Biscuits prepared from 100 percent wheat flour.
T_1 = Biscuits prepared from composite flour containing 80 percent wheat flour and 20 percent untreated full fat soyflour.
T_2 = Biscuits prepared from composite flour containing 80 percent wheat flour and 20 percent untreated partially defatted soyflour.
T_3 = Biscuits prepared from composite flour containing 80 percent wheat flour and 20 percent treated full fat soyflour.
T_4 = Biscuits prepared from composite flour containing 80 percent wheat flour and 20 percent treated partially defatted soyflour.

Sensory evaluation

Biscuits were subjected to sensory evaluation by a panel of 5 judges. It was observed that quality score (with respect to colour, taste, flavour, texture and overall acceptability) decreased with increasing protein content as compared to standard biscuits (T_0). Soy blended biscuits remained 'good' even after 3 months of storage. The results of sensory evaluation (on zero day) showed that the judges had preference for full fat soyflour biscuits (Table 3). But after 3 month, storage, full fat soyflour biscuits scored lower than partially defatted soyflour biscuits due to higher PV and FFA contents.

Statistical analysis revealed a significant difference during storage and among treatments. Quality score of all biscuits decreased slightly after storage. White flour biscuits got higher quality score than soy blended biscuits because judges are used to consume biscuits made from white flour. Hence, when biscuits were supplemented with soyflour, the quality score fell down. A fall in quality score of biscuits during supplementation was found by Shakoor (1995) and during storage by Rao *et al.* (1995).

Table 3. Average Quality Score of Biscuits.

	T_0	T_1	T_2	T_3	T_4
Colour	7.41	7.06	7.09	7.00	7.07
Taste	7.04	6.86	6.87	6.81	6.63
Flavour	7.03	6.85	6.86	6.85	6.80
Texture	6.99	6.62	6.61	6.64	6.57
Overall acceptability	6.08	6.86	6.86	6.82	6.77

T_0 = Biscuits prepared from 100 percent wheat flour.
T_1 = Biscuits prepared from composite flour containing 80 percent wheat flour and 20 percent untreated full fat soyflour.
T_2 = Biscuits prepared from composite flour containing 80 percent wheat flour and 20 percent untreated partially defatted soyflour.
T_3 = Biscuits prepared from composite flour containing 80 percent wheat flour and 20 percent treated full fat soyflour.
T_4 = Biscuits prepared from composite flour containing 80 percent wheat flour and 20 percent treated partially defatted soyflour.

Table 4. Biological Evaluation of Experimental Diets (percent).

Category	Digestibility	NPU	BV
A	87.05	83.06	95.40
B	87.20	81.80	93.80
C	89.13	79.30	88.97

A = Diet containing biscuits prepared from composite flour containing 80 percent wheat and 20 percent untreated full fat soyflour
B = Diet containing biscuits prepared from composite flour containing 80 percent wheat and 20 percent treated full fat soyflour
C = Diet containing skim milk (standard diet).

Biological studies

On the basis of sensory evaluation (on zero day) two best treatments (untreated full fat and treated full fat soyflour biscuits) were selected for biological studies. Results of these studies are presented in Table 4. It is obvious from the Table that diet C (containing skin milk) obtained highest digestibility value followed by diets B (containing 80% wheat and 20% treated full fat soyflour) and A (containing 80% wheat and 20% untreated full fat soyflour). Soy blended diets had high net protein utilization values than standard diet (skin milk). Biological value also increased with supplementation with soyflour. Similar results were recorded by Hussain (1993) and Shakoor (1995).

These studies revealed that mixture of wheat and soy protein is better with respect to NPU and BV than casein. It may be due to these two ingredients which complemented each other's deficient nutrient to such an extent that this mixture excels in quality of protein than that of milk protein (casein).

Conclusion

To make up nutrient deficiency in wheat flour, soyflour was blended at 20 percent level to develop composite flours for the production of biscuits. Owing to this supplementation, protein, fat, fibre and ash contents increased in the product with a fall in width and spread factor. Biological studies revealed a decrease in digestibility with an increase in NPU and BV. Sensory characteristics remained within acceptable range till 90 days of storage.

It may be concluded that soyflour (particularly untreated partially defatted T_2) can satisfactorily be blended with wheat flour at 20 percent level for the production of biscuits and the product thus formed will complement the deficiency of one or more of the essential nutrients.

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EFFECT OF BARLEY MALT SUPPLEMENTATION ON THE QUALITY OF BREAD

Faqir M. Anjum, M. Siddique Awan*, Ijaz Ahmad, Shahid Latif and M. Ashraf
Department of Food Technology, University of Agriculture, Faisalabad.
**Department of Food Technology, University of Azad Jammu and Kashmir, College of Agriculture, Rawalakot.*

ABSTRACT

Malted flour was prepared from barley varieties Haider 93 and Jau 87. Malted barley was obtained from Murree Brewery, Rawalpindi. The malted flour was supplemented in flour of two wheat varieties viz. Punjab 85 and Inqulab 91 to test the quality of bread. Falling number values showed a linear increase in α-amylase with progressive rise in malt concentration, hence improving the diastatic activity of wheat flours. The supplementation of whole barley malted flour of Haider 93 in wheat flour showed the lowest falling number value (116 seconds) indicating the highest α-amylase activity. Hence low α-amylase activity in wheat can be improved with the supplementation of malted barley flour. The mixing time decreased by the addition of malted barley flours especially at levels of 0.75% and 1.0%, while peak height rose from 62 to 73% by various concentrations of malts. There was significant improvement in bread loaf volume due to malt supplementation. The highest loaf volume and weight to volume ratio was observed in bread loaves of Inqulab 91. Whole barley malted flour of Haider 93 proved to be the best supplement for bread baking quality when used at levels of 0.75% and 1.0%.

Key words: Barley, Malt, Supplementation, Falling Number, Mixograph, Bread Preparation and Evaluation.

INTRODUCTION

Malt and malt extracts contribute maltose, minerals, salt, soluble protein, dough conditioning enzymes, flavor, colour and nutritive materials which promote vigorous yeast activity, and accelerate flavor and aroma to the baked products (Pyler, 1988). In dough, α-amylase activity can be enhanced by the use of malt flour (Warchalewski *et al.*, 1989) to get optimum bread production (Zawistowska & Bushuk, 1988).

The research studies conducted by different workers, (Anjum, 1991; Ahmad 1993) have indicated that commercial wheat varieties grown in Pakistan are generally low in amylase activity especially α-amylase and recommended the use of malt for bread production. The present study was undertaken to delineate the supplementation of different barley malt flours on bread quality.

MATERIALS AND METHODS

Wheat varieties namely Inqulab 91 and Punjab 85 and barley varieties Haider 93 and Jau 87 were collected from Wheat Research Institute, Faisalabad. Five kilogram sample of each wheat variety was tempered and milled according to AACC (1983). Break flour and reduction flours were mixed to get straight grade flour. Two types of malted barley were used in this study. First prepared in the laboratory and the second obtained from Murree Brewery, Rawalpindi. In the laboratory, malted barley was prepared and then ground by using Udy Cyclone Mill and Quadrumate Senior Mill.

Falling number, mixographic studies and bread baking qualities were determined according to their respective procedures described in AACC (1983). The instructions of Blish *et al.* (1928) were followed to compute baking quality scores.

Details of Treatments

M1 Malt of Murree Brewery milled in Quadrumate Senior Mill.
M2 Malt of Murree Brewery milled in Udy Cyclone Mill.
M3 Malt of Jau 87 milled in Quadrumate Senior Mill.
M4 Malt of Jau 87 milled in Udy Cyclone Mill.
M5 Malt of Haider 93 milled in Quadrumate Senior Mill.
M6 Malt of Haider 93 milled in Udy Cyclone Mill.

T0		Control
T1	0.25%	Straight grade malted barley flour of Murree Brewery
T2	0.50%	Straight grade malted barley flour of Murree Brewery
T3	0.75%	Straight grade malted barley flour of Murree Brewery
T4	1.00%	Straight grade malted barley flour of Murree Brewery
T5	0.25%	Straight grade malted barley flour of Jau 87
T6	0.50%	Straight grade malted barley flour of Jau 87
T7	0.75%	Straight grade malted barley flour of Jau 87
T8	1.00%	Straight grade malted barley flour of Jau 87
T9	0.25%	Straight grade malted barley flour of Haider 93
T10	0.50%	Straight grade malted barley flour of Haider 93
T11	0.75%	Straight grade malted barley flour of Haider 93
T12	1.00%	Straight grade malted barley flour of Haider 93
T13	0.25%	Whole barley malted flour of Murree Brewery
T14	0.50%	Whole barley malted flour of Murree Brewery
T15	0.75%	Whole barley malted flour of Murree Brewery
T16	1.00%	Whole barley malted flour of Murree Brewery
T17	0.25%	Whole barley malted flour of Jau 87
T18	0.50%	Whole barley malted flour of Jau 87
T19	0.75%	Whole barley malted flour of Jau 87
T20	1.00%	Whole barley malted flour of Jau 87
T21	0.25%	Whole barley malted flour of Haider 93
T22	0.50%	Whole barley malted flour of Haider 93
T23	0.75%	Whole barley malted flour of Haider 93
T24	1.00%	Whole barley malted flour of Haider 93

RESULTS AND DISCUSSION

Falling number (FN)

The straight grade flour showed highest falling number (FN) value (606 sec), while subsequent decrease in FN was observed due to supplementation of different concentrations of various malts (showing low α-amylase activity) as indicated in Table 1.

Gradual decrease in FN was observed with an increase in malt concentration from 0.25% to 1.0%. All types of malted barley flours showed a positive effect of improving α-amylase activity. The highest α-amylase activity was observed when whole barley malted flour of Haider 93 was used at a level of 1.0%. According to Mailhot and Patton (1988) falling number value should be between 200 to 300 seconds for all types of breads.

Mixographic characteristics

Mixograph bowl of 10g capacity was used. In case of mixing time, the influence of barley supplementation was less apparent. The highest mixing time (3.5 min) and the highest peak height (74%) was observed when straight grade malted barley flour of Haider 93 was used at 0.25% and 0.50% levels respectively (Table 2). The peak height varied widely among different malt levels. Hence there was a pronounced effect on peak height due to different concentrations of malts. Angelino (1988) concluded that barley malt flour within desirable limits improves rheological properties of wheat flour which was also observed in the present investigation.

Table 1. Falling number values of different malt supplemented wheat flours.

Types of malt	Concentration of Malt				
	0.00%	0.25%	0.50%	0.75%	1.0%
	Falling number values (Seconds)				
M1	606	296	234	197	178
M2	606	413	323	295	256
M3	606	443	352	311	255
M4	606	330	221	192	192
M5	606	264	222	207	198
M6	606	336	288	251	116

Table 2. Mixographic characteristics of flour by using different levels of malted barley flours.

Types of malt	Concentration of Malt				
	0.00%	0.25%	0.50%	0.75%	1.0%
			1. Mixing Time (min.)		
M1	2.8	2.7	2.6	2.8	2.8
M2	2.8	2.8	2.9	2.4	2.8
M3	2.8	2.7	2.0	2.5	2.8
M4	2.8	2.6	2.9	2.7	2.8
M5	2.8	3.5	2.8	2.8	2.7
M6	2.8	3.3	3.0	2.6	2.7
			2. Peak Height (%)		
M1	60	73	72	69	71
M2	60	68	62	69	70
M3	60	70	72	70	70
M4	60	68	72	68	67
M5	60	73	74	69	70
M6	60	67	68	67	69

Table 3: Loaf weight, volume, weight to volume ratio and bread baking quality score of different malt levels on Punjab 85.

Treatment	Weight(g)	Volume (cc)	Wt. to Vol. ratio	Baking Quality score
T0	142.61	450	3.15	61.0
T1	137.34	425	3.09	56.5
T2	136.33	450	3.30	61.0
T3	128.03	475	3.71	63.5
T4	138.74	475	3.42	66.5
T5	145.77	450	3.08	62.0
T6	143.18	450	3.14	65.0
T7	142.84	475	3.32	66.5
T8	140.36	500	3.56	68.0
T9	141.39	450	3.18	64.0
T10	135.12	475	3.50	65.5
T11	148.80	500	3.36	69.0
T12	150.31	500	3.32	70.0
T13	143.51	475	3.30	71.5
T14	141.24	500	3.54	67.5
T15	138.27	500	3.61	71.0
T16	141.57	525	3.70	72.5
T17	146.28	500	3.24	74.5
T18	139.35	500	3.58	78.5
T19	145.29	550	3.78	74.0
T20	148.27	550	3.69	72.5
T21	142.87	500	3.49	78.0
T22	146.39	525	3.58	79.5
T23	143.17	525	3.66	81.5
T24	139.28	575	4.12	83.0

Bread baking quality

The data on loaf volume, loaf weight and weight to volume ratio of bread prepared from Punjab 85 and Inqulab 91 have been presented in Tables 3 and 4 respectively. Loaf volume and weight to volume ratio was higher in Inqulab 91 as compared to Punjab 85.

In case of bread baking quality, wide variation was noticed as a result of supplementation of different types and concentrations of malted barely flour. The highest baking quality score (91.5) was given to bread of Inqulab 91 when whole barley malted flour of Haider 93 was used at 1.0% level followed by 0.75% and 0.50% (Tables 3, 4). Punjab 85 showed lower score. Lower score of the control sample (T0) was due to the absence or very low α-amylase activity.

In general, wheat varieties showed improvement, specially in bread loaf volume, due to effect of both types and levels of malted barley flours. Whole barley malted flours have given better results on bread loaf volume. Highest bread loaf volume was observed when whole barley malted flour of Haider 93 was used at the rate of 1.0% for Punjab-85 and Inqulab 91 (Tables 3, 4). The major effect of malt supplementation was on bread volume which resulted due to increase in α-amylase activity. Both wheat varieties showed significant improvement on bread loaf volume due to the addition of malted barley flours at different concentrations. The results are in concordance with workers like Warchalewski *et al.* (1989) and Seibel *et al.* (1968). Generally there was a gradual increase in loaf volume score with an increase in malted flour supplementation in wheat flour (Tables 3, 4). It was further observed that whole barely flour yields bread with an improved loaf volume as compared to other types and sources of malted flour. The bread weight obtained from flours supplemented with different types and concentrations of malts showed erratic behaviour.

Inqulab 91 showed highest overall scores, while whole barley malted flours of Haider 93 proved to be the best supplement for both wheat varieties when used at levels of 0.75% and 1.0%. The results revealed that supplementation of whole barley malted flour of Haider 93 improved the quality of bread measured in terms of loaf volume, weight to volume ratio and overall bread baking quality scores. It may be concluded that wheat flour may be supplemented with malt to get better quality bread.

Table 4: Loaf weight, volume, weight to volume ratio and bread baking quality score of different malt levels on Inqulab 91.

Treatment score	Weight(g)	Volume(cc)	Wt. to Vol. ratio	Baking Quality
T0	143.37	475	3.30	65.0
T1	141.71	475	3.35	72.0
T2	142.34	500	3.51	73.5
T3	138.29	500	3.61	74.0
T4	142.74	525	3.67	73.0
T5	145.19	500	3.51	69.5
T6	143.29	500	3.48	71.0
T7	144.84	525	3.62	71.5
T8	143.42	575	4.00	72.0
T9	141.74	500	3.52	68.5
T10	139.29	525	3.76	72.5
T11	148.71	525	3.53	73.5
T12	146.81	550	3.74	77.0
T13	145.72	525	3.60	73.0
T14	143.71	550	3.86	78.0
T15	150.21	575	3.82	81.5
T16	146.92	575	3.91	83.5
T17	138.44	525	3.79	79.5
T18	145.42	575	3.95	83.5
T19	147.75	600	4.06	85.0
T20	147.24	600	4.11	84.5
T21	145.71	550	3.77	87.5
T22	146.07	600	3.10	89.5
T23	147.37	600	3.07	90.5
T24	141.43	625	4.38	91.5

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PHYSICOCHEMICAL CHARACTERISTICS OF OIL FROM THE SEEDS OF *CRAMBE ABYSSINICA*

Shahnaz Hamid, Hamid Ahmad & K.H. Naqvi*
PCSIR Laboratories Complex, Lahore.
**Department of Chemistry,*
Govt. Dyal Singh College, Lahore.

ABSTRACT

The seed oil of *Crambe abyssinica* brought from United States has been analyzed for its physicochemical properties. The seeds upon extraction with n-hexane and chloroform-methanol (2:1) mixture yielded 35.82 per cent and 32.33 per cent oil, respectively. The saponification value of 168.30 shows that more fatty acids present in the oil have a higher number of carbon atoms whereas the iodine value of 95.00 indicates the extent of the unsaturated fatty acids. Erucic acid (58.52%) is the dominant fatty acid followed by oleic acid (18.20%). The oil was composed as hydrocarbon (1.80%), wax esters (3.10%), sterol esters (0.92%), triglycerides (64.20%), free fatty acids (4.90%), 1, 3-diglycerides (6.40%), 1, 2-diglycerides (2.50%) and monoglycerides (4.40%). The presence of erucic acid (58.52%) in the oil of *Crambe abyssinica* indicates its importance in lubricants, plastic and nylon.

Key words: oil; *Crambe abyssinica*; physicochemical properties; saponification value; fatty acids.

INTRODUCTION

Crucifereae or mustard family is a large family including about 220 genera and 3000 species. They are widely distributed but are much more abundant in warm and cold parts of the world than in the tropics. Many of them are alpine plants. Most plants of the family have been reported to grow in Pakistan. Familiar plants are *Brassica compestris* (*sarson*), *B. rapus* (turnip), *Raphnus sativus* (reddish), *B. oleracea* and *botrytis* (cauliflower). These plants are annual or perennial herbs, rarely shrubby with water juice which is often acidic.

Despite all known uses and properties, very little is known about the quality and chemical composition of their fatty acids and essential oils in Pakistan. Vegetable oils and proteins are highly essential to our national economy. In view of the increasingly large demand for oil and protein to support a burgeoning population, oil seeds are being considered as primary nutritional and economical sources (Weber, 1997). The seeds of *Crambe abyssinica* render it fit for many industrial uses. Its oil has been utilized successfully for the manufacture of lubricants (Nieschlag, 1977), plasticizer (Mod, 1969; Neischlag, 1969), nylon (Carlson, 1977) and others (Neischlag, 1971). It has been shown that proper conditions during seed processing make the meal useful for beef cattle feeding (Vanetten, 1977). Leading steel producers have found Crambe oil superior to others in continuous casting of steel (Nieschlag, 1971). A vulcanized product made from Crambe oil has given excellent results in commercial evaluation (Vanetten, 1977). The interest in Crambe stems from high erucic acid contents of the seed oil with its usefulness as a raw material from which other chemicals can be made. However, the contents of this acid may limit the use of Crambe oil for edible purposes because recent investigations have shown that the oil with high erucic acid content causes changes in heart tissues of experimental animals (Perry, 1979; Nieschlag, 1964). Due to the coordinated research efforts between the government and industrial sector. *Crambe abyssinica* has emerged as a new industrial crop in Canada. (Carlson, 1985).

Carlson (1985) reported Crambe meal as protein source for cattle feed. The *C. abyssinica* and *Brassica rapa rabifora* hybrid (typhon) is a new material for the production of oil and proteins. This paper describes some physicochemical characteristics of the oil from the seeds of *Crambe abyssinica*.

MATERIALS AND METHODS

The seeds of *C. abyssinica* were brought from the United States. These were cleaned by removing pebbles, sticks and other unwanted matter. The pale yellow seed coats were removed. The seeds were then ground to a

fine particle size. Crude oil contents were determined from 10 g sample by extracting the oil using Soxhlet apparatus. The extraction was brought about using n-hexane and chloroform-methanol (2:1) mixture. The solvent was removed by rotary evaporator to get the oil. The yields of the oil with n-hexane and chloroform-methanol (2:1) mixture are reported in Table 1.

Table 1. Percentage recovery of oils from seeds of *Crambe abyssinica*

Seed	Solvent used in extraction	Weight of seeds (g)	Yield of oil (g)	Percentage of oil
Crambe abyssinica	n-hexane	10.00	3.582	35.82
Crambe abyssinica	Methanol	10.00	2.233	32.33

Physicochemical properties

The physicochemical properties like moisture, refractive index, specific gravity, colour, acid value, free-fatty acids (FFA), iodine value, unsaponification value, saponification value, etc. of the lipids were determined by standard AOAC methods (Horvitz, 1970) and the results are given in Table 2.

Table 2. Physicochemical characteristics of oil of *Crambe abyssinica*

Moisture content	5.94%
Refractive index at:	
25°C	1.466
40°C	1.462
60°C	1.459
Specific gravity at 25°C	0.90
Colour	Pale yellow
Acid value	0.56
Free fatty acid value	0.282
Iodine value	96.00
Saponification value	168.30
Unsaponification (%)	0.50
Fixed seed oil (%)	35.82
Smoke point at 520°C	269

Thin layer chromatography (TLC)

The thin layer chromatograms of 0.5 mm thickness were prepared by using 50 g silica gel and 100 mL water. These were activated at 105°C in thermostated oven for one hour and later used for the separation of neutral and polar lipids by using n-hexane, ether and acetic acid in 60:20:2 v/v, respectively. The saturated solution of antimony trichloride in chloroform was used for the identification of sterols as well as sterol esters. Appearance of red violet spots on thin layer chromatograms when kept at 100°C for 10 minutes confirmed the presence of these compounds (Raie *et al.*, 1989).

The general composition of the neutral lipids component as revealed by TLC is given in Table 4.

Identification of fatty acids

Methyl esters of fatty acids were prepared (Raie *et al.*, 1983) and analyzed on Schimadzn. GC 14A gas chromatograph with flame ionization detector using 1.6 m 3 mm (id) glass column packed with diethylene glycol succinate 15% coated on Scimalite AW 201 (60-80 mesh) column. Temperature was programmed at 153°C for low and then with a rise of 5°C per minute to 200°C. Injector and detector temperatures were 250°C and 300°C, respectively. Nitrogen was used as carrier gas with a flow rate 40 mL per minute. The methyl esters were identified by comparing their retention times with those of authentic methyl fatty esters under the same conditions. The percentage of various acids were determined by Scimadzn-CR4, chromatopack computing integrator. The results are given in Table 3.

Table 3. Fatty acid composition of *Crambe abyssinica* seed oil

Palmitic	$C_{16:1}$	2.76
Palmitoleic	$C_{16:1}$	0.19
Stearic	$C_{18:0}$	0.16
Oleic	$C_{18:1}$	18.20
Linoleic	$C_{18:2}$	11.23
Linolenic	$C_{18:3}$	7.50
Erucic	$C_{18:3}$	58.52

Table 4. Percentage composition of neutral lipids of *Crambe abyssinica* by TLC

Lipid	Percentage
Hydrocarbons	1.80
Wax esters	3.10
Sterol esters	0.92
Triglycerides	64.20
Free fatty acids	4.90
1, 3-diglycerides	6.40
1, 2-diglycerides	2.50
Monoglycerides	4.40

RESULTS AND DISCUSSION

The results presented in the Tables show that the values of various physical properties of Crambe oil are generally on the higher side as compared to other domestic vegetable oils. The physical properties are summarized in Table 2. Some properties along with the visible characteristics are compared with those of other common Crucifer seeds. All other parameters are comparable except the acid value which is less in case of *Crambe* seeds. On the other hand, erucic acid is higher in it.

Glucosinulates are natural toxicants common in vegetable and seed meals from the plants of the family Cruciferae. It has been noted that proper conditions during seed processing can make the meal useful for animal feeding. The glucosinulate content of the processing can make the meal useful for animal feeding. The glucosinulate content of the fraction is about 8 per cent which is too high to feed to monogastric animals. However, if it is maintained at 5-9 per cent level, weight gains in ruminants are satisfactory. Glucosinulates and their breakdown products are known to effect humans and animals in various detrimental ways. They have been linked with thyroid disturbance, liver damage, throat abscesses, appetite depression, tongue swelling and abortion in animals (Princon, 1984).

The saponification value of 168.3 shows that more fatty acids present in the oil have a higher number of carbon. The presence of erucic acid (55-60%) is comparable to the rape seed oil which has 40-55 per cent erucic acid by weight. Rapeseed oil is already being used as lubricant in steel manufacturing. Erucic acid or its derivatives are used in plastics, foam suppressants and lubricants. Use of Crambe oil in all these applications has been found quite satisfactory or even superior and hence it seems to be a good substitute for higher erucic acid rapeseed oil which has been reported in one of the most novel applications (Carlson, 1977).

Thin layer chromatography of the oil shows that its major constituents are mainly the triglycerides (84.2%). The neutral as well as the polar lipids were identified by comparing the Rf values with standard. The results of thin layer chromatography analysis of the oil are given in Table 4. The lipids are classified by thin layer chromatography into various neutral lipids such as hydrocarbons, sterol esters, triglycerides, 1, 3-triglycerides, 1, 2-triglycerides, monoglycerides and free fatty acids by using solvent system as hexane-ether and acetic acid.

The results indicate that the percentage of polar lipids were very low as compared to neutral lipids. So, like other unsaturated vegetable oils, Crambe oil may be vulcanized reacting with sulphur or sulphur-derivatives. The familiar art gum eraser is made of vulcanized vegetable oil. The vulcanized vegetable oils are also blended with neutral and synthetic rubbers to facilitate processing to provide soft elastic products with improved resistance to light and ozone and to increase tolerance towards liquid plasticizers (Bailey, 1964). A vulcanized product made from Crambe oil has given excellent results in commercial evaluation (Nieschlag, 1971).

Leading steel producers have found Crambe oil to be superior to other oils for continuous casting of steel. It should be beneficial in formulating lubricants since addition of high erucic triglycerides to mineral oil is known to increase oiliness of base stock and hence improve durability under high speed and high pressure operation (Nieschlag, 1977). The high content of erucic acid limits the use of Crambe oil for edible purposes because investigations have shown that a high erucic acid content causes damage to the heart of experimental animals (Perry, 1979).

Current trends in most countries especially in Canada, towards production of low erucic acid rapeseed variables for improved nutritional quality of the oil, have increased the importance of Crambe oil as a domestic source of high erucic oil for industrial purposes. It is interesting to mention here that as a result of cooperative research between Government and the industry, undertaken in Canada, *Crambe abyssinica* has now emerged as a new industrial crop in that country.

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FATTY ACID ANALYSIS OF TOTAL LIPID IN CHICKEN LIVERS

Khalid Zaheer, Rizwan Maqbool, Zahid Hussain, Ali Abbas Qazilbash & Salma Jahan*
Department of Biological Sciences, Quaid-i-Azam University, Islamabad.
**Fisheries & Livestock Department, Government of Sindh, Karachi.*

ABSTRACT

Studies were undertaken to analyze liver lipids and fatty acid composition of total lipids of broiler and domestic (*desi*) chicken by thin layer and gas liquid chromatography and to whether there are significant differences in the fatty acid composition of both the chicken varieties. Although, both types of chicken are used as protein source for human but present studies reported higher degree of unsaturation in broiler than domestic chicken.

INTRODUCTION

The chicken is a useful model for conducting studies in lipid chromatography because of the changes in lipid metabolism during development (Pfeifer *et al.*, 1993). The composition of the adipose tissue of simple stomach animals of which pigs and poultry are economically the most important and is markedly affected by the fat in the diet (Gurr & James, 1980). The fatty acid is made up of hydrocarbon chain from which the properties of lipid solubility derive and is terminal carboxyl group giving acidic properties (Gunstone & Norris, 1981).

The present study was undertaken to analyze liver lipids and fatty acid composition of total lipids of broiler and domestic (*desi*) chicken and to know whether there are significant differences in the fatty acid composition of both the chicken varieties.

MATERIALS AND METHODS

Extraction of total lipids was accomplished with chloroform methanol (2:1 v/v), 20-folds the volume of pre-weighed test samples according to the procedure of Folch *et al.* (1957).

The lipid classes were determined by spotting an aliquot of the extract on thin layer silica gel HF254, type 60 (0.5 mm thick) and activated for an hour at 110°C. Zones representing lipid classes were visualized with iodine vapours (Malins & Mangold, 1960). Reference standards were chromatographed along with the samples. Following solvent systems were used during the analysis of total lipids:

Petroleum ether	Diethyl ether	Acetic acid
90	10	1 (v/v Malins & Mangold, 1960).
80	20	1 (v/v Khan & Khalid, 1970).

Fatty acid methyl esters were analysed isothermally on Packard Model 430 Gas Chromatograph equipped with flame ionization detector. Methyl esters were separated on glass column (1 mm x 2 mm) packed with 10% SE on 80/100 mesh gas chrom and recorded on Packard Model 613 Recorder. The temperature of injection port, detector and oven was 270°C. Nitrogen, hydrogen and air flow rates were 25 mL minute^{-1} and 1 atmosphere. Chart speed was 0.1 mm second^{-1}.

RESULTS AND DISCUSSION

The percentage composition of total lipids in the livers of both domestic and broiler chicken are presented in Table 1 which showed higher percentage of lipids in broiler chicken livers than the domestic one. In both varieties, the saponifiable matter gave higher percentage compared to non-saponifiable. Further, broiler chicken sample showed increased amount of saponified (56.40%) and non-saponifiable (27.00%) matter than the domestic chicken. The percentage composition of saturated and unsaturated fatty acids of total lipids in both the varieties showed variation and were calculated from the results of gas liquid chromatography.

Figure 1 represents the thin layer chromatographic analysis of lipids extracted from livers of broiler and domestic chicken. Also the spots of standards are visible which were run along the samples. Hydrocarbons, sterol esters, triglycerides, free fatty acids, 1,3-diglycerides, 1,2-diglycerides, sterols, monoglycerides and phospholipids appeared as lipid classes in both the samples.

Table 2 showed the saturated and unsaturated fatty acid composition of total lipid in chicken livers of both varieties as determined by the gas liquid chromatography. It is found that in both the cases

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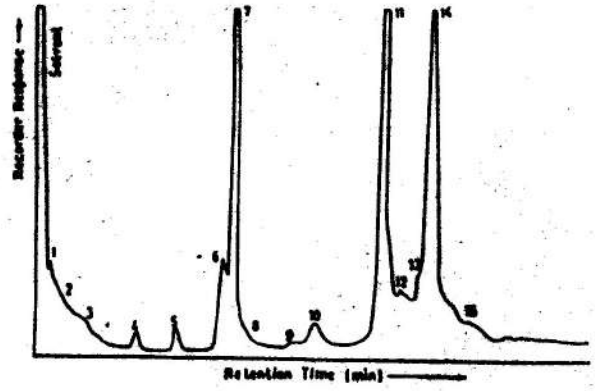

Fig. 2. Gas chromatograph of fatty acid methyl esters derived from total lipid of broiler chicken.

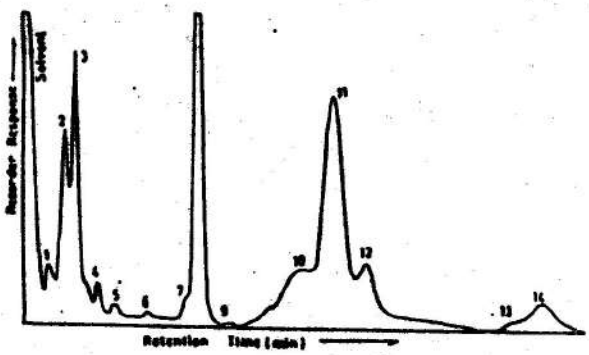

Fig. 3. Gas chromatograph of fatty acid methyl esters derived from total lipid of domestic chicken.

The percentage of lipids in broiler and domestic chicken livers is 8.08 and 6.12, respectively. This difference of percentage in total lipids may be due to defined/specific diet of the broiler chicken. From the data, it is evident that in domestic chicken the unsaturated fatty acids of total lipids were 58.43% as compared to 75.11% of broiler chicken. Similarly, 41.53% of saturated fatty acids in domestic chicken as compared to 11.75% of broiler chicken. Whereas Kaitrante and Reino (1979) reported that chloroform:methanol extraction of white flesh gave an average lipid content of 3%. David and Paul (1969) reported the presence of saturated fatty acids in the liver lipids of broilers as 47% and unsaturated fatty acids ranged from 13% to 19%. Number of factors are responsible for variation in the proportion of fatty acids present in the chicken liver and flesh. Gomez and Fernandez (1988) reported that during chick embryo development there is slight increase in the ratio of unsaturated to saturated fatty acids. Number of researchers have reported that the activity and concentration of the animal fatty acid synthase vary depending upon the nutritional, hormonal and developmental status (Fischer & Goodridge, 1978; Joshi & Aranda, 1979; Kasturi & Joshi, 1982; Evangelista *et al.*, 1993; Maria *et al.*, 1991).

The lipids were separated by thin layer chromatography. In both varieties of chicken, phospholipids and hydrocarbons constitute major part of the total lipids, whereas sterol esters, free fatty acids and monoglycerides were present in less concentration. These findings are supported by Aleksandrova (1992) who used densitometric method for the quantitative determination of natural lipid composition. Gas liquid chromatographic patterns of both chicken samples (Fig. 2-3) revealed differences in saturated and unsaturated fatty acid proportion of the total lipids. Similar pattern of results were obtained by Kang *et al.* (1994) who studied effect of dietary energy level on the fatty acid composition.

The purpose of the current study was to determine the fatty acid composition of both broiler and domestic chicken. Data presented here is extensive and provide an insight into the range and proportions of fatty acids present in both types of chicken. Although, both types of chicken are used as protein source for humans but current studies reported higher degree of unsaturation in broiler than the domestic chicken.

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EFFECT OF TALLOW ON THE QUALITY OF FRESH BEEF SAUSAGES DURING STORAGE

Zubair Farooq, Nuzhat Huma, Salim-ur-Rehman & M. Asghar*
Department of Food Technology,
**Department of Biochemistry, University of Agriculture, Faisalabad.*

ABSTRACT

Sausages are prepared from a mixture of minced meat/meats, seasoned, spiced and stuffed into casing. Shrinkage of sausages during cooking is a problem which could be reduced by the addition of tallow into lean meat. Sausages containing 0, 10, 20 and 30% tallow were prepared, frozen and stored for 60 days at -20°C. After 15 days interval, each batch was thawed and subjected to chemical analysis and then deep fat fried in corn oil for sensory evaluation. Analysis of variance showed that addition of tallow and storage periods affected the quality of sausages highly significantly and had a considerable range of variability. Sausages having 10% tallow level were found highly acceptable.

INTRODUCTION

Sausage is a form of processed meat, deriving its name from the Roman word *Salas*, meaning preserved meat (Smith *et al.*, 1982). There are many kinds of sausages differentiated on the basis of their formulations and processing procedures. Fresh sausages are made from fresh and uncured/cured meat which are usually cooked before eating.

The recipes and preparation methods of the sausages vary according to locality and taste of the people. In Pakistan, sausages are imported from various Islamic countries. Meat processors are least interested in their manufacturing due to a number of factors such as technical know how, higher price of the product, taste and eating habits of the people.

Some of the important merits of the sausages including ease of preparation for meal, nutritive value, taste and new product suggest that they have all the potentialities of finding popularly among our masses especially during the days of famine and in armed forces as a new addition to their diet.

Sausages made from a mixture of beef and pork are preferred than beef only because beef is less fatty. So, fat reduces shrinkage in cooking (Ashbrook, 1954). As pork is unlawful in Islam so tallow could be added to coup with the problem of shrikage in fresh sausages, but some adjustments were needed in the original formulations considering the taste and flavour suit to our consumers.

There is a genuine need among processors for scientific information on the most desirable fat content for maximum consumer acceptance (Huffman & Powell, 1970). Consumer interest in reduction of fat consumption has led to the development of low-fat hamburgers (Egbert *et al.*, 1991). So, in order to produce fat-reduced meat products, Wirth (1988) has developed methods for manufacture of fat-reduced dry sausage, Frankfurter type sausage, liver sausage, brawn and spreadable sausage.

The objectives of this study were to know the acceptance of consumer and effect of storage on the quality of sausage.

MATERIALS AND METHODS

Beef and other ingredients were procured from the local market. Fresh sheep casings (Ashbrook, 1954) were obtained from the slaughterhouse, washed thoroughly with water and then used for stuffing.

Lean meat was trimmed, deboned, washed, cut into small pieces and then minced under the conditions as described by Romans and Ziegler (1974).

Sausages were prepared according to the method described by Shabbir (1974). All the ingredients were mixed together thoroughly at 10°C to attain uniformity by hand and stuffed into natural casing with the help of syringe. The stuffed sausages were stored at -20°C for 60 days for chemical and sensory evaluation.

The slow frozen sausages were analysed after 15 days intervals for moisture, ash, protein and fat contents according to the methods described in AOAC (1990). Acidity, peroxide value and free fatty acid contents were determined by modified AOAC's methods (Koniecko, 1985). The pH was determined by the method as described by Acton *et al.* (1972).

For sensory evaluation, sausages were thawed, then deep fat fried (in corn oil) individually in cooking pan

for 5 minutes at 232°C and presented to a panel of 5 judges to evaluate colour, flavour, texture, juiciness, tenderness, chewability and taste following 0-10 point intensity rating scale (Land & Shepherd, 1988).

The data obtained was analysed by analysis of variance technique using completely randomised factorial design according to the methods described by Steel and Torrie (1980) and Smith (1988).

RESULTS AND DISCUSSION

The results of chemical analysis of sausages having different treatments are shown in Table 1. Moisture, ash and protein contents decreased with the increase in tallow level. Decrease in moisture was same as found by Huffman *et al.* (1970) while using fat percentages of 15, 25 and 35. Decrease in the ash contents and protein was supported by Theodore *et al.* (1967). Fat percentage increased due to added levels of tallow. Values for free fatty acids and peroxide increased with increasing tallow but they were within the range as given by FAO (1986). The increase in pH values due to increase in tallow were within the acceptable range proposed by Koniecko (1985). Acidity of the sausages varied non-significantly with increasing tallow percentages. Graphical representation as shown by Fig. 1 and 2 makes the results clear.

Effect of storage on sausage is shown in Table 2. Moisture, ash, protein, fat and pH values decreased as storage period increased from 0-60 days. Moisture decreased due to evaporation as narrated by Shabbir (1974). Ash contents decreased due to leaching effect.

Decrease in protein took place due to degradation as reported by Theodore *et al.* (1967). Slight decrease in the fat percentage occurred due to oxidation of fat like that reported by Shabbir (1974). The pH changed due to chemical and enzymic changes (Shabbir, 1974). Free fatty acids increased due to fat hydrolysis (FAO, 1986). The results of peroxide value are in line with Shabbir (1974). Acidity of sausages during storage increased as a result of peroxide formation at the double bonds by the atmospheric oxygen and hydrolysis by microorganisms (Plummer, 1988). Graphs shown in Fig. 3 and 4 describe the effect of storage on chemical characteristics of sausages.

Table 1. Effect of treatments on the quality of sausages

	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	FFA (%)	PV (meq/kg)	pH	Acidity (%)
T1 (Control)	70.37 a	0.97 a	20.38 a	2.88 d	0.40 a	0.10 d	5.81 b	0.08 a
T2 (10%)	65.12 b	0.84 b	18.99 b	11.62 c	0.41 b	0.16 c	5.83 b	0.08 a
T3 (20%)	63.92 c	0.75 c	17.01 c	17.73 b	0.71 c	0.20 b	5.83 b	0.08 a
T4 (30%)	59.65 d	0.68 d	15.03 d	28.41 a	0.77 c	0.31 a	5.87 a	0.09 a

Values sharing the same letter are non-significant.

Table 2. Effect of storage on the chemical characteristics of sausages

	Moisture (%)	Ash (%)	Protein (%)	Fat (%)	FFA (%)	PV (mEq/kg)	pH	Acidity (%)
0 day	70.37 a	0.97 a	20.38 a	2.88 d	0.40 a	0.10 d	5.81 b	0.08 a
15 days	65.12 b	0.84 b	18.99 b	11.62 c	0.41 b	0.16 c	5.83 b	0.08 a
30 days	63.92 c	0.75 c	17.01 c	17.73 b	0.71 c	0.20 b	5.83 b	0.08 a
60 days	59.65 d	0.68 d	15.03 d	28.41 a	0.77 c	0.31 a	5.87 a	0.09 a

Values sharing the same letter are non-significant.

Table 3. Effect of treatments on the quality of sausages

	Colour	Flavour	Texture	Taste	Tenderness	Juiciness	Chewability
0 day	8.20 a	6.20 b	7.20 b	7.40 b	5.80 c	6.40 b	6.80 b
15 days	7.40 b	7.20 a	8.00 a	7.80 a	7.60 a	7.20 a	7.40 a
30 days	7.40 b	6.20 b	7.20 b	7.00 c	6.76 b	6.60 b	5.80 c
60 days	5.80 c	6.20 b	6.40 c	6.60 d	6.76 b	6.60 b	5.80 c

Values sharing the same letter are non-significant.

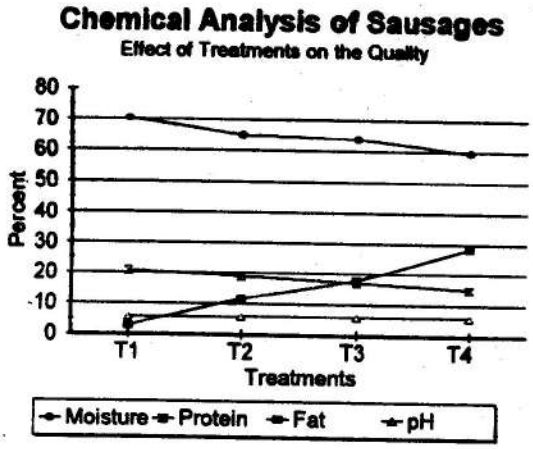

Fig. 1. Chemical analysis of sausages (Effect of treatments on the quality).

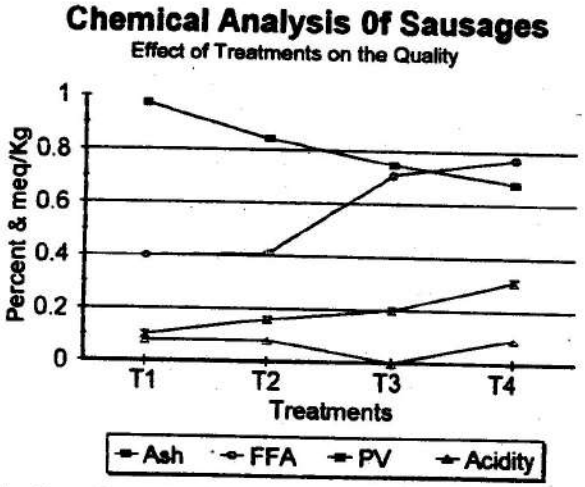

Fig. 2. Chemical analysis of sausages (Effect of treatments on the quality).

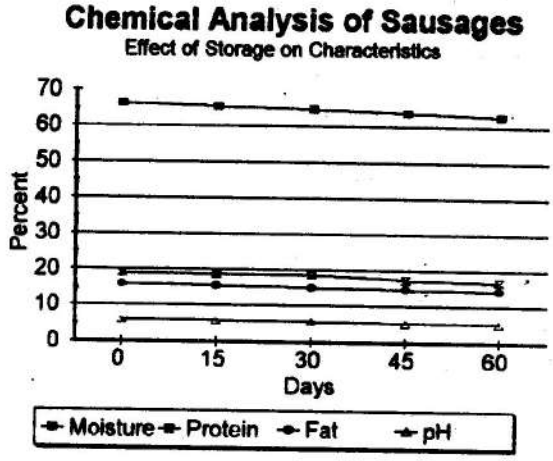

Fig. 3. Chemical analysis of sausages (Effect of storage on characteristics).

Table 4 shows the effect of storage on the sensory characteristics of the sausages. The values for colour, flavour, taste, tenderness, juiciness and chewability decreases with the increase in storage period. Decrease in colour score took place due to fat oxidation (Watts & Lehman, 1954). Flavour scores decreased due to oxidative deterioration of fat (Freeman *et al.*, 1954). As regards texture of the sausages, the highest quality score was obtained after a storage period of 30 days. Decrease in taste score also occurred due to fat oxidation. Tenderness and juiciness of the sausages were reduced due to the moisture evaporation during storage (Egbert *et al.*, 1991). The values for chewability decreased because of protein degradation (Becker *et al.*, 1969). Fig. 6 explains this effect graphically.

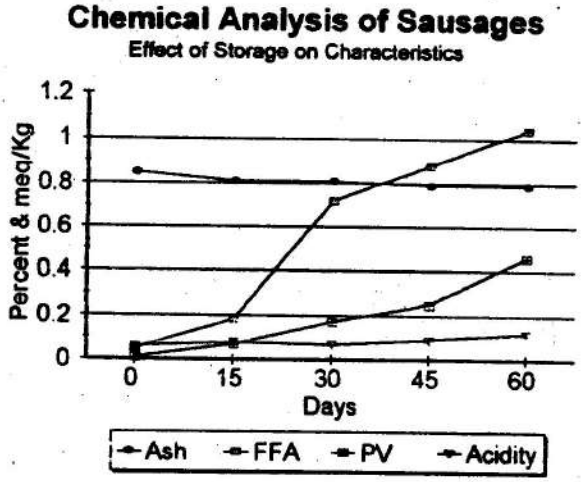

Fig. 4. Chemical analysis of sausages (Effect of storage on characteristics).

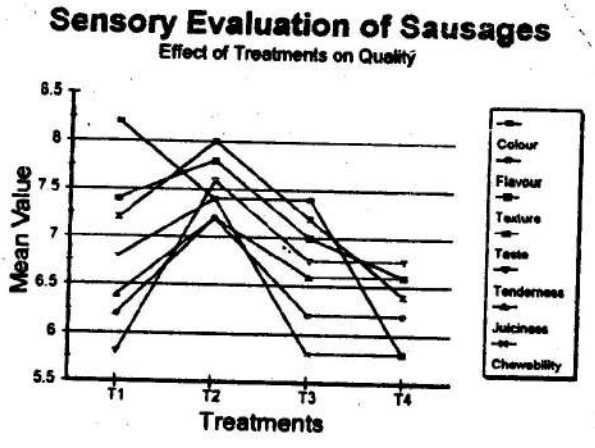

Fig. 5. Chemical analysis of sausages (Effect of treatments on quality).

Table 4. Effect of storage on the quality of sausages

	Colour	Flavour	Texture	Taste	Tenderness	Juiciness	Chewability
0 day	7.65 a	7.25 a	7.00 b	7.75 a	7.75 a	7.75 a	7.50 a
15 days	7.65 a	7.25 a	7.00 b	7.50 a	7.00 b	7.00 b	6.50 b
30 days	7.65 a	6.25 b	7.75 a	7.50 a	7.00 b	6.50 c	6.50 b
45 days	7.00 b	6.25 b	7.25 b	6.50 b	6.00 c	6.25 cd	5.75 c
60 days	6.00 c	5.25 c	7.00 b	6.75 b	5.90 c	6.00 d	5.75 c

Values sharing the same letter are non-significant.

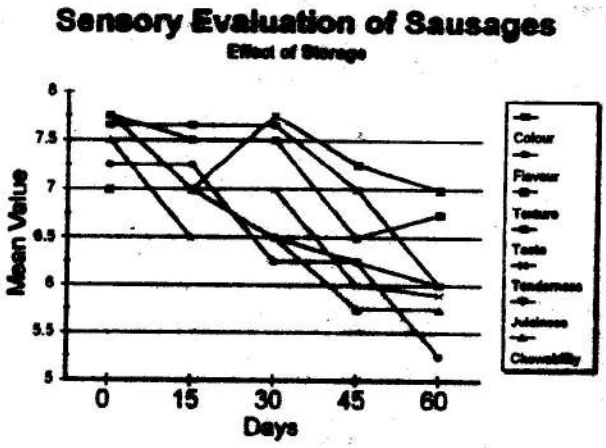

Fig. 6. Chemical analysis of sausages (Effect of storage).

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EFFECT OF TRADITIONAL STORAGE ON THE EXTRACTABILITY OF SOYMILK FROM SOYBEAN

Razia Adil, M. Aslam, and M. Arshad
Biotechnology and Food Research Centre,
PCSIR Laboratories Complex,
Lahore 54600, Pakistan.

ABSTRACT

During traditional storage of soybeans, no significant effect was found on the extractability of soymilk solids. Overall losses of nutrients ranged from 14-15 per cent. Proteins and carbohydrates are the major components that are lost during processing.

INTRODUCTION

Soymilk is the water extract of dry mature soybean. It is generally prepared by boiling water grind method and filtering the residue. The product is a milky-looking liquid and in no way a new product. It is manufactured or home-made in most countries of East Asia and is consumed as a general drink.

Soymilk has nearly a protein content and quality that is quite comparable to cow's milk. However, the composition of soymilk varies depending upon the variety of soybean and process of manufacturing. It has been a subject of interest to agencies that intend to prepare milk-like beverages on commercial scale, especially for those areas of the world where protein malnutrition is serious problem. Unfortunately, soymilk has a strong characteristic 'beany' flavour (Hand *et al.*, 1964) which makes it unacceptable to non-oriental people. Studies on beany flavour in soymilk and flour (Mustakas *et al.*, 1967; Badenhop & Hackler, 1970) effect of processing (Khaleque *et al.*, 1967; Wilkens *et al.*, 1967) and model storage of soybean (Saio *et al.*, 1982; Sono *et al.*, 1985) were carried out but not much published work on extractability of soymilk from soybean stored at ambient conditions is available. Since soymilk is of commercial value, therefore, present studies were carried out to find out the effect of traditional storage of beans on the extraction of soymilk.

MATERIALS AND METHODS

Soybean (light yellow variety) was directly procured from Seed Division, Ghee Corporation of Pakistan and stored in gunny bags at ambient conditions.

Soybean (200 g) on moisture free basis was treated with sodium bicarbonate solution (0.1%) according to the method described earlier (Arshad *et al.*, 1980). Treated soybeans were ground in soybean chopper with the addition of 1.2 L hot water (80-90°C) for 12 minutes exactly. The milky extract was filtered through a nylon cloth (150 mesh). Residue was squeezed out to the last drop of eater and total volume of water-extract was measured. Total solids extracted in the milk were calculated in total volume. The residue left over was also dried and weighed. Experiments were repeated after regular intervals of 2 months for a period of ten months. Percentage of milk solids, soy residue and losses due to the treatment and processing were calculated on moisture free basis.

Chemical composition of soybean, milk solids and residue were determined according to AOAC method (AOAC, 1984).

RESULTS AND DISCUSSION

The results of analysis of fresh soybeans are shown in Table-1, while that of soymilk solid and soy residue are shown in Table-3 and 4, respectively. Dispersive break-up of each experiment is also shown in Table-2. Overall extraction of soymilk solids ranged from 43.2 to 44.9 per cent of soymilk solids was found when the moisture content of seeds was 11-12 per cent as the seeds at this stage were tender. The lower yield 43.2-43.5 per cent of soymilk solids was found during dry months (April to June), when the moisture content of the seeds was 7-8 per cent. Hence it was concluded that there was no significant effect on the extraction of soymilk solids as a result of storage of soybeans. It was also observed that overall losses during treatment and processing of soybeans ranged from 14-15 per cent with average loss of 14.8 per cent (Table-2). The average losses (Table-5) of proteins, fat, ash and carbohydrates were 6.0, 2.0, 1.0, and 5.8 per cent, respectively. Proteins and carbohydrates are the major components that are lost during treatment and processing.

Table 1. Analysis of fresh soybeans g/100 g (results on moisture free basis)

Contents	Percentage
Protein	35.5
Fat	25.5
Ash	6.0
Fibre	6.5
Carbohydrates (by difference)	26.5

Table 2. Break-up of each experiment g/100 g (results on moisture free basis)

	Storage (in months)					
	0	2	4	6	8	10
Moisture % in seeds	12.0	11.1	8.0	7.0	11.0	11.9
Soymilk solids	44.5	44.6	43.5	43.2	44.4	44.9
Soy residue	40.5	40.4	42.5	42.0	40.6	40.1
Losses	15.0	15.0	14.0	14.8	15.0	15.0

Table 3. Analysis of soymilk solids g/100 g (results on moisture free basis)

	Months					
	0	2	4	6	8	10
Proteins	41.5	41.4	40.6	40.5	41.3	41.4
Fat	33.5	33.6	33.2	33.4	33.7	33.6
Ash	5.5	5.3	5.8	5.8	5.6	5.8
Carbohydrates (by difference)	19.5	19.7	20.4	20.3	19.4	19.2

Table 4. Analysis of soy residue g/100 g (results on moisture free basis)

	Months					
	0	2	4	6	8	10
Proteins	28.3	27.0	29.4	29.5	29.5	29.3
Fat	22.2	22.1	20.5	21.3	21.0	21.4
Ash	5.7	4.7	4.9	4.4	5.1	4.7
Fibre	14.0	14.2	13.5	12.9	15.8	14.1
Carbohydrates (by difference)	29.8	32.0	31.7	31.9	28.6	30.5

Table 5 Average losses of components g/100 g (results on moisture free basis)

Contents	Percentage
Protein	6.0
Fat	2.0
Ash	1.0
Carbohydrates (by difference)	5.8
Total	14.8

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EXTENSION ARTICLE

TESTING MILK AND MEAT FOR ANTIBIOTIC RESIDUES

Ghulam Muhammad, Anil K. Anal*, M. Athar, M. Saleem, and Surya L. Bajracharya
Department of Clinical Medicine & Surgery, Faculty of Veterinary Science
University of Agriculture, Faisalabad 38040, Pakistan.
**Asian Institute of Technology, Bangkok, Thailand.*

INTRODUCTION

With the burgeoning human population associated with an ever increasing demand of food of animal origin, more and more reliance is being placed on the use of antibiotics as therapeutic, preventative and growth promoting agents in food animal production. This widespread use of antibiotics is associated with a problem of antibiotic residues which may remain in the tissues of the medicated animals and ultimately become a part of the human diet. The prevention of antibiotic residues in milk and meat is a concern shared by consumers, food processors, drug manufacturers as well as the veterinarians and general public. It is pertinent to mention that except for some tetracyclines, most therapeutic antibiotics are relatively heat stable and resist both pasteurization and cooking processes. The present paper reviews some information of the sources of antibiotic residues, problems associated with these and the methods of testing milk and meat for antibiotic residues.

Reasons of antibiotic residues in milk and meat:

1. Failure to observe drug withdrawal period.
2. Extended usage or excessive dosages of antibiotics.
3. Non-existence of restrictive legislations or their inadequate enforcement (this is single most important reason for the apparent widespread violative levels of antibiotics residues in milk and meat in Pakistan).
4. Poor records of treatment.
5. Failure to identify treated animals.
6. Lack of advice on withdrawal periods.
7. Extra-label use of antibiotics.
8. Availability of antibiotics to lay persons as over-the-counter drugs in the developing countries.
9. The addition of antibiotics as milk preservative during hauling from the center of production (villages) to the centres of consumption (cities).
10. Lack of consumer awareness about the magnitude and human health hazards associated with antibiotic residues in the food of animal origin.

Reasons for testing milk and meat for antibiotic residues:

The need for testing milk or meat for antibiotic residues stems from the following reasons:

1. Hypersensitivities of human beings to antibiotics: Among almost all antibiotics, penicillin is without doubt the most frequent sensitizing antibiotic. About 21-80% of all drug allergies are caused by penicillin alone. The allergic effect may vary with the kind of preparation, duration of the treatment, daily dosage, route of administration or frequency of repetition of therapy. The frequency of allergic reactions to penicillin vary form 0.7 to 10% in various reported series, out of whom 0.015-0.2% developed an anaphylactic type reaction. The fatality rate of penicillin allergy has been estimated as 0.0015 to 0.01%. Orally administered penicillin preparations have a reaction incidence of about 0.3%. It has been reported that the incidence of penicillin sensitivity is about equal in adults of both sexes, and that children of both sexes under 12 years of age are more resistant than adults.

The most serious hypersensitivity reactions produced by penicillin are angioderma, serum sickness, anaphylaxis and the Arthus phenomenon. Angioderma with marked swelling of lips, tongue, face, and periorbital tissues, not infrequently accompanied by asthmatic breathing has been observed after oral consumption even with low quantities (such as those present in milk and meat as residues) as well as after topical or systemic administration of different kinds of penicillin. Serum sickness follows sensitization, especially to the repository penicillin. The illness varies in severity from mild fever, rash and leukopenia to severe arthralgia, purpura, lymphadenopathy, splenomegaly, mental changes, myocarditis, generalized oedema and hematuria. These reactions generally occur after consumption of penicillin for one week or longer. In few instances, patients repeated exposures to penicillin may develop allergic vesiculitis, disseminated lupus erythematosus or polyarthritis.

Anaphylactic reactions may occur with the ingestion of penicillin contaminated milk. The most dramatic is sudden, severe hypotension and rapid death

while in most instances, bronchoconstriction with severe asthma, abdominal pain, nausea, vomiting, extreme weakness, hypotension, diarrhoea and purpuric skin eruptions characterize the anaphylactic episodes.

2. ***Development of antibiotic resistance among the gut flora and enteric pathogens of consumers:*** The consumption of milk and meat containing traces of antibiotics over a protracted period of time may lead to emergence of resistance among gut flora and pathogens of consumers. The development of resistance to an antibiotic involves a stable genetic change, heritable from generation to generation. Any of the mechanisms that result in alteration of bacterial genetic composition can operate and bacteria may thus become antimicrobial resistant by mutation, transduction, transformation, or conjugation. The first three mechanisms i.e. mutation, transduction, transformation are particularly involved in the development of drug insensitivity in Gram-positive cocci, while all four may be responsible for the acquisition of resistance by Gram-negative bacilli. Regardless of the genetic mechanisms involved in the development of resistance, the basic alterations in susceptibility are related to (i) elaboration of drug-metabolizing enzymes such as penicillinase, cephalosporinase, and adenylylating, phosphorylating and acetylating enzymes, (ii) alteration of the permeability of the bacterial cell to drug, (iii) increased amount of an endogenous antagonist of drug action, or and (iv) alteration of the amount of drug receptor or the binding characteristics of the compound to its critical target.

Nonpathogenic *E. coli* in the human gut flora have the ability to acquire drug resistance and to transfer this resistance to sensitive pathogenic salmonellae. These latter organisms may then become refractory to a range of antibiotic compounds without ever contacting them directly.

3. ***The problem of superinfection:*** Superinfection refers to a fresh invasion or reinfection added to an already existing infection. Some definitions limit the use of the term to organisms of the same species that caused the initial infection. Candidiasis is a classical example of the untoward consequence of the use of antibiotics. The causative organism, i.e. *Candida albicans* occurs normally in small numbers saprophytically on the mucus membranes of the respiratory, gastrointestinal and uro-genital tracts of many healthy humans and animals. Although this organism is non-pathogenic in healthy individuals, it may become invasive and behave as opportunistic pathogen in persons who have immunosuppressive diseases or are consuming antibiotics continuously either as therapy or through milk and meat as residues. By a continuous consumption of antibiotic-tainted milk or meat, candidiasis characterized by superficial lesions on skin (cutaneous candidiasis) and on mucus membranes (oral and vaginal thrush) may develop.

4. ***Fermentation problems in milk processing:*** Antibiotics in milk prevent souring by lactic acid bacteria (e.g., *Streptococcus cremoris* and *Lactobacillus bulgaricus* etc.) and so inhibit starter growth in cheese making and the growth of lactic organisms in yoghurt and other cultured milks. In view of these reasons, in countries where dairy and food processing industries are well developed (like in Europe, USA, Australia etc.), antibiotics-tainted milk is considered to be unfit for processing. Therefore, antibiotic residues can result in substantial penalties being levied against the producers, if care is not taken to follow recommended withdrawal times for the particular antibiotic products. When an antimicrobial is used, it is incumbent upon the practitioner to notify the owner that the animal cannot be slaughtered (or milk sent for human consumption) before withdrawal (or withholding) periods has expired.

5. ***Adverse effect on the development of teeth and bones:*** Reports in the literature suggest that prolonged ingestion of tetracycline from any source, including food, has detrimental effects on teeth and bones in growing children. Similarly, quinolones (e.g., nor-floxacin, pefloxacin, enrofloxacin, flumequine, ciprofloxacin, nalidixic acid, fleroxacin, etc.) interfere with the cartilage development in children and their use in children should therefore be deprecated.

6. ***Chloramphenicol-associated bone marrow depression in human beings:*** Shortly after the introduction of chloramphenicol in 1950, researchers reported a causal relationship between the use of this drug and development of bone marrow depression. The bone marrow depression and resulting anaemia have been divided into 2 types; a reversible, dose-related anaemia and an irreversible fatal aplastic anaemia (which is idiosyncratic and genetically based predisposition) that is not dose-related. Instances are described in literature whereby (a) patients developed aplastic anaemia following topical ocular preparations of chloramphenicol or (b) accident exposure of a farmer to this drug while administrating it to cattle. Conceivable, therefore, aplastic anaemia could occur in susceptible individuals who are exposed to concentrations of chloramphenicol that approach those that might be secreted in milk or remain in meat of chloramphenicol-treated food-

producing animals. As such the code of Federal Regulations of USA prohibits the use of this drug in animals raised for food production.

The mechanisms of chloramphenicol-induced aplastic anaemia is uncertain. Bone marrow stem cells are believed to be involved. Formation of a nitroso reduction production of the p-nitro group of chloramphenicol can irreversibly inhibit growth of bone marrow precursor cells. This has been verified under *in vitro* conditions. Thiamphenicol and florfenicol, the analogs of chloramphenicol (devoid of the p-nitro group, although sharing many of the clinical antibacterial and toxicologic properties of chloramphenicol) do not cause aplastic anaemia.

Tests to determine antibiotics residues in milk:

The most commonly used tests to determine antibiotic residues in milk are *Sarcina lutea* cylinder plate assay, *Bacillus stearothermophillus* disc assay, Delvotest-P, Penase, and Charm test. A brief description of these tests follows:

1. ***Sarcina lutea* cylinder plate assay:** It is highly sensitive test for penicillin detection (0.0125 IU per mL milk) and actually allows for quantitating the amount of antibiotic present in the milk. For this test, a sample of milk is placed inside a stainless steel cylinder on a plate previously streaked with *Sarcina lutea*. The plate is then incubated for about 16 hours. Standards of known antibiotic concentrations in milk are also placed in cylinders and used to create a standard curve. The zone sizes that develop allow for determination of the amount of antibiotic present in the sample.

2. ***Bacillus stearothermophilus* disc assay:** This test is six times more sensitive than the *Sarcina lutea* cylinder plate assay. It allows for quantitating the amount of antibiotic (0.002 to 0.004 IU of penicillin per mL milk). The *Bacillus stearothermophilus* is highly sensitive to penicillin and is thermophilic. In this test, a disc is dipped in the milk sample, placed on an agar plate that is coated with *Bacillus stearothermophilus*. The plate is then incubated for about three hours.

Interpretation: The presence of an inhibitory substance is determined on the basis of the inhibition of bacterial growth around the disc.

Negative = No zone of inhibition or a zone of inhibition upto 16 mm.

Positive = Zone of inhibition greater than 16 mm.

Limitation: There is a lack of uniformity noted relative to the materials, methods and interpretation of test results between laboratories.

3. Delvotest-P: The sensitivity of Delvotest-P is 0.006 IU penicillin per mL milk. For conducting this test, 0.01 mL milk sample is added to ampoules which contain medium and a penicillin sensitive bacteria (like *Bacillus stearothermophilus* var. *calidolactis*). This ampoule is then incubated for about 2.5 hours at about $64 \pm 0.5°C$.

Interpretation: The Delvotest-P is actually based on the production of acid as a result of metabolism during the growth of *Bacillus stearothermophilus* var. *calidolactis*.

Positive = Deep purple colour in ampoule.

Negative = Yellow colour in ampoule.

Limitations: A problem with the Delvotest-P occur when an intermediate colour change develops. This results in uncertainty regarding the presence or absence of penicillin. Hence, there is always chance of false positive results.

4. Penase test: Penicillinase is used in Penase test to confirm the presence of penicillin activity in milk. Penicillinase neutralizes the antibacterial properties of penicillin, inactivating upto 25 IU penicillin per mL milk. This inactivation allows bacteria to grow. In this test, a disc is dipped in the milk sample and then placed on a bacteria-streaked agar plate. Penicillinase is added to the same sample and another disc is dipped in it, then placed on the same agar plate. The plate is then incubated. If penicillin is present, it will be hydrolyzed by the penicillinase and the bacteria would grow up to the disc.

Interpretation:

Positive = No zone of inhibition.

Negative = Zone of inhibition.

5. Charm test: This test takes about eight minutes to conduct. It needs radioactive carbon for measuring the presence or absence of penicillin and is very accurate for quantitating the amount of antibiotic residues in milk (sensitivity of 0.005 to 0.025 IU of penicillin per mL milk). The charm test also identifies tetracycline, some aminoglycosides and other commonly used antibiotics. Radioactive carbon (C_{14}), the milk sample and a binder are mixed, incubated, centrifuged, and the radioactivity is determined.

Interpretation: The low radioactive value shows the presence of penicillin because penicillin forms complex with most of the radioactive carbon (C_{14}) and is discarded with liquid following centrifugation.

Tests to determine antibiotic residues in meat:

The most common and accurate method to determine antibiotic residues in meat is Live Animal Swab Test (LAST), developed by scientists in USDA's Food

Safety and Inspection Service. LAST is adopted for testing live animals on the farm. It should be used on animals which have been treated with antibiotics and are ready for slaughter and should be performed only after the antibiotics have been withdrawn for the prescribed period. LAST is designed for detecting antibiotics in urine in live animals, but in near future, it may be possible to run the test on blood of animals, too. When the urine is free of antibiotics, the tissue levels also have been decreased to acceptable levels and the animal can be safely marketed.

Procedure:

Collect the urine specimen after the animal has been off medication for the prescribed withdrawal period. Refrigerate the sample until the test has been started. For the test to work properly, adjust the incubator between 28 and 30°C. To assure proper humidity, a cup or glass of water is placed in the incubator. The test plates for LAST are 50 mm in diameter and contain agar gel, which supports bacterial growth. An X is marked on the side of the plate which is used as a guidepost to show the beginning of streaks over plate. The tightly capped bottle should be shaken to mix the *Bacillus subtilis* suspension uniformly. The swab is inserted into the bacterial suspension and taken gently out and streak the material of swab over the agar plate gently. N_5 disc (containing 5 μg of Neomycin) is gently placed on the surface of gel. A fresh swab dipped in urine sample is put carefully next to the N_5 disc in a rabbit-ear configuration. Incubate the plate for 18-24 hours at 28-30°C.

Interpretation:

Positive for antibiotic residues in meat = Clear zones around swab tips or clear zone around N_5 disc less than 16 mm (10/16").

Negative of antibiotic residues = Opaque bacterial growth right up to the swab tips or a clear zone around N_5 disc greater than 24 mm (15/16").

SUGGESTED FURTHER READINGS

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OPINION COLUMN

THE LEGAL ASPECTS RELATING FOOD COLOURS IN PAKISTAN

Abdul Hameed Blund
Rainbow Dyetech (Pvt) Ltd., Mandi Bahauddin.

Colour is as common in our environment as the air, we breath. We are not aware of it. Whereas, we use colours for identification. Likewise food colours are important to identify foods and quality control.

Some decades ago, in USA and European community colours containing lead dichromate were used to dye food. Now-a-days, in Pakistan the situation is far better. However, here is a common practice to use sub-standard colours which contain heavy metals. Acid and basic colours are used which are not safe for human.

There is a need that the Government should revise West Pakistan Pure Food Rules, 1965 and take necessary measures to implement them. I have suggestions which are as follows:

1. Sec. 4 of West Pakistan Pure Food Rules, 1965 should be amended.
2. Food Violet 2, Food Violet 3, Food Green 1, Food Green 3, should be deleted from the permitted list of food colours.
3. Food Yellow 13 (E104), Food Red 3 (E122), Food Red 7 (E124), Food Red 10 (E128), Food Red (E129), and Food Green 4 (E142) should be added in permitted list of food colours.
4. Sec. 11 of the rules should be amended, the word food additive should be added here and it is to be read as "Any article of food and food additive shall be considered as injurious to health and unfit for human consumption within the meaning of Sec. 5".

Now, I would like to discuss about Amaranth. In 1968, 1969 and 1970, reports of studies in USSR raised concerns about possible carcinogenic and reproductive effects of Amaranth and other food colours. Scientists from the USA, Canada, UK, the WHO and the FAO showed this work to be faulty because no tumours were reported. This report was published in BBRA Reports in 1972 by British Industrial Biological Research Association under head Significance of Recent Studies on Amaranth. Canadian Government released a press note on February 2, 1976 about the safety of Amaranth, too. However, FDA terminated Amaranth from the approved list on February 12, 1976 which was presumed a political decision. Amaranth is still permitted in Europe as a food colour (E123).

In my opinion, Amaranth is safe for human consumption and a cheap source for colouring foods having a good tintorial value.