EFFECT OF ULTRA VIOLET RADIATION AND METALS ON LIPID PEROXIDATION IN FRESH MILK OF DIFFERENT SPECIES

Tahir Zahoor, Faqir M. Anjum and Nuzhat Huma
Department of Food Technology University of Agriculture, Faisalabad 38040, Pakistan

ABSTRACT

Milk has a very complex chemical composition, which differs between and within species and even among individuals within these species. Milk, like other fatty foods, undergoes chemical changes that are indicated by development of undesirable flavours. Such changes are generally due to chemical reactions involving oxygen and unsaturated fatty acids loosely designated as autoxidation. These reactions are catalysed by exposure to light and also by metal contamination, particularly by Cu^{2+} and Fe^{3+}. The present study was carried out to determine the effect of different metals (Cu^{2+} and Fe^{3+}) and ultraviolet (UV) radiation on lipid peroxidation of milk from various species (cow, goat, sheep, buffalo and camel) by using the method of Sinnhuber and Yu (1957). The results showed a significant increase in lipid peroxidation when milk samples of all species were exposed to UV radiation at 254 nm and a further increase was also observed when the milk was treated with metals (Cu^{2+}, Fe^{3+}). Goat milk exposed to UV radiation and metals alone or in combination with UV radiation showed highest increase in peroxide value. Undue exposure to light and metallic contamination (Cu^{2+}, Fe^{3+}) should be avoided when attempting to extend the shelf life of milk.

INTRODUCTION

The dairy industry is expanding steadily in Pakistan. Total milk production during 1997 was 20950 thousand tonnes obtained from cow, buffalo, sheep and goat and increased to 22039 thousand tonnes during 1998 (Anonymous, 1998).

Milk is an important foodstuff consumed virtually without processing throughout man's life. Milk has some of its constituents in form of a true solution, some are in an emulsified state and others are colloidally dispersed. It has a very complex chemical composition, which differs not only among various species, but also within species, and even among individuals of the same species. Its constituents, fat, carbohydrate, protein, vitamins and minerals, are well balanced and other foods have difficulty in serving as a substitute for milk in the diet.

Milk is perishable food, and like other fatty foods, it undergoes certain chemical changes that are indicated by the development of undesirable flavours. Such changes are generally due to chemical reactions involving oxygen and unsaturated fatty acids loosely designated as autoxidation. These changes are greatly favoured by exposure to light, contact with air, high temperature, increased unsaturation in the fatty acids present, and metal contaminants, such as Cu^{2} and $Fe3^{}$

The present study was an attempt to determine for different species the susceptibility of milk lipid to peroxidation in relation to UV radiation confined with metallic contamination.

MATERIALS AND METHODS

Milk

Samples of fresh milk of different species of animals, i.e., cow, buffalo, sheep and goat were obtained from the University of Agriculture, Faisalabad, Pakistan. Camel milk was obtained from the local market of Faisalabad, Pakistan.

Metals

Ferric and copper chlorides were obtained from E. Merck, Darmstadt for this study.

UV Radiation

Covered culture hood from microbiological laboratory containing one tube rod 2527^{0} A for one hour at room temperature was used for incubation

purpose to study the effect of UV radiation on fresh milk samples.

Peroxide Value

Peroxide value was determined by using the 2-thiobarbituric acid method (TBA) according to method of Sinnhuber and Yu (1957).

The red compound formed by the reaction of 2-thiobarbituric acid with oxidized lipids has been demonstrated to be a measure of the extent of oxidation in fatty foods, being based on the condensation of two molecules of TBA with one of malondialdehyde to form a red colour with an absorption maximum at 532 nm.

Reagents

i 2-Thiobarbituric acid: A fresh aqueous solution of thiobarbituric acid (0.75 w/v) was made each time using a hot water bath.

ii- Trichloroacetic acid (20%): This was prepared by dissolving 20g of trichloroacetic acid in water and making the volume to 100 ml.

Procedure

To a test tube, one-ml representative sample and 4 ml of 20% trichloroacetic acid were added. After addition of 1 ml of 0.75% TBA reagent the test tubes were placed in a boiling water bath. The test tubes were covered with glass marbles to avoid evaporation losses and heated for 20 minutes. After cooling, the samples were centrifuged for two minutes at 8000 rpm to clarify the sample and the absorbance measured at 532 nm wavelength. The peroxide value was calculated according to the following formula

$C = A/E$

Where

C = Concentration of peroxide in moles/litre.
A = Absorbance of the sample.
E = Molar absorbance of the TBA-derived chromophore, which was shown by Sinnuber and Yu (1987) to be 156,000 cm^{-2} mol^{-1}

RESULTS AND DISCUSSION

Fresh Milk Samples of Various Species Simultaneously Incubated

The fresh milk samples were incubated firstly in the presence of UV radiation and secondly in the presence of UV radiation in combination with metals.

In presence of UV radiation

The results for this study are shown in Tables 1 and 2. The statistical results revealed that the peroxide value was affected significantly due to differences in milk sources, treatments as well as the interaction between milk sources and treatments (Table 1). The effect of UV radiation on lipid peroxidation is clearly profound, the peroxide values of all the samples were significantly ($P<0.01$) more than doubling on radiation for one hour (Table 2).

Table 1: Analysis of variance showing effect of UV radiation on lipid peroxidation of milk from various species

S.O.V.	D.F	S.S	Error mean square	F. Value	Prob.
Milk source	4	92194.69	23048.672	60.55**	0.000
Treatments	1	994642.28	994642.28	2612.76**	0.000
Source x Treatments	4	74602.80	18650.700	48.99**	0.000
Error	40	15227.43	380.686		

Coefficient of variation = 6.44%

Table 2: Effect of UV light on lipid peroxidation of milk from various species

	Peroxide value (micromoles/liter)		
Milk	Fresh Milk	UV radiation	Mean
Cow	143 ± 14	306 ± 24	224d
Buffalo	146 ± 4	455 ± 12	301c
Sheep	147 ± 8	485 ± 41	316bc
Goat	167 ± 7	541 ± 27	356a
Camel	208 ± 7	435 ± 10	322b

Means carrying same letters are not significantly different ($P < 0.01$)

These findings are in agreement with those of Webb *et al* (1974), Godzheva and Miteva (1985) and Richardson and Dahl (1983) who suggested that UV radiation gave a higher peroxide value depending upon the wavelength involved, the intensity of the source and the length of exposure. Maximum peroxide formation was obtained in goat's milk and minimum in cow's milk. The reason might be the complex phenomenon involving many factors like structure of fatty acids, effect of feed on composition of milk fat, interaction of micro and macro nutrients in milk.

Peroxide Value in the Presence of UV Radiation and Metal Ions

Effect of ferric chloride with UV radiation on peroxide value of milk from various species

Results indicating the effect of ferric chloride along with UV radiation are presented in Tables 3 and 4. The peroxide values were found to be significantly different due to variation in milk source, treatments and interaction between these two (Table 3). The peroxide values obtained for control samples of different milk sources were compared to the values obtained with the addition of ferric chloride alone and

Table 3: Analysis of variance showing effect of UV radiation in presence of ferric chloride on lipid peroxidation of milk from various species

S.O.V.	D.F	S.S	Error mean square	F. Value	Prob.
Milk source	4	4338.63.36	108465.840	160.57**	0.000
Treatments	2	682692.28	314346.140	505.33**	0.000
Source x Treatments	8	369215.87	46151.984	68.32**	0.000
Error	60	40529.35	675.489		

Coefficient of variation = 9.30%

Table 4: Effect of UV radiation in presence of ferric chloride on lipid peroxidation of milk from various species

Milk source	Peroxide value (micromoles/liter)			
	Control(a)	Ferric Chloride (20 µm)		
		Normal light(b)	UV radiation(c)	Mean(a+b+c)
Cow	214 ± 17	162 ± 21	243 ± 20	206d
Buffalo	164 ± 9	183 ± 20	232 ± 31	193d
Sheep	239 ± 5	318 ± 33	475 ± 19	344b
Goat	239± 22	241 ± 40	683 ± 44	388a
Camel	190 ± 2	164 ± 05	435 ± 41	263c
Mean	209b	217b	414a	

The means carrying same letters are not significantly different P<0.01)

in combination with UV radiation. In case of cow and camel, control milk samples showed 214m/l and 191 m/l peroxide values which are higher than 162 m/l and 164 m/l respectively, obtained from milk samples treated with ferric chloride, but this values were increased to 243 and 435 m/l respectively with the combined effect of ferric chloride plus UV light (Table 4). The reason for the increase in peroxide values in the milk sample treated with ferric chloride might be the speed of reaction as reported by Unnikrishnan and Rao, (1977) who stated that peroxide value development was slower in cow ghee than in buffalo ghee. Time for incubation might be the limiting factor in this case, due to which the reaction proceeded slowly. The peroxide values for buffalo, sheep and goat were 164 ± 9; 239 ± 5 and 239 ± 22 *u*m/l which increased to 183± 20; 318 ± 33 and 241± 40 with ferric chloride alone and 232 ± 31; 475 ± 19 and 683 ± 44 with the effect of ferric chloride in combination with UV radiation, respectively. Though the difference in peroxide value in milk of different species due to metal alone and in combination with UV radiation was existed but the trend was found to be similar to the result obtained in UV light alone and was significant statistically ($P < 0.01$). The results obtained are in agreement with Webb et al. (1974) Pal and Mulay (1985); Richardson and Dhal (1983); Min and Wen (1983); Ke and Ackman (1976); and Kajimoto and Yoshida (1974) who observed that peroxide value is greatly influenced by metallic contamination and light exposure.

Effect of copper chloride with UV radiation on peroxide value of milk from various species

The results regarding statistical analysis have shown that the species, treatments and the species x treatments significantly affected the peroxide value (Table 5).

Table 5: Analysis of variance showing effect of UV radiation in presence of copper chloride on lipid peroxidation of milk from various species

S.O.V.	D.F	S.S	Error mean square	F. Value	Prob.
Milk source	4	279289.95	69822.488	42.62**	0.000
Treatments	2	928050.32	464025.161	283.26**	0.000
Source x Treatments	8	352014.90	44001.863	26.86**	0.000
Error	60	98287.94	1638.132		

Coefficient of variation = 15.09%

The peroxide values for control milk samples were 153±12; 177 ± 9; 185 ± 14; 198 ± 12 and 164 ± 12 m/l which increased to 186± 1; 207 ± 16; 200 ± 15; 243 ± 28 and 181 ± 7 m/l with the addition of copper chloride and further increased with the effect of copper chloride plus UV light to 271 ± 12; 297 ± 13; 356±15; 694 ± 84 and 505 ± 111 m/l in milk sample of cow, buffalo, sheep, goat and camel respectively (Table 6). Maximum effect of both the treatments was found in milk of goat, which may be due to the presence of higher amount of free fatty acid in goat's milk as stated by Webb et al. (1974). Free fatty acids are more prone to autoxidation. Minimum peroxide formation was found in case of buffalo milk sample. There might be other reasons including i. effect of feed, ii. lactation number, iii. structure of fatty acids as stated elsewhere. UV radiation was found to be more significant than metals when they were individually investigated and there was more peroxide formation when UV radiation along with copper chloride was applied as shown in Table 6.

Table 6: Effect of UV radiation in presence of copper chloride on lipid peroxidation of milk from various species.

Milk source	Peroxide value (micromoles/liter)			
	Control	Ferric Chloride (20 µm)		Mean
		Normal light	UV radiation	
Cow	153 ± 12	186 ± 1	271 ± 12	204d
Buffalo	177 ± 9	207 ± 16	297 ± 13	227cd
Sheep	185 ± 14	200 ± 15	356 ± 6	247c
Goat	198 ± 12	243 ± 28	694 ± 84	378a
Camel	164 ± 12	181 ± 7	505 ± 111	282b
Mean	175c	204b	424a	

The means carrying same letters are not significantly different P<0.01)

The results are in accordance with those of Webb et al. (1974); Pal and Mulay, (1985); Richardson and Dhal, (1983), Ke and Ackman, (1976); Rudolph and Odell, (1970) and Patton and

Kurtz, (1955) who also stated that the exposure of light and metals may enhance the peroxide values.

SUMMARY

Milk is a dynamically balanced mixture of protein, fats, carbohydrates, salts and water co-existing as part emulsion, part colloidal suspension and part true solution. Interest in the study of milk stems largely from its use as human food. Milk lipid plays an important role in maintaining the good quality of milk, peroxidation being a major deteriorative reaction. Moreover, the contribution of various factors for peroxide formation, such as metal contaminants from containers and exposure of bottled milk to UV radiation are serious problems for the dairy industry. Iron and copper both act as pro-oxidants. They catalyse the rate of formation and breakdown of peroxide, thus leading to the more pronounced odour and flavour changes. The results also indicated the UV radiation also has a pronounced effect on lipid peroxidation. In practice the undue exposure to light and to metallic contamination (Cu^{2+}, Fe^{3+}) should be avoided to maximize the shelf life of the milk.

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STUDIES ON WHEAT ATTA FORTIFIED WITH ELEMENTAL IRON USED FOR CHAPATI PRODUCTION

Misfa Hinnai, Salim-ur-Rehman, Nuzhat Huma and Faisal Rafique
Department of Food Technology, University of Agriculture, Faisalabad 38040, Pakistan

ABSTRACT

Wheat atta was fortified with elemental iron (Iron folic acid premix containing 30% elemental iron, 1.5% folic acid and 68.5% starch) @ 30, 45 and 60 ppm levels and stored for 42 days. Chapaties were prepared from this atta at weekly intervals and analysed. It was observed that in atta with 60 ppm iron level, iron retention was maximum. Both iron and phytic acid decreased gradually during storage. Iron level also affected the sensory characteristics of chapaties except colour and flavour.

INTRODUCTION

Over two billion people world wide suffer from iron deficiency with a total prevalence estimated at about 40% of the world population (Anon., 1991). Incidence of iron deficiency anaemia among pregnant women is 51%, infants and two years old children 48% and nonpregnant women 35%.

Iron deficiency is the main cause of anaemia in the world. Iron deficiency usually results due to poor intake of dietary iron, malabsorption, increased demand during pregnancy, lactation and growth, due to blood loss in mensturation, hook worms infestation and malaria (Edwards, 1995).

Iron deficiency anaemia increases the risk of maternal mortality, preterm delivery, inadequate gestational weight gain and perinatal mortality (MacGregor, 1963). Iron deficiency anaemia causes damage to featel brain during early pregnancy and also impairs mental development in infants (Agarwal and Agarwal 1991). It also decreases the physical activity, performances and work capacity of children and adults (Bothwell and Charlton, 1981).

Wheat is one of the cheapest sources of calories in most developing countries. In Pakistan more than 80% of wheat is consumed in the form of chapati. Wheat atta is thus an ideal vehicle for fortification because chapati is the staple and affordable food consumed by all age groups.

To overcome iron deficiency in wheat atta, it was fortified with iron folic acid premix at different levels. The objective was to determine the stability of iron fortified atta during storage and to ascertain the impact of fortificant on the sensory characteristics of chapati.

MATERIALS AND METHODS

Wheat atta was purchased from Usman Flour Mills, Faisalabad. Proximate analysis of wheat atta and chapati was carried out according to the methods described in AACC (1983). Iron folic acid premix was obtained from Pharmabiotics Inc., Mississauga Canada. Wheat atta was fortified with iron folic acid premix at 30 (T_1), 45 (T_2) and 60 ppm (T_3) iron levels. Control i.e. no premix added was designated as To.

Atta and fortificant were blended to a uniform distribution. The fortified samples were stored at ambient temperature in cotton bags for 42 days. Chapaties were prepared from stored atta at weekly intervals. These chapati samples and atta were analysed for iron and phytic acid contents.

Iron was determined by using spectrophotometer according to the Drablkin's method as described by Benjamin (1978). Phytic acid was determined by subtracting free phosphate from total phosphorus. Total phosphorus was determined by the method of Oser (1976) and free phosphate was determined by the method of Fiske and Sublarrow (1925).

Sensory evaluation of chapaties was conducted by a panel of six judges according to method described by Land and Shepherd (1988). The characteristics evaluated were colour, taste, flavour, texture, foldingability and chewability by using two factors factorial in randomized complete block design. The means were compared for their

significance by Duncan's Multiple Range Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Chemical Composition

Proximate analysis of wheat atta showed that it contained moisture 10.95%, crude protein 12.30%, crude fat 2.57%, crude fibre 2.45, ash 1.68% and NFE 81.00% (Table 1). These results are similar with the findings of Akhtar (1998).

Table 1: Proximate composition of wheat atta and chapati

Composition	Wheat atta (%)	Chapati (%)
Moisture	10.95	20.01
Crude protein (d b)	12.30	11.19
Crude fat (d b)	2.57	2.11
Crude fibre (d b)	2.45	2.28
Ash (d b)	1.68	1.73
NFE (d b)	81.00	82.69

d b = dry baris NFE= Nitrogen Free Extract

Effects of Storage on Iron Content of Wheat Atta and Chapati

Comparison of means showing the effect of treatments and storage on iron content of atta and chapaties is presented in Tables 2 and 3.

Table 2: Effect of storage period on iron (%) content of atta and chapaties.

Storage Periods	Atta	Chapati
S_0	0.0072a	0.0078b
S_1	0.0069b	0.0076a
S_2	0.0074a	0.0074b
S_3	0.0072a	0.0019c
S_4	0.0068b	0.0073b
S_5	0.0069b	0.0073c
S_6	0.0069b	0.0065d

Means sharing similar letters are statistically non-significant.

S_0 = 0 day storage S_1 = 1st week storage
S_2 = 2nd week storage S_3 = 3rd week storage
S_4 = 4th week storage S_5 = 5th week storage
S_6 = 6th week storage

Table 3: Effect of different treatments (%) on Iron content of atta and chapaties.

Treatment	T3	T2	T1	T0
Flour	0.0096a	0.0080b	0.0068c	0.0039d
Chapati	0.0098a	0.0077b	0.0072c	0.0040d

Means sharing similar letters are statistically non-significant.

These show that iron content decreased gradually during storage in all treatments both in atta and chapati. Lowest iron content was observed after six weeks of storage. During storage, iron may have decreased due to formation of complexes with phytic acid (Akhtar, 1998). Oxidation may also have decreasing effect (Ittefaq, 1997). Hallberge *et al.*, (1987) stated that phytate inhibits trace minerals especially iron phytate which is the main cause of inhibitory action of bran on iron absorption.

Effect of storage on phytic acid of atta and chapati.

The comparison of means showing the effect of storage on phytic acid content of atta and chapati has shown in Table 4.

Table 4: Effect of storage period on phytic acid (%) content of atta and chapaties.

Storage Period	Atta	Chapati
S_0	0.893a	0.476a
S_1	0.898a	0.477a
S_2	0.986a	0.470a
S_3	0.883ab	0.470a
S_4	0.877bc	0.472a
S_5	0.873bc	0.460ab
S_6	0.867cd	0.442a

Means sharing similar letters are statistically non-significant.

Phytic acid in atta was significantly affected during prolonged storage. It gradually decreased with increasing storage intervals, because at high temperature and in humid environment, phytase becomes active and degrades phytic acid. The data further showed a substantial loss in phytate content of chapati during storage and processing. During processing of chapati, wet heating enhanced phytase activity which resulted in lower phytic acid content in chapti as compared to its respective atta (Svanberge, 1995). These findings are in close relation with Ittefaq (1997) and Tariq (1990).

Sensory Evaluation

Chapties were prepared wheat atta at weekly intervals from unfortified and fortified. These chapati samples were evaluated for sensory characteristics i.e. colour, taste, flavour, texture, foldingability and chewability.

Statistical analysis of the data for treatments and storage periods is given in Tables 5 and 6.

Table 5: Effect of iron levels on the sensory characteristics of chapaties.

Characteristics	To	T1	T2	T3
Texture	7.21ab	7.17 b	7.30 a	6.66 c
Foldingability	7.18ab	7.11 b	7.20 a	6.83 c
Chewwability	7.19a	7.10 b	7.03 c	6.93 d

Means Sharing Similar letters are statistically non-significent

Table 6: Effect of storage on sensory characteristics of chapaties.

Storage period	Taste	Flavour	Texture	Folding-ability	Chewabi lity
S0	7.57a	7.62a	7.67a	7.64a	7.70a
S1	7.54a	7.64a	7.55ab	7.49b	7.55b
S2	7.49ab	7.26b	7.52b	7.45b	7.44b
S3	7.26d	7.20b	7.36c	7.39b	7.24c
S4	7.40cd	6.78c	7.08d	6.89c	6.72d
S5	7.28cd	6.42d	6.29e	6.39d	6.66d
S6	6.2e	6.17e	6.11f	6.29d	6.14e

Means Sharing Similar letters one statistically non-significant.

It is clear from Table 5 that different iron levels affected texture, foldingability and chewability of the chapaties. According to the data presented in Table 5, T3 obtained lowest score which reflected that 60 ppm iron level affected the texture and made it tough. This might be due to electrolytic effect of iron on protein of dough.

Increasing iron level showed no effect on colour, flavour and taste of chapati. The data shown in Table 6 clearly indicates that taste, flavour, texture, foldingability and cheweability were significantly affected by storage. Highest scores by chapati prepared at 0 day and lowest scores were obtained by chapati prepared after six weeks of storage.

It was, thus, conclueded that 30 ppm iron level was most acceptable and long storage adversely affected the sensory characteristics of chapaties.

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PRODUCTION OF PAN BREAD FROM WHEAT FLOUR FORTIFIED WITH IRON FOLIC ACID PREMIX

Farkhanda Sohail, Javaid A. Awan, Nuzhat Huma and Ghazala Qamar
Department of Food Technology, University of Agriculture, Faisalabad

ABSTRACT

Elemental iron was added @ 30, 45 and 60 ppm in wheat flour for the preparation of pan bread. Iron retention and rancidity development in flour and bread were tested weekly. Iron retention was affected as a function of storage. Maximum iron retention was found in samples containing 60 ppm iron. Rancidity development was affected by iron level and storage period. Least rancidity was found in flour fortified with 60 ppm iron. Rancidity increased with the length of storage period. The sensory characteristics of bread prepared from fortified wheat flour were adversely affected both by iron levels and storage periods except evenness of bake.

INTRODUCTION

Iron deficiency anaemia is a prevalent nutritional problem in Pakistan. It is particularly high among pregnant women, young girls and children. Severe anaemia in pregnancy is associated with increased risk of maternal mortality, premature delivery and low birth weight (Menon, 1968). Anaemia, in its milder form, may manifest itself in symptoms like fatigue and lethargy, thereby affecting individual's ability to work. Anaemia is caused by insufficient iron intake, malabsorption and iron losses through bleeding.

Some positive steps are necessary to prevent the anaemic conditions. The three different measures to control iron deficiency are supplementation, fortification and dietary diversification. Fortification of foods with iron is considered to be an approach to supplement iron and minimise the risks of anaemia in the population (Cook and Reusser, 1983). Wheat flour is the staple food consumed by all segments of the population. It has been used as a suitable vehicle for fortification (Awan *et al.*, 1996). For this purpose, baked products particularly pan bread, fortified with iron, can be an efficient mode, especially due to the current popularity of convenience foods. In Pakistan, pan bread is being manufactured at small scale by petty bakers and at large scale by various plants in most big cities.

Bread is primarily consumed at breakfast. Large amounts are consumed in the form of snacks such as sandwiches, French toasts, rolls, etc. These snacks are used during working hours in the work place and by children in schools and elsewhere. In today's fast and busy life, consumers prefer to eat such light foods instead of taking heavy lunch or dinner. Bread is also recommended as a solid diet for infants.

It was, therefore, planned to fortify pan bread with iron to reduce iron deficiency disorders among the population, especially in school going children. The present study is aimed at determining the effect of elemental iron on the keeping quality of flour, quality of bread and consumer acceptability of the product.

MATERIALS AND METHODS

White patent flour was procured from a local flourmill in Faisalabad. Proximate composition was determined according to the methods described in AACC (1983). Elemental iron was added to the flour at the following levels: -

Treatment	Elemental iron (ppm)
T_0	0
T_1	30
T_2	45
T_3	60

Iron fortificant was mixed in flour with the help of a laboratory mixer. The fortified flour samples were packed in cotton bags and stored for 42 days at ambient temperature inside a laboratory shelf.

Bread was prepared weekly from the stored flour starting on the zero day according to standard method of preparation. Proximate analysis of bread was conducted following the standard methods of AACC (1983). Iron content and rancidity development in the flour and bread were also

determined at weekly intervals. Flour and bread were analysed for retention of iron using spectrophotometer according to the Drabkin's method as described by Benjamin (1978). Rancidity development in bread and flour was determined in terms of free fatty acids by the method described in AOAC (1984).

Bread was evaluated by a panel of 6 to 8 judges for external and internal characteristics according to Matz (1972). Data obtained were analysed statistically by using 2-factors factorial in Randomised Complete Design. The mean values were compared for their significance by Duncan's Multiple Range Test (Steel and Torrie, 1980).

RESULTS AND DISCUSSION

Chemical Composition

The results of chemical composition of flour revealed that it contained moisture 13.5%, crude protein 11.0%, crude fibre 0.5%, crude fat 1.4%, and ash 0.6% (Table 1). The results of this study correlate with the findings of other workers such as Bashir (1997) and Ayaz (1998).

Table 1: Proximate composition of white patent flour and bread

Composition	Flour	Bread
Moisture (%)	13.5	36.5
Crude protein (%)	11.0	10.9
Crude fibre (%)	0.5	0.5
Crude fat (%)	1.4	2.8
Ash (%)	0.6	0.6
Nitrogen free extract (%)	73.0	48.7

The proximate composition of bread (Table 1) showed that it contained moisture 36.5%, crude protein 10.9%, crude fibre 0.5%, crude fat 2.8%, ash 0.6% and nitrogen free extract 48.7%.

Effect of Storage Periods on Iron Content in Flour and Bread

Effect of storage period on iron content in flour and bread is presented in Table 2.

The statistical analysis of this data is given in Table 3. From Table 2 it is clear that length of storage affects iron content significantly. Iron content in all treatments remained stable upto 3rd week of storage. Then it decreased gradually. Lowest iron content was found in flour and bread at 6th week of storage in all the treatments. During storage, iron forms insoluble polymers with phytic acid and polyphenols, thus decreasing over all solubility in the bread.

Table 2: Effect of storage time on iron content of flour and bread

Storage Week	T_0	T_1	T_2	T_3
		Flour		
0	0.0016	0.0040	0.0054	0.0071
1	0.0016	0.0040	0.0054	0.0070
2	0.0016	0.0039	0.0054	0.0069
3	0.0015	0.0037	0.0053	0.0068
4	0.0012	0.0035	0.0053	0.0067
5	0.0011	0.0034	0.0052	0.0066
6	0.00096	0.0033	0.0051	0.0064
		Bread		
0	0.0016	0.0046	0.0055	0.0071
1	0.0013	0.0044	0.0054	0.0070
2	0.0013	0.0043	0.0053	0.0069
3	0.0012	0.0042	0.0053	0.0067
4	0.0011	0.0041	0.0052	0.0067
5	0.0010	0.0039	0.0052	0.0066
6	0.0010	0.0036	0.0051	0.0063

Table 3: Analysis of variance for iron content in flour and bread

(SOV)	Df	SS	F. Value
Wheat flour			
Treatments	3	0.000328	2720.5162**
Storage	6	0.0000016	6.7544**
Bread			
Treatment	3	0.0003388	1820.709**
Storages	6	0.000017	4.5489**

Rancidity Studies in Flour and Bread

Rancidity is a very useful indicator of quality of the product. The effect of storage on rancidity development in flour and bread is presented in Table 4.

Table 4: Effect of storage on rancidity content of flour and bread

Storage Week	T_0	T_1	T_2	T_3
		Flour		
0	1.41	1.40	1.30	1.29
1	1.55	1.54	1.42	1.40
2	2.22	1.94	1.86	1.68
3	2.39	2.22	1.98	1.70
4	2.82	2.80	2.58	2.33
5	3.24	2.82	2.80	2.46
6	3.92	3.64	3.37	2.96
		Bread		
0	1.67	1.65	1.39	1.30
1	1.95	1.94	1.53	1.40
2	2.50	2.24	1.97	1.20
3	2.80	2.49	2.20	1.95
4	2.34	3.10	2.82	2.55
5	3.50	3.36	3.11	2.80
6	4.20	3.92	3.64	3.38

characteristics prepared at zero-day and reduced gradually with storage period. Evenness of bake was not affected both by storage periods and fortification levels. Best bread was prepared from flour fortified with 45 ppm iron level.

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The results show that there is significant increase in rancidity in flour and bread with the length of storage period. Storage of flour at room temperature and humidity hasten the development of rancidity (Hansen and Rose, 1996). Rancidity in flour decreased significantly as the level of elemental iron increased. Minimum rancidity was observed in T_3 (60 ppm iron) and maximum was found in T_0 without iron in both flour and bread. Added iron oxidises due to available oxygen present in flour and bread. The reduction in oxygen content renders these materials more stable towards development of rancidity.

Table 5: Analysis of variance for rancidity in flour and bread

(SOV)	df	SS	F. Value
Wheat flour			
Treatment	3	3.127	1762.639**
Storage	6	42.232	11570.317**
Bread			
Treatment	3	6.273	4785.727**
Storage	6	49.043	18708.302**

Sensory evaluation

Bread prepared at weekly intervals from stored fortified and unfortified wheat flours was evaluated for external and internal characteristics. Scores for this evaluation of bread are given in Table 6. Statistical analysis of the data is presented in Table 7.

Tables 6 and 7 reveal that the scores for iron content and storage period significantly affected the grain, aroma, colour of crumb, taste and texture of breads prepared from fortified flours stored for 42 days. The scores for various characteristics of bread decreased as the storage period increased. The highest scores for crumb grain, taste and texture of bread were awarded to bread prepared from flour fortified with 45 ppm iron. Fortification did not affect the aroma, as it was affected by storage interval. Least scores for aroma were observed in breads prepared from fortified flour samples at sixth week in all treatments. This was due to rancidity development in stored flour.

The results for external characteristics of bread revealed that colour of crust, volume, character of crust and symmetry of form were affected significantly by length of storage period of fortified wheat flour. It is clear from Table 6 that the fortification level had highly significant effect on volume of bread. Highest score for volume was assigned to bread prepared from flour with 45ppm iron and stored for two weeks.

Colour of crust, symmetry of form and character of crust were not affected by iron levels but by length of storage of fortified flour. Highest scores were assigned to breads for all these

Table 6: Effect of iron on sensory characteristics of bread.

Characteristics (score)	T_0	T_1	T_2	T_3
Volume (10)	7.19	7.88	8.04	7.82
Crust colour (8)	6.50	6.69	6.92	6.85
Symmetry of form (5)	3.50	3.85	3.83	3.85
Evenness of back (3)	2.16	2.40	2.33	2.23
Character of crust (4)	2.64	2.97	2.95	2.88
Grain of bread (15)	11.90	12.38	12.90	12.09
Colour of crumb (10)	7.88	7.85	8.16	7.83
Aroma (10)	7.78	7.88	7.81	7.71
Taste (20)	15.69	16.19	17.26	16.50
Texture (15)	12.45	13.23	13.26	13.21

Table 7: Effect of storage on the sensory characteristics of iron fortified bread upto 2 days.

Characteristics	Storage period						
	S_0	S_1	S_2	S_3	S_4	S_5	S_6
Volume	8.33 a	8.12 ab	7.91 abc	7.87 abc	7.62 bc	7.37 cd	7.08 d
Crust colour	7.41 a	7.08 a	7.08 a	6.917 b	6.375 b	6.375 b	5.955 c
Symmetry of form	4.12 a	4.00 ab	3.95 ab	3.79 abc	3.58 bc	3.58 c	3.417 c
Character of crust	3.33 a	3.33 a	3.00 ab	2.83 b	2.83 b	2.66 b	2.04 c
Grain of bread	13.33 a	13.00 ab	12.58 ab	12.42 bc	11.92 cd	11.75 cd	11.25 d
Colour of crumb	8.458a	8.208b	8.171b	8.04c	8.042c	7.542d	7.083e
Aroma	8.383 a	8.167 ab	7.94 bc	7.615 c	7.50 cd	7.375 d	7.375 d
Taste	18.04 a	17.29 ab	16.83 ab	16.46 b	16.17 bc	15.08 c	15.00 c
Texture	13.67 a	13.75 a	13.33 ab	13.33 ab	13.00 ab	12.46 bc	11.75 c

PRODUCTION AND CHARACTERIZATION OF BAKING POWDER

*Faisal Rafique, Salim-Ur-Rehman, Masood S. Butt, *Muhammad Amin Javaid and Muhammad Azam*

Department of Food Technology, University of Agriculture, Faisalabad.

** Livestock Agronomy Section, Ayub Agricultural Research Institute, Faisalabad.*

ABSTRACT

Baking powders were prepared with locally available acidic ingredients. These were blended in various combinations with sodium bicarbonate and their performance in biscuits was evaluated and compared with imported baking powder. Total, residual and available carbon dioxide of baking powders ranged from 15.8 to 21.5%, 0.5 to 2.1% and 14.9 to 19.4%, respectively. Biscuits wee also subjected to objective and subjective tests. Biscuits prepared by the use of mono calcium phosphate (5%) and sodium aluminum sulphate (25.5%) in sodium bicarbonate were of good quality and appealed to the consumers.

INTRODUCTION

Baking powder is a leavening agent produced by mixing of sodium bicarbonate, one or more leavening acids and with or without starch or flour. It yields not less than 12% available carbon dioxide upon complete reaction. Carbon dioxide is the gas responsible for leavening most of bakery products (Matz, 1968). When product is placed in the oven the chemical leavening agents release Co_2 gas and results in low density, desired volume, grain and texture of baked product (Sultan, 1969).

The amount of acid required in the formulation of baking powder depends upon the neutralizing value of the acid against sodium bicarbonate to generate maximum amount of carbon dioxide (Pomeranz, 1988; Lips, 1994). The amount of carbon dioxide required to leaven the final product is very important because over or under leavened product is not acceptable.

Biscuits are good source of sodium and potassium due to leavening agents (Beduarcyk, 1987). Baking powder has 5-10 ppm fluoride in products in which it is used (Farkas, 1977).

Baking powder of different types are available in the market in which mostly are imported and not economical. This project was designed to prepare a baking powder of comparable strength consisting locally available ingredients.

MATERIALS AND METHODS

Patent commercial flour, four commercial baking powders (Picture pure, Aziz group, Holiday and Econo Bake baking powder) and ingredients including starch, sodium bicarbonate, tartaric acid, cream of tartar, mono calcium phosphate, sodium acid pyrophosphate and aluminum sulphate were purchased from local market for the preparation of different formulations of baking powders, which were prepared by using various ingredients and are presented in Table 1.

Baking powder of Aziz group was found best during preliminary trials and hence it was selected as a control in this study.

Table 1: Preparation of Baking Powders

Treatments	Acids	Acids %	Sodium bicarb-onate %	Starch %
T_1	Control			
T_2	Tartaric Acid Cream of Tartar	5.97 44.90	26.73	23.40
T_3	Cream of Tartar	50.00	25.00	25.00
T_4	Sodium Aluminum Sulphate	25.60	26.73	47.67
T_5	Sodium Acid Pyrophosphate	40.40	30.58	29.02
T_6	Mono Calcium Phosphate	33.50	26.80	39.70
T_7	Mono Calcium Phosphate Sodium Aluminum Sulphate	12.50 17.30	36.00 -	34.20 -
T_8	Mono Calcium Phosphate Sodium Aluminum Sulphate	5.00 25.50	29.50 -	40.00 -

Flour was analysed for moisture, ash, crude protein, crude fat and crude fibre and farinogrpahic studies were carried out by using Brabender Farinograph as described in AACC (1983).

Total, available and residual carbon dioxide were determined by methods as described by Pearson (1976).

Biscuits were prepared by modified method as described in AACC (1983). Sensory evaluation of biscuits was carried out by a panel of judges for colour, flavour, taste, texture and overall acceptability as described by Land and Shepherd (1988). Width, thickness and spread factor of biscuits were also determined by following the method of AACC (1983). The data obtained for different treatments of baking powder were analysed by using complete randomized design as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

This study was conducted to evaluate the commercially available baking powders and to compare with those prepared with locally available raw materials.

Patent flour used in this study contained 11.35 percent moisture, 11.52 percent crude protein, 1.45 percent crude fat, 0.52 percent ash, 0.66 percent crude fibre and 74.50 percent NFE and its farinogram revealed water absorptoon 60 percent, arrival time 1.0 min, departure time 8.5 min, dough stability 7.5 min, peak time 3.0 min, tolerance index 44 BU and softening of dough 124 BU.

The total, residual and available carbon dioxide of the baking powders are given in Table 2.

Table 2: Carbon Dioxide of Baking Powders.

Treatments	Total Co_2	Residual Co_2	Available Co_2
T1	16.3cd	1.00d	15.3f
T2	16.4cd	0.70f	15.7e
T3	19.1b	0.80ef	18.3b
T4	21.5a	2.10a	19.4a
T5	16.6cd	1.50b	15.1fg
T6	15.8d	1.20c	16.3d
T7	15.8d	0.90de	14.9g
T8	18.0bc	0.50g	17.5c

Means carrying same letters in a column are not significantly different from each other.

T1 = Control
T2 = Tartaric Acid + Cream of Tartar.
T3 = Tartaric Acid
T4 = Sodium Aluminum Sulfate (SAS)
T5 = Sodium Acid Pyrophosphate
T6 = Mono Calcium Phosphate (MCP)
T7 = MCP (12.5%) + SAS (17.3%)
T8 = MCP (5%) + SAS (25.5%)

Data revealed that total carbon dioxide of baking powders ranged from 15.8 percent (T_6, T_7,) to 21.5 percent (T_4). Treatments T_1, T_2, T_5, and T_7 were not significantly different from each other, although these were prepared with different chemicals. These results are supported by the findings of Kichline and Conn (1970) who stated that sodium aluminium sulphate has very slow reactivity at room temperature. Maximum residual carbon dioxide was 2.10 percent (T_4) followed by 1.5 percent (T_5), 1.2 percent (T_6) and minimum 0.5 percent (T_8) in biscuits. T_7 was not significantly different than the control. These results are in agreement with the findings of Pearson (1976) who stated that the samples which contained an excess amount of residual carbon dioxide produced a soda taste in the products. T_8 was good in taste due to less amount of residual carbon dioxide. Available carbon dioxide of baking powders ranged from 14.9 percent (T_7) to 19.4 percent (T_4) in biscuits. T_8 contained the highest amount of available carbon dioxide while T_4 contained the lowest amount of vailable carbon dioxide. T_1, T_5 and T_7 were not significantly different from each other. The results are similar to those reported by Raymond and Donald (1948) who stated that baking powder should contain less than 12% carbon dioxide and as much as 15% or more available carbon dioxide.

Width, thickness and spread factor of biscuits prepared with different baking powders are presented in Table 3. The width of biscuits ranged from 260 mm (T_4) to 273 mm (T_5) and the thickness from 51.3 mm (T_4) to 64 mm (T_7) while the spread factor ranged from 42.54 (T_7) to 51.65 (T_4) for ten cookies respectively.

Table 3: Width Thickness and Spread Factor of Biscuits Prepared from Different Baking Powders.

Treatments	Width (mm)*	Thickness (mm)*	Spread factor *
T1	266.3	60.3	44.16
T2	268.6	58.3	46.07
T3	260.0	53.0	49.05
T4	266.0	51.3	51.85
T5	273.0	62.0	44.03
T6	267.0	56.6	47.17
T7	272.3	64.0	42.54
T8	263.3	54.6	48.22

* Six biscuits

The biscuits were evaluated by a panel of judges to determine the effect of various ingredients of baking powder on the colour, taste, flavour, texture and overall acceptability and the results are given in Table 4. The results revealed that score of colour ranged from 5.5 (T_4) to 8.1 (T_8). The highest score for taste were obtained by T_8 followed by T_2, T_3, T_7, T_6,

T_1, T_4, and T_5. Flavour score ranged from 8 (T_2) to 5.9 (T_4) while texture score 7.9 in T_6 followed by T_2 (7), T_3 (6.6), T_7 (6.3), T_1 (6), T_6 (5.6), T_4 (5.3) and T_5 (5). These results are similar to the findings of Hui (1992) and Matz (1991).

Table 4: Quality Score of Biscuits

	T1	T2	T3	T4	T5	T6	T7	T8
Colour	6.5bcd	7.5ab	7.1abc	5.5d	6.1cd	6.1cd	6.5bcd	8.1a
Taste	6.8bc	7.5ab	7.5ab	6.1c	6.1bc	6.8bc	7.0abc	8.0a
Flavour	7.3ab	8.0a	7.5ab	5.8d	6.3cd	6.5bcd	7.1abc	7.9a
Texture	6.6bcd	7.5ab	7.5ab	5.9d	6.3cd	6.6bcd	7.1abc	7.9a
Overall Acceptability	6.0cd	7.0b	6.6bc	5.3ef	5.0f	5.6def	6.3bcd	8.0a

Means carrying same letters in a column are not significantly different from each other.

Biscuits containing more available carbon dioxide showed better spread factor while those containing more residual carbon dioxide were not liked by the judges. T_2 that contained tartaric acid and cream of tartar was also liked but T_6 was more acceptable. It was concluded from the present research that biscuits prepared by the use of mono calcium phosphate (5%) and sodium aluminum sulphate (25.5%) had good quality and were preferred by the judges.

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EFFECT OF DIFFERENT SWEETENERS ON THE QUALITY OF BISCUITS

Muhammad Shafiq, Tahir Zahoor and Masood S. Butt

Department of Food Technology, University of Agriculture, Faisalabad 38040, Pakistan

ABSTRACT

The effect of three different sweeteners i.e. dextrose, golden syrup and hydrol on the quality of biscuits was studied. Four levels (100, 75, 50 and 25%) of each sweetener were used, depending on their sweetness. The biscuits were evaluated for acceptability test after 0, 15, 30, 45 and 60 days interval of storage. Substitution of 25 % sucrose with corn sweetener improved the physical and sensory characteristics of biscuits. The results pertaining to overall acceptability of biscuits indicated that 25 percent replacement of sucrose with dextrose got the highest scores for fresh biscuits that was subsequently decreased but remained highest during storage after 60 days. Physical test of biscuits showed that width was decreased with increased levels of dextrose and hydrol while in case of golden syrup the trend was not same. Increasing level of dextrose also increased thickness, but decreased the spreadability of biscuits. Highest calorific value of biscuits was obtained by 25% replacement of sucrose with golden syrup i.e. 5833.83 cal/gm but varied to 5549.09 cal/g with 100 percent golden syrup. The calorific value of biscuits was increased by substitution of sucrose with corn sweeteners. During storage although there was a decreasing trend in acceptability of biscuits however the biscuits remained acceptable even after 60 days.

INTRODUCTION

Sugars in cookies generate flavour, affect cookie spread and control crispness and surface characteristics. Sucrose is rarely used as the sole sugar in biscuit recipes. Relatively small amounts of reducing sugars, typically 10-20 % by weight of the total sugars, are added to most recipes. These reducing sugars are usually added in the form of syrups containing 70-80 % dry solids. The addition of liquid sugars to biscuit dough reduces the amount of water required in the dough (Wade, 1988).

Cookie surface characteristics are influenced mainly by the presence of sucrose in the formula. Symmetrical surface cracking was observed with sucrose but it was absent or greatly diminished when sucrose was replaced by or blended with glucose or fructose. (Doescher and Hoseney, 1985). The advantage of using liquid sweeteners may be due to the handling on a continuous flow basis through a pumping system as the efficiency of liquid state ingredients over dry form is notable. (Elmer, 1971).

The baking industry which accounts for about one fourth of all sweeteners used has shown a growing preference for liquid sugars and sweetener blends because of the easier and more sanitary handling offered by the bulk handling system. (Pyler, 1988).

Controlling the spread factor is also an important one as it was reviewed that the spread of cookie during baking affected by the choice of sweeteners and whether it was solid or liquid (Mulvihill 1992). Use of coarse particle size and low total saccharides retarded the spread. Dextrose and regular conversion glucose syrup could be used in cookies to get the acceptable quality spread factor.

Keeping in view the factors like improvement in sweetness, flavour, crust colour, spread, tenderness, keeping quality, nutrition and general appearance of biscuits and crackers. The plan was made to improve the quality of biscuits by using different kind of sweeteners as dextrose, golden syrup and hydrol. The experiments were performed to check the effect of these sweeteners on several factors stated above. The idea was supported by the studies of Hickenbottom (1977) who also studied the same factors to strengthen the idea under different conditions.

MATERIALS AND METHODS

Procurement of Samples

Commercial flour sample was obtained from Akbar Flour Mill, Faisalabad. Different sweeteners i.e. dextrose, golden syrup and hydrol were obtained from CPC Rafhan Ltd, Faisalabad.

Proximate Analysis of Wheat Flour

The wheat flour sample was analysed for moisture, crude protein, crude fat, crude fibre, nitrogen free extract and total ash contents according to the methods described in AACC (1983).

Preparation of Biscuits

Biscuits were prepared with some modifications in the method given in AACC (1983)

In the formulation of biscuits, sucrose was replaced with different sweeteners. If the sweetness of sucrose is 100 then the sweetness of dextrose, golden syrup and hydrol is 75, 70 and 75 respectively. The proportions of the sweeteners used are mentioned in the Table 1.

Table 1: Table showing replacement of sucrose by different sweeteners according to sweetness level

Treatment	Sucrose (g)	Dextrose (g)	Golden Syrup (g)	Hydrol (g)
T1	100	-	-	-
T2	75	33.33	-	-
T3	50	66.66	-	-
T4	25	100	-	-
T5	-	133.32	-	-
T6	75	-	35.71	-
T7	50	-	71.42	-
T8	25	-	107.13	-
T9	-	-	142.84	-
T10	75	-	-	33.33
T11	50	-	-	66.66
T12	25	-	-	100.00
T13	-	-	-	133.32

After accurate weighing of the ingredients creaming of vegetable ghee and sugar was done, followed by the addition of eggs. Creaming was continued till foaming occurred. The flour and baking powder were added to the creamy mass and mixed to form a homogenous phase. The batter was then rolled out to 7mm thickness and was cut with the help of a 36mm diameter biscuit cutter. The biscuits were placed on baking trays at a proper distance and were baked at 425 °F in the baking oven for 10-12 minutes. After baking, the biscuits were cooled at room temperature and packed in polythene bags for further studies.

Physical Analysis

The width, thickness and spread factor of biscuits were evaluated according to the method described in AACC (1983).

Sensory Evaluation

The biscuits were evaluated by a panel of judges for colour, taste, flavour, texture and overall acceptability at 0, 15, 30, 45 and 60 days intervals of storage according to the procedure described by Larmond (1977).

Gross Energy

Calorific Value (C.V) of the biscuits was estimated by using Parr Oxygen Bomb Calorimeter method as described by Krishna and Ranjhan (1981).

Statistical Analysis

The data obtained for each parameter was subjected to statistical analysis to determine the level of significance within the treatments and storage intervals by using the procedure as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Chemical Composition of Wheat Flour

Results regarding chemical composition of wheat flour indicated that the flour contained moisture contents 10.36%, crude protein 10.09%, crude fat 1.24%, crude fiber 0.45%, ash 0.57% and nitrogen free extract 77.28%.

Sensory Evaluation of Biscuits

The biscuits prepared from different sweeteners were subjected to sensory evaluation for colour, taste, flavour, texture and overall acceptability at 0, 15, 30, 45 and 60 days interval of storage.

The results pertaining to overall acceptability of biscuits are presented in Table 2. It is evident from the data that at zero days the judges placed T2 at the highest position with 7.88 score while T13 was placed at the lowest position with 6.0 score, T6 and T7 got fairly high scores when fresh biscuits were evaluated. During storage there was a decreasing trend in acceptability of biscuits. After 60 days of storage, T2 which was equivalent to the controlled one and then T6 achieved maximum score while T5 got the minimum score. However, all the biscuits remained acceptable even after 60 days. The result obtained could be supported by studies of Elahi (1997), Who in earlier studies found, a

decreasing trend in overall acceptability of biscuits and attributed it to the moisture absorption and increase in peroxide value and free fatty acid contents in the biscuits. Rao *et al* (1995) also reported similar trend. They found that colour, taste, aroma, texture and overall acceptability of whole egg incorporated biscuits were adversely affected during six months storage in various packaging materials.

Table 2: Effect of storage period on the overall acceptability of biscuits prepared with different sweeteners.

Treatments/Storage period (Daye)	0	15	30	45	60	Mean
T1	7.38	7.33	7.25	7.13	7.00	7.22
T2	7.88	7.88	7.38	7.25	7.00	7.48
T3	7.00	6.63	6.5	6.25	6.00	6.48
T4	6.88	6.63	6.5	6.25	6.00	6.45
T5	5.75	5.50	5.13	5.00	5.00	5.28
T6	7.75	7.63	7.25	7.00	6.88	7.30
T7	7.75	6.38	6.38	5.75	5.50	6.35
T8	7.25	6.13	6.00	5.38	5.25	6.00
T9	6.63	6.13	6.00	5.38	5.25	5.88
T10	7.5	7.38	7.13	6.75	6.50	7.05
T11	6.63	6.5	6.38	6.25	6.25	6.40
T12	6.63	5.75	5.63	5.50	5.50	5.80
T13	6.00	5.63	5.38	5.25	5.25	5.50
Mean	7.00	6.58	6.38	6.09	5.95	

Physical Tests of Biscuits

The results pertaining to physical tests of biscuits are presented in Table 3. It was indicated that the width of biscuits vary from 25.85 mm in T7 to 21.90 mm in case of T5. A decreasing trend in width of biscuits was observed with increasing levels of dextrose and hydrol, while with the use of golden syrup first it was increased and then decreased the width of biscuits in comparison to the width of control treatment that was 25.05 mm.

Table 3: Effect of sweeteners on the physical characteristics of biscuits

Treatments	Width (mm)	Thickness (mm)	Spread Factor
T1	25.05	6.4	39.14
T2	25.35	6.35	39.92
T3	23.4	6.4	37.97
T4	23.15	6.7	34.55
T5	21.90	7.5	29.20
T6	25.5	5.9	43.22
T7	25.85	5.7	45.35
T8	25.2	6.15	40.98
T9	22.5	5.95	37.81
T10	25.85	5.45	47.43
T11	25.75	5.35	48.13
T12	23.5	5.35	43.92
T13	23.4	5.25	44.57

The thickness of biscuits ranged from as high as 7.5 mm in T5 to as low as 5.25 mm in T13. It was noted that increasing levels of dextrose resulted in an increase in the thickness of biscuits but thickness of biscuits was decreased with increasing levels of hydrol. Increase in thickness was followed by decrease in thickness due to increasing levels of golden syrup and comparable to the thickness of controlled treatment that was 6.4 mm.

The spread factor as is evident from the Table 3 was highest 48.13 in T11 and lowest 29.20 in T5.

Increasing levels of dextrose decreased the spread while increasing levels of golden syrup and hydrol first increased and then decreased the spread factor.

The results obtained could be compared with those of Alvi (1994) who used different sweeteners in biscuits and concluded that liquid glucose increased the width and spread factor, while honey decreased the width and thickness of biscuits. It can also be supported through the studies of Siddique (1995) who reported that use of artificial sweeteners in biscuits decreased the width and thickness of biscuits irrespective of the concentration of the sweeteners. However, the spread factor of biscuits increased progressively with the increase in concentration of the sweeteners in general.

Calorific Value of Biscuits

The calorific value of biscuits prepared from different sweeteners has been presented in Table 4. Highest calorific value 5833.82 cal / g was given by T6 while the lowest were found in T5 i.e 5128.03 cal/g i.e comparable to calories 4803.80 cal / g given by the control treatment T1. It was observed that increasing levels of dextrose decreased the caiorific value placing T2 5209.69 cal / g at the top and T5 5128.03 cal / g at the bottom. Similar trend was observed with golden syrup. Calorific value of biscuits containing golden syrup varied from as high as 5833.82 cal / g in T6 to as

low as 5549.07 cal / g in T9. On the other hand, an increasing trend in C.V. of biscuits was observed with increasing levels of hydrol, placing T13 5629.41 cal / g at the top and T10 5156.69 cal / g at the bottom.

Table 4: Effect of sweeteners on the calorific value of biscuits

Treatments	Gross Energy (cal / g)
T1	4803.80
T2	5209.69
T3	5171.70
T4	5138.25
T5	5128.03
T6	5833.82
T7	5799.95
T8	5609.96
T9	5549.07
T10	5156.69
T11	5328.55
T12	5509.81
T13	5629.41

The results obtained in these studies could be related to the findings of Siddique (1995) who reported that increasing levels of artificial sweeteners in biscuits progressively decreased the calorific value of biscuits, which support the conclusions found in this manuscript.

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PREPARATION AND EVALUTION OF DRIED APRICOT DIET JAM

Faqir M. Anjum, Maqam-ud-Din, Ijaz Ahmad and A.R. Pasha
Department of Food Technology, University of Agriculture, Faisalabad

ABSRACT

Dried apricot diet jam was prepared by incorporating a suitable combination of sorbitol, cyclamate and aspartame instead of sucrose and glucose syrup on the equivalent solid basis. The treatments were analyzed for physico-chemical and sensory evaluation fortnightly for two months. Significant results were obtained for TSS, pH, acidity and reducing sugars with regard to treatments and storage periods. All the sensory characteristics affected significantly due to the differences in sweetener combinations while the effect of storage period was found to be non-significant. There was no effect of treatment and storage period on ash contents of apricot jam. The total soluble solids, acidity and reducing sugars increased while pH decreased during storage. Total soluble solids increased gradually in all treatments during storage periods. The mean of TSS was 68.95 at 0 days which rose to 69.60 after 60 days of storage. Minimum percent acidity i.e. 0.69 was observed in sample containing 85% sorbitol, 7.5% cyclamate, 7.5% aspartame. Minimum reducing sugars 2.43% were recorded in sample containing 80% sorbitol, 10% cyclamate and 10% aspartame in their compositions. In organoleptic evaluation, all treatments remained acceptable during 60 days of storage. Samples containing sorbitol, aspartame and cyclamate in the ratio 85: 7.5: 7.5 and 80:10:10, respectively could be prepared successfully on commercial scale manufacturing due to attractive colour, good taste, charming flavour and low calories.

INTRODUCTION

Apricot (*Prunus armeniaca L.*) is an attractive, delicious, highly nutritious and important fruit of Pakistan. The annual production of apricot in Pakistan was 76,200 tonnes in 1989-90 which rose to 190,000 tonnes in 1998-99 (Anonymous, 1998-99).

The apricot flesh contains about 84.2g water, 0.8g protein, 0.6 g fat, 13.8g carbohydrate, 1.1g fiber, 0.6g ash, 30mg calcium, 32mg phosphorus, 1.1mg iron, 670µ vitamin A, 0.4mg niacin, 0.6mg riboflavin, 0.4mg thiamine and 10mg ascorbic acid per 100g (Woot, 1960).

Many products are prepared from apricot. A fully ripened apricot is excellent dessert fruit. It is frozen, dried and is also processed into a number of products such as jam and nectar.

Sugar is a major ingredient being used in jam as a source of sweetness. The excessive intake of sugar results serious like cardiovascular disease, hyperglycemia, diabetes and tooth decay. There has been a growing desire to utilize artificial sweeteners other than sucrose for nutritional and heath purposes (Salminen and Hallikainen, 1989).

With the help of non-nutritive sweeteners, sugar can be replaced and new product can be developed with low calories to avoid the risk of heart disease and other lethal conditions. The demand of low caloric products without scarifying the sweet taste is increasing rapidly (Hyvonen and Torma, 1983).

Alcoholic and artificial sweeteners like sorbitol, manitol, xylitol, cyclamate, saccharine and aspartame respectively are available. In developed countries these alternative sweeteners are being used in different reduced or sugar free products which give sweet taste to the product with no calorific value (Mulinari and Bileski, 1995).

Diabetic products may also be prepared without sugar and can be sweetened with artificial sweeteners. The present study was conducted to provide a guideline for low caloric content apricot diet jam with an equi-sweet taste.

MATERIALS AND METHODS

Diet jams were prepared by using sorbitol, cyclamate and aspartame by replacing sucrose and glucose syrups. The pulp was homogenized and cooked for ten minutes with sorbitol, cyclamates and aspartame in the combinations given in Table 1.

Table 1: Formulation of different treatments for the preparation of dried Apricot diet jam.

Treatment	Pulp %	Sugar %	Sorbitol %	Cyclamate %	Aspartame %	Pectin %	Citric acid %	S. Benzoate %	Color and Flavor
T0	39.28	59.21	0	0	0	0.55		0.1	As desired
T1	29.13	0	70	0	0	0.45	0.63	0.1	As desired
T2	30.17	0	68.79	0.038	0.0056	0.45	0.48	0.1	As desired
T3	31.29	0	67.59	0.078	0.012	0.45	0.48	0.1	As desired
T4	32.49	0	66.29	0.12	0.018	0.45	0.48	0.1	As desired
T5	33.79	0	64.89	0.17	0.025	0.45	0.48	0.1	As desired

Pectin was dissolved in sorbitol using high speed mixer. These were added to different lots near the end point 64-66 Brix. After boiling sodium benzoate, citric acid, color and flavour were added. The cooking was ended when the final temperature range between 104-108 °C and the jam was filled in pre-sterilized wide mouth glass jars.

TSS, pH, acidity, ash content and reducing sugars were determined according to the procedures as given by Ruck (1963). Sensory evaluation was made on 9 point hedonic scale as described by Larmond (1977). Statistical analyses were done as described by Steel and Torrie (1980).

RESULS AND DISCUSSION

Total Soluble Solids (TSS)

The TSS was higher with non significant different among T_2, T_4 and T_1 (Table 2).

Table 2: Effect of sweeteners on various chemical characteristics of dried apricot diet jam.

Treatments	TSS	pH	Acidity (%)	Ash (%)	Reducing sugars (%)
T0	69.12b	3.30d	0.69a	7.20a	3.51a
T1	69.74a	3.33b	0.67b	7.13a	3.07b
T2	69.92a	3.33b	0.67b	7.13a	2.66d
T3	68.46c	3.34a	0.67b	7.13a	2.79c
T4	69.86a	3.34a	0.66c	7.13a	2.66d
T5	68.60c	3.32c	0.66c	7.11a	2.43e

Mean values carrying same letters are not significantly different

TSS of all the treatments showed an increasing tend with increase in storage days (Table 3). The initial TSS content values were 68.95 on 0 day, which rose to 69.60, (Table 3) after 60 days of storage. The Increase in TSS may be attributed to the formation of water soluble pectin from protopectin during storage (Bindra, 1974, Pandit, 1991).

pH

pH of jam differed significantly by treatments and storage intervals while their interaction was found to be non significant. The pH of jam samples ranged from 3.37-3.33 on 0 day which decreased to 3.25-3.1 after 60 days storage. The pH of the samples decreased due to an increase in acidity during the storage period. This decrease in pH might be due to the formation of acidic compounds (Lindroth, 1980, Lodhi, 1989).

Acidity

The data showed that there was an increase in acidity of all samples with the increase in storage days (Table 3). The data indicates the maximum mean percent acidity i.e. 0.69 was recorded for To (Table 2). Increase in acidity might be due to the formation of acidic compounds by degradation or oxidation of carbohydrate compounds present in jam as reported by Bindra (1974), Komitet (1980).

Ash

The effect of treatments and storage was found to be non-significant on ash percentage of jam. The results obtained were in accordance with finding of Winton (1935) who reported that there was no effect of treatments and storage on ash content of jam.

Reducing Sugars

During storage maximum reducing sugars were recorded in T_1 and minimum reducing sugars were observed in T_5 (Table 3). The maximum reducing sugar contents in diet jam were recorded 3.51% in T_o and minimum reducing sugar percentage were observed 2.43% in T_5 after 60 days of storage.

Table 3: Effect of storage periods on different characteristics of dried apricot diet jam.

Storage period	TSS	pH	Acidity (%)	Ash (%)	Reducing sugar (%)
S1 (0 days)	68.95c	3.35a	0.65e	0.48a	7.13e
S2 (15days)	69.15bc	3.34b	0.66d	0.48a	7.46d
S3 (30days)	69.28abc	3.32c	0.67c	0.48a	7.84c
S4 (45 days0	69.43ab	3.31d	0.68b	0.48a	8.23b
S5 (60 days)	69.60a	3.29e	0.70a	0.48a	8.61a

Mean values carrying same letters are not significantly different.

The increase in reducing sugars might be due to the prolong storage time, high storage temperature, increased catalytic oxidation and hydrolysis of sugars with increase in acidity and decrease in pH. The similar results were given by Bindra (1974) Pandit (1991) and Saleemi (1999).

Sensory Evaluation

Sensory characteristics were evaluated on the basis of 9 point hedonic scale method by the panel of 5 judges. Jam prepared from different sweeteners were evaluated organoleptically for colour, taste, flavour, texture and over all acceptability.

The statistical analysis revealed that all sensory characteristics differed significantly by the treatments while the effect of storage periods was found to be non-significant. The interaction of treatments and storage period was found to be significant for flavour and overall acceptability while non-significant for colour, taste and texture of apricot diet jam.

Treatment 5 was ranked the best for flavour, taste, texture and overall acceptability (Table 4). The

Table 4: Effect of treatments on sensory characteristics of dried apricot diet jam

Treatments	Colour	Taste	Texture	Flavor	Overall acceptability
T0	7.20d	6.00f	6.10f	6.43e	6.13e
T1	7.35c	7.11d	2.28e	7.03d	7.16d
T2	7.05e	7.01e	7.10d	7.10c	7.27c
T3	6.96f	7.32b	7.14c	7.25b	7.31c
T4	7.45b	7.21c	7.23b	7.47a	7.37b
T5	7.77a	7.55a	7.51a	7.51a	7.46a

Mean values carrying same letters are not significantly different

differences in flavour were non-significant between T_5 and T_4. The differences in sensory characteristics may be due to differences in formulations of treatments (Pasha *et al.*, 1994, Saleemi, 1999).

Calorific value of apricot diet jam

The calorific value of diet jam prepared by replacing sucrose with sorbitol, cyclamate and aspartame has been shown in Table 5. The highest calorific value was recorded in T_0 in which sugar was used. It was found that decreasing the sorbitol in treatment there was a decrease in the calorific value of jam. The lowest calorific value 2.39 k. cal/g was observed in T_5.

Table 5: Effects of sorbitol, cyclamate and aspartame on the calorific value of apricot diet jam.

Treatments	Gross energy kcal/g
T0	2.63
T1	2.49
T2	2.47
T3	2.44
T4	2.42
T5	2.39

In conclusion, it may be stated that apricot diet jam prepared from treatments T_5 and T_4 containing sorbitol, cyclamate and aspartame in the ratio of 85:7.5:7.5 and 80:10:10 respectively could be prepared successfully on commercial scale marketing due to attractive colour, good taste, charming flavour and low calories.

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EFFECT OF SEPTIC TANK EFFLUENTS ON QUALITY OF GROUND WATER

Musa Kaleem Baloch, Inam Ullah Jan and Sheikma T. Ashour*

Department of Chemistry, Gomal University, D.I. Khan, N.W.F.P., Pakistan.

*Umm Al-Qura University, Makkah Al-Mukarramah, Saudi-Arabia.

ABSTRACT

The present work deals with the pollution of domestic drinking water due to vicinal septic tanks in the houses of Dera Ismail Khan and Bannu cities of N.W.F.P. Fourteen water samples were collected from hand pumps of D.I. Khan city, while five samples were taken from small water tanks made underneath the house floors in Bannu city. The physical parameters like TSS, TDS, pH, conductance, alkalinity, absorbance and total hardness were measured. It was found that their levels were mostly higher than those recommended by WHO. Li^{+}, K^{+}, Fe^{++}, Ca^{++}, Mg^{+}, Cl^{-}, SO^{-}_{4}, CO^{-}_{3}, HCO^{-}_{3}, were also analysed. The bacterial contamination was also assessed, using nutrient agar media and MacConkey's broth for detection of Gram +ve and Gram -ve microorganisms, respectively. It was observed that the samples from Bannu city were 60% more contaminated than those of D.I. Khan. The prominent microorganisms found were *staphylococci* and coliform bacteria. It was concluded that the extent of pollution was more near the septic tanks and increases with its age. A model has also been proposed to explain the observed results. The deviations are explained in terms of soil properties, where the septic tanks and hand pumps are built/fixed.

INTRODUCTION

Water is considered as one of the nutrients, although it yields no calories, yet it enters into structural composition of cell and is the essential component of diet (Awan, 2000). The increase in population and industrialization has deteriorated the quality of water and about 0.25 % of the total deaths are caused due to water based diseases (Kiss *et al.*, 1991). These data have forced the scientists to look into the quality of the water and causes for its pollution. Therefore, a lot of work have been done to analyze different sources of water with reference to these aspects. For example, Follet and Walker (1989) and Saxena and Kavita (1991) found an increase in nitrates in ground water due to excessive use of N-fertilizers. Cova *et al.*, (1990) found some chemicals in ground water. Whereas Dikshith et al (1990) found DDT and HCN residues in many of the Indian water sources. Others (Mirkin *et al.*, 1990; Bendar and Kies, 1991; Kiss *et al.*, 1991; Hussain, 1989) investigated the pollution of ground water caused due to bacteria, pesticides, industrial effluents, etc. and found the results as positive. Keeping in view the global changes, the increase in population and industrialization in Pakistan, the scientists of Pakistan also started working on similer aspects. As a result a number of reports have been published, which are mostly on analysis of ground water and its contamination due to different factors/parameters (Hussain., 1989; Khan. *et al.*, 1993; Akhtar and Khan 1995; Ibrar and Waqar, 1996; Shah, 1997 and Khan *et al.*, 2000). These reports reveal that pollution of ground water is increasing due to increase in population, low standard of waste water management, and seepage of industrial effluents, etc.

The literature shows that there are different factors which can contaminate/pollute the ground water. Further, no systematic study has been carried out upto now. On the other hand, the ground water is the major source of drinking water not only in Pakistan but all over the world. Therefore our objective was to determine the effect of presence of septic tanks on the quality of ground water samples from D.I. Khan and Bannu cities

EXPERIMENTAL

SAMPLING: Approximately 1 liter water sample was taken from each source in a clean plastic bottle. These samples were air tightened and stored in the refrigerator until complete analysis was made. Before sampling, the bottles were washed with detergent followed by tap water, chromic mixture and finally several times with double distilled water. The bottles were rinsed thrice with the sample water. The water was allowed to flow for some time, after that the bottles were filled with the samples (water) and stoppered.

Nineteen sites were randomly selected. Out of these, fourteen were taken from D.I. Khan city hand pumps, which were installed in the vicinity of septic tank and/or marsh streams. The other five were taken from Bannu city. In Bannu the drinking water is stored in small tanks built underneath the floor of the houses. The details about the sources of the water are given in Table1.

Table 1: Detail about the source of water samples from D.I.Khan and Bannu .

Sample No	Depth of HP/WT (m)	Age of HP/WT (Years)	Depth of ST (m)	Age of ST (m)	Distance b/w HP/WT &ST (years) (m)
1	8.4	12	1.6	4	3.6
2	12.0	11	1.2	10	0.9
3	12.0	8	1.8	8	3.0
4	13.5	6	1.8	3	2.4
5	11.4	60	0.5	25	0.3
6.	15.0	28	1.5	13	2.1
7	15.0	6	1.8	6	1.5
8	10.5	6	1.8	6	6.0
9	12.0	11	1.8	4	1.8
10	12.0	14	1.8	4	1.8
11	10.5	12	1.6	11	0.9
12	10.5	12	1.8	11	3.6
13	12.0	16	1.5	11	4.5
14	12.0	16	1.8	14	3.9
15	1.5	20	1.8	10	1.8
16	1.8	8	2.0	8	4.2
17	1.2	6	2.4	6	0.8
18	1.5	8	1.8	8	0.6
19	1.5	4	2.0	4	2.1

HP = Hand pump, WT = Water Tank, ST = Septic Tank
Samples No.1-14 were taken from D.I.Khan whereas 15-19 from Bannu.

MATERIALS AND METHODS

The following measurements were made as soon as the samples were brought to the laboratory:

pH

It was measured using Corning EEL-12 England pH meter.

Conductivity

The electrical conductance of ionized constituents present was measured, using HINA Instrument HI 8820, Italy conductometer.

Total Dissolved Solids (TDS)

Total dissolved solids were determined by drying a known amount of water and then keeping it in an oven at 103-105 ^{0}C upto a constant weight which required normally one hour.

Total Suspended Particles (TSP)

A known amount of water was filtered through a clean, washed and weighed filter paper. After filtration, the paper was dried to a constant weight at a temperature of 103-105^{0}C in an oven. Weight of TSP was calculated.

Total Hardness as $CaCO_3$

Total hardness and Ca contents were analyzed using complexometric titration (APHA-1995). The total hardness was calculated in the following way: Total hardness as $CaCO_3$ = [Volume of EDTA used (mL)] x (100 x 1000) / (Aliquote of sample (mL) taken).

Alkalinity

It was determined using standard method of titration and consulting Table-2 given by (APHA, 1995).

Table 2: Relationship between OH^-, CO_3^{-2} and HCO_3^- Alkalinity

Value of Phenolphthalein Alkanlity	OH^- Alkanlity	CO_3^{-2} Alakalinity as $CaCO_3$	HCO_3^- Alkalinity as $CaCO_3$
0	0	0	T
< 1/2 T	0	2P	T-2P
1/2 T	0	2P	0
>1/2 T	2 (P-T)	2 (7-P)	0
T	T	0	0

T = Total alkalinity

Chloride

Chloride was determined by titrating acidified (adding H_2SO_4 equal to that required for total alkalinity) sample against $AgNO_3$, using potassium chromate as indicator (APHA, 1995).

Sulphate

The amount of sulphate present in the samples was determined through the usual method of volumetric titration against EDTA (APHA, 1995).

Sodium, Potassium and Lithium

These were determined using flame photometer (Vogel, 1961).

Iron

Iron in the samples was determined colourimetrically. For this purpose the colour was developed using $FeNH_4(SO_4)_2$ and NH_4 SCN and the intensity was measured using spectrophotometer (Vogel, 1961).

Magnisium

Mg was determined using flameless atomic absorption spectroscopy. For the purpose first the standard curve was obtained and then the samples were investigated. All the parameters were kept same as required for usual procedure.

Bacterial Analysis

Bacterial analysis was carried out by plate counting method (APHA, 1971) and the total viable count as well as coliform count were determined.

RESULTS AND DISCUSSION

Before discussing the results obtained, the following facts have to be noted:

1) The composition of the soil has a great influence upon the quality of ground water. That is, some soils show more salts whereas others less. This property can not be dealt separately from the influence of septic tank upon quality of water.
2) The morphology of the soil can also play an important role in diffusing ions from one place to another.
3) The material used to pump out water and to construct the septic tanks or water storage tanks in case of Bannu city also plays part. If the quality of the material is good, then rusting will be low and leakage will also be less from tanks. As a result, the pollution caused by leakage will be low and vice versa.
4) In addition the following forces will be involved:-

(i) Gravitational force: This will facilitate/ force the water of septic tank to flow down the earth and ultimately mix up with the ground water.
(ii) Capillary forces: These forces are responsible to take out the water from the tanks and bring close to the hand pump or water tanks. In this case soil texture plays an important role.
(iii) Osmotic forces: These forces are generated due to a huge difference in ionic strength of ground/ tank water and the septic tank water.
(iv) Brownian forces. Brownian and other forces created in ground water help in mixing pure and polluted (leaked out from septic tanks) waters.

5) There can be several other factors like the quality of sewerage system of the area, mechanism of handling of waste water in neighbourhood, etc.

When there is no self made ideal system for the study purpose and all earlier mentioned factors are there, then one can simply expect a trend from the results rather than to develop an exact model for the problem.

Since all earlier mentioned factors are unknown and cannot be determined easily, therefore for the purpose of data analysis, we can consider these to be same. The factors which also play role in the extent of contamination/pollution "P" and can be determined or known are the distance "d", between the source of water/water storage tank, and septic tank and age of the septic tank "t". Further, if we define the extent of pollution in water at present as "P_t" then the pollution caused by septic tank, will be $P_t - P_o$. Where P_o is the extent of pollution before building the tank. Therefore, P will be a net increase in pollution and depends upon, in addition to other factors, the time and the distance between the septic tank and source of water. Mathematically:

$P = P_t - P_o$

$P \propto t/d$

$P = f\ t/d$

putting the value of P, we get

$P_t - Po = f\ t/d$

$P_t = P_o + f\ t/d$ (1)

If we take

$P_t = P$ then $P = P_o + f\ t/d$

considering $f\ t = f'$

$P = P_o + f'/d$ (2)

Here f is proportionality constant and depends upon all the factors stated earlier. f' is a product of f and t, which is also considered to be constant for the time being. In this discussion we supposed that all the

factors represented by f' may be considered as similar which is not a true supposition. It means that the value of constant f' depends upon the soil in which the pump/ tank is built, and other related factors.

The equation (1) tells us that the contamination will be P_o if t = 0 or d is very large or it will be very high if 'd ' approaches to zero. This is what one can expect. Hence as per equation (2) the P vs 1/d should give a straight line. However, the value of slope and its sign should depend upon the contribution of age and distance. To verify this equation the data obtained through analysis are plotted vs distance, and t/d. The observations made in this respect are discussed in detail. It is also pointed out that the life of the pump and/or septic tank, t, was not provided exactly by the owners and there may be an error of several years (i.e from zero to 10 years). This error becomes significant, when the age is less.

To start with the discussion, let us consider the present contamination of the samples with reference to values of different parameters recommended by World Heallth Organization (WHO). The results obtained through analysis are reported in Tables-3 and 4 and discussed below.

Total Suspended Solids

The results obtained for TSS vary from 2 to 500ppm (Table 3) and about one third of these are above the limits of WHO i.e. 200 ppm. High values of suspended solids are also reported by others (Khan *et al.*, 1993; Shah, 1997). The probable reason being rusting of hand pump, and rusted material remaining suspended in water. The data are also plotted versus distance, d from septic tank and age of the tank, t. The

Table 3: Results of physio-chemical analysis of water samples from D.I.Khan & Bannu.

Sample No.	TSS (ppm)	TDS (ppm)	pH	Conducta-nce (ms)	Absorban-ce x1000	Alkalinity as $CaCO_3$ (ppm)	Hardness (ppm)
1	50	156	8.2	2.15	4	200	288
2	75	170	7.0	2.24	5	300	272
3	150	18	8.1	2.41	1	196	428
4	500	304	7.7	4.05	8	200	500
5	3	351	8.1	3.67	6	150	388
6	48	274	7.9	3.10	8	304	524
7	126	233	8.0	2.71	2	300	488
8	2	200	8.0	2.53	25	160	320
9	76	267	7.9	2.90	20	204	432
10	180	352	8.2	3.53	9	240	404
11	42	120	8.2	1.96	7	144	264
12	65	296	7.9	3.47	23	248	368
13	7	148	8.1	1.95	25	244	260
14	47	150	7.9	1.86	26	248	224
15	13	354	7.1	3.30	47	376	560
16	26	262	7.9	2.20	39	292	480
17	80	274	7.8	3.18	25	320	492
18	34	297	7.4	3.12	41	300	548
19	24	267	7.4	3.33	45	384	512

Table 4: Inorganic materials in water samples from D.I.Khan & Bannu.

Sample No.	Lith (ppm)	Sodium (ppm)	Potas (ppm)	Iron (ppm)	Magni (ppm)	Sulph (ppm)	Chloride (ppm)
1	1	15.5	11.0	8.0	44.0	9.8	80.0
2	1	17.5	12.5	8.5	43.0	13.5	66.0
3	1	20.0	12.5	10.0	45.0	18.2	100.0
4	2	28.0	18.0	15.8	53.0	24.0	190.0
5	2	25.0	20.0	4.4	49.0	15.1	188.0
6	2	17.5	20.0	15.8	51.0	17.6	190.0
7	1	17.5	18.8	2.0	50.0	17.9	250.0
8	1	25.0	14.3	21.0	48.0	13.3	150.0
9	1	17.5	17.3	9.0	49.0	16.3	134.0
10	1	27.5	18.5	8.0	48.0	15.9	214.0
11	1	16.0	15.0	8.0	44.0	10.8	262.0
12	1	27.5	16.5	19.0	46.0	13.5	148.0
13	1	15.5	9.0	21.0	41.0	11.3	100.0
14	1	15.0	12.5	20.0	46.0	11.4	136.0
15	1	25.5	2.0	40.0	46.0	18.2	86.0
16	1	29.0	2.0	31.0	42.0	19.8	46.0
17	1	25.0	3.5	21.0	45.0	19.9	42.0
18		1	25.0	3.5	32.0	49.0	19.2
19	1	25.0	2.0	38.0	46.0	16.1	85.0

results obtained for linear regression are shown in Table 5

Table-5: Different parameters obtained for linear regression, when a sample is plotted vs distance between the water source and the septic tank.

Property	Standard Deviation	Correlation Coefficient	Slope
T.S.S	59.49	-0.28	-10.69
T.D.S	76.11	-0.17	- 8.32
pH	0.30	+0.21	+ 0.04
Conductance	0.69	-0.29	- 0.13
Absorbance	9.08	+0.67	+ 3.95
Alkalinity	52.15	-0.09	- 2.99
Hardness	104.76	-0.29	-19.76
Sodium	4.68	-0.07	- 0.20
Potassium	3.40	-0.54	- 1.20
Iron	6.63	+0.80	+ 3.46
Magnisium	3.24	-0.23	- 0.48
Sulphate	3.49	-0.29	- 0.65
Chloride	58.82	-0.33	- 2.68
T.V.Count	17.72	-0.91	-10.56

which shows that the slope is negative, which means T.S.S. decreases with distance from the septic tank, both for D.I. Khan and Bannu city samples. However, the data is very much dispersed. The extent of dispension decreases when T.S.S. is plotted versus t/d (Table-6). However, the rate of decrease is less than that of distance only. From these observations we can conclude that the equation proposed by us works well for T.S.S.

Table 6: Different parameters obtained for linear regression, when a sample is plotted versus age / distance (t / d)

Property	Standard Deviation	Correlation Coefficient	Slope
T.S.S	122.76	- 0.16	-4.48
T.D.S	77.70	+ 0.02	+0.37
pH	0.30	- 2.70	-0.20
Conductance	0.07	- 0.20	+0.03
Absorbance	9.08	- 0.54	-1.10
Hardness	95.13	- 0.16	-3.43
Sodium	4.83	- 0.70	-0.80
Potassium	3.40	+ 0.20	-1.48
Iron	6.43	- 0.82	-1.19
Magnisium	3.57	- 0.25	-0.20
Sulphate	3.66	- 0.18	-0.02
Chloride	58.47	- 0.15	-2.00
T.V.Count	17.52	- 0.51	-5.06

Total Dissolved Solids

The values obtained for total dissolved solids are also reported in Table 3. This table shows that the samples have high value of dissolved solids and reached to an alarming stage, though they are under the limit provided by WHO for the purpose. The Bannu samples have got low T.S.S. but high T.D.S and all the samples have almost the same level of T.D.S. This is because the primary source of water is the same and is not pure. Further, the values decrease with the distance and increase with t/d as expected (Tables 5, 6).

pH

The results for pH indicate that the values are about to cross the acceptable limit (6.5). Most of the samples show very high value of pH, like 8.2. Some of the samples have high value of T.D.S., as well as pH which clearly indicate pollution due to septic tanks. This will become more clear, after going through all the results obtained. Though the slope in case of pH versus distance is very small but the values obtained for average pH (8.94) and maximum (8.8) for D.I. Khan is very high and have to be considered seriously as the level of contamination is very high.

Conductance

Most of the samples analyzed show high value of conductance even upto 4.05 ms, whereas the permissible limit (provided by WHO.) is 2ms. Plot of conductance versus distance shows a decrease in conductance versus distance from the septic tank (Tables 5, 6) The high value of conductance may not be only due to septic tank as N.W.F.P. water otherwise show high value of conductance due to high dissolved amount of minerals (Akhtar *et al.*, 1995).

Absorbance

Normally, turbidity is measured to estimate the concentration of suspended particles in water, but we have measured the absorbance, since pure water shows no absorbance and the decrease in light transmittance (i.e. increase in absorbance) will be only due to turbidity/impurity of the water. The results so obtained show very low values but it decreases very sharply with the increase in distance.

Alkalinity

The alkalinity determined as phenolphthalein ranges between 100-280 ppm for D.I. Khan samples and 268-364 ppm for Bannu samples. Though the values are well below the maximum permissible (500 mg) limit yet are very high and can be considered in relation to as pollution which is very high, at least in Bannu samples. Though the water of Bannu as such is highly alkaline, but septic tanks are also contributing a lot as is clear from the slope, which is negative, i.e. alkalinity decreases with the increase in distance for the septic tank (Table 5).

Hardness

The values obtained for hardness are very high i.e. 224-680 ppm for D.I. Khan and 480-492 ppm for Bannu samples, whereas the acceptable limit provided by WHO for drinking water is 250 mg/L to 500 mg/L. Though it has been reported by several investigaters (Hussain, 1989 and Khan *et al.*, 1993) that hardness is high in N.W.F.P., specially in D.I. Khan and Bannu water samples, but this hardness decreases with the increase in distance from the septic tank and Tables 5, 6). Hence the major source of high hardness can also be the septic tanks.

Sodium

The important metal like sodium has also been determined and its values range between 15-30 ppm for D.I. Khan and 25-28 ppm for Bannu samples. Though these values are under permissible limits yet are otherwise higer than for a normal drinking water. Further the data show a very low decreasing order with the increase in distance from the septic tank.

Potassium

The values for potassium range from 11-22 ppm for D.I. Khan and 2-3.5 ppm for Bannu samples. These are in the same range as determined by others (Khan *et al.*, 1993 and Akhtar *et al.*, 1995). These values are presently very low but their dependance upon distance from septic tank is very high and Tables 5, 6) for both D.I. Khan and Bannu cities.

Iron

Iron contents are found to be 2-22 ppm for D.I. Khan and 32-40 ppm for Bannu water samples. The WHO acceptable limit is 0.3 ppm. The high values of iron contents are found due to two following reasons.

a. The material used for the water to be pumped out from the earth or to be delivered gets rusted and the iron contents are increased.

b. The soil of this area is very rich in iron and hence iron is found to be in abundance (Shah, 1997) in this area.

The data plotted versus distance show that the iron content decreases with the reduction in distance for the septic tank. This trend can be explained on the basis that water becomes alkaline due to increase in pollution from the septic tanks and the solubility of iron goes down. That is why, it decreases with increase in distance from septic tank.

Magnesium

The analysis shows that the range for D.I.Khan samples is ppm 41-53 and 34-49 ppm for Bannu. Such range was also observed by Ibrar and Waqar (1996). The WHO permissisible limit for magnesium is 30 ppm. However, this limit can be increased to 250 ppm if sulphates are present as the magnesium gets precipitated in such medium and water becomes suitable for drinking. Though the level of magnesium is under admissible limit, but it is otherwise high and indicates level of pollution.

Sulphates

The results are in the range of 3.8-24.4 ppm for D.I. Khan and 16-19 ppm for Bannu. These are under the permissible limit. The data indicate that the values decrease with decrease in distance from the septic tank, both for D.I. Khan as well as Bannu. This may be due to formation of such compounds/complexes which are insoluble in alkaline media and hence are precipitated down and do not appear in water.

Chlorides

The data obtained for chlorides show that their concentration range is 66-262 ppm for D.I. Khan and 86-180 ppm for Bannu. The D.I. Khan samples range is quite high and is approaching very fast to permissible (200-500 ppm) limit. It is clearly due to the pollution caused by septic tank, as it decreases at high rate with increase in distance from the tank and same is true for Bannu. (Tables 5, 6).

Bacterial Analysis

Bacterial analysis shows that the septic tanks are affecting the quality of ground water. The total viable count ranges from 5.3×10^3 to 1.28×10^5. The total viable count decreases very rapidly with the increase in distance from the septic tank and increase with the age of the tanks both for D.I. Khan and Bannu (Tables 5, 6). It was found that the water samples taken from under ground storage tanks located at Bannu were more contaminated than water sample of hand pumps located at D.I. Khan. The coliform count was detected only in two waer samoples collected from Bannu

The results obtained in this study of ground water analysis in D.I. Khan and Bannu conclude that the contamination due to septic tank is increasing very fast and that the hypothesis/ model given by us works very well. Further Bannu samples are more polluted than D.I. Khan samples.

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EXTRACTABILITY AND STORAGE STABILITY OF RAPESEED PHENOLICS

Nizakat, Bibi M. Ashraf Chaudry, Zahid Ali and Abdus Sattar
Nuclear Institute for Food and Agriculture, Peshawar

ABSTRACT

Rapeseed variety "Salam" was used to extract different polyphenols, with water adjusted to pH 5, 6, 7, 8 and in ether and ethanol at room conditions for subsequent analysis and their stability. All the phenolics were found to be more stable in alkaline pH (8 and 9) than in acidic ones. Maximum loss of 28.4%, 31.5%, 38.0% and 77.8% were recorded in samples extracted at pH 5 in sinapine, total phenols, leucoanthocyanidine and procyanidine contents, respectively, during 7 weeks storage period. Leucoanthocyanidine, precursor of procyanidine, was more stable than procyanidine in alkaline media. Extractability of sinapine, leucoanthocyanidine and procyanidine was more in ether solvent than ethanol whereas extractability of total phenols was almost same in both solvents. Maximum loss of 82.5%, 65.71% and 89.26% were in sinapine, total phenols, procyanidine and leucoanthocyanidine in ether extractable fraction, respectively, Leucoanthocyanidine extractable in ethanol (89.26%) was lost more than other phenolics during storage at room conditions.

INTRODUCTION

Rapeseed is one of the major oilseed crops grown in the world. It contains substantial amount of polyphenols which thwart its utilization as feed or food. Polyphenols consist of two groups i.e. flavonoid and cinnamic acid derivatives (Ranganna, 1977) Flavonoids, which are the major group, are characterized by the presence of a specific carbon skeleton consisting of two aromatic rings linked by an aliphatic three carbon chain. The polymerized phenolics are called tannins, which can be growth depressant factors because of their inhibitory effect on protein digestion. Quebracho tannin and tannic acid have astringent properties, whereas gambier tannins and myrobalan tannins are non-astringent and catechin is questionable (Peterson, 1977). The main phenolic constituent of rapeseed appears to be sinapine (Fenton *et al.*, 1980; Krygier et al; 1982) and is the choline ester of sinapic acid, which is a bitter tasting compound. Hobson *et al.*, (1973) and Buttler *et al.*, (1982) have reported the involvement of phenolics in fishy odour or taste in eggs of certain brown layers. High tannin levels produce marked inhibition of enzymatic reactions, growth of yeast and certain parasitic fungi. Great health value has been ascribed to tannins by Chinese scholars especially to those of tea leaves, which is a controversy. Elagi-tannins have shown to contribute to resistance radioactivity damage in rats (Peterson, 1977). The presence of tannins is associated with decreased bird preference (Bullard and Elias, 1980) and lower protein digestibility (Miturn *et al.*, 1984) of the grain. Because certain phenolics (Appelquist. 1972) are bitter tasting and/or have the ability to precipitate plant and animal proteins (McManus *et al.*, 1981), they have been considered by Ritta (1985) as defence compounds.

MATERIALS AND METHODS

In view of the importance of phenolics, the present studies were undertaken to extract and store phenolic extracts and study their stability at room condition.

High glucosinolate rapeseed variety "Salam" was obtained from the rapeseed group of this Institute. Clean and healthy seeds were ground to 40 mesh. The extracts were prepared with distilled water and pH was adjusted to 5, 6, 7, 8 and 9 with ether and ethanol solvents by shaking twice in a mechanical shaker at room temperature for 30 minutes. These extracts were centrifuged (3000rpm, 10 min) and the supernatants stored at room temperature ($27^{\circ}\pm C^{\circ}2$) in screw capped test tubes for subsequent analysis for sinapine, total phenols, leucoanthocyanidine and procyanidine earlier (Bibi *et al.*, 1991).

RESULTS AND DISCUSSIONS

The sinapine extracted at all pH levels showed minimum changes during 7 weeks storage at room temperature (Table 1). The mean values for pH 5, 6, 7, 8 and 9 extracts were 0.63, 0.59, 0.63, 0.63 and 0.67%, respectively. The maximum loss of sinapine (28.4%) was recorded after 7 weeks storage in samples extracted at pH 5 and minimum (4.3%) in samples extracted at pH 9 during the same storage period. About 10% loss in sinapine content was observed after first week of storage followed by inconsistent changes. In case of total phenols, maximum loss was recorded in samples extracted at pH 5 (31.48%) and pH 6 (30.6%) and minimum loss (9.3%) was recorded in samples extracted at pH 7. In case of pH 9, the values decreased from 1.37 to 1.19 after 5 weeks followed by increase to 1.52 after 7 weeks, which could be due to interconversion of different phenolies at alkaline pH.

Table 1: Effect of different pH levels on the stability of extracted phenolics* of rapeseed during storage.

	Storage-weeks									
pH	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**Mean**	**CV%**
Sinapine (%)										
5	0.74	0.66	0.70	0.64	0.60	0.59	0.55	0.53	0.63	10.83
6	0.70	0.63	0.62	0.64	0.58	0.52	0.54	0.50	0.59	10.81
7	0.69	0.62	0.63	0.63	0.65	0.65	0.58	0.59	0.63	5.20
8	0.68	0.64	0.64	0.63	0.67	0.63	0.61	0.54	0.63	6.35
9	0.69	0.62	0.66	0.68	0.67	0.66	0.71	0.66	0.67	0.70
Mean	0.70	0.63	0.65	0.64	0.63	0.61	0.59	0.56		
CV	2.99	2.36	4.35	2.88	5.87	8.36	10.22	9.94		
Total Phenols (%)										
5	1.62	1.74	1.39	1.26	1.26	1.26	1.11	1.11	1.31	12.62
6	1.44	1.39	1.26	1.16	1.16	1.15	1.05	1.00	1.20	11.97
7	1.40	1.38	1.34	1.30	1.30	1.31	1.26	1.27	1.32	3.53
8	1.37	1.34	1.31	1.26	1.25	1.19	1.58	1.52	1.35	9.34
9	1.39	1.38	1.32	1.31	1.37	1.27	1.44	1.52	1.38	5.38
Mean	1.44	1.39	1.32	1.26	1.27	1.23	1.29	1.28		
CV	6.30	3.06	3.19	4.22	5.40	4.60	15.43	16.43		
Leucoanthocyanidine (ΔA550/g)										
5	2.50	2.25	2.12	2.01	2.02	1.93	1.63	1.55	2.00	14.48
6	2.63	2.53	2.48	2.43	2.22	2.20	1.88	1.77	2.27	12.82
7	2.25	2.25	2.12	2.10	2.06	1.87	1.55	1.43	1.95	14.92
8	2.25	2.25	2.21	2.18	2.14	2.07	2.02	1.96	2.14	4.75
9	2.38	2.35	2.32	2.27	2.28	2.21	2.16	2.02	2.27	3.24
Mean	2.40	2.30	2.20	2.20	2.14	2.07	1.85	1.78		
CV	6.13	4.69	2.49	6.57	4.51	6.71	12.44	1.54		
Procyanidine (ΔA550/g)										
5	2.25	1.38	0.95	0.83	2.78	0.65	2.62	0.50	0.99	53.93
6	2.50	1.75	1.02	0.98	0.98	0.88	0.75	0.85	1.21	46.49
7	2.75	1.88	1.28	1.20	1.12	1.00	0.94	0.82	1.37	43.74
8	2.50	1.88	1.28	1.25	1.12	1.09	1.01	1.01	1.39	53.51
9	2.25	2.00	1.75	1.28	1.17	1.17	1.02	1.02	1.46	30.56
Mean	2.45	1.78	1.26	1.11	1.03	0.96	0.87	0.84		
CV	7.64	12.04	22.37	15.73	13.73	18.97	18.13	22.42		

*Phenolics were extracted with water, adjusted to different pH levels.
cv-cerficient of variability

The other two important phenolics determined were leucoanthocyanidine and procyanidine. The leucoanthocyanidine extracted at pH 5, 6, 7, 8 and 9 decreased progressing during the storage period (Table 1). The initial values ranged between 2.38 to 2.63 and after 7 weeks storage, these values ranged between 1.55 to 2.19 ΔA 550/g. Maximum loss of 38.0% was recorded in samples extracted at pH 5 followed by 36.44% in samples

extracted in alkaline region pH 7.0 and minimum loss of 12.89% and 15.12% was recorded in samples extracted in alkaline region (pH 8 and 9). This showed that the leucoanthocyanidin was almost equal at all pH levels used. The extractability for procyanidine was also identical at all pH levels and the initial values ranged between 2.25 to 2.75 ΔA550/g. Like leucoanthocyanidine, maximum loss was recorded in samples extracted in acidic range and the values ranged between 66.0% (pH 6) to 77.8% (pH 5) after 7 weeks storage. It was also observed that leucoanthocyanidine (which is precursor of procyanidine) was more stable in alkaline region (loss 12.89 – 15.12%) than procyanidine (loss 54.7 to 59.6%) after 7 weeks storage which could again be due to interconversion of various phenolics.

Table 2 shows results on extraction and stability of rapeseed phenolics extracted in ether and ethanol without dilution. More sinapine was extractable in ether (0.4%) than in ethanol (0.24%). Ether extractable sinapine was less stable (82.5% loss) than ethanol extractable (45.8% loss) during 7 weeks storage at room temperature. The extractability of total phenols was almost equal in ether and ethanol but ethanol extractable total phenols were less stable (45.6% loss) than ether extractable phenols (57.1% loss) during the same storage period.

(42.2%) during same storage period (Table 1). More loss was recorded in case of ether extractable and ethanol extractable lucoanthocyanidine (89.3 and 82.5% respectively) than procyanidine during 7 weeks storage indicating more stability of procyanidine than lucoanthocyanidine. This is reverse to extraction of these phenolics at different pH levels.

Literature on such extractability and storage stability of rapeseed phenolics is lacking. However, high content of sinapine in *Brassica napus* rapeseed cultivar was noticed (1.65 to 2.26%) than in *Brassica compestris* cultivars (1.22 to 1.54%). Blair and Robert 1984. and Bibi *et al.*, (1991) found 0.87 to 1.49% and 0.98 to 1.62% and 0.42 to 0.71% in seed cotylendons and hulls in different cultivars of rapeseed.

Ramamurthi *et al.* (1986) found that the concerntration of flavan-4-ols in methanol and acidified methanol extracts of the grains of mold resistant sorghum cultivars were much higher than those of the mold susceptible cultivars. Similar trends were observed, although of a lower magnitude, in the leucoanthocyanidine assay. The data indicated that in addition to the protective roleplayed by proanthocyanidine, other phenolic compounds in plants may also play a role in their defence mechanism.

Table 2: Storage stability of rapeseed phenolics extracts (ether and ethanol soluble) at room temperature.

Storage Weeks	Sinapine %		Total phenols %		Procynadine (ΔA550/g)		Leucoanthoc ynidine (ΔA550.g)	
	A	B	A	B	A	B	A	B
0	0.40	0.24	0.70	0.68	3.60	2.90	5.03	2.36
1	0.26	0.14	0.67	0.65	3.52	2.78	1.18	1.86
2	0.20	0.12	0.41	0.67	3.48	2.34	1.14	1.14
3	40.13	0.11	0.39	0.50	3.19	2.24	1.04	1.24
4	-	0.11	0.36	0.48	3.19	2.08	0.40	1.18
5	-	-	0.29	0.44	3.16	2.05	0.59	1.06
6	0.09	0.10	0.27	0.37	2.28	2.03	0.56	0.62
7	0.07	0.09	0.24	0.37	2.08	1.86	0.54	0.46
% loss	82.50	62.50	65.71	45.58	42.22	35.86	89.76	80.51
Mean	0.20	0.13	0.42	0.52	3.04	2.30	1.50	1.24
CV%	27	36	39	23	19	15	110	47

More procyanidine was extractable in ether (3.60 ΔA550/g) than in ethanol (2.90 ΔA550g). Extractability of leucoanthocyanidine was almost double in ether (5.03 ΔA 550/g) than in ethanol (2.36 ΔA550/g). Loss of procyanidine extractable in ether (52.4%) was more than ethanol extractable

However, stability of sinapine, total phenols and leucoanthocyanidine was more in the alkaline medium i.e. pH 8 and 9 during 7 weeks storage. Therefore such extracts in alkaline media can be stored at room temperature. These extracts will be used to study their effect on insect repellency in future.

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OCCURRENCE OF PATHOGENIC MICROORGANISMS IN FOOD AND WATER SUPPLIES IN DIFFERENT AREAS OF PESHAWAR, NOWSHERA AND CHARSADDA

Misal Khan, Ihsanullah, T. Sharafat, Fazal Mehmood and Abdus Sattar
Nuclear Institute for Food and Agriculture (NIFA), Tarnab-Peshawar

ABSTRACT

Among various pollutants in the environment, pathogenic bacteria are directly related to diseases in humans. Different samples of beef, poultry meat, milk and drinking water were collected from various locations in Peshawar. The samples were analyzed for total bacterial count, total coliforms and E. coli. The data revealed that more than 50% water samples were highly contaminated and were considered unfit for human consumption. In case of food samples, almost, 58%, 42% and 30% samples of poultry meat, beef and milk respectively showed presence of salmonella, as well as E. coli. High level of total bacterial counts in beef (3.2x 10^7/g), and milk. (1.0x10^6/mL) were observed.

INTRODUCTION

The growing urbanization and industrialization are creating a variety of problems; pollution being the most serious one. Environmental, water as well as food pollution is very serious problem in under-developed countries including Pakistan. Wastes from farms, factories, households and hospitals pollute water. Contaminated drinking water has direct effect on human beings while sewerage and industrial effluents, when used for irrigation purposes, have indirect effect through consumption of crops, vegetables and fruits. Contamination is transferred to animals via direct exposure and through polluted water and crops. Biological contamination of foods due to sewerage water, industrial effluents, pesticides, fertilizers and air pollution is proving to be a serious hazard in Pakistan (Khan, 1986)

According to World Health Organization (WHO) more than 80% human diseases are water borne. In the developing countries, 60% population has no access to pure drinking water. Bacteria especially enteric gram-negative rods are considered more dangerous because their natural habitat is the intestinal tracts of humans and animals. The microbiological quality is normally determined by testing a group of organisms known as coliforms. Among various species of bacteria, *E. coli* is the best indicator of pathogenic bacteria. Microbiological standards for drinking water in most developed countries rely on the detection of total coliforms and *E. coli* as markers for enteric organisms (Kratz *et al.*, 1999).

Supplies of drinking water contaminated with sewerage from human and animals may cause diseases like typhoid-fever, cholera, bacteriosis, amoebiasis and behlminthiasis (IDRC, 1988, Awan, 1983). Various workers studied different pollutants in water samples (Ahmad and Saleem, 1983; Ahmad *et al.*, 1982; Masaaki *et al.*, 1985; Wadud *et al.*, 1992). Bacteriological status of different food items were studied earlier (Saleem *et al.*, 1989; Farber *et al.*, 1990; Khalaf and Shareef, 1985). Salmonella was reported in poultry and meat products (Wafa *et al.*, 1986) and other foods (Memon, 1985). Details on safety and sanitation of slaughtering, meat processing, inspection, preservation and judging/evaluation are available in literature (Romans *et al.*, 1979). Gamma irradiation was applied for irradiation of poultry meat (Mahmood *et al.*, 1993; Wakeford *et al.*, 1991).

Provision of regular supply of clean drinking water is a birth right of all the citizens of the country. Infected or contaminated water endangers health and impairs quality of life. Pakistan is struggling hard to provide its citizens with basic amenities but clean drinking water is not available to a great number of people mainly because of rising level of pollution in the environment, poor upkeep of water supply lines and faulty drainage system. These result in frequent mixing of human and animal excreta in drinking water, leading to outbreak of water borne diseases. Apart from that, food poisoning and other food borne diseases caused by pathogenic microorganisms are well documented (Khalaf and Shareef, 1985; Collin and Patricia,

1976). Hence, the present study was conducted to assess the existing microbiological status of various drinking sources and different types of food samples.

Generally, beef and poultry meat are consumed in Peshawar. In view of the nutritional and health aspects of meat, it was considered important to assess the quality of meat sold at selected places in Peshawar. Little or no effort has been made to assess or monitor the pollution of meat in Peshawar. There are numerous centers of meat production and sale, which are located on the main city roads and there is every likelihood that the meat we eat in Peshawar is not of desired quality. Another basic food consumed by us is milk and its products. Milk is considered a complete diet containing all essential nutrients. Hence special attention has been focussed on the analysis of meat and milk samples.

Keeping in view the hazardous impact of microbiological contaminants, it is necessary to investigate present level of these pollutants in the environment. The principal aim of this study was to determine total bacterial counts (TBC), coliforms and presence of salmonella and *E. coli* in drinking water, milk and different types of meat in Peshawar.

MATERIALS AND METHODS

Sixteen water samples from hand pumps and thirteen from tube wells were collected in sterilized bottles from different localities in Peshawar. Figure I shows various sampling sites. Food samples such as beef, poultry meat and milk were collected likewise from different sites in Peshawar City.

All samples were analyzed for total bacterial and coliform counts. Some biochemical tests such as indole, citrate, urease and deoxycholate citrate media were used for identification of *E. coli* and *Salmonella*. For total bacterial counts and coliform counts, nutrient agar and MacConkey Broth were used respectively as culture media. Plate count method was used for total bacterial counts and the most probable number (MPN) method for coliforms. Respective incubation temperatures water 37 and 28 $\pm$ 1°C for pathogenic and total bacterial counts. Using statistical tables, the MPN of coliform bacteria per 100 mL was determined from the pattern of positive sample dilutions. Identification of *Salmonella/E. coli* was carried out by chemical tests (Collin and Patricia, 1976).

RESULTS AND DISCUSSION

Quantitative tests were performed for total coliform count only whereas for *Salmonella* and *E. coli* only qualitative (presence or absence) tests were carried out. As per WHO requirements, the coliform counts should be less than 10 per 100 mL while presence of *E. coli* and *Salmonella* must be negative in the samples (WHO, 1994;). The most common water sources in NWFP are as follows and in order of common use:

1. Dug wells
2. Ponds, canals, streams, lakes, rivers
3. Hand pumps (drilled wells)
4. Springs (open)

In Peshawar, Nowshera, Charsadda and the nearby surrounding areas, the principal source of the drinking water is the ground water including hand pump and tube well water. The water from deep wells is generally administered and supplied by the Municipal Corporation. In order to determine microbiological contamination level in the ground water, samples were analyzed and the results are given in Table 1.

Table 1: Bacteriological level of ground water.

Source	TBC/mL	Coliforms/ 100mL	*E. coli*
Hand pump			
Julozai	4000	350	+
Akora Khattak	5200	1800	+
Pabbi	350	-	-
Khudrazai	845	8	-
Akbarpura	335	5	-
ARI Hostel Tarnab	560	8	-
Madina Colony Peshawar	2300	5	-
Warisabad Peshawar	1840	450	+
Gulbahar Peshawar	843	20	-
Bashir Abad-II	120	66	+
Bashir Abad-II	415	71	-
Danish Abad-I	400	25	+
Danishabad-II	430	40	-
Danishabad-III	1206	80	-
Charsadda-I	350	900	+
Charsadda-II	2200	1200	+
Tube-Well			
Hayatabad-II	230	20	-
Hayatabad-III	830	65	+
Palosi	140	8	-
Bashirabad	213	30	-
Danishabad	418	5	-
Municipa Corporation Peshawar	1135	120	+
ASC Nowshera	740	10	-
AS Cnowshera Officer's Mess	340	7	-
Risalpur	1275	540	+
Sufaid Dheri	2580	215	+
Pabbi	1830	250	+
Pirpiai	2275	80	-
Hayatabad-I	1020	5	-

TBC= Total bacterial counts, += positive, -= negative

The results of the analyses of the drinking water samples were compared with the WHO standards. Drinking water samples were highly contaminated with coliform bacteria in case of hand pump and tube wells. These samples were unfit for human consumption. In some cases, *E. coli* was also detected. The highly contaminated water was that collected from Akora Khattak containing more than 1800 coliforms/100 mL followed by Charsadda I, II and Warisabad, Peshawar having 1200, 900 and 450 coliforms/100mL respectively. Out of 29, only few samples were found within the required standard and fit for human consumption. Among the probable reasons for high level of bacteria is the sewage water. The sewage effluents are discharged into normal water bodies. The water in these ponds either evaporates to the open atmosphere or is leached down into the groundwater, hence causing a serious source of drinking water pollution.

Results of bacteriological analyses of beef, poultry meat and milk are given in Table 2. The total bacterial counts ranged $3.1 \times 10^3 - 3.2 \times 10^7$/g in beef and 1.6×10^4-2.6×10^7/g in poultry meat indicating that some of the samples had high levels of bacterial counts. In milk samples, the total bacterial counts ranged from 2.9×10^3 to 1.6×10^6/mL. The percentage of food samples contaminated with *Salmonella/E. coli* was 42.0, 58.0 and 30 in beef, poultry meat and milk respectively.

Similarly milk may be contaminated from animals fed and on contaminated feed/water. In addition, milk can also be contaminated with pathogens from dirty places where it is obtained and use of polluted water for washing of utensils.

E. coli is a normal inhabitant of the intestinal tract of humans and other warm-blooded animals. Salmonella produces clinically recognizable specific disease i.e. enteric (typhoid) fever. Salmonella also causes food poisoning and enteritis in human beings (Awan, 1983). Some samples in present study were found highly polluted with the *E. coli* and *Salmonella.*

The data differed widely depending upon the location from where that samples were taken. However, these initial investigations indicate the need for continuous monitoring of the status of pollution of water, meat and milk supplies. The present study can be used as base line by health management authorities. In this regard, an effective programme to control the biological quality of drinking water, milk and different meats is highly recommended. Health education is also necessary to educate the public on the health effects of biological constituents present in water and food items.

Table 2: Bacteriological examination of different food samples collected from Peshawar city.

Sample	Beef TBC/g	Salmonella/ *E.coli*	Poultry TBC/g	Meat Salmonella/ *E.coli*	TCB/g	Milk Salmonella/ *E.coli*
1	7.5×10^5	+	4.3×10^6	+	7.3×10^3	-
2	1.5×10^5	+	2.5×10^4	-	4.8×10^5	-
3	2.7×10^6	+	1.6×10^4	+	2.9×10^3	-
4	8.0×10^5	+	7.0×10^5	+	9.0×10^4	+
5	4.7×10^4	-	8.2×10^4	-	1.2×10^5	+
6	3.2×10^7	+	6.5×10^6	+	3.9×10^5	-
7	1.6×10^6	-	8.9×10^5	-	9.0×10^4	-
8	3.1×10^3	-	2.6×10^7	+	1.6×10^6	-
9	9.2×10^5	-	3.9×10^6	+	7.3×10^5	+
10	4.8×10^4	-	4.0×10^5	-	8.2×10^5	-
11	2.0×10^7	+	7.2×10^5	+	-	-
12	2.4×10^6	-	8.5×10^4	-	-	-

The contamination may be transferred to animals from dirty places and polluted water used through crops irrigated with sewage water. Other factors include deposition of contaminants from roadside air and from dirty slaughter places. The shopkeepers sell most of the meat consumed by the public in the open place and even on roadside.

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STUDIES ON THE DETOXIFICATION OF CONTAMINATED FEED ON THE PERFORMANCE OF BROILER CHICKS

Sakhawat Ali, N. Ejaz, Tahira Firdos and Wazir H. Shah
Biotechnolgoy and Food Research Centre, PCSIR Laboratories Complex, Lahore

ABSTRACT

Poultry feed contaminated with aflatoxin was procured from commercial source and detoxified with aqueous ammonia at 38°C. Detoxified feed was fed to broiler chicks for 49 days. Feeding trial results showed that decontamination process degraded aflatoxin to non-detectable limits, while feeding on untreated materials resulted in watered belly, enlarged liver with haemorrhages, lowered weight gain and reduced feed efficiency.

INTRODUCATION

Poultry industry in Pakistan is facing problems of disease, high mortality rate and poor feed efficiency. The major cause of these problems has been attributed to aflatoxin contamination of feed and feed stuffs. Shah *et al.*, (1985, 1986) reported that maize, maize gluten and other poultry feed ingredients were highly contaminated with aflatoxin (133-800 µg/kg). It has been reported (Pasteriner, 1977) that contamination (mycotoxin) affected as much as 25% of the world crop each year. Mycotoxin contamination is caused by molds in ingredients of feed and food (Dale, 1998) and results in disease and death in humans and animals (Feit, 1991). Aflatoxin is most common and poisonous. It is carcinogenic and causes liver damage (Jafery, 1996). According to Richard (1992) aflatoxins are group of toxic metabolites produced by *Aspergillus flavus* and *Aspergillus arasiticus* and have high potential to contaminate feed. A number of attempts have been made to reduce/degrade aflatoxin from food and feed. Samarajewa *el al.*, (1990) reported that aflatoxin can be degraded with physical and chemical treatments. Hamid and Shah (1983) conducted studies on the decontamination of aflatoxin from feed with ammoniation and observed encouraging results. Ali *et al.*, (1989) also reported that decontamination of aflatoxin could be done with ammonia treatment for broiler chicks.

MATERIALS AND METHODS

One hundred day-old broiler chicks were procured from commercial hatchery and divided into two groups having 50 chicks each. Experimental room was white washed, cleaned and disinfected before starting the experiments. Wood shavings were used as bedding material. Clean water was made available round the clock. Feed was offered *ad-libitum*.

Temperature of the experimental room was maintained at 37°C. Subsequently it was decreased by 2°C after every week till it reached at par with ambient temperature. Composition of experimental feeds is given in Table I, while chemical composition of the feed is reported in Table II.

Tale 1: Composition of experimental feeds

Ingredients	Feeds	
	A	B
Corn	40.00	10.75
Rice broken	10.75	10.75
Rice polishing	12.00	12.00
Rape seed meal	3.00	3.00
Cotton seed meal	3.00	3.00
Corn gluten meal 60%	2.00	2.00
Soya bean meal	15.10	15.10
Fish meal	3.5	3.5
Poultry offal meal	1.00	1.00
Canola meal	4.00	4.00
Molasses (cane)	2.00	2.00
Ground limestone	1.25	1.25
Dicalcium phosphate	0.70	0.70
Blood meal	0.0	0.3
Vitamin + Minerals	0.3	0.3
Lysine	0.11	0.11
Methionine	0.175	0.175
NaCl	0.115	0.115
Aflatoxin (ppb)	80	- ve

The composition was estimated by following the methods reported in A.O.A.C. (1984). Detoxification of contaminated feed was carried out by the method of Hamid and Shah (1983). Aflatoxins were determined by the method of Romer (1976) and quantitative estimation was carried out by T.P.I standard procedure (Coomes and Fenell, 1965). Microbiological analysis was done by

following DIFCO Manual method (Anonymous, 1953). Viable count was estimated on nutrient agar whereas for faecal count McConkey's broth was used. Fungus was isolated on malt extract media. Following parameters were noted during experimental period:

1. Initial weight / chick (g)
2. Final weight / chick (g)
3. Total feed consumed / chick (g)
4. Feed efficiency ratio
5. Dressing percentage
6. Weight of internal organs.

Data collected were subjected to statistical analysis as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

Results regarding chemical composition and microbial load before and after decontamination are reported in Tables 2 and 3, respectively.

Table 2: Chemical composition of experimental rations

Constituents	Estimations	
	A (%)	B (%)
Moisture *	10±0.05	9.8±0.80
Protein (N x 6.25)*	20.00±0.8	20.5±0.58
Ether Extract*	4.00±0.3	4.5±0.40
Ash*	6.00±1.00	6.3±0.85
Fibre*	4.50±0.9	4.25±0.8
Energy Kcal/kg**	2800	2800
Calcium**	0.94	0.90
Phosphorus (available)**	0.45	0.45
Lysine**	1.00	1.00
Methionine	0.45	0.45

Analyzed values
* Calculated values

These observations indicate that there was higher microbial load on untreated feed. However, this load was reduced to non-detectable level after ammonia treatment. These results indicated that ammonia treatment reduced the microbial load upto zero level. Aflatoxins were also determined before and after the decontamination process (Table 3).

Results indicate that high contents of aflatoxins decreased on treatment with ammonia to non-detectable limits. These observations are in agreement with the findings of Hamid and Shah (1983) who reported that ammonia treatment reduced aflatoxin to non-detectable limits.

Results of feeding trials are presented in Table 4. Average weight of chicks fed on contaminated and treated feed was 1900 and 2200 g per chick, respectively. It is clear from these observations that aflatoxin contaminated feed depressed growth of broiler chicks. These findings are in agreement with Doerr *et al.* (1983) and Campbell *et al.* (1983) who reported that ingestion of aflatoxin tended to decrease the body weight.

Table 4: Average weight gain, feed consumption, feed efficiency, dressing percentage, mortality and eight of internal organs of broiler chicks fed on treated and untreated feeds

Description	Groups	
	A (untreated)	B (treated)
Number of chicks	50	50
Length of experiment (days)	49	49
Average weight at the start of experiment (g/chick)	39.00	39.00
Average weight at the end of experiment (g/chick)	1900	2200
Total feed consumed/ chicks (g)	4800	4850
Feed efficiency ratio	2.40	2.20
Dressing percentage	58.00	60.00
Weight of heart (g)	10.00	10.00
Weight of liver (g)	55.00	45.00
Weight of gizzard (g)	48.00	46.00
Liver hermorrhage	+ ve	- ve
Dropping condition Bloody diarrhea Ist week Loose dropping Later on	 + ve + ve	 - ve - ve
Water belly	+ ve	- ve

Jafery (1996) and Lakshamirajan *et al.*, (1984) also reported similar observations that optimum growth in layer/broiler was achieved with corn treated with ammonia and heat treatments. Feed intake data indicated that there were no adverse

Table 3: Effect of Ammoniation on the Mierobial and Aflatoxin Level of Feeds.

	Untreated				Treated			
Feed	Aflatoxin (ppb)	Viable count/g	Fecal count/g	Fungi/g	Aflatoxin (ppb)	Viable count/g	Fecal count/g	Fungi/g
A	80	$9.8X10^{8}$	$3X10^{3}$	Fusarium mucor Aspergilus niger	N.D	- ve	- ve	-
B	82	4.5X108	$2.1X10^{3}$	Aspergilus flavus Rhizopus Penicillum	N.D	- ve	- ve	-
C	79	$5.4X10^{8}$	$2.6X10^{3}$	-do-	N.D	- ve	- ve	-
D	81	$7.2X10^{8}$	$1.9X10^{3}$	-do-	N.D	- ve	- ve	-
E	80	$4.3X10^{8}$	$1.0X10^{3}$	-do-	N.D	- ve	- ve	-

ND = not detectable

effects on the palatability of feed due to ammonia treatment. It was also observed from the feed efficiency data that aflatoxin contamination adversely affected feed conversion ratio, however, detoxification of feed with ammonia improved this ratio. These findings are in line with Ali *et al.* (1987). Aflatoxin contamination also affected the dressing percentage of birds as had been reported by Doerr *et al.* (1983) who observed that aflatoxin tended to decrease parts yield with a low meat to bone ratio. In general it was observed in the present investigation that decontamination technique improved weight gain, feed efficiency and dressing percentage.

Weight of internal organs i.e. heart and gizzard was reduced, while liver was enlarged on feeding the contaminated material as compared with the birds fed on detoxified material. These observations are in agreement with the results of Jafery (1996).

Spots on livers were noted in all the birds receiving contaminated feed. This indicated liver damage due to aflatoxicosis while there was normal spotless liver in birds receiving the detoxified feed. Thus it may be concluded that 1.5% ammonia treatment successfully degraded aflatoxin which just passed through the body without adversely affecting general performance of the birds.

Droppings of the birds fed on contaminated feed were found loose while these were normal in the case of birds given detoxified feed. Moreover, water belly cases (due to toxicity) were also noted in the birds fed on contaminated feed as compared to the birds fed on detoxified feed.

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CHEMICAL AND IN VITRO BIOLOGICAL EVALUATION OF BIOMASS PROTEIN FROM *ASPERGILLUS NIGER NRRL 567*

Muhammad Ishfaq Ghori, Muhammad Aslam Malana, Muhammad Yaqub**

Department of Chemistry, Bahauddin Zakariya University, Multan.
*** Department of Chemistry, University of Agriculture, Faisalabad.*

ABSTRACT

An efficient cellulolytic fungus, *Aspergillus niger NRRL-567* was used to prduce biomass protein from cornstover (4%) in optimized medium containing $CaCl_2.2H_2$) (0.02%), $MgSO_4.7H_2O$ (0.03%), urea (0.1%), KH_2PO_4 (0.3%), cane molasses (0.75%) and yeast sludge (1%). Microbial biomass obtained after 96 hours of fermentation with continuous shaking (120 rpm) incubation at pH 3.5 and temperature 30°C was evaluated for its various quality components. It contained 10.5% crude protein, 6.12% true protein, an essential amino acids profile giving higher Threonine and Leucine contents but low quality values for lysine. Biological evaluation shows 75.04% in vitro digestibility. This biomass protein can supplement the feed components of ruminants and other monogastric animals.

INTRODUCTION

With the outset of exploding population, the traditional sources of protein and energy are no longer appreciable. Cereals which were thought to be the major nutritive elements for both the humans and livestocks lack good quality protein and essential amino acids like lysine, arginine and threonine (Saima, 1996), supplementation with animal protein seems to be the most appropriate in this regard, but it results in higher feeding costs (Dasilva *et al.*, 1987).

According to a survey conducted in Punjab (Anonymous, 1987), the consumption of protein from animal origin by human beings was 16.3 gm against the minimum requirement of 24 gms/capita/day. This shows that there is a critical shortage of conventional protein source in our country. It is, therefore, imperative to produce economical good quality proteins from non conventional sources.

In Pakistan, total production of cereals is 19395 thousand tonnes, out of which production of corn grains is 1179 thousand tonnes. Corn stover being 20% of corn grains its production comes to 235.68 thousand tones annually (FAO, 1993). In recent years, the utilization of agriculturally based raw material have received greater attention to increase the substrate range for single cell protein production by microorganism which can play povital role in this regard, due to its high nutritive value and low cost of production (Khan, 1992). The cycling and recycling of agricultural wastes would not only reduce the pollutants but may also serve as a potential source of energy for the production of microbial protein. This high quality protein can be fed to animals and human beings.

Due to high production rate, microorganisms are capable for the conversion of agricultural wastes into biomass protein through fermentation. Process moreover microorganisms have ability to tailor high fiberous material like corn-stover into high quality protein rich in essential amino acids (Gohl, 1991). Due to this reason study was carried out to produce biomass protein from corn stover using *Aspergillus niger* NRRL-567.

MATERIALS AND METHODS

Substrate

Cornstover was obtained from local market of Faisalabad. It was dried in sun shine and then kept in an oven at 100°C to a constant weight. The dried substrate was ground to 40 mm mesh seive and used as a subsrate.

Fermentive Organisms

Aspergillus niger NRRL 567 after certification was procured from NIBGE Faisalabad. It was maintained on substrate agar slant for sporulation medium at pH 4 and temperature 30°C.

Inoculum Preparation

Inoculum medium was prepared in 500 mL Erlenmyer flask. pH was adjusted at 4 using 1 M HCl/1M NaOH. Flask was sterilized by autoclaving at 121°C (15 Lb/In^2) for 15 minutes. Spores of the fungus were transferred aseptically from substrate agar slant and flask was incubated on rota shaker working at 120 rpm for five days at 30°C. The mycelia were removed through sterilized cotton and the filterate containing homogenous suspension of fungal spore was diluted with distilled water. This fungal suspension was used as inoculum to study the quality of biomass protein produced on large scale.

Production on Large Scale

Biomass protein was produced on large scale in 10 litre fermenter under optimum conditions.

Biomass Harvesting and Analysis

After 96 hours (optimum fermentation period) the biomass sample was harvested by steaming (121°C for 5 minutes), filtered and residues collected as protein biomass. The biomass residues were dried and analyzed for crude protein-contents (Hiller 1948) and true protein (Munro and Fleck, 1966).

Chemical and Biological Evaluation

After hydrolyzing, the sample of biomass protein was injected in-to amino acid analyzer to study amino acid contents (Moore & Stein 1958) and essential amino acids profile (mg/g of protein) was compared with reference hens egg protein (Block & Mitchell, 1946). In vitro digestibility of biomass protein was estimated by three enzyme system (Pepsin, chymotrypsin and peptidase) (FAO, 1991). This digestibility was compared with standard sodium caseinate processed under the same conditions.

RESULTS AND DISCUSSIONS

Chemical Evaluation

Essential amino acid profile was compared with that of reference protein for the chemical grading of protein quality as given in Table 1. Biomass protein contains 10.5% crude protein and 6.12% true protein. It was observed that almost all the amino acids were present in the biomass protein.

Table 1: Essential amino acid profile and chemical score of biomass protein produced by *Aspergillus niger* NRRL 567 using hen's egg protein amino acid pattern as reference (Block and Mitchell, 1946)

Amino acids	Hen's egg protein amino acid pattern (mg /g protein)	Biomass amino acids (mg/g protein)	Percentage availablility of amino acids
Isoleucine	54	-	-
Leucine	86	23.00	26.74
Lysine	70	7.02	10.02
Methionine and cystine	57	10.94	19.19
Phenyl alanine and tyrosine	93	12.90	13.87
Threonine	47	24.67	52.48
Tryptophane	17	-	-
Valine	66	20.91	31.68
Chemical score Limiting amino acid	I II		10.02 Lysine Phenylalanine & tyrosine

Comparison of essential amino acid profile with that of reference hens egg protein amino acids pattern showed that sulphur containing amino acids (Cystine and Methionine) were found to be lower in quantity. Table (1) indicated that the biomass was limiting in lysine (I) phenylalanine and tyrosin (II) with a chemical score 10.02 and 13.87 respectively. It was also observed that the biomass was deficient in isoleucine and tryptophan. These results are in accordance with those of (Akhtar *et al.* 1997) who produced SCP (smigle cell protein) from wheat bran (W) and gram bran (G). *Arachmiotus sp.* Produced 15.31% and 10.93% crude protein containing 6.75% and 3.28% true protein. The biomass protein from different sources were compared with FAO standard with respect to amino acids profile. Methionine was the first limiting amino acid and the chemical scores were 56.41 and 28.82% respectively. Second limiting amino acids were lysine and Isoleucine respectively. *Arachinotus sp* cultured on corn stover medium produced microbial biomass containing 22.75% crude protein with all essential amino acids. Lysine and Methionine were found to be the limiting amino acid I and II respectively (Bajwa *et al.*, 1998).

Biological Evaluation

The protein digestion by proteases is an excellent measure of protein quality. The biomass protein obtained from corn stover by *Aspergillus niger* NRRL 567 under optimum conditions proved 75.04% in vitro digestibility as compared to 98.2%

digestibility of standard sodium caseinate. The results are very much closer with those of (Guo and Guo, 1989) who found in vitro protein digestibility by pepsin 83%. (Singh *et al.*, 1991) reported 77% in vitro digestibility of SCP produced from alkali treated corn cob by *Aspergillus niger* AS-101. (Bajwa *et al.*, 1998) produced biomass having 68.29% in vitro digestibility as compared with standard sodium caseinate.

CONCLUSION

The biomass protein prepared under optimum conditions contain 10.5% crude protein and 6.12% ture protein. The essential amino acids are shown in Table (1). In vitro digestibility was found to be 75.04%, favourably comparable with other quality proteins. Thus biomass protein may be used to supplement the feed component of ruminant and other monogastric animals.

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