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Effect of Different Concentrations of Sucrose and Honey on the Physiochemical and Sensory Properties of Strawberry Leather

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Abstract. The aim of the present research work was to study the effect of different levels of sucrose and honey content on strawberry leather stored at room temperature for a total period of 90 days. The sucrose and honey was added at the level of 300:0, 250:50, 200:100, 150:150, 100:200, 50:250 and 0:300, representing each treatment. The prepared strawberry leather were analysed physiochemically for pH, acidity, ascorbic acid, reducing and non-reducing sugar content, sugar acid ration and organoleptically for colour, taste, texture and overall acceptability for a total period of 90 days. Statistical results revealed that treatment and storage interval had a significant ($P< 0.05$) effect on both physiochemical and organoleptic evaluation. Results also revealed that the decrease occurred in moisture content from (18.17-13.53%), pH (3.58 to 3.43), ascorbic acid (34.41 to 25.53), non-reducing sugar (5.09 to 4.85), sugar-acid ratio (71.03-65.37%), and sensory evaluation included colour (8.17 to 5.10), texture (8.31 to 5.69), taste (8.37 to 6.04) and overall acceptability (8.37 to 5.61), while increase was found in total acidity (1.137-1.267%), total soluble solid (80.96 to 83.04 °Brix) and reducing sugar (19.09-19.45%) during storage. The maximum mean values were observed for moisture in SL_5 (16.37), ascorbic acid SL_4 (31.59), pH SL_4 (3.57), titratable acidity SL_4 (1.230), total soluble solid SL_4 (82.80), total solid SL_4 (82.80), reducing sugar SL_4 (26.47), non-reducing sugar SL_1 (6.20), colour SL_4 (7.13), texture SL_4 (7.54), taste SL_4 (7.79), overall acceptability SL_4 (7.5) and sugar-acid ratio SL_6 (70.76). Among all the treatments, T_4 was found to be the best both physiochemically and organoleptically.

Keywords: strawberry fruit, leather, sucrose, honey

Introduction

Among all berries, strawberry is one of the most important berry fruit and different wild species of strawberry are grown all over the world (John, 1994). Strawberry (*Fragaria* spp.) is a herbaceous member of family Rosaceae and with more than six hundred varieties have different taste, texture and sizes (Childer, 1983). According to Food and Agriculture Organization, the world production of strawberries exceeded 4 million tonnes, with United States being major contributor with 28% production (FAO, 2009).

In Pakistan, strawberries are grown in Punjab and Khyber Pakhtunkhwa (Amin, 1996). The favourable varieties grown are Tuft, Mission, Corona, Sweet Charlie, Super Faction and Festival. During 2009-2010 the production of strawberry was 274 tonnes on the area of about 193 acres in Pakistan (Aslam and Rasool, 2012). The production of strawberries has increased due to its nutritional values.

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From the nutritional point of view, one cup of strawberry contain 10.5 g carbohydrate, 1.6 g fibre, 0.6 g fats, 84.5 mg vitamin C, 1 g protein, 26.4 g folic acid, 0.1 g thiamine, 0.4 g niacin, 0.6 g iron, 0.1 g riboflavin, 21 g calcium, 2 g sodium, 16 g magnesium and 45 Kcal. The level of vitamin C in strawberry is more the in oranges and strawberry also gives rare supply of vitamin K, B5, B6, manganese, potassium, copper and omega 3 fatty acids (USDA, 2011; 1998). The main organic acids which contribute in aroma are malic and citric acid. The non-nutrients which are present in strawberry fruit are phenol and flavonoids as compared to other berries (Hakkinen and Torronen, 2000). Strawberry fruit possess different attributes such as juicy, tiny, flavourful, nourishing, syruping flavour, diuretic, remineralizing and tonic (Johnson and Peterson, 1974).

Strawberry fruit is normally used in fresh form and can be preserved as jellies, jams, and squashes that can be utilized throught out the year (Galleta and Bringhurst, 1995). The shelf life of the product can be increased by

using different chemical preservatives. Among them most important preservatives are sodium benzoate, potassium sorbate, potassium bisulphate, ascorbic acid, and sulphur dioxide (Krebs *et al*., 1983).

Fruit leathers are dehydrated fruit products which are eaten as snacks or desserts. They are flexible sheets that have a concentrated fruit flavour and nutritional aspects. Most fruit leathers are prepared by mixing fruit puree and other additives like sugar, pectin, acid, glucose syrup, colour and potassium metabisulphite then dehydrating them under specific conditions (Diamante *et al*., 2014).

Sweetener add the taste of food items and in earlier times foods were sweetened with honey and molasses as desired by the consumer. There are two types of sweeteners used in foods i.e., nutritive and non nutritive sweeteners (Mitchell and Pearson, 1991). Sucrose provides sweet taste and flavour to the product. It also provides freshness and contribute to the product quality (Ayub *et al*., 2005). The addition of simplest sugars to jams and jellies holds growth of microorganism and later on spoilage. Sucrose has a long lasting connection for water reduction, and to reduce moisture loss in baked products therefore, increased the life of these products. The addition of sugars to canned vegetables and fruits helps in maintaining soundness and reduces oxidation, also shield changes in texture, flavour and colour resulting from the breakdown of different substances (Clarke, 1997).

In last decade the popularity of fruit leather increased significantly due to its richness in Vitamin C (Naz, 2012). Nonetheless, the future of strawberry production and processing is helpful in boosting the economy of developing agricultural countries, as it fetches high economic returns. The overall aim of this study was to prepare strawberry bar with addition of sucrose and honey in different levels and to study their effect on strawberry bar quality. Effect of honey addition on storage duration of these leather will be studied for commercial transportation of these products to long distance market.

Materials and Methods

Selection of fruits. Strawberry fruit at optimum maturity was purchased from the local market of Peshawar and was brought to the Laboratory of Food Science and Technology section, Agriculture Research Institute Tarnab, Peshawar for preparation of strawberry leather.

Pre-treatment of fruits. Strawberry fruit were carefully sorted to discard diseased, damaged, bruised and immature fruits. The sorted fruits were thoroughly washed with tap water to remove dirt and clay. Washing was repeated for two to three times for complete washing of strawberry fruit. After washing the strawberry fruits were crushed with the help of pulper machine (Model.35027 Rochdale, England) to prepare the pulp.

Preparation of strawberry leather. Strawberry leather was an intermediate food (IMF) product prepared by blending all the ingredients in appropriate amount (Table 1). The homogenised material was first cooked and then spread on stainless steel tray covered with aluminium foil and were kept in dehydrator (Mitchell dryer, model 7230/60) at a temperature of 50 °C for 24 h. After drying, the leather samples were cooled and then packed in polyethylene bags to be studied for physiochemical characteristics and sensory attributes for three months at room storage with 15 days of interval (Khan *et al*., 2014).

Physiochemical analysis. All the samples were analysed physiochemically for pH, total soluble solids, % acidity, ascorbic acid, reducing sugar and non reducing sugar by method of AOAC (2012).

Sensory evalution. The samples of strawberry leather were sensory evaluated for colour, texture, flavour and overall acceptability by 10 trained judge's panel. Organoleptic study was carried out at each 15 days interval during 3 months storage. The evaluation was conceded out by using 9 points hedonic scale of Larmond (1977). The results are of scoring rate 1-9 awarded by panel of judges.

Statistical analysis. All the data concerning treatments and storage interval were statistically analysed by means of Completely Randomized Design (CRD) 2 Factorial and means were separated by applying least significant difference (LSD) test at 5% possibility level as defined by Steel and Torrie (1997).

Table 1. Proposed plan of study for research

Sample	Strawberry pulp (g)	Sucrose (g)	Honey (g)	Pectin (%)	Sodium benzoate (g)
SL_0	700	300	_	0.5	2
SL_1	700	250	50	0.5	2
SL_2	700	200	100	0.5	2
SL_3	700	150	150	0.5	2
SL_4	700	100	200	0.5	2
SL_5	700	50	250	0.5	2
SL_6	700	_	300	0.5	2

Results and Discussion

Moisture (%). Both treatment and storage have significant ($p<0.05$) influence on the moisture content of strawberry leather (Table 2). This is revealed from treatment means that higher moisture content was observed in SL_5 (16.37%) and SL_0 contained the lower moisture content (14.26%). During storage the maximum moisture content decreased as observed in SL_0 (34.41%), while minimum moisture content decreased was found in SL_4 (20.26%) during 90 days of storage. However, microbial growth and activity was inhibited below this moisture content range with exception of Europhilic mold and osmophilic yeast (Jay *et al*., 2005; Raab and Oehler, 1976). On the other hand, Chavan and Shaik (2015) reported that the moisture content of guava leather decreased during storage from 15.85 to 14.67%. They further reported that due to ambient temperature moisture loss was higher in guava leather (Shakoor *et al*., 2015). Hence, it was concluded that guava leather moisture content was well below the range where no microbial growth occurs and due to room temperature moisture slightly reduces and ensure safety of the product during storage.

Ascorbic acid. The ascorbic acid content of strawberry leather was significantly ($p<0.05$) affected by storage period and treatment (Table 3). Mean data revealed that highest ascorbic acid content was observed in SL_4 (31.59%) and SL_0 contained the lowest ascorbic acid content (28.73%). However, data presented here showed that sample SL_1 has more ascorbic acid content (30.36%) than rest of the strawberry leather samples. In most liable nutrients Vitamin C is very important because of its degradation property that is used as an indicator of quality. The losses of ascorbic acid may be attributable to oxidation of ascorbic acid to dehydroascorbic acid followed by hydrolysis of the final 2,3-diketogluconic acid, which undergoes polymerization to other nutritionally inactive products (Shakoor *et al*., 2015; Dewanto *et al*., 2002). Similar results were found by Chavan and Shaik (2015). They reported that there was a decrease

Table 2. Effect of different levels of sucrose and honey on moisture content (%) of strawberry leather

Treatment	storage interval							% decrease	Mean
	0	15	30	45	60	75	90		
SL_0	17.0	16.4	15.25	14.36	13.42	12.22	11.15	34.41	14.26c
SL_1	18.12	17.7	15.83	14.18	13.09	12.88	12.27	32.28	14.87b
SL_2	18.53	18.0	17.31	16.45	15.03	14.86	14.23	23.21	16.34a
SL_3	18.40	17.4	16.83	15.38	15	14.99	14.56	20.87	16.08a
SL_4	18.31	17.79	16.21	15.68	15.99	14.88	14.6	20.26	16.21a
SL_5	18.55	18.0	17.73	16.01	15.42	14.49	14.4	22.37	16.37a
SL_6	18.26	17.57	17.06	16.36	15.85	14	13.5	26.07	16.09c
Mean	18.17a	17.55b	16.60c	15.49d	14.83e	14.05f	13.53f		

Values with different letters are significantly ($p < 0.05$) different from each other.

Table 3. Effect of different levels of sucrose and honey on ascorbic acid (mg/100 g) of strawberry leather

Treatment	storage interval							% decrease	Mean
	0	15	30	45	60	75	90		
SL_0	33.1	31.33	30.43	29.41	27.87	25.78	23.19	29.94	28.73c
SL_1	35.08	33.77	31.67	29.98	27.65	26.13	24.43	30.36	29.82b
SL_2	34.56	33.12	31.13	29.89	28.75	26.1	25.34	26.68	29.84a
SL_3	33.21	31.12	30.08	29.91	28.61	26.24	25.91	21.98	29.30a
SL_4	35.22	34.33	32.55	31.87	30.76	28.98	27.41	22.17	31.59a
SL_5	35.23	34.4	32.82	30.77	28.83	27.67	26.91	23.62	30.95a
SL_6	34.44	32.13	30.12	28.76	27.88	26.87	25.55	25.81	29.39c
Mean	34.41a	32.89b	31.26c	30.08d	28.62e	26.82f	25.53g		

Values with different letters are significantly ($p < 0.05$) different from each other.

in the ascorbic acid of guava leather samples during 3 months of storage. Ascorbic acid decreased in the range of 99.36 to 73.09 mg/100 g at ambient temperature. It was to be noted that ascorbic acid of the samples was at lower level when stored at ambient temperature. Ascorbic acid content may be decreased due to oxidation of ascorbic acid at high storage temperature or due to light.

pH. Statistical analysis showed that the pH of strawberry leather samples was significantly ($p<0.05$) effected during storage (Table 4). Mean data showed that higher pH was observed in SL_4 (3.57) and SL_6 contained the lower pH content (3.40). During storage the maximum pH (3.58) was observed at day one, whereas minimum pH (3.43) was recorded after 90 days of storage. However, data presented here showed that sample SL_1 has lost more pH (4.40) than rest of the strawberry leather samples. Similar result was found by Offia-Olua *et al*. (2015). They reported that pH of the guava leathers was decreased when packed in different packing materials during storage period. Shakoor *et al*. (2015) also revealed decrease in pH value of guava leather with storage. Variation in pH is directly related to change in acidity of samples. Comparable increase in acidity and decrease in pH were also reported by Jain and Nema (2007).

Total acidity (%). Total acidity of strawberry leather was affected significantly ($p<0.05$) during storage (Table 5). This is revealed from the mean values of data that higher total acidity was observed in SL_4 (1.230%) and SL_6 contained the lower total acidity (1.171%). During storage the maximum total acidity (1.267%) was observed at day one, whereas minimum total acidity (1.137%) was recorded after 90 days of storage. However, data presented here showed that sample SL_0 revealed maximum increase in total acidity (11.72%) than rest of the strawberry leather samples. Similar results were found by Chavan and Shaik (2015) who reported increase in the acidity of the guava leather samples as a whole. At ambient temperature it increased from 0.476 to 0.518% while in refrigerated temperature it increased from 0.476 to 0.506% during three months of storage

Table 4. Effect of different levels of sucrose and honey on pH of strawberry leather

Treatment	Storage interval							% Decrease	Mean
	0	15	30	45	60	75	90		
SL_0	3.54	3.52	3.49	3.47	3.45	3.43	3.41	3.67	3.47e
SL_1	3.64	3.61	3.59	3.56	3.53	3.51	3.48	4.40	3.56b
SL_2	3.56	3.54	3.51	3.49	3.47	3.44	3.41	4.21	3.49d
SL_3	3.57	3.55	3.52	3.51	3.48	3.46	3.43	3.92	3.50c
SL_4	3.65	3.64	3.59	3.56	3.54	3.52	3.5	4.11	3.57a
SL_5	3.55	3.51	3.49	3.48	3.46	3.44	3.41	3.94	3.48e
SL_6	3.48	3.45	3.42	3.39	3.38	3.36	3.34	4.02	3.40f
Mean	3.58a	3.55b	3.52c	3.50d	3.48e	3.46f	3.43g		

Values with different letters are significantly ($p < 0.05$) different from each other.

Table 5. Effect of different levels of sucrose and honey on total acidity (%) of strawberry leather

Treatment	storage interval							% Increase	Mean
	0	15	30	45	60	75	90		
SL_0	1.13	1.15	1.18	1.2	1.23	1.26	1.28	11.72	1.204cd
SL_1	1.15	1.17	1.19	1.21	1.23	1.25	1.27	9.45	1.210bc
SL_2	1.14	1.17	1.2	1.22	1.24	1.25	1.28	10.94	1.214b
SL_3	1.14	1.16	1.18	1.2	1.22	1.24	1.26	9.52	1.200d
SL_4	1.15	1.18	1.21	1.24	1.26	1.28	1.29	10.85	1.230a
SL_5	1.13	1.15	1.17	1.19	1.21	1.23	1.25	9.60	1.190e
SL_6	1.12	1.12	1.14	1.17	1.19	1.22	1.24	9.68	1.171f
Mean	1.137g	1.157f	1.181e	1.204d	1.226c	1.247b	1.267a		

Values with different letters are significantly ($p < 0.05$) different from each other.

period. The increase in acidity might be due to development of acidic substances by the degradation of pectic bodies or breakdown and also attributed to hydrolysis of polysaccharides (Shakoor *et al*., 2015).

Total soluble solid (TSS). Total soluble solid content of strawberry leather was affected significantly ($p<0.05$) during storage (Table 6). This is revealed from the mean data that higher TSS content was observed in SL_4 (82.80) and SL_0 contained the lower TSS content (79.54). During storage the minimum TSS content (80.96) was observed at day one, whereas maximum TSS content (83.04) was recorded after 90 days of storage. However, data presented here showed that increased higher percent was observed in treatment SL_0 (2.97%) than rest of the strawberry leather samples. Similar result was reported by Chavan and Shaik (2015) that increase in TSS of the guava leather might be due to decrease in the moisture content. It may also increase in the TSS content. Shakoor *et al*. (2015) observed increase in total soluble content of guava leather due to the conversion of non-soluble polysaccharides to soluble di and mono-saccharides.

Reducing sugar. The reducing sugar content of strawberry leather was affected significantly ($p<0.05$) during storage (Table 7). The statistical results showed that higher reducing sugar content was observed in SL_4 (26.47%) and SL_0 contained lower reducing content (9.54%). During storage the maximum reducing sugar content (19.45%) was observed at 90 days of storage, whereas minimum reducing sugar content (19.09%) was recorded at day first. However, data presented here showed that sample SL_0 has lost more reducing content (5.77%) than the rest of the strawberry leather samples. Chavan and Shaik (2015) reported that reducing sugar content of the guava leather increased with the progress of the storage period. During storage period the guava leather samples increase the reducing sugar, it might be due to the inversion of added sugar. At ambient temperature range of the reducing sugar content, means value increased from 13.88 to 16.35% while at refrigerated temperature it ranges from 13.88 to 16.02% during three months of storage. Increase in reducing sugar of ambient temperature was more than the refrigerated temperature.

Table 6. Effect of different levels of sucrose and honey on total soluble solid of strawberry leather

Treatment	storage interval							% Increase	Mean
	0	15	30	45	60	75	90		
SL_0	78.3	78.8	79.3	79.6	79.9	80.2	80.7	2.97	79.54e
SL_1	81.4	81.9	82.3	82.5	82.8	83.1	83.5	2.51	82.50b
SL_2	81.5	81.8	82.1	82.4	82.7	83	83.5	2.40	82.43b
SL_3	81.8	82.2	82.5	82.8	83.1	83.4	83.7	2.27	82.79a
SL_4	81.8	82.2	82.5	82.8	83.1	83.4	83.8	2.39	82.80a
SL_5	80.5	80.7	81	81.3	81.6	81.9	82.3	2.19	81.33c
SL_6	80.1	80.4	80.7	81.1	81.4	81.7	82.2	2.55	81.09d
Mean	80.96g	81.38f	81.74e	82.02d	82.32c	82.62b	83.04a		

Values with different letters are significantly ($p < 0.05$) different from each other.

Table 7. Effect of different levels of sucrose and honey on reducing sugar of strawberry leather

Treatment	storage interval							% decrease	Mean
	0	15	30	45	60	75	90		
SL_0	9.31	9.34	9.4	9.51	9.63	9.72	9.88	5.77	9.54g
SL_1	15.57	15.59	15.62	15.66	15.69	15.75	15.85	1.77	15.68f
SL_2	17.76	17.79	17.82	17.88	17.96	18.03	18.09	1.82	17.90e
SL_3	19.36	19.39	19.45	19.51	19.55	19.59	19.64	1.43	19.50d
SL_4	26.26	26.36	26.41	26.47	26.52	26.59	26.67	1.54	26.47a
SL_5	23.38	23.42	23.49	23.56	23.61	23.67	23.73	1.47	23.55b
SL_6	21.99	22.05	22.12	22.16	22.19	22.24	22.29	1.35	22.15c
Mean	19.09f	19.13f	19.19e	19.25d	19.31c	19.37b	19.45a		

Values with different letters are significantly ($p < 0.05$) different from each other.

Non reducing sugar. The non-reducing sugar content of strawberry leather was affected significantly ($p<0.05$) during storage (Table 8). Mean data revealed that higher non reducing sugar content was observed in SL_4 (6.20%) and SL_6 contained the lower non reducing sugar content (4.21%). During storage the maximum non reducing sugar content (5.09%) was observed at day one, whereas minimum non reducing content (4.85%) was recorded after 90 days of storage. However, data presented here showed that sample SL_6 has lost more non reducing sugar content (5.10%) than rest of the strawberry leather samples. Comparatively greater decrease was noted in SL_0 due to the use of pure sucrose alone. The changes occur on the sample containing sugars in the form of honey (reducing sugar) and sucrose (non-reducing sugar). The reason for reduction of the non-reducing sugar was due to the inversion of sucrose in the presence of acid and temperature. Similarly, Hussain *et al*. (2004) also concluded that the degradation in non-reducing sugar range from (8.82 to 7.3). The same results were reported by Sharma *et al*. (2013), mango leather by Rao and Roy (1980), apricot-soy toffees and papaya leather by Thakur *et al*. (2007) and Phimpharian *et al*. (2011), guava leather by Duangmal and Khachonsakmetee (2009) and sapota papaya bar by Sreemathi *et al*. (2008).

Sensory evaluation. ***Colour.*** The colour of strawberry leather was affected significantly ($p<0.05$) during storage (Table 9). Mean values of data showed that higher colour score was observed in SL_4 (7.13) and SL_0 assumed the lower colour score (5.07). During storage the maximum colour (8.17) was observed at day one, whereas minimum colour (5.10) was recorded after 90 days of storage. However, data presented here showed that sample SL_0 has lost more colour (47.06%) than rest of the strawberry leather samples. Similar result was found by Chavan and Shaik (2015) who reported that there was a gradual decrease in colour range from 8.35 to 7.45 at ambient temperature, whereas in refrigerated temperature 8.35 to 7.80 was noted at 3 months of storage. The score 8.35 was noted in guava leather sample stored at refrigerated condition. Related trend for colour and appearance of guava leathers was noted at ambient condition but values were at lower level then the refri-gerated storage. The deterioration of the colour was more in guava leather at ambient condition when it was stored. The deterioration of colour may be due to the degradation of pigments that might have occurred ambient temperature. A decrease in colour score with storage interval might be due to Millard reaction that occur during drying at high temperature (Shakoor *et al*., 2015).

Texture. The texture of strawberry leather was affected significantly ($p<0.05$) during storage (Table 10). The mean data showed that higher texture score was observed in SL_4 (7.54) and SL_0 contained the lower texture score (5.2). Statistical results revealed that mean value of the texture score decreased from (8.31 to 5.69) during 90 days of storage. However, data presented here showed that higher percent decrease in texture was found in sample SL_0 (51.39%) than the rest of the strawberry leather samples. Similar result was found by Chavan and Shaik (2015) who reported that there was a gradual decrease in the texture of guava leather. At ambient temperature the decrease in texture score ranged from 8.27 to 7.56 while in refrigerated temperature it decreased in the range of 8.27 to 7.80. Score of the texture decreased significantly at ambient temperature during storage period than stored at refrigerated temperature. During

Table 8. Effect of different levels of sucrose and honey on non-reducing sugar of strawberry leather

Treatment	storage interval							% decrease	Mean
	0	15	30	45	60	75	90		
SL_0	4.75	4.72	4.69	4.66	4.63	4.6	4.57	3.79	4.66e
SL_1	5.36	5.31	5.25	5.21	5.17	5.14	5.1	4.85	5.22c
SL_2	5.51	5.46	5.41	5.37	5.32	5.27	5.24	4.90	5.37b
SL_3	4.88	4.85	4.81	4.76	4.72	4.68	4.65	4.71	4.76d
SL_4	6.36	6.29	6.24	6.19	6.15	6.11	6.04	5.03	6.20a
SL_5	4.44	4.39	4.37	4.35	4.32	4.28	4.23	4.73	4.34f
SL_6	4.31	4.28	4.24	4.21	4.17	4.14	4.09	5.10	4.21g
Mean	5.09a	5.04b	5.00c	4.96d	4.93e	4.89f	4.85g		

Values with different letters are significantly ($p < 0.05$) different from each other.

storage period a gradual decrease in the texture score is due to the hardening effect resulting from the loss of moisture content (Shakoor *et al*., 2015).

***Taste*.** The taste of strawberry leather was affected significantly ($p<0.05$) during storage (Table 11). The mean data showed that higher taste score was observed in SL_4 (7.79) and SL_0 assumed the lower taste score (5.71). The mean value of the taste score decreased from (8.37 to 6.04) after 90 days of storage. However, data presented here showed that sample SL_0 has lower score (48.68%) than rest of the strawberry leather samples. Similar result was found by Chavan and Shaik (2015), who reported that there was a gradual decrease in the taste score of guava leather from 8.30 to 7.49 at ambient temperature and from 8.30 to 7.98 at refrigerated temperature was noted. In guava leather the taste deterioration was more at ambient condition than that of refrigerated temperature. This might be due to the consistency of guava leather or proper blending of sugar and acidity. For guava leather both storage condition gave acceptable taste score. It is reported that the taste score decreased during storage with respect to storage condition and storage period in sweet potato leather (Collins and Hutsell, 1987) and in guava leather (Shakoor *et al*., 2015).

Overall acceptability. The overall acceptability of strawberry leather was affected significantly ($p<0.05$) during storage (Table 12). This is revealed from the mean data that higher overall acceptability score was observed in SL_4 (7.5) and SL_0 contained the lower overall acceptability (5.4). Mean data in the table showed that the maximum overall acceptability (8.37) was observed at day one, whereas minimum overall acceptability (5.61) was recorded after 90 days of storage. However, data presented here showed that sample SL_0 has more overall acceptability loss (48.61%) than rest of the strawberry leather samples. Generally all sensory characteristics are related to overall acceptability and similar result was found by Chavan and Shaik (2015). They reported that there was a gradual decrease in the overall acceptability score from 8.38 to 7.53 at ambient temperature and from 8.38 to 7.78 at refrigerated temperature. It was noted

Table 9. Effect of different levels of sucrose and honey on colour of strawberry leather

Treatment	storage interval							% decrease	Mean
	0	15	30	45	60	75	90		
SL_0	6.8	6	5.4	5	4.6	4.1	3.6	47.06	5.07f
SL_1	8.4	7.4	6.9	6.5	6	5.6	4.9	41.67	6.53de
SL_2	8.4	7.3	6.9	6.4	6	5.5	5.1	39.29	6.51e
SL_3	8.4	7.7	7.2	6.8	6.3	6	5.7	32.14	6.87b
SL_4	8.4	7.8	7.4	7	6.7	6.5	6.1	27.38	7.13a
SL_5	8.4	7.5	7.1	6.7	6.2	5.7	5.2	38.1	6.69cd
SL_6	8.4	7.6	7.2	6.7	6.2	5.7	5.1	39.29	6.7c
Mean	8.17a	7.33b	6.87c	6.44d	6.00e	5.59f	5.10g		

Values with different letters are significantly ($p < 0.05$) different from each other.

Table 10. Effect of different levels of sucrose and honey on texture of strawberry leather

Treatment	storage interval							% decrease	Mean
	0	15	30	45	60	75	90		
SL_0	7.2	6.4	5.7	5.1	4.6	3.9	3.5	51.39	5.2f
SL_1	8.5	7.6	7.2	6.8	6.3	5.9	5.7	32.94	6.86de
SL_2	8.5	7.3	6.9	6.6	6.1	5.7	5.4	36.47	6.64c
SL_3	8.5	8	7.7	7.3	7	6.7	6.4	24.71	7.37ab
SL_4	8.5	8.1	7.8	7.6	7.2	6.9	6.7	21.18	7.54a
SL_5	8.5	7.8	7.3	6.9	6.6	6.3	6.1	28.24	7.07cd
SL_6	8.5	7.9	7.4	7	6.7	6.4	6	29.41	7.13bc
Mean	8.31a	7.59b	7.14c	6.76d	6.36e	5.97f	5.69g		

Values with different letters are significantly ($p < 0.05$) different from each other.

that decrease in the ambient temperature was faster than at refrigerated temperature in overall acceptability. Refrigerated temperature depicted highest overall acceptability score than the ambient temperature. It may be due to quicker deterioration in relation with taste, texture flavour and colour at higher temperature during ambient condition. It is reported that decrease in the score of the overall acceptability during storage is retaled to the storage condition and period. It is reported that fruits and vegetable acceptability is subjected to their odour (Karmas and Harris, 1998). Similar results were found by Iman *et al*. (2011) during physiochemical analysis and quality evaluation of intermediate moisture in apple slices.

Table 11. Effect of different levels of sucrose and honey on taste of strawberry leather

Treatment	storage interval							% decrease	Mean
	0	15	30	45	60	75	90		
SL_0	7.6	6.9	6.4	5.7	5	4.5	3.9	48.68	5.71d
SL_1	8.5	7.9	7.5	7.2	6.9	6.7	6.3	25.88	7.29b
SL_2	8.5	8.1	7.8	7.5	7.1	6.5	6.1	28.24	7.37b
SL_3	8.5	8	7.6	7.3	7	6.8	6.6	22.35	7.4b
SL_4	8.5	8.3	8	7.8	7.6	7.3	7	17.65	7.79a
SL_5	8.5	8.1	7.7	7.4	7.1	6.8	6.5	23.53	7.44b
SL_6	8.5	7.7	7.3	6.9	6.5	6.1	5.9	30.59	6.99c
Mean	8.37a	7.86b	7.47c	7.11d	6.74e	6.39f	6.04g		

Values with different letters are significantly ($p < 0.05$) different from each other.

Table 12. Effect different levels of sucrose and honey on overall acceptability of strawberry leather

Treatment	storage interval							% decrease	Mean
	0	15	30	45	60	75	90		
SL_0	7.2	6.4	5.8	5.3	4.7	4.7	3.7	48.61	5.4e
SL_1	8.6	7.6	7	6.7	6.2	5.8	5.4	37.21	6.76d
SL_2	8.6	7.6	7.3	6.9	6.5	6	5.6	34.88	6.93cd
SL_3	8.6	7.9	7.5	7.1	6.8	6.5	6.2	27.91	7.23b
SL_4	8.5	8.1	7.7	7.5	7.2	6.9	6.6	22.35	7.5a
SL_5	8.5	7.5	7.4	7	6.7	6.3	5.9	30.59	7.04bc
SL_6	8.6	7.7	7.	6.9	6.6	6.2	5.9	31.4	7.03bc
Mean	8.37a	7.54b	7.14c	6.77d	6.39e	6.06f	5.61g		

Values with different letters are significantly ($p < 0.05$) different from each.

Table 13. Effect of different levels of sucrose and honey on sugar acid ratio of strawberry leather

Treatment	storage interval							% decrease	Mean
	0	15	30	45	60	75	90		
SL_0	69.29	68.52	67.20	66.33	64.96	63.65	63.05	9.01	66.14e
SL_1	70.78	69.41	68.02	66.53	65.71	64.92	64.73	8.55	67.16d
SL_2	71.49	69.91	68.42	67.54	66.69	66.40	65.23	8.75	67.96c
SL_3	70.26	69.31	68.39	67.58	66.72	65.89	65.24	7.15	67.63c
SL_4	71.13	70.26	69.33	68.43	67.56	66.72	65.91	7.34	68.48b
SL_5	71.24	70.17	69.23	68.32	67.44	66.59	65.84	7.58	68.40b
SL_6	73.04	73.39	72.37	70.77	69.83	68.36	67.58	7.47	70.76a
Mean	71.03a	70.14b	68.99c	67.93d	66.99e	66.08f	65.37g		

Values with different letters are significantly ($p < 0.05$) different from each other.

Sugar acid ratio. The sugar acid ratio of strawberry leather was affected significantly ($p<0.05$) during storage (Table 13). This is revealed from the mean data that higher sugar acid ratio was observed in SL_4 (70.76) and SL_0 contained the lower sugar acid ratio (66.14%). Maximum sugar acid ratio (71.03) was observed at the initial day, whereas minimum sugar acid ratio (65.37) was recorded after 90 days of storage. However, data presented here showed that sample SL_0 has lost more sugar acid ratio (9.01%) than rest of the strawberry leather samples. These results are found in agreement with the results of Chyau *et al*. (1992).They found that during the ripening of guava fruit, the contents of total pectin, total sugar, reducing sugar and acidity dropped obviously from the mature to the ripe stage but the sugar acid ratio increased inversely.

Conclusion

Drying of strawberry fruit into intermediate moisture food i.e., leather will help to reduce the post-harvest loss due to the high perishability of strawberry fruit as it contain higher moisture content. The physiochemical and organoleptic evaluation showed that treatment SL_4 (sucrose 100: 200 honey) was found adequate among all other treatments. Results also revealed that as the concentration of honey increases from 0 to 300 g/kg, the structure characteristic of the strawberry leather become more sticky and shows low textural property. The results also indicated the stability of strawberry leather up to 90 days under ambient temperature.

It is recommended to study the effect of different packaging materials on the overall quality of strawberry leather. Further, the effect of different gums on keeping quality of strawberry leather, needs to be studied.

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Development and Quality Evaluation of Banana Mushroom Blended Jam

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Abstract. The effect of various blends of banana (B) and mushroom (M) as well as storage time on the overall quality characteristics of jam at ambient temperature were studied for three months of storage period. All the treatments were analysed for physicochemical properties (total soluble solids (°Brix), pH, reducing sugars (%), non-reducing sugars (%), ascorbic acid (mg/100 g) and percent acidity) and sensory properties (taste, colour, texture and overall acceptability). Significant ($P < 0.05$) increase were examined in total soluble solids (67.94-69.78 °brix), percent acidity (0.71-0.87%) and reducing sugars (18.17-29.33%) during the storage period. While, significant ($P < 0.05$) reduction in pH (3.45 to 3.26), non reducing sugars (44.90-30.83%), ascorbic acid (7.81 to 5.52 mg/100 g), colour (7.34 to 4.84), taste (7.27 to 4.51), texture (7.06 to 4.60) and overall acceptability (7.17 to 4.69) were observed. Physicochemical and sensory analyses showed that jam prepared from BM_6 (400 g banana + 600 g mushroom + 1kg sugar + 2 g citric acid) was of good quality attributes among the treatments.

Keywords: banana, mushroom, jam, storage time, physicochemical properties, sensory properties

Introduction

Banana is a seedless fruit, which is valued for its sweet taste, aroma, sticky texture and high vitamin contents. It is native to tropical Southeast Asia (Frison and Sharrock, 1999). Banana is the second major fruit produced after citrus, which comprises of about 16% of the total fruit produced in the world (FAO, 2009). It is very rich source of carbohydrates, minerals (potassium and calcium) as well as vitamins (A, B_1, B_2 and C) and provides significant amount of energy (100 Cal/ 100g) to the body. It is deficient in protein, so can be fortified with other protein sources to develop a new product (Viana *et al*., 2014; Mohapatra *et al*., 2010; Yousaf *et al*., 2006). It contains antioxidants such as dopamine and also has citric acid, malic acid and ascorbic acid that enhance the flavour when mixed with fruit juices and other products, by providing a synergistic effect (Mohapatra *et al*., 2010).

Applications of various processing and preservation techniques have significantly improved the value of the fruit and make it available to the consumers even in the off season (Emaga *et al*., 2007). The carbohydrate of banana consists of resistant starches and non-starch polysaccharides, which have low digestibility or glycemic index values (Lehmann and Robin, 2007). It contain pectin that has the ability to form gel, hence utilized in the development of jams, marmalades and jellies, as well as used as a thickener, emulsifier, texturizer and sugar/fat replacer (Prasanna *et al*., 2007). The choices in the food industrial sector are diversified by substitution or fortification, thus developing new products with enhanced nutritional and functional properties. In addition, they must be affordable, practical, attractive and shelf stable (Leistner, 2011).

The world population is suffering from the shortage of food and Pakistan is also not exempted from it. More than 50% of the diet of the world population is deficient in protein. Due to the high protein content of mushroom, it can be supplemented to bridge the protein malnutrition gap (Wani *et al*., 2010; Ahmad *et al*., 2003). Mushrooms are the simple form of life known as fungus. It cannot produce its own food and depends on other living organisms as well as dead plants and organic matters; hence, it is commercially grown on agricultural wastes (Chang and Miles, 1991). It is considered as the most important food product for its significant role in nutrition and disease control. Mushroom has tremendous scope for applications in the food industry (Adeniji *et al*., 2007).

Mushroom is one of the world greatest untapped resources of nutrition in regards to its palatability and medicinal value for the future (Bahl, 1983). It has been recognized effective against cancer, hypercholesterolemia conditions, asthma, stress, hypertension, insomnia,

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allergies, infections and diabetes (Wang *et al*., 2001; Bahl, 1983). Recently, mushroom is gaining significant importance as a vegetable meat. It is delicate, nutritious and delicious and also used as a flavour enhancer in other foods. Other than protein, it is also a very good source of folic acid, niacin, biotin, B complex, A, C and D as well as mineral i.e., potassium, phosphorus, calcium, zinc, iron, sodium and magnesium. The fat content of mushroom normally consists of linoleic acid, containing little starches and has no cholesterol; hence considered ideal for hypertensive and diabetic patients (Ahmad *et al*., 2003).

Jam is a traditional food item, commonly used as desserts, cake toppings and bread spreads. It is an intermediate moisture food, which is prepared by using fruit pulp, sugar, acid, pectin and other ingredients hence, has a very sweet taste (Baker *et al*., 2005). Mixed jams associate the characteristics of two or more fruits, allowing the achievement of a product with higher nutritional value and pleasant sensory properties, thereby creating the possibility of achieving a larger space in the consumer market (Kvikliene *et al*., 2006; Wicklund *et al*., 2005). Various ingredients give new flavours and have enhanced the storage life, which depends on high sugar content (68-72%) combined with the acidic nature of the fruit that prevent microbial spoilage. The quality characteristics of jam depends upon the raw materials, processing conditions, recipe selections, preserving methods and storage conditions (Redalen and Haffner, 2002).

As banana is deficient in protein, so has been blended with a protein rich source i.e., mushroom. This study was undertaken with the objective to develop a value added jam having health beneficial aspects, by using various blends of banana and mushrooms, as well as to study their physicochemical and sensory properties. In addition, it provides opportunity to combat the post-harvest losses of banana and mushroom, as both of them are highly perishable, thus assist in the betterment of the farmers' economy.

Materials and Methods

The research work was conducted in the laboratory of Food Science and Technology, Agriculture Research Institute (ARI) Tarnab, Peshawar. Optimally ripe banana and Oyster mushroom (*Pleurotus ostreatus*) were procured from the local market of Tarnab.

Banana mushroom mixed jam preparation. Banana and mushroom were thoroughly washed by using tap water to reduce plant soil and debris load. Clean and undamaged samples with no symptoms of visible discolouration were selected and cut into slices. The slices were dipped in warm water (80 °C) for 2 min containing citric acid to reduce microbial load and to avoid oxidation. Materials were put into the pulping machine to get pulp. The pulp was mixed in six different ratios by using the procedure as described by Awan and Rehman (1999) for jam preparation. The various blending formulations are presented in Table 1. All the batches were subjected to cooking at 104 °C for preparation of jam to reasonable TSS of 68 to 70 °Brix.

Packaging and storage of banana and mushroom blended jam. Thereafter, the hot jam samples were filled into pre-sterilised (autoclaved at 121 °C for 15 min) glass bottles, which were closed air-tight and stored at room temperature (31 °C). The jam samples were stored for 90 days and examined after 15 days interval for physicochemical and sensory properties.

Physicochemical analysis. Physicochemical properties such as total soluble solids, pH, acidity, reducing sugar, and non-reducing sugar were analyzed by the standard method of AOAC (2012).

Sensory analysis. The jam samples were examined for sensory attributes such as colour, taste, texture and overall acceptability. The analysis was performed by 10 judges using 9 point hedonic as suggested by Larmond (1977).

Statistical analysis. The data was analysed by using CRD two factorial suggested by Gomez and Gomez (1984). The mean values were separated by applying LSD test at 0.05% significant level as described by Steel and Torrie (1997).

Table 1. Blending formulation of banana and mushroom blended jam

Treatments	Banana	Mushroom	Sugar	Citric acid
		(g)		
BM_0	1000	Nil	1000	Nil
BM_1	800	200	1000	Nil
BM_2	800	200	1000	2
BM_3	600	400	1000	Nil
BM_4	600	400	1000	2
BM_5	400	600	1000	Nil
BM_6	400	600	1000	2

Results and Discussion

Physicochemical analysis. Physicochemical properties i.e., total soluble solids, pH, titratable acidity, reducing sugar and non-reducing sugar are shown in Table 2 -3.

Total soluble solids (°brix). The total soluble solids (TSS) of banana mushroom blended jam samples increased significantly ($P<0.05$) during storage (Table 2). TSS of banana mushroom blended jam samples increased gradually during storage period. The TSS of jam samples at an initial day of storage period ranged from 66.0 °brix (MB_0) to 68.1 °brix (MB_6), which gradually increased to 69.4 °brix (MB_4) to 70.8 °brix (MB_0) during three months of storage. The mean total soluble solids value was 67.94 at initial day, which increased to 69.78 at 90 day of storage. The maximum mean value for treatment was observed for BM_0 (69.19 °brix), while minimum was observed for BM_3 (68.86 °brix). The result of the present study was similar to the findings of Shakir *et al*. (2007), who observed an increase in TSS of apple pear fruit jam during storage. Likewise, Khan *et al*. (2012) reported an increase in TSS (66.5-68.8 °brix) of jam prepared from apple and apricot. However, Ehsan *et al*., (2003) observed an increase in TSS of watermelon lemon mixed jam (68.6-68.9 °brix) and apple grape fruit marmalade (70.0 to 70.8 °brix) during storage.

pH. The pH values of the jam samples at an initial day ranged from 3.46 to 3.44, which gradually decreased during storage. The mean pH value for storage noted at initial day was 3.45, which decreased to 3.26. The pH value of banana mushroom blended jam samples reduced gradually during storage. The maximum mean value of treatment was observed for BM_4 (3.39) while minimum was observed for BM_0 (3.20). Maximum % decrease was observed for BM_0 (6.94) followed by BM_1 (5.51), while minimum % decrease was observed for

Table 2. Effect of storage period on total soluble solids, pH and acidity% of banana mushroom blended jam

Variables	Storage intervals (days)	Treatments							
		BM_0	BM_1	BM_2	BM_3	BM_4	BM_5	BM_6	Means
Total soluble solide	1	68.0	67.9	68.0	68.0	67.8	67.8	68.1	67.94g
	15	68.3	68.1	68.1	68.2	68.0	68.0	68.2	68.10f
	30	68.6	68.4	68.3	68.4	68.2	68.3	68.4	68.33e
	45	69.0	68.7	68.6	68.7	68.5	68.6	68.6	68.62d
	60	69.5	69.1	68.9	69.1	68.7	69.0	68.9	68.95c
	75	70.1	69.5	69.3	69.5	69.0	69.4	69.2	69.32b
	90	70.8	70.1	69.8	70.1	69.4	69.8	69.5	69.78a
	% Increase	3.95	3.14	2.58	3.00	2.31	2.87	2.01	
	Means	69.19a	68.83b	68.71b	68.86b	68.51c	68.70b	68.70b	
pH	1	3.46	3.45	3.44	3.43	3.46	3.44	3.44	3.45a
	15	3.43	3.43	3.42	3.41	3.44	3.42	3.43	3.43a
	30	3.39	3.40	3.40	3.38	3.42	3.40	3.41	3.40ab
	45	3.35	3.37	3.38	3.35	3.40	3.37	3.39	3.37ab
	60	3.31	3.33	3.35	3.32	3.37	3.32	3.36	3.34ab
	75	2.27	3.00	3.31	3.29	3.34	3.29	3.33	3.04c
	90	3.22	3.26	3.27	3.25	3.31	3.26	3.30	3.26bc
	% Decrease	6.94	5.51	4.94	5.25	4.34	5.23	4.07	
	Means	3.20b	3.32ab	3.37a	3.35ab	3.39a	3.36ab	3.38a	
%Acidity	1	0.70	0.71	0.70	0.70	0.72	0.72	0.71	0.71f
	15	0.73	0.73	0.72	0.72	0.74	0.74	0.72	0.73e
	30	0.76	0.76	0.74	0.75	0.76	0.77	0.73	0.75d
	45	0.69	0.79	0.76	0.78	0.79	0.80	0.75	0.77d
	60	0.83	0.82	0.79	0.81	0.82	0.83	0.77	0.81c
	75	0.87	0.85	0.82	0.84	0.85	0.86	0.80	0.84b
	90	0.90	0.89	0.86	0.87	0.88	0.89	0.83	0.87a
	% Increase	22.22	20.22	18.60	19.54	18.18	19.10	14.46	-
	Means	0.78abc	0.79ab	0.77cd	0.78bc	0.79ab	0.80a	0.76d	-

Mean values followed by different letters are significantly ($P<0.05$) different from each other.

BM_6 (4.07) followed by BM_4 (4.34). The observed data is in accordance with the findings of Ehsan *et al*. (2002), who found decrease in the pH value of watermelon and lemon blended jam samples during storage. In contrast, the pH of apricot and apple jam determined by Hussain and Shakir (2010) was slightly higher than the present findings. Similarly, Ayub *et al*. (2010) and Shakir *et al*., (2007) investigated a decline in pH of the jam samples upon storage. pH is an important factor to acquire an optimum gel condition. The acidity of fruit jam increased during storage hence, reducing the pH which might occur due to the acidic compounds formations (Ayub *et al*., 2010; Hussain and Shakir, 2010).

Titratable acidity. Statistically analysed data shows that % acidity of banana mushroom blended jam samples increased significantly ($P<0.05$) among storage and treatments. The acidity of banana mushroom jam at initial day ranged from 0.70 to 0.72%, which gradually increased during storage. The mean acidity value of 0.71% was observed at initial day, which increased to 0.87% as the storage period prolonged. The maximum mean value for treatment was observed for BM_5 (0.80%), while minimum was observed for BM_6 (0.76%). Maximum % increase was observed for BM_0 (22.22), while minimum % increase was observed for BM_6 (14.46). The present findings are supported by the work of Anjum *et al*. (2000), who found an increase in the acidity of apricot jam from 0.65 to 0.70% after storage interval. Shakir *et al*. (2007) also reported increase in acidity (0.60-0.78%) in apple pear mixed jam during storage. However, Khan *et al*. (2012) analysed increase in acidity (0.68-0.86%) of strawberry jam during storage. The degradation of ascorbic acid and hydrolysis of pectin results in higher acidity of the fruit jam. Increase in TSS and sugar breakdown also resulted in the increase of acidity (Ehsan *et al*., 2002; Sogi and Singh, 2001).

Reducing sugar. The data revealed that reducing sugar of banana mushroom blended jam samples increased significantly ($P<0.05$) on storage (Table 3). The reducing sugars of banana mushroom jam at initial day from BM_0 to BM_6 were 17.7 to 18.50%, which gradually increased from 31.90 to 27.60%, respectively, throughout storage. The mean reducing sugar value of 18.17% was noted at initial day, which gradually increased to 29.33% during storage. The maximum mean value for treatment was observed for BM_0 (24.56%), while minimum was observed for BM_6 (22.53%). Maximum % increase was observed for BM_0 (44.51%), while minimum % increase was observed for BM_6 (32.97%). The present results are in agreement with the work of Anjum *et al*. *(*2000) and Riaz *et al*. (1999), who examined gradual increase in reducing sugar content of strawberry jam and apricot jam respectively, throughout storage. Ehsan *et al*. (2003) also observed increase in the reducing sugar content

Table 3. Effect of storage period on reducing sugar and non reducing sugar of banana mushroom blended jam

Variables	Storage intervals (days)	Treatments							
		BM_0	BM_1	BM_2	BM_3	BM_4	BM_5	BM_6	Means
Reducing sugar	1	17.7	18.6	17.9	18.5	18.2	17.8	18.5	18.17g
	15	20.1	20.2	19.2	20.1	19.3	19.3	19.4	19.66f
	30	22.5	22.1	20.5	21.7	20.9	20.7	20.7	21.93d
	45	24.2	23.7	22.1	23.3	22.7	22.3	22.2	22.93d
	60	26.6	26.0	24.3	25.7	24.1	24.1	23.9	24.96c
	75	28.9	28.4	26.6	27.8	25.9	25.9	25.4	26.99b
	90	31.9	32.3	27.8	30.6	27.8	28.3	27.6	29.33a
	% Increase	44.51	40.58	35.61	39.54	34.53	37.10	32.97	
	Means	24.56a	24.33a	22.63b	23.96a	22.70b	22.63b	22.53b	
Non reducing sugar	1	45.4	45.6	44.5	44.8	44.6	45.1	44.3	44.90a
	15	42.8	43.5	42.3	42.3	42.4	43.7	42.9	42.84b
	30	40.1	41.1	40.1	41.7	40.6	42.2	41.1	40.99c
	45	37.2	38.8	37.5	39.9	38.8	40.3	39.5	38.86d
	60	34.3	36.5	36.1	37.3	37.3	37.5	37.9	36.70e
	75	31.1	34.1	34.2	34.5	35.1	34.8	35.5	34.19f
	90	27.7	30.0	31.5	30.4	32.4	30.7	33.1	30.83g
	% Decrease	38.99	34.21	29.21	32.14	27.35	31.93	25.28	
	Means	36.94c	38.51ab	38.03b	38.70ab	38.74ab	39.93	39.19a	

Mean values followed by different letters are significantly ($P<0.05$) different from each other.

during storage of grape apple marmalade (16.55 to 31.36%). During storage the increase in reducing sugar may be due to the inversion of sucrose to glucose plus fructose due to high temperature and acid.

Non-reducing sugar. Influence of both, treatment and storage on non-reducing sugar of banana mushroom jam samples are shown in Table 3. Statistically analysed data showed that non-reducing sugar value of the jam samples decreased significantly ($P<0.05$) during storage. The non-reducing sugars of various samples ranged from 45.6 to 44.30% at initial day, which gradually decreased from 27.7 to 33.10% correspondingly during storage. The mean value of 44.90% was recorded for non-reducing sugar, which decreased to 30.83% during storage. The maximum mean value for treatment was observed for BM_6 (39.19%), while minimum was observed for T_0 (36.94%). Maximum % decrease was observed for BM_0 (38.99%), while minimum % decrease was observed for BM_6 (25.28%). The present findings are in accordance with the results of Shakir *et al*. (2007) and Riaz *et al.* (1999). They found decrease in non-reducing sugars content of strawberry jam (44.64-32.35%) and apple pear blended jam (44.24-17.08%), respectively, throughout the storage period. Likewise, Ehsan *et al*. (2003) observed decline in non-reducing sugar in grape apple marmalade.

Sensory analysis. The sensory properties of various jam samples including colour, taste, texture and overall acceptability are presented in Table 4.

Colour. It was examined from the observed sensory scores that colour of banana mushroom blended jam samples decreased significantly ($P<0.05$) on storage. The colour of various jam samples at initial day from BM_0 to BM_6 ranged from 7.1 to 7.6, which gradually decreased (1.6-6.0) during storage. The mean colour value for storage at initial day was 7.34, which decreased to 4.84. The maximum mean value of treatment was observed for BM_6 (6.90) while minimum was observed for BM_0 (4.89). Maximum % decrease was observed for BM_0 (77.46%), while minimum % decrease was observed for BM_6 (21.05%). Similarly, Ehsan *et al*. (2003) analysed decrease in colour from 7.8 to 6.8 during storage period of grape apple marmalade. Likewise, Khan *et al*. (2012) also observed decrease in colour of strawberry jam from 9.00 to 7.00. From consumer's point of view colour is one of the significant parameter of food products. In food industries, degradation in colour was observed during storage (Gimenez *et al*., 2001).

Taste. The taste value of banana mushroom blended jam samples decreased significantly ($P<0.05$) on storage. The taste of banana mushroom jam at initial day from BM_0 to BM_6 was 7.2 to 7.40, which gradually decreased from 1.5 to 5.5, respectively, during storage. The mean taste value of 7.27 was recorded at initial day, which decreased to 4.51 during storage. The maximum mean score for treatment was observed for BM_6 (6.60), while minimum score was observed for BM_0 (4.83). Maximum % decrease was observed for BM_0 (79.17%), while minimum % decrease was observed for BM_6 (25.68%). The results of this study were similar to that of Muhammad *et al*. (2009) who found decline in taste value from 8.60 to 5.90 in apple jam during storage. Similarly, Ehsan *et al*. (2002) analysed decrease (6.2 to 4.0) in sensory score for taste of watermelon and lemon jam during storage.

Texture. It was examined from statistically analysed data that texture value of banana mushroom blended jam samples decreased significantly ($P<0.05$) on storage. The texture of banana mushroom jam at initial day from BM_0 to BM_6 ranged from 6.9 to 7.2, which decreased gradually to 1.3 to 5.7 correspondingly during storage. The mean texture value for storage was noted at initial day (7.06), which decreased to 4.60. The maximum mean value of treatment was observed for BM_6 (6.54) while minimum was observed for BM_0 (4.76). Maximum % decrease was observed for BM_0 (75.36%) followed by BM_1 (35.71%), while minimum % decrease was observed for BM_6 (20.83%) followed by BM_4 (23.94%). Suutarinen *et al*. (2000) examined sensory properties of strawberry jam and determined gradual decrease in texture profile during storage phase. The present scores for texture are slightly lower than Ehsan *et al.* (2003), who experienced decrease in texture from 8.80 to 7.96 during storage of grape fruit apple marmalade. Decrease in sensory score (9.00 to 6.70) for apple jam texture was observed by Muhammad *et al*. (2009).

Overall acceptability. The overall acceptability value of banana mushroom blended jam samples decreased significantly ($P<0.05$) during storage period. The overall acceptability of banana mushroom jam at initial day from BM_0 to BM_6 ranged from 7.0 to 7.3, which decreased gradually to 1.8 and 5.8, respectively, throughout storage. The mean overall acceptability value of 7.17 was observed at initial day, which decreased to 4.69 during storage. The maximum mean score was observed for BM_6 (6.69), while minimum score was

Table 4. Effect of storage period on sensory properties of banana mushroom blended jam

Variables	Storage intervals (days)	Treatments							
		BM_0	BM_1	BM_2	BM_3	BM_4	BM_5	BM_6	Means
Colour	1	7.1	7.2	7.4	7.3	7.5	7.3	7.6	7.34a
	15	6.7	6.9	7.2	7.0	7.3	7.0	7.4	7.07a
	30	6.3	6.6	6.9	6.6	7.1	6.7	7.2	6.77ab
	45	5.5	6.2	6.6	6.3	6.8	6.4	7.0	6.40bc
	60	4.1	5.7	6.3	5.9	6.5	6.1	6.7	5.90cd
	75	2.9	5.3	5.9	5.5	6.2	5.7	6.4	3.41de
	90	1.6	4.8	5.5	5.0	6.8	5.2	6.0	4.84e
	% Decrease	77.46	33.33	25.68	31.51	22.67	28.77	21.05	
	Means	4.89d	6.10c	6.54abc	6.23bc	6.74ab	6.34abc	6.90a	
Taste	1	7.2	7.2	7.3	7.2	7.3	7.3	7.4	7.27a
	15	6.8	6.9	7.0	6.9	7.1	7.0	7.2	6.99ab
	30	6.1	6.5	6.7	6.6	6.9	6.7	7.0	6.64bc
	45	5.2	6.1	6.4	6.2	6.5	6.3	6.7	6.20cd
	60	4.1	5.7	6.0	5.8	6.1	5.9	6.4	5.71d
	75	2.9	5.1	5.6	5.3	5.7	5.4	6.0	5.14e
	90	1.5	4.6	5.1	4.8	5.2	4.9	5.5	4.51f
	% Decrease	79.17	36.11	30.14	33.33	28.77	32.88	25.68	
	Means	4.83c	6.01b	6.30ab	6.11ab	6.40ab	6.21ab	6.60a	
Texture	1	6.9	7.0	7.1	7.0	7.1	7.1	7.2	7.06a
	15	6.5	6.8	6.9	6.8	6.9	6.9	7.0	6.83ab
	30	5.9	6.4	6.7	6.5	6.7	6.6	6.8	6.51bc
	45	5.1	6.0	6.5	6.1	6.5	6.3	6.6	6.16cd
	60	4.2	5.5	6.2	5.7	6.2	5.9	6.4	5.73d
	75	3.0	5.0	5.8	5.2	5.9	5.4	6.1	5.20e
	90	1.7	4.5	5.3	4.7	5.4	4.9	5.7	4.60f
	% Decrease	75.36	35.71	25.35	32.86	23.94	30.99	20.83	
	Means	4.76c	5.89b	6.36ab	6.00b	6.39ab	6.16ab	6.54a	
Overall acceptability	1	7.0	7.1	7.2	7.1	7.3	7.2	7.3	7.17a
	15	6.5	6.8	7.0	6.8	7.1	7.0	7.2	6.91ag
	30	5.8	6.4	6.8	6.5	6.9	6.7	7.0	6.59bc
	45	5.1	6.0	6.5	6.1	6.7	6.3	6.8	6.21cd
	60	4.4	5.6	6.2	6.7	6.4	6.0	6.5	5.83d
	75	3.1	6.1	5.8	5.2	6.0	5.6	6.2	5.29e
	90	1.8	4.6	5.3	4.7	5.6	5.0	5.8	4.69f
	% Decrease	74.29	35.21	26.39	33.80	23.29	30.56	20.55	
	Means	4.81c	5.94b	6.40ab	6.01b	6.57a	6.26ab	6.69a	

Mean values followed by different letters are significantly ($P<0.05$) different from each other.

observed for BM_0 (4.81). Maximum % decrease was observed for BM_0 (74.29%) followed by BM_1 (35.21%), while minimum % decrease was observed for BM_6 (20.55%) followed by BM_4 (23.29%). Similarly, Ehsan *et al*. (2003) examined decrease in overall acceptability of grape apple marmalade from 8.8 to 7.96 throughout storage. During storage, Khan *et al*. (2012) also observed gradual decrease (9.0-7.0) in overall acceptability. While, Ehsan *et al*. (2002) examined decrease in overall acceptability of watermelon and lemon jam on storage.

Conclusion

The study revealed that banana and mushroom blended jam as prepared successfully showed acceptable quality attributes during storage. It was observed that storage has significant impact on the quality and stability of the banana mushroom jam. Physicochemical analysis showed that the total soluble solids, percent acidity and reducing sugar increased, while pH and non-reducing sugar decreased significantly during storage period. The sensory analysis of prepared jam samples showed

acceptable colour, taste, texture and overall acceptability, which degrades upto certain extent during storage. From the analysis it was observed that BM_6 followed by BM_4 retain acceptable quality attributes during storage. This successful attempt sums up the use of blends of banana and mushroom for jam preparation, thus increasing its market value.

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Barley and Oat Meal Supplemented Chapaties and its Impact on Serum Biochemical Profile in Normal Individuals

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Abstract. The present study was taken to prepare barley and oat meal supplemented flours with special reference to chapati making quality. For this purpose, nine treatments of supplemented flours were prepared by gradually replacing whole wheat. Chapaties were prepared from all compositions along with control (100% whole wheat flour) and analysed for dietary fibre content and sensory attributes like colour, taste, aroma, texture, breakability, folding ability, chewability and overall acceptability at stated intervals. Efficacy study was carried out on healthy individuals to explore the hypocholesterolemic and hypoglycemic worth of chapaties prepared from supplemented flours. Results of dietary fibre analysis showed that there was significant increase in level of soluble 0.96 to 3.13%, insoluble 1.92 to 5.57% and total dietary fibre content 2.88 to 8.13% with increase in the supplementation level of barley and oat meal. The highest soluble (3.13), insoluble (5.57) and total dietary fibre (8.13) content were found in wheat flour supplemented with 7.5% oat meal and 7.5% barley flour while their concentration was not changed during storage. Sensory attributes showed that chapaties prepared from wheat flour supplemented with 7.5% oat meal and 7.5% barley flour were liked the most due to better overall acceptability. On the basis of nutritional and sensory characteristics, three best chapaties along with control were served to normal humans. The results revealed that consumption of chapaties supplemented with 15% oat meal greatly reduced serum cholesterol (7.7%), low density lipoprotein (6.8%), triglycerides (28.5%), blood glucose (5.5%) and weight (4.7%) while increased high density lipoprotein (2.0%), serum protein (12.5%) and albumin protein (15.7%) as compared to other two chapaties. It is concluded that the consumption of barley and oat meal supplemented chapaties tackle the hyperglycemia and hypercholesterolemia in healthy humans as well as in obese persons because it deliver three times more dietary fibre as compared to chapaties prepared from wheat flour only.

Keywords: barley, oat meal, β-glucan, chapaties, hyperglycemia

Introduction

The consumption of healthy food products such as sugar-free, low caloric and high fibre products is increasing in modern era to overcome health complications. Cereal grains provide protein, dietary fibre, energy, antioxidants and vitamins required for human health. Cereal grain is mainly comprised of endosperm, germ and bran portion. However, after milling of these grains many of the nutrients present in the bran and germ portion are lost leaving behind carbohydrates only. Cereal grains including wheat, sorghum, barley, rice and corn contribute a huge share (68%) in the world food supplies. Consumption of fruits, vegetables and whole grains increase the energy and nutritional profile of humans. The high fibre whole grain cereals contain low sugar contents which makes the foods more nutritive. The nutritionists suggest that consumption of cereal foods daily is better for health instead of rely on just one food (Anjum *et al*., 2005; Slavin, 2004).

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Wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and oat (*Avena sativa* L.) are important cereal food crops. Wheat a major dietary staple supplies instant energy and proteins to the world population as contrast to other cereal crops. The health benefit of whole grain products are mainly due to the occurrence of bioactive compounds. Multifunctional and nutritional profile of oat and barley is unique among cereals (Butt *et al*., 2011; Sramkova *et al*., 2009; Thondre and Henry, 2009). Barley and oat meal are rich sources of soluble dietary fibre which provide protection against many health ailments like gastrointestinal diseases, colon cancer, diabetes, overweight and obesity. These cereals can be supplemented with whole wheat flour to form a wide range of healthful baked foodstuff.

Dietary fibres are very complex carbohydrates and lignins that are not broken down in the upper gut due to absence of intestinal enzymes. Dietary fibre β-glucan content imparts potential beneficial effects when used as part of the human diet. The number of people suffering

from elevated blood glucose and cholesterol, especially low density lipoprotein is increasing briskly in Pakistan. To overcome the menace, little efforts have been flinching towards diet diversification, modification and improvement in the nutritional value of commonly used foods.

The US Food and Drug Administration endorsed daily intake of 3 g dietary fibre which contains 0.75 g of β-glucan of barley to abate the development of heart diseases and other metabolic disorders. About 85-90% of wheat produced in world is used in the form of unleavened flat bread locally known as chapati mostly prepared from the whole wheat flour. Typically, it is consumed hot along with other sauces, gravies and chutneys (Hemalatha *et al*., 2010; Akhtar *et al*., 2008).

The present study was designed to augment the consumption of dietary fibre in Pakistan by supplementing innumerable levels of underutilised dietary fibre rich sources. The main objectives of the study were to develop barley and oat meal supplemented flours with special reference to chapati making quality and compute the acceptability of chapaties through chemical analysis and sensory evaluation. Another notable point of study was to explore the influence of chapaties prepared from supplemented flours on serum biochemical profile in normal human beings.

Materials and Methods

Preparation of chapaties. Commercially available varieties of barley (Haider) and wheat variety (Galaxy, 2013) were procured in the year 2014 from Ayub Agricultural Research Institute, Faisalabad, Pakistan. Oat grains were acquired from Pakistan Agricultural Research Center (PARC), Islamabad, Pakistan. Barley and oat meal flour were supplemented in whole wheat flour by gradually replacing wheat flour to prepare composite flours (Table 1) and stored in polyethylene zip bags at room temp (25 °C±5) for 45 days. Additionally, chapaties were prepared from all compositions following the method of Haridas *et al*. (1986) with slight amendments. Dough was prepared by mixing 200 g flour with 135 mL water in mixer and covered to give rest time. Then it was divided into balls followed by molding into a smooth disc on a flour dusted wooden board using a rolling pin to get chapaties with uniform diameter and thickness. The uncooked chapaties were quickly transferred to a pre heated hot plate at 240 °C. After 30 sec the chapati was turned and pressed with the soft cloth to spread the steam uniformly. Chapaties were cooled and packed in individual polyethylene bags for further analysis.

Total dietary fibre analysis. The chapaties were analysed for total dietary fibre, soluble and insoluble dietary fibre content according to method No. 32-05, 32-07 and 32-20, respectively as described in AACC by employing Megazyme Assay Kit (AACC, 2000). The samples were dispersed in a buffer solution and incubated with heat-stable α-amylase at 95-100 °C for 35 min. After cooling the samples were conceived at 60 °C for 30 min by adding 100 µL protease solution. Furthermore, α-amylase and protease treated samples were incubated with amyloglucosidase at 60 °C for 30 min. The fibre contents were precipitated by the addition of alcohol in 1:4 ratios and filtered to eliminate other substances. The remnant was washed with alcohol and acetone. A blank was run in a similar manner.

Sensory evaluation. Sensory evaluation of barley and oat meal supplemented chapaties was performed for various attributes like colour, texture, folding ability, chewability, taste and overall acceptability by a penal

Table 1. Treatments used in study for barley and oat meal supplemented flours

Treatments	Wheat flour	Oat meal	Barley flour
		(%)	
WF	100	-	-
WF-OF-5	95	5	-
WF-OF-10	90	10	-
WF-OF-15	85	15	-
WF-BF-5	95	-	5
WF-BF-10	90	-	10
WF-BF-15	85	-	15
WF-OBF-2.5	95	2.5	2.5
WF-OBF-5	90	5	5
WF-OBF-7.5	85	7.5	7.5

WF = 100% whole wheat flour; WF-OF-5 = wheat flour supplemented with 5% oat meal; WF-OF-10 = wheat flour supplemented with 10% oat meal; WF-OF-15 = wheat flour supplemented with 15% oat meal; WF-BF-5 = wheat flour supplemented with 5% barley flour; WF-BF-10 = wheat flour supplemented with 10% barley flour; WF-BF-15 = wheat flour supplemented with 15% barley flour; WF-OBF-2.5 = wheat flour supplemented with 2.5% oat meal and 2.5% barley flour; WF-OBF-5 = wheat flour supplemented with 5% oat meal and 5% barley flour; WF-OBF-7.5 = wheat flour supplemented with 7.5% oat meal and 7.5% barley flour.

of 8 judges. They used 9-point Hedonic score system to evaluate the chapaties following the procedure of Meilgaard *et al*. (2007).

Efficacy studies. On the basis of chemical assay and sensory attributes three chapaties comprising of (i) 15% oat meal, (ii) 15% barley flour and (iii) 7.5% oat meal and 7.5% barley flour showing suitability for product development along with control were selected for efficacy purposes. Efficacy trail was conducted on healthy individuals to determine the impact of selected chapaties on serum biochemical profile including serum cholesterol, high density lipoprotein, low density lipoprotein, triglycerides, glucose, total protein and albumin concentration during 30 days study interval. For this purpose, 18 healthy persons (male and female) of age period 25-40 years were randomly divided into three groups having six in each and were feed on two chapaties per day. Body weight of the subjects were measured fortnightly throughout the experimental period. Blood samples were collected at the baseline, mid and end of the study.

Statistical analysis. The data obtained from all parameters were subjected to the analysis of variance and two way factorial design using statistical package Statisix 8.1 (Steel *et al*., 1997). The calculated mean values were compared using Turkey test with significance defined at $p>0.05$.

Results and Discussion

Dietary fibre analysis. The mean values for soluble, insoluble and total dietary fibre content of whole wheat flour supplemented with barley and oat meal revealed highly significant effect of different flour compositions and storage intervals. Wheat flour was supplemented with 7.5% oat meal and 7.5% barley flour having total dietary fibre (8.13%) comprising of soluble (3.13%) and insoluble (5.57) fibre. Results of analysis showed that there was significant increase in soluble fibre (0.96 to 3.13%), insoluble fibre (1.92 to 5.57%) and total dietary fibre content (2.88 to 8.13%) with increase in the supplementation level (Table 2). Soluble dietary fibre content was 2.78% at 0 day which was gradually decreased to 2.76, 2.73 and 2.70% at 15, 30 and 45 days, respectively during the storage.

At the beginning of the study, the insoluble and total dietary fibre content were 4.68% and 7.36% which was gradually increased to 4.71, 4.74 and 4.76% in insoluble fibre and 7.36, 7.38 and 8.13% in total dietary fibre content, respectively as the study continued (Table 3). Total dietary fibre content of oat and barley especially β-glucan ranged from 6.5 to 8.5% correspondingly (Sudha *et al*., 2007). There was a progressive increase in dietary fibre with the addition of barley and oat meal which control glucose and cholesterol level in the body. It is suggested from the present study that consumption of chapaties prepared from composite flours containing barley and oat meal provide an additional dietary fibre that would be helpful for the diabetic and obese patients.

Table 2. Mean values of dietary fibre content of barley and oat meal supplemented chapaties

Treatments	T D F	S D F	I D F
WF	6.04 ± 0.01^{j}	0.96 ± 0.09^{j}	1.92 ± 0.04^{j}
WF-OF-5	7.51 ± 0.05^{i}	2.65 ± 0.05^{i}	4.56 ± 0.02^{i}
WF-OF-10	7.60 ± 0.08^{h}	2.73 ± 0.05^{h}	4.65 ± 0.03^{h}
WF-OF-15	7.72 ± 0.08^{g}	2.78 ± 0.04^{g}	4.79 ± 0.05^{g}
WF-BF-5	7.83 ± 0.06^{f}	2.92 ± 0.04^{f}	4.90 ± 0.03^{f}
WF-BF-10	7.92 ± 0.06^{e}	3.00 ± 0.04^{e}	5.00 ± 0.04^{e}
WF-BF-15	8.01 ± 0.04^{d}	3.06 ± 0.05^{d}	5.09 ± 0.11^{d}
WF-OBF-2.5	8.04 ± 0.03^{c}	3.10 ± 0.02^{c}	5.30 ± 0.09^{c}
WF-OBF-5	8.06 ± 0.13^{b}	3.11 ± 0.06^{b}	5.46 ± 0.07^{b}
WF-OBF-7.5	8.24 ± 0.05^{a}	3.13 ± 0.07^{a}	5.57 ± 0.70^{a}

TDF = total dietary fibre; SDF = soluble dietary fibre; IDF = insoluble dietary fibre; values are Mean ± SD for samples analysed individually in triplicate; superscripts indicate the implementation of statistical technique (Tukey's Test).

Table 3. Effect of storage on dietary fiber content of barley and oat meal supplemented chapaties

Storage	T D F	S D F	I D F
S_0	7.35±3.66	2.78±1.52	4.68±2.57
S_{15}	7.36±3.70	2.76±1.54	4.71±2.57
S_{30}	7.38±3.73	2.73±1.52	4.74±2.58
S_{45}	7.38±3.73	2.70±1.54	4.76±2.60

TDF = total dietary fibre; SDF = soluble dietary fibre; IDF = insoluble dietary fibre; values are Mean ± SD for samples analysed individually in triplicate; superscripts indicate the implementation of statistical technique (Tukey's Test); S_0 = storage at 0 day; S_{15} = storage at 15 days; S_{30} = storage at 30 days, S_{45}= storage at 45 days; values are mean ± SD for samples analysed individually in triplicate.

Sensory evaluation. The mean squares of colour, texture, folding ability, chewiness, taste and overall acceptability revealed significant differences among all composition and storage intervals. The maximum score of 9 for the

above mentioned attributes was found in chapaties prepared from wheat flour supplemented with 7.5% oat meal and 7.5% barley flour. The second highest score of 8.4 was awarded to chapaties prepared by wheat flour supplemented with 15% barley flour due to its attractive and appealing light brown colour, soft texture, chewable nature and pleasant taste. The lowest score of 4.5 and least overall acceptability was given to chapaties prepared from wheat flour supplemented with 15% oat meal. The second minimum score of 5.0 was assigned to chapaties prepared from 10% oat meal (Table 4). Whole wheat flour chapaties was scored 7 due to its good colour, taste and soft texture. From the mean values for storage, it was observed that colour, texture, folding ability, chewiness, taste and overall acceptability of chapaties decreased during storage.

The mean values of colour of chapaties at 0, 15, 30 and 45 days interval were 7.22, 7.20, 6.68 and 6.66 and of texture of chapaties were at 0, 15, 30 and 45 day interval were 6.9, 6.8, 6.7 and 6.6. The mean values of folding ability at 0, 15, 30 and 45 day interval were 5.97, 5.95, 5.93 and 5.88 and of chewability at 0, 15, 30 and 45 day interval were 6.4, 6.32, 6.3 and 6.29, while the mean values of taste of chapaties at 0, 15, 30 and 45 day interval were 6.78, 6.74, 6.71 and 6.6. The mean values of overall acceptability at 0, 15, 30 and 45 day interval were 6.78, 6.74, 6.71 and 6.6 (Table 5). The migration and absorption of moisture, oxidation of fat and increased mold count result in loss of colour and palatability in chapaties during storage (Butt *et al*., 2007; Sharif *et al*., 2005).

The decrease in quality score for texture was due to absorption of moisture that has negative impact on texture. The dwindling trend in folding ability, chewiness and taste of chapaties was certainly due to dryness. Escalation in moisture has an inverse correlation with the chewiness. Lowest acceptability of oat meal chapaties was due to little change in taste and texture. Similarly, declining trend in scores for overall acceptability was observed in oat meal chapaties during various storage intervals that might be due to the development of rancidity, protein and lipid breakdown and mold infestation (Sharma *et al*., 2012; Wade, 1998).

Table 4. Mean values of sensory evaluation of barley and oat meal supplemented chapaties

Treatments	Colour	Texture	Folding ability	Chewability	Taste	Overall acceptability
WF	7.40 ± 0.09^{e}	7.40 ± 0.09^{e}	5.9 ± 0.09^{e}	6.5 ± 0.04^{e}	6.90 ± 0.06^{e}	7.00 ± 0.06^{e}
WF-OF-5	5.90 ± 0.09^{h}	5.90 ± 0.09^{h}	4.9 ± 0.11^{h}	6.2 ± 0.34^{h}	5.50 ± 0.06^{h}	5.50 ± 0.06^{h}
WF-OF-10	5.40 ± 0.09^{i}	5.40 ± 0.09^{i}	4.5 ± 0.08^{i}	4.5 ± 0.07^{i}	5.00 ± 0.43^{i}	5.00 ± 0.43^{i}
WF-OF-15	4.90 ± 0.09^{j}	4.90 ± 0.09^{j}	4.4 ± 0.19^{j}	3.9 ± 0.51^{j}	4.50 ± 0.08^{j}	4.50 ± 0.08^{j}
WF-BF-5	7.90 ± 0.09^{d}	7.90 ± 0.09^{d}	5.45 ± 0.09^{d}	7.4 ± 0.33^{d}	7.50 ± 0.10^{d}	7.50 ± 0.10^{d}
WF-BF-10	8.40 ± 0.07^{c}	8.40 ± 0.07^{c}	7.0 ± 0.09^{c}	7.5 ± 0.12^{c}	7.90 ± 0.24^{c}	7.90 ± 0.24^{c}
WF-BF-15	8.70 ± 0.10^{b}	8.60 ± 0.10^{b}	7.5 ± 0.07^{b}	8.15 ± 0.09^{b}	8.40 ± 0.09^{b}	8.40 ± 0.09^{b}
WF-OBF-2.5	6.90 ± 0.09^{f}	6.90 ± 0.09^{f}	6.5 ± 0.07^{d}	5.25 ± 0.09^{g}	6.10 ± 0.36^{g}	6.20 ± 0.36^{g}
WF-OBF-5	6.50 ± 0.09^{g}	6.50 ± 0.09^{g}	5.4 ± 0.09^{f}	5.5 ± 0.09^{f}	6.60 ± 0.07^{f}	6.50 ± 0.07^{f}
WF-OBF-7.5	8.80 ± 0.09^{a}	8.70 ± 0.09^{a}	8.0 ± 0.09^{a}	8.5 ± 0.07^{a}	8.75 ± 0.09^{a}	8.80 ± 0.09^{a}

Values are mean ± SD for samples analysed individually in triplicate; Means within a row with different letters are significantly different ($p < 0.05$); superscripts indicate the implementation of statistical technique (Tukey's Test).

Table 5. Effect of storage on sensory evaluation of barley and oat meal supplemented chapaties

Storage	Colour	Texture	Folding ability	Chew ability	Taste	Overall acceptability
S_0	7.22±1.41	6.9±1.41	5.97±1.42	6.40±1.41	6.78±1.40	6.78 ±1.40a
S_{15}	7.20±1.41	6.8±1.41	5.95±1.40	6.32±1.41	6.74±1.37	6.74 ±1.37ab
S_{30}	6.68±1.39	6.7±1.39	5.93±1.39	6.30±1.39	6.71±1.37	6.71 ±1.37c
S_{45}	6.66±1.41	6.6±1.41	5.88±1.41	6.29±1.39	6.66±1.37	6.60±1.37d

S_0 = storage at 0 day; S_{15} = storage at 15 days; S_{30} = storage at 30 days, S_{45} = storage at 45 days; values are mean ± SD for samples analysed individually in triplicate.

Efficacy results. Mean values for serum biochemical profile of normal individuals consuming dietary fibre enriched chapaties exhibited significant effect of different flour compositions and efficacy trial intervals. The maximum cholesterol concentration (189 mg/dL) was found in normal individuals fed on chapaties containing wheat flour only followed by 174 and 155 mg/dL in groups fed on chapaties supplemented with 15% barley and 15% oat meal, respectively. The maximum high density lipoprotein concentration (46.1 mg/dL) was found in normal individuals fed on chapaties containing wheat flour only followed by 44.4 and 43 mg/dL in groups fed on chapaties supplemented with 15% barley (WF-BF-15) and 15% oat meals and maximum low density lipoprotein concentration (101 mg/dL) was found in normal individuals fed on chapaties containing wheat flour followed by 99.8 and 77.7 mg/dL in groups fed on chapaties supplemented with 15% barley and 15% oat meal, respectively.

The maximum triglycerides concentration (141 mg/dL) was found in normal individuals fed on chapaties containing wheat flour followed by 130 and 114 mg/dL in groups fed on chapaties supplemented with 15% barley and 15% oat meal and maximum glucose concentration (90 mg/dL) was found in normal individuals fed on chapaties containing wheat flour only followed by 87 and 84 mg/dL in groups fed on chapaties supplemented with 15% barley and 15% oat meal, respectively. The minimum serum protein concentration (6.7 g/dL) was found in normal individuals fed on chapaties containing wheat flour followed by 6.8 and 6.9 g/dL in groups fed on chapaties supplemented with 15% barley and 15% oat meal and albumin protein concentration (4.5 g/dL) was found in normal individuals fed on chapaties containing wheat flour only followed by 4 and 3.8 mg/dL in groups fed on chapaties supplemented with 15% barley and 15% oat meal, respectively. It was revealed that maximum weight (70 kg) was found in normal individuals fed on chapaties containing wheat flour followed by 67 and 64 kg in groups fed on chapaties supplemented with 15% barley and 15% oat meal, respectively.

At the initiation of the study, mean values of cholesterol was 179.3 mg/dL which gradually decreased to 174.1 and 164.3 mg/dL. At the 0 day of the study, mean values of high density lipoprotein were 45.3 mg/dL which gradually decreased to 45 and 44.5 mg/dL, values of low density lipoprotein was 98.1 mg/dL which gradually decreased to 95.1 and 91.4 mg/dL by consuming supplemented chapaties. At the initiation of the study, mean values of triglycerides was 165 mg/dL which gradually decreased to 143.6 and 138.4 mg/dL and mean values of glucose level were 83.16 mg/dL, mean values of serum protein concentration were 6.5 g/dL and mean values of albumin protein were 4.1 g/dL which gradually increased to 4.2 and 4.5 g/dL during 30 days trail. At the initiation of the study, the mean values of weight were 64.1 kg which gradually decreased to 63.2 and 62.4 kg.

The individuals consuming chapaties made from wheat flour supplemented with 15% oat meal showed maximum cholesterol reduction (8%) followed by 5.2% in consuming chapaties prepared from 15% barley flour supplemented in whole wheat flour whereas chapaties made from 7.5% oat and 7.5% barley flours induced 4% reduction as compared to control group (Fig. 1). It is obvious from the present findings that consumption of chapaties supplemented with barely flour and oat meal alone as well as in combination may be helpful in controlling serum cholesterol in hypercholesterolemic as well as in healthy individuals. In a study it was reported that reduction in cholesterol level after a meal containing oat meal are mainly due to the viscosity caused by β-glucan content of oat dietary fibre which decreases the concentration of bile acids in the body. Results of the present study was closely related to the findings of researchers which showed that by incorporation of barley and oat meal dietary fibre frequent reduction in the serum cholesterol concentration was occurred because dietary fibre bind with bile acids and decreased fat absorption in the body (El-Rabey *et al*., 2013; Hooda *et al*., 2009).

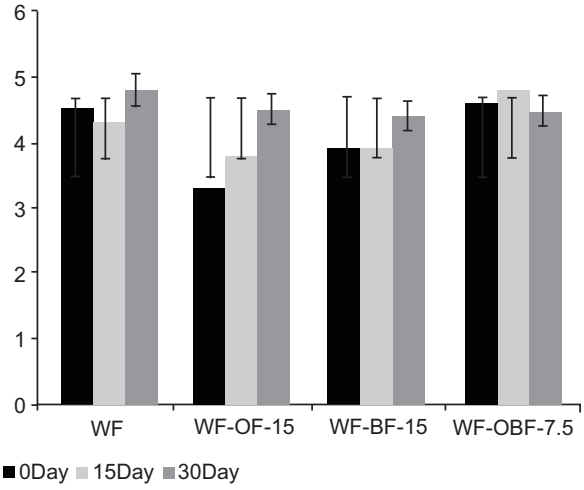

Fig. 1. Percent decrease in cholesterol.

In a previous study it was explored that guar gum supplementation in the flour (10% and 20% concentration) decreased blood total cholesterol significantly. The human volunteers consuming chapaties made from wheat flour supplemented with barley and oat meal showed little or no reduction in high density lipoprotein reduction as compared to control group (Tiwari and Cummins, 2011). The chapaties made from wheat flour supplemented with 15% oat meal showed maximum low density lipoprotein reduction (6.8%) followed by 1.8% in 15% barley flour supplemented chapaties whereas chapaties made from 7.5% oat and 7.5% barley flours induced 1.5% reduction as compared to control group (Fig. 2-3). The healthy objects consuming chapaties made from wheat flour supplemented with 15% oat meal showed maximum triglycerides reduction (28.5%) followed by 3.8% in consuming chapaties prepared from 15% barley flour supplemented in whole wheat flour whereas, chapaties made from 7.5% oat and 7.5% barley flours induced 2.5% reduction (Fig. 4). The consumption of such chapaties had slight effect on high density lipoprotein concentration in the healthy individuals. The consumption of dietary chapaties was helpful in controlling LDL level and blood triglycerides in heart patients. Results of the present study were closely related to earlier findings which showed (16.33%) reduction in triglycerides level of rats fed on chapaties prepared from chick pea flour 5% + guar gum 1%, (12.31%) and (2.76%) followed by guar gum 3% and 2%, respectively as compared to control diet.

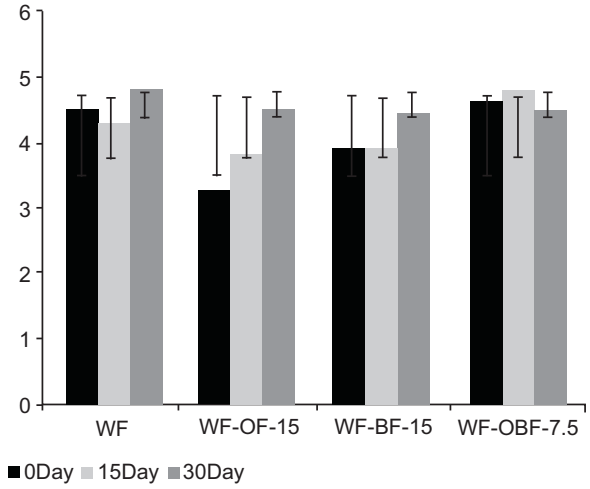

Fig. 2. Percent increase in HDL cholesterol.

The chapaties made from wheat flour supplemented with 15% oat meal showed maximum glucose reduction (5.5%) in persons followed by 3.5% in consuming chapaties prepared from 15% barley flour supplemented in whole wheat flour whilst chapaties made from 7.5% oat and 7.5% barley flours caused 2% reduction as compared to wheat flour chapaties (Fig. 5). Dietary flour had significant effect on controlling blood glucose in hyperglycemic persons. Results of the present study were closely related to earlier findings which showed (14.57%) reduction in glucose level of rats fed on chapaties prepared from chick pea flour 5% + guar gum 1%, followed by 11.64% and 9.60% reduction due to guar gum 3% and 2%, respectively as compared to control diet. Reduction in glucose and insulin responses

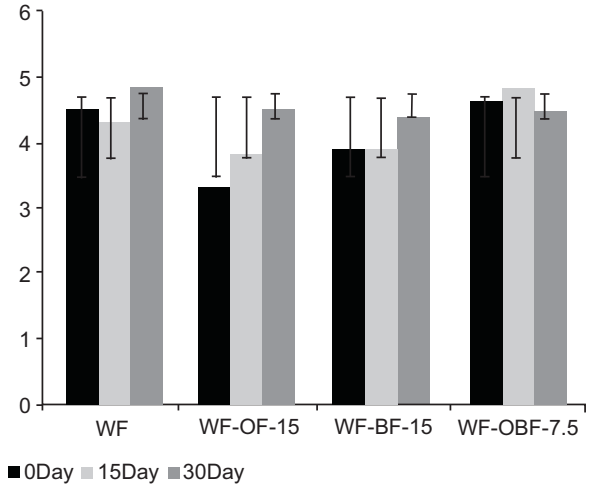

Fig. 3. Percent decrease in LDL cholesterol.

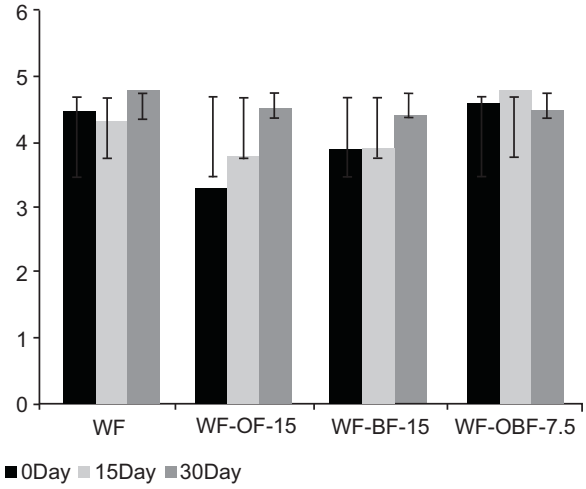

Fig. 4. Percent decrease in triglyceride.

after a meal containing barley and oat meal are mainly due to the viscosity caused by β-glucan content of dietary fibre which accelerates the movement of food throughout the body.

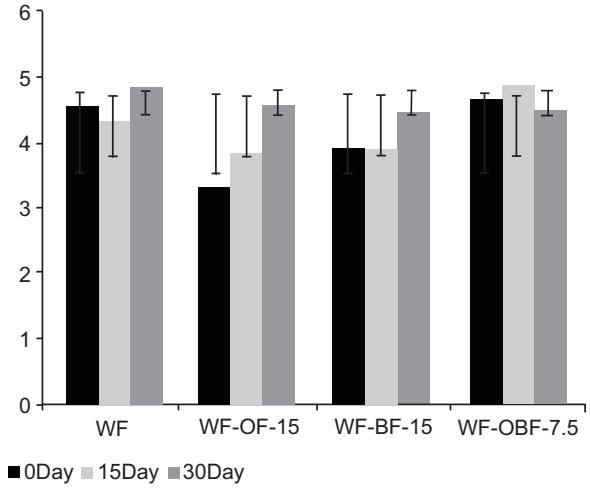

Fig. 5. Percent decrease in glucose.

The individuals consuming chapaties made from wheat flour supplemented with 15% oat meal showed maximum protein (12.5%) and albumin protein concentration (15.7%) followed by 11.1% and 12% in consuming chapaties prepared from 15% barley flour supplemented in whole wheat flour, respectively. Whereas, chapaties made from 7.5% oat meal and 7.5% barley flour induced 10% increase in protein and 11% in albumin protein concentration as compared to control group (Fig. 6-7).

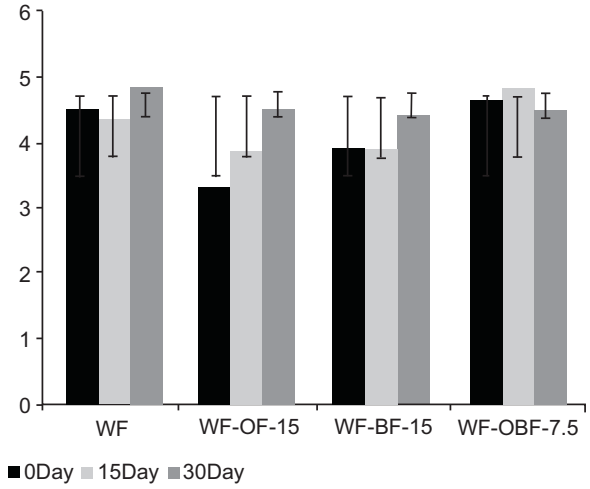

Fig. 6. Percent increase in serum protein.

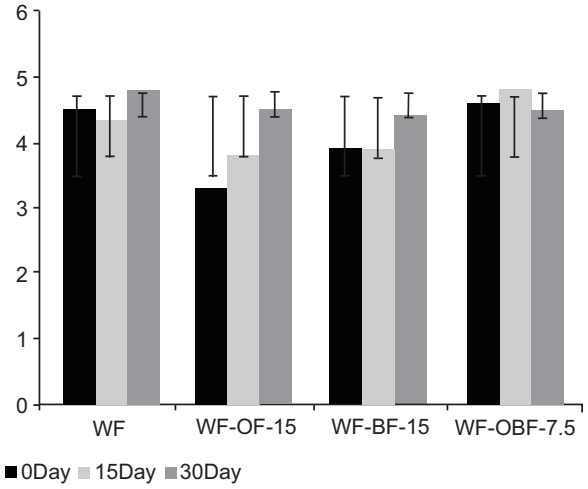

Fig. 7. Percent increase in albumin protein.

The chapaties supplemented with barely flour and oat meal increased the serum protein and albumin protein level of the individuals due to presence of high protein content. Results of this study were closely related to the findings of earlier scientists who reported that maximum serum protein 6.39 g/dL and serum albumin protein 3.63 g/dL were found in rats fed on chapaties prepared from blend of chickpea 5% and guar gum 1% flour as compared to control chapaties (6.33 g/dL) and (3.60 g/dL), respectively. The individuals consuming supplemented chapaties made from 15% oat meal showed maximum weight reduction (4.7 %) followed by 4.1% in consuming chapaties prepared from 15% barley flour whereas, chapaties made from 7.5% oat

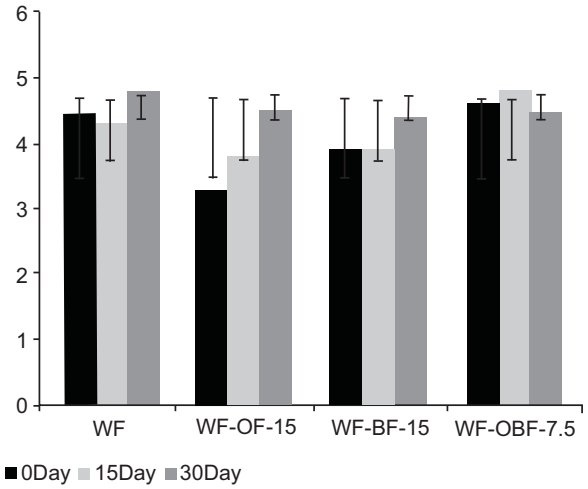

Fig. 8. Percent decrease in weight.

meal and 7.5% barley flours prompted 3% reduction as compared to control group (Fig. 8). The researchers reported that reduction in weight of normal and obese persons by consuming dietary fibre rich chapaties was mainly due to their effect of bulkiness but not given any caloric values.

Conclusion

From the present study it is concluded that oat and barley based chapaties should be made part of a regular diet in order to achieve health benefits associated with dietary fibre content. Composite flour technology must be encouraged for its nutritional and therapeutic effects on human heatlh. Dietary fibre rich chapaties prepared from selected compositions of barley and oat meal supplemented flours would be supportive for hypercholesterolemic and hyperglycemic individuals as well as normal humans.

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Development of Buckwheat Cookies Supplemented with Wheat Flour

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Abstract. The present study was conducted to develop buckwheat cookies supplemented with wheat flour. Buckwheat and wheat flour were examined for their proximate composition. Buckwheat flour contained 11.6% moisture, 15.79% crude protein, 1.81% crude fat, 1.83% ash, 0.70% crude fibre content and 68.27% NFE, while wheat flour contained moisture content 13.12%, crude fibre content 1.93%, crude fat 1.42%, crude protein content 12.53%, ash content 1.57% and 69.43% NFE, respectively. Wheat flour was incorporated into buckwheat flour at 10, 20, 30, 40 and 50% ratio to make composite flour and the developed cookies were analysed for quality evaluation. Supplementation of wheat flour significantly influenced the proximate and mineral composition of buckwheat flour based cookies. Moisture contents, crude fibre contents and NFE (Nitrogen Free Extract) increased, whereas crude fat, crude protein and ash contents decreased. Mineral contents (Fe, Ca, K, Zn and Mg) of developed buckwheat cookies decreased with increase in wheat flour supplementation levels. Sensory characteristics of supplemented cookies increased with increase in supplementation levels of wheat flour and were acceptable by judges in terms of test, colour, texture and overall acceptability. Cookies developed from C 50% C supplementation level of wheat flour got maximum scored points while C_0 control C_0 was found to be more nutritious and gluten free having more crude protein and mineral contents when compared to supplemented cookies.

Keywords: buckwheat cookies, chemical quality, sensory quality, wheat flour

Introduction

Cookies and biscuits are very vital bakery products. Both are liked and eaten by all age groups especially school going kids who need more energy like proteins per unit body weight when compared to adults (Shahzad *et al*., 2006). These are ideal for availability of essential nutrients. The term cookies comes from the Dutch word koekje (little cake) and the name biscuit is the Latin word which means biscoctum (Macrae *et al*., 1993). Cookies and biscuits are different from other bakery items such as cakes and bread because these contain lower moisture contents as compared to other bakery foods and are relatively free from microbial spoilage and have longer shelf life (Wade, 1988). Cookies are prepared by supplementing different low priced sources like pulses and legumes flour with wheat flour (Akubor and Onimawo, 2003). In supplementation, proteins giving constituent for biscuits should have sweet flavour, high protein efficiency ratio and low water absorption capacity. It should neither have negative influence on the dough spread ratio and texture nor cause any significant changes in the consistency of dough (Lorenz, 1983). The challenge of selecting the best-suited protein source has made the baking factory to examine such components that assign desirable functional and nutritional characteristics to the baked items (Tyagi *et al*., 2007). Common buckwheat *Fagopyrum esculentum* Moench (sweet buckwheat) is a broad leafy herbaceous crop that belongs to the family Polygonaceae. Its seeds structurally and chemically resemble that of wheat grains; therefore, it is considered as pseudo cereal. It originated from East Asia and then shifted into European countries in the 15th century. The cultivation of this miracle crop has spread to many other countries of the world such as The United States of America, Canada, China, Latin America and Africa, with an

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annual production of about one million tonnes (Eggum *et al*., 1980; Pomeranz and Robbins, 1972).

There are various species of buckwheat grown throughout the world, but only nine of them have nutritional and agricultural value (Krkoskova and Mrazova, 2005). Mostly, two types of buckwheat (Common buckwheat and Tartary buckwheat) are used as a source of food throughout the world. These two species of buckwheat are cultivated in mountainous regions of Pakistan, mostly in Gilgit-Baltistan at the area of 948 hectares with an annual production of 1798 metric tonnes (SSR, 2007). Because of high nutritional and medicinal value, its production has been increased in recent years. Being gluten free, it has medicinal value and is used in gluten free food preparation for those who have gluten allergy (celiac patients) (Bonafaccia *et al*., 2003). It is naturally gluten free and contains various kinds of essential nutrients including easily digestible protein, starch, essential minerals (Zn, Fe, K, Ca, Mg, Mn, and Cu), amino acids (lysine) and rutin. It is low in saturated fat, sodium, and cholesterol (Bonafaccia *et al*., 2003). Common buckwheat that is mostly consumed can be compared to other species because it is sweet in taste and easy to dehul unlike tartary buckwheat that has bitter taste and is small in seed size with tough seed coating hence, it is hard to dehull (Jiang *et al*., 2007). Buckwheat is an excellent source of micronutrients like potassium, manganese, copper, iron, and zinc (Ikeda and Yamashita, 1994). In contrast with cereals, buckwheat contained more crude protein, high in lysine content and gluten free that makes it important from medicinal and nutritional viewpoint. Therefore, it is used to prepare an alternate gluten free food for celiac patients (Javornik and Kreft, 1984; Eggum, 1980). Buckwheat foodstuffs are considered as a good nutritional and medicinal value food (Bonafaccia and Kreft, 1998; Mazza, 1989). It has been reported that there is high concentration of amino acid in buckwheat (Kato *et al*., 2001; Liu *et al*., 2001).

Present experiment was therefore conducted to develope buckwheat cookies supplemented with wheat flour to determine the level of buckwheat and to evaluate the gluten free biscuits for patients.

Materials and Methods

The present research work was carried out at (PCSIR) Pakistan Council of Scientific and Industrial Research Laboratories, Skardu during 2012-2013.

Collection of raw materials. Common buckwheat (sweet) *Fagopyrum esculentum* and wheat flour were selected for the development of buckwheat cookies. Whole buckwheat was procured from District Ghancha Baltistan while wheat flour, sugar, industrial fat and other ingredients used in cookies preparation were purchased from local market and brought to PCSIR (Pakistan Council of Scientific and Industrial Research) Laboratory, Skardu. Dehulling and milling of buck wheat was conducted to obtain flour. The hulls were removed through blower to obtain dehulled buckwheat. The dehulled grains were milled by using laboratory mill. The flour was sealed in polyethylene bags and stored in refrigerator for further use.

Preparation of buckwheat cookies. according to the official method of AACC (2000) different % level (10, 20, 30, 40, and 50) of wheat flour were prepared for buckwheat. The recipe used to prepare buckwheat cookies is shown in Table 1.

Proximate composition. Proximate composition includes moisture, crude protein, crude fat, crude fibre, ash and nitrogen free extract. Moisture occurred by oven dehydration method at 105 °C up to constant weight. Crude protein was evaluated by using Kjeldhal method and crude fat extracted by ether extraction method using Soxhlet apparatus. Crude fibre was known through acid digestion and alkali digestion method. Ash content was determined in muffle furnace at 550 °C for 6 h. For all these determinations powdered and oven dried samples were used in triplicate in accordance with standard procedures. NFE was calculated by difference (AACC, 2000).

Mineral estimation. The developed buckwheat cookies were analysed for minerals (Ca, Fe, Zn, K and Mg) through wet digestion. Iron, calcium, magnesium and zinc contents were calculated by using atomic absorption spectrophotometer, while potassium was estimated by

Table 1. Ingredients used in cookies preparation

Ingredients	Weight (g)
Flour	500
Sugar	250
Industrial fat	250
Baking powder	6.50
Salt	0.040
Egg	1

the use of flame photometer; according to the recommended method of AACC (2000).

Minerals (Fe, Zn, Ca and Mn) were calculated in cookies. About 1 g of finely ground sample was kept in digestion tube and 10 mL concentrated nitric acid was added and kept at room temperature overnight. Then the mixture was treated with 4 mL concentrated perchloric acid and sample was kept on magnetic hot plate for digestion. The process was completed in about 1-2 h. The sample was then allowed to cool down to room temperature, transferred to 200 mL volume flask and filtered by using filter paper. The volume of sample in flask was made up to 100 mL with distilled water and absorbance was estimated through atomic absorption spectrophotometer (Model GBC 932 PLUS, UK).

Sensory evaluation. Supplemented buckwheat cookies were sensory evaluated in terms of taste, colour, texture and overall acceptability by presenting developed cookies to a panel of six judges using 9 points hedonic scale as recommended by Larmond (1977).

Statistical analysis. The data achieved from different treatments were statistically evaluated in MSTAT-C software using completely randomized design (CRD) and least significant difference (LSD) test (at 5% level of significance) to separate means according to the method described by Steel and Torrie (1997).

Results and Discussion

The product was prepared using different ratios of ingredients in different trials. The samples were analysed for their proximate (crude fat, ash, crude fibre, crude protein, moisture and NFE (nitrogen free extract) contents and mineral composition (iron, zinc, magnesium, calcium and potassium). The developed buckwheat cookies were presented to a panel of six expert judges for estimation of organoleptic characteristics according to 9 point hedonic scale.

Proximate composition of buckwheat flour and wheat flour. Buckwheat (common buckwheat) flour and wheat flour used in the present research work were investigated for their chemical composition. Percentage chemical compositions are presented in Table 2.

The data shows that buckwheat flour contained 11.6% moisture, 15.79% crude protein, 1.81% crude fat, 1.83% ash, 0.70% crude fibre content and 68.27% NFE. These results are in close conformity with the outcomes of Bonafaccia *et al*. (2003), who confirmed that buckwheat flour contained 7.89 to 10% moisture, 10.23 to 17% crude protein, 1.3 to 2.8% ash, 1.1 to 3.5% crude fat, 0.7 to 1.8% crude fibre and 64 to 73% NFE (Bilgicli, 2009; Fessas *et al.* 2008). Buckwheat flour contained 8.5 to 19% of crude protein content depending on the variety, fertilizer and pesticides used that probably affect the overall concentration of buckwheat protein contents (Fornal, 1999). Data shows that wheat flour contained 13.12% moisture, 12.53% crude protein, 1.42% crude fat, 1.93% crude fibre, 1.57% ash content and NFE 69.43%. These outcomes are in close agreement with the study of Wahab (2001), who reported that wheat flour contain 7.38% moisture, 10.40% protein, 2.15% crud fat, 2.80% crude fibre, 1.47% ash and 75.80% NFE. Similar result are also reported in the outcomes of Ahmad *et al*. (2005) who examined that commercially available flour contained 9.95-11.58% moisture, 0.52-0.68%, ash, 0.94-1.51% fat, 10.32-11.58% protein, 0.40-0.60% crude fibre and 74.62-77.74% NFE.

Table 2. Proximate composition of buckwheat flour and wheat flour

Sample type	Moisture	Crude protein	Crude fat	Crude fibre	Ash	NFE
			%			
Buck-wheat flour	11.60	15.79	1.81	0.70	1.83	68.27
Wheat flour	13.12	12.53	1.42	1.93	1.57	69.43

NFE = nitrogen free extract.

Proximate composition of different treatments of developed buckwheat cookies. The products prepared with different formulations were analysed for proximate composition. The data is shown in Table 3.

Moisture content. Analysis of variance showed that supplementation with wheat flour had significant effect on moisture content of common buckwheat flour based cookies. Data reveals that moisture content of supplemented buckwheat cookies increased with increase incorporation of wheat flour. High moisture content of supplemented cookies may be credited to high moisture content of wheat flour in contrast to buckwheat flour. This may be due to relatively higher amount of fibre content in wheat flour than that of common buckwheat flour. The results achieved are in complete confirmation with the finding of Eastwood (1986), who reported that

Table 3. Proximate composition of different treatments of developed buckwheat cookies

Treatments	Moisture	Crude fat	Crude fibre	Crude protein	Ash content	NFE
C_0	2.88^{d}	24.44^{a}	0.72^{e}	15.87^{a}	1.70^{a}	54.39^{f}
C_1	3.08^{c}	24.24^{b}	0.88^{d}	15.31^{b}	1.59^{ab}	54.91^{e}
C_2	3.16^{bc}	24.10^{bc}	1.01^{c}	14.99^{c}	1.50^{b}	55.24^{d}
C_3	3.27^{b}	23.96^{c}	1.16^{b}	14.67^{d}	1.33^{c}	55.61^{c}
C_4	3.42^{a}	23.79^{d}	1.29^{a}	14.37^{e}	1.05^{d}	56.08^{b}
C_5	3.50^{a}	23.68^{d}	1.35^{a}	13.93^{f}	0.95^{d}	56.59^{a}

C_0 = Control 100% common buckwheat flour; C_1 = 90% buckwheat + 10% wheat flour; C_2 = 80% buckwheat + 20% wheat flour; C_3 = 70% buckwheat + 30% wheat flour; C_4 = 60% buckwheat + 40% wheat flour; C_5 = 50% buckwheat + 50% wheat flour; NFE = nitrogen free extract.

the incorporation of rice bran and wheat in bakery products preparation retain more moisture in developed products because of the existence of cellulose and hemicellulose. Other researchers (Pflaumer *et al*., 1990; French and Hill, 1988) also reported that the incorporation of guar gum and CMC hold more moisture amount in baked products because of their high water holding capacity.

Crude protein content. Significant differences were examined in protein content of buckwheat flour based cookies. Data indicates that protein content of wheat flour supplemented buckwheat cookies decreased with increase in wheat flour incorporation. The mean crude protein content results of test cookies were C_0 (15.87%), C_1 (15.30%), C_2 (14.99%), C_3 (14.67%), C_4 (14.37%) and C_5 (13.93%). The highest mean value (15.87%) was recorded in C_0, while lowest mean value (13.93%) in C_5 (Table 3). High protein content of supplemented cookies may be credited to high protein content of common buckwheat flour contrasted to wheat flour. The outcomes achieved are in confirmation with the results of Baljeet *et al.* (2010) who reported a decrease in crude protein content with increase in a mixture of different flours from cereal, legume, or root crops that is created to satisfy specific functional characteristics and nutrient composition. This work also supported the finding of Dhingra and Jood (2001) who reported a decrease in the crude protein.

Crude fat content. Supplementation of wheat flour significantly influenced the crude fat content of common buckwheat flour based cookies. Data explained that crude fat content of wheat flour supplemented buckwheat cookies decreased with gradual increase in wheat flour incorporation. The mean crude fat content results of test cookies were (24.44%), C_1 (24.24%), C_2 (24.08%), C_3 (23.96%), C_4 (23.79%) and C_5 (23.68%). The highest mean value (24.44%) was recorded in C_0, while lowest mean value (23.68%) in C_5 (Table 3). Higher crude fat content may be credited to high crude fat content of wheat flour contrasted to buckwheat flour. The outcomes of the study are in agreement with the results of Khan *et al.* (2012) who estimated decreasing fat contents in gluten free ready to serve buckwheat product (1.01%), (0.71%), (0.59%), (0.59%) and (0.34%). It is clear that by the addition of buckwheat flour, fat contents also increased.

Crude fibre content. Supplementation of wheat flour significantly affected the crude fibre content of common buckwheat flour based cookies. It is found from the results that crude fibre content of wheat flour supplemented buckwheat cookies increased with gradual increase in wheat flour incorporation. The mean crude fibre content results of test cookies were C_0 (0.72%), C_1 (0.88%), C_2 (1.01%), C_3 (1.16%), C_4 (1.29%) and C_5 (1.35%). The highest mean value (1.35%) was recorded in C_5, while lowest mean value (0.72%) in C_0 (Table 3). High crude fibre content of supplemented biscuits may be credited to higher crude fibre in wheat flour in contrast to buckwheat flour. The present data achieved are in complete confirmation with the findings of Baljeet *et al.* (2010), who observed that incorporation of buckwheat flour had significant effect on crude fibre content. Hooda and Jood (2005) reported similar result on increase in dietary fibre with 10% replacement of wheat flour with fenugreek flour. Our findings also agree with the study of Hamid and Luan (2000) and French and Hill (1988), who found that incorporation of CMC in baked biscuits had significant effect.

Ash content. Supplementation of wheat flour significantly affects the ash content of common buckwheat flour based cookies. From the result it is found that ash content of buckwheat cookies decreased with gradual increase in wheat flour incorporation. The mean ash content results of test buckwheat cookies were C_0 (1.70%), C_1 (1.59%), C_2 (1.50%), C_3 (1.33%), C_4 (1.05%) and C_5 (0.95%). The highest mean value (1.70%) was recorded in C_0, whereas lowest mean value was found in C_5 (0.95%) as shown in Table 3. Decrease in ash content of supplemented buckwheat cookies with the increase in incorporation level of wheat flour is evidently due to the presence of higher ash content in buckwheat flour when compared to wheat flour. The outcomes

achieved are contrary to the finding of Rani *et al.* (2008), where the addition of soya bean flour resulted in increased ash content in biscuits developed from wheat flour. Ndife *et al.* (2011), also points out an increase in ash content in whole wheat flour based bread with increase in different supplementation levels of soybean flour. These are contrary to the findings of the present studies.

Nitrogen free extract (NFE). Supplementation of wheat flour had significant effect on nitrogen free extract contents of common buckwheat flour based cookies. The findings shows that carbohydrate content of buckwheat cookies increased with gradual increase in wheat flour incorporation. The mean carbohydrate content of test cookies were C_0 (54.39%), C_1 (54.91%), C_2 (55.24%), C_3 (55.61%), C_4 (56.08%) and C_5 (56.59%). The highest mean value (56.59%) was observed in C_5, while lowest mean value was found in C_0 (54.39%) as shown in Table 3. Increase in nitrogen free extract (NFE) was observed when the supplementation with wheat flour increased. It might be due to the fact that common buckwheat contains higher crude protein content, ash content and crude fat than that of wheat flour, thus as the supplementation level of common buckwheat decreased, NFE increased. The results achieved are in complete confirmation with the results of Balajeet *et al.* (2010), reporting increase in the NFE content by the addition of buckwheat in wheat supplemented flour.

Mineral composition of different treatments of developed buckwheat cookies. The products prepared with different supplementation levels of wheat flour were analysed for proximate mineral composition. The data is shown in Table 4.

Table 4. Mineral composition of different treatments of developed buckwheat cookies

Treatments	Iron	Zinc	Calcium	Potassium	Magnesium
	(mg/100 g)				
C_0	20.35^a	3.36^a	50.89^a	695.33^a	368.33^a
C_1	18.65^b	3.25^b	48.19^b	661.67^b	347.67^b
C_2	17.20^c	3.15^c	46.70^c	634.33^c	337.00^b
C_3	15.77^d	3.09^c	44.95^d	593.67^d	315.00^c
C_4	14.02^e	2.97^d	44.27^e	564.00^e	300.33^d
C_5	12.27^f	2.94^d	43.77^f	535.67^f	281.33^e

C_0 = Control 100% common buckwheat flour; C_1 = 90% buckwheat + 10% wheat flour; C_2 = 80% buckwheat + 20% wheat flour; C_3 = 70% buckwheat + 30% wheat flour; C_4 = 60% buckwheat + 40% wheat flour; C_5 = 50% buckwheat + 50% wheat flour.

Potassium content. Supplementation of wheat flour significantly effect potassium (K) content of common buckwheat flour based cookies. Data disclosed that potassium content of wheat flour supplemented buckwheat cookies decreased with gradual increase of wheat flour incorporation. The mean potassium content results of test cookies were as mg/100 g C_0 (695.33), C_1 (661.67), C_2 (634.33), C_3 (593.67), C_4 (564.00) and C_5 (535.67). The highest mean value (695.33 mg/100 g) was recorded in C_0, while lowest mean value (535.67 mg/100 g) in C_5 (Table 4). High potassium content of supplemented cookies may be credited to high potassium content of buckwheat flour contrasted to wheat buckwheat flour.

Calcium content. Supplementation of wheat flour significantly effect calcium (Ca) content of common buckwheat flour based cookies. Results obtained showed that calcium content of wheat flour supplemented buckwheat cookies decreased with gradual increase of wheat flour incorporation. The mean calcium content results of test cookies were as mg/100 g C_0 (50.89), C_1 (48.19), C_2 (46.70), C_3 (44.95), C_4 (44.27) and C_5 (43.77). The highest mean value (50.89 mg/100 g) was recorded in C_0, while lowest mean value (43.77 mg/100 g) in C_5 (Table 4). Decrease in calcium content of supplemented buckwheat cookies may be credited to high calcium content of buckwheat flour contrasted to wheat flour.

Iron content. Supplementation of wheat flour significantly effect iron (Fe) content of common buckwheat flour based cookies. Results showed that iron content of wheat flour supplemented buckwheat cookies decreased with gradual increase of wheat flour incorporation. The mean iron content results of test cookies were as mg/ 100 g C_0 (20.35), C_1 (18.65), C_2 (17.20), C_3 (15.77), C_4 (14.02) and C_5 (12.27). The highest mean value (20.35 mg/100 g) was recorded in C_0, while lowest mean value (12.27 mg/100 g) in C_5 (Table 4). High iron content of supplemented biscuits may be credited to high iron content of buckwheat flour contrasted to wheat flour. The end results achieved are in complete confirmation with the findings of Khan *et al.* (2012). Kashlan *et al.* (1991) also reported that during baking significant loss of most of minerals such as iron content was found when baked bread was compared to wheat flour.

Zinc content. Supplementation of wheat to common buckwheat flour based cookies had significant ($p<0.005$) effect on zinc (Zn) content. It was observed that zinc

content decreased with gradual increase in wheat flour supplementation levels. The mean results of test cookies were as mg/100 g C_0 (3.36), C_1 (3.25), C_2 (3.15), C_3 (3.09), C_4 (2.97) and C_5 (2.94). The highest mean value (3.36 mg/100 g) was recorded in C_0, while lowest mean value (2.94 mg/100 g) was in C_5 (Table 4). Khan *et al.* (2005) and Steadman *et al*. (2001) noticed increase in zinc contents with increase in the addition of buckwheat flour to wheat flour bur contrasting results are observed in the present study.

Magnesium content. Supplementation of wheat flour significantly affected magnesium (Mg) content of common buckwheat flour based cookies. Results revealed that magnesium content of wheat flour supplemented buckwheat cookies decreased with gradual increase in wheat flour supplementation. The mean magnesium content of test cookies were as mg/100 g C_0 (368.33), C_1 (347.67), C_2 (337.00), C_3 (315.00), C_4 (300.33) and C_5 (281.33). The highest mean value (368.33 mg/100 g) was recorded in C_0, while lowest mean value was found (281.33 mg/100 g) in C_5 (Table 4). High magnesium content of supplemented buckwheat cookies may be credited to high magnesium content of buckwheat flour contrasted to wheat flour. When the supplementation level of wheat flour increased and buckwheat concentration decreased then the magnesium contents in cookies also decreased. The results achieved are in complete confirmation with the finding of Khan *et al*. (2012); Ikeda *et al.* (2006) and DeFrancischi *et al*. (1994) who found increase in magnesium content of tartary buckwheat flour than in whole-wheat flour. In the present study decrease in magnesium content was recorded, probably because of the addition of wheat flour to common buckwheat flour.

Sensory/organoleptic evaluation of different treatments of developed buckwheat cookies. The results of sensory/organoleptic evaluation of all treatments of buckwheat cookies are presented in Table 5.

Taste. Supplementation of wheat flour has significant effect on quality score in terms of taste. The minimum scored point (5.26) was recorded in C_0, while maximum point scored (7.54) recorded in C_5 (Table 5). The data shows that with the increase in wheat flour and reduction in buckwheat flour content, taste of developed cookies improved. The results achieved are in close confirmation with the findings of Tyagi *et al.* (2007), who reported the same result when products of biscuit incorporated with mustard flour was evaluated. Eneche (1999) also estimated maximum sensory scores for taste and overall acceptability for the cookies developed from 65% millet flour incorporating with 35% pigeon pea flour.

Colour. Supplementation of wheat flour significantly effect on quality score in terms of colour of common buckwheat flour based cookies. Results showed that quality score of buckwheat cookies increased with gradual increase of wheat flour incorporation. The lowest scored point (5.11) was recorded in C_0, while maximum point scored (8.15) recorded in C_5 (Table 5). The data shows that with the increase in wheat flour supplementation levels and reduction in buckwheat flour content, colour of developed cookies improved. The results achieved are in close confirmation with the findings of Tyagi *et al*. (2007) who reported that maximum colour score of 7.70 was observed of biscuits containing 15% defatted mustard flour. Khouryieh *et al*. (2006) also reported highest score points for colour in noodles formulated with soy flour and whole eggs. Our findings are also similar with the findings of Singh *et al*. (2005), who indicated maximum score points for colour at 15% supplementation of green gram and bengal gram.

Texture. Supplementation of wheat flour significantly ($p<0.005$) effect quality score in terms of texture of common buckwheat flour based cookies. From these results it was observed that with gradual increase of wheat flour incorporation the quality score of buckwheat cookies also increased. The maximum point score (7.84) was recorded in C_5, while lowest point scored (5.41) recorded in C_0 (Table 5). The results revealed that with the increase in wheat flour and reduction in buckwheat flour, content of developed cookies improved. The

Table 5. Colour, taste, texture and overall acceptability of different treatments of developed buckwheat cookies

Treatments	Colour	Taste	Texture	Overall acceptability
C_0	5.11^f	5.26^e	5.41^d	5.26^f
C_1	5.75^e	5.66^e	5.62^d	5.67^e
C_2	6.31^d	5.98^d	6.00^c	6.10^d
C_3	6.88^c	6.38^c	6.74^b	6.67^c
C_4	7.53^b	6.91^b	7.00^b	7.15^b
C_5	8.15^a	7.54^a	7.84^a	7.85^a

9-point hedonic scale (points: likeness/dislike); 9. Like extremely; 8. Like very much; 7. Like moderately; 6. Like slightly; 5. Neither like nor dislike; 4. Dislike slightly; 3. Dislike moderately; 2. Dislike very much; 1. Dislike extrem.

results achieved are in close confirmation with the finding of Tyagi *et al.* (2007) who reported same result when incorporation of biscuits with mustard flour was evaluated. Eneche (1999) also indicated maximum sensory score points for texture and overall acceptability for the biscuits developed from incorporation of 65% millet flour with 35% pigeon pea flour.

Overall acceptability. Supplementation of wheat flour significantly effect quality score in terms of overall acceptability of common buckwheat flour based cookies. Results showed that quality score of buckwheat cookies increased with gradual increase of wheat flour incorporation. The maximum point score (7.85) was received in C_5, while lowest point scored (5.26) recorded in C_0 (Table 5). From these results it is clear that with the increase in wheat flour and reduction in buckwheat flour content, overall acceptability of developed cookies improved. The results achieved are in close confirmation with the findings of Tyagi *et al.* (2007) who reported the same result when the end product of mustard flour incorporation into biscuits was evaluated. Singh *et al.* (1993) also observed maximum mean score points for overall acceptability at 30% incorporation level of soy flour.

Conclusion

It is concluded from this research work that supplementation of wheat flour with buckwheat flour could produce acceptable cookies having nutritional value. Supplementation of wheat flour significantly influenced the proximate and mineral composition of buckwheat flour based cookies. Moisture contents, crude fibre contents and NFE (nitrogen free extract) increased whereas crude fat contents, crude protein contents and ash content decreased. Mineral contents (Fe, Ca, K, Zn and Mg) of developed buckwheat cookies decreased with the increase in wheat flour supplementation levels. Sensory evaluation of buckwheat cookies in terms of colour, texture, test and overall acceptability increased with the increase in wheat flour supplementation levels. Buckwheat leaves are used as green tea in many countries of the world so it is recommended that research work be carried out on green tea. It is also recommended that other varieties of buckwheat grown in Pakistan be looked into for its utilization.

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A Study on Molecular Diagnosis of *Theileria* Species Infection by PCR Amplification in Sheep and Goats in Multan, Pakistan

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Abstract. In this present study polymerase chain reaction (PCR) assay was used for identification and differentiation of *Theileria* species infection in Multan, Pakistan. Out of 220 blood samples collected from sheep and goats, 31.2% (70/220) were found positive for *Theileria* species by PCR amplification compared to only 9.1% (20/220) on blood smear. *Theileria* infection was observed in 39.3% (57/145) of sheep and 18.6% (13/75) of goats sampled. The prevalence of *Theileria ovis* and *Theileria lestoquardi* in the 70 positive samples was found to be 57% (40/70) and 30% (21/70), respectively with only 12.3% (9/70) of blood samples having a mixed infection of both *T. ovis* and *T. lestoquardi.* Overall the prevalence of *T. ovis* infection was higher than *T. lestoquardi* in both sheep and goats. Herds with sheep only had significantly higher parasitic prevalence. The results confirm that PCR is direct, specific and sensitive tool for diagnosis of ovine theileriosis.

Keywords: sheep, goats, DNA extraction, PCR amplification, *Theileria ovis, Theileria lestoquardi*

Introduction

The role of livestock in the national economy of Pakistan is very important. They provide nutrition, energy and organic fertilizers for crops (Eyduran *et al.*, 2009; Ahmad *et al.*, 2007; Manan *et al.*, 2006). This role is most clearly appreciated in the rural community, where thirty million people are involved in subsistence production, with an average each family having 2-3 cattle/buffaloes and 5-6 small ruminants (sheep or goats). From just these few animals each family derives 30 and 40% of their annual income (Bakhsh *et al.*, 2014; Irshad *et al*., 2010; Duranni *et al.*, 2006). According to an economic survey conducted in 2013-14, livestock expansion can improve economic conditions and reduce poverty of rural inhabitants in Pakistan through the sale of surplus animals and their byproducts (Irshad *et al*., 2010). Small ruminants are also an important animal's protein source in the national diet (Nusrullah *et al.*, 2013). Pakistan after China and India, is the more reliant on small ruminants livestock with more than two dozen indigenous breeds of goats and sheep (Khan *et al.*, 2007). Despite the importance of small ruminants to the rural economy the production by these animals is not as efficient as it could be (Shahzad *et al.*, 2013). One of the main causes of this inefficiency is the tick borne haemoparasitic diseases. These diseases are responsible for high morbidity and mortality rates in the affected animals and negatively impacts the economic performance of rural sector in Pakistan (Aktas *et al.*, 2005). The climate of Pakistan is subtropical and highly suitable for the survival of ticks which transmit many ticks borne diseases including theileriosis and babesiosis in cattle and small ruminants (Gosh *et al.*, 2007).

Both domestic and wild small ruminants in tropical and subtropical regions of the world are infected with *Theileria* species. In Pakistan it is believed that two *Theileria* species, *Theileria ovis* and *Theileria lestoquardi* are responsible for ovine theileriosis. However, there are few reports on ovine theileriosis in Pakistan and further research is needed to understand its epidemiology.

Ovine theileriosis is caused by several *Theileria* species which are transmitted by ticks in wild and domestic ruminants (Irshad *et al.*, 2010; Heiderpour *et al.*, 2009). Among the *Theileria* associated with ovine theileriosis *Theileria lestoquardi*, *T. luwenshuni* and *T. uilenbergi* are considered extremely pathogenic (Schnittger *et al.*, 2000) whilst *T. separata*, *T. ovis* and *T. recondite* are less pathogenic or benign and cause subclinical infections in sheep and goats (Ahmad *et al.*, 2006). *Theileria lestoquardi* and *T. ovis* are the main *Theileria* species reported in small ruminants from Pakistan (Rehman *et al.*, 2012; Irshad *et al.*, 2010). *Theileria lestoquardi* (*T. leastquardi*) causes malignant ovine theileriosis (MOT) in sheep and goats (Heiderpour *et al*., 2009),

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which is equivalent to tropical theileriosis in cattle (Tageldin *et al.*, 1992). The main signs of MOT are enlarged superficial lymph nodes, pale and icteric mucous membranes, diarrhoea or constipation, high fever, listlessness and emaciation (Naz *et al.*, 2012; Rehman *et al.*, 2010). *Theileria ovis* (*T. ovis*) is the cause of ovine theileriosis with fever, weight loss, reduce production and eventually death of infected animals are the commonly observed symptoms (Shahzad *et al.*, 2013; Durrani *et al.*, 2011).

Several reports on the basis of microscopic screening in Pakistan showed that *Theileria* infection ranges from 7.4-16.5% in sheep and 3.8-8.2% in goats (Shahzad *et al*., 2013; Durrani *et al*., 2012; Naz *et al.*, 2012; Durrani *et al*., 2011; Irshad *et al.*, 2010; Rehman *et al.,*2010). PCR amplification method revealed 35% prevalence of *Theileria* species in sheep during a study at Lahore (Durrani *et al.*, 2011); 6% incidence of *Theileria ovis* observed in sheep and goats from Punjab and Khyber Pukhtoonkhwa, Pakistan (Durrani *et al.*, 2012) and 37% prevalence of *T. ovis* in Lohi sheep breed during a study in Okara, Pakistan (Shahzad *et al.*, 2013). All these reports confirm that ovine theileriosis is endemic in Pakistan and potentially damaging livestock production. Giemsa stained thin blood smears examination and clinical symptoms based detection are the conventional methods used to study theileriosis in small ruminants (Telmadarriy *et al.*, 2012; Inci *et al.*, 2010). However, in some cases, recovered animals frequently sustain subclinical infections which are often not detectable through microscopy. Serological methods such as immunoflurescent antibody test (IFAT) are also used to determine subclinical infections (Sayin *et al.*, 2009). However, these methods are not reliable in recovered animals due to low parasitemia level in these animals (Aktas *et al.*, 2007; Schnittger *et al.*, 2004). In order to implement disease control programmes the exact status of *Theileria* infection in sheep and goat herds need to be established. The use of ploymerase chain reaction (PCR) amplification to diagnose *Theileria* species infection in epidemiological studies is therefore ideal since this technique is more sensitive than microscopy and allows detection of hemoprotozoans at very low parasitemia levels.

No previous epidemiological studies have been conducted on ovine theileriosis in tehsil Jalalpur, district Multan, Punjab, Pakistan. Hence, the aim of this study was to identify the *Theileria* species infecting sheep and goats in tehsil Jalalpur and estimate the prevalence of infection in a sample of sheep and goat flocks.

Materials and Methods

Animals and blood sampling. A total of 220 apparently healthy small ruminants (sheep = 145 and goats = 75) were selected from 12 herds using a multistage sampling method (Thrusfield, 2005) during 2013 from different localities of tehsil Jalalpur, district Multan. Sheep and goats of both sexes were sampled. Approximately 10% animals from each herd were bled from the jugular vein into 5 mL Eppendorf tubes containing few drops of 0.5 M EDTA as a preservative for DNA extraction. Data were collected from each individual sampled animal including species, sex, age, and breed and from each herds, data including its location, size and species of animal. The data was collected using a questionnaires completed by investigator during sample collection. The collected samples were transported to Institute of Pure and Applied Biology at Bahauddin Zakariya University, Multan, Pakistan in a cold pack and stored at -20 °C until DNA extraction. All the experiments were approved by the ethical committee of Institute of Pure and Applied Biology at Bahauddin Zakariya University, Multan, Pakistan.

Microscopic study of blood samples. Thin blood smears were prepared in the field as stated in the manual of Tick Fever Research Center, Australia (TFRC, 1996), dried in air, used methanol as fixative and stained with 10% giemsa solution. For removal of extra stain, the stained smears were rinsed with tap water for 2-3 times and then dried in air. Binocular microscope was used for examining blood smears with an oil immersion lens of 1000× magnification. *Theileria* parasites were recognized as described by Zajac and Canboy (2000).

DNA extraction. DNA was extracted from collected blood samples following inorganic method (Shaikh *et al.*, 2004). The quality of extracted DNA was assessed by spectrophotometer analysis at 260/280 nm density constant and gel electrophoresis method. The extracted DNA was either used for PCR amplification or stored at -20 °C.

PCR amplification. For PCR reaction three primer's sets (Table 1) were used to amplify different gene segments for genus *Theileria* and *Theileria* species during present study. The first primers set was used for the amplification 1098 base pair (bp) region of rRNA (ssu rRNA) gene fragment from *Theileria* genomic

Table 1. Primers used for the detection of genus *Theileria*, *T. ovis* and *T. lestoquardi* in sheep and goats in district Multan, Pakistan

	Target gene	Primer specificity	Product size (bp)	Reference
Theileria specific	18S rRNA	F. 5'-AGTTTCTGACCTATCAG-3' R. 5'-TTGCCTTAAACTTCCTTG-3'	1098	(Allsopp *et al*., 1993)
Theileria ovis	18S rRNA	F. 5'-TCGAGACCTTCGGGT-3', R. 5'-TCCGGACATTGTAAAACAAA-3'	520	(Altay *et al*., 2005)
Theileria lestoquardi	18S rRNA	F. 5'-GTGCCGCAAGTGAGTCA-3' R.5'GGACTGATGAGAAGACGATGAG3'	785	(Kirvar *et al*., 1998)

DNA. For PCR amplification, the 50 µL reaction volume composed of 5 µL of template DNA, 5 µL of 10 × PCR buffer (100 mMTris–HCl (pH 9) 500 mMKCl, 1% Triton ×-100), 5 µL of 50 Mm $MgCl_2$, 6 µL of 250 M each of the four dNTPs, 4 µL of each primer (Penicon) at a concentration of 10 pmol/µL, 2 U of Taq DNA polymerase (Vivintas) and 20.5 µL of PCR water. PCR was carried out in a BIORAD touchdown thermocycler with following cycling programme for genus *Theleria* at 94 °C for 3 min., followed by 35 cycles, 1 min. for denaturation at 94 °C, 1 min for annealing at 60 °C and 7 min. for extension at 72 °C, with a final extension step of 7 min at 72 °C.

The second primer set was used for amplification 785 bp gene coding for the 30 kDa *T. lestoquardi* merozoite surface antigen. Total reaction volume used for PCR amplification was 25 µL comprising 4 µL of template DNA, 2.5 µL of 10 × PCR buffer (100 mMTris–HCl (pH 9) 500 mMKCl, 1% Triton ×-100), 2 µL of 25 Mm $MgCl_2$, 2 µL of 250 M each of the four dNTPs, 1.5 µL of each primer (Penicon) at 10 pmol/µL concentration, 2 U of Taq DNA (Vivantus) and 12.5 µL of PCR water. For *Theileria lestoquardi* Thermo profile was used at 94 °C for 3 min, followed by 35 cycles at 94 °C for 1 min, 56 °C for 1 min. and 72 °C for 1 min. with a final extension step of 72 °C for 7 min. The third primers set was used for amplification 520 bp gene fragment of 18SS rRNA gene of *Theileria ovis* (Altay *et al*., 2005). The final reaction volume was 25 µL containing 3 µL of template DNA, 2.5 µL of 10 × PCR buffer (100 mMTris–HCl (pH 9) 500 mMKCl, 1% Triton ×-100), 2 µL of 25 Mm $MgCl_2$, 2 µL of 250 M each of the four dNTPs, 2 µL of each primer (Penicon) at a concentration of 10 pmol/µL, 2 U of Taq DNA (Vivantus) and 11 µL of PCR water. Cycling conditions for *Theileria ovis* was consisting of 3 min at 96 °C for 3 min followed by 5 cycles, 94 °C for 30s, at 56 °C for 30s and 72 °C for 1 min. The 5 cycles were again followed by 30 cycles. Each cycle consisted of 94 °C for 30s at 54 °C for 30s and at 72 °C for 1 min. The PCR programme was ended with a final extension step of at 72 °C for 7 min. 1.5% concentration agarose gel was used for separation of amplified PCR products by using gel electrophoresis. PCR products (5 µL) admixed with 6× loading dye (Vivantus) was loaded on gel with 100-1500 bp (Vivantus) ladder as molecular marker. After running the gel, UV trans illuminator was used for assessment of PCR product. Positive control genomic DNA of *Theileria ovis* and *T. Lestoquardi* was provided by Professor Urike Seitzer (VIIRC, Borstel, Germany).

Statistical analysis. Age of the animals was categorised into three groups <1 year, 1-2 years and >2 years old. Three categories of herd size were made i.e., 1-30, 31-60 and more than 60 animals/herd.

Also, herds were grouped based on composition i.e., herds with sheep only or goats only and mixed herds. The Chi square test or Fisher's exact test was used to test independence between variables and the odds ratio was used to measure the association between variables and risk of ovine theileriosis. MiniTab (Version 16) was used for statistical analysis.

Results and Discussion

The sampling sites, total number of samples collected and the number of *Theileria* positive samples along with prevalence details are presented in Table 2. Microscopy revealed 20 (9.1%) blood samples positive while PCR identified 70 (31.8%) blood samples positive. All samples positive with blood smear examination were also positive with PCR amplification. The highest prevalence of *Theileria* infection based on PCR diagnosis was observed in Shujatpur (54.1%) and the lowest was

Table 2. Sampling sites and total number of samples collected for prevalence of theileriosis in sheep and goats in district Multan, Pakistan

Area	No. of samples	Test				P*value
		Microscopic examination		PCR examination		
		Positive	(%)	Positive	(%)	
Basti Mehwala	21	3	14.3	11	52.4	
Shujatpur	37	4	10.8	20	54.1	
Baileawala	26	2	7.7	3	11.5	
Basti Matam	43	5	11.6	11	25.6	
Basti Boherai	52	2	3.8	14	26.9	
Chalk M 65	41	4	9.8	11	26.8	0.001[a]
Total	220	20	9.1	70	31.8	0.000[b]

[a] = Chi square test; [b] = Fisher exact test.

in Mozza Bailae wala (11.5%). The prevalence of *Theileria* infection between different sampling sites was found statistically significant ($p < 0.05$) (Table 2).

Two strains of *Theileria* species i.e., *T. ovis* and *T. lestoquardi* were identified. From the 70 *Theileria* sp. positive samples, 40 (57.2%) were identified as *T. ovis,* 21(30.0%) as *T. lestoquardi* while mixed infection was diagnosed in 9 (12.8%) blood samples (Table 3). No such PCR amplified fragment was detected in negative control samples.

In the present study, prevalence of theileriosis has been detected by PCR amplification in blood samples collected from different locations of tehsil Jalalpur, district Multan, Pakistan, along with risk factors associated with ovine theileriosis. It has been observed that 31.8% samples were positive for theileriosis through PCR. Based on microscopy, Rehman *et al.* (2010) found 16.5% *Theileria* infection in sheep from Okara, Pakistan while Naz *et al.* (2012) reported 11.2% positive blood samples in small ruminants during a study at Lahore, Pakistan. These results are similar to those based on microscopic examination in the present study. However, Irshad *et al.* (2010) recorded much lower *Theileria* infection rate (5%) in small ruminants at two livestock stations in Pakistan as compared to the present study. The difference in prevalence may be due to variation in the geoclimatic conditions and parasitemia level in the infected animals. Durrani *et al.* (2011) has reported 35% *Theileria* infection in small ruminants in Lahore district, Pakistan which was high as compared to present findings in tehsil Jalalpur, Multan district indicating that climatic conditions and animals distribution affects the prevalence of ovine theileriosis.

From 70 piroplasm positive samples including 57 sheep (39.3%) and 13 goats (17.3%) indicated that sheep are more prone to theileriosis than goats ($p < 0.05$). Naz *et al.* (2012) reported 13.9% and 8.2% prevalence of theileriosis in sheep and goats which is in agreement with the present study that sheep are more susceptible to *Theileria* sp. infection. Similar differences in species susceptibility between goats and sheep have also been reported in China (Guo *et al*., 2002) and Turkey (Altay *et al*., 2012). It is suggested that difference in species susceptibility could be due to goat skins being thinner

Table 3. Molecular prevalence of *Theileria* species in sheep and goats by PCR amplification in district Multan, Pakistan

Specie		*Theileria* sp. primers	*T. ovis*	*T. lestoquardi*	*T. ovis* + *T. lestoquardi*	Total *T. ovis*	Total *T. lestoquardi*
					N		
Sheep	145	57(39.3%)	36(24.8%)	13(8.9%)	8 (5.5%)	44(30.3%)	21(14.4%)
Goats	75	13(17.3%)	4 (5.3%)	8 (10.6)	1 (1.3%)	5 (6.6%)	6(12.0%)
Total	220	70(31.8%)	40(18.1%)	21(9.5%)	9 (4.0%)	49(22.2%)	27(12.2%)
		P=0.008[a]*	P=0.002[a]*	P=0.81[a]	P=0.17[a]	P=0.002[a]*	P=0.1[a]

[a] = fisher exact test; * = statistical significant.

and more easily resistant to attachment of ticks as compared to skin of sheep. Also due to presence of sheep wool, the ticks could easily become entangled in sheep skin and transmit tick borne diseases (Naz, 2012). The low prevalence in goats may also reflect the habitats where they graze which may be less suitable for tick survival or that the steep and inaccessible pastures means that goats have minimal contact with animals infested with ticks (Alessandra and Santo, 2012).

The same *Theileria* species found in this study have been reported by many researchers from different parts of the world (Yaghfoori *et al*., 2013; Heiderpour *et al*., 2010; Heiderpour *et al*., 2009) from Iran and Durrani *et al.* (2011) from Pakistan. The overall prevalence of *T. ovis* infection was higher (30.3%) as compared to *T. lestoquardi* (14.4%) by PCR testing during present study. Iqbal *et al.* (2013) reported 43.7% and 37.5% prevalence of *Theileria* species infection in sheep and goats, respectively during a study in Pakistan. The difference in infection rate may be due to difference of animal breeds under study, genetic resistance against *Theileria* species, difference of immunity level and abundance of tick vectors.

In sheep, the prevalence of *T. ovis* infection was found significantly ($p < 0.05$) higher (24.8%) as compared to *T. lestoquardi* (9 %) in the present study. Shahzad *et al.* (2013) recorded 37.0% infection of *T. ovis* in Lohi sheep in Okara district while Durrani *et al.* (2011) reported 27.50% infection of *T. ovis* through PCR amplification in sheep at Lahore district which was higher than the present study. But contrary to present study, Durrani *et al*. (2012) revealed lower prevalence (6.0%) of *T. ovis* in sheep by using the same primers in two provinces of Pakistan. The variation in prevalence rate may be due to difference of tick infestation, breed of animals, management practices and techniques used for diagnosis.

In goats, the incidence of *T. lestoquardi* was observed higher (10.7%) as compared to *T. ovis* (5.3%) but no significant association was found ($p > 0.05$) (Table 3). Similar reports of higher incidence of *T. lestoquardi* in goats was reported as 40.0% in China by Luo and Yin (1997); 20.8% in Iran by Zangana and Naqid (2011) and 78.3% in Nubian goats in Sudan by Hussein *et al.* (2013). The difference in prevalence of *T. lestoquardi* may be due to higher vector availability for *T. lestoquardi*

Table 4. *Theileria* parasites presence in sheep and goats along with studied parameters of animal characteristics in district Multan, Pakistan

Animal type	Parameters		No. of samples (%)	Piroplasms positive (%)	Piroplasms negative	P* Value
Sheep and goats (Combined)	Sex	Male	40	16 (40.0)	24 (60.0)	
		Female	180	54 (30.0)	126 (70.0)	0.26[a]
	Age	≤ 1 year	53	21 (39.6)	32 (60.4)	
		≤ 2 year	85	25 (29.4)	60 (70.6)	
		≤ 3 year	82	24 (29.2)	58 (70.8)	0.37[b]
Sheep	Sex	Male	21	11 (52.3)	10 (47.7)	
		Female	124	46 (37.0)	78 (63.0)	0.22[a]
	Age	≤ 1 year	33	16 (48.4)	17 (51.6)	
		≤ 2 year	59	20 (33.9)	39 (67.1)	
		≤ 3 year	53	21(39.6)	32 (60.4)	0.49[b]
	Breed	Lohi	132	55 (41.6)	87 (58.4)	
		Kajli	13	2 (15.3)	11 (84.7)	0.13[a]
Goats	Sex	Male	19	5 (26.3)	14 (73.7)	
		Female	56	8 (14.2)	48 (85.8)	0.29[a]
	Age	≤ 1 year	20	5 (25.0)	15 (75.0)	
		≤ 2 year	26	5 (19.2)	21 (80.8)	
		≤ 3 year	29	3 (10.3)	26 (89.7)	0.39[b]
	Breed	Nachi	53	9 (16.9)	44 (83.1)	
		Beetal	14	2 (14.2)	12 (85.8)	
		Teddy	8	2 (25.0)	6 (75.0)	0.89[b]

[a] = fisher exact test; [b] = Chi square test.

and resistance against *T. ovis* in that areas. There was no association of gender in small ruminants with *Theileria* parasitic presence. *Theileria* species infection was found higher in males (40.0%) as compared to females (30.0%) as stated in Table 4. This current study is consistent with findings of Iqbal *et al.* (2013) who reported that male had higher infection (30.7%) as compared to females i.e. (12.7%) during a study in Pakistan. The difference in prevalence of theileriosis between males and females might be endorsed due to influence of hormones in determining hemoprotozoans infections in small ruminants. The effect of age on theileriosis revealed that age group <1 year was more infected (39.6%) followed by age group 1-2 years (29.4%) and lower in >3 years (29.2%) in small ruminants (Table 4) but no significant association was found between different age groups on theileriosis. Iqbal *et al.* (2013) observed higher infection of theileriosis in small ruminants of age less than one year which is in accordance to present study. Similar results have been found by Guo *et al.* (2002), when higher incidence and death was found in young and adult small ruminants during a study in Ganan region, China. The higher prevalence of theileriosis in younger animals may be due to the fact that younger small ruminants have less immunity against this parasite. The breed of sheep and goats has no significant effect on the prevalence of theileriosis in small ruminants.

By comparing the odd ratio there was significant association ($p < 0.05$) of *T.ovis* infection between breeds of sheep whereas *T. lestoquardi* infection was found significant ($p < 0.05$) in overall gender of small ruminants (Table 5). Herd size showed no significant effect ($p > 0.05$) on prevalence of ovine theileriosis but smaller herd size 1-30 animals/herd were more infected (37.5%) as compared to larger herds (Table 6). Present results are in agreement to Durrani *et al.* (2012) who reported that prevalence of theileriosis was not affected by the herd size in small ruminants during a study from Punjab and Khyber Pukhtoonkhwa provinces in Pakistan. Herd composition was significantly associated ($p < 0.05$) with presence of theileriosis as herds composed of sheep only had higher parasitic prevalence (50.0%) than herds composed of goats only (32.3%) or having both sheep and goats kept together (23.7%) as stated in Table 6.

Table 5. Prevalence of *Theileria* species in sheep and goats and association of different parameters describing animal characteristics based on PCR in district Multan, Pakistan

Animals	Parameters		*Theileria ovis*			*Theileria lestoquardi*		
			Pos./exam.	OR(95%CI)	P value	Pos./exam.	or(95%CI)	P value
Sheep	Gender	Male	7/21			2/21		
		Female	29/124	1.63(0.60-4.44)	0.32	11/124	1.08(0.22-6.22)	0.92
	Breed	Lohi	0/13			2/13		
		Kajli	36/132	0.04(0.00-0.68)	0.02*	11/132	2.0(0.39-10.2)	0.40
	Age	<1 year	9/33			5/33		
		1-2 year	12/59	1.46(0.54-3.97)	0.44	3/59	3.3(0.73-14,9)	0.11
		>2 year	15/53	1.00(0.35-2.51)	0.91	5/53	1.7(0.45-6.44)	0.42
Goat	Gender	Male	2/19			3/19		
		Female	2/56	3.14 (0.44-24.2)	0.26	5/56	1.9(0.41-8.8)	0.40
	Breed	Teddy	0/8			1/8		
		Beetal	1/14	0.52(0.01-14.5)	0.70	3/14	0.52(0.04-6.04)	0.60
		Nachi	3/53	0.84(0.04-17.9)	0.91	4/53	1.7(0.17-18.0)	0.63
	Age	<1 year	1/20			2/20		
		1-2 year	1/26	1.2(0.07-21.5)	0.87	2/26	1.33(0.17-10.38)	0.78
		>2 year	2/29	1.4(0.11-16.6)	0.78	4/29	1.4(0.23-8.72)	0.69
Overall	Gender	Male	9/40			7/40		
		Female	31/180	1.4(0.60-3.22)	0.43	14/180	2.5(0.94-6.70)	0.05*
	Age	<1 year	10/53			5/53		
		1-2 year	13/85	1.2(0.52-3.18)	0.50	7/85	0.86(0.25-2.86)	0.80
		>2 year	17/82	0.88(0.37-2.12)	0.79	9/82	1.18(0.37-3.74)	0.77

OR = odd ratio.

Table 6. Association between presence of *Theileria* spp. infection identified by PCR in sheep and goats and the studied parameters describing animal and herd characteristics of Tehsil Jalalpur, Multan, Pakistan

Parameters		No. of samples	PCR positive	PCR negative	P* value
Size of herd	1-30	40	15 (37.5)	25 (62.5)	
	30-60	70	23 (32.8)	47 (68.2)	
	More than 60	110	32 (29.0)	68 (71.0)	0.82
Herd composition	Sheep herd only	60	30 (50.0)	30 (50.0)	
	Goats herd only	30	10 (33.3)	20 (64.7)	0.002*
	Sheep and goats mixed herd	130	30 (23.1)	95 (748.3)	

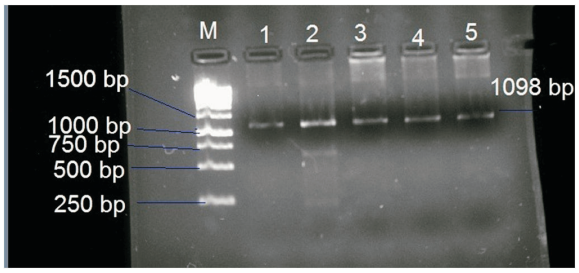

Fig. 1. Agarose gel electrophoresis of amplified PCR products obtained from *Theileria* species genomic DNA using *Theileria* specific primers; Lane: M, 100-1500 bp DNA marker; Lane: 1, positive control; 2 to 5, parasite positive blood sample.

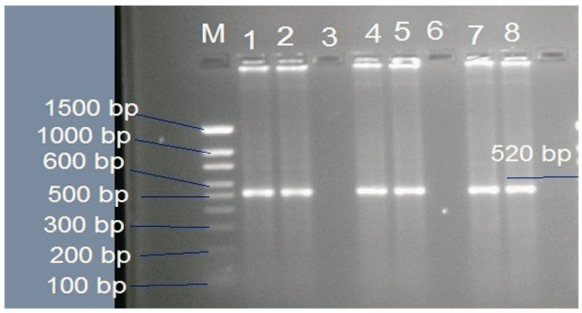

Fig. 2. Agarose gel electrophoresis of amplified PCR products obtained from *Theileria ovis* genomic DNA using *Theileria ovis* primers; Lane: M, 100 bp DNA marker; Lane: 1, positive control; 2, parasite positive blood sample; 3, negative control (distilled water); 4, parasite positive blood sample; 5, parasite negative blood sample; 6, parasite positive blood sample; 7 to 8, parasite positive blood samples.

Earlier studies confirmed that PCR amplification is better technique as compared to microscopic examination to identify carrier small ruminants infected with theileriosis without any obvious signs of the disease (Sayin *et al.*, 2009; Razmi *et al.*, 2006; Aktas *et al.*, 2005). Lower prevalence of *Theileria* infection (9.1%) has been observed by microscopic screening than PCR (31.8%) which was in accordance with previous studies that microscopic screening is only effective during acute infection when parasitemia level is high but during low parasitemia level only PCR amplification is effective for diagnosis of infection. Durrani *et al.* (2011) in Lahore, Pakistan reported 35% prevalence of *Theileria* species infection in small ruminants by PCR amplification and 22% detected by blood smear screening while Durrani *et al.* (2012) found only 1% prevalence of theileriosis by microscopy as compared to 6% by PCR amplification

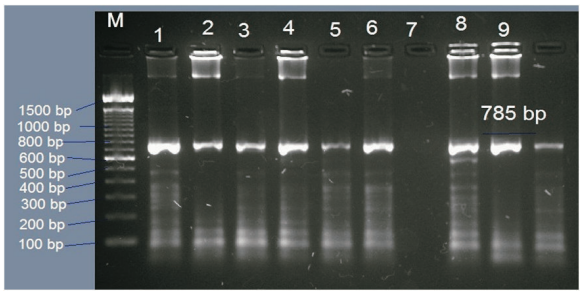

Fig. 3. Agarose gel electrophoresis of amplified PCR products obtained from *Theileria lestoquardi* genomic DNA using *Theileria lestoquardi* primers. Lane: M, 100 bp DNA marker; Lane: 1, positive control; 2 to 6, parasite positive blood sample; 7, negative control (distilled water); 8 to 10, parasite positive blood sample.

in small ruminants during a study from two provinces of Pakistan indicating that PCR is better than microscopy. There are only few reports on prevalence of theileriosis in sheep and goats in Punjab, Pakistan but to our knowledge this is first report describing the status on *Theileria* species infection in tehsil Jalalpur, distric Multan, Pakistan. Presence of *Theileria* species infection indicates that ovine theileriosis is endemic in this region.

Conclusion

PCR amplification method for the detection of ovine *Theileria* infections is sensitive and specific for tracing *Theileria* infection in carrier animals and provides validated measures that are valuable for studying the epidemiology of theileriosis in small ruminants. The present study revealed that sheep as compared to goats are more susceptible to *Theileria* infection. *T. ovis* is more important cause of ovine theileriosis in small ruminants. The herds comprising only sheep have higher risk of *Theileria* infection and prevalence rate of theileriosis in small ruminants in tehsil Jalalpur, district Multan cannot be ignored. In order to enhance livestock production in the studied areas special attention should be given to the control of *Theileria* piroplasms in sheep and goats.

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Short Communication

Photo-oxidation of Pasteurized Milk in Polyethylene Pouch Packs

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Abstract. In the present study photo-oxidative stability of pasteurized milk packaged in polyethylene pouches was investigated. Milk packaged in three layer polyethylene pouch packs was exposed to 400, 600 and 800 l× florescent light at 4 °C for 6 days, compared with a control (stored in dark). Light had a pronounced effect on fat content of milk with no effect on protein, lactose and ash content. Photo-oxidative stability of milk decreased as the intensity of light increased, peroxide value, anisidine value and conjugated dienes increased during the storage period of 6 days, higher values were observed in samples exposed to 800 l× florescent light. After 6 days of storage period, milk exposed to 400 l× florescent light did not reveal any oxidized flavour. The results of this study depicted that polyethylene pouches have the capability to resist photo-oxidation up to 400 l× light.

Keywords: pasteurized milk, photo-oxidation, polyethylene pouches, fluorescent light

Oxidation of unsaturated fatty acids and its results have been studied by Brien and Connor (2011); Richmond (2007); McSweeney and Fox (2003) and Fox and McSweeeney (1998). Photo-oxidation of milk leads to the boost of acetaldehyde and reduction of riboflavin. Oxidation of polyunsaturated fatty acids leads to the formation of numerous volatile and potentially toxic oxidation products (Cladman *et al*., 1998; Jeng *et al*., 1988). The photo-oxidative stability of pasteurized milk in polyethylene pouches has not been studied so far. This study aimed to determine the effect of light of various intensities on photo-oxidation of pasteurized milk in polyethylene pouches.

Collection of milk samples and experimental plan. Fresh pasteurized milk samples of same batch (3.5% fat) packed in 3 layer polyethylene pouch packs (low density polyethylene, linear low density polyethylene and high density polyethylene) of one liter capacity were procured from Lahore and exposed to 400 l×, 600 l× and 800 l× fluorescent light at 4 °C for 6 days, compared with control (3.5% fat) packed in polyethylene pouches stored in dark at same temperature and similar length of storage period. The effect of photo-oxidation on milk composition was determined for 6 days at the interval of 2 days using lactoscan (Julie Z-7, Slovakia). Fat from milk was extracted by the standard method of AOAC (2000). Peroxide value, anisidine value, iodine value and refractive index were determined for 6 days at the frequency of 2 days (AOCS, 1995). Specific extinctions were measured at 232 nm in the ultraviolet region of the spectrum on a double beam spectrophotometer (Anwar *et al*., 2010). Sensory evaluation of pasteurized milk exposed to photo-oxidation was performed by a panel of 10 trained judges on a 9 point scale (1-worst, 9-excellent). The judges were asked to determine oxidized flavour (Larmond, 1987). The experiment was planned in a Completely Randomized Design. The collected data were analyzed through Analysis of Variance Technique. To determine the effect of treatments, storage and their interaction, two way analysis of variance was used. Means of the treatments were compared by Duncan's Multiple Range Test (Steel *et al*., 1997).

Effect of light on milk composition. Light exposed samples at different light intensities (300 l×, 600 l× and 900 l× fluorescent light showed significant changes in different parameters. Light had a pronounced effect on fat content of pasteurized milk but to varying extents (Table 1). The drop in fat content of the experimental samples and control was in the order of $T_3 > T_2 > T_1 > T_0$. A linear correlation between fat content and light strength was observed. The harmful effect of light on oxidation of milk has been well established (Shahidi, 2005). It is evident from the results that light did not

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Table 1. Fat, protein, lactose and ash contents of pasteurized milk in polyethylene pouch packs exposed to different intensities

Treatments	0-Day	2-Day	4-Day	6-Days
Fat contents				
T_o	3.92±0.02a	3.87±0.06a	3.87±0.06a	3.84±0.04a
T_1	3.89±0.02a	3.85±0.06a	3.83±0.24a	3.83±0.24a
T_2	3.84±0.02a	3.82±0.24a	3.79±0.4a	3.77±0.04a
T_3	3.74±0.02a	3.65±0.01a	3.51±0.04b	3.35±0.27c
Protein contents				
T_o	3.18±0.03	3.18±0.03	3.17±0.02	3.16±0.02
T_1	3.18±0.03	3.17±0.003	3.17±0.02	3.15±0.01
T_2	3.17±0.02	3.16±0.02	3.15±0.01	3.14±0.01
T_3	3.18±0.03	3.16±0.02	3.14±0.01	3.12±0.009
Lactose contents				
T_o	4.33±0.03	4.31±0.03	4.27±0.02	4.24±0.02
T_1	4.33±0.03	4.30±0.03	4.26±0.02	4.21±0.02
T_2	4.32±0.03	4.27±0.02	4.24±0.02	4.22±0.02
T_3	4.33±0.03	4.29±0.02	4.25±0.02	4.25±0.02
Ash contents				
T_o	0.66±0.02	0.65±0.01	0.65±0.01	0.64±0.05
T_1	0.66±0.02	0.66±0.02	0.65±0.01	0.64±0.05
T_2	0.66±0.02	0.65±0.01	0.64±0.05	0.64±0.05
T_3	0.66±0.02	0.65±0.01	0.65±0.01	0.64±0.05

Within rows & columns means denoted by different letters are different. T_o: Control; T_1: Pasteurized milk stored in 300 l× fluorescent light; T_2: Pasteurized milk stored in 600 l× fluorescent light; T_3: Pasteurized milk stored in 900 l× fluorescent light; All the figures present in Table 1 are statistically non-significant.

Table 2. Anisidine, conjugated dienes, peroxide value and iodine value of pasteurized milk in polyethylene pouch packs exposed to different intensities

Treatments	0-Day	2-Day	4-Day	6-Days
Anisidine value				
T_o	3.76±0.04efg	4.71±0.41cdef	5.76±0.64bc	5.88±0.25g
T_1	3.76±0.04efg	6.37±0.33b	8.22±0.05a	7.62±0.50a
T_2	3.76±0.04efg	5.47±0.56bcd	7.62±0.04g	7.68±0.37fg
T_3	3.76±0.04efg	6.35±0.83def	8.41±0.14a	8.71±0.02a
Conjugated dienes value				
T_o	0.24±0.02i	0.13±0.01i	0.23±0.008i	0.27±0.40a
T_1	0.24±0.02i	0.21±0.06i	0.64±0.03h	0.85±0.01gh
T_2	0.24±0.02i	1.09±0.04efg	1.28±0.01de	1.21±0.008ef
T_3	0.24±0.02i	1.58±0.03cd	1.81±0.03bc	1.99±0.008ab
Peroxide value				
T_o	0.18±0.02f	0.25±0.03e	0.30±0.05e	0.37±0.05e
T_1	0.20±0.02f	0.27±0.03e	0.45±0.06d	0.52±0.07d
T_2	0.17±0.02f	0.30±0.03e	0.67±0.08c	1.34±0.01b
T_3	0.22±0.02f	0.35±0.05e	0.79±0.09c	1.92±0.15a
Iodine value				
T_o	33.10±0.20a	32.56±0.14b	32.43±0.23b	31.40±0.05b
T_1	33.10±0.20a	30.46±0.14c	30.20±0.05c	29.43±0.12c
T_2	33.10±0.20a	30.20±0.12c	29.53±0.17c	28.96±0.08d
T_3	33.10±0.20a	28.23±0.12d	27.53±0.14e	26.96±0.14f

Within rows & columns means denoted by different letters are different; T_o : Control; T_1: Pasteurized milk stored in 300 l× fluorescent light; T_2: Pasteurized milk stored in 600 l× fluorescent light; T_3: Pasteurized milk stored in 900 l× fluorescent light.

have any significant effect on protein content of fresh milk (Table 1). Non-significant changes in lactose and ash content were observed when pasteurized milk was exposed to different light intensities (Table 1).

Anisidine value. Anisidine value of all the experimental samples increased during 6 days of storage period (Table 2). The increase in anisidine value depends upon intensity of light, storage time and the type of packaging material. Storage time and packaging material was same for all the treatments. The rise in anisidine value during the storage period of 9 days was in the order of $T_3 > T_2 > T_1 > T_0$.

Conjugated dienes. Conjugated dienes of all the samples increased during the storage period of 9 days (Table 2). Moyssiadi *et al*. (2004) reported an increase in conjugated dienes value when the milk was exposed to different light intensities in PET containers; however, little is known regarding the photo-oxidative stability of milk in polyethylene pouch packs. With the progression of storage period in photo-oxidation conditions, sensory score deteriorated. The rise of conjugated dienes at all the determination frequencies were in the order of $T_3 > T_2 > T_1 > T_0$.

Peroxide value. Peroxide value of all the samples increased throughout the storage period of 9 days (Table 2). The rise in peroxide value during the storage period was due to the photo-oxidation. The peroxide value increased as a function of light intensity, the effect of treatments, storage and their interaction was also significant. Peroxide value also had a great effect on the sensory characteristics of milk. The strong correlation between peroxide value and sensory score has been reported by Shiota *et al*. (2004) when ice cream was exposed to light, the length of exposure was directly related to the peroxide value.

Iodine value. Milk fat is composed of higher concentration of saturated fatty acids and lower extent of unsaturated fatty acids. Iodine value of all the treatments and control decreased during storage period of 9 days (Table 2). Iodine value of stored fats and oils is lower than the fresh stuffs (Anwar *et al*., 2010). Chatha *et al*. (2011) and Gulla and Wagahary (2011) also recorded

a similar fashion of decline in iodine value when canola oil was stored at ambient temperature for longer period of time.

Flavour score. Light exposure had a great effect on flavour score of pasteurized milk, changes in flavour score was dependent upon the intensity of light and storage period, both the factors had a great effect on flavour score of pasteurized milk (Table 3).

Table 3. Oxidized flavour score of pasteurized milk in polyethylene pouch packs exposed to different intensities

Treatments	0-Day	2-Day	4-Day	6-Days
T_o	8.2±0.26a	8.2±0.21a	7.5±0.15c	6.8±0.31d
T_1	8.2±0.26a	7.8±0.14b	7.0±0.16d	6.6±0.08e
T_2	8.2±0.26a	7.4±0.11d	6.9±0.13d	6.1±0.17g
T_3	8.2±0.26a	7.1±0.19d	6.4±0.24f	5.5±0.09h

Within rows & columns means denoted by different letters are different.

The correlation between the flavour score and light intensity was linear (R^2= 0.952). The decline in flavour score of treatments and control was in the order of $T_3 > T_2 > T_1 > T_0$. The strong correlation between light intensity and flavour score of ice cream has already been established (Shiota *et al*., 2004). The deterioration in flavour score was caused by the photo-oxidation of milk i.e., higher peroxide value yielded lower flavour score.

Conclusion

The results showed that exposure of pasteurized milk packed in polyethylene pouches at 4 °C had no effect on compositional attributes except fat content, which decreased as a function of photo-oxidation. Experimental samples exposed to 600 and 800 l× light yielded the higher extents of oxidation products, as compared to 400 l× light. Milk exposed to 400 l× light florescent light, for 6 days in three layered polyethylene pouch did not develop oxidised flavour.

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Review

Control of Avian Coccidiosis: Present and Future Strategies for Natural Alternatives of Therapeutics

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Abstract. Avian coccidiosis has great economic impacts on poultry enterprise. Coccidiosis is caused by *Eimeria* species mostly affecting epithelium of the bird's intestines causing enteric problems. Prominent clinical outcomes are bloody diarrhoea, poor FCR, weight gain and growth rate with high morbidity and mortality rate. For the control of coccidiosis various strategies have been adapted including chemical agents and feed additives. But due to their repeated use, drug resistance to *Eimeria* species emerged thus badly affecting their efficacy. Moreover, these chemical agents have adverse effects on bird's health and meat quality. Therefore, alternatives are used nowadays including natural and herbal products having the desired efficacy without harmful effects. Natural products and their anticoccidial activity have been reviewed in this study. This group comprises herbal extracts, fatty acids, fungal extracts, probiotics and immune response immunomodulators with proven anticoccidial activity. Additionally, poultry industry and economic cost of coccidiosis as well as classical strategies used in the control of avian coccidiosis are also reviewed.

Keywords: coccidiosis, natural alternatives, poultry, probiotics, herbal extracts

Introduction

Commercial poultry farming is one of the most flourishing industries in the world and it provides the cheapest source of animal proteins to human beings (Ahmad *et al*., 2011). In Pakistan, out of total meat production, 19% is contributed by poultry (Ahmad *et al*., 2010). Poultry farming is increasing day by day at commercial level however, due to certain diseases like coccidiosis, its development is affected in Pakistan and other countries of the world (Saima *et al*., 2010). Coccidiosis is mainly caused by single celled protozoan of genus *Eimeria* of phylum Apicomplexa with complex life cycle. In commercial poultry farming, the incidence of coccidiosis ranges from 5 to 70% (Du and Hu, 2004). *Eimeria* species mostly affect the epithelial cells of bird's intestine, resulting in enteric problems leading to bloody diarrhoea, poor growth rate and high mortality with heavy economic losses up to three billion dollar per annum worldwide (Dalloul and Lillehoj, 2006). So far seven species of genus *Eimeria* (*E. mitis*, *E. maxima*, *E. acervulina*, *E. necatrix*, *E. brunetti*, *E. tenella* and *E. praecox*) have been identified that are responsible for coccidiosis in chickens by residing in different parts of their intestines. The most devastating specie is *E. tenella* that causes ceacal coccidiosis in chickens. Ceacum is the predilection site for *E. tenella* sporozoites that invades its mucosa through villi of epithelial cells and results in severe damage to epithelium, reduced weight gain, blood in feaces, poor feed efficiency and ultimately death of birds that leads to heavy economic losses (Zaman *et al*., 2012). Sub-clinical form of avian coccidiosis manifests as immunosuppressant in the birds that result in outbreak of secondary bacterial diseases. During rainy seasons, there is high incidence of coccidiosis (Etuk, 2010). The hot and humid weather positively hastens prevalence of disease by providing favourable conditions to the infective oocyst.

Various strategies in the past have been adopted to overcome avian coccidiosis including use of synthetic chemicals and anticoccidial feed additives. However, with the passage of time and repeated use of these drugs there is emergence of anticoccidial drug resistance in *Eimeria* species (Abbas *et al*., 2012). Along with this Nogueira *et al*. (2009) has also reported that harmful effects of these chemicals on bird's health status and

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consumption of affected meat by humans are the major constraints. Another technique for the control of coccidiosis is the use of live vaccines but this practice may lead to development of clinical disease in the broiler under poor management (Chapman, 2000). Owing to increasing drug-resistant parasite strains, the use of these chemical agents proved ineffective and there is need of time to introduce alternatives for the control of disease (Abbas *et al*., 2012; Tacconelli, 2009). Resistance development in target parasites, high cost, and toxic effects of chemotherapeutic drugs are the main constraints. Therefore, for the prevention of coccidiosis, current circumstances demands alternatives. Natural and synthetic biological compounds considered as appropriate agents (Patwardhan and Gautum, 2005), to sustain bird health without affecting performance. Plant based biological active compounds are thought to be promising candidates due to their availability and powerful efficacy with no or minimal residual effect in consumers (Patwardhan and Gautam, 2005). To cure health related issues almost 64% of human population use plant based drugs worldwide (Farnsworth, 1999).

During last decade, various authors have reported that plant and herbal products have protective and therapeutic effects against experimental coccidial infections in birds (Lorrain *et al*., 2010; Nweze and Obiwulu, 2009). It has been reported that 50% synthetic drugs have been made from compounds of plants. In this regard, one of the important source is cereals. In humans and other animal models, studies have shown that dietary cereal fibres may have a great effect on different physiological parameters. Cereals based carbohydrates are mainly reported as antitussive, antioxidant, antimutagenic, anti-inflammatory, anti-cancerous and immunomodulators (Zhou *et al*., 2010). Cereals, fungi and yeast cell wall contain β-glucans as principle structural components with molecular weight 2000 kDa approximately. These polysaccharides enable the host to develop resistance against viral, parasitic, fungal and bacterial diseases by enhancing immune system, lysosomal enzyme activity, phagocytosis and IL-1 production (Estrada *et al*., 1997). Botanicals may act as best and safe alternative source to anticoccidial drugs for the prevention of coccidiosis (Abbas *et al*., 2012). A variety of botanicals including *Triticum aestivum*, *Hordeum vulgare*, *Emblica officinalis*, *Aloe vera* and *Avena sativa* have shown effective biological responses. *Oryza sativa* also known as Asian rice, belonging to family *Poaceae* is an annual plant, which has shown immunostimulant effect through increased allergic reactions, inhibited mast cell degradation in rat (Ghatak and Panchal, 2012; Oka *et al*., 2010), increased nitric oxide, TNF-α and interleukins in murine macrophages (Park *et al*., 2013) as well as increased lymphocytic proliferation, TNF-α and interferon-γ (Ghoneum and Jewett, 2000). The denatured bran of this plant results in arabinoxylan by means of enzymatic hydrolysis, a polymer of xylose and arabinose (Ghoneum, 1998). Alternatives are not only natural products but they also contain valuable molecules to which resistance has not yet developed. Herbal and medicinal plants, fungal extracts and probiotics could have additional significant benefits such as palatability, low cost, low toxicity and non-residual properties (Table 1-3). Currently interest has been developing in the use of these natural products to reduce the chances of coccidiosis.

Review of literature. Favourable results have been obtained by using natural alternatives in avian coccidiosis as described in the following review literature:

Antioxidants. ***Fats and oils.*** Deficiency of vitamin A in poultry increases susceptibility to coccidiosis and other enteric infections. Alterations in intraepithelial lymphocytes due to vitamin A deficiency increase susceptibility of birds to coccidiosis (Dalloul *et al*., 2003). Fats contained higher concentrations of linolenic acid, eicosapentanoic acid and reduced severity of coccidiosis in young broilers. Feed supplemented with 10% linseed oil, 10% flax seed oil and 10% fish oil markedly reduced ceacal lesions. Fats decreased parasitic infestation and provided immunity against *Eimeria* (Allen *et al*., 2000). Antioxidants have been divided into two categories; i.e., fat soluble (Vitamin E and carotenoids) and water soluble (Vitamin C, uric acid, glutathione and lipoic acid). Fat soluble antioxidants hinder lipid peroxidation and protect cell membranes while water soluble reacts with free redicals existing in cytosol and blood plasma (Surai, 2007). Utilisation of botanical antioxidants is correlated in control of coccidiosis because coccidial infections are associated with lipid peroxidation of mucosa of intestines and antioxidants prevent such lipid peroxidation (Naidoo *et al*., 2008). Coccidiosis infections promote lipid peroxidation in broilers and dietary alpha tocopherols at 316 mg/kg improved oxidant/

antioxidant system in victim birds (Masood *et al*., 2013). Antioxidant nature of ground corn showed that corn inhibited generation of lipid hydro peroxides and their secondary products. This activity was assumed due to bound hydroxyl-cinnamates like *p*-coumaric acid and ferulic acid (Bauer *et al*., 2013).

Antioxidant potential of corn bran. Corn husk derived arabinoxylans have been reported to possess an immunostimulatory effect, characterised by increased cytokines production and natural killer cells activity (Ogawa *et al*., 2005; Zhang *et al*., 2004). Bauer *et al*. (2013) reported the antioxidant potential of ground untreated corn fibre and wheat bran against lipid oxidation based on oxidative products inhibition. It was concluded that corn fibre had higher antioxidative effect at 800 mg/kg emulsion concentration which inhibited lipid hydroperoxides formation than wheat bran based on molar formulated oligosaccharides products through enzymatic treatment and methanolic extraction.

Herbal extracts and medicinal plants. Cereals derived β-glucans not only have antitussive and adipogenic activity, but they are also very important in tumor regression. Morikawa *et al*. (1985) conducted a study on wheat derived β-glucans. Results of their study showed about 100% increased polymorphonuclear leukocytes activity against tumor cells. Immuno-modulatory properties of oat derived β-glucans are not new, about two decades earlier a study was conducted by Estrada *et al*. (1997), that investigated the role of β-glucans on mice immune system. Results of their study revealed the release of TNF-α, IFN-γ, IL-4, IL-2 and IL-1 from peritoneal macrophages. Oat is an important plant that has an extensive list of biological effects. It contains proteins, fats, total digestible nutrients, minerals and vitamin (thiamine). Oat is one of the beneficial grains, which is used as nutritional compound in both humans and animals. Oat can be used as an antioxidant compound due to abundant amount of two main enzymes; alcalase and tryptic that actively transform proteins into peptides and these peptides perform antioxidant activity (Hussain *et al*., 2002). In emergency circumstances and scarcity period oat can be used to form hay or silage. Due to this reasons it is commonly used in the diet of young animals, poultry, dairy cows and horses (Hussain *et al*., 2002).

In another study, Yun *et al*. (2003) reported that oat β-glucans were dynamically responsible for the enhancement of resistance in mice, against *E. vermiformis* and *S. aureus*. To support his idea he conducted *in vitro* and *in vivo* studies that enabled the immune system to increase phagocytic activity. Results of their study were satisfactory that fecal oocyst shedding reduced by 28% in intragastric and 39% in intraperitoneal group of mice (C57BL/6) that were initially infected with *E. vermiformis* as compared to control group. Moreover, oat cell wall polysaccharide, arabinoxylans and β-glucans are much important regarding health point of view. These carbohydrates are mainly effective against tumor growth and stimulate immune system (Vetvicka, 2011). *Avena sativa* locally known as jodar, javi or jai is an important cereal grain that has positive impact on consumer's health. Regarding their chemical structure, in recent years Havrlentova *et al*. (2011) reported that oat contained an important polysaccharides, β-glucans. It was concluded that one of the characteristic feature was their chemical structure β, 1-4 linkage due to which these were effective against bacteria, viruses and other pathogens. Additionally, these had anti-tumor activity and also act as free radical scavengers. Oat derived β-glucans may have some additional properties. A study was conducted by Vetvicka (2011), investigating the immunomodulatory and adipogenic activity of oat β-glucans. Moreover, it was found that these were helpful in wound healing process and also decreased the skin irritation.

Beta vulgaris. *Beta vulgaris* belongs to the family Chenopodiaceae and commonly known as red beet. *B. vulgaris* is a traditional plant in India and many countries including Pakistan and extensively used to treat as a therapeautic agent in hypertension, in diabetes and cancer due to its antioxidant properties. Roots of *B. vulgaris* have also been reported to be rich in antioxidant compounds (Shrishailappa *et al*., 2007). Its juice has been found to reduce the xenobiotic-oxidative stress in rats by rejuvenating the activity of the majority of antioxidant enzymes in liver (Kujawska *et al*., 2009). The active compounds present in *B. vulgaris* root include folic acid (15.8 mg/g), betacyanins and betaxanthines prevent active oxygen-induced, free radical scavengers and help in radical-mediated oxidation of biological molecules. *B. vulgaris* methanolic extract bears anticancerous and immunomodulatory activities (Tripathy and Pradhan, 2013).

Camellia sinensis. *Camellia sinensis* (green tea) is an important plant which contains natural flavonoids.

These flavonoids are known to have antioxidant properties due to which it can be used as an excellent anticoccidial agent. Furthermore, it also contains lipids, volatiles compounds, amino acids (especially l-theanine), carbohydrates, alkaloids, carotenoids and minerals which are helpful against several diseases. It reduced fecal oocyst shedding and improved weight gain when supplemented in feed of broilers (Jang *et al*., 2007). *Camellia sinensis* also has anti-inflammatory antiproliferative anticancer antibacterial, antiviral trypanocidal agent and also showed inhibitory effect against ovine Babesia (Chen *et al*., 2008).

***Eclipta alba*.** *Eclipta alba* which is also known as *Eclipta prostate* having white flowers is renowned to use as diuretic and tonic agent in treatment of various hepatic diseases. In an experiment, there was appreciable weight gain observed in broiler birds infected with *Eimeria* which were orally treated with *Coumestans* obtained from *Eclipta alba* at the rate of 120 ppm. It also resulted in 80% reduction in excretion of oocysts and treated birds did not show any signs of toxicity such as hepatic and muscular lesions but, at higher dose rate (180 ppm), signs of toxicity were observed in birds (Michels *et al*., 2011).

Aloe vera. Aloes are known medically most important plants and are used to treat many disease conditions due to their therapeutic properties. More than 360 species of aloes are identified but *A. excelsa* is well known species that has excellent anticoccidial properties, comparable with sulphachlopyrazine sodium monohydrate. Supplementation of *A. excelsa* in feed improved mean weight gain and reduced oocysts expulsion in broiler birds (Gadzirayi *et al*., 2005). Another important species of aloe plant is *Aloe vera* which is also known to have anticoccidial properties. Recently, Akhtar *et al*. (2012) has conducted a study to evaluate the anticoccidial and immunomodulatory effects of both aqueous and ethanolic *Aloe vera* extracts against coccidiosis in broiler birds and reported excellent results of *Aloe vera* in terms of improved immune response and increased weight gain in birds against mixed *Eimeria* infection. So, it can be effectively used as an immunomodulatory agent against avian coccidosis. In a study, supplementation of *A. vera* in feed showed remarkable effect in lowering gut lesions and oocyst excretion of *Eimeria* in faeces of broiler chickens (Yim *et al*., 2011).

***Pinus radiata*.** *Pinus radiata* belongs to family Pinaceae which is commonly used as Monterey pine or radiata pine, has phenolic compounds due to which it has been as traditional medicinal plant in various countries such as Europe and North America. Molan *et al*. (2009) evaluated the *in vitro* efficacy of *Pinus radiata* aqueous extracts on prevention of oocysts sporulation of different *Eimeria* species. Inhibition bioassay was used to investigate the effect of PBE on the sporulation of *Eimeria* oocysts. The results of study revealed that incubation of unsporulated oocysts in water containing 500 μg PBE per mL caused the prevention of sporulation of these oocysts by 28-84% as compared to control tubes. Furthermore, 12% of *Eimeria* oocysts showed abnormal sporocysts in size, shape and number. This study suggests that pine bark extract can be used for control of coccidiosis in broiler birds.

***Ageratum conyzoides*.** The Asteraceae or Compositae (referred to as the aster, daisy, sunflower family) is a widespread family of flowering plants having phenolic compounds. Due to this property it has been used against ceacal coccidiosis as well as acute toxicity and given orally at dose rate (250-300 mg/kg to 500-1000 mg/kg). Reportedly casing increased weight gain and increased red blood cell count (Nweze and Obivulu, 2009).

***Curcuma longa*.** Turmeric (*Curcuma longa*) is a rhizomatous herbaceous perennial plant of ginger family. Effectiveness of turmeric (*C. longa*) crude powder and salinomycin sodium against occurrence of coccidiosis in broiler has been reported that feed consumption, body weight gain and feed conversion ratio were better in birds supplemented with 3% turmeric powder than untreated group (Mashhadan, 2015). Reduction in oocyst excretion and bloody diarrhoea was almost similar to the anticoccidial drug "salinomycin sodium" (Abbas *et al*., 2010).

***Saccharum officinarum*.** Sugarcane (*Saccharum officinarum*) belonging to the genus *Saccharum* is one of the several species of tall perennial true grasses of warm temperate to tropical regions of South Asia Melanesia. The effectiveness of sugar cane juice and baggase were reported to counter broiler coccidiosis. Water and ethanolic extracts were used to evaluate the therapeutic efficacy of sugar cane. It showed good results in terms of reduced oocyst shedding and mortality rate was also low (Akhtar *et al*., 2012). Similarly, higher

weight gain and antibodies response observed in challenged birds but relative lymphoid organ weight ratio was not significant. However, ethanolic extract was more effective than water extract (Awais *et al*., 2011).

***Ocimum basilicum*.** Plants belonging to genus *Ocimum* have 50-150 species of shrubs and herbs found in different sub-tropical and tropical areas of Africa, Asia, South and Central America. Among these species, *Ocimum basilicum* has over 50 therapeutic activities and has been reported to treat large number of disease conditions. It is antiseptic, antibacterial, febrifuge and aneurostimulant (Onwurah *et al*., 2011). Another study was conducted to determine the effect of basil in broiler coccidiosis. The study revealed that provision of this plant extract to diseased birds showed good results with respect to feed conversion ratio, live weight gain, oocyst count and hematological parameters. However, the effect on mortality and daily feed intake were not statistically significant. This study recommended prophylactic and curative dose as 5 and 15 g, respectively in feed and water (Onwurah *et al*., 2011).

***Artemisia annua*.** *Artemisia annua*, also known as sweet wormwood or sweet annie. The dietary effect of *Azadirachta indica* and *Artemisia annua* has been observed on broilers performance and anticoccidial potency against *E. tenella* post infection trial. Three parameters; feed conversion ratio, average body weight gain and feed intake were focused for the evaluation of anti-coccidial activity of both plant extracts in challenged groups. Significant results were observed in orally infected bird's group with *A. indica* (10%) or *A. annua* (5%) incorporation in broiler diet (Hady and Zaki, 2012).

***Thonningia sanguinea*.** *Thonningia sanguinea* is a renowned plant used as traditional medicine in African countries. Antioxidant and antiprotozoal activity of this plant has been observed against different diseases in birds. The results of study showed that dose more than 2.5 mg/mL significantly inhibited the invasion of bovine kidney cells by the sporozoites of *E. necartix* and *E. tenella*. This study demonstrated that *Thonningia sanguinea* can be effectively used in feed for the control of coccidiosis in broiler birds (Severin *et al*., 2012).

Immune response modulators. ***Immunostimulatory and therapeutic potential of rice bran*.** Rice is also known as an important immunostimulant and therapeutic agent against avian coccidiosis. Martinez *et al*. (2015) described the increased cytotoxic potential of natural killer (NK) cells by means of rice bran derived Arabinoxylans (MGN-3) against murine neuroblastoma. For this study, NK cells activity by means of MGN-3 addition was evaluated for their phenotypic, cytotoxic and cytometric bead potential on cultured cell lines both *in vivo* and *in vitro*, respectively for two weeks. MGN-3 showed significantly increased activity for CD25 and CD69 receptors without alteration of induced self antigens and other non-catalytic receptors. Moreover, carcinoma cells growth was also inhibited due to increased NK cells cytotoxicity. These results concluded the immunoregulatory efficacy of NK cells and their beneficial remedial application for neural carcinoma. Fang *et al*. (2012) elaborated the anti-inflammatory and therapeutic results of acid hydrolysed feurloylated oligosacchrides (FO) from rice bran at dose rate of 0.1-100 µg/mL through increased TNF-α, IL-1β, IL-6 and PGE2 production in RAW264.7 macrophages. Ani *et al*. (2013) concluded that feeding rice milling waste (RMW) improved growth rate through increased feed intake in broiler chicks. Rice is also known as an important immunostimulant and therapeutic agent against avian coccidiosis. Choi *et al*. (2014) reported the immunomodulatory activity of rice bran derived arabinoxylans through increased natural killer (NK) cell activity and modulated cytokine production in humans. Ghoneum *et al*. (2014) evaluated anticancer effects of rice bran derived arabinoxylan on cultured nonmetastatic MCF-7 human and metastatic 4T1 mice breast cancer cells. MGN-3 increased apoptosis, DNA damage and inhibition of cellular proliferation in 4T1cells. It was also concluded that rice bran derived arabinoxylans have a potent chemotherapeutic effect against metastatic breast cancer.

Ani *et al*. (2013) conducted a study to evaluate comparative effects of feeding rice milling waste (RMW) and Roxazyme G2® enzyme on the performance of broiler chicks. Enzyme supplementation significantly reduced feed intake and improved the performance of birds in terms of average daily weight gain, protein efficiency ratio and feed cost. Enzyme supplementation significantly reduced feed intake and improved the performance of birds in terms of average daily weight gain, protein efficiency ratio and feed cost. These results

concluded that feeding rice milling waste (RMW) improved growth rate through increased feed intake in broiler chicks. Rohman *et al*. (2014) described the nutritional significance of rice for diabetic patients in Asia. Besides other cereals, there is an increased dietary intake of rice due to glucose rich contents. Similarly, rice derivatives including its bran and oil in traces have also been reported to exhibit beneficial effects on health. Fat free rice bran would be resulted on further processing of rice husk. Fat free fraction of rice bran contained carbohydrates rich contents. These contents of rice derivatives exhibit their therapeutic potential against cardiac diseases and cancer in terms of hypocholestrolemic and hypoglycemic effects.

Immunomodulatory potential of barley. Yun *et al*. (1997) evaluated the immunostimulant potential of oat derived β-glucans (1→ 3, 1→ 4) at dose rate of 0.2 mL intraperitoneally in CD-1 mice in terms of increased number of macrophages in peritoneal fluid and improved survival in 3 days pre-exposure to *S. aureus*. Moreover, its subcutaneous dose of 0.l mL or administered intragluteally 0.3 mL daily for 10 days in immunosuppressed and infected C57BL/6 mice with coccidiosis reduced fecal oocyst output by 23 and 34%, as well as increased total antibody titers against sporozoites and merozoites. On the whole oat β-glucans showed immunostimulatory effects in terms of increased humoral and cellular immune responses against *E. verniformis* infection in mice.

These results suggested an immunostimulant potential of oat β-glucan against coccidiosis. Pelizon *et al*. (2005) evaluated the immunostimulatory effect of *Sacchromyces cerevisiae* derived β-glucans at dose rate of 20 µg I.P. in female C57BL/6 mice by increased IL-12, TNF-α and natural killer spleen cells. β-glucans are soluble non-starch and complex polysaccharides abundantly found in bacterial, fungal, oats and barley cell walls which play their vital role in anti-microbial immunomodulation. Angeli *et al*. (2009) examined the mutagenicity of barley derived β-glucans in hep G2 hepatocytes at concentrations of 100 and 200 mg/mL, respectively. Chan *et al*. (2009a) concluded the receptor based mode of action of β-glucans with respect to natural or acquired immunity in terms of lymphocytic proliferation and differentiation in reticuloendothelial system (RES). Talati *et al*. (2009) suggested that barley supplementation could reduce total cholesterol, triglycerides and the low density lipoprotein levels in humans. Takeshi and John (2010) described anti-inflammatory and immunostimulant potentials of 1 g barley derived arabinoxylans against irritable bowel syndrome (IBS) in humans in terms of improved gastrointestinal symptoms and increased NK cell activity. Samuelsen *et al*. (2011) concluded the low immunomodulatory response of barley derived arabinoxylans and β-glucans *in vitro*. Besides other cereals, barley is also known to have immunomodulatory potential against various diseases. Khoury *et al*. (2012) concluded the therapeutic significance of oats and barley derived β-glucans against obesity and metabolic syndrome through maintenance of enteric health in humans. Jacob and Pescatore (2012) reported the improved feeding values of barley litter through reduced intestinal viscosity while poultry farming. Gao *et al*. (2012) suggested that oat derived β-glucans supplementation decreased cholesterol level of serum and less enteric microbial activity with low immunomodulation in humans.

Immunomodulatory and therapeutic potential of wheat bran. Vahouny *et al*. (1985) evaluated an increased intestinal goblet cell secretary potential through 10% wheat bran dietary supplementation. Kruis *et al*. (1986) evaluated the supportive therapeutic efficacy of wheat bran in terms of superior symptomatic effect against human irritable bowel syndrome (IBS). Watzl *et al*. (1990) evaluated the phagocytic stimulant potential of wheat bran derived polysaccharides extract at dose rate of 10^{-2} mg/mL for human polymorphonuclear leukocytes. Roccatello *et al*. (1990) examined an increased *in vitro* cytotoxic activity of human peripheral blood mononuclear cells particularly of neutrophil chemokinesis by means of gliadin and glyc-gali at dose rate of 5 and 50 µg/mL, respectively. Takahashi *et al*. (1999) examined the inhibitory effect of microfibril wheat bran on azoxy-methane at dosage rate of 20% (w/w) and 10 mg/kg against colon carcinogenesis in female CF1 mice, respectively. Neyrinck *et al*. (2008) suggested the therapeutic value of wheat bran derived arabinoxylans against obesity and metabolic disorders at dose rate of 10% with decreased macrophages, IL-6 and CD68 mRNA in adipose tissues in mice. Cao *et al*. (2011) reported the antitumor effect of wheat bran arabinoxylans in S180 mice by promoting cell mediated immunity with peripheral leukocytosis and increased haemopeisis. Among these cereals wheat is an important

Table 1. Overview of the anticoccidial effect of botanicals against avian coccidiosis

Botanical and English names	Active ingredients	Dose	Mode of action	Species studied	Affected parameters	Sources
Agele marmelos (Bael)	Unknown	Orally @ 2 mL for 5 consecutive days	Unknown	Mixed infection	OC↓, FC↑, WG↑	Khan *et al.*, 2008
Ageratum conyzoides (Ageratum)	Flavonoids	Orally 500-1000 mg/kg b.w.	Oxidative stress	E.t	OC↓, WG↑, PCV↑, RBC↑, OC↑	Nweze and Obiwulu, 2009
Carica papaya (Papaw)	Papaine	Dry leaf powder 15% of feed	Sporozoits digestion in the caeca	E.t	-	Al-Fifi, 2007
Cyamopsis tetragonoloba (Guar)	Glacactomannans and saponins	5% of feed	Binding with sterol molecules present on protozoal cell membrane surfaces	E.t	OC↓, BD↓	Hassan *et al.*, 2008
Eclipta alba (Bhringaraj)	Coumestans	120 pp in feed	Unknown	E.t	OC↓, FC↑, WG↑	Michels *et al.*, 2011
Linum usitatissimum (Flaxseed)	N-3 fatty acids	15% of feed	Oxidative stress	E.t	LS↓, degree of parasitization↓	Allen *et al.*, 1997
Lentinus edodes and *Tremella fuciformis* (Mushrooms)	Polysaccharide extracts	1 g/kg feed	Immune stimulation	E.t	WG↑	Guo *et al.*, 2004; 2005
Musa paradisiacal (Banana)	Unknown	Methanolic extract @ 1,000 mg/kg b.w.	Unknown	E.t	OC↓, CS↓, PCV↑	Anosa and Okoro, 2011
Olea europaea (Olive tree)	Maslinic acid (2-α, 3-β-dihydroxiolean-12-en-28-oic acid)	90 ppm of maslinic acid in feed	Anti-inflammatory & antioxidant properties	E.t	OC↓, LS↓, WG↑	De Pablos *et al.*, 2010
Pinus radiate (Monterey pine)	35% condensed tannins	Oocysts exposed to 500-1000 mg pine bark/mL	Damage of cytoplasm (sporont)	E.t; E.m; E.a	Sporulation↓	Molan *et al.*, 2009
Pasum sativum (Pea plant)	Antibody fragments	-	Inhibition of sporozoites reproduction	E.t	Sporozoite infectivity and reproduction↓	Khalafalla and, Daugschies 2010
Sophora flavescens aiton (Sophora)	Unknown	6-30 g/L Drinking water	Unknown	E.t	M↓, LS↓, OC↓, WG↑	Youn and Noh, 2001
Tulbaghia violacea (Society garlic, sweet garlic)	Antioxidant compounds (S-(methylthiomethyl) Cysteine sulphoxide (marasmine), bis[(methylthio) methyl]disulphide and various derivatives)	Aqueous extract 35 mg/kg feed	Oxidative stress	Mixed infection	FCR↑, OC↓	Naidoo *et al.*, 2008
Vitis vinifera (Grape seed)	Tannins	10-20 mg/kg Feed	Oxidative stress	E.t	M↓, LS↓, WG↑	Wang *et al.*, 2008
Vernonia amygdalina (Vernonia tree)	Vernoside	Dry leaf powder @ 15% of feed	Oxidative stress	E.t	OC↓	Al-Fifi, 2007

↑ = improvement/increase/higher; ↓ = decrease/lower; E.t = *Eimeria tenella*; E.a = *Eimeria acervulina*; E.m = *Eimeria maxima*; E.b = *Eimeria brunetti*; E.n = *Eimeria necatrix*; E.miv = *Eimeria mivati*; WG = body weight gain; FC = feed consumption; FCR = feed conversion ratio; LS = lesion scores; OC = oocyst count; BD = bloody diarrhea; M = mortality; PCV = packed cell volume; RBC = red blood cells; CS = clinical signs.

Table 2. Overview of the anticoccidial effect of herbal complexes against avian coccidiosis

Herbal complex	Composition	Dose	Mode of action	Species studied	Affected parameters	Sources
Herbal complex	*Uncariae* ramulus cum Uncis, *Agrimoniae herba*, *Sanguisorbae radix*, *Eclipta prostrate herba*, *Pulsatillae radix*, *Sophorae feed flavescentis radix*, *Rehmanniaea radix*, *Glycyrrhizae radix*	2 g/mL drinking water & 10 g/kg	Unknown	E.t WG↑	LS↓	Du and Hu, 2004
Muscadine pomace	By-product of the production of wine and juice from *Vitis rotundifolia*	0.5-2.0% of feed	Unknown	E.a; E.m; E.t	M↓, LS↓, WG↑	McDougald *et al.,* 2008
Herbal complex	*Solanum nigrum* (35%), *Aloe vera* (15%), *Moringa indica* (35%) and *Mentha arvensis* (15%)	10% in feed for 7 days continuously	Unknown	E.t	LS↓, WG↑, FCR↑	Chandrakesan *et al.,* 2009
Herbal complex	*Allium sativum* 4 g, *Zingiber officinale* 6 g, *Azadirachta indica* 3 g and *Berberis lyceum* 10 g mixed per liter drinking water	Treated water for 14 days	Unknown	Mixed infection	OC↓	Nidaullah *et al.,* 2010
Herbal complex	*Azadirachta indica*, *Nicotiana tabacum*, *Calotropis procera* and *Trachyspermum ammi*	Orally (2-6 g once a day) for 7 Days	Unknown	E.t	M↓, LS↓, WG↑, FCR↑, OC↓	Zaman *et al.,* 2011

LS = lesion score; WG = weight gain; FCR = food consumption ratio; OC = oocyst count; M = mortality.

Table 3. Some natural products commercially available for prevention and treatment of coccidiosis

Product	Ingredients	Supplier
Avihicox	Clove and *Bocconia cordata* extract	Centaur
Nutrimin	Apple cider vinegar	Chicken lickin
Kocci Free	Olive leaf, mustard seed, black seed, cloves, grapefruit seed extract	Amber Technology
Oil of oregan	Oregan extra virgin olive oil (80% carvacrol)	Natural factors
Oilis	Natural vegetal extracts	Engormix
Oreganico	Oregan oil and essential oils	Flyte so fancy
Garlic granules	Garlic	Flyte so fancy
Poultry Provita	Probiotics and prebiotic inulin	Vets Plus
CitriStim	Mannan oligosaccharides and beta glucans	ADM
Orego Stim	Carvacrol (82%) and Thymol (2.4%)	Saife vetmed
Herban	Etheric oils, soya oils, oregan oils	Uncle Ted Organics Ltd
Herb 'n' thrive	Concentrated blend of herbs and essential oils	Chicken lickin
Eimericox	Several essential oils	Phytosynthese/Trouw Nutrition
Natustat	Several essential oils and yeast cell walls	Alltech
Enteroguard	Garlic and cinnamon	Orffa
Xtract Immunocox	Spanish pepper and turmeric	Pancosma
Coxynil	*Allium sativum* Linn 15%, *Cinnamonum camphora* Nees & Eberum 15%, *Elephantopus scaber* Linn 15%, *Valeriana wallicgii* DC 15%, Sulphur dioxide 25% and NaCl 15%.	Growell India
Ropadiar solution	Oregan oil (on diatomite)	Ropapharm

immuno-modulator against various diseases. Singh *et al*. (2015) described the immunomodulation of potent prebiotic, xylooligosacchrides (XOS) resulting from hydrolysis of agricultural byproducts. These XOS improved gut epithelial health for microflora and regulated antioxidant activity. Akhtar *et al*. (2012) evaluated the

immuno-stimulatory and anticoccidial potentials of wheat (*Triticum aestivum*) bran derived arabinoxylans (AXs) in chickens. These showed an improved daily weight gain, organ body weight ratio for thymus and cecal tonsils with lower oocyst count and lesion scores and higher antibody titers against sheep red blood cells.

Fungal extracts. Fungal, *Sclerotinia sclerotiorum* derived beta glucans are used to treat many disease conditions due to their therapeutic effects. Suzuki *et al*. (1989) conducted a study in mice and found that fungal derived β-glucans were responsible to modulate immune system. Higher spleen cell proliferation was noted in response to concanavalin A (T-cell mitogen) and lipopolysaccharide (B-cell mitogen) with comparison to control group of mice. Moreover, oral administration of *S. sclerotiorum* glucans also improved the function of NK (natural killer) cells and lysosomal enzyme that actively fight back against numerous pathogens. In addition to these, antitumor activity was also observed.

This study suggests that *S. sclerotiorum* derived beta glucans can be used to enhance immune system. *Saccharomyces cerevisiae* is an important fungus that may have immunostimulatory effect by increasing different interleukins and cytokines production. In a study, Pelizon *et al*. (2005) investigated that *S. cerevisiae* derived β-glucans were responsible for increasing the fungicidal and natural killer cell activity. Moreover, increased cytokine production, IL-12 and TNF-α was also observed. Among body's complex system one is the immune system that is broadly categorized into adaptive and innate immune system. It is proved experimentally that both these system can be modulated by β-glucans. Chan *et al*. (2009b), suggested that these polysaccharides initiated their response through immune receptors. These receptors included CR3 (complement receptor), TLR-2/6, Dectin-1 and activated a set of immune cells including dendritic cells, monocytes, neutrophils, NK cells and macrophages. These polysaccharides with specific linear backbone 1→3 beta-glycosidic chain could not be digested orally. Some beta glucans were initially internalized by macrophages and then fragmented inside the cells. After that these fragmented β-glucans were released and eventually picked up by other immune cells. Moreover, β-glucans with variable branching patterns and sizes had different immune potency.

In recent years, an experiment was performed by Mahdi and Al-Abass (2012), and they concluded that *S. cerevisiae* derived β-glucans had immunomodulatory effect in broilers. Results of their study showed that administered group had faster clearance rate of carbon particles than untreated control group. The fast clearance rate might be due to increased phagocytic activity. Moreover, it was also observed that β-glucans administered group had improved their body weight gain than control group. Conclusions of their study suggested that β-glucans can be used to improve weight gain in broilers.

Conclusion

Over a time, anticoccidial drugs development has increased in response to the urgent need to control avian coccidiosis. Today, we have many strategies available, many of which currently widely used in chickens farms. Moreover, new alternatives are emerging, as is the case with anticoccidial is obtained from plants, fungal or microorganisms. One of advantages of using natural extracts is the lower risks of developing resistance, such as that observed with chemical drugs. It is widely known that availability of raw materials and cost of production could be high in the development of natural extracts alternatives. However, the cost is well worth it if we consider that these alternatives are friendly to the environment, producers and consumers.

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Erratum

Author's names Dr. Safdar Ali Mirza and Shahjehan Baig were inadvertently omitted in PJSIR Vol. 58, Ser. B: Biol. Sci. No. 1, pp.23-29 (2015). The inconvenience caused to the authors is highly regretted.

Executive Editor