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Effect of Storage Period on the Microbiological and Sensory Characteristics of Cooked Low Salt White Soft Cheese (Gebna Beyda)

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Abstract: This study was carried out to evaluate the microbial and sensory characteristics of cooked low salt white soft cheese during storage. Low salt cheese was made from pasteurized (72°C/1 min) cow's milk. After complete coagulation, the curd was cooked at 35°C for 30 min. Cheese was preserved in 2% brine solution for 48 h and then transferred to plastic bags and stored in the refrigerator at 4°C for 60 days. Microbiological examination as well as sensory characteristics was carried out at 1, 20, 40 and 60 day intervals. The results indicated that, during storage period the total viable bacteria count did not show significant change, while psychrotrophic bacteria and yeasts and moulds counts significantly increased throughout storage period. Storage period did not significantly affect the colour, while the flavour, taste, body, saltiness and overall acceptability were significantly affected by the storage period.

Key words: Cooked white soft cheese, low salt, storage period, cow milk

INTRODUCTION

The dominant and the most popular type of cheese manufactured in Sudan is Gebna Bayda, the local name for white soft cheese (Khateeb, 1997), which may be consumed fresh but more commonly after maturation in salt brine or salted whey (Abdalla and Abdel Razig, 1997). The cheese is traditionally made by adding 6-20% salt without the use of starter culture (Abdalla and Davidson, 1990; Abdalla *et al*., 1993). Salting process in cheese not only contributes to the flavour of cheese, but can also have an impact on the growth of microorganisms with the viability of probiotic bacteria being inversely proportional to salt concentration (Vinderola *et al*., 2000). Therefore the salting process may also lead to poor survival of bacteria in cheese. Coagulation time increases with increasing sodium chloride concentration (Patel and Bayoumi, 1988). Lifton *et al*. (2001) reported that all mendelian diseases associated with hypertension include defects that cause increase in salt absorption by the kidney.

Cooking process helps to destroy spoilage microorganisms, improves the shelf life of the processed cheese, contracts the particles and drives out the free whey, influences texture and gives more time for production of lactic acid that suppresses the growth of spoilage organisms (Nuser, 2001; Siew *et al*., 2004).

The high salt added to this cheese might affect the hypernsive people and deprives them from consuming this cheese, therefore this investigation aimed at producing cheese with low salt content that suits the hypertensive people and determining its acceptability in the market in addition to being safe during storage till consumption.

MATERIALS AND METHODS

The present study was conducted during the period February to May, 2009. Fresh cow's milk was brought from Khartoum University Farm at Shambat. Rennet powder was obtained from Chr. Hansen's Lab. (Denmark). The plastic bags (HiDiSpo bag™-10; size 10 cm x 6 cm) were obtained from HiMedia Laboratories Pvt. Limited, Mumbai, India. Salt was purchased from the local market.

Cheese manufacture: Cheese was manufactured according to the method described by Ibrahim (2003). Fresh cow's milk (25 L) was laboratory pasteurized at 72°C for 1 min and then cooled to 40°C. Rennet powder (1.3 gm) was dissolved in 50 ml distilled water and added to milk at 40°C. Milk was stirred for 5 min and left undisturbed to develop a curd. After complete coagulation, the curd was cut into small cubes (2.5 x 2.5 x 2 cm) and cooked at 35°C for 30 min. The curd was then poured into small wooden moulds lined with cheese cloth and pressed by weight (2.5 kg) overnight.

The next day, brine solution was prepared by adding salt to the collected whey (2%w/w), laboratory-pasteurized at 72°C for 1 min and cooled to 40°C. The pressed cheese was cut into small cubes and immersed into brine solution for 48 h. Cheese was then transferred to plastic bags and stored without whey in the refrigerator at 4°C for 60 days. A representative sample (20 g) was taken and subjected to microbiological examination and sensory evaluation at 1, 20, 40 and 60 day intervals. The analysis was carried out in triplicate.

Microbiological examination: The total viable bacteria count was determined according to Houghtby *et al*.

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(1992) using Standard Plate Count Agar medium. The plates were incubated at 32°C for 48 h and colonies were counted. Psychrotrophic bacteria count was determined according to Frank *et al.* (1992) using Standard Plate Count Agar medium. The plates were incubated at 7±1°C for 10 days. Yeasts and moulds count was determined according to Frank *et al.* (1992) using Potato Dextrose Agar medium. The plates were incubated at 25°C for 5 days.

Sensory evaluation: A panel of 10 untrained panellists were chosen and asked to judge on the quality of cheese (colour, flavour, body, taste, saltiness and overall acceptability) using an evaluation sheet, where colour ranged from 1 = not acceptable to 4 = acceptable; flavour from 1 = bland to 4 = extremely intense; taste from 1 = absent to 4 = excessive acid; body from 1 = smooth to 4 = pasty; saltiness from 1 = moderate to 4 = too salty; overall acceptability from 1 = unacceptable to 4 = acceptable.

Statistical analysis: Statistical analyses were performed using Statistical Package for Social Sciences (SPSS, 2004) program. Analysis of Variance (ANOVA) was used to estimate the effect of storage period (1, 20, 40, 60 days) on the microbial quality and sensory characteristics. Mean separation was carried out using Duncan's Multiple Test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Storage period did not significantly ($p>0.05$) affect the total viable bacteria count (Table 1), although a slight increase was noticed at the end. These findings are in agreement with those of Hamed *et al.* (1992), Ahmed (1997) and Warsama *et al.* (2006) who reported that the total bacteria count of soft white cheese increased until day 60 of storage then gradually decreased. The increase can be explained by the sufficient change in the environmental conditions happened during the ripening period which allowed the growth and multiplication of microorganisms (Ibrahim, 2003; El Owni and Hamid, 2008). On the other hand, highly significant increase ($p<0.001$) due to the storage period was observed in the psychrotrophic bacteria count, gradually increasing from $\log_{10}$ 5.73±0.043 cfu/gm at day 1 to the $\log_{10}$ 6.20±0.043 cfu/gm at the end of storage (Table 1). This result is in accord with the findings of Nour Eldiam and El Zubeir (2006) who found that the storage period of soft white cheese showed significant differences ($p<0.05$) with psychrotrophic bacteria count. The yeasts and moulds count was significantly ($p<0.001$) affected by storage period as they showed a continuous increase with progress in storage. The counts were $\log_{10}$ 6.78±0.057, $\log_{10}$ 6.87±0.057, $\log_{10}$ 7.07±0.057 and $\log_{10}$ 7.20±0.057 cfu/gm at days 1, 20, 40 and 60 respectively (Table 1). These findings are in line with the results of Aly and Galal (2002); El Owni and Hamid (2008) who reported that yeasts and moulds increased as storage period progressed.

During storage the colour did not significantly change ($p>0.05$), while the flavour was moderately intense (2.93±0.133) at day 60 and slightly intense (2.30±0.133) at day 20 ($p<0.05$). The taste was slightly acidic (2.25±0.115) at day 60 and acid taste was absent (1.55±0.115) at day 40 ($p<0.001$). The body was harsh (2.98±0.163) at day 60 and slightly smooth (1.80±0.163) at day 20 ($p<0.001$). The cheese scored between salted and over salted (1.50±0.079) at day 20 and moderately salted (1.08±0.079) at day 60 ($p<0.001$). Overall, the cheese was slightly acceptable (3.10±0.134) at the beginning of storage, the quality was then decreased to half way between not acceptable and moderately unacceptable (1.40±0.134) at the end of storage ($p<0.001$) (Table 2).

Table 1: Effect of storage period on the microbial quality (Log_{10} cfu/ gm) of cooked low salt white soft cheese (Mean±SE)

	Storage period (days)				
Type of bacteria	1	20	40	60	SL
Total viable bacteria	8.10±0.047^{a}	8.16±0.047^{a}	8.09±0.047^{a}	8.20±0.047^{a}	NS
Psychrotrophic bacteria	5.73±0.043^{b}	6.07±0.043^{a}	6.13±0.043^{a}	6.20±0.043^{a}	***
Yeast and moulds	6.78±0.057^{c}	6.87±0.057^{b}	7.07±0.057^{a}	7.20±0.057^{a}	***

Means in the row bearing the same superscripts are not significantly different ($p>0.05$). *** = $p<0.001$.
NS = Not Significant, SL = Significance Level

Table 2: Effect of storage period on the sensory characteristics of cooked low salt (2%w/w) white soft cheese (Mean±SE)

	Storage period (days)				
Organoleptic parameters	1	20	40	60	SL
Colour	3.18±0.151^{a}	3.78±0.151^{a}	2.93±0.151^{a}	2.83±0.151^{a}	NS
Flavour	2.65±0.133^{ab}	2.30±0.133^{b}	2.55±0.133^{ab}	2.93±0.133^{a}	*
Taste	1.98±0.115^{b}	1.80±0.115^{b}	1.55±0.115^{b}	2.25±0.115^{a}	***
Body	2.15±0.163^{b}	1.80±0.163^{b}	2.18±0.163^{b}	2.98±0.163^{a}	***
Saltiness	1.35±0.079^{a}	1.50±0.079^{a}	1.13±0.079^{b}	1.08±0.079^{b}	***
Overall acceptability	3.10±0.0134^{a}	2.33±0.134^{b}	2.40±0.134^{b}	1.40±0.134^{c}	***

Means in the row bearing the same superscripts are not significantly different ($p>0.05$). *** = $p<0.001$. * = $p<0.05$.
NS = Not Significant, SL = Significance Level

The findings of this investigation are in agreement with those of Abdalla and Mohamed (2009) and Mohammed (2009) who reported that colour of cheese did not significantly change during storage period, while flavour and taste gradually improved throughout the storage, and Nuser (2001) who reported a significant change in body score during storage. The findings agree with those of Hamid and El Owni (2007) who reported that the flavour score of cheese and saltiness were significantly different. However, the results are in disagreement with those of Khalid (1990) who found that cheese developed acceptable flavour and acid taste after two weeks of storage. This might be attributed to low bacteria count of heat treated milk which resulted in slower acidity and flavour development (Abdalla, 1992). On the other hand, Hamed *et al*. (1992) reported that the composition and quality were affected more by storage temperature than by heat treatment of cheese milk. Cheese made from pasteurized milk received the organoleptic score of 92 and 88 (out of 100) after 90 days at room and refrigerator temperatures respectively, while cheese made from raw milk received the highest organoleptic score after 60 days at room temperature (Hamed *et al*., 1992).

Conclusion: In conclusion, the total viable bacteria, psychrotrophic bacteria and yeasts and moulds counts increased as the storage period progressed. During storage the colour, saltiness and overall acceptability of cheese deteriorated, while flavour, body and taste improved.

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Chemical Composition of Mish "A Traditional Fermented Dairy Product" from Different Plants During Storage

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Abstract: This study was conducted to evaluate the chemical composition of mish during storage. Ninety samples of mish were collected on the day of manufacture (day one) from three different dairy plants (DP1, DP2, DP3), transported to the laboratory of the Department of Dairy Production, Faculty of Animal Production in ice box and stored at 7°C for 28 days. Samples were analyzed for fat, protein, total solids, ash and titratable acidity at 1, 7, 14, 21 and 28 days. The results showed that fat, protein, total solids, ash and titratable acidity were high in DP1 and low in DP2, except for fat content which was low in DP3. During storage fat, protein, total solids, ash contents and titratable acidity increased to a maximum at day 21 and then decreased thereafter, while titratable acidity steadily increased towards the end. Towards the end of storage, the fat content slightly decreased in DP1 and increased in DP2 and DP3. The protein content slightly decreased towards the end in DP1 and DP2 and increased in DP3. The total solids and ash contents slightly decreased in all three plants at the end of storage, while titratable acidity increased towards the end of storage in all plants.

Key words: Mish, dairy plant, chemical composition, storage period

INTRODUCTION

Fermented products generally have a large shelf life than their original substrate and their ultimate spoilage is different in character. The antimicrobial effects of fermentation are not confined to spoilage organisms alone and can also affect pathogens that might be present. Thus, traditional food fermentation can take potentially hazardous substances as raw materials, such as raw milk and transform them into products with both improved keeping qualities and reduced risk of causing illness (Keller and Jordan, 1990; Mitchell, 2000; Beukes *et al*., 2001).

The fermented dairy products of the Sudan are divided into two major groups; the truly indigenous which include roub, gariss and mish and the quasi-indigenous which include zabadi and jibna beida. Mish is a fermented milk product, which like other dairy products such as cheese, yoghurt, butter and cream, is manufactured in Sudan and in the rural areas where plenty of milk is available during the rainy season. Surplus milk is utilized for the manufacture of fermented dairy products but recently, mish is being produced in the modern dairy plants for consumption in urban areas. The intensity of spicing in mish may differ from region to another and even from family to another within the same district as it depends on spices availability and the taste of the people (El Mardi, 1988; Dirar, 1993). The bulk of milk in the country is produced by nomadic herds of cattle and these produce plenty of milk supply in the rainy season, which is fermented by souring into dairy products some of which are spread in the country whereas others are confined to certain geographic areas (Abdel Gadir *et al*., 1998).

Spontaneous food fermentation has a long history in Africa and relies on indigenous knowledge of the majority of the population, only seldom are fermentation processes fully industrialized and many food production fermentations still occur at the household-scale or at small enterprise scale (Mathara *et al*., 2004). The nature of fermented products is different from one region to another and this depends on the local indigenous microflora, which in turn reflects the climatic conditions of the area (Savadogo *et al*., 2004). Many people throughout Africa enjoy soured milk products, in which the lactic acid bacteria play an essential role in preserving a highly nutritious food product. Fermented milk products are also of great significance for their therapeutic and social values, alleviating lactose intolerance and as a means of generating income (Beukes *et al*., 2001).

This study was carried out to evaluate the fermented dairy product "mish" locally produced by three dairy plants chemically during storage period of 28 days.

MATERIALS AND METHODS

The experiment was carried out in the Department of Dairy production, Faculty of Animal production, University of Khartoum during the period February to June 2009.

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Collection of samples: Ninety samples of mish were collected from DP1, DP2 and DP3 (30 samples from each) in 250 gm size plastic cups, transported to the laboratory in ice box and kept in the refrigerator (7°C) for 28 days. The samples were analyzed for fat, protein, total solids, ash and titratable acidity at 1, 7, 14, 21 and 28 day intervals.

Chemical analysis: Fat content was determined by Gerber method, while protein content was determined by Kjeldahl method (AOAC, 2000).

Total solids content was determined according to the modified method of AOAC (2000) as follows; three grams of mish were placed in a clean dried flat-bottomed aluminum dish and heated on a steam bath for 10 min. The dishes were then dried in an air oven at 100°C for 3 h, after which they were transferred to a desiccator to cool and then weighed. Heating, cooling and weighing were repeated several times until the difference between two successive weighings was less than 0.5 mg. The total solids content was calculated as follows:

$$\text{Total solids (\%)} = \frac{W1}{W2} \times 100$$

Where:

W1 = Weight of sample after drying

W2 = Weight of the original sample

The ash content and titratable acidity were determined according to AOAC (2000).

Statistical analysis: The data were statistically analyzed using Statistical Package for Social Sciences (SPSS, ver. 13). Completely randomized design was used for statistical analysis and means were separated by Duncan Multiple Range Test at $p \leq 0.05$.

RESULTS AND DISCUSSION

Table 1 shows the chemical composition of mish. Fat, protein, total solids, ash and titratable acidity were significantly affected by the dairy plant. The highest fat content was in DP1 (6.82±0.103), while the lowest content was in DP3 (5.27±0.103). The protein content was high in DP1 (8.38±0.169) and low in DP2 (7.44±0.169). The total solids content was high in DP1 (30.93±0.187) and low in DP2 (18.59±0.187). The ash content and acidity were high in DP1 (2.00±0.074 and 3.96±0.035 respectively), while lowest values were obtained from DP2 (1.31±0.074 and 2.48±0.035 respectively).

Table 2 shows the chemical composition of mish during storage period of 28 days. The fat content regularly increased from day one (5.18±0.133) to a maximum at day 21 (6.08±0.133), beyond which the content decreased ($p<0.001$).

The protein content steadily increased to a maximum at day 14 (8.72±0.219) and then decreased towards the end of storage (7.21±0.219) ($p<0.001$). The total solids content followed the same trend as protein content increasing to a maximum at day 14 (23.67±0.242) then decreased to 23.01±0.242 at the end ($p<0.01$). The ash content decreased from 2.03±0.096 at day one to 1.38±0.096 at the end ($p<0.001$). The titratable acidity showed a gradual increase from 2.70±0.046 at day one to 3.84±0.046 at day 28 ($p<0.001$).

The chemical composition of mish from each plant during storage period is presented in Table 3. The results show that fat content showed irregular pattern during storage period slightly decreasing towards the end in DP1, while in DP2 and DP3 the content slightly increased. The protein content slightly decreased towards the end in DP1 and DP2 and increased in DP3. The total solids and ash contents slightly decreased in

Table 1: Chemical composition of mish from different plants at the end of storage period (Mean±SE)

	Manufacturer			
Chemical composition	DP1	DP2	DP3	SL
Fat (%)	6.82^{a}±0.103	5.31^{b}±0.103	5.27^{b}±0.103	***
Protein (%)	8.38^{a}±0.169	7.44^{b}±0.169	7.84^{b}±0.167	**
Total solids (%)	30.93^{a}±0.187	18.59^{c}±0.187	20.75^{b}±0.187	***
Ash (%)	2.00^{a}±0.074	1.31^{b}±0.074	1.48^{b}±0.074	***
Acidity (%)	3.96^{a}±0.035	2.48^{c}±0.035	3.23^{b}±0.035	***

Means in the row bearing the same superscripts are not significantly different ($p>0.05$). *** = $p<0.001$, ** = $p<0.01$.
SL = Significance Level, DP1, DP2, DP3 = Dairy plants 1, 2 and 3 respectively

Table 2: Changes in chemical composition of mish during storage (mean from the three dairy plants) (Mean±SE)

	Storage period (days)					
Parameters	1	7	14	21	28	SL
Fat (%)	5.18^{b}±0.133	6.24^{a}±0.133	5.45^{b}±0.133	6.08^{a}±0.133	6.03^{a}±0.133	***
Protein (%)	7.66b^{c}±.219	8.21^{ab}±0.219	8.72^{a}±0.219	7.64^{bc}±0.219	7.21^{c}±0.219	***
Total solids (%)	23.51^{ab}±0.242	23.91^{a}±0.242	23.67^{ab}±0.242	23.04^{b}±0.242	23.01^{b}±0.242	**
Ash (%)	2.03^{a}±0.096	1.49^{b}±0.096	1.56^{b}±0.096	1.47^{b}±0.096	1.38^{b}±0.096	***
Acidity (%)	2.70^{d}±0.046	3.04^{c}±0.046	3.14^{c}±0.046	3.39^{b}±0.046	3.84^{a}±0.046	***

Means in the row bearing the same superscripts are not significantly different ($p>0.05$). ** = $p<0.01$. *** = $p<0.001$.
SL = Significance Level

Table 3: Chemical composition of mish from each dairy plant during storage (Mean±SE)

	DP1					DP2					DP3				
	Storage period (days)														
Parameters	1	7	14	21	28	1	7	14	21	28	1	7	14	21	28
Fat (%)	6.88±	7.10±	6.45±	7.17±	6.49±	4.78±	5.64±	4.93±	5.51±	5.68±	3.88±	5.99±	4.97±	5.57±	5.93±
	0.230	0.230	0.230	0.230	0.230	0.230	0.230	0.230	0.230	0.230	0.230	0.230	0.230	0.230	0.230
Protein (%)	8.06±	7.74±	10.92±	8.09±	7.11±	6.15±	9.40±	7.13±	7.42±	7.13±	8.76±	7.48±	8.12±	7.42±	7.83±
	0.379	0.379	0.379	0.379	0.379	0.379	0.379	0.379	0.379	0.379	0.379	0.379	0.379	0.379	0.379
Total solids (%)	30.99±	31.78±	30.89±	30.52±	30.49±	19.37±	18.93±	18.49±	18.15±	18.02±	20.16±	21.03±	21.63±	20.44±	20.51±
	0.418	0.418	0.418	0.418	0.148	0.148	0.148	0.148	0.148	0.148	0.148	0.148	0.148	0.148	0.148
Ash (%)	3.30±	1.52±	1.93±	1.65±	1.58±	1.30±	1.32±	1.50±	1.18±	1.18±	1.48±	1.63±	1.26±	1.58±	1.38±
	0.166	0.166	0.166	0.166	0.166	0.166	0.166	0.166	0.166	0.166	0.166	0.166	0.166	0.166	0.166
Acidity (%)	3.63±	3.72±	3.48±	4.20±	4.75±	1.93±	2.26±	2.58±	2.66±	2.97±	2.53±	3.16±	3.38±	3.30±	3.79±
	0.079	0.079	0.079	0.079	0.079	0.079	0.079	0.079	0.079	0.079	0.079	0.079	0.079	0.079	0.079

DP1, DP2, DP3= Dairy plants 1, 2 and 3 respectively

all three plants at the end of storage. The titratable acidity showed an increase towards the end of storage period in all plants.

From the results of chemical composition it was obvious that mish product from DP1 was higher in all chemical components studied, while that of DP2 was of lower composition. This might be attributed to the raw materials used by different plants, since DP1and DP2 use reconstituted whole milk products and DP3 uses fresh cow's milk.

The decrease in protein content during storage might be due to protein degradation leading to formation of soluble compounds (Abdalla *et al*., 1993). Decrease in total solids content was mainly due to degradation of total protein and decrease in fat content during storage period (Hayaloglu *et al*., 2005). Ash in mish was high which might be due to addition of spices (El-Erian *et al*., 1975). Increase in acidity towards the end of storage period was mainly due to increase in the number of lactic acid bacteria which converted lactose into lactic acid (Bozamic and Tratnik, 2001; Hayaloglu *et al*., 2005; Tarakci and Kucukoner, 2006; Cais-Skolinska *et al*., 2008; El Owni and Hamid, 2008).

The findings in this investigation are higher than those reported by El Mardi (1988); Ali *et al*. (2002); Aly *et al*. (2004); El Zubeir *et al*. (2005); Uzeh *et al*. (2006); Adam (2008); Hassan *et al*. (2008). However, the results of ash content in DP2 and DP3 reported in this study are lower than those reported by Ali *et al*. (2002) and Uzeh *et al*. (2006).

From the results it could be observed that from chemical composition point of view, mish deteriorated after the storage period of 21 days, in addition to increase in titratable acidity meaning that the product turned into highly acidic. These results are in accordance with Al-Otaibi and El-Demerdash (2008) who reported maximum fat, total solids and acidity at 21 day of storage of Labneh. However, these results are in disagreement with Haj *et al*. (2007) who reported decreasing chemical composition of stirred yoghurt during storage period of 10 days.

Conclusion: The results of this study concluded that, mish from different dairy plants showed a significant variation in the chemical composition. The product kept good quality chemically up to 21 days.

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Assessment of Toxic Trace Metals in Selected Fish Species and Parts of Domestic Animals

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Abstract: The Cd, As, Pb, Cr and Se contents of fillets of ten fish species and As, Cd and Pb contents of some parts of cow and goat meats were determined by Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES), after wet digestion of powdered samples with 1:1 HNO_3,/H_2O_2. The ranges obtained for the metals analyzed in fish (mg/kg, dry weight) are as follows; As (0.02-4.58), Se (0.66-1.66), Pb (0.45-4.78), Cd (0.22-2.02) and Cr (0.18-1.96). While the ranges obtained for the metals analyzed in cow meat (mg/kg, dry weight) are as follows: As (0.82-2.09), Cd (0.28-1.50) and Pb (0.80-1.42) and the range of metals analyzed in goat meat are as follows: As (0.02-4.88), Cd (0.04-0.93) and Pb (0.02-1.36). Of all the samples analyzed, the goat intestine contained the highest amount of As (4.88±0.015). Mean Pb concentrations (mg/kg) in *Hydrocynus forskahlii* (3.51±0.06), *Clarias anguillaris* (3.43±0.021), *Scomber japonicus* (4.61±0.043) and *Scomber scombrus* (4.78±0.045) exceeded the recommended limits specified by most food regulatory bodies. It is concluded that fish samples in the study area have heavy metal accumulations a little above recommended safety standards.

Key words: Assessment, toxic metals, fish, domestic animals

INTRODUCTION

Animal protein intake remains the surest way to furnish the body with a complete assay of all the needed amino acids required for proper tissue formation, growth and repair. The common animal protein sources in Nigeria include fish, beef and mutton. The habitat of these animals are continually been polluted with heavy metals discharged as a result of industrial activities. These metals find their way into the food chain of these animals and consequently build up in these animal products. When these animal products are consumed, the heavy metals in them produce pathologies relative to quantity and period of time consumed. This explains why the presence of heavy metals in animal products has continued to receive a lot of attention from nutritionists and environmental scientists. The main threats to human health from heavy metals are associated to exposure to lead, cadmium, mercury and arsenic (Lars, 2003). Excessive intake of these toxic heavy metals can lead to several diseases such as organ failure, cancer and retarded mental development, most especially in children and foetus in pregnant women. It has been reported that acute large dose of arsenic causes gastrointestinal damage with profuse watery diarrhea, bleeding and death (Dodd, 1984). Pratter, 1981 observed that cadmium is not a biological essential or beneficial element, but it is associated with various deleterious effects. Cadmium has been found to be teratogenic, carcinogenic and possibly mutagenic (Young and Bevins, 1981). Hauser and Hauser, 2009 reported that high doses of cadmium can lead to kidney failure, damage to testicles and liver. Acute lead poisoning usually manifest itself in gastro intestinal effects, anorexia, dyspepsia, constipation, attack of colic with intense paroxysmal abdominal pain, bone pain, brain damage, confusion, convulsions, dizziness drowsiness, fatigue, headaches, hypertension, memory difficulties, inability to concentrate, indigestion and irritability (Blood, 1969; Anonymous 2002a,b). Excessive intake of lead can also lead to damage to the brain, liver, kidney and reproductive systems (Hauser and Hauser, 2009). The present study was carried out to assess the levels of Cd, As, Pb, Cr and Se in some fish samples and Cd, As and Pb in parts of cow and goat meats consumed in the study area.

MATERIALS AND METHODS

The meat samples which include the liver, heart, muscle, intestine and kidney of goat and cow meats were purchased from Abattoirs in Nsukka and Enugu, Nigeria while the fish samples were purchased from the banks of River Niger at Onitsha or the Onitsha market, Nigeria. Ten species of fish viz: Osteogloid (*Heterotis niloticus*), moon fish (*Citharinus citharus*), grass eater (*Distichodus rostratus*), catfish (*Hemisynodontis membranaceus*), African carp (*Labeo coubie*), tilapia (*Oreochromis niloticus*), tiger fish (*Hydrocynus forskahlii*), mudfish (*Clarias anguillaris*), mackerel

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Table 1: Mean concentration (mg/kg) of heavy metals in cow meat parts

Metals	Heart	Kidney	Muscle	Liver	Intestine
As	N.D	1.40^{b}±0.016	0.82^{c}±0.010	1.23^{b}±0.015	2.09^{a}±0.046
Pb	0.80^{b}±0.055	1.16^{a}±0.012	0.29^{c}±0.023	0.87^{b}±0.020	1.42^{a}±0.017
Cd	0.28^{d}±0.018	1.50^{a}±0.015	0.81^{c}±0.010	0.88^{c}±0.014	1.25^{b}±0.010

^{abcd}Row means with different superscript are significantly different (p<0.05), ND = Not-detectable

Table 2: Mean concentration (mg/kg) of heavy metals in goat meat parts

Metals	Heart	Kidney	Muscle	Liver	Intestine
As	N.D	0.02^{c}±0.010	2.22^{b}±0.011	1.53^{b}±0.070	4.88^{a}±0.015
Pb	0.56^{b}±0.50	0.04^{d}±0.061	N.D	0.44^{c}±0.040	0.93^{a}±0.070
Cd	0.76^{b}±0.041	0.02^{c}±0.070	0.55^{b}±0.060	1.30^{a}±0.031	1.36^{a}±0.012

^{abcd}Row means with different superscript are significantly different (p<0.05), ND = Not-detectable

(*Scomber scombrus*) and mackerel (*Scomber japonicus*) were analyzed along with the parts of cow and goat meats.

Sample preparation: Samples (fillets) for analysis were extracted from descaled (where necessary) fish after other necessary treatment. Meat and fish samples were dried to a constant weight at 105°C. The dry samples were ground using plastic mortar and pestle and thereafter stored in desiccators. Wet digestion of samples was done using method reported in literature (FAO, 1983). This was done by digesting 10.00 g of dried samples in 60.00 cm^3 of freshly prepared 1:1 HNO_3/ H_2O_2 solution at 160°C on a hot plate for about one hour untill the contents came to about 5.00 cm^3. This was transferred to a standard flask and made up to 25.00 cm^3 with distilled water. The solution were aspirated into an optima 2000DU Perkin Elmer ICP-OES for the determination of the concentrations of the relevant metals. Appropriate standards supplied by Perkin Elmer were used for all metals determined.

Statistical analysis: The data obtained were subjected to a one-way Analysis of Variance (ANOVA) according to the procedure of Steel and Torrie (1980). Significantly different means were separated using the methods of Duncan (1955). The values obtained were presented as Least Significance Differences (LSD) of means at (p<0.05).

RESULTS AND DISCUSSION

Results showed that the highest accumulation of As and Pb in cow occurs in the small intestine whereas Cd had a higher significant (p<0.05) accumulation in the kidney. The concentration of As in the small intestine was 2.09±0.046 which differed significantly from the values observed for the other cut parts. The least concentration of As was found in the muscles. These concentrations differ significantly from the quantity of As found in the kidney (p<0.05). The intestine also had a Pb concentration of 1.42±0.017 which was significantly (p<0.05) higher than values found in the other parts. In goat parts, mean concentration of As, Pb and Cd were statistically higher (p<0.05) in the intestine. The least values of all these heavy metals were recorded in the kidney. The observed high concentration of As, Pb and Cd found in the small intestine of cow and goat may be related to the physiological role of the small intestine as the active site of digestion. The small intestine is lined up with finger like projections called villi. The villi allow the assimilation of digestive end products to the body. It appears there is a 'trap' in the small intestine that accumulates heavy metals. The mean concentration of As was significantly (p<0.05) higher in *Clarias anguillaris* (4.58±0.14). Cd concentration was significantly (p<0.05) higher in *Hemisynodontis membranaceus* (2.02±0.019). *Scomber scombrus* and *Scomber japonicus* had significantly (p<0.05) higher accumulation of Pb. Se concentration in *Labeo* coubie was significantly (p<0.05) higher than concentration found in *Heterotis niloticus, Hemisynodontis membranaceus, Oreochromis niloticus, Hydrocynus forskahlii* and *Scomber scombrus*. *Heterotis niloticus* had highest concentration of Cr (1.96±0.011), which did not differ significantly (P>0.05) from the value of 1.63±0.014 observed for *Districhodus rostratus*. The habitat and feeding pattern of the fish could account for varying concentration of metals. *Hydrocynus forskahlii* feeds on phytoplankton, insects, water beetle, larvae and plants. *Clarias anguillaris* is an omnivore which can feed on anything found in the river for example fish, mud, rotten vegetable, insects and even occasionally zooplankton while *Hemisynodontis membranaceus* is a voracious, predatory, bottom dwelling fish and thrives more in dirtier waters where more food is available (Welcome, 1955). The level of Pb obtained for the fish in the present study are below the levels reported in previous works by Okoye (1994) and Odoemelam (2005). However the concentrations of Cd reported in this study were close to previously reported values for fish in Oguta lake (Odoemelam, 2005). The concentrations (mg/kg) of Pb recorded for *Hydrocynus forskahlii* (3.51), *Clarias auguillaris* (3.43), *Scomber japonicus* (4.61) and *Scomber scombrus* (4.78) were above the MAFF and the Australian National Health and Medical Research Council recommended limits of 2.0 mg/kg as observed by Bebbinton *et al.* (1977). Of all the fish species studied only *Hemisynodontis*

Table 3: Mean concentration (mg/kg) of heavy metals in fish samples

Metals	Osteoglid (*Heterotis niloticus*)	Moonfish (*Citharinus citharus*)	Grasseater (*Districhodus rostratus*)	Catfish (*Hemisynodontis membranaceus*)	Africancap fish (*Labeo coubie*)
As	0.58^{c}±0.010	0.02^{d}±0.092	0.26^{d}±0.071	0.06^{d}±0.012	0.16^{d}±0.011
Cd	0.60^{c}±0.013	0.61^{c}±0.017	0.43^{d}±0.020	2.02^{a}±0.019	0.92^{c}±0.010
Pb	0.74^{d}±0.014	0.45^{c}±0.015	0.49^{c}±0.010	0.97^{b}±0.013	0.68^{c}±0.011
Se	1.12^{d}±0.010	1.61^{a}±0.121	1.60^{a}±0.011	1.32^{b}±0.010	1.90^{a}±0.011
Cr	1.96^{a}±0.011	0.50^{c}±0.014	1.63^{a}±0.010	1.00^{ab}±0.101	0.78^{b}±0.015
Metals	Tilapia (*Oreochromic niloticus*)	Tiger fish (*Hydrocynus forskahllii*)	Mud fish (*Clarias auguillaris*)	Mackerel (*Scomber japonicusl*	Mackerel (*Scomber scombrusl*)
As	0.39^{d}±0.012	1.58^{b}±0.060	4.56^{a}±0.014	0.60^{c}±0.015	1.10^{b}±0.011
Cd	0.28^{e}±0.014	0.55^{d}±0.013	0.22^{e}±0.011	1.00^{b}±0.010	1.06^{b}±0.015
Pb	0.28^{d}±0.013	3.51^{ab}±0.011	3.43^{ab}±0.021	4.61^{a}±0.043	4.78^{a}±0.045
Se	0.66^{c}±0.012	1.00^{b}±0.010	1.66^{a}±0.012	N.D	1.20^{b}±0.010
Cr	0.92^{b}±0.017	0.52^{c}±0.013	0.18^{e}±0.011	0.71^{b}±0.010	0.38^{d}±0.021

^{abcd}Row means with different superscript are significantly different ($p<0.05$), ND = Not-detectable

membranaceus recorded concentration of Cd exceeding the Australian National Health and Medical Research Council Recommended limits of 2.0 mg/kg. Most of the fish had concentration of Cd exceeding the Codex Committee of Food Additives and Contaminants draft guideline of 0.05 mg/kg. The levels of Cr recorded for fish in this study are below the values reported for fish caught from rivers in Ikot-Ekpene, Nigeria (Ibok *et al*., 1989). A concentration of 2.86 mgCr/kg was reported for *Auchenoglanis occidentalis* in a previous study (Odoemelam, 2005). The highest concentration of Cr in the present study is 1.96 mg/kg recorded for *Heterotis niloticus*. The results of the present study have not shown that there is great danger of heavy metal poisoning from the studied fish and meats. This is evident from the fact that heavy metal accumulation in the studied samples merely exceeded recommended limits by negligible amount. However periodic surveillance of heavy metals levels in fish and meats is highly necessary, since heavy metals tend to accumulate in these foods.

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Knowledge of the Nutritional and Medicinal Use of Some Vegetables among a Cross Section of Market Women in Two Major Food Markets in Lagos State, South West Nigeria

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Abstract: The knowledge of the nutritional and medicinal benefits of some vegetables found around the south west-Nigeria was tested among cross section of market women in Lagos state Nigeria, using questionnaires and coloured pictures of seventeen of these vegetables. This study was carried out in Lagos state which houses a cross section of the nation ethnic groups and also serves as the commercial nerve centre of Nigeria. One thousand market women with varying level of education were selected and tested on proper identification and their knowledge on nutritional and medicinal benefits of seventeen vegetables found around them. Sociodemographic data such as age, educational status were also recorded. The results revealed high percentage school dropout among the market women. There is also a level of ignorance about the available vegetables around among the market women thou there seems to be a good appreciation of their medicinal values. Considering the capital base of these women especially the largest age bracket of 21-45 years, we suggested that financially empowering them will reduce the vicious circle of poverty associated with school dropout among girls in Nigeria. The populace need to be educated about the readily available vegetables around them to reducing the incidence of macronutrient deficiencies.

Key words: Vegetable, knowledge, medicinal plants, girls education

INTRODUCTION

The vegetation of the south western Nigeria being in the rainforest is very rich in fruits and leafy vegetables. A closer look at the vegetable content of the diet in this geographical area of Nigeria however, revealed that very few vegetables are routinely included in the diet compared to the abundance of vegetables in the area. This can be attributed to the inadequate knowledge of dietary and medicinal use of some of these plants. This knowledge varies from place to place. While the knowledge of the nutritive benefits of some vegetables are general to all communities there are also strong discrimination about dietary and nutritional values of several other vegetables in different communities due to cultural background. This has greatly affected the cultivation, preservation and utilization of many vegetables that are found in abundance around us. In the light of the global food crisis, this phenomenon has also reduced the available nutritional sources of such communities, making it a situation of suffering in the midst of plenty or malnutrition in the midst of abundant food source.

The joint FAO/WHO (2004) report on a Global Strategy on Diet, Physical Activity and Health, recommended a minimum daily intake of 400 g of fruits and vegetables. At the 2004 joint Kobe workshop, WHO and FAO also developed a framework that proposes ways to promote increased production, availability and access and adequate consumption of fruits and vegetables. In addition, there has been an increased awareness of the health protecting properties of non-nutrient bio-active compounds found in fruits and vegetables and this has directed immense attention to vegetables as vital components of daily diets. The effect is global reorientation and a gradual shift towards the consumption of vegetables and herbs. More people are getting aware of the benefits of consumption of vegetable such as the Cruciferous vegetables (Cabbage and Mustard) on prevention of prostate cancer (Genoveva and Rajendra, 2001). The reported high antioxidant activities of vegetables also help in protecting cell membrane integrity and reducing rate of aging (Cook *et al*., 1998). In this study, we tried to determine the extent of the knowledge of a cross section of market women in Lagos state on the various vegetables available around their locality as well as the knowledge about nutritional and medicinal values of these vegetables.

There has been several attempt and concerted efforts by both individual researchers and International organizations towards combating the problem of malnutrition, hunger and food scarcity by a way of reorientation of the populace on the available nutritional vegetables around them (Fleuret, 1979; Nordeide *et al*.,

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1996; Sena *et al*., 1998; Jansen *et al*., 2004). Researchers have tried to enumerate and emphasized several vegetables whose nutritional values were not previously appreciated and so not eaten in such a way that the populace can start including them in their diet as well as making some economic gain from their cultivation. In a research conducted in Bangladesh by Hellen Keller International Foundation it was discovered that teaching women and men about the nutritional value of certain foods, such as green leafy vegetables and encouraging them to grow and eat these is an effective way of improving nutrition and preventing micronutrient deficiencies (Helen Keller International (HKI)/AVRDC, 1993).

Vegetables are known to be the cheapest source of Vitamins especially the water soluble vitamins and micro nutrients. To this effect, there has been advocacy for extension of nutrition education to different groups in rural area in Nigeria taking into consideration local custom, food production pattern, post-harvest handling of agricultural produce and extent of malnutrition and the dietary needs of different age groups (Sylvester *et al*., 1991). A regional workshop at the African Institute for Capacity Development, Juja, Nairobi, in December 6-9, 2005 focused extensively on developing African leafy vegetables for improved nutrition. Other authors in the field of Agriculture, Food Biochemistry and Nutrition have tried to highlight the beneficial and nutritional values of some of the vegetables with the view to encouraging cultivation and consumption of such vegetables in different parts of Africa (Eggum, 1970; Muhammad and Amusa, 2005; Richard *et al*., 2007; Voster *et al*., 2007). Others have described the medicinal benefits derivable from some of these vegetables. The list of such beneficial effects that identified with the major health problems peculiar to Africa and different localities is inexhaustible.

MATERIALS AND METHODS

Questionnaires were administered to collect data from the market. A pilot study was first carried out to test the questionnaire and also to perfect areas of ambiguity. This market was randomly picked out of the markets in Lagos state. A total of eighty (80) questionnaires was administered by trained Interviewers who have previously been briefed about the aim and objectives of the study. Our target interviewees were selected using our predetermined inclusive and exclusive criteria. The criteria are:

- Any woman who has been selling in the market for more than 12 months
- Any woman that sells unprocessed food stuff or herbs in the market.

The exclusion criteria are:

- Any woman that has not been selling in the market for the past 12 months
- Any woman that does not sell unprocessed food stuff or herbs.

Women were chosen as our target audience because they were directly involved with planning of meal for the home as well as preparing the various dishes in which these vegetables are used. The result of the pilot study was used to fine tune the questionnaire for the full study There are two groups of questions in the questionnaire designed to take care of sociodemographic patterns as well as knowledge and attitude of our target population on the nutritional and medicinal values of some of the available vegetables around them. The first group of questions which are five in number took care of the sociodemographic patterns which are the age bracket, level of educational attainment, marital status, religion affiliation, type of wares sold in the market. The second group of questions which were twelve in number involved identification of the vegetable plants from the pictures as well as the knowledge of the medicinal use of the plants in treatment of some common diseases and disorders such as fever, diarrhoea, rashes, cold, sexually transmitted infections, anaemia, epilepsy, infertility, high blood pressure, diabetes mellitus and ulcer. Questions on identification require giving the local name of the plant which interviewers will check out with the correct local name(s) on their records Other questions on medicinal use of the plants require either yes or no while sociodemographic questions requires specific answer from the interviewee.

The second phase was the main study. A thousand questionnaires (3000) were administered to our focused audience at Ojuwoye market in Mushin local government area and Mile 12 market at kosofe local government area of Lagos state. The questionnaires were administered by trained interviewers who have been properly briefed about the study. Rough maps of the markets were drawn with the aid of the Interviewer and the two markets were divided into six areas prior to the administration of the questionnaire to prevent overlap in the administration. The questionnaires were administered for 4 weeks.

This research work does not require any institutional review board approval.

Data collected were analyzed using SPSS version 11 and presented as percentage response. Histogram and pie chart were also used for graphical presentation.

A total number of seventeen vegetable plants that were randomly selected from a total number of fifty vegetable plants were used in this study. Sharp clear and coloured pictures of the vegetables (A4 size) were taken, printed and laminated to make them more durable. The pictures were given only numbers that corresponds to names that were only known by the Interviewers. The following vegetables were used (Table 1). The leaves and the stems of all plants are consumed locally except for *Xylopia Acthiopica* (erunje eeru) where the fruit is the edible portion.

Table 1: Botanical name (common name); % correctly identified; % knowledge of nutritional values of some vegetables among a cross section of some market women in Lagos state, south west Nigeria

Botanical Name of Vegetable	Part used in this study	Vernacular names (Yoruba)	Identification (%)		Response on nutritional value %	
			Correct	Incorrect	Edible and nutritious	Not edible/No knowledge/ Ignorance
Piper Guineese	Seed	Iyere	61.4	38.6	61.4	38.6
Psidium Guajava	Plant leaves and stem	Guava	42.6	57.4	42.6	57.4
Allium sativum	Bulb	Ayuu	93.1	6.9	93.1	6.9
Celosia Argentea	Plant leaves and stem	Efo Sokoyokoto	57.4	42.6	57.4	42.6
Cucurbita pepo	Plant leaves and stem	Elegede	48.5	51.5	48.5	51.5
Hibiscus Sabdarifa	Plant leaves and stem	Sobo	5.0	95.0	5.0	95.0
Manihot Esculenta	Plant leaves and stem	Ege/Gbaguda	63.4	36.6	63.4	36.6
Ocium Basilicum	Plant leaves and stem	Efirin	41.6	58.4	41.6	58.4
Telfaria Occidentalis	Plant leaves and stem	Ugwu	63.4	36.6	63.4	36.6
Xanthosoma Mafaffa	Plant leaves and stem	Koko	86.1	13.9	86.1	13.9
Solanum Macrocarpon	Plant leaves and stem	Efo Igbo	59.4	40.6	59.4	40.6
Talinum Triangulare	Plant leaves and stem	Efo Gbure	87.1	12.9	87.1	12.9
Lactuca capensis	Plant leaves and stem	Iyarin oko	1.0	99.0	1.0	99.0
Lycopersicon Esculentum	Plant leaves and stem	Tomati	55.4	44.6	55.4	44.6
Cnidoscolus Asconitifolius	Plant leaves and stem	Efo Iyana Ipaja	19.8	80.2	19.8	80.2
Corchorus Olitorus	Plant leaves and stem	Ewedu/Ayoo	72.3	27.7	72.3	27.7
Xylopia Acthiopica	Fruit	Erunje eeru	86.1	13.9	86.1	13.9

RESULTS

Eleven out of the seventeen vegetables in this study have above 50% correct identification mark with *Lactuca capensis* being the least recognized (1.0%) followed by *Hibiscus Sabdarifa* (5.0%). This calls for the need to educate the people about the available vegetable around them. Interestingly, *Hibiscus sabdarifa* (sobo), the petals of which are used for the nutritious sobo drink commonly found in Nigeria had 5.0% correct identification. This is a typical example of how some part of a vegetable can be known while other parts that are equally nutritious and useful are not known. There was good sense of appreciation of those vegetables that were properly identified. The results of the appreciation of the medicinal use of the plants showed that the market women have knowledge of the medicinal values of several of the vegetables used in this study. The vegetable with the largest percentage use for fever was *Psidium guajava* (27.0%) and *Corchorus olitorius* (25.7%) while for diarrhoea it was *Piper guineese* (100%). *Allium sativa* has the highest for lowering blood pressure (53.5%). For anaemia it was *Telfaria occidentalis* (49.5%) while *xylopia acthiopica* has the highest percentage (41.6%) for treating rashes. Most of the women responded that the vegetables are not used for treating diabetesmellitus.

Majority of the women fell between the age brackets of 21-45 which happens to be the productive age. Investing into their life during this period will be a good way to reduce poverty and reduce the vicious circle of girls drop out from school. Also the largest population of the women happened to be Muslim followed by Christians the two major.

Majority of the market women were only able to the level of secondary school education before they retire to trading. This might not be unconnected to poverty level in the society as well as the cultural believe about the place of women in the society. The largest percentages of these women were involved in the sales of perishable foods which include vegetables. The capital base for such business is low and the risk is high due to lack of storage facility.

Table 2: Age bracket of the cross section of market women from Lagos state, south west Nigeria, that were interviewed in this study as well as their religious affiliations

Age of the market women		Religious affiliation of the market women	
Age bracket	Frequency	Status	Frequency
15-20	2.0	Christianity	36.0
21-25	4.0	Islam	60.0
26-30	14.0	Traditional religion	4.0
31-35	19.0	Others	0.0
36-40	16.0		
41-45	11.0		
46-50	9.0		
51-55	10.0		
56-60	6.0		
61-65	5.0		
66-70	1.0		
>70	5.0		

DISCUSSION

In the face of the global food crisis all hands must be on deck to ensure that relief is brought to as many that have been affected. These come inform of food aids, agricultural loans and sub cede. However, the long term remedies will not only be in the encouragement of modern commercial agriculture but also in an inward search into the available nutritional food crops and vegetable in each community. This will go a long way in utilization of the available recourses in achieving far reaching benefits for the communities.

Table 3: Level of formal education attained as well as the type of wares sold in the market by cross section of the market women from Lagos, south west Nigeria that were interviewed in this study

Level of formal education		Types of wares sold in the market	
Level	Frequency	Wares	Frequency
Nil/No formal education	19.0	Perishable foods	50.5
Primary School Uncompleted	7.4	Non perishable foods	26.8
Primary School Completed	22.3	Herbs	19.6
Secondary School Uncompleted	16.0	Others	3.1
Secondary School Completed	29.0		
Tertiary Institution Uncompleted	1.1		
Tertiary Institution Completed	4.3		

The disparity between the abundance of vegetable around compared to the number been included in our daily diet has led to the concept of Neglected Underutilized Specie (NUS). Bioversity International (formerly IPGRI) defines these species as neglected and underutilized plant species, not crops, since wild, managed and cultivated species are taken into consideration. These plant species may belong to any category, from fruit and nut trees to leafy vegetables, from functional herbs (or medicinal and aromatic plants (MAPs)) to cereals, from legumes to forest trees, from forages to roots and tubers. The importance of NUS is that they require only limited external inputs for production, they grow well on poor soil and also offer multiple uses raging from nutrition to medicinal (Alessandra Giuliani, 2007). Mohammad and Amusa (2005) highlighted the important food crops of the northwest Nigeria among which are a lot of fruits and vegetables. As far back as 1970, Eggum had documented the protein content of some vegetable leaves from Nigeria among which is cassava and had proven that the protein content rages from 30-40% with digestibility that ranges from 70-80% (Eggum, 1970). Taiga *et al.* (2008) reported that *Telfairia. Occidentalis* contains 13.33% protein and 63.64%. Carbohydrate. They also reported the carbohydrate content of *Piper guineese* to be 77.17%. It has been well argued that edible wild plants play a major role in augmenting the macronutrient requirements of people all over the world and that they account for over 80% of the leafy vegetables consumed all over the world (Grivette and Ogle, 2000).

The results of the demographic pattern from this study showed that there have been problems with girls' education in Lagos state in the past. This was made evident by the results of the number of drop out both at the primary school level and Secondary school level which was 7.4% and 16% respectively. This result corroborates the 30% result given recently by UNICEF on the number of drop out among girls at primary and secondary school level in Nigeria (UNICEF, 2002). This may not be unconnected with poverty level which was placed at 70% (Percentage of people living below poverty line), Teenage pregnancy, early marriage as well as cultural and religious bias (UNICEF, 2002). Until recently, the place of women in Nation building was relegated to the background due to cultural and religious bias. Women's place was said to be in the kitchen and at home raising children. In addition, the imbalance in the appointment of women into top Government positions as well as in top private establishment made mentorships and motivation of girls' education a difficult task. The ages of most of the women interviewed in the market were between 21-45 years which accounted for 64% of total number of women interviewed. This happens to be the productive age beyond which most workers retire to a less strenuous activity. The percentage of the women that were above 45 years of age was 33% with majority falling between 56-60 years of age. Considering the asset base of this set of women, one tends to imagine how and when the vicious circle of the problem with girl's education will stop. Therefore, we are suggesting that these women can be empowered financially by making available soft loans which will go a long way in reducing the poverty level that has been one of the causes of increase in girls drop out from schools.

The results of the identification of the vegetable plants showed that eleven out of the seventeen vegetables have correct identification above 50% among the population studied. That is not to conclude that our audience have no knowledge of the vegetables at all but the parts specified may not be known. To emphasize this, it will be surprising to know for example that while the audience were able to recognize the petals of *Hibiscus sabdariffa* (Zobo) only 5% were able to correctly identify the plant itself which is a good vegetable. This buttress the need to educate the populace about the available nutritious vegetables around them so that they can be included in the daily diet. Nigeria is blessed with vas vegetation with abundance of edible but undiscovered vegetable plants. Even those that were discovered were grossly underutilized due to poor knowledge of their nutritional benefits and religious and tradition bias. It was reported by Maziya-Dixon *et al.* (2004) that leafy vegetables in Nigeria are relatively available and affordable particularly during the rainy seasons but were found to be among the least

consumed foods. The least correctly identified vegetable out of the seventeen vegetables used in this study was Lactuca carpensis (Iyarin oko).

From the response to the medicinal importance of these vegetables, it was evident that the medicinal values of majority of these medicinal plants are locally appreciated. For the treatment of fever *Psidium guajava* leaf was scored highest (27.7%) followed by *Corchorus olitorus* (25.7%) and then *Piper guineense* (10.9%). For the treatment of rashes *Xylopia Aethiopica* ranked highest (41.6%), followed by *Ccucubita pepo* (17.8%). For the treatment of sexually transmitted infections *Cucubita pepo* was the only suggested vegetable with percentage yes response of (6.7%). For the treatment of anaemic condition, *Telfaira occidentalis* ranked highest (49.5%), followed by *Celosia argentea* (14.9%) and *Talinium triangulare* (13.9%). As contraceptive, *Piper guineese* was the only one with highest yes response of 6.9%. Allium satuum ranked highest with 53.5% in treatment of high blood pressure. For the treatment of Ulcerative colitis *Telfaria occidentalis* raked highest with percentage yes response of 9.9%, followed by Allium satiuum (4.0%).

Some of the acclaimed medicinal uses of these vegetables in folk law medicine have been investigated to ascertain their beneficial and detrimental effects. Ajayi *et al*. (2000) reported that two weeks oral administration of water extract of *Telfaira occidentalis* to Rabbits increased their haematocrit and red blood cell count. We have earlier reported also that administration of methanol seed extract of *Piper guineese* for 28 days to female rats adversely affected several female reproductive parameters including hormonal profile, oestrus cycle pattern and ovulation. In addition, we have also reported the reversible deleterious effects of Aqueous Extract of *Spondias mombin* bark and methanol fruit extract of *Abelmoschus esculentus* leaf (okra) on male reproduction (Raji *et al*., 2006; Olatunji-Bello *et al*., 2007a) and also the disruption of estrus cyle pattern in rats administered aqueous leaf extract of *Magnifera indica* (mango leaf) (Olatunji-Bello *et al*., 2007b). These are some of the reportedly consumed vegetables for such benefits as contraceptives in male and females.

Conclusion: The results of the present study has revealed that there is need for public education on the available vegetables around us which can serve as herbal medicine, sources of nutrients and micro nutrients that may salvage the population from the incidence of malnutrition. In addition the results highlighted the fact that urgent attention still needs to be directed towards girls' education in Nigeria. It also pointed out the need to financially empower the market women whose capital base are small to eradicate poverty that has been the major cause of increased number of girls that drop out from school.

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Safety Assessment of Functional Drinks Prepard From Green Tea Catechins and Epigallocatechin Gallate

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Abstract: Increasing awareness regarding natural ingredients has led to utilization of functional beverages in diet based therapy. Present project was designed to evaluate safe use of functional drink prepared from green tea active ingredients. Efficacy trial was conducted in male Sprague Dawley rats for period of eight weeks. Functional drinks were prepared by adding catechins and Epigallocatechin Gallate (EGCG) @ 550 mg/500 mL and provided to rats for the period of eight weeks. Four types of studies were conducted consisting of different types of diets i.e. study I (normal diet), study II (high cholesterol diet), study III (high sucrose diet), study IV (high cholesterol+high sucrose diet). The results revealed safety of functional drinks as values for liver and kidney function tests and serum proteins remained in normal range. Organs to body weight ratio were non-significantly effected by functional drinks. Conclusively it can be suggested that functional drinks carrying green tea catechins and EGCG are safe and could be a part of diet therapy for treatments of lifestyle related disorders.

Key words: Functional drink, safety, green tea, liver, catechins, EGCG

INTRODUCTION

Recently, pivotal linkages ascertained between health and nutrition has turned away the human inclination towards plant based natural products to treat various disorders. In this milieu, green tea has gained popularity because of its health enhancing prospective. Green tea was accidentally discovered by Shen Nung a Chinese emperor in 2737 B.C (Wheeler and Wheeler, 2004) and is one of most widely consumed beverages in Asian countries (Zaveri, 2006). Tea is grown over 30 countries (Graham, 1992) and occupies about 2.7 million hectares of cultivable area of the world (Mondal *et al*., 2004). China, Japan, Taiwan, India, Bangladesh, Sri Lanka and Kenya are the major producers (Shaheen *et al*., 2006). Worldwide per capita consumption of tea is 40 L per year (Vinson *et al*., 2004), approximately 3 million metric tons of tea is produced annually, increasing at rate of 2.1% (Yang and Landau, 2000).

Polyphenols are the main constituents of green tea, accounting for 25-35% on dry weight basis (Balentine *et al*., 1997; Shaheen *et al*., 2006; Yao *et al*., 2006). Health claims of green tea are attributed to its polyphenolic fractions known as catechins, including Epicatechins (EC), Epicatechin Gallate (ECG), Epigallocatechin (EGC) and Epigallocatechin Gallate (EGCG). Among catechins, EGCG is the most promising component (Demeule *et al*., 2002; Kovacs *et al*., 2004; Bettuzzi *et al*., 2006; Wang *et al*., 2006) constituting 48-55% of total polyphenols (Ho *et al*., 1997) and is responsible for majority of the health benefits of green tea (Nagle *et al*., 2000; Lambert and Yang, 2003; Wolfram *et al*., 2006). The chemical composition of tea varies with the growing conditions like climate, season, agricultural practices, variety, age and position of the leaf (Katiyar and Mukhtar, 1996a,b; Aherne and O'Brien, 2002; Fernandez *et al*., 2002; Lin *et al*., 2003). In green tea, catechins are present in higher amounts than that of black or oolong tea, because of the processing differences (Zaveri, 2006). Some other sources of catechins are red wine, fruits like plum, apples, peach, strawberry, cherry, broad bean, lentil and cocoa (Scalbert *et al*., 2005; Yilmaz, 2006).

Green tea possesses antioxidative (Yoshino *et al*., 1994; Miura *et al*., 2001; Hakim *et al*., 2003; Suzuki *et al*., 2004), antiallergic (Sano *et al*., 1999), anti-inflammatory (Dona *et al*., 2003; Lee *et al*., 2005) and hypolipidemic (Yoshino *et al*., 1994; Imai and Nakachi, 1995; Murase *et al*., 2002; Raederstorff *et al*., 2003; Zheng *et al*., 2004) properties.

Diets rich in cholesterol lead to higher production of Reactive Oxygen Species (ROS) resulting in oxidative stress. ROS attack polyunsaturated fatty acids in cell membrane resulting in lipid peroxidation products leading to structural and functional cell damage (Kuper *et al*., 2000). The levels of ALT, ALP, AST and bilirubin are altered thereby damaged structural integrity of the liver, as they are present in cytoplasm and are released in blood circulation after cellular damage (Recknagel *et al*., 1989; Dobrzynska *et al*., 2004).

MATERIALS AND METHODS

Present research project was conducted in the Postgraduate Research Laboratory, National Institute of

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Food Science and Technology (NIFSAT). Green tea leaves of Qi-Men variety were obtained from National Tea Research Institute (NTRI), Shinkiari, Mansehra. Reagents (analytical and HPLC grade) and standards were purchased from Merck (Merck KGaA, Darmstadt, Germany) and Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan). Sprague Dawley rats used in the efficacy trials were acquired from National Institute of Health (NIH) Islamabad. Diagnostic Kits used were from Sigma-Aldrich, Bioassay (Bioassays Chemical Co. Germany) and Cayman Chemicals (Cayman Europe, Estonia).

Safety assessment of functional drink: Functional drinks (T_0, T_1, T_2) were prepared by adding catechins and Epigallocatechin Gallate (EGCG) @ 550 mg/500 mL in respective drink and a control was also prepared for comparison purpose.

Experimental animals and housing conditions: One hundred and twenty male Sprague Dawley rats (seven weeks old) weighing 125±10 g were procured from National Institute of Health (NIH), Islamabad and housed in the Animal Room of National Institute of Food Science and Technology. The animals were acclimatized by feeding basal diet (AIN-76A) for a period of one week. The temperature (23±2°C) and relative humidity (55±5%) were maintained throughout the experiment period with 12 h light-dark period.

Experimental design: After one week of wash out period rats were divided into four groups according to four different types of diet i.e. normal diet, high cholesterol diet, high sucrose diet and high cholesterol + high sucrose diet. In each group rats were further divided into three subgroups (Table 1). Functional drink was provided in polypropylene bottles with stainless steel sipper tubes. The experimental diets comprised of corn oil (10%), protein (10%), corn starch (66%) and cellulose (10%), mineral (3%) and vitamin mixture (1%). In high cholesterol diet and sucrose diets, cholesterol and sucrose were added at 1 and 40%, respectively. The overnight fasted rats were sacrificed after eight weeks of feeding with simultaneous intake of functional drinks. Body organs including heart, liver, left and right kidney, spleen, lungs and pancreas were weighed to calculate organ to body weigh ratio. Blood samples of rats were collected through cardiac puncture; EDTA coated tubes were employed for serum collection and further used to perform various assays through Microlab-300, Merck, Germany.

Organs weight: Organs i.e. liver, heart, kidney, spleen, lungs and pancreas were collected after dissection to determine the effect of test diets on organ weights of rats. The organs were properly cleaned and weighed on electronic balance (Dyer *et al*., 2008). The results were expressed as organ to body weight ratios (g/100 g of body weight).

Table 1: Diet plan used in the studies

Study No.	Rats groups	Drinks
Normal diet (I)	1	T_0
	2	T_1
	3	T_2
High cholesterol diet (II)	1	T_0
	2	T_1
	3	T_2
High sucrose diet (III)	1	T_0
	2	T_1
	3	T_2
High cholesterol +	1	T_0
high sucrose diet (IV)	2	T_1
	3	T_2

Liver and renal function tests: Liver function tests including Aspartate Aminotransferase (AST), Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP) and bilirubin total were assessed. Levels of AST and ALT were measured by the Dinitrophenylhydrazene (DNPH) method using Sigma Kits 59-50 and 58-50, respectively and ALP by Alkaline Phosphates-DGKC method (Thomas, 1998; Moss and Handerson, 1999). Bilirubin total was determined by Jendrassik-Grof method (Tolman and Rej, 1999). The serum urea (GLDH-method) and creatinine (Jaffe-method) were determined using commercial kits (Jacobs *et al*., 1996; Thomas, 1998) to assess the renal functionality of different rats groups in each study.

Serum proteins: Serum total proteins, albumins, globulin and A/G ratio were estimated using respective kits of Sigma-Aldrich Chemicals Co. (Bradford, 1976).

Statistical analysis: Completely Randomized Design (CRD) was applied and resultant data was subjected to statistical analysis using Cohort version 6.1 (Costat-2003). Analysis of Variance technique (ANOVA) was used to determine the level of significance (Steel *et al*., 1997).

RESULTS

Organs to body weight ratio: Organs weight was non-momentously affected by functional drinks in all studies (Table 2). Means for heart to body weight ratio of rats in different studies ranged from 0.32±0.03 to 0.44±0.04 g/100 g body weight. Likewise, non-momentous effect for liver weight was noted that ranged from 4.01±0.04 to 4.57±0.03 g/100 g body weight. Similarly, weight of right and left kidney of rats in different studies was affected non-significantly by functional drinks i.e. 0.40±0.03 to 0.46±0.02 and 0.38±0.02 to 0.47±0.03 g/100 g body weight, respectively. Spleen weight varied non-substantially from 0.31±0.03 to 0.36±0.03 g/100 g body

Table 2: Organs to body weight ratio (g/100 g body weight)

		Studies			
Parameters	Treatments	Study I	Study II	Study III	Study IV
Heart	T_0	0.34±0.02^{NS}	0.35±0.03^{NS}	0.36±0.03^{NS}	0.41±0.04^{NS}
	T_1	0.35±0.03^{NS}	0.38±0.02^{NS}	0.40±0.03^{NS}	0.44±0.04^{NS}
	T_2	0.32±0.03^{NS}	0.37±0.03^{NS}	0.38±0.02^{NS}	0.42±0.02^{NS}
Liver	T_0	4.01±0.04^{NS}	4.51±0.04^{NS}	4.52±0.03^{NS}	4.57±0.03^{NS}
	T_1	4.17±0.03^{NS}	4.32±0.04^{NS}	4.30±0.03^{NS}	4.33±0.04^{NS}
	T_2	4.20±0.03^{NS}	4.15±0.03^{NS}	4.14±0.04^{NS}	4.20±0.03^{NS}
Right kidney	T_0	0.43±0.04^{NS}	0.44±0.03^{NS}	0.44±0.04^{NS}	0.45±0.04^{NS}
	T_1	0.46±0.02^{NS}	0.42±0.04^{NS}	0.41±0.04^{NS}	0.42±0.02^{NS}
	T_2	0.46±0.02^{NS}	0.42±0.04^{NS}	0.40±0.03^{NS}	0.43±0.03^{NS}
Left kidney	T_0	0.43±0.03^{NS}	0.41±0.04^{NS}	0.43±0.04^{NS}	0.45±0.02^{NS}
	T_1	0.47±0.03^{NS}	0.41±0.04^{NS}	0.39±0.03^{NS}	0.38±0.02^{NS}
	T_2	0.46±0.03^{NS}	0.42±0.04^{NS}	0.40±0.02^{NS}	0.40±0.03^{NS}
Spleen	T_0	0.36±0.02^{NS}	0.34±0.02^{NS}	0.35±0.03^{NS}	0.36±0.03^{NS}
	T_1	0.35±0.03^{NS}	0.32±0.02^{NS}	0.31±0.03^{NS}	0.31±0.03^{NS}
	T_2	0.36±0.03^{NS}	0.32±0.02^{NS}	0.34±0.02^{NS}	0.32±0.03^{NS}
Lungs	T_0	1.16±0.10^{NS}	1.14±0.09^{NS}	1.15±0.09^{NS}	1.18±0.08^{NS}
	T_1	1.19±0.07^{NS}	1.10±0.06^{NS}	1.09±0.01^{NS}	1.12±0.08^{NS}
	T_2	1.19±0.09^{NS}	1.09±0.10^{NS}	1.10±0.10^{NS}	1.13±0.06^{NS}
Pancreas	T_0	0.60±0.05^{NS}	0.58±0.04^{NS}	0.58±0.03^{NS}	0.61±0.05^{NS}
	T_1	0.61±0.05^{NS}	0.56±0.04^{NS}	0.55±0.03^{NS}	0.59±0.04^{NS}
	T_2	0.61±0.05^{NS}	0.55±0.04^{NS}	0.56±0.03^{NS}	0.57±0.04^{NS}

weight in different studies. Mean values for lungs ranged from 1.09±0.01 to 1.19±0.09 g/100 g body weight in the entire efficacy trial. Means pertaining to the pancreas to body weight ratio were 0.55±0.03 to 0.61±0.05 g/100 g.

Liver and kidney functioning tests: Alanine Transaminase (ALT) values were non-significantly affected by functional drinks in T_0, T_1 and T_2 groups in study I. However in study II, higher ALT value (52.32±3.72 IU/L) was noted in T_0 group consuming control drink that reduced in T_1 (42.57±2.44 IU/L) and T_2 (41.33±2.12 IU/L) groups taking functional drinks. Likewise in study III, mean for ALT in T_0 was 48.09±2.25 IU/L that decreased to 42.57±1.44 IU/L in T_1 and 43.14±2.26 IU/L in T_2. In study IV, mean for T_0 was 54.28±3.93 IU/L whereas T_1 and T_2 groups provided functional drink showed significant reduction in ALT level i.e. 46.55±2.76 and 45.07±3.41 IU/L, respectively (Table 3).

Mean AST values for T_0, T_1 and T_2 groups in study I were 122.29±4.44, 125.66±5.83 and 121.46±5.89 IU/L, respectively. Means pertaining to AST level in study II, showed high value in T_0 (176.29±9.34 IU/L) as compared to T_1 (133.22±6.39 IU/L) and T_2 (130.85±6.24 IU/L). High sucrose diet (study III) given to rats resulted in elevated AST level in T_0 (149.10±7.20 IU/L) group provided drink without any active ingredients whilst its level was comparatively low in T_1 (132.54±5.32 IU/L) and T_2 (134.71±7.60 IU/L) groups consuming enriched functional drinks. Likewise in study IV, AST value for T_0 was 186.56±9.52 IU/L followed by T_1 and T_2 groups having mean values 137.56±8.86 and 128.39±5.01 IU/L, respectively for this trait.

Mean ALP values in study I (normal diet) for T_0, T_1 and T_2 groups were 169.60±8.47, 168.25±10.13 and 170.72±7.58 IU/L, respectively. In study II, supply of high cholesterol diet to rats lifted their ALP level to 266.14±7.69 IU/L in T_0 group consuming control drink whereas its value was decreased in T_1 and T_2 groups consuming catechins and EGCG enriched drinks to 203.86±6.10 and 196.51±8.34 IU/L, respectively. Likewise in study III, higher ALP value was recorded in T_0 (255.00±9.53 IU/L) as compared to T_1 (192.45±8.75 IU/L) and T_2 (195.46±9.24 IU/L) groups. Mean ALP value in study IV for T_0 group was 306.63±11.90 followed by T_1 (235.61±7.86 IU/L) and T_2 (234.79±6.91 IU/L) groups.

Mean values in T_0, T_1 and T_2 groups for bilirubin in study I were 0.71±0.03, 0.72±0.04 and 0.74±0.05 mg/dL, respectively. However in study II, higher bilirubin value (1.21±0.09 mg/dL) noted in T_0 group was reduced in T_1 (0.89±0.07 mg/dL) and T_2 (1.03±0.08 mg/dL) groups consuming functional drinks. Likewise in study III, bilirubin in T_0 was 1.02±0.07 mg/dL that decreased to 0.69±0.04 mg/dL in T_1 and 0.73±0.06 mg/dL in T_2. In study IV, mean for bilirubin in T_0 was 1.26±0.08 mg/dL whereas T_1 and T_2 groups showed significant reduction i.e. 0.79±0.05 and 0.96±0.08 mg/dL, respectively.

In study I, mean values for urea in T_0, T_1 and T_2 groups were 25.72±1.72, 24.93±0.86 and 25.41±1.43 mg/dL, respectively. In study II, there was noted high urea level 33.98±1.52 mg/dL in T_0 group that reduced to 26.85±0.50 mg/dL in T_1 and 28.73±1.18 mg/dL in T_2 group with concurrent intake of functional drinks containing active ingredients. Similarly in study III, rats showed uplifted urea level (30.70±2.18 mg/dL) in T_0

Table 3: Effect of functional drinks on liver and kidney functioning in different studies

		Studies			
Parameters	Treatments	Study I	Study II	Study III	Study IV
ALT (IU/L)	T_0	42.04±1.24^{NS}	52.32±3.72^{NS}	48.09±2.25^{NS}	54.28±3.93^{NS}
	T_1	42.85±2.52^{NS}	42.57±2.44**	42.57±1.44**	46.55±2.76**
	T_2	41.50±1.76^{NS}	41.33±2.12**	43.14±2.26**	45.07±3.41**
AST (IU/L)	T_0	122.29±4.44^{NS}	176.29±9.34^{NS}	149.10±7.20^{NS}	186.56±9.52^{NS}
	T_1	125.66±5.83^{NS}	133.22±6.39**	132.54±5.32*	137.56±8.86**
	T_2	121.46±5.89^{NS}	130.85±6.24**	134.71±7.60*	128.39±5.01**
ALP (IU/L)	T_0	169.60±8.47^{NS}	266.14±7.69^{NS}	255.00±9.53^{NS}	306.63±11.90^{NS}
	T_1	168.25±10.13^{NS}	203.86±6.10**	192.45±8.75**	235.61±7.86**
	T_2	170.72±7.58^{NS}	196.51±8.34**	195.46±9.24**	234.79±6.91**
Bilirubin (mg/dL)	T_0	0.71±0.03^{NS}	1.21±0.09^{NS}	1.02±0.07^{NS}	1.26±0.08^{NS}
	T_1	0.72±0.04^{NS}	0.89±0.07**	0.69±0.04**	0.79±0.05**
	T_2	0.74±0.05^{NS}	1.03±0.08**	0.73±0.06**	0.96±0.08**
Urea (mg/dL)	T_0	25.72±1.72^{NS}	33.98±1.52^{NS}	30.70±2.18^{NS}	38.26±1.96^{NS}
	T_1	24.93±0.86^{NS}	26.85±0.50**	27.03±1.52**	31.92±2.58**
	T_2	25.41±1.43^{NS}	28.73±1.18**	25.76±0.85**	34.33±2.80**
Creatinine (mg/dL)	T_0	0.81±0.05^{NS}	1.16±0.09^{NS}	0.99±0.07^{NS}	1.25±0.09^{NS}
	T_1	0.78±0.04^{NS}	0.82±0.05**	0.76±0.04**	0.87±0.06**
	T_2	0.80±0.05^{NS}	0.97±0.06**	0.85±0.05**	0.94±0.07**

group whereas its level reduced to 27.03±1.52 and 25.76±0.85 mg/dL in T_1 and T_2 groups, respectively. Maximum urea was in T_0 group (38.26±1.96 mg/dL) followed by T_1 (31.92±2.58 mg/dL) and T_2 (34.33±2.80 mg/dL) in study IV.

In study I, mean values for creatinine were 0.81±0.05, 0.78±0.04 and 0.80±0.05 mg/dL for T_0, T_1 and T_2 groups, respectively. Likewise in study II, means for creatinine in T_0 was 1.16±0.09 mg/dL followed by significant reduction in T_1 (0.82±0.05 mg/dL) and T_2 (0.97±0.06 mg/dL). In study III comprising of high sucrose diet, T_0 showed highest creatinine level (0.99±0.07 mg/dL) that momentously decreased to 0.76±0.04 and 0.85±0.05 mg/dL in T_1 and T_2 groups, respectively. Considering the results of study IV, maximum creatinine 1.25±0.09 mg/dL was recorded in T_0 group (control drink) that significantly reduced to 0.87±0.06 mg/dL in T_1 (drink containing catechins) and 0.94±0.07 mg/dL in T_2 (drink containing EGCG) groups.

Serum proteins: Serum proteins include total proteins, albumin, globulins and A/G ratio were estimated to establish safety of product (Table 4).

In study I, value for total proteins were 6.48±0.33, 6.29±0.49 and 6.40±0.02 g/dL in T_0, T_1 and T_2 groups, respectively. Likewise in study II, level of total proteins was 6.82±0.56 g/dL in T_0, 7.02±0.57 g/dL in T_1 and 6.85±0.34 g/dL in T_2. In study III, protein values for T_0, T_1 and T_2 groups were 7.26±0.30, 7.09±0.48 and 7.20±0.49 g/dL whereas 7.06±0.58, 7.38±0.42 and 7.35±0.31 g/dL, respectively in study IV.

In study I, mean albumin values were 3.18±0.24, 3.11±0.22 and 3.12±0.15 g/dL in T_0, T_1 and T_2 groups, respectively. Albumin level for T_0 group in study II was 3.05±0.14 g/dL that raised significantly in T_1 and T_2 groups to 3.86±0.11 and 3.62±0.25 g/dL, respectively. In study III, albumin values for T_0, T_1 and T_2 groups were 3.52±0.27, 3.52±0.13 and 3.46±0.29 g/dL, respectively. In study IV, albumin level (3.17±0.12 g/dL) in T_0 group was comparatively lower than T_1 (3.85±0.20 g/dL) and T_2 (3.70±0.21 g/dL) groups consuming functional drinks.

In study I, mean values for globulin were 2.79±0.13, 2.71±0.17 and 2.80±0.15 g/dL for T_0, T_1 and T_2 groups, respectively. Globulin level for T_0 group in study II was 3.25±0.24 g/dL that reduced momentously in T_1 and T_2 groups to 2.65±0.16 and 2.72±0.13 g/dL, respectively. In study III groups T_0, T_1 and T_2 showed globulin level of 3.20±0.22, 3.03±0.26 and 3.17±0.28 g/dL, respectively. Likewise in study IV, globulin level for T_0, T_1 and T_2 groups was 3.29±0.22, 2.95±0.15 and 3.02±0.25 g/dL, correspondingly.

Mean values for A/G ratio for T_0, T_1 and T_2 groups in study I were 1.14±0.07, 1.15±0.09 and 1.12±0.06, respectively. In study II, A/G ratio in T_0 group was 0.95±0.04 that momentously increased to 1.47±0.05 in T_1 and 1.34±0.02 in T_2 groups. In study III, mean values for A/G ratio were 1.12±0.05, 1.18±0.04 and 1.10±0.06 for T_0, T_1 and T_2 groups, respectively. Likewise in study IV, A/G ratio was 0.98±0.07 in T_0 group that substantially increased to 1.32±0.09 in T_1 and 1.23±0.07 in T_2.

DISCUSSION

Morita *et al.* (2009) delineated non-substantial effect of different green tea doses on rats organs weight like heart spleen and brain except for liver and kidney of rats. Likewise, Chengelis *et al.* (2008) mentioned that rat's organs like liver, kidneys, heart and spleen were not affected significantly by orally given green tea catechins up to dose of 2000 mg/kg/day for 28 days. In a research study Takami *et al.* (2008) reported similar non-momentous effect of green tea catechins (1.25%) on lungs, heart, spleen, liver and kidneys of rats. The

Table 4: Effect of functional drinks on serum proteins

		Studies			
Parameters	Treatments	Study I	Study II	Study III	Study IV
Total proteins (g/dL)	T_0	6.48±0.33^{NS}	6.82±0.56^{NS}	7.26±0.30^{NS}	7.06±0.58^{NS}
	T_1	6.29±0.49^{NS}	7.02±0.57^{NS}	7.09±0.48^{NS}	7.38±0.42^{NS}
	T_2	6.40±0.02^{NS}	6.85±0.34^{NS}	7.20±0.49^{NS}	7.35±0.31^{NS}
Albumin (g/dL)	T_0	3.18±0.24^{NS}	3.05±0.14^{NS}	3.52±0.27^{NS}	3.17±0.12^{NS}
	T_1	3.11±0.22^{NS}	3.86±0.11**	3.52±0.13^{NS}	3.85±0.20*
	T_2	3.12±0.15^{NS}	3.62±0.25**	3.46±0.29^{NS}	3.70±0.21*
Globulin (g/dL)	T_0	2.79±0.13^{NS}	3.25±0.24^{NS}	3.20±0.22^{NS}	3.29±0.22^{NS}
	T_1	2.71±0.17^{NS}	2.65±0.16**	3.03±0.26^{NS}	2.95±0.15^{NS}
	T_2	2.80±0.15^{NS}	2.72±0.13**	3.17±0.28^{NS}	3.02±0.25^{NS}
A/G ratio	T_0	1.14±0.07^{NS}	0.95±0.04^{NS}	1.12±0.05^{NS}	0.98±0.07^{NS}
	T_1	1.15±0.09^{NS}	1.47±0.05**	1.18±0.04^{NS}	1.32±0.09**
	T_2	1.12±0.06^{NS}	1.34±0.02**	1.10±0.06^{NS}	1.23±0.07**

results regarding organ to body weight ratio showed that green tea did not impart any hazardous effect on these organs as the values were within normal ranges proving the safe use of functional drink.

Liver is an important organ for the detoxification and deposition of endogenous and exogenous substances. ALT and AST are important serum enzymes as their varied concentrations indicate liver dysfunctioning (Wang *et al*., 2007). A number of natural/herbal products used against liver injury possess one or combination of antioxidant, antifibrotic, immune modulatory or antiviral activities (Seeff *et al*., 2001; Lee and Jeong, 2002; Shin *et al*., 2006). Recently, Noori *et al*. (2009) investigated the effect of green tea against carbon tetrachloride (CCL_4) induced liver cirrhosis in rodents modeling. Plasma Alanine Aminotransferase (ALT) was much lower in orally treated green tea group confirming its vitality against liver dysfunctions. Yasuda *et al*. (2009) revealed that 0.1% solution of EGCG in drinking water decreases serum AST and ALT raised by CCL_4 in rat modeling thus cures liver complications. Bose *et al*. (2008) also illustrated that ALT concentrations were reduced in EGCG treated high fat diet obese mice group. Likewise, Kuzu *et al*. (2008) mentioned that EGCG administration to Sprague Dawley rats for six weeks along with High Fat Diet (HFD) caused significant reduction in plasma ALT.

Feillet-Coudray *et al*. (2009) elucidated that diet rich in sucrose and fat leads to increased lipid peroxidation products resulting in oxidative stress however, green tea provides protection against oxidative damage thereby lowering Aspartate Aminotransferase (AST) activity (Panza *et al*., 2008). Earlier, Hassan *et al*. (2007) used carbon tetrachloride induced hepatotoxic rats to evaluate the protective role of green tea. They reported reduction in liver AST level with green tea.

In diabetic rats, treatment of green tea extract (300 mg/kgbodyweight/day) significantly lowers serum AST, proving its worth as therapeutic agent in diabetes complications (Babu *et al*., 2007). EGCG is an effective antioxidant (Yin *et al*., 2008) and its 0.1% solution decreases serum AST and ALT raised by CCL_4 in rat modeling, thus acts as remedy for liver complications (Yasuda *et al*., 2009).

Ramesh *et al*. (2009) determined the role of tea catechins in rats with hepatic oxidative abnormalities and highlighted that intraperitoneal injection of tea catechins decreased activities of serum AST, ALT and ALP.

Increased serum activities of total bilirubin, AST, ALP and ALT reveal cellular leakage and loss of functional integrity of cell membrane in liver (Mukherjee, 2003). The reduction in their levels confirms stabilization of plasma membranes as well as restoration of hepatic tissue damage (Lin *et al*., 2008). Recently, Morita *et al*. (2009) reported non-momentous effect of green tea catechins on serum chemistry including bilirubin. Likewise, Chengelis *et al*. (2008) also affirmed the safety issues of green tea catechins using up to 2000mg/kg/day; reported non-significant effect on markers of liver toxicity including AST, ALP, ALT and bilirubin.

High serum urea and creatinine concentrations reflect abnormal kidney functioning (Kataya and Hamza, 2008). Renno *et al*. (2008) proved the ability of tea catechins to normalize elevated level of urea. They mentioned significantly high urea nitrogen in serum of diabetic Sprague Dawley rats that reduced substantially by provision of green tea. In present findings though effect of functional drinks was significant on serum urea of rats provided cholesterol and sucrose rich diets but values were within normal limits.

The work of Sabu *et al*. (2002) supported the present finding of reduced creatinine by function drinks as they recorded significant reduction in serum creatinine level of diabetic rats by administration of green tea polyphenols. Likewise, Renno *et al*. (2008) observed similar declining trend in serum creatinine level by use of green tea solution as drinking source in diabetic rats. Morita *et al*. (2009) reported non-significant effect of green tea catechins up to dose of 1200 mg/kg/day on serum total proteins of Sprague Dawley rats. Likewise, Kao *et al*. (2000) expounded non-momentous effect of green tea epigallocatechin gallate on this trait.

Chengelis *et al.* (2008) mentioned non-momentous effect on serum albumin content of male and female rats during 28 days study period. Malley *et al.* (2007) reported the values i.e. 6.8-7.9, 3.8-4.2, 3.1-3.7 g/dL for total proteins, albumin and globulin, respectively that are in line with instant findings as serum albumin was in range from 3.05±0.34 to 3.86±0.61 g/dL. In present investigation though albumin level was increased momentously in study II and IV but values were within normal range.

In present investigation effect of functional ingredient on serum globulin was non-substantial except for study II in which declining trend was recorded nonetheless, values were within safe limit.

From present exploration it is observed that values for liver and kidney functioning tests were within normal range showing the acceptability of product. Moreover, protein related parameters showed non-momentous differences though some of values behaved substantially but were within normal range proving the functional worth of prepared drinks. Considering above all results, it is concluded that functional drinks are risk free and could be used against various ailments.

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Changes in Endogenous Cytokinins and *in vitro* Photoperiodic Flowering Induction in *Cichorium intybus* L.

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Abstract: Cytokinins were extracted from the explants of *Cichorium intybus* L. roots grown under flowering inductive (long-day and red light), or non inductive conditions (short-day). Flowering was expressed as percent after 8 weeks of their development. Overall, cytokinins levels exhibited biphasic trends with initial increasing concentrations followed by a decrease over time. The highest content of cytokinins was observed in explants exposed to either red light or long-day periods. Measured maximum levels of up to 525 ng.g^{-1} dry weight were obtained under red light exposure conditions. Cytokinins concentrations measured in materials cultured under long-day conditions were slightly lower by quite similar to those obtained under red-light growth conditions. In contrast, much lower peak values (not exceeding 155 ng.g^{-1} dry weight) were obtained under short-day experimental conditions. The *in vitro* development of *C. intybus* under both long-day periods and exposure to red light resulted in percent flowering of 55.75% and 64.28%, respectively. No flowering was observed under short-day experimental conditions. Overall, our results show that both endogenous content of cytokinins and flowering of *C. intybus* grown *in vitro* are dependent on both light quality and length of the photoperiod.

Key words: *Cichorium intybus* L., cytokinins, flowering, *in vitro* culture, photoperiodicity

INTRODUCTION

The use of *in vitro* cultures in agriculture, arboriculture, forestry and horticulture plays a significant role in meeting the continuously growing needs of human populations (Dubos, 2001; El Kbiach *et al*., 2002). Despite current efforts for the development and improvement of *in vitro* techniques by both academic and industrial institutions, knowledge on the physiology of plant materials cultivated *in vitro* remains rather limited (El Kbiach *et al*., 2002). This is because the neo-formation of plant materials such as floral buds cultured *in vitro* depends on several and complex physicochemical parameters that includes photoperiods and climatic variations and which promote different types of biochemical modifications. For instance, these parameters have been linked to changes in cellular differentiation, which in turn impacts floral organogenesis (Attibayéba and Paulet, 2004). For of *Cichorium intybus* L., the inductive process of *in vitro* flowering is a key factor for reproductive development, which is associated with important biochemical changes such as variations in enzymatic activities (Attibayéba, 1992), accumulation of chlorogenic acid (Badila and Paulet, 1986) and changes in endogenous contents of ß-indolylacetic acid (Gaspar *et al*., 1982). Therefore, understanding the different physiological mechanisms involved in the flowering process would help close current knowledge gaps.

In this paper, we investigated the potential relations between cytokinins levels and flowering induction under *in vitro* culture conditions. The study emphasizes the effect of changes in endogenous cytokinins levels on floral metabolism of explants of *Cichorium intybus* L.

MATERIALS AND METHODS

Plant materials: Roots of *Cichorium intybus* L. C.V. Witloof were used in all experiments. Under natural conditions, *C. intybus* exhibits a port in bow in a vegetative state and flowering under such conditions requires exposure to cold temperatures and diurnal cycles with long photoperiods. After collection of the plant's roots from the field and hand cleaning, they were placed horizontally in tubs filled with slightly humidified vermiculite and preserved at 4°C for about three months.

***In vitro* cultures:** At the time of their use, roots, freed of their snares, were divided into three zones from the snare. These zones are representative of the hormonal distribution gradient (Vardjan and Nitsch, 1961). Explants were taken in the median third of the root. After sowing on culture medium of Margara and Rancillac (1961a), the cultures of tissues were arranged in premises air-conditioned, maintained at 24°C during daytime and 22°C at night. In the air-conditioned premises, white light with an intensity of 56 µmoles m^{-2}s^{-1} (Mazda Fluor TFR 20 L 5L) was used for 16 h (L-D:

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long-day) or 8 h (S-D: short-days) for every 24 h. Additionally, for cultures under short-day conditions, the 16 h of darkness was followed by an illuminated by red light or R (3 µmoles $m^{-2}s^{-1}$; Mazda Fluor TF 40W/15) from the 8th day of incubation to the 18th day. Red plastic filters (Rodhoïd 227 P2F 0.3 mm, COFRAMAP, France) were used in combination with the above described fluorescent red light.

For every treatment, explants were removed from growth media starting on the 5th day of the experiment up to the 30th day. Removed explants were freeze-dried immediately until analysis for cytokinins. After 8 weeks of *in vitro* development and for the number N of replicates corresponding to a given treatment type, the number of explants with visually identifiable neoformation of floral organs or n was determined. The ratio n/N was then used to report the results on a 100% scale.

Extraction of cytokinins: A method adapted from Palni and Horgan (1983) was used. First, 10 g of freeze-dried root explants were finely crushed at room temperature using a Dangoumau grinder for 30 sec. The obtained powdered material was extracted overnight with a 100 ml of an 80% methanol solution at 4°C and without shaking to solubilize the nucleotides. The supernatant was then filtered using a Büchner the following day and the above described methanol extraction repeated twice on the same powdered material. For each sample, the three methanol extracts were combined and evapo-concentrated using a rotary evaporator at 35°C to about 20 ml. The concentrated sample was centrifuged at 9000g at 5°C for 15 min. The supernatant was recovered and its pH adjusted to 3.5. The surfactant Polyvinylpyrrolindone (PVP) was added to the sample (80 mg/mL of extract), mixed and then filtered using a Büchner to remove the excess PVP. At this stage, the obtained filtrate contains the cytokinins. The pH of the extract is next adjusted to 8.5 and a volume of water-saturated n-butanol, with a water content equivalent to that of the extract, is added to it. The mixture is shaken for 45 min (Tay and Palni, 1987) and decanted by sedimentation. The aqueous phase is then extracted 3 times with the n-butanol solution as described above to transfer the nucleotidic cytokinins to the organic phase. The butanolic supernatants are combined and evaporated to dryness under controlled pressure at 40°C. Two mL of a 80% methanol solution in water was used to resuspend the active components extracted from the samples. The pH of this obtained solution was adjusted to 8.2 and brought to 10 ml. Next, the 4 mg of alkaline phosphatase was added to the sample (Van Staden and Dimalla, 1980), then centrifuged at 40,000g at 3°C for 20 min. The recovered supernatant was adjusted to a known final volume, split in n- butanol (Palni and Horgan, 1983), before being purified using PVP following the procedure described above.

Purification of the extracts of cytokinins: The free cytokinins stemming from the butanolic phase and the bound cytokinins were separated by chromatography on whatman paper 3 mm according to the method of Van Staden *et al.* (1983), after sample treatment with ß-glucosidase. They are purified on Sephadex column LH 20 (21 x 2.5 cm) and then on Thin Layer Chromatography (TLC) before identification by Gas Chromatography (GC).

Gas Chromatography (GC): Prior to GC identification, the purified solutions of cytokinins were evaporated and dried on P_2O_5. The residues were dissolved in 10 ml of pyridine. The derivatives TMS (trimethylsylilyed) were prepared by addition of 20 ml of BSTFA (twice trimethyltrifluoroacetamid). The sylilation was conducted in conical tubes for 30 min at 90°C. Residues transformed into TMS by BSTFA were analyzed by GC (Delsi DI-700, equipped with a FID detector and a 2.5 m long Pyrex column with a 2 mm ID). The column packing material was chromosorb WHP with 3% silicon. Helium was used as carrier gas at a flow rate of 40 ml/min. The detector and the injector temperatures were maintained at 300°C. The cytokinins were analyzed using different temperature conditions. The riboylzeatine was analyzed under isotherm conditions with a column temperature of 280°C. In contrast, temperature gradient was used for the separation of zeatine (first 240°C for 5 min and increase to 260°C at a rate of 30°C per minute) and isopentenyladenosine (245°C for 5 min and up to 260°C at a rate of 30°C per minute).

For the identification of the cytokinins, 10 ng of Benzylaminopurine (BAP) were used as internal standard and co-injected with the extract (v/v). The co-chromatography is obtained by mixing cytokinins from commercial sources with those extracted from samples, volume per volume.

Analysis of the cytokinins: From a mother solution of cytokinins with a concentration of 1 mg/mL, standards were prepared. Cytokinins concentrations in samples are expressed in ng/g dry weight.

RESULTS AND DISCUSSION

Our results show that the levels of cytokinins in analyzed plant extracts increase from the 5th day during *in vitro* development, to reach their maximum on the 15th day. This initial growth phase is then followed by a decrease up to the 30th day of incubation. Figure 1 shows that explants exposed to Red Light (R) have Ribosylzeatine

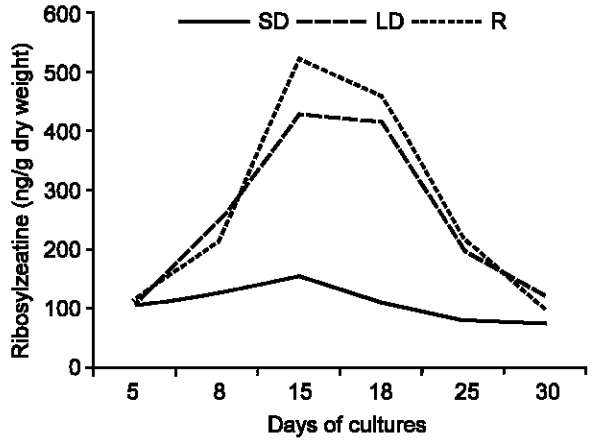

Fig. 1: Effect of photoperiodic variations on ribosylzeatine levels over time (SD = Short Day, LD = Long-Day and R = Exposure to Red Light)

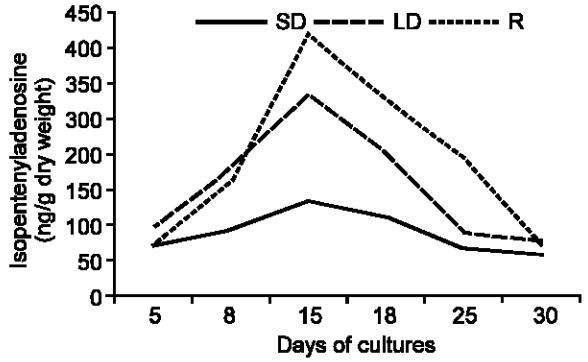

Fig. 2: Effect of photoperiodic variations on isopentenyladenosine levels (SD = Short Day, LD = Long-Day and R = Exposure to Red Light)

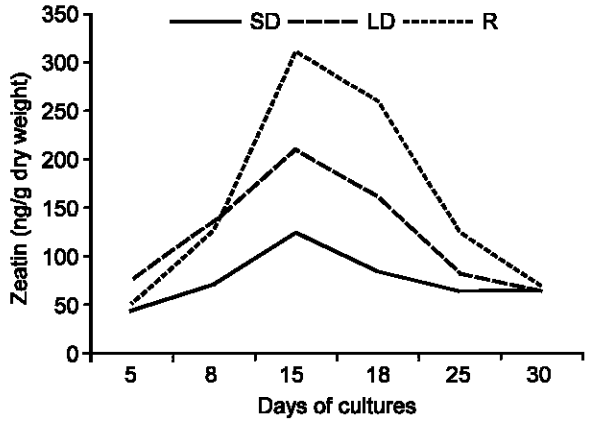

Fig. 3: Effect of photoperiodic variations on zeatine concentrations (SD = Short Day, LD = Long-Day and R = Exposure to Red Light)

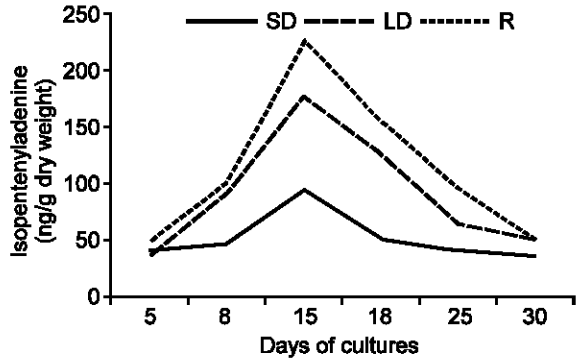

Fig. 4: Effect of photoperiodic variations on concentrations of isopentenyladenine (SD = Short Day, LD = Long-Day and R = Exposure to Red Light)

(RZ) contents that vary from 109, 525 and 100 ng.g^{-1} dry weight to 100, 430 and 120 ng.g^{-1} dry weight in L-D experiments and to 100, 155 and 75 ng.g^{-1} dry weight in S-D experiments. The contents of Isopentenyladenosine (IPA) varied from 80, 415 and 85 ng.g^{-1} dry weight in the R; to 110, 320 and 90) ng.g^{-1} dry weight in L-D incubations; to 80, 150 and 65 ng.g^{-1} dry weight in the S-D tests (Fig. 2). Also, the curves of endogenous zeatine (Z) varied from 52, 312 and 70 ng.g^{-1} dry weight in the R; to 75, 210 and 64 ng.g^{-1} dry weight in the L-D and to 44, 124 and 64 ng.g^{-1} dry weight in the S-D (Fig. 3). The Fig. 4 shows that, the curves of endogenous Isopentenyladenine (IP) changes to 48, 225 and 50 ng.g^{-1} dry weight in explants exposed to the red light; to 40, 176 and 50 ng.g^{-1} dry weight in those developing in the L-D and to 36, 94 and 36 ng.g^{-1} dry weight in the S-D. In the same way, in the R, the contents of Ribosylzeatine Glucoside (RZG) changes to 32, 112 and 34 ng.g^{-1} dry weight; to 42, 90 and 38 ng.g^{-1} dry weight in the L-D and to 30, 46 and 26 ng.g^{-1} dry weight in the S-D (Fig. 5). Finally, the Fig. 6 shows that, the contents of Zeatine Glucoside (ZG) changes to 36, 138 and 36 ng.g^{-1} dry weight in the R; to 45, 112 and 36 ng.g^{-1} dry weight in the L-D and to 36, 60 and 25 ng.g^{-1} dry weight in the S-D.

Generally, we note that, the content in RZ and IPA is more important with regard to those in Z and IP or RG and RZG. In the R and in the L-D the contents of endogenous cytokinins are higher than in S-D explants. What seems to justify itself because, in the L-D and the R, explants differentiates respectively 55.75% and 64.28% of flowers; in the S-D, no culture differentiates *in vitro* flowers (Table 1). Light conditions of the study led to an increase of cytokinins and the capacity to flowering. The analysis of these cytokinins revealed that more Ribosides (RZ, IPA) than bases (Z, IP) as commonly found in plants (Dyson and Hall, 1972; Miller, 1975; Joseph, 1984). This can be explained by the higher ability of bases to bind to the receivers. Such fixed

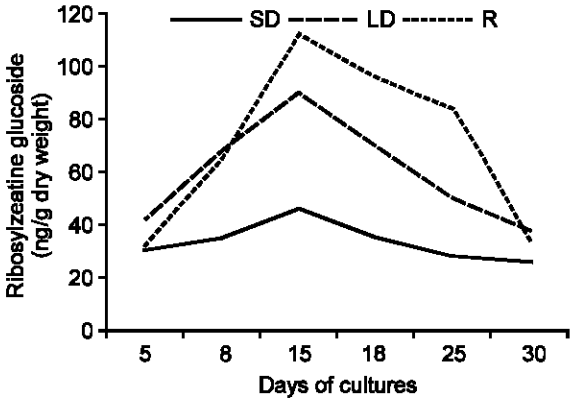

Fig. 5: Changes in ribosylzeatine glucoside levels versus photoperiodic conditions (SD = Short Day, LD = Long-Day and R = Exposure to Red Light)

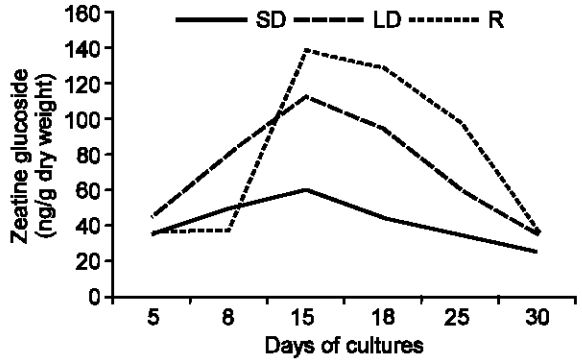

Fig. 6: Changes in zeatine glucoside levels versus photoperiodic conditions (SD = Short Day, LD = Long-Day and R = Exposure to Red Light)

Table 1: Effect of different photoperiodic treatments on *in vitro* flowering of fragment roots of *Cichorium intybus* L.

Photoperiodic conditions	Percentage of flowering (%)
Short day	00.00±0.00
Long day	55.75±0.91
Red light	64.28±3.21

molecules would become inaccessible by the alcoholic extraction used in this study and extracts would consequently be relatively poor in bases. Nucleotides were present in small quantities and in the form of ribosylzeatine only. Glucosides were present only in the form of glucoside of RZ and Z. Some authors have reported that these glucosides have a weak biological activity (Laloué and Pethe, 1982; Badenoch *et al*., 1984b). In *C. intybus* L., favourable conditions for *in vitro* flowering and the formation of meristematic structures generator of floral buds are preceded by an important production of cytokinins, followed by a decrease during the floral expression's phase. On the contrary, the conditions putting obstacle to the flowering decrease strikingly these contents. Overall, we note 3 distinct phases of evolution of levels of endogenous cytokinins during *in vitro* flowering. (1) A pre-inductive phase, of a week approximately from the stake in culture. It is the phase of formation of meristematic nodules which get organized in buds. It seems to have a little dependence on photoperiod, and the contents of cytokinins in explants are low. (2) A photo-inductive phase also of a week approximately, where neo-formed meristems present a stage of development to which the susceptibility in the illumination is maximal. An important production of cytokinins is observed. Finally (3), a phase of initiation and floral development, with little sensitivity to day length and during which the quality previously acquired expresses itself outside by the elaboration of floral organs. Here, the contents of cytokinins fall again. It proves well that, the *in vitro* culture of explants of roots leads to a differentiation of tissues, to phenomena of embryogenesis and allows obtaining a plantlet (El Kbiach *et al*., 2002; Azeqour *et al*., 2002). The light modulates the morphogenetic process which leads to the regeneration this plantlet (Baaziz *et al*., 1996; Majourhat, 2002). The only difference between the inductive and non-inductive conditions of flowering results in the intensity of production of cytokinins, which is raised in the conditions founders of the flowering. What lets augur the existence of a correlation between the cytokinins contents and the neoformation of the *in vitro* floral buds. These same relations of causality exist between the enzymatic activities and the primary events of the photoperiodic induction of the flowering (Attibayéba and Paulet, 2004). What shows that, the photoperiodic induction acts no only on cytokinins, but also on other metabolites and regulators which have an indisputable role on the flowering.

Conclusion: In this study, the measurable changes during the photoperiodic treatments are the successive increase and decrease of the contents of cytokinins as the experiments reach the switching point from photo-induction to floral expression phases. Levels of cytokinins are more important under conditions that are inductive to flowering than in non-inductive conditions. GC-analyses reveal that cytokinins are present mostly as nucleosides (isopentenyladenosine and ribosylzeatine), bases (zeatine and isopentenyladenine), glucosides (ribosylzeatine, zeatine), while ribosylzeatine was the only identified nucleotide. Photo-period appears to be a necessary condition for the stake with flowers bound to the long or short, daily duration of the illumination according to the sorts. Future work should (1) focus on the characterization of the physiological changes of the photo receiving systems, and biochemical sites involved in the perception of the stimulus in the floral induction and in the production of

cytokinins; particularly to bring to light put back Red Light-Far Red (R-FR) which allow to involve the phytochrom in the morphogenesis; (2) use short periods of illumination for photophase and (3) bring to light the possible role of blue, green and yellow lights. Such a comprehensive study would allow a better understanding of the modulation of morphogenetic process by light. This could have positive implications for increased quality and productivity in relevant farming activities.

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Description of Different Growth Stages of *Sesamum indicum* L. Using the Extended BBCH Scale

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Abstract: The extended Biologische Bundesantalt and CHemische (BBCH) scale and its associated decimal code were used to describe the different growth stages of *Sesamum indicum* L. The study focused on different primary and secondary plant growth phases from germination to senescence. The use of a two-digit decimal code allowed the identification of the principal growth stages and their respective secondary stages. This approach suggests that this could be of great help to sesame growers and researchers for efficient planning of both management practices and experimental designs.

Key words: Congo-Brazzaville, plant growth phases, extended BBCH scale, fruit, germination, phenology, senescence, *Sesamum indicum* L., ripening

INTRODUCTION

The Pedaliaceae includes about 16 genus and 60 species, of which *Sesamum* is the major genus group. The sesame is an annual plant that is 10-20 cm high and rarely reaching 180 cm. The stem is obtusely quadrangular and finely pubescent to glabrescent. The leaves are heteromorphic and grow on either opposite or alternate position on branches. Flowers come in different colors and the most common are either white, pink, or mauve-pink. Fruits are oblong capsules with small oval and yellowish seeds (Kafiriti and Deckers, 2001). Sesame is self-pollinating, although differing rates of cross pollination have been reported by Yermanos (1980), Ashri (2007) and Sarker (2004). The pollinisation process occurs at the time the flowers open (Kafiriti and Deckers, 2001; Langham, 2007). The species *Sesamun indicum* L. is cultivated in Europe, Asia, America and Africa. By 2001, the estimated land area used for sesame production was about 2-2.5 million ha (India), 1 million ha (China), 0.6 million ha (Birmania) 0.5 million ha (Soudan) and 0.25 million ha (Mexico). In Africa, Soudan leads in sesame production, followed by Nigeria, Somalia, Ouganda and Ethiopia (Kafiriti and Deckers, 2001).

Sesame is an important oil seed crop. The seed has excellent nutritional value having high and unique protein composition making them a nearly perfect food. The seeds by expression yield a fixed oil consisting essentially of the glycerides of oleic acid and linoleic acid with preparations of stearin, palmitin and myristin. Liquid fatty acids are present to about 70% solid fatty acid 12-14% (Rakipov, 1987; Kafiriti and Deckers, 2001). Knowledge of the phenology of the crop is important for the correct timing of management practices such as fertilizer application and disease, pest and weed control. Several descriptions of the growth stages of this plant can be found in the literature (Kang *et al*., 1985; Mulkey *et al*., 1987; Langham, 2007) and as is the case for the majority of cultivated plants, there is no unified and standard description approach. Most papers refer to specific growth stages, but no effort is usually made to establish a full description that could lead a framework of growth stages for general use.

Since the proposal of Zadoks *et al*. (1974) of a decimal code for the description of growth stages for cereals, there has been a growing interest in the extension of these general principles for the description of the growth stages of many other crops (Agusti *et al*., 1997; Gonzales *et al*., 2002; Cautin and Augusti, 2005). In recent years and based on the above mentioned proposal, a uniform decimal code, known as the BBCH-scale, was developed by Bleiholder *et al*. (1991) and Lancashire *et al*. (1991). A more advanced scale, the extended BBCH-scale was proposed later by Hack *et al*. (1992) and Hess *et al*. (1997). Subsequently, Meier (1997) published the "BBCH-monograph" for 27 crops and weeds. According to this universal scale, by using phonological criteria and a consistent set of numeric codes, it is possible to establish a uniform coding that describes the growth stages of a large number of plant

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species. In this paper, we take advantage of the extended BBCH-scale and its decimal code to provide a description of the growth stages of the sesame plant.

MATERIALS AND METHODS

This study was conducted using S. *indicum*, an annual plant with a wide variety of specimen and sizes ranging from 0.5-2 m. In this research effort, seeds from *S. indicum* were first collected from Okouesse in the central portion of the Republic of Congo and taken to the experimental site located in Brazzaville, the capital city of the Republic of Congo (4°168; 15°110). Experimental studies were conducted on a study site located on the University of Brazzaville's campus. The study area is characterized by a short dry season from June to September and a rainy season from October to May. It is worth to note that the rainy season is not uniform as it is interrupted by a 2-month period (January and February) of very little to no precipitation. Overall, the annual precipitation averages 1370 mm per year, while the average temperature is about 25.3°C.

Prior to planting, the top 10cm of surface soil was well mixed with a hoe. Then, furrows of about 9.30 x 1.70 m were made using shovels. Furrows were separated by empty spaces of about 0.7 m and the surface of each furrow was "leveled" using a rack. To avoid the formation of deep depressions in which water could accumulate and lead to the death of growing plants, experimental plots were well-homogenized and leveled following the method described by Kafiriti and Deckers (2001). The planting was finally done along well-defined transacts in the month of April. Approximately 4 seeds were placed in small holes and covered with loose soil. For each furrow, three transacts were used. Finally, after germination, a total of 50 plants were chosen for observational studies.

RESULTS AND DISCUSSION

Scale characteristics: The extended BBCH scale [16, 18] considers 10 principal growth stages numbered from 0-9. For the sesame plant, we begin with the germination of seeds, or shoot development in cuttings or stumps (stage 0). This stage is then followed by vegetative growth considered under 3 macro-stages corresponding to leaf development in seedlings in the nursery or on branches (stage 1), formation of branches (stage 2) and elongation of branches (stage 3). Next are inflorescence emergence and flower development (stage 5), flowering (stage 6), development of fruit (stage 7), ripening of the fruit and seed (stage 8) and senescence (stage 9). Note that the development of vegetative harvestable parts corresponding to stage 4 was not considered because it does not apply to the sesame plant.

The secondary growth stages are also numbered from 0-9 and are related to ordinal or percentage values of growth. For example, for leaf development (stage 1), the fifth true pair is assigned a value of 5 and its identification in the scale will be 15; for branch elongation (stage 3), when 20 nodes are present, a value of 2 is given and its identification in the scale will be 32. In the sesame manner, if 10% of flowers are open, this characteristic is given a value of 1 within the principal stage 6 (flowering) and will be defined as 61 in the scale. For fruit ripening, the change of color was the criteria chosen, thus, stage 88 corresponds to fruits fully yellow and ready for picking and the stage 89 means that fruits are over ripe or decaying.

The descriptions presented below are valid for sesame growth under average climatic conditions in the study area: that is a temperature of 17-23°C, monthly rainfall of at least 120 mm and less than 13.5 h of day light. Although some variation in the timing of the growth stages occurs among cultivated species and regions, the scale can still be applied under these generalized circumstances.

Description of the phenological growth stages for the studied sesame plants

Principal growth stage 0: germination, vegetative propagation, bud development:

00: Dry seed (2.5 mm long, 2 mm large and 3 mg weight); beige color (Fig. 1).
01: Beginning of seed imbibitions, beige color, no radical visible, no shoots and no callus visible.
03: Seed imbibitions complete; small swelling and whitish in color and the radical is not yet visible.
05: Seed radical protrusion and hooking are visible (Fig. 1).
06: Elongation of radical; formation of root hairs and lateral roots on germinated seeds; formation of root hairs and lateral roots on cuttings.
07: Hypocotyls with cotyledons breaking through the seed coat; cuttings have formed shoots and branched roots.
09: Emergence: seedlings have emerged from soil and show the hypocotyls with cotyledons still enclosed in the parchment (Fig. 1).

Principal growth stage 1: leaf development on main shoot of the young plant and branches:

10: Cotyledons completely unfolded. First pair of true leaves separating on branch (see Fig. 1).
11: First leaf pair unfolded, not yet at full size and exhibit a light green color.
12: 2 leaf pairs unfolded, not yet full size; the third leaf pair from apex is light green.
13: 3 leaf pairs unfolded, not yet full size; the third leaf pair from apex is still light green.
14: 4 leaf pairs unfolded. The fourth leaf pair from apex is light green and has reached full size.
15-18: Five to 8 leaf pairs unfolded.
19: Nine or more leaf pairs unfolded (see Fig. 1).

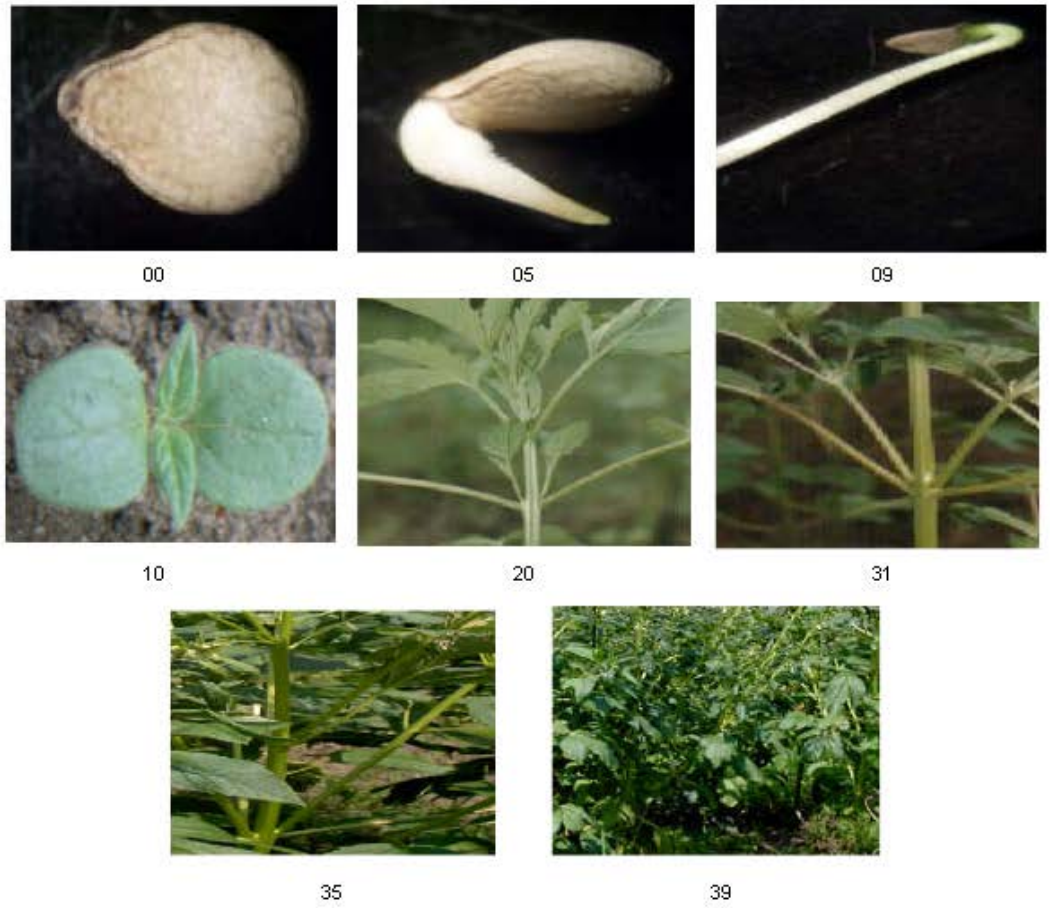

Fig. 1: Vegetative growth stages (00-39) of *Sesamum indicum* L. plant

Principal growth stage 2: formation of branches:

20: First pair of primary branches is visible (Fig. 2)
21: Ten pairs of primary branches visible
22: Twenty pairs of primary branches visible
23: Thirty pairs of primary branches visible

Principal growth stage 3: branch elongation:

30: Beginning of branch growth; axes of developing shoots visible.
31: Branches about 10% of final size (see Fig. 1).
32: Branches about 20% of final size.
35: Branches about 50% of final size (Fig. 1).
39: Branches about 90% of final size (Fig. 1).

Principal growth stage 5: Inflorescence emergence:

50: Inflorescence buds are closed in leaf axils.
51: Inflorescence buds swelling in leaf axils (Fig. 2).
53: Inflorescence buds burst; no flowers visible
57: Flowers visible, still closed (Fig. 2)
58: Flowers visible, still closed, petal 1.5 cm long and white.
59: Flowers with petals elongated (3 cm long), still closed and white in color (Fig. 2).

Principal growth stage 6: flowering:

60: First flowers open and white color (Fig. 2)
61: 10% of flowers open
63: 30% of flowers open
65: 50% of flowers open
67: 70% of flowers open
69: 90% of flowers open

Principal growth stage 7: fruit development:

70: Fruit set; fruit visible as small capsule.
71: Beginning of capsule growth. Fruit have reached 10% of final size (Fig. 2).
73: Fruit about 30% of final size
75: Fruit about 50% of final size
77: Fruit about 70% of final size (Fig. 2).
79: Fruit about 90% of final size. Physiological maturity is complete (Fig. 2).

Principal growth stage 8: ripening of fruit and seed:

81: Beginning of fruit color change from pale green to yellow (Fig. 3).
85: Increase in fruit color intensity, fruit not yet ready for picking.

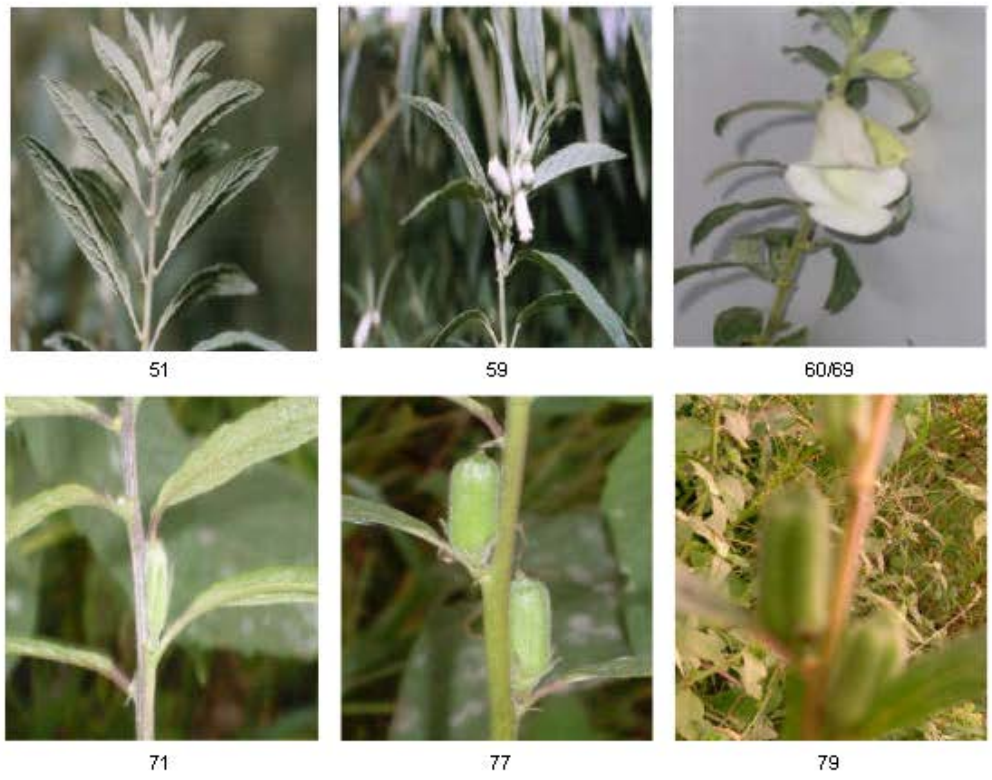

Fig. 2: Reproductive growth stages (51-79) of *Sesamum indicum* L. plant

Fig. 3: Ripening and drying phases (79-98) of *Sesamum indicum* L.

88: Fruit is fully-ripe and ready for picking.
89: Over ripe; beginning drying (Fig. 3).

Principal growth stage 9: senescence:
90: The shoots have completed their development (Fig. 3).
93: Older leaves change from deep green to yellow (Fig. 3).
94-98: Leaves falling (Fig. 3).
99: Storage treatments

Conclusion: The use of the extended BBCH scale and a two-digit decimal code allowed the identification of the principal growth stages and their respective secondary stages. This method can be used by sesame growers as well as researchers for efficient planning in both management practices and experimental designs.

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Fruit Properties and Nutritional Composition of Some Walnut Cultivars Grown in Pakistan

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Abstract: Walnuts (*Juglans regia* L.) are rich source of a number of important nutrients that have a very positive effect on the human health. In this study nuts of six different walnut cultivars grown in Pakistan namely Chitral-1, SW-1, Chitral-3, Chitral-2, SW-3 and Dir-2 were selected and evaluated for their physical properties, proximate and mineral composition. The nut length was found in the range of 35.17-41.37 mm, nut diameter (31.72 mm-34.32 mm), Nut thickness (32.21-35.10 mm), nut weight (10.30 g-19.22 g), Kernel weight (5.81 g-9.24 g), Kernel ratio (43.19-65.14%), shell thickness (0.81-1.35 mm), moisture content (2.76-4.20%), ash content (1.27-1.95%), fats (63.54-69.92%), protein (15.96-19.15%) and total carbohydrates (8.04-12.14%). Kernels of Dir-2 and Chitral-2 cultivars were determined to contain high protein content (>18% protein) while Chitral-1 and SW-1 contain high carbohydrates content of >10%. The energy value of the kernels of these cultivars was determined in the range of 698.10-732.44 Kcal/100 g, which shows that the fruits of these varieties are rich source of energy. In minerals the level of sodium is 40.9-64.5 ppm, K (3551-4827ppm), Ca (925-1250 ppm), Fe (30.08-41.20 ppm), Zn (11.75-25.5 ppm), Mg (1059-1765 ppm), Cu (1.96-2.75 ppm) and Pb (0.69-1.06 ppm). The fruit properties indicates that Chitral-3, Chitral-2 and SW-3 are superior walnut cultivars in terms of their physical properties than the rest of the cultivars and they could be cultivated and developed as standard varieties in Pakistan. Moreover, kernels of all the cultivars studied are rich in K, Ca, Mg, Na and Fe.

Key words: *Juglans regia* L., fruit properties, nutritional composition, proximate composition, energy content

INTRODUCTION

Walnut (*Juglans regia* L) a member of *Juglandaceae* family is one of the finest nuts of temperate regions (Ozcan, 2009). Walnuts are of a high economic interest for the food industry (Martinez *et al*., 2008) and its nuts are highly appreciated for its unique organoleptic characteristics (Lopez *et al*., 1995), hypocholesterolemic effects (Sabate and Fraser, 1994; Abbey *et al*., 1994; Savage, 2005; Dogan and Akgulb, 2005; Pereira *et al*., 2008) and antihypertensive effect (Sabate and Fraser, 1994; Mexis *et al*., 2008; Arranz *et al*., 2008). The bark of *Juglans regia* (locally called Dandasa) is regularly used as miswaks for teeth cleaning (Ibrar *et al*., 2007). Walnut species are important sources of nuts and timbers in the temperate zones across the world (Zhang *et al*., 2009; Li *et al*., 2007; Khan and Khatoon, 2007). A valuable edible nuts produced by walnut trees are well appreciated because they are enriched with unsaturated fat (linoleic, oleic acid). The walnut plant has a high nutritional value and high-quality wood. In addition, walnuts have significant economical value and medicinal importance for human health because of their biochemical composition of polyunsaturated fatty acids, especially 18:2 and 18:3 and protein value (Savage *et al*., 2001). They also contain other beneficial components like plant protein (e.g. arginine, leucine), carbohydrates (e.g. dietary fibre), vitamins (e.g. vitamin A, E), pectic substances, minerals (magnesium, potassium, phosphorus, sulphur, copper and iron), plant sterols and phytochemicals (Kris-Etherton *et al*., 1999; Savage *et al*., 2001; Prasad, 2003; Colaric *et al*., 2006). In Pakistan there are up to 50 different walnut cultivars growing in different regions of Pakistan like Chitral, Dir, Swat, Gilgit, Kaghan, Baltistan, Kurrum and Muzafarabad. However, the literature describing physical properties, proximate and mineral composition of walnut fruits is very limited. The present study was designed to evaluate the fruits of walnut cultivars grown in the Chitral, Swat and Dir districts of Pakistan for their physical parameters, proximate composition and mineral composition.

MATERIALS AND METHODS

Walnut seed from six walnut cultivars namely Chitral-1, SW-1, Chitral-3, Chitral-2, SW-3, and Dir-2, were collected from the selected areas of Swat, Chitral and Dir (Lower and Upper) districts of Pakistan in September, 2008 from the trees which were 25-40 years

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old. These areas are at altitudes of 1550-1750 meters. The storage conditions and time until analysis were similar for all cultivars. Physical analysis was quickly determined and kernel samples were kept at -18°C before chemical analyses. There were at least three repetitions in proximate chemical analyses and ten repetitions in physical analyses.

Physical analysis: Physical analysis include nut fruit properties (i.e. nut diameter, nut length, nut thickness, nut shape, nut size, nut weight, shell thickness, shell roughness) and kernel properties (kernel weight and Kernel ratio) were determined according to the Turkish Standard Institute (TSI, 1990 and 1991).

- Shape of the nuts was determined by the following formula:

$$\text{Nut index} = \frac{\text{Nut length (mm)}}{(\text{Nut diameter} + \text{nut thickness})/2}$$

Nut index < 1.25 were taken as sphere shape
Nut index ≥ 1.25 indicate the oval shape of the nuts

- Kernal ratio was determined by the formula:

$$\text{Kernel ratio (\%)} = \frac{\text{Kernel Wt. (g)}}{\text{Nut Wt. (g)}} \times 100$$

- Size "extra" for the nuts if,

Nut diameter ≥ 27 mm for sphere
Nut diameter ≥ 26 mm for oval

All these analysis were determined at least for 10 of each of the samples from each Cultivar.

Chemical analysis: For the Chemical analysis AOAC methods were used. Fats contents were determined by using AOAC 22.034, protein content AOAC, PN-75/A-04018 and percent moisture by AOAC 22.003 (AOAC, 1990). Percent ash was determined as described by Ali *et al.* (2008). % carbohaydrates were determined by difference.

For mineral composition, the concentration of Na+ and K+ was determined with the help of flame photometer (Jenway PFP7) by the method describe by Khan and Zeb (2007). Heavy metals like Ca, Cu, Fe, Mg, Zn and Pb were determined with help of Atomic Adsorption Spectrometer (Perkin Elmer, model Analyst 700) with air/acetylene flame at 2200-2400K (photo multiplier tube detector), against the standard as described by Hanlon (1992).

Total energy values were calculated by multiplying the amounts of protein and carbohydrate by the factor of 4 kcal/g and lipid by the factor of 9 kcal/g as described by Ullah *et al.* (2010) and Colak *et al.* (2009).

All the parameters were determined at least in triplicate and the results were presented in mean±Standard Deviation (SD).

RESULTS AND DISCUSSION

Fruit properties of the walnuts: Walnut cultivars and their locations from where they were collected are shown in Table 1. The fruit dimensions and shape properties of the six walnut (as shown in Table 2) shows the maximum nut length of 41.37±0.72 mm for SW-3 while the minimum (35.17±0.86 mm) was recorded for the SW-1. The maximum nut diameter of 34.32±1.17 was determined for the Chitral-1 while minimum (31.72±0.83) was found for SW-3. The nut thickness was in the range of 35.10±1.13 mm (Chitral-1) to 32.21±1.25 (SW-3). Nut shape was determined to be spherical for Chitral-1, Swa-1, Chitral-2 and Dir-2 while oval for Chitral-3 and SW-3. Nut size was determined extra for all the cultivars.

Table 1: Walnut verities and their locations used in this study

Name of cultivar	Local market name	Location of cultivar (District)
Chitral -1	Chatral No. 1	Chitral, NWFP
Swat-1 (Kaghzai)	Kaghazi No. 1	Swat, NWFP
Chitral-3	Chitral 3	Chitral, NWFP
Chitral-2	Chitral 2	Chitral, NWFP
SW-3 (Kaghazi)	Kaghzi No. 2	Swat and Dir Upper, NWFP
Dir-2	Dir 2	Sawat and Dir Lower, NWFP

The results regarding the fruit properties of the walnut cultivars are shown in Table 3. Nut weights were in the ranged of 19.22±0.73 g (Chitral-3) to 10.3±0.86 (Swat-1). Kernel weight ranged form 5.81±0.59 g (Dir-2) to 9.24±0.47 g (Chitral-2). Kernel ratio (%) was in the range of 43.19±2.56 % (Dir-2) to 65.14±2.31% (Swat-1). Kernel ratios were <50% for Chitral-1 and Dir-2 while the rest of the cultivars were >50%. Shell thickness of were in the range of 0.81±0.09mm to 1.35±0.72mm for Swat-1 and Chitral-1, respectively. The Swat-1 and SW-3 Cultivar is locally known as kaghazi variety and is valued for its thin shelled nuts.

Akca and Sen (1995) showed nut length as 39.97 mm, nut diameter as 33.59 mm and nut thickness as 34.75 of the promising walnut genotype. This notion is in agreement with our results. Khattak *et al.* (2000) determined the nut weight (12.30-1.90 g), kernel weight (5.43-8.94 g) and kernel ratio (43-47%) for the four walnut cultivars (Kurrum-1, Kurrum-2, Kurrum-3 and Kurrum-4) grown in Kurrum agency, Pakistan. Our results are better than that shown by Ozkan and Koyuncu (2005), who studied the walnut cultivars grown in Turkey and found nut weights (11.09 g-8.43 g), kernel weight (6.32 g-4.35 g), kernel ratio (57.41%-48.89%), shell thickness (0.83 mm-1.47 mm), nut thickness (33.45 mm-29.24 mm), maximum nut length (37.88) and maximum nut diameter (31.12 mm). This could be due to differences in the ecological and genetic properties of walnut cultivars growing in Pakistan and Turkey.

The results of the fruit properties shows that fruits of Chitral-3, Chitral-2 and SW-3 cultivars have superior physical properties and they could be cultivated and developed as standard cultivars in Pakistan.

Table 2: Fruit dimension and shape properties

	Chitral-1	SW-1	Chitral-3	Chitral-2	SW-3	Dir-2	Mean±SD
Nut length	39.51±1.52	35.17±0.86	41.25±1.37	39.71±0.86	41.37±0.72	36.56±1.39	38.93±2.531
Nut diameter	34.32±1.17	32.71±0.65	32.86±1.34	33.25±0.96	31.72±0.83	33.68±1.27	33.09±0.89
Nut thickness	35.10±1.13	33.05±0.76	32.89±0.89	33.47±0.83	32.21±1.25	34.14±1.17	33.47±1.02
Shape	Spherical	Spherical	Oval	Spherical	Oval	Spherical	-
Size	Extra	Extra	Extra	Extra	Extra	Extra	-

Mean±Standard Deviation (SD)

Table 3: Fruit properties of walnut cultivars

	Chitral-1	SW-1	Chit. 3	Chit. 20	SW-3	Dir-2	Mean±SD
Nut wt. (g)	13.51±0.15	10.3±0.86	18.22±0.73	18.15±0.41	16.13±0.69	13.45±0.86	14.96±3.107
Kernal wt. (g)	6.34±0.52	6.71±0.35	9.15±0.41	9.24±0.47	8.20±0.53	5.81±0.59	7.575±1.485
Kernal ratio (%)	46.92±1.96	65.14±2.31	50.21±2.1	50.90±2.41	50.3±1.9	43.19±2.56	51.11±7.465
Shell thickness (mm)	1.35±0.72	0.81±0.09	0.98±0.18	0.95±0.25	0.92±0.07	1.27±0.76	1.046±0.213
Shell roughness	Rough	Median and Porous	Smooth	Medium	Smooth	Rough	-

Mean±Standard Deviation (SD)

Table 4: Proximate composition of the walnut Cultivars

	Chitral-1	SW-1	Chitral-3	Chitral-2	SW-3	Dir-2	Mean±SD
Moisture (%)	3.34±0.12	3.56±0.37	3.42±0.37	2.76±0.51	2.83±0.31	4.2±0.35	3.352±0.527
% Ash	1.56±0.02	1.95±0.009	1.36±0.15	1.63±0.13	1.27±0.08	1.38±0.39	1.525±0.247
Fat (%)	63.54±2.53	66.72±2.25	69.25±3.72	68.85±2.53	69.92±3.46	67.35±3.35	6.76±2.323
Protein (%)	19.15±1.19	15.96±1.06	17.84±1.08	18.66±2.17	17.75±1.17	18.92±2.16	1.804±1.169
Carbohydrates (%)	12.41±1.03	11.81±0.95	9.22±0.83	8.10±1.17	8.04±1.07	9.24±0.97	9.803±1.87
Energy (kcal/100 g)	698.10±11.05	711.56±12.04	731.49±10.06	726.69±9.75	732.44±12.79	718.79±11.05	719.845±13.29

Mean±Standard Deviation (SD)

Proximate composition: Proximate composition is shown in the Table 4. Moisture content was found to be in the range of 2.76±0.51% (Chitral-2) to 4.20±0.35% (Dir-2). Ash content was in the range of 1.27±0.08% (SW-3) to 1.95±0.009% (Swat-1). Fat content (%) was determined to be in the range of 63.54±2.53 % (Chitral-1) to 69.25±3.72% (Chitral-3). Protein content was in the range of 15.96±1.06% (SW-1) to 19.15±1.19% (Chitral-1). Carbohydrate content was found in the range of 8.04±1.07% (SW-3) to 12.41±1.03% (Chitral-1). The proximate composition shows that the fats were the highest constituent and ash was present in the lowest quantity in the nuts. The results of the present study show that Chitral-3, Chitral-2 and SW-3 are physiochemically superior walnut cultivars than the rest of the cultivars.

Pereira *et al.* (2008) determined fats (68.83%-72.14%), Proteins (14.38%-18.03%), Carbohydrates (3.75%-7.16), moisture (3.85%-4.50) and ash (4.26%-3.31%) for the six walnut cultivars grown in Purtagal. The results of this study are in good agreement with this notion except for the percent carbohydrates and ash contents. Al-Bachir, (2004) determined the moisture content (3.48%), proteins (22.85%), fats (67.35%) and ash (1.26%). The results of the moisture content, fats content and ash content are in agreement with the results of this study but there is variation in term of protein content. These differences and variations can be attributed to environmental conditions, horticulture procedures and genetic parameters which influence the chemical composition of walnut fruits.

Energy content: The energy value of the kernels of these cultivars (shown in Table 4) was determined in the range of 698.10 Kcal/100 g (Chitral-1) to 732.44 Kcal/100 g (SW-3), which shows that the fruits of these varieties are rich source of energy. The differences in the energy level are due to differences in the proximate composition of the varieties.

Mineral composition: The results of the mineral composition are shown in Table 5. It shows the level of sodium is 40.9-64.5 ppm, K (3551-4827 ppm), Ca (925-1250 ppm), Fe (30.08-41.20 ppm), Zn (11.75-25.5 ppm), Mg (1059-1765 ppm), Cu (1.96-2.75 ppm) and Pb (0.69-1.06 ppm). Mineral composition of six Pakistani walnut cultivars showed high levels of Potassium, Magnesium, Calcium, Sodium and Iron while that of lead was the lowest. The lowest concentration of the toxic metal lead is an advantage. Ozcan (2009) determined the mineral content of the walnut fruits grown in Turkey and found Ca (1108.6 mg/kg), K (4627.6 mg/kg), Na (44.7 mg/kg) and Fe (32.4 mg/kg), Mg (1089.9 mg/kg), Zn (26.4 mg/kg), and Cu (3.8 mg/kg). The concentration of Na, K, Ca, Mg and Fe are in agreement with the results of the present study while that of the Zn and Cu shows higher values in Turkish walnut fruits as compared to the present study. Our data of mineral composition is in agreement with Lavedrinea *et al.* (2000) except for the concentration of Ca, Fe, Cu and Pb.

Table 5: Mineral Composition of the Walnut kernels. All the values are given in ppm

	Chitral-1	Swat-1	Chitral-3	Chitral-2	SW-3	Dir-2
Na	64.5±5.5	52.6±5.2	40.9±4.7	45.2±4.12	60.12±4.51	45.7±3.1
K	3586±32.2	3551±55.7	4500±54.1	4827±30.11	4541±32.06	3825±40.85
Ca	1250±25.07	1125±45.12	925±40.24	1170±41.03	1204±15.04	1249±12.5
Fe	38.5±2.10	41.20±1.54	36.14±3.75	30.51±1.06	31.26±2.09	30.08±1.40
Zn	25.5±2.15	12.26±1.12	11.75±1.09	17.39±1.68	18.05±2.46	17.85±2.76
Mg	1532±27.6	1765±25.09	1059±20.08	1065±25.14	1270±20.49	1530±25.57
Cu	2.12±0.09	2.17±0.81	2.09±0.56	1.96±0.84	1.98±0.99	2.75±1.08
Pb (Lead)	1.06±0.009	0.81±0.001	0.97±0.05	0.94±0.01	0.69±0.04	1.01±0.005

Mean±Standard Deviation (SD)

Conclusion: The data indicate that fruits nuts of these cultivars vary greatly in term of nut weight, kernel weight, kernel ratio, shell thickness, moisture content, protein content, carbohydrates content, energy contents and mineral content. The variability observed in these parameters is due to both genetic and environmental factors which may influence the individual parameter describing fruit properties, mineral composition and nutritional parameters including chemical composition and weight distribution of the endosperm. Moreover, kernels of Dir-2 and Chitral-2 cultivars were determined to contain high protein content (>18% protein) while Chitral-1 and SW-1 contain high carbohydrates content of >10%. In minerals the concentration of K, Ca, Mg, Na and Fe were found high while that of Pb was the lowest. These results will be useful to know about the nutritional properties of the local walnut cultivars and may guide us in designing strategies that maximize the utility of walnut germplasm.

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Effects of Low Carbohydrate High Fat Nigerian-Like Diet on Biochemical Indices in Rabbits

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Abstract: The effects of a low carbohydrate high fat Nigerian like diet on lipoprotein levels, serum electrolytes, liver and renal functions were investigated. The diet consist of 28% carbohydrate and 35% fat as percentage of total energy in a wholly compounded form. Twelve rabbits were randomly divided into two groups. The groups were: Group I that was fed the control diet and the second group II were fed with the low carbohydrate high fat diet. At the end of eight weeks, biochemical analyses were done on blood samples of the animals. There were significant ($p \leq 0.05$) decrease in weight for the experimental animals when compared with the control group. Total protein, albumin and globulin did not change significantly ($p \leq 0.05$), for the rabbits fed on the low carbohydrate high fat diet. Aspartate Transaminase (AST) and Alanine Transaminase (ALT) levels increased significantly ($p \leq 0.05$) while Alkaline Phosphatase (ALP) levels decreased significantly ($p \leq 0.05$). The bilirubin level for rabbits on the experimental diet did not change significantly ($p \leq 0.05$). Serum electrolyte concentrations showed no significant change in sodium, potassium, chloride and bicarbonate ions when compared with control. Creatinine and urea values showed no significant ($p \leq 0.05$) changes. Total Cholesterol levels were significantly ($p \leq 0.05$) reduced in the experimental diet. The lipoprotein fractions showed significant increases ($p \leq 0.05$) in High Density Lipoprotein (HDL) and Triacylglycerol (TAG) levels when compared with controls. Low Density Lipoprotein (LDL)-Cholesterol did not change significantly ($p \leq 0.05$). The results showed that the Low Carbohydrate High Fat (LCHF) Nigerian-like diet promotes hypertriglyceridemia and weight loss. This could also increase the risk of ischemic heart disease.

Key words: Low carbohydrate high fat diet, serum lipids, electrolytes, liver function, renal function

INTRODUCTION

Food is important to the physical well-being, energy needs, growth and repair of damaged tissues and the regulation of body function in humans. A balanced diet containing adequate amount of nutrient should include a wide variety of foods from different food groups. The food guide pyramid outlines the group of foods for which recommendations have been established. The diet type consumed by individuals is a major factor that can determine the propensity to develop cardiovascular disease or are likely to be diagnosed with adult-onset diabetes (Krauss *et al*., 2006).

Reducing calories is a proven way to make humans lose weight (Rocette *et al*., 2006). In Nigeria; this has become the practice among those who are overweight. It facilitates weight loss by promoting the metabolism of adipose tissue. The campaign for low calorie diet was due to a massive increase in the proportion of people who are overweight or obese. The benefits of a low carbohydrate diet includes weight loss, improved values in conventional tests for hyperlipidemia (Volek and Westman, 2002), fewer free radicals (Westman *et al*., 2003), a boost in antioxidant enzymes (Westman *et al*., 2002), strengthening of the immune systems, reduction in blood glucose and insulin levels, anticancer and antiaging effect (Bravata *et al*., 2003). The Atkins Diet consists of a low carbohydrate, high fat and protein diet. It has been recommended to be safe and effective, producing weight loss despite ad-libitum consumption of fatty meat, butter, other high-fat dairy and protein products (Parker *et al*., 2002). The efficacy of the Atkins Diet is raising medical and nutrition questions; the adverse effect of a high protein diet is negative on renal and hepatic function, calcium balance and insulin sensitivity (Astrup *et al*., 2004).

The guidelines for fat consumption have undergone a dramatic shift and are currently being evaluated. Restricted intakes of animal fats are recommended, because of their content of saturated fats and cholesterol (Ravnskov, 2000) Although, previous research has not determined the amount of dietary saturated fat that actually benefits a person's health, a high fat or ketogenic diet could lead to Obesity, high blood pressure, heart disease, diabetes, immunosuppression, atherosclerosis and chronic fatigue (Yancy *et al*., 2004).

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Several reports show that various groups existed that consumed relatively high amounts of fat yet were free of heart disease (Bang *et al*., 1980). The associated risk of a high fat diet was the amount of trans fat and not saturated fat consumed (Ghafoorunissa, 2008). Trans fatty acids are formed when vegetable oil is hydrogenated or heated to high temperature in deep oil frying. Trans fatty acids raise serum low density lipoprotein and lower high density lipoprotein in humans (Mozaffarian and Clarke, 2009). Therefore, the effects of trans fatty acids on risk profile for cardiovascular disease is unfavorable. Labels of food products should state the trans fatty acid content as part of nutritional information.

Palm oil is widely consumed in Nigeria for use in cooking soup, stew and some traditional foods. It is obtained from the mesocarp (pulp) of the fruit Palm-*Elaeis guineensis* by squeezing the pulp of boiled and pounded palm fruit. The pulp is discarded and the liquid obtained is boiled to give thickened red liquid, called Red Palm oil (RPO). The oil is consumed fresh. It is rich in beta-carotene which can be used to prevent vitamin A deficiency. The presence of tocols (tocopherols and tocotrienols) makes palm oil act as an antioxidant. (Schroeder *et al*., 2006).

The typical Nigerian-like diet is consumed by combining a carbohydrate based meal (Cassava flour, rice, cocoyam, potatoes, yam or plantain) with soup or stew cooked in palm oil and very small amount of protein. Proteins (meat, fish, milk and eggs) are expensive and out of the reach of a very large number of the populace. Fruits and vegetables which have high quantity of dietary fiber and numerous nutrients (vitamins, minerals and antioxidants) are in abundance in Nigeria. They promote good health and prevent diseases. However, Nigerians do not consume enough quantity.

Several studies have reported the effects of either a low carbohydrate or high fat/protein diet fed to humans (Volek *et al*., 2004). This was done by the utilization of refined processed food products. Such diets may be metabolized differently. Nigerians do not consume their food in this form. This study was designed to examine the effects of combining a low carbohydrate, high fat wholly compounded Nigerian-like diet on biochemical indices in rabbits. The experimental animals have similar lipid metabolism with humans (Zhang *et al*., 2008).

MATERIALS AND METHODS

Animals and management: New Zealand white rabbits (Initial mean weight 1.75 kg), three months old, were used in the present study.

The rabbits were housed in individual stainless steel animal cages with wire mesh floors to prevent coprography. Light was a 12 h-light and 12 h-dark cycle and the room temperature was uniform. The animals were acclimatized on growers' mash for two weeks. Prior to the study, food and water were given *ad libitum*. The rabbits were divided into two groups of six rabbits per group, each group having animals of similar weight after the adaptation period. One group was fed the control diet and the second group was fed the low carbohydrate high fat diet for a total period of eight weeks.

Diet: The composition of both diets is shown in Table 1. Red Palm Oil (RPO) was obtained from the Nigerian Institute for oil Palm Research (NIFOR), Benin, Benin City, Nigeria. Garri and fish were obtained from an open air market in Benin City, Nigeria. The fish was oven dried (Gallenkamp, UK) for 16hr to a constant weight, skinned and deboned. The dried fish was cooled in a dessicator and milled to powder in warring blender. The different components of the diet were stored in air-tight containers at -10^{0}C until used as feed for the rabbits. Fresh feed was provided on daily basis while stale remnants were discarded after weighing. On the average each rabbit received about 150 g/feed/day. Clean drinking water was provided. During this period, feed intake, water intake and dry fecal output were measured daily. Weight gain was recorded weekly.

Animal management and experimental procedures were performed in strict accordance with the requirements of the National Research Council's Guide for the use of Laboratory Animals (NRC, 1985).

Blood samples, collection and analysis: The animals were fasted for 18 h and baseline blood samples were drawn from the rabbit ear marginal veins using 21-gauge syringes. After eight weeks of feeding, the rabbits were anesthetized with pentobarbital (60 mg/Kg body weight). Insertion was made into the heart region for collection of blood samples with the use of a needle and syringe. The blood samples were collected into labeled bijou bottles containing heparin as anticoagulant and centrifuged immediately (3,000 x g for 10 min), to obtain the serum. The serum samples were stored in the biofreezer until analyzed. Duplicate serum samples for each animal group were analyzed for total proteins, albumin, globulins, Baertl *et al*. (1974), electrolytes (Na^{+}, K^{+}, $HC0_{3}^{-}$, Cl^{-}) Kinsley and Schaffert (1953), lipoprotein profile, LDL-Cholesterol (LDL-C), HDL-Cholesterol (HDL-C), Triacylglycerol (TAG) and Total Cholesterol (TC). Alkaline Phosphatase (ALP), Aspartate Transaminase (AST), Alanine Transaminase (ALT) Anderson *et al*. (1971) urea and creatinine Carr (1959) were measured using commercial kits (Boehringer, Mannheim).

Statistical analysis: Data were expressed as Standard Error of the Mean (SEM) for each group of rabbits. Significant differences between the control and

experimental set of data were analyzed by the student's t-test. P ≤ values 0.05 were indicative of significance. The statistical analyses were done with INSTAT statistical (2000, GraphPad 3.06 software, Inc. San Diego, CA).

RESULTS

Table 1 shows the composition of control and experimental diet fed to the rabbits. The weight gain, feed and water intake, feed efficiency and dry fecal output of the rabbits in the control and experimental groups are presented in Table 2. Statistical analyses showed that there were significant ($p \leq 0.05$) decreases in weight gain, feed and water intake and feed efficiency in the experimental diet when compared with the control diet.

Table 1: Composition of experimental diets

	Diet type	
Dietary components	Control	LC/HF
Garri	65.0 g	28.2 g
Fish	28.4 g	35.2 g
Mineral and vitamin mix (*Opimix premix)	1.0 g	1.0 g
Palm oil	5.0 g	35.0 g
Methionine	0.6 g	0.6 g

Note: Garri is a cassava based meal, rich in carbohydrate and commonly consumed in Nigeria. It contributed to the fiber in the Nigerian- like diet.

*Opimix premix:

Vitamin A = 8,000,000IU	Copper = 5 gm
Vitamin D = 1,600,000IU	Iron = 20 gm
Vitamin E = 5,000IU	Iodine = 1.2 gm
Vitamin K 2,000 mg	Selenium = 200 mg
Thiamine (B_1) = 1500 mg	Cobalt = 200 mg
Riboflavin (B_2) = 4,000 mg	Cholin chloride = 200 gm
Pyridoxine (B_6) = 1500 mg	Anti oxidant = 125 gm
Niacin = 15,000 mg	Manganese = 80 gm
Vitamin (B_{12}) = 10 mg	Zinc = 50 gm
Pathothenic acid = 5,000 mg	Biotin = 20 mg
Folic acid = 5,000 mg	

Table 2: Weight gain, feed intake, water intake, feed efficiency and dry fecal output of rabbits in the control and experimental groups

Groups	Control diet	Experimental diet
Weight gain (g/rabbit)	650±8.0[a]	150±6.0[a]
Feed intake (g/rabbit/day)	53.8	42.5
Water intake(ml/rabbit/day)	20.50±2.0[a]	16.80±2.50[a]
Feed efficiency (g/body weight/g feed)	12.08	3.52
Dry fecal output (g/rabbit/day)	5.24±1.14[a]	3.25±1.02[a]

Values are mean±SEM of six rabbits. Means of the same row followed by different letters differ significantly ($p<0.05$)

The values observed for total protein (0.73 mg/dl), albumin (0.05 mg/dl) and globulin (1.75 mg/dl) in the experimental diet are given in Table 3. There were no significant differences ($p \leq 0.05$) between the control and experimental diet. The values obtained for AST, ALT, ALP and bilirubin are shown in Table 4. The AST and ALT

Table 3: Mean concentrations of total proteins, albumin and globulins of rabbits fed on the control and experimental diet

Parameters	Control diet	Experimental diet
Total protein (mg/dl)	1.28±0.41[a]	0.73±0.32[a]
Albumin (mg/dl)	0.26±0.15[a]	0.05±0.27[a]
Globulin (mg/dl)	1.75±0.52[a]	1.75±0.32[a]

Values are mean±SEM of six rabbits. Means of the same row followed by different letters differ significantly ($p<0.05$)

Table 4: Mean Concentrations of serum enzymes and bilirubin of rabbits fed on the control and experimental diet

Parameters	Control diet	Experimental diet
AST (IU/L)	2.90±0.07[a]	9.75±3.43[b]
ALT (IU/L)	7.65±0.47[a]	11.50±2.60[b]
ALP (IU/L)	32.5±16.48[a]	4.75±1.25[b]
Bilirubin (mg/dl)	0.28±0.09[a]	0.24±0.02[a]

Values are mean±SEM of six rabbits. Means of the same row followed by different letters differ significantly ($p<0.05$)

increased significantly ($p \leq 0.05$) and ALP levels decreased significantly ($p \leq 0.05$). The bilirubin levels for rabbits in the control and experimental diet showed no significant difference ($p \leq 0.05$).

Table 5 shows the serum levels of electrolytes, creatinine and urea in rabbits. The rabbits fed with the LCHF diet had no significant difference at ($p \leq 0.05$) in the creatinine, urea, sodium, potassium, bicarbonate and chloride ions content when compared with the control samples.

The values of serum lipids of rabbits fed the control and experimental diet are presented in Table 6. Serum cholesterol levels were significantly ($p \leq 0.05$) reduced. The lipoprotein fractions were also significantly altered. HDL and TAG levels were significantly elevated ($p \leq 0.05$) when compared with the control. LDL- cholesterol did not have a significant change ($p \leq 0.05$) in the experimental groups when compared with the control diet.

DISCUSSION

The 28% carbohydrate and 35% fat diet utilized in this study was supplied by *garri* (a processed form of cassava) and palm oil, respectively. The rabbits fed the Nigerian like LCHF diet showed significant weight loss when compared to the control animals. Significant body weight reduction was observed in humans on the LCHF. (Meckling *et al*., 2002). There were no significant changes in total protein, albumin and globulin levels in rabbits fed the experimental diet. This showed that the percentage of protein in the diet was adequate for the synthesis of plasma proteins.

There were significant increases in the serum AST and ALT levels of the experimental animals. Increases in serum levels of these enzymes are usually indicative of possible liver damage but the levels observed in this present study is lower than the normal physiological range of 20-90 IU/L. This can be correlated to the fact

Table 5: Mean concentrations of electrolytes, creatinine and urea of rabbits fed on the control and experimental diet

Parameters	Control diet	Experimental diet
Potassium (mM/L)	0.47±0.11[a]	0.28±0.47[a]
Sodium (mM/L)	17.0±2.55[a]	17.25±4.70[a]
Bicarbonate (mM/L)	3.67±0.58[a]	2.6±0.41[a]
Chloride (mM/L)	12.00±0.58[a]	12.73±2.59[a]
Creatinine (mg/dl)	0.46±0.06[a]	0.25±0.05[a]
Urea (mg/dl)	8.33±3.69[a]	8.5±10.97[a]

Values are mean±SEM of six rabbits. Means of the same row followed by different letters differ significantly ($p<0.05$)

Table 6: Mean Concentrations of plasma lipids of rabbits fed on the control and experimental diet

Parameters	Control diet	Experimental diet
Total Cholesterol (mg/dl)	50.18±7.75[a]	35.00±17.9[b]
HDL-Cholesterol (mg/dl)	6.5±0.96[a]	10.50±2.06[b]
LDL-Cholesterol (mg/dl)	32.18±7.22[a]	30.76±1.49[a]
Triacylglycerol (mg/dl)	15.5±7.59[a]	52.5±8.27[b]

Values are mean±SEM of six rabbits. Means of the same row followed by different letters differ significantly ($p<0.05$)

that the bilirubin levels had no significant change in comparison to the control. This indicates that the experimental did not affect the maintenance of the normal excretory function of the liver or caused damage of liver cells. Moderate consumption of palm oil supports normal enzyme levels (Jones, 1975). The decreased concentration of ALP in the rabbits fed the experimental diet could indicate that the levels of fat used in this study did not adversely affect metabolic activities as mediated by ALP.

Creatinine levels for rabbits fed the experimental diet did not change significantly and this is an indication of normal kidney function. The levels of electrolytes in rabbits fed the experimental diet had no significant changes.

The total serum cholesterol decreased significantly. LDL Cholesterol did not show significant changes, while HDL and TAG increased significantly. Similar results have been observed in HDL and total cholesterol levels, when palm oil was used as the source of fat in the diet (Karaji-Bani *et al*., 2006). In this present study, the total cholesterol and HDL Cholesterol levels tend to improve with weight loss, but hypertriglyceridemia was manifested.

The improvement of total cholesterol and HDL levels accompanied by weight loss could be due to the low carbohydrate content of the diet and increased fat intake of 35%. This may have caused the body cells to use all the dietary fats and then breakdown fats (lipolysis) in skeletal and adipose tissues for energy, leading to hypertriglyceridemia and weight loss as observed in the study.

The HDL increase may be due to down regulation of HDL-Cholesterol receptors which bind HDL-Cholesterol and facilitate reverse cholesterol transport to the liver, this may be regulated by the dietary fat (Tan *et al*., 1991).

The hypercholesterolemic risk of consuming high levels of palm oil has been investigated extensively in experimental animals and in human subjects in various countries with different types of diets. All these studies have established that palm oil does not behave like a saturated fat in its effects on blood cholesterol or blood clotting, as might be anticipated from its fatty acid composition (Chong, 1991). The vitamin E tocotrienols present in palm oil are known to reduce circulating cholesterol concentrations in humans, this effect is attributed to a dose-dependent inhibition by tocotrienols of 3-hydroxy-3-methylglutaryl coenzyme A (HMG CoA) reductase (Haave *et al*., 1990). Thus inhibiting the in vivo synthesis of cholesterol in the liver and thereby lowering serum cholesterol, particularly of the Low-Density Lipoprotein (LDL) fraction. Although, in this study, the LDL fraction did not change in the experimental animals. Palm oil, like many other vegetable oils, is rich in the Mono-Unsaturated Fatty Acid (MUFA) oleic acid (40%), which has recently been shown to have a hypocholesterolaemic influence (Kamisah *et al*., 2005). This is believed to be the cardio-protective fraction of palm oil.

The potential of any dietary saturated fat for causing hypercholesterolemia is particularly related to the level of its consumption. When dietary saturated fats are consumed at high levels, they can become important risk factor in the development of hypercholesterolemia and cardiovascular disease. At low to moderate levels of palm oil consumption, such as prevailing among the bulk of the Nigerian population that kind of risk may not apply. There may be a risk in populations with a high fat intake. The typical Nigerian diet contains significant amounts of invisible fats rich in PUFA, high dietary fiber which has hypocholesterolemic factors.

Conclusion: Low Carbohydrate High Fat (LCHF) Nigerian like diet leads to improved atherogenic dyslipidemia in the presence of weight loss but with the appearance of hypertriglyceridemia and increased HDL levels. The use of human volunteers on this LCHF Nigerian like diet could give more accurate responses on formulating dietary management for people suffering from metabolic diseases.

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Practicing of Women Reproductive Health Rights: A Road Map for HIV Prevention

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Abstract: Denying of reproductive health rights encompasses the problems of HIV and STDs/STIs, unintended pregnancy and abortion and infertility. The present study was designed to identify vulnerability (towards HIV/AIDS) and violence against women due to lack of knowledge at reproductive health rights. It was found that those married women who were agreed that HIV/AIDS and STD/STIs are preventable had 'highly consistent' attitude towards the RHR practice. Similarly, statistically highly significant positive relationship was observed from the correlation coefficient Somers'd ($p \leq 0.0001$) between the perception of married women about the RH-Security and their attitude towards the practices of RHR. It was suggested that in addition to the information that HIV/AIDS and STD/STIs are preventable diseases married women must have information and knowledge about the preventive measures. Only then they can insist for the adoption of any RH-Service to her partner or spouse.

Key words: Reproductive health, reproductive health rights, reproductive health security

INTRODUCTION

Reproductive health is affected by a multifaceted web of factors. Reproductive health rights embrace human rights that are already recognized in national and international human rights documents. Correa (1997) described health akin to a right and the right of health like a human right. To this scope, the World Health Organization defines health, the Cairo Program delineates the reproductive health as: "A state of complete physical, mental and social well being and…not merely the absence of disease or infirmity, in all matters relating to the reproductive system and to its functions and processes" (ACPD, 2004; WHO, 2002). "Reproductive health therefore implies that people are able to have a satisfying and safe sex life and that they have the ability to reproduce and the freedom to decide if, when and how often do so. Implicit in this last condition are the right of man and women to be informed and to have access to safe, effective, affordable and acceptable methods of family planning of their choice, as well as other methods of their choice for regulation of fertility which are not against the law and the right of access to appropriate health care services that will enable women to go safely through pregnancy and childbirth and provide couples which the best chance of having a healthy infant" (UNDP, 1994; Social Watch, 1995; Correa, 1997; ACPD, 2004; Sadik, 1997; Hunt and De Mesquita, 2005).

Denying of reproductive health rights encompasses the problems of HIV and STDs/STIs, unintended pregnancy and abortion and infertility. The rates of HIV infection among women continues to grow. Over the last ten years, discussions around reproductive health rights at the international level have begun and special consideration is now being given to the reproductive health rights of women in the context of HIV/AIDS (Roseman *et al*., 2004). Sometimes couples give little consideration to their risk of acquiring STIs/HIV. Because, young women's primary incentive for becoming sexually involved with older partners is financial and material gain (Longfield *et al*., 2004; WHO, 2005). Majority of men preferred younger partners because they deem that young women are a low-risk group because they are "innocent", sexually inexperienced, or have had few sexual partners.

Reproductive health security methods kept away from the risk of pregnancy and HIV infection. In general, however, condoms were seen as more effective at preventive HIV infection than pregnancy (Maharaj and Cleland, 2004). The present study was carried out to identify vulnerability (towards HIV/AIDS) and violence against women due to lack of knowledge at reproductive health rights.

MATERIALS AND METHODS

The study was carried out in urban and rural areas of three districts of Punjab province i.e. Toba Tek Singh, Rawalpindi, Bahwalpur. In the present study 700 married women of age 15-49 years having at least one child were selected through Multistage Sampling

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technique. At the first stage, three districts, Toba Tek Singh, Rawalpindi and Bahawalpur were selected through simple random sampling technique. At the second stage, from each district one Tehsil was selected by simple random selection. At the third stage, three urban and three rural union councils were selected *randomly*. At the fourth stage, rural and urban localities were selected for the selection of household. Finally, systematic sampling technique was used to select each nth household. A well designed interviewing schedule was used to collect data and draw inferences and collected information was analyzed by using SPSS/PC+ 15.0 Statistical Package for Social Sciences (Nachmias and Nachmias, 1992) and relationship between two variables was observed by applying Chi-Square, Somers' d and Gamma tests. Researcher pooled the respondents' opinion regarding different aspects of RHR and HIV/AIDS prevention by sets of statements on semantic differential rating scale (bi-polar) of seven points from one to seven where four marked the neutral position (Ahmad, 1995; Nachmias and Nachmias, 1992) and Likert Scale which were merged by indexation after measuring their reliability through Crown Bach Alpha-Test (α = 0.78-0.9). The ordinal regression method was also used to model the relationship between the ordinal outcome variable e.g., married women's attitude towards practice of reproductive health rights and the explanatory variables. The outcome variable was measured on an ordered, categorical and three-point Likert scale- "highly inconsistent", "moderately consistent" and "highly consistent".

RESULTS AND DISCUSSION

The information presented in Table 1 show that reflect that a bit more than three fourth (75.4%) of the respondents heard about HIV/AIDS and had knowledge about STD/STIs. Amongst them an enormous majority (92.4%) of those respondents who ever heard about HIV/AIDS and STD/STIs had knowledge that these diseases could be transmitted from one person to another person and a healthy person could be infected from these diseases by some way. Finally, it can also be revealed that a big majority (85.0%) of those respondents who had awareness that HIV/AID and STD/STIs were transferable diseases also had knowledge that these diseases could be prevented from by some ways.

The data presented in Table 2 reflect that more than four fifth amongst those respondents who had knowledge that HIV/AIDS and STD/STIs were preventable diseases and could be prevented by using new blades (83.1%) at barber shop or beauty salon, testing of blood (80.5%) and by avoid extra-marital sex (80.5%). Furthermore, it can also be revealed that more than three fourth (78.1%) of the respondents were agreed that a healthy person could get rid of these transmittable diseases by using condoms, while having sex with one's marital partner or some extra-marital partner. Similarly, Maharaj and Cleland (2004) found that condoms protect against the risk of pregnancy and HIV infection. In general, however, condoms were seen as more effective against preventive HIV infection than pregnancy.

It is depicting from the Table 3 that almost two third (41.0%) of married women amongst those who were

Table 1: Distribution of the respondents according to their awareness about HIV/AIDS and STD/STIs

Response	Frequency	Valid percentage
Ever heard about HIV/AIDS and STD/STIs		
Heard	528	75.4
Not heard	172	24.6
Total	700	100.0
Knowledge that HIV/AIDS and STD/STIs are transmitted from one person to another		
Knowledge has	488	92.4
Knowledge hasn't	40	7.6
Total	528*	100.0
Knowledge about the prevention from HIV/AIDS and STDs/STIs		
Yes	415	85.0
No	73	15.0
Total	488**	100.0

*172 Respondents have no knowledge about HIV/AIDS and STD/STIs.
**40 Respondents have no knowledge that these are transferable diseases

Table 2: Distribution of the respondents according to their responses regarding prevention from HIV/AIDS and STD/STIs

	Agree	Disagree	No opinion	Total
HIV/AIDs and STD/STIs can be prevented by	Count valid N %	Count valid N %	Count valid N %	Count valid N %
Avoiding to share needles with others	323 (77.8%)	46 (11.1%)	46 (11.1%)	415 (100%)
Testing blood before transfer	334 (80.5%)	49 (11.8%)	32 (7.7%)	415 (100%)
Using new blades at barber shop or beauty salon	345 (83.1%)	33 (8.0%)	37 (8.9%)	415 (100%)
Avoiding extra-marital sex	334 (80.5%)	39 (9.4%)	42 (10.1%)	415 (100%)
Using condom during sex	324 (78.1%)	40 (9.6%)	51 (12.3%)	415 (100%)
Homo-sexuality	342 (82.4%)	31 (7.5%)	42 (10.1%)	415 (100%)

Table 3: Association between knowledge about transmittable diseases and respondent's attitude towards practices of RHR

		Respondents' attitude towards RHR-practices			
Awareness about HIV/AIDS and STDs/STIS	Attributes	Inconsistent	Moderately consistent	Highly consistent	Total
Perception about the prevention from	No opinion	10 (2.7%)	37 (9.9%)	21 (5.6%)	68 (18.2%)
HIV/AIDS STDs/STIs	Disagree	9 (2.4%)	24 (6.4%)	28 (7.5%)	61 (16.4%)
	Agree	11 (2.9%)	80 (21.4%)	153 (41.0%)	244 (65.4%)
	Total	30 (8.0%)	141 (37.8%)	202 (54.2%)	373 (100.0%)

Statistics: Chi-Square≤0.0001, Somers' d≤0.0001,Gamma≤0.0001 (0.427)

agree HIV/AIDS and STD/STIs are preventable (65.4%) had 'highly consistent' attitude towards the practice of RHR. Whereas, more than a half (9.9% of 18.2%) of those married women who has 'no opinion' or had neutral attitude in this regard were 'moderately consistently' in their attitude towards the RHR-Practices. On the basis of this discussion it can be evaluated that in addition to the information that HIV/AIDS and STD/STIs are preventable diseases one must have information and knowledge about the preventive measures. Only then one can insist for the adoption of any RH-Service to her partner or spouse. Likewise, Germain and Jennifer (2005); Prata *et al.* (2005); Maharaj and Cleland (2005); Erulkar (2004) presented the that in order to protect one selves, men and women need to be well informed about the means available. Awareness about the risk of HIV infection is also strongly related to an increase in self-protective behavior.

It is evident from the Table 4 that an enormous majority (91.0%) of the respondents had positive attitude regarding the adoption of RH-Methods because from their point of view 'contraceptives are effective to have small family and to maintain good health by avoiding frequent births (83.0%). According to more than a half (57.14%) of them expressed that use of RH-Methods or contraceptive methods was a source of creating lack of trust among spouses and 55.0% married women were the of view that it could be a source to create conflict among spouse. Similarly, Singh *et al.* (2005) and Prata *et al.* (2005) found that amongst the important impediment which stop women from practicing of RH-Methods are the dread and tension that use or even negotiations of family planning entailed with unfaithfulness or lack of commitment to marriage.

It can be extracted from the above discussion that majority of married women had favorable attitude towards the adoption of RH-Methods and were well aware of the benefits of the practice of RH-Services but at the same time they also had fear that it might be a cause of creating conflict and lack of trust among spouse.

It is evident from the table that statistically highly significant positive relationship was observed from the correlation coefficient Somers'd ($p \leq 0.0001$) between the perception of married women about the RH-Security and their attitude towards the practices of RHR. The average value showed that majority of married women (68.3%) were 'not against' the use/adoption of contraception. To whom more than a half (35.3%) was 'highly consistent' in their attitude towards RHR-Practices. Whereas, more than three fourth of those married women who were 'against' (16.3%) to the use of contraception were either 'moderately consistent' (6.7%) or 'inconsistent' (6.0%) in their attitude towards the practice of RHR. This means that perceived favorable perception towards the adoption/use of contraception has novel role in determining their attitude towards RHR-Practices. Prata *et al.* (2005) also concluded that about three quarters of respondents thoughts that condom were safe, one-forth equated condom use with lack of trust and one-third said they are difficult to use with new partners. Consistent use was less likely among females who were married or cohabiting and among those who associated the condom use with lack of trust.

Data presented in Table 6 clearly shows that those married women who have 'no knowledge' about HIV/AIDS and STD/STIs and its preventive measures were statistically significantly ($p<0.0001$) less likely to incline towards the RHR-Practices. Likewise, the women who have 'no opinion' or disagreed with preventive measures of such transmissible diseases were also significantly ($p=0.015$ and $p=0.001$ respectively) less likely to prone to the practice of RHR than those who not only have awareness but also *agreed* with the preventive measures while controlling the effect of all other factors by regression analysis. It shows that relative chance of denying the RHR-Practice is higher among those married women who have no knowledge about HIV/AIDS and STD/STIs and its preventive measures. The results of the present study are in accordance with the results of international studies. Prata *et al.* (2005) found that men were more knowledgeable than women about preventing pregnancy (85% vs. 80%) and HIV infection (86% vs. 81%). Similarly, Maharaj and Cleland (2004) found that condoms were more effective at preventive HIV infection than pregnancy. Women with a high perceived self-efficacy were more likely to report using a condom than were those with a low perceived self-efficacy.

The data presented in Table 6 indicate that those respondents who disagreed or had neutral opinion regarding the hurdles i.e. RH security measures were expensive, neither easily available, nor available on regular basis and not accessible were more likely to be practicing RHR in their marital lives as compared to those married women who considered the cost of RH

Table 4: Distribution of the respondents according to their opinion towards different aspects of contraceptive use

Different aspects of RH-Services/contraceptives use	Response	Count	N %
It has lesser health hazards as compared to repeated pregnancies	Not Hazards	550	78.57
	Health Hazards	97	13.58
	Neutral	53	7.57
It is against Islam	Not Against	459	65.57
	Against	151	21.57
	Neutral	90	12.86
Contraceptive methods are effective to have a small family	Effective	637	91.00
	Ineffective	34	4.86
	Neutral	29	4.14
Acceptable for the birth control in our society	Acceptable	616	88.00
	Not Acceptable	34	4.86
	Neutral	50	7.14
Acceptable to get benefits of small family in our Religion/Islam	Acceptable	439	62.71
	Not Acceptable	104	14.86
	Neutral	157	22.43
Contraceptive methods are effective in maintaining good health	Effective	581	83.00
by avoiding frequent births	Ineffective	48	6.86
	Neutral	71	10.14
Use of contraceptive is against human nature	Not Against	523	74.71
	Against	61	8.72
	Neutral	116	16.57
Use of contraceptive creates conflict among spouses	Not Create	385	55.00
	Create Conflict	250	35.71
	Neutral	65	9.28
Use of contraceptive is creating lack of trust among spouse	Trust	400	57.14
	Lack of trust	193	27.57
	Neutral	107	15.29

Table 5: Association between respondent's reproductive health security and their attitude towards practices of RHR

		Respondents' attitude towards RHR-practices			
RH-Security	Attributes	Inconsistent	Moderately consistent	Highly consistent	Total
Perception about the Reproductive	Against	35 (6.0%)	39 (6.7%)	21 (3.6%)	95 (16.3%)
Health (RH) security	Neutral	17 (2.9%)	50 (8.6%)	23 (3.9%)	90 (15.4%)
	Not Against	45 (7.7%)	148 (25.3%)	206 (35.3%)	399 (68.3%)
	Total	97 (16.6%)	237 (40.6%)	250 (42.8%)	584 (100.0%)

Statistics: Chi-Square≤0.0001, Somers' d≤0.0001, Gamma≤0.0001 (0.468)

Table 6: Ordinal regression of socio-economic and cultural determinants of attitude towards the practice of Reproductive Health Rights (RHR)

Parameters	Regression coefficients	Std. Error	p-value
Awareness about HIV/AIDS and STD/STIS			
No Knowledge	-0.691	0.194	0.000
No opinion	-0.524	0.216	0.015
Disagree	-0.759	0.235	0.001
Agree	0(a)	-	-
Reproductive Health Security			
Perception about RH Security			
Against	-0.316	0.200	0.113
Neutral	-0.226	0.184	0.219
Not Against	0(a)	-	-
Cost of RH security measures			
Disagree	0.537	0.229	0.019
Neutral	0.794	0.198	0.000
Agree	0(a)	-	-
Agree	0(a)	-	-
Structural/environmental factors effect on RH security measures			
Disagree	-0.246	0.189	0.193
Neutral	-0.318	0.177	0.073
Agree	0(a)	-	-

security measures as a stumbling block were less likely to be prone to the practice of RHR. Therefore, it can be concluded that 'cost of RH security measures' appeared as an important determinant in facilitating women for using their RH-Rights. The result of the present study is aligned with the results of some international studies. Singh *et al.* (2005) explored the important barriers that prevent women from practicing of RH-Method. Among those the important constraints were fear of side effects, cost, inconvenience and the fear that use or even discussions of RHR may cause unfaithfulness or lack of commitment to marriage. Germain and Francoise (2000) explored that reproductive health should be measured not but a single indicator- contraceptive use- as in the past, but by empowerment of women in decisions regarding family formation, monitoring provision of, access to and use of fundamental services i.e. achievable range of safe and effective planning and contraception. As "Family Care International" (2005) indicated that access to comprehensive sexuality education, information and services can help them to establish healthy attitudes and behaviors among adolescent.

Conclusion: The results of Ordinal Regression Analysis showed that relative chance of denying the RHR-Practice is higher among those married women who have no knowledge about HIV/AIDS and STD/STIs and its preventive measures. Additionally, the married women who considered the cost of RH security measures as a stumbling block were less likely to be prone to the practice of RHR than those who didn't. Therefore, there is a need to publicize that in addition to the information that HIV/AIDS and STD/STIs are preventable diseases one must have information and knowledge about the preventive measures. Only then one can insist for the adoption of any RH-Service to her partner or spouse.

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The Nutrient Composition of Seeds of the African Pear (*Dacryodes edulis*) and its Implications for Non-Ruminant Nutrition

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Abstract: In an investigation conducted to determine the nutrient composition of seeds of the African Pear (*Dacryodes edulis* G. Don, H.J.Lam) and their potential as a feed ingredient in non-ruminant nutrition, samples of sun-dried, ground seeds of the African pear (*Dacryodes Edulis* Seed Meal) (DESM) were analyzed using standard laboratory procedures. The gross energy and metabolizable energy contents of DESM (3820.74 kcal/kg and 3368.04 kcal/kg respectively) and its crude protein (6.98%), ether extracts (8.98%) crude fibre (7.42%) ash (3.36%) and nitrogen-free extracts (73.26%) indicate that DESM can be classified as an energy feed. The anti-nutrients present were tannins (6.37 x 10^{-4} mg/100g), phytates (0.49 mg/100 g) and oxalates (1.68 x 10^{-15} mg/100 g) while its amino acid profile revealed only trace amounts of the essential and non-essential amino acids and vitamins. DESM contained reasonable amounts of iron, manganese, zinc, sodium and iodine while its calcium, copper and selenium levels were low. It was concluded that DESM may act as a close substitute for maize, especially in poultry diets, on account of its similarity in soluble carbohydrate content to maize and its protein content. This is expected to reduce production costs to poultry farmers in Nigeria and make poultry products available to consumers at more affordable costs.

Key words: Seeds, African pear, *Dacryodes edulis*, nutrient composition, non-ruminants

INTRODUCTION

Maize has, over the years, been a basic ingredient in non-ruminant feeds in Nigeria for providing the energy component of their finished feeds and usually constitutes 50% or more of most commercial poultry feeds (Adebiyi *et al*., 2005, Farinu *et al*., 2005). However, shortfalls in domestic production of maize, high tariffs on its importation, seasonal fluctuations in its availability and its use as an important staple food by Nigeria's human population and as an industrial raw material in the brewery, pharmaceutical and baby foods industries among others have led to an escalation in its domestic price. Since it constitutes over 50% of the finished feeds of most non-ruminants, its continued use invariably increases the costs of production, as feed costs account for up to 80% of production costs and makes the cost poultry products derived therefrom high. If the poultry industry in Nigeria must survive and live up to its responsibility of making poultry meat and eggs available to the populace at affordable prices, the cost of finished poultry feeds in Nigeria must be brought down drastically.

Although some poultry nutritionist have, in the past, explored the possibility of replacing maize partially or wholly with sweet potato, (Fetuga and Oluyemi, 1976), palm oil slurry (Atteh and Ologbenla, 1993), water hyacinth (Dairo, 1997), cassava root meal (Eruvbetine, 2000) and maize offal and cashew nut meal (Oyero *et al*., 2005) among others, most of these new ingredients have the disadvantage of not being readily available, or requiring considerable effort and resources to obtain and process. Some of them are equally useful as human food or industrial raw materials. The seeds of the African pear, *Dacryodes edulis* (G. Don, H.J. Lam) which, according to Leaky (1999), are discarded after the pulp has been consumed by man, are usually available in large quantities during the fruiting season (May to October) in Nigeria. They can be picked up and collected in large quantities at little or no cost. They require little or no further processing, are not consumed by man and are not currently in use as an industrial raw material in Nigeria. Preliminary work on the seed of the African pear by Obasi and Okolie (1993) suggests that it contains as much as 76 g kg^{-1} carbohydrates (other than crude fibre), 126g kg^{-1} of lipids, has no toxic principles and may be high in crude fibre (273 g kg^{-1}). So far, no work is available on the potentials of these high-energy seeds of African pear as a feed ingredient for livestock.

The objective of this work was to ascertain the chemical composition of *Dacryodes edulis* seeds with a view to determining their potentials in non-ruminant nutrition.

MATERIALS AND METHODS

The experimental site: The study was carried out at the Poultry Unit of the Teaching and Research Farm, Delta State University, Asaba Campus, Asaba, Nigeria (6° 45' E and latitude and 6° 12' N). Annual rainfall ranges from 1800-3000mm while the maximum day temperatures range from 27.5°C to 30.9°C.

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Preparation and chemical analysis of the african pear seeds: Seeds of the African pear, *Dacryodes edulis* (G. Don, H.J. Lam), commonly called 'Ube' in southeast Nigeria, which are usually discarded during the fruiting season, were picked up from Asaba and its environs, washed in water to remove all sand particles and de-hulled by removing the tough leathery coat to expose the cotyledons. The cotyledons were carefully separated by hand and spread out to dry under the sun for several days until a safe moisture level of 10-13% was attained. The dry cotyledons were then winnowed to remove all seed chaff and ground with a hammer mill to obtain the seed meal of *Dacryodes Edulis* (DESM). Samples of DESM were sent to the Institute of Agricultural Research and Training (I A R and T), Ibadan, Nigeria for laboratory analyses to ascertain its proximate composition including its energy content and the presence and quantities of anti-nutritional factors. A portion of the samples was also sent to the International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria for determination of its mineral, vitamin and amino acid profiles using AOAC (1990) procedures. Percent oxalates was obtained using the method of Talapatra and Price (1948) by comparing the absorbance of water extracts of DESM samples with standard solutions of oxalic acid using a spectrophotometer set at 420 nm. Percent phytic acid was determined using the method of titration with a standard iron III chloride solution as described by Ler (1983) while % tannic acid was determined by comparing tannin extracts of DESM samples with those of prepared standards using a spectrophotometer at 500nm (Gbedioh *et al*., 1994).

RESULTS AND DISCUSSION

The proximate, energy and anti-nutrient composition, amino acid profile and mineral and vitamin contents of the test ingredient, *Dacryodes Edulis* Seed Meal (DESM) are presented in Tables 1, 2 and 3 respectively. The gross energy and metabolizable energy contents of the test ingredient (DESM) were 3820.74 kcal/kg and 3368.04 kcal/kg respectively, while its soluble carbohydrate content was over 60%. DESM was low in crude protein (6.98%), contained fairly high amounts of ether extracts (8.98%), crude fibre (7.42%) and ash (3.36%) and had small amounts of tannins (6.37×10^{-4} mg/100 g), phytates (0.49 mg/100 g) and oxalates (1.68×10^{-15} mg/100 g) (Table 1).

The amino acid profile of the test ingredient (Table 2) revealed only trace amounts of the essential and non-essential amino acids. Except for its biotin content (0.1 ppm), only trace amounts of the vitamins assayed were present in the test ingredient (Table 3).

Its Crude Protein (CP) content of 6.98% was much lower than that reported by Obasi and Okolie (1993) (33.8%), and higher than those reported by ICRAF (2001) (3.3%) and Ajayi and Oderinde (2002) (1.4%) for *Dacryodes edulis* seeds, but slightly lower than standards published for yellow maize (8.50-8.90%) by Aduku (1993), NRC (1994) and Olomu (1995). Although the Crude Fibre (CF) content of 7.42% obtained for DESM in this study is quite high, compared to yellow maize whose CF content is about 2.70% (Aduku, 1993), its CF content was considerably lower than values earlier reported for *D. edulis* seeds by Obasi and Okolie (1993) (27.3%) and Ajayi and Oderinde (2002) (48.5%). The fat content of DESM obtained in this study (8.98%) was higher than that of yellow maize (4.10%) (Olomu, 1995) but lower than values reported for *D. edulis* seeds by Obasi and Okolie (1993) and Ajayi and Oderinde (2002) (11.0% and 10.44% respectively). With its soluble carbohydrate (NFE) content of 73.26%, which was close to the 78.50% reported for yellow maize by Olomu (1995), its low crude protein and low crude fibre contents, DESM can safely be classified as an energy feed and used for non-ruminant feeding (Olomu, 1995, Banerjee, 1998). Its ash content of 3.36%, which is higher than values reported for yellow maize by Aduku (1993) and Olomu (1995) is an indication that DESM may be in a position to provide some of the minerals required by non-ruminants. Details of its mineral composition (Table 3) indicated that *Dacryodes edulis* Seed Meal (DESM) contained 0.08% Ca, 0.62% P, 0.39% K and 0.52% Mg. The corresponding values for maize, which are 0.02, 0.28, 0.30 and 0.12% respectively (NRC, 1994), indicate that DESM possesses higher quantities of these macro-nutrients than maize. DESM, however, had only trace amounts of Na, Fe, Mn, Zn, Cu, Se and I as well as the essential amino acids and vitamins (Tables 2 and 3).

Table 1: Proximate, Energy and Anti-nutrient Composition of the Test Ingredient, *Dacryodes edulis* Seed Meal (DESM)

Fraction/Component	Mean
Dry Matter (%)	89.53
Crude Protein (%)	6.98
Ether Extract (%)	8.98
Crude Fibre (%)	7.42
Ash (%)	3.36
Nitrogen Free Extract (%)	73.26
Gross Energy (kcal/kg)	3820.74
Metabolizable Energy (kcal/kg)	3368.04
Tannins (mg/100g)	6.37×10^{-4}
Phytates (mg/100g)	0.49
Oxalates (mg/100g)	1.68×10^{-15}

The presence of small amounts of the anti-nutritional factors, tannins, phytate and oxalates in DESM (Table 1) which, as chelating agents, tend to render some ions, vitamins and proteins unavailable to farm animals (Olomu, 1995) suggests that DESM may have to be subjected to further processing in order to make available to non-ruminant farm animals appreciable amounts of the amino acids, vitamins and minerals

Table 2: Amino Acid Content (ppm) of *Dacryodes edulis* Seed Meal (DESM)

Amino Acid	Mean±SE
1. Essential Amino Acids	
Phenylalanine	1.105±0.01
Valine	0.225±0.19
Thyreonine	0.085±0.00
Tryptophan	0.544±0.02
Isoleucine	0.556±0.05
Methionine	0.082±0.00
Histidine	0.095±0.00
Arginine	0.048±0.00
Leucine	0.994±0.00
Lysine	1.087±0.03
2. Non-Essential Amino Acids	
Cystine	0.038±0.00
Alanine	0.050±0.00
Tyrosine	0.010±0.00
Glycine	0.089±0.01
Serine	0.099±0.01
Aspartic Acid	4.048±0.06
Glutamic Acid	3.165±0.15
Asparadine	0.020±0.01
Glutamine	0.027±0.02
Proline	0.258±0.05

SE = Standard Error; ppm = Parts per million

Table 3: Vitamin and mineral contents of *Dacryodes edulis* Seed Meal (DESM)

Parameters	Mean±SE
1. Vitamins (ppm)	
A	0.0035±0.0005
D	0.0015±0.0005
E	0.0025±0.0005
K	0.0020±0.0010
Thiamine (B_1)	0.0065±0.0005
Riboflavin (B_2)	0.0170±0.0010
Pantothenic Acid	0.0120±0.0010
Niacin (B_3)	0.0790±0.0045
Pyridoxine (B_6)	0.0150±0.0010
Cobalamine (B_{12})	0.0085±0.0005
Biotin	0.1000±0.0010
Choline	0.0025±0.0005
Folic Acid	0.0020±0.0010
2. Minerals	
Calcium (%)	0.08±0.04
Phosphorus (%)	0.62±0.01
Potassium (%)	0.39±0.00
Magnesium (%)	0.52±0.01
Sodium (ppm)	18.58±0.53
Iron (ppm)	36.02±0.13
Manganese (ppm)	16.77±0.26
Zinc (ppm)	7.16±0.18
Copper (ppm)	0.78±0.04
Selenium (ppm)	0.01±0.00
Iodine (ppm)	0.62±0.01

SE = Standard Error; ppm = parts per million

contained in it, or used in conjunction with conventional supplements such as the oil-seed cakes, fish meal and mineral/vitamin premixes to guarantee availability of these nutrients to non-ruminants.

Conclusion: The closeness of the energy value of *Dacryodes Edulis* Seed Meal (DESM) to that of maize, and its crude protein content of almost 7% makes DESM a choice replacement for maize in the diets of non-ruminants. This will afford non-ruminant animal farmers, especially poultry farmers, in Nigeria the opportunity to reduce their production costs and make poultry products available to consumers at more affordable prices.

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Comparative Antioxidant, Phytochemical and Proximate Analysis of Aqueous and Methanolic Extracts of *Vernonia amygdalina* and *Talinum triangulare*

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Abstract: This study evaluated and compared *in vitro* antioxidant activity, phytochemical constituent and proximate analysis of aqueous and methanolic leaf extracts of *Vernonia amygdalina* and *Talinum triangulare* to determine their acceptability in folkloric medicine. The total phenolic content of aqueous and methanolic extracts of *V. amygdalina* (0.822±0.050 and 0.681±0.050 mg gallic acid equivalent GAE/g respectively) was higher than aqueous and methanolic extract of *T. triangulare* (0.456±0.040 and 0.288±0.020 mg GAE/g respectively). Furthermore, DPPH scavenging activity of *V. amygdalina* was significantly higher ($p<0.05$) than *T. triangulare* at all levels of concentration (100, 200 and 300 µg/ml). Lipid peroxidation was inhibited by all samples, although there was no significant difference ($p>0.05$). Aqueous extracts of leaf tested positive to tannin, phlobatannins, cardiac glycosides, saponins, phenols, flavonoids and alkaloids. The proximate composition of *V. amygdalina* leaf showed higher percentage crude fibre, fat, protein and total carbohydrate content than *T. triangulare* except moisture and ash contents. Methanolic and aqueous leaf extracts of *V. amygdalina* possess higher antioxidant properties, phyto-nutrients and longer shelf life than *T. triangulare* and hence it's pervading use and acceptability in folkloric and trado-medicine.

Key words: Aqueous and methanolic leaf extracts, *Vernonia amygdalina*, *Talinum triangulare*

INTRODUCTION

Antioxidants have been known to play protective role in human body against deleterious effects of reactive free radicals and it has been defined as any substance that when present at low concentrations compared to those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate (Halliwell, 1990). They are chemical compounds that can prevent, stop, or reduce reactive effect of free radicals. These effects include oxidative damage to membranes and enhanced susceptibility to lipid peroxidation or enzyme inactivation (Farombi and Fakoya, 2005; Sathishsekar and Subramanian, 2005). Free radicals are formed from molecules via the breakage of a chemical bond such that each fragment keeps one electron, by cleavage of a radical to give another radical and also via redox reactions (Halliwell and Gutteridge, 2008; Bahorun *et al*., 2006). Free radicals include hydroxyl ($OH^{\bullet}$), superoxide ($O_2^{\bullet -}$), nitric oxide ($NO^{\bullet}$), nitrogen dioxide ($NO_2^{\bullet}$), peroxyl ($ROO^{\bullet}$) and lipid peroxyl ($LOO^{\bullet}$). Also, hydrogen peroxide (H_2O_2), ozone (O_3), singlet oxygen ($^1O_2$), hypochlorous acid (HOCl), nitrous acid (HNO_2), peroxynitrite ($ONOO^-$), dinitrogen trioxide (N_2O_3), lipid peroxide (LOOH), are not free radicals but generally, they are called oxidants, although, they can easily lead to free radical reactions in living organisms (Genestra, 2007). Biological free radicals are thus highly unstable molecules that have electrons available to react with various organic substrates such as lipids, proteins, DNA and eventually progress to oxidative stress. Thus, antioxidant containing foods like fruits and vegetables could have strong protective effect against the risk of major diseases such as cancer and cardiovascular diseases (Kaur and Kapoor, 2001; Amic *et al*., 2003). Vegetables and fruits extracts are often employed in folkloric medicine to treat several ailments (Wang *et al*., 2007).

Vernonia amygdalina Del. (Asteraceae) commonly called bitter leaf contains anti-nutritional factors such as alkaloids, saponins, tannins and glycosides responsible for its bitter taste (Buttler and Bailey, 1973; Ologunde *et al*., 1992). It is consumed as a vegetable in Nigeria and many areas of East Africa (Mensah *et al*., 2008). However, extract of bitter leaf had been reported to exert antibiotic action against drug resistant microorganisms and possesses antioxidant, anticancer, antiviral, anti-helminthic and anti-inflammatory activities (Akinpelu, 1999; Dahanukar *et al*., 2000). Furthermore, the root provides one of the commonly used chew sticks in Nigeria due to alleged beneficial effect on dental caries (Aregheore *et al*., 1998). The leaves and bark in Ethiopian local medicine are used as purgative, against menstrual pain and wound dressing (Akah and Okafor, 1992; Uhegbu and Ogbuchi, 2004).

Talinum triangulare (Jacq.) Wild (Portulacaceae) is a herb with fleshy green leaves, succulent stem and pink

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flowers which are rarely white (Keay, 1981). It is an all season vegetable, grown mostly in West Africa from seed or by vegetative propagation (Akobundu and Agyakwa, 1998; Imoh and Julia, 2000). *T. triangulare* leaf is used in folkloric medicine to treat diuretic and gastro-intestinal disorders (Mensah *et al*., 2008) and oedema. Furthermore, the leaves serve as sauce, condiment, spice or flavorings in foods (Mbang *et al*., 2008). Therefore, this study was designed to compare the antioxidant properties, phytochemical and proximate composition of *V. amygdalina* and *T. triangulare* in order to gain insight into their acceptability in therapeutic and folkloric use.

MATERIALS AND METHODS

Chemicals: DPPH (2,2 diphenyl-1- picrylhydrazyl hydrate), gallic acid, ascorbic acid and Folin-Ciocalteu's reagent were purchased from Sigma Aldrich, USA. All other chemicals and reagents used were of analytical grade.

Collection of plant materials: *Vernonia amygdalina* and *T. triangulare* were collected from a farmland at Ilisan Remo, Ogun State, South-Western Nigeria. Both plants were authenticated by Prof. Edward B. Esan, a plant scientist in the Department of Chemical and Environmental Sciences, Babcock University, Ogun State, Nigeria.

Extraction of plant material: Fresh leaves of *V. amygdalina* and *T. triangulare* were air dried and then ground to fine powder. The pulverized samples (20 g) were soaked in 150 ml of 100 % methanol and 300 ml of distilled water for 72 h before extraction. The methanolic and/or aqueous extracts were concentrated to dryness in a rotary evaporator and thereafter preserved in a refrigerator at 4°C until further use.

Determination of total phenolic content: The total phenolic content was estimated as described by Singleton and Rossi (1965) and modified by Gulcin *et al*. (2003). One ml aliquot of extracts or standard solution of gallic acid (10, 20, 30, 40 and 50 mg/l) was added in a volumetric flask containing 9 ml of water. One ml of Folin-Ciocalteu's reagent was added to the mixture and vortexed. After 5 min, 10 ml of 7% sodium carbonate was added to the mixture and incubated for 90 min at 25°C. The absorbance against reagent blank was determined at 750 nm. A reagent blank was prepared and the amount of phenolic compound in the extract was determined from the standard curve. The total phenolic content of the plant was then calculated as shown in the equation below and expressed as mg Gallic Acid Equivalent (GAE)/g fresh weight. All samples were analyzed in duplicates.

$$C = c.\ m/V$$

Where:

C = Total content of phenolic compound in gallic acid equivalent (GAE)/g
c = The concentration of gallic acid established from the calibration curve (µg/ml)
V = Volume of extract (ml)
m = Weight of the crude plant extract (g)

Antioxidant assay: Rapid Thin Layer Chromatography (TLC) screening for antioxidant activity was carried out by spotting a concentrated methanolic solution of the extract on silica gel plates. The plates were developed in methanol: ethyl acetate (2:1, v/v) afterwards air-dried and sprayed with 0.2% w/v DPPH spray in methanol. The plates were visualized for the presence of yellow spots. The radical scavenging activity of leaf extracts was performed according to the DPPH spectrophotometric method of Mensor *et al*. (2001). One ml of a 0.3 mM DPPH methanol solution was added to a 2.5 ml solution of the extract or standard (100 µg/ml, 200 µg/ml, 300 µg/ml) and allowed to react at room temperature for 30 min. The absorbance of the resulting mixture was measured at 518 nm and converted to percentage Antioxidant Activity (AA %), using the formula:

$$AA\% = [1-(Abs_{sample}/Abs_{control})] \times 100$$

Methanol (1.0 ml) plus extract solution (2.5 ml) was used as blank. 1 ml of 0.3 mM DPPH plus methanol (2.5 ml) was used as a negative control. Solutions of ascorbic acid and gallic acid served as positive controls. This assay was carried out in triplicates for each concentration.

Inhibition of lipid peroxidation: A modified Thiobarbituric Acid Reactive Substances (TBARS) assay was used to measure the lipid peroxide formed using egg yolk homogenate as lipid-rich media (Ruberto and Baratta, 2000). Egg homogenate (0.5 ml, 10 % v/v) was added to 0.1 ml of extract (1 mg/ml) and the volume made up to 1 ml with distilled water. Thereafter, 0.05 ml of $FeSO_4$ was added and the mixture incubated for 30 min. 1.5 ml of acetic acid was then added followed by 1.5 ml of TBA in SDS. The resulting mixture was vortexed and heated at 95°C for 60 min. After cooling, 5 ml of butan-1-ol was added and the mixture centrifuged at 3000 rpm for 10 min. The absorbance of the organic upper layer was measured at 532 nm and converted to percentage inhibition using the formula:

$$(1 - E / C) \times 100$$

Where:

C = Absorbance of fully oxidized control and
E = Absorbance in the presence of extract

Phytochemical screening: Chemical tests were carried out on the aqueous and methanolic extracts for the

qualitative determination of phytochemical constituents using standard procedures as described by Harbone (1973) and Sofowora (1993).

Proximate analysis: The chemical tests were carried out on the plant samples for the quantitative determination of physico-chemical constituent using standard procedures as described by Pearson (1976).

Statistical analysis: This was done with the aid of Windows Microsoft Excel and SPSS for windows; SPSS Inc., Chicago, standard version 14.0 to determine differences between means using Analysis of Variance (ANOVA). Data were reported as Mean±Standard deviation.

RESULTS

The total phenolic content of crude extracts was obtained from the regression equation for the calibration curve of standard gallic acid ($y = 0.001x + 0.007$, $R^2 = 0.996$) and expressed as Gallic Acid Equivalent (GAE). The GAE indicated that aqueous and methanolic extracts of *V. amygdalina* had higher phenolic content than those of *T. triangulare* (Table 1).

Rapid TLC screening for antioxidant activity in the plant extracts was positive. The percentage DPPH radical scavenging activity of the extracts showed that methanolic extracts of *V. amygdalina* and *T. triangulare* had high antioxidant activity than aqueous extract of *V. amygdalina* and *T. triangulare* respectively. However, the antioxidant activity of aqueous and methanolic extracts of *V. amygdalina* was slightly higher than those of aqueous and methanolic extract of *T. triangulare*. Nevertheless, the antioxidant activity of both *V. amygdalina* and *T. triangulare* extracts was low compared with standard gallic and ascorbic acid (Fig. 1-3).

Percentage inhibition of lipid peroxidation by extracts tested showed no significant difference ($p>0.05$) (Table 1).

Phytochemical analysis of extracts tested positive to tannins, saponins, flavonoids, phenols and alkaloids, excluding methanolic extract of *T. triangulare* that tested negative for tannin. Only the aqueous extracts tested positive for tannins, phlobatannins and cardiac glycosides. However, all extracts tested negative for terpenoids, cardenolides and anthraquinones (Table 2).

The proximate composition of *V. amygdalina* leaf indicates higher percentage of crude fibre, crude fat, crude protein, and carbohydrate than *T. triangulare* except the moisture and ash contents (Table 3).

DISCUSSION

Recently, there have been increased scientific interests in the study of antioxidants, particularly those intended to prevent the presumed deleterious effects of free radicals

Table 1: Quantitative determination percentage inhibition of lipid peroxidation and total phenol content of aqueous and methanol extracts of *V. amygdalina* and *T. triangulare*

Sample	Concentration (µg/ml)	Percentage Inhibition of lipid peroxidation	Total phenol (mg/g GAE)
Aqueous extract of *V. amygdalina*	100	72.85±0.495*	0.822±0.05
Methanolic extract of *V. amygdalina*	100	72.40±0.424	0.681±0.05
Aqueous extract of *T. triangulare*	100	72.25±0.919	0.456±0.04
Methanolic extract of *T. triangulare*	100	73.85±0.636	0.288±0.02

*Indicates mean±standard deviation

Table 2: Phytochemical constituent of aqueous and methanolic extracts of *Vernonia amygdalina* and *Talinum triangulare* leaves

	V. amygdalina		*T. triangulare*	
Phytochemical	Aqueous extract	Methanolic extract	Aqueous extract	Methanolic extract
Terpenoids	-	-	-	-
Tannins	++	+	+	-
Saponins	++	++	++	+
Phlobatannins	++	-	++	-
Cardiac glycoside	++	-	++	-
Flavonoids	++	++	++	++
Cardenolides	-	-	-	-
Anthraquinones	-	-	-	-
Phenol	+	+	+	+
Alkaloid	++	++	+	+

+ = Indicates trace; ++ = Indicates abundant; - = Indicates absent

Table 3: Proximate compositions of *Vernonia amygdalina and Talinum triangulare*

Plant	Moisture (%)	Fiber (%)	Fat (%)	Protein (%)	Ash (%)	Carbohydrate (%)
V. amygdalina	18.6±0.6	13.8±0.06	14.9±0.2	4.56±0.4	21.6±0.14	26.5±0.6
T. triangulare	23.1±0.2	12.5±0.04	9.4±0.1	1.88±0.2	47.6±0.07	5.52±0.4

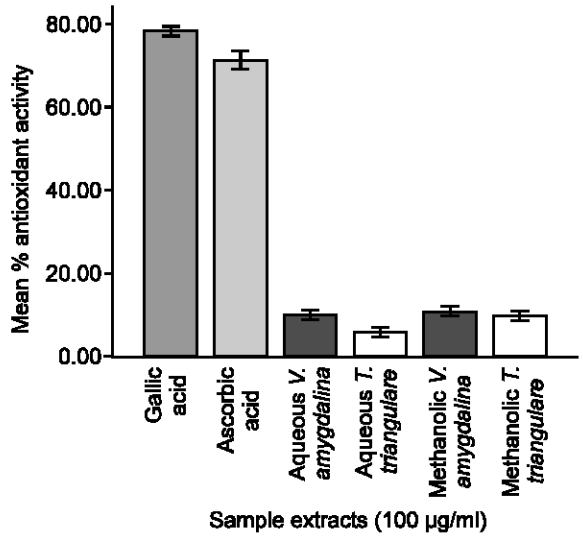

Fig. 1: Percentage scavenging activity of DPPH against 100 µg/ml sample extracts

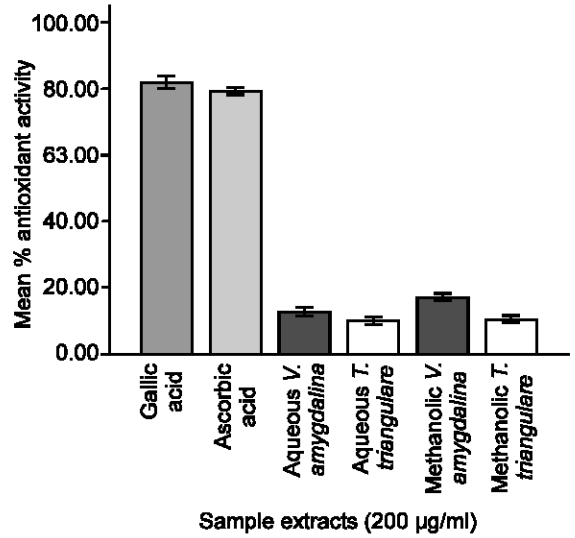

Fig. 2: Percentage scavenging activity of DPPH against 200 µg/ml sample extracts

in human body and deterioration of fats and other constituents of foodstuff. In both cases, antioxidants from natural rather than synthetic sources are preferred (Abdalla and Roozen, 1999).

The result demonstrated that the aqueous extract of *V. amygdalina* showed higher phenolic content than other extracts. Plant phenolics are major group of compounds acting as primary antioxidants or free radical scavengers (Kahkonen *et al*., 1999). All sample extracts tested positive using the rapid Thin Layer Chromatography (TLC) screening for the presence of antioxidant activity. The change in color of DPPH spray from deep violet to yellowish spots suggests presence of free radical scavengers. Furthermore, the free radical scavenging

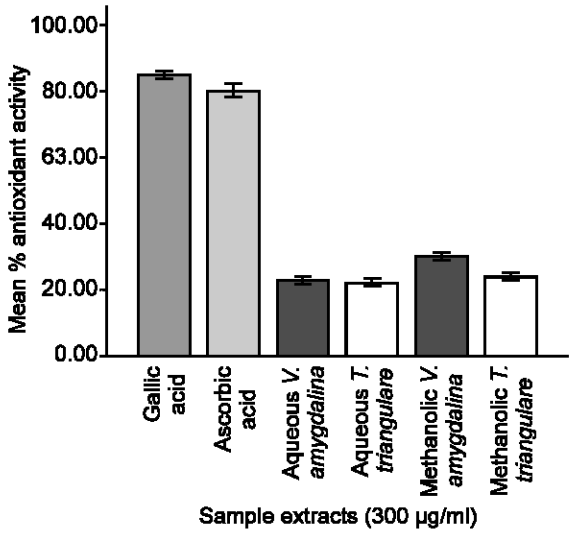

Fig. 3: Percentage scavenging activity of DPPH against 300 µg/ml sample extracts

power of the leave extracts of *V. amygdalina* and *T. triangulare* increased with increasing concentration of the extracts as evident in the rapid reduction of the stable DPPH radical and thus, may be considered good sources of antioxidants for nutrition, medicine and commercial use (Ayoola *et al*., 2008). Lipid peroxidation was prevented by 100 µg/ml extracts of both *V. amygdalina* and *T. triangulare*. Reports have shown that the oxidation index is a good indicator of degradation and lack of an antioxidant property. This result however, indicates inhibition of lipid peroxidation by leave extracts in the biological system and it is in close agreement with the work of Odukoya *et al*. (2006) that reported the antiperoxidative effect of leaves of *V. amygdalina* using the linoleic acid model system.

Phytochemical composition in leave extracts of *V. amygdalina* and *T. triangulare* include bioactive compounds such as saponins, phenols, flavonoids, alkaloids, phlobatannins, cardiac glycosides and tannins. This suggests stimulatory, antiseptic, anti-inflammatory and mild anti-hypertensive properties of the leave extracts (Ayoola *et al*., 2008; Mensah *et al*., 2008).

Proximate analysis result revealed that *V. amygdalina* leaf had higher total carbohydrate, crude fat, crude protein and crude fibre contents than *T. triangulare*. This suggests that *V. amygdalina* rather than *T. triangulare* could serve as a better source of dietary carbohydrate, protein and lipids. Hence, *V. amygdalina* adds to the calorific value of food and possesses odour and flavor carrying ability thereby enhancing the palatability of food. Mensah *et al*. (2008) also reported that crude fibre adds bulk to the food and prevents the intake of excess starchy food. However, *T. triangulare* had higher ash and moisture content than *V. amygdalina* leaf suggesting the

presence of high total inorganic residue, an indicator of the mineral content. High moisture content reduces the shelf life of food substances (Ruberto and Baratta, 2000). Hence, *V. amygdalina* leaf could have a higher shelf life than *T. triangulare* leaf.

Conclusion: Aqueous leaf extracts of *V. amygdalina* and *T. triangulare* contain more phytochemicals (tannins, phlobatannins and cardiac glycosides) in addition to methanolic extracts' saponins, phenols, flavonoids and alkaloids. The methanolic and aqueous leaf extracts of *V. amygdalina* possess higher antioxidant properties, phyto-nutrients and longer shelf life than *T. triangulare*. This may explain the wide acceptability and uniqueness of *V. amygdalina* in folkloric medicine to prevent or slow down the progress of various oxidative stress-related diseases.

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Development and Quality Characteristics Studies of Tomato Paste Stored at Different Temperatures

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Abstract: A comparative study was done to determine the most suitable storage temperature for tomato paste at which there would be minimum damage to the product quality. Tomato paste samples were prepared, 0.1% sodium benzoate preservative added, stored at 25°C, 6°C and -10°C and were analyzed for chemical parameters as well as subjected to sensory evaluation at 30 days storage intervals till 240 days. A gradual increase in Total Soluble Solids (TSS) and acidity was observed during storage whereas pH and ascorbic acid were decreased. These changes were more pronounced at 25°C than at 6°C and -10°C. Results of sensory evaluation revealed that samples stored at lower temperatures such as 6°C and -10°C remained acceptable after 240 days storage. However, samples were rejected organoleptically at higher temperature storage i.e. 25°C.

Key words: Tomato paste, chemical analysis, sensory evaluation, temperature, storage studies

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.*)* is considered as prize vegetable in western countries and extensively used in fresh condition as well as in the preparation of various food products. In Pakistan, tomato is one of the major vegetables grown at an area of 47.1 thousand hectre with total production 502.3 metric tons during 2006-07 (GOP, 2008). Tomato represents an essential part of human diet. It is a good nutritional resource rich in vitamin C and antioxidant mainly lycopene, carotenes, organic acids and phenolics (Giovanelli and Paradiso, 2002). Consumption of beta-carotene and lycopene, has been related to lower incidence of cardiovascular disease and prostate, gastrointestinal and epithelial cell cancer (Ishida and Chapman, 2004; Rao and Rao, 2007). Organic acids which influence its shelf-life and organoleptic properties such as colour brightness and texture contribute to acid-base balance for the consumer (Adedeji *et al*., 2006); while plant phenolics can have anti-inflammatory, anti-allergic and anti-thrombotic properties and may be beneficial in cardiovascular, neoplastic and neurological pathologies (Kuskoski *et al*., 2005).

In Pakistan, about 20% of the tomato produce are wasted due to negligence, defective marketing system and lack of processing facilities. During the peak season tomato sells at low prices due to larger supplies "glut" resulting in less return to growers. With the spread of education, change in habits of populace, growth in working women force and increase in per capita income and urbanization, the demand for processed vegetable/fruit products is increasing progressively. Tomato is processed in the forms of pulp, paste, juice, ketchup, sauce and purée (Hayes *et al*., 1998). It has a limited storage life and cannot be stored over extended periods. The problem is further compounded by lack of cold chain system. Conversion of tomato into paste provides a way out for extended shelf life/storage periods. Food processors store tomato pulp under conditions available in their premises. It has been observed that temperatures varying from as high as 20-40°C, refrigeration (4-10°C) to as low as -20°C are employed for storage purpose. High temperature storage is detrimental to product quality while lower temperature adds cost to the product (Jamil, 1990). Therefore a study had been designed to determine the suitable temperature for tomato paste storage at which there would be minimum damage to the product quality.

MATERIALS AND METHODS

Fully matured tomatoes of "Roma" variety were procured from wholesale market and taken to Food Quality and Nutrition Program (FQNP) Lab. National Agricultural Research Centre (NARC). Fresh tomatoes were sorted, washed and blanched at 90°C for 5 min, passed through a pulper using sieve of 0.058 cm dia holes and vacuum concentrated at 85°C for 34 min to 26 °brix paste as described by Apaiah and Barringer (2007). Sodium benzoate @ 0.1% was added as preservative. Tomato paste was immediately filled in pre-sterilized glass bottles, capped airtight, processed in boiling water for ten minutes and subsequently cooled. It was stored at room temperature (25°C), refrigerated (6°C) and freezing (-10°C) temperature. Chemical and sensory analysis of tomato paste was carried out just after processing and at 30 days intervals till storage period of 240 days.

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Chemical analysis: Tomato paste samples were analyzed for Total Soluble Solids (TSS), percent acidity, pH and ascorbic acid according to standard procedures described by AOAC (2007). Total Soluble Solids of tomato paste were estimated by using Abbe refractometer (ATAGO 3T) and the readings were corrected at 20°C whereas, pH was measured by digital pH meter (Orion 420 A^+).

Sensory evaluation: Sensory evaluation of tomato paste was carried out for colour, taste, flavour and overall acceptability by a panel of seven judges at intervals of 0, 30, 60, 90, 120, 150, 180, 210 and 240 days. Samples were presented in succession and panelists were asked to rate evaluation variables according to 9- point Hedonic scale as described by Larmond (1977).

Statistical analysis: The data obtained for each parameter was subjected to statistical analysis according to methods described by Steel *et al.* (1996) using Statisca software.

RESULTS AND DISCUSSION

Tomato paste samples stored at three different temperatures i.e. 25°C, 6°C and -10°C were analyzed for changes in total soluble solids, percent acidity, pH, ascorbic acid as well as sensory attributes. The composition of tomato paste (Table 1) was found within limits prescribed by Codex Alimentarius Commission (1981).

Chemical characteristics: Data regarding changes in total soluble solids of tomato paste during storage reveals that there was a gradual increase in T.S.S. throughout the storage period at all the storage temperatures (Table 1). Maximum increase (5.36%) was observed in samples stored at 25°C, whereas samples stored at -10°C had the minimum increase (2.28%). Thus the increase in T.S.S. was more in tomato paste samples stored at higher temperature than at lower temperature. Increase in T.S.S. during storage may be due to acid hydrolysis of polysaccharides especially gums and pectin (Luh and Woodroof, 1975).

Percent acidity of tomato paste stored at different temperatures exhibited a gradual increase throughout the storage period (Table 1). However, rise in acidity was more at higher temperature than at lower temperature. Highest increase (18.39%) was recorded in samples stored at 25°C followed by 6°C storage conditions (10.34%), while least increase (7.47%) occurred in samples stored at -10°C. Increase in titratable acidity of tomato paste may be due to acids produced by *Bacilus coagulans*, *Clostridium butyricum* and as a result of phenolic compounds produced by *Bacillus coagulans*. It may also be due to oxidation of alcohol and aldehyde during processing and is influenced by storage temperature, higher the temperature greater the increase in acidity (Gould, 1992).

As regards pH of tomato paste held at different temperatures, a decreasing trend was observed during the storage period (Table 1). The overall decrease in pH was maximum (4.63%) and minimum (2.19%) in samples stored at 25°C and at -10°C respectively. The temperature influences the decrease in pH. Change in pH is directly related to change in acidity of samples. Similar findings were reported by Ahmad (1997) during his study on tomato concentrate.

It is evident from the data on ascorbic acid content of tomato paste stored at different temperatures that there was a gradual and considerable decline in ascorbic acid throughout the storage period at all temperatures (Table 1). The declining trend was more pronounced (87.10%) at higher temperature i.e. 25°C and least (32.25%) in paste samples stored at -10°C. Ascorbic acid may be destroyed by oxidation, especially at higher temperature. Its stability is greatly influenced by temperature, oxygen and metal ion content. Vitamin C is the most labile of the nutrients, so its degradation is used as an indicator of quality (Smith and Hui, 2004). The losses of ascorbic acid is probably attributable to oxidation of ascorbic acid to dehydroascorbic acid followed by hydrolysis of the latter to 2,3-diketogluconic acid, which then undergoes polymerization to other nutritionally inactive products (Dewanto *et al.*, 2002).

Sensory evaluation: Tomato paste samples stored at different temperatures were sensory evaluated at 30 days interval for colour, taste, flavour and overall acceptability (Table 2). A decreasing trend in sensory scores of tomato paste samples was observed at all temperatures with the length of time. However, decline was more prominent in samples stored at higher temperature. As regards colour, a gradual decrease was observed with highest loss in colour (47.29%) in samples stored at 25°C and least loss (22.77%) at -10°C. The colour score of tomato paste samples reveals that after 240 days storage, samples stored at 25°C were rejected (4.19), at 6°C neither liked nor disliked (5.57), whereas samples were still acceptable (6.14) at -10°C storage condition. In addition to degradation of lycopene by an auto catalytic mechanism, darkening occurs during storage due to non-enzymatic browning (Mudahar *et al.*, 1986).

In case of changes in taste as affected by storage conditions, a gradual decline was observed throughout the storage period being maximum (46.85%) and minimum (21.26%) in samples stored at 25°C and -10°C respectively. Mean score for taste (4.05) was below acceptable range and thus rejected at 25°C while samples stored at -10°C got the acceptable taste score (6.00) after 240 days storage. With respect to flavour of

Table 1: Effect of storage time and temperature on TSS, acidity, pH and ascorbic acid content of tomato paste

Storage interval	TSS (%)			Acidity (%)			pH			Ascorbic acid (mg/100g)		
(Days)	25°C	6°C	-10°C	25°C	6°C	-10°C	25°C	6°C	-10°C	25°C	6°C	-10°C
0	26.30	26.30	26.30	1.74	1.74	1.74	4.10	4.10	4.10	31.00	31.00	31.00
	±0.10	±0.10	±0.10	±0.03	±0.03	±0.03	±0.02	±0.02	±0.02	±3.00	±3.00	±3.00
30	26.41	26.37	26.35	1.77	1.75	1.75	4.08	4.09	4.09	26.00	29.00	30.00
	±0.09	±0.08	±0.07	±0.02	±0.01	±0.01	±0.02	±0.01	±0.01	±2.64	±2.00	±2.00
60	26.62	26.48	26.42	1.81	1.77	1.76	4.06	4.07	4.08	23.00	27.00	29.00
	±0.06	±0.05	±0.05	±0.02	±0.02	±0.01	±0.02	±0.02	±0.02	±2.00	±3.00	±2.64
90	26.79	26.63	26.48	1.84	1.79	1.78	4.05	4.06	4.08	19.00	25.00	29.00
	±0.06	±0.06	±0.06	±0.02	±0.01	±0.02	±0.01	±0.02	±0.01	±4.00	±2.00	±2.00
120	26.90	26.72	26.58	1.86	1.82	1.80	4.03	4.05	4.07	15.00	24.00	28.00
	±0.05	±0.07	±0.08	±0.01	±0.02	±0.01	±0.02	±0.02	±0.01	±3.00	±1.00	±1.53
150	27.02	26.80	26.65	1.90	1.84	1.82	4.01	4.04	4.06	12.00	22.00	26.00
	±0.06	±0.05	±0.10	±0.02	±0.01	±0.01	±0.02	±0.01	±0.01	±3.00	±2.00	±2.00
180	27.23	26.90	26.74	1.95	1.86	1.83	3.98	4.02	4.04	9.00	21.00	25.00
	±0.07	±0.05	±0.08	±0.01	±0.02	±0.01	±0.03	±0.02	±0.02	±2.00	±2.64	±1.53
210	27.48	27.08	26.80	2.00	1.89	1.85	3.95	4.00	4.02	7.00	18.00	23.00
	±0.07	±0.08	±0.10	±0.03	±0.02	±0.02	±0.02	±0.02	±0.01	±1.00	±2.00	±2.00
240	27.71	27.20	26.90	2.06	1.92	1.87	3.91	3.99	4.01	4.00	17.00	21.00
	±0.08	±0.09	±0.12	±0.02	±0.01	±0.01	±0.03	±0.01	±0.01	±1.53	±2.00	±1.00

All values are mean of three replications

Table 2: Effect of storage time and temperature on sensory attributes of tomato paste

Storage interval	Colour			Taste			Flavour			Overall acceptability		
Days	25°C	6°C	-10°C	25°C	6°C	-10°C	25°C	6°C	-10°C	25°C	6°C	-10°C
0	7.95	7.95	7.95	7.62	7.62	7.62	7.71	7.71	7.71	7.67	7.67	7.67
	±0.22	±0.22	±0.22	±0.22	±0.22	±0.22	±0.14	±0.14	±0.14	±0.22	±0.22	±0.22
30	7.71	7.71	7.90	7.38	7.48	7.57	7.43	7.57	7.62	7.43	7.57	7.62
	±0.14	±0.14	±0.17	±0.09	±0.08	±0.14	±0.14	±0.14	±0.16	±0.14	±0.14	±0.16
60	7.09	7.48	7.71	7.00	7.33	7.43	6.91	7.28	7.43	7.00	7.38	7.48
	±0.21	±0.08	±0.14	±0.28	±0.09	±0.14	±0.08	±0.28	±0.14	±0.14	±0.17	±0.08
90	6.76	7.28	7.43	6.62	7.14	7.28	6.67	7.00	7.19	6.67	7.14	7.28
	±0.30	±0.14	±0.14	±0.16	±0.14	±0.14	±0.17	±0.14	±0.16	±0.17	±0.14	±0.14
120	6.38	6.95	7.14	6.24	6.86	7.09	6.43	6.71	6.95	6.33	6.81	7.05
	±0.22	±0.22	±0.28	±0.17	±0.28	±0.16	±0.14	±0.14	±0.22	±0.09	±0.17	±0.08
150	6.14	6.81	7.00	6.00	6.67	6.81	6.19	6.48	6.71	6.14	6.67	6.86
	±0.14	±0.22	±0.14	±0.14	±0.17	±0.17	±0.16	±0.08	±0.14	±0.14	±0.17	±0.14
180	5.62	6.47	6.67	5.19	6.28	6.62	5.57	6.14	6.38	5.47	6.38	6.57
	±0.22	±0.22	±0.22	±0.22	±0.14	±0.22	±0.29	±0.14	±0.09	±0.22	±0.17	±0.14
210	5.00	6.05	6.43	4.38	6.00	6.33	4.66	5.86	6.14	4.71	6.00	6.33
	±0.14	±0.21	±0.14	±0.17	±0.14	±0.09	±0.08	±0.14	±0.28	±0.14	±0.28	±0.09
240	4.19	5.57	6.14	4.05	5.33	6.00	4.00	5.47	5.90	4.05	5.52	6.00
	±0.16	±0.29	±0.14	±0.21	±0.09	±0.14	±0.14	±0.17	±0.17	±0.16	±0.16	±0.14

All values are mean of three replications

tomato paste held at different storage conditions, a gradual loss was observed throughout the storage period. Flavour loss was highest (48.12%) in samples stored at 25°C followed by samples (29.05%) stored at 6°C. The flavour of tomato paste stored at 25°C was disliked (4.00) and therefore rejected by sensory panel after 240 days storage. The Maillard reaction is the major mode of deterioration during storage of canned fruit and vegetable products and leads to a bitter off-flavour (OTA, 1979) Changes in flavour are the most sensitive index to quality deterioration during storage followed by colour (Eckerle *et al.*, 1984). For overall acceptability score of tomato paste, the decreasing trend was more rapid (47.20%) and slower (21.77%) in samples stored at 25°C and -10°C respectively. The overall acceptability score of tomato paste samples shows that after 240 days storage, at 25°C samples stored were rejected (4.05), at 6°C neither liked nor disliked (5.52), whereas samples remained acceptable (6.00) at -10°C storage condition.

Conclusion: It was concluded that tomato paste could be stored at varying storage condition for 240 days with minimum damage to the product quality at lowest possible cost. It may be stored even for longer periods at low temperature such as 6°C and -10°C. During sensory studies, it was observed that samples stored at lower temperatures such as 6°C and -10°C remained acceptable after 240 days storage. However, samples were rejected organoleptically at higher temperature storage i.e. 25°C.

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Nutritional Status and the Risk for Colorectal Adenomas: A Case-Control Study in Hospital Kuala Lumpur, Malaysia

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Abstract: The most important and possible risk factor of colorectal adenomas is the individual's nutritional status. The role of nutritional status in the aetiology of colorectal adenomas remains an area of active investigation, as the exact relationship between nutritional status and colorectal adenomas remains unclear. The objective of this case-control was to determine the nutritional status of subjects with colorectal adenomas as compared with healthy subjects at Hospital Kuala Lumpur over a period of one year. A total of 118 subjects were recruited (n case = 59 and n control = 59). A pre-tested questionnaire was used to collect socio-demographic information and dietary intake. Lipid profile was determined using standard Roche diagnostic kits. The data were analyzed using SPSS version 12.0. The intake of beta-carotene, alpha-carotene, lycopene, vitamin A and crude fiber found to be significantly different between the groups ($p<0.05$), while beta-carotene, alpha-carotene, lycopene, vitamin C, vitamin D, vitamin E and crude fiber significantly reduced the risk. Although the percentages intake of nutrient achieved according to RNI were below the recommended value, percentages of RNI achieved for vitamin C, D, E and folate were found to be significantly different between male subjects ($p<0.05$). Our results support the notion that certain nutritional factors are of importance for the development of these pre-cancerous lesions. Identification of risk factors through this case-control study should be able to supplement the available data in order to develop an intervention package that focuses on multiple risk factors to reduce the chances for developing colorectal adenomas or colorectal cancer.

Key words: Nutritional status, dietary intake, anthropometric measurements, colorectal adenoma

INTRODUCTION

Colorectal Cancer (CRC) was estimated to be the third and fourth most commonly occurring cancer worldwide among men and women respectively in the year 2002 (IARC, 2002). Colorectal cancer was estimated to contribute to 9.5% and 9.3% of total cancer cases among males and females respectively in the same year. Among Malaysians, colon cancer ranked third among cancers reported, accounting for 7.8% and 6.0% of all cancer cases in males and females respectively in 2003 (NCR, 2004). The age-standardized rate for colon cancer in males and females were 13.9 and 11.2 respectively. Cancer of the rectum, on the other hand, ranked fifth among cancers reported in Malaysian males (6.8%) and females (4.1%) respectively.

Colorectal cancer develops over a period of several years and nearly all arise from benign, neoplastic adenomatous polyps (Bond, 2000; Kahn *et al*., 1998). The progress of adenoma to cancer may take five to ten years (Young *et al*., 2002). These polyps are benign growths that protrude from the inner walls of the colon and rectum and are relatively common in people over the age of 50. It is estimated that the average 60 year-old without special risk factors for polyps had a 25% chance of having a polyp (ASGE, 2006). Data on the incidence of Colorectal Adenoma (CRA) in the Malaysian population is yet to be reported. The closest available study done in Singapore showed that the prevalence of CRA amongst males was 20.4% for Chinese, 4.6% for Malays and 7.9% for Indians (Lee, 1987).

The most important and possible risk factor of CRC and CRA is the individual's diet. The role of diet in the aetiology of CRA remains an area of active investigation, as the exact relationship between diet and CRA remains unclear.

Diet and lifestyle factors have been implicated in the development of the sporadic adenomatous polyps (Larsen *et al*., 2006; Wark *et al*., 2006) while mutation in genes and DNA may also cause conditions known as Familial Adenomatous Polyposis Syndrome (FAP) or Hereditary Non-Polyposis Colorectal Cancer (HNPCC) syndrome, which lead to development of multiple polyps (Burt, 2000).

Results from several observational studies have suggested that three main dietary factors may or may not protective against cancer of the large bowel, i.e. low fat

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intake, high fiber intake and high fruits and vegetables intake (Schatzkin *et al*., 2000). An inverse relationship was also reported between level of total physical activity and the risk for adenomatous polyps of the colon and rectum (Sandler *et al*., 1995). Tobacco smoking and alcohol consumption may increase the risk of colonic polyps (Kahn *et al*., 1998; Todoroki *et al*., 1995; Otani *et al*., 2003).

MATERIALS AND METHODS

Selection of subjects: Cognitively sound men and women who were at least 30 years of age and had completed a colonoscopy between January 2005 and December 2005 were invited to participate in the study with informed consent. Hospital Kuala Lumpur (HKL) served as the clinical centre and the source of participants for this study. Ethical approval was obtained prior commencement of the research.

The inclusion criteria for selection of cases were patients newly diagnosed with one or more histologically confirmed CRA removed through polypectomy; had no other types of polyps (hyperplastic polyps, FAP and HNPCC); free from other chronic diseases and who are not involved in other studies. It is vital for individuals with other polyps and chronic diseases to be excluded from the study, as the risk factors for such conditions have already been established and thus may interfere with the results of the study. The exclusion criteria included: history of colorectal and/or any other cancers or, bowel resection, polyposis syndrome, or inflammatory bowel disease; unsatisfactory colon preparation or incomplete colonoscopy; taking cholesterol-lowering drugs and have chronic medical conditions or dietary restrictions that would substantially limit their ability to complete the study.

Three hundred and forty three patients who fulfilled these criteria were selected by the surgeon in the Surgical Department of HKL. Of these patients, 157 responded to the invitation letter that was sent and attended a briefing session. Seventy five percent or 118 of those who attended the briefing session agreed to take part in this study and gave informed consent. Fifty nine subjects who had histologically confirmed adenomatous polyps removed were recruited as cases and an equal number of subjects who were found to be negative for colorectal adenomatous polyps upon colonoscopy and fulfilled the other inclusion criteria were recruited as control subjects.

Data collection: All data were collected during a face to face interview with the subjects at HKL. A pre-tested, structured questionnaire was used to record the socio-economic information (age, ethnicity, marital status, educational status, occupation and income).

Three days 24 h diet recalls (1 weekend and 2 weekdays) were obtained from the subjects. Details of foods and drinks taken each day were recorded. It is important to have repeated 24 h diet recalls, as the food intake by subjects differs every day. This is mainly because the respondents are free-living subjects and not subjected to any dietary restriction. It is important that consecutive days were not selected and one weekend was included to make sure the variety of food consumed is recorded (Gibson, 2005).

A food album on commonly consumed food in household measures was used to facilitate subjects to improve accuracy of the recalls and household measurements of the food and drink consumed by the subjects. The first and the second recalls were done during the face-to-face interview, and the subjects were followed up through phone calls for the third dietary recall. The data were then entered into Nutritionist Pro 2.4 to be analyzed.

Nutritionist Pro (First Data Bank, USA) single version 2.4 dietary software was used to analyze nutrient intakes based on the 24 h diet recalls. The foods and beverages consumed by the respondents for 3 days were entered in the program. Wherever necessary recipes were obtained and each ingredient was entered into the software. The software was then used to calculate the average energy and nutrient intakes of the subjects. The data obtained were entered into SPSS to be analyzed.

Anthropometric measurements: Anthropometric measurements were taken directly after the interview session. Height was measured to the nearest to 0.1 cm using a body meter (GIMA) and weight was measured to the nearest to 0.1 kg using an electronic weighing scale (TANITA). Body Mass Index (BMI) was calculated and was classified according to recommendation by WHO (1995). Waist circumferences were measured using a non-elastic measuring tape and measured to the nearest 0.1 cm and categorized according to the respective classification of WHO (1995).

The Omron body fat monitor HBF-302 (Omron Healthcare Co., Ltd., UK) was used to obtain subjects' body fat percentage and total body fat mass with accuracy up to 0.1% and 0.1 kg respectively. The measurement of body fat using the Omron body fat monitor is based on the bioelectric impedance principle which is a non-invasive method based on the principle that the resistance to an applied minor electric current is inversely related to the amount of fat-free mass within the body (Lukaski *et al*., 1985). The cut-off points for percentage of body fat were those recommended by Omron HBF-302. The OMRON's body fat monitor has been validated in previous studies (Martín-Moreno *et al*., 2001; Lintsi *et al*., 2004).

Determination of biomarkers: Venous blood was collected by a trained and qualified nurse from the Faculty of Medicine and Health Science, Hospital Kuala Lumpur. Fifteen millilitres of fasting blood samples was drawn into empty tubes. Tubes were protected from light

with aluminium foil and were centrifuged within three hours after collection at 3000 rpm at 4°C for 10 min. The plasma was then separated and transferred into polypropylene micro centrifuge tube and was kept at -80°C up to three months until further analysis.

Plasma lipids and lipoproteins were quantified using enzymatic methods by using Roche-Diagnostics standards and kits (Warnick *et al*., 1982; Carr *et al*., 1993) with an auto analyzer Hitachi 747. This method uses the combined action of polymers, polyanions, and detergent to solubilise cholesterol from HDL but not from very LDL and chylomicrons.

Data analysis: All statistical analyses were performed using SPSS version 15.0. Descriptive statistics such as frequencies, percentages, means and standard deviations were used to describe the data. Independent *t*-test was used to determine differences between case and control groups for continuous variables. The data on nutrient intake was then categorized into tertiles to facilitate the risk calculation. The crude and adjusted odds ratios and their corresponding 95% confidence intervals were determined using binary logistic regression using the enter method. The odds ratio was adjusted for variables such as age, ethnicity, income, alcohol consumption, smoking status and energy intake based on previous studies (Bowers *et al*., 2006; Otani *et al*., 2006). p-value of <0.05 was considered as significant.

RESULTS AND DISCUSSION

Socio-demographic characteristics: The majority of the study participants were Chinese and married (Table 1) though there were no significant differences between the study groups. The majority of the male subjects were in the 60-69 years old age bracket, while female subjects were in the 50-59 years age group. Rajendra *et al*. (2005) have reported higher incidence of CRA in Malaysians as the age increases. Peters *et al*. (2004), for example, reported that the mean age of participants with histologically verified adenoma was 63.5 years, which was significantly different from the mean age of healthy controls (62.7 years). Another study (Erhardt *et al*., 2003) reported that the mean age of patients with CRA (59 years) was significantly higher than the healthy controls (54 years) and the data is very much closer to the mean age reported here. Rajendra *et al*. (2005) also reported that race does not have any association with CRA.

A similar distribution of subjects in both groups was seen for educational status categories. The majority of the participants in either group were either unemployed or retired. The next biggest occupational group was the blue collar job category, which is mainly made up of drivers, tailors, labourers and general workers. The majority of the respondents were found in the low-income group with monthly personal income of <RM500. No previous study has correlated these variables with the risk for CRA.

Nutrient intakes: Of all the important macro and micronutrients studied, beta-carotene, alpha-carotene, lutien, retinol and crude fibre were found to significantly differ in their intakes between the study groups (Table 2). Vitamin C intake was found to differ significantly only among the male subjects. The percentages of RNI achieved by the males (Fig. 1) and the females (Fig. 2) revealed that the intake of most macro and micronutrients were way below the recommended values. Among the male subjects, only protein was consumed way above its recommendation. The male controls achieved more of the RNI for calcium, while the cases had less. However, the percentages achieved by the males for vitamin C, vitamin E and folate were significantly different at $p<0.05$. Among the female subjects, the percentages of RNI achieved for protein and calcium were higher than the recommended value, while the female controls achieved more of the RNI for vitamin A, while the cases had lower than what is recommended. The percentages of RNI achieved for other nutrients were below the recommended value and none were significantly different.

Highest tertile of intake of carotenoids such as b-carotene, a-carotene and lycopene were found to be protective of CRA in all subjects, regardless of gender (Table 3). There were reductions in the risk by 73-80% when highest tertile of intakes compared to the lowest. In a previous study, an increase intake of carotene was suggested to lower the risk for CRA by 40% when the highest tertile of intake compared with the lowest (Lubin *et al*., 1997). However, Senesse *et al*. (2005) found that although beta-carotene seems to be protective in non-smokers, the adverse effect of the nutrient in smokers should be taken as a caution. Some studies suggested gender difference in response to CRA risk. The risk of CRA in the highest quartile was approximately half of that of men in the lowest quartile for alpha-carotene (OR = 0.38, 95% CI = 0.18-0.84), beta-carotene (OR = 0.51, 95% CI = 0.24-1.07) and total carotenoids (OR = 0.48, 95% CI = 0.22-1.03) (Jiang *et al*., 2005). Conversely, Malila *et al*. (1999) found no significant association between carotenoids and the risk for CRA.

Vitamin C intake also found to be protective with reduction of risk by 72% in all subjects. However, the reduction in risk was found to be exclusive to men (Table 3). In previous study by Enger *et al*. (1996), dietary intake of vitamin C showed a weaker inverse association (OR, 0.8; 95% CI, 0.5-1.5). Tseng *et al*. (1994) however, suggested the reduction in the risk is only confined to women, in contrary to the findings on this study. Supplemented vitamin C has shown mixed results in its relationship with CRA (Greenberg *et al*., 1994; Senesse *et al*., 2005).

Table 1: Socio-demographic characteristics of the participants

	Males		Females		All	
Variables	Case (n = 42)	Control (n = 33)	Case (n = 17)	Control (n = 26)	Case (n = 59)	Control (n = 59)
Age (years)						
<40	1 (2.4)	1 (3.0)	0 (0.0)	3 (11.5)	1 (1.7)	4 (6.8)
40-49	6 (14.3)	7 (21.2)	2 (11.8)	8 (30.8)	8 (13.6)	15 (25.4)
50-59	10 (23.8)	11 (33.3)	7 (41.2)	9 (34.6)	17 (28.8)	20 (33.9)
60-69	18 (42.9)	11 (33.3)	7 (41.2)	4 (15.6)	25 (42.4)	15(25.4)
>70	7 (16.7)	3 (9.1)	1 (5.9)	2 (7.7)	8 (13.6)	5 (8.5)
Mean±SD	59.8±9.8	57.4±11.0	57.9±8.3	52.2±10.9	59.3±9.3*	55.0±11.2*
Ethnicity						
Malays	15 (35.7)	9 (27.3)	3 (17.6)	9 (34.6)	18 (30.5)	18 (30.5)
Chinese	17 (40.5)	13 (39.4)	10 (58.8)	8 (30.8)	27 (45.8)	21 (35.6)
Indians	10 (23.8)	10 (30.3)	3 (17.6)	9 (34.6)	13 (22.0)	19 (32.2)
Others	0 (0.0)	1 (3.0)	1 (5.9)	0 (0.0)	1(1.7)	1 (1.7)
Marital status						
Single	2 (4.8)	4 (12.1)	3 (17.6)	1 (3.8)	5 (8.5)	5 (8.5)
Married	39 (92.9)	28 (84.9)	11 (64.7)	24 (92.4)	50 (84.7)	51 (86.4)
Widowed/Divorced	1 (2.4)	1 (3.0)	3 (17.7)	1 (3.8)	4 (6.8)	2 (5.1)
Education status						
Primary	12 (28.6)	10 (30.3)	7 (41.2)	8 (30.8)	19 (32.2)	18 (30.5)
Secondary	16 (38.1)	13 (48.5)	8 (47.0)	10 (38.5)	24 (40.7)	26 (44.0)
Pre-U/Tertiary	14 (33.4)	10 (30.3)	2 (11.8)	8 (30.8)	16 (27.1)	18 (25.5)
Occupation						
Unemployed/retired	26 (63.7)	18 (54.6)	13 (76.4)	17 (65.4)	39 (66.1)	35 (59.3)
Blue collar	7 (16.7)	7 (21.2)	2 (11.8)	6 (23.1)	9 (15.3)	13 (22.0)
Businessmen	3 (7.1)	1 (3.0)	1 (5.9)	0 (0.0)	3 (5.1)	1 (1.7)
Government	1 (2.4)	2 (6.1)	0 (0.0)	1 (3.8)	2 (3.4)	4 (6.8)
Professionals	3 (7.1)	4 (12.1)	0 (0.0)	1 (3.8)	3 (5.1)	5 (8.5)
Others	1 (3.0)	1 (3.0)	1 (5.9)	0 (0.0)	3 (5.1)	1 (1.7)
Personal income (RM)						
<500	16 (38.1)	15 (45.5)	12 (70.6)	16 (61.5)	28 (47.5)	31 (52.5)
500-999	5 (11.9)	2 (6.1)	4 (23.9)	3 (11.5)	9 (15.3)	5 (8.5)
1000-1999	13 (31.0)	9 (27.3)	0 (0.0)	4 (15.4)	13 (22.0)	13 (22.0)
2000-2999	2 (4.8)	3 (9.1)	0 (0.0)	3 (11.5)	2 (3.4)	6 (10.2)
>3000	6 (14.3)	4 (12.1)	1 (5.9)	0 (0.0)	7(11.9)	4 (6.8)
Mean±SD	1721.6±360.9	2936.1±1045.1	770.1±366.9	4786.9±570.1	1113.0±938.0	1214.2±399.2

*p<0.05

Vitamin D, but not calcium was found to lower the risk for CRA by 69% (Table 3). However, vitamin D and calcium are generally studied together and few studies have shown the protective effect of both micronutrients in subjects with CRA. Oh *et al.* (2005) suggested that higher total calcium and vitamin D intakes were associated with reduced risk, though the intakes of vitamin D may be attenuated by high retinol intake. Hartman *et al.* (2005) also reported that dietary calcium and total vitamin D may be inversely associated with adenoma recurrence. Grau *et al.* (2003) further elaborated that calcium supplementation actually work together vitamin D states to reduce the risk.

The highest tertile of vitamin E was found to reduce the risk by 67% compared to the lowest tertile after adjusting for confounders (Table 3). Similar findings were reported by McKeown *et al.* (1998) where group supplemented vitamin E together with vitamin C resulted in significant reduction in risk. The antioxidant properties of vitamin E may be a reason for the reduction in the risk. However, there was no evidence that vitamin E reduced the incidence of adenomas (RR = 1.08, 95% CI = 0.91-1.29) (Greenberg *et al.*, 1994). The limited available evidence suggests vitamin E has conflicting association with CRA. Dietary intake vitamin E had shown either to have protective effect or no significant relationship with risk for CRA.

Crude fibre was found to be protective of colorectal adenoma in all subjects, when compared to the lowest tertile (Table 3). As the Malaysian Food Composition Table does not provide values for dietary fibre, crude fibre intake has been used as a measure of fibre intake in the subjects. Evidence for an association between dietary fibre and colorectal neoplasia has been equivocal and some data suggest that there may be sex differences in response to fibre. Pooled analysis of data from two large randomized controlled trials; the Wheat Bran Fibre Trial and the Polyp Prevention Trial indicated that the reduction in risk is more evident in men compared to women. (Jacobs *et al.*, 2006). A prospective

Table 2: Nutrients intake of the participants

Variables (Mean±SD)	Males		Females		All	
	Case (n = 42)	Control (n = 33)	Case (n = 17)	Control (n = 26)	Case (n = 59)	Control (n = 59)
Energy (kcal)	1461±431.1	1562.2±458.8	1447.3±381.3	1501.3±385.6	1457.06±414.12	1535.39±425.62
Macronutrient						
Carbohydrate (g)	196.7±64.5	268.7±55.0	198.2±57.2	198.5±62.9	197.13±62.02	204.20±58.3
Protein (g)	62.9±17.1	65.7±18.7	58.5±14.2	58.5±14.2	61.67±16.34	66.98±19.95
Fat (g)	48.2±18.4	53.2±24.1	47.9±15.0	48.8±17.5	48.08±17.40	51.29±21.38
Vitamins						
beta-carotene (µg)	873.3±591.5*	1555.4±1416.5*	802.2±645.6**	1631.1±1033.5**	852.78±602.76**	1588.80±1248.98**
alpha-carotene (µg)	171.5±109.9*	354.5±276.3*	136.6±106.1*	435.7±346.3*	161.07±108.81**	390.38±307.17**
Lutien (µg)	901.8±321.1	1813.8±679.4	197.3±145.6	689.9±518.3	764.13±285.43	1423.60±608.39
Lycopene (µg)	402.0±236.9*	2638.2±1315.4*	1323.9±626.1	3272.4±2121.2	793.34±349.04**	2936.74±670.49**
Vitamin A (µg)	4731.0±2127.3**	6944.6±3902.9**	5008.3±2007.5	6304.4±2730.5	4810.9±2080.1	662.5±3423.5
Retinol (µg)	1055.9±505.6*	1457.9±736.4*	1049.3±451.6	1107.5±567.6	1053.98±486.82*	1303.45±684.72*
Vitamin C (mg)	44.0±29.1*	66.1±44.8*	63.6±109.4	84.2±95.1	49.65±63.09	74.10±71.35
Vitamin D (mg)	1.4±1.0	1.2±1.0	0.7±0.6	0.9±0.6	1.3±0.9	1.1±0.8
Vitamin E (mg)	3.2±1.2*	5.1±4.7*	3.8±2.1	5.0±3.8	3.39±1.53*	5.08±4.14*
Folate (mg)	80.6±34.6**	126.2±77.2**	83.4±44.0	112.0±69.8	81.4±37.7	119.9±73.7
Minerals						
Calcium (mg)	538.0±287.7	567.5±257.2	425.8±171.5	521.0±265.1	505.65±263.15	547.03±256.19
Iron (mg)	12.0±5.1	15.6±13.8	13.7±6.2	14.4±8.2	12.47±5.43	15.04±11.57
Crude fiber (g)	3.8±2.3**	6.6±4.3**	4.2±2.7*	7.6±6.5*	3.93±2.42**	7.07±5.34**

*p<0.05; **p<0.01

Table 3: Crude and adjusted odds ratio of nutrient intakes of participants

Variables	Males		Females		All	
	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)
Macronutrient						
Carbohydrate						
T1	1.00	1.00	1.00	1.00	1.00	1.00
T2	1.20 (0.36-3.92)	1.42 (0.39-5.18)	2.86 (0.61-3.84)	2.44 (0.66-3.89)	1.53 (0.22-2.32)	1.45 (0.16-2.23)
T3	1.36 (0.11-2.16)	0.36 (0.10-1.32)	1.11 (0.21-5.80)	1.90 (0.27-13.54)	1.58 (0.65-3.88)	1.52 (0.56-4.16)
Protein						
T1	1.00	1.00	1.00	1.00	1.00	1.00
T2	2.43 (0.74-8.0)	2.98 (0.76-11.70)	1.19 (0.02-2.10)	1.25 (0.02-3.53)	1.55 (0.21-1.42)	1.42 (0.14-2.22)
T3	1.85 (0.27-4.6)	1.98 (0.27-3.54)	1.47 (0.10-2.30)	1.60 (0.09-4.41)	1.80 (0.75-4.34)	1.66 (0.61-4.53)
Fat						
T1	1.00	1.00	1.00	1.00	1.00	1.00
T2	1.80 (0.54-5.89)	1.65 (0.47-5.82)	1.23 (0.27-5.67)	2.30 (0.39-4.74)	1.63 (0.24-3.60)	1.51 (0.18-3.44)
T3	1.60 (0.19-2.89)	1.59 (0.17-3.04)	1.62 (0.12-3.22)	1.32 (0.17-5.00)	1.64 (0.68-3.94)	1.54 (0.60-3.98)
Vitamins						
Beta-carotene						
T1	1.00	1.00	1.00	1.00	1.00	1.00
T2	0.83 (0.25-2.76)	0.17 (0.29-3.66)	0.43 (0.27-2.52)	0.64 (0.24-2.24)	0.96 (0.38-2.43)	1.11 (0.41-3.02)
T3	0.25 (0.08-0.80)*	0.23 (0.06-0.86)*	0.08 (0.01-0.54)*	0.07 (0.01-0.58)*	0.18 (0.07-0.48)*	0.21(0.07-0.59)*
Alpha-carotene						
T1	1.00	1.00	1.00	1.00	1.00	1.00
T2	0.11 (0.06-3.47)	0.33 (0.06-4.97)	0.60 (0.33-7.85)	0.31 (0.19-8.92)	0.26 (0.05-3.15)	0.32 (0.04-3.69)
T3	0.21 (0.06-0.74)	0.22 (0.06-0.80)*	0.20 (0.04-1.07)	0.09 (0.01-0.81)*	0.20 (0.07-0.53)*	0.20 (0.07-0.55)*
Lutien						
T1	1.00	1.00	1.00	1.00	1.00	1.00
T2	0.60 (0.19-1.94)	0.66 (0.18-2.41)	0.17 (0.02-1.91)	0.05 (0.00-0.99)*	0.62 (0.23-1.66)	0.46 (0.15-1.37)
T3	0.25 (0.07-0.95)*	0.28 (0.07-1.10)	0.51 (0.13-2.02)	0.35 (0.07-1.88)	0.72 (0.31-1.65)	0.73 (0.29-1.81)
Lycopene						
T1	1.00	1.00	1.00	1.00	1.00	1.00
T2	0.79 (0.05-13.50)	0.93 (0.03-30.68)	1.02 (0.23-4.53)	0.44 (0.07-2.84)	0.88 (0.29-0.95)*	0.73 (0.37-0.99)*
T3	0.37 (0.12-1.14)	0.35 (0.10-1.25)	0.14 (0.02-0.82)	0.08 (0.01-0.77)	0.24 (0.10-0.60)*	0.27 (0.10-0.75)*
Vitamin A						
T1	1.00	1.00	1.00	1.00	1.00	1.00
T2	0.31 (0.10-1.00)	0.28 (0.08-1.06)	0.40 (0.07-2.37)	0.75 (0.09-6.05)	0.15 (0.06-0.38)*	0.13 (0.04-0.38)*
T3	1.11 (0.63-5.13)	1.99 (0.69-5.07)	1.75 (0.40-3.88)	1.62 (0.29-3.49)	0.64 (0.24-1.68)	0.54 (0.17-1.73)
Retinol						
T1	1.00	1.00	1.00	1.00	1.00	1.00
T2	1.00 (0.31-3.28)	0.89 (0.24-3.31)	0.22 (0.05-1.09)	0.15 (0.02-1.01)	1.59 (0.64-3.93)	1.45 (0.54-3.88)
T3	0.82 (0.07-2.73)	0.77 (0.04-3.66)	0.80 (0.15-4.25)	0.73 (0.04-2.76)	0.38 (0.15-0.96)*	0.36 (0.13-1.04)

Table 3: Crude and adjusted odds ratio of nutrient intakes of participants (Continued)

	Males		Females		All	
Variables	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)	Crude OR (95% CI)	Adjusted OR (95% CI)
Vitamin C						
T1	1.00	1.00	1.00	1.00	1.00	1.00
T2	0.75 (0.24-2.39)	0.55 (0.15-2.01)	1.02 (0.46-8.78)	0.95 (0.68-3.76)	0.96 (0.38-2.41)	0.90 (0.33-2.44)
T3	0.28 (0.09-0.92)*	0.19 (0.05-0.73)*	0.24 (0.04-1.53)	0.35 (0.05-2.68)	0.32 (0.13-0.78)*	0.28 (0.11-0.77)*
Vitamin D						
T1	1.00	1.00	1.00	1.00	1.00	1.00
T2	0.15 (0.04-0.59)*	0.11 (0.02-0.60)*	0.47 (0.28-1.63)	0.96 (0.13-7.17)	0.21 (0.08-0.54)*	0.17 (0.06-0.51)*
T3	0.43 (0.13-1.42)	0.34 (0.09-1.35)	0.40 (0.89-2.78)	0.33 (0.71-4.11)	0.45 (0.18-1.13)	0.31 (0.11-0.89)*
Vitamin E						
T1	1.00	1.00	1.00	1.00	1.00	1.00
T2	0.29 (0.39-4.24)	0.46 (0.40-5.30)	0.36 (0.08-1.64)	0.51 (0.10-2.58)	0.63 (0.35-4.07)	0.41 (0.07-5.33)
T3	0.20 (0.06-0.69)	0.23 (0.07-0.84)	0.15 (0.03-0.85)	0.23 (0.03-1.76)	0.23 (0.09-0.61)*	0.33 (0.12-0.92)*
Folate						
T1	1.00	1.00	1.00	1.00	1.00	1.00
T2	0.38 (0.10-1.46)	0.19 (0.04-0.99)	0.88 (0.20-3.76)	2.37 (0.30-18.74)	0.40 (0.14-1.17)	0.32 (0.10-1.08)
T3	0.21 (0.06-0.70)	0.14 (0.03-0.60)	0.27 (0.05-1.42)	0.32 (0.05-2.00)	0.67 (0.30-1.53)	0.63 (0.26-1.53)
Minerals						
Calcium						
T1	1.00	1.00	1.00	1.00	1.00	1.00
T2	3.43 (1.03-11.45)	3.14 (0.83-11.91)	2.00 (0.46-8.78)	1.86 (0.38-9.08)	1.43 (0.58-3.48)	1.16 (0.44-3.10)
T3	0.77 (0.25-2.93)	0.67 (0.18-2.43)	0.22 (0.04-1.39)	0.33 (0.04-2.65)	0.59 (0.24-1.46)	0.49 (0.17-1.42)
Iron						
T1	1.00	1.00	1.00	1.00	1.00	1.00
T2	0.42 (0.17-2.98)	1.00 (0.75-3.18)	0.60 (0.12-3.90)*	0.72 (0.09-6.82)*	0.88 (0.24-3.41)	0.86 (0.31-4.82)
T3	0.41 (0.13-1.29)	0.65 (0.17-2.48)	0.37 (0.27-6.87)	0.73 (0.40-4.68)	0.96 (0.38-5.66)	1.00 (0.68-3.91)
Other						
Crude fibre						
T1	1.00	1.00	1.00	1.00	1.00	1.00
T2	1.14 (0.34-3.86)	1.11 (0.28-4.45)	0.29 (0.04-1.98)	0.06 (0.03-1.12)	0.21 (0.08-0.55)*	0.19 (0.06-0.57)*
T3	0.19 (0.06-0.65)*	0.15 (0.04-0.62)*	0.17 (0.03-1.09)	0.07 (0.01-0.95)*	0.14 (0.05-0.40)*	0.20 (0.04-0.36)*

*p<0.05

study of 88,757 women (Fuchs *et al*., 1999) for example, also did not find any significant protective effect of dietary fibre on CRA in women.

Anthropometric measurements: The mean BMI was slightly higher among the case subjects as compared to the controls, regardless of the gender (Table 4). The majority of the participants fell in the overweight and obese category. A study using a larger number of samples found that men in the upper quartiles of BMI have higher risk of recurrent adenomas, but there was no effect on women (Davidow *et al*., 1996). Yet, Sass *et al*. (2004) found no relationship between weight and BMI and recurrence of adenomas. Despite this, Chung *et al*. (2006) found that higher BMI was strongly associated with increased risk of advanced adenoma (OR = 10.8, 95% CI = 4.6-25.3). Incidence of colorectal polyps was also found to be related to obesity (Tashiro *et al*., 2004). The cross-sectional study found that 51% of obese patients have polyps as compared to 29% in non-obese. While the mean waist circumference suggested the case subjects to have higher mean than the controls, only a minority of them had abdominal obesity (≥102 cm for men and ≥88 cm for women). Almendingen *et al*. (2001) proposed that high body fatness is a promoter of adenoma growth. This is based on their findings where increasing percentage of body fat (p = 0.02) and BMI (p = 0.006) highly associated with adenomas growth.

Although the mean percentages of body fat were almost equal between groups, a majority of the male respondents had high percentage of body fat though this pattern was not seen in the female groups. The percentage of body fat also found to be insignificantly different between study group, although there were other studies that found otherwise (Giovannucci *et al*., 1996; Morimoto *et al*., 2002). A study found that Japanese patients with CRA showed significantly more visceral fat area in comparison with the controls [OR = 2.19; 95% CI = 1.47-3.28). These results suggest an association of visceral fat accumulation (Otake *et al*., 2005).

Lipid profile: The mean total cholesterol and LDL-C were found to be in undesirable range in men, while mean triglyceride, total cholesterol and LDL-C were in the undesirable range in women (Table 5). There were significant differences in the plasma HDL-C in females, with cases having lower mean HDL-C compared to the controls. Conversely, mean plasma LDL-C was higher among female cases than controls.

A study by Bayerdorffer *et al*. (1993) supported these findings, where the authors concluded that patients with CRA have lower HDL cholesterol levels and higher LDL

Table 4: Anthropometrical measurements of the participants

Variables	Males		Females		All	
	Case (n = 42)	Control (n = 33)	Case (n = 17)	Control (n = 26)	Case (n = 59)	Control (n = 59)
Body Mass Index (kg/m^2)						
Underweight	1 (2.5)	3 (9.1)	1 (6.3)	0 (0.0)	2 (3.4)	3 (5.1)
Normal	19 (46.3)	15 (42.5)	9 (56.3)	12 (46.2)	28 (47.5)	27 (45.8)
Overweight	20 (48.8)	13 (39.4)	5 (31.3)	7 (26.9)	25 (42.4)	20 (33.9)
Obese	2 (4.9)	2 (6.1)	1 (6.3)	7 (26.9)	3 (5.1)	9 (15.3)
Mean±SD	25.1±3.1	24.4±3.8	25.6±4.9	23.9±3.7	24.8±4.5	24.9±4.3
Waist circumference (cm)						
Normal	38 (90.5)	30 (90.9)	8 (47.2)	19 (73.1)	46 (78.0)	49 (83.1)
High risk	4 (9.5)	3 (9.1)	9 (52.9)	7 (26.9)	13 (22.0)	10 (16.9)
Mean±SD	90.3±9.4	88.3±9.3	84.3±9.3	82.5±12.9	88.6±9.7	85.6±11.8
Percentage of body fat (%)						
Low/normal	6 (14.3)	3 (9.1)	5 (29.4)	9 (34.6)	11 (18.6)	12 (20.3)
Moderate	4 (9.5)	6 (18.2)	6 (35.3)	8 (30.8)	10 (16.9)	14 (23.7)
High	32 (76.2)	24 (72.7)	6 (35.3)	9 (34.6)	38 (64.4)	32 (54.2)
Mean±SD	27.1±5.4	27.4±6.5	33.5±5.2	33.0±5.6	28.9±6.1	30.1±6.7

Table 5: Lipid profile of the participants

Variables	Males		Females		All	
	Case (n = 42)	Control (n = 33)	Case (n = 17)	Control (n = 26)	Case (n = 59)	Control (n = 59)
Lipid profile						
Triglycerides (mmol/L)						
Desirable (<1.73)	12 (28.6)	13 (39.4)	6 (35.3)	11 (42.3)	18 (30.5)	24 (40.7)
Borderline (1.73-2.29)	20 (47.6)	11 (33.3)	3 (17.6)	8 (30.8)	23 (39.0)	19 (32.2)
Increased risk (>2.29)	10 (23.8)	9 (27.3)	8 (47.1)	7 (26.9)	18 (30.5)	16 (27.1)
Mean±D	2.0±1.1	2.3±1.1	2.6±2.1	2.2±1.1	2.2±1.5	2.3±1.1
Total cholesterol (mmol/L)						
Desirable (<5.2)	10 (23.8)	0 (0.0)	4 (23.5)	0 (0.0)	15 (25.4)	0 (0.0)
Borderline (5.2-6.19)	2 (4.8)	0 (0.0)	0 (0.0)	1 (3.8)	2 (3.4)	1 (1.7)
Increased risk (>6.2)	30 (71.4)	33 (100.0)	13 (76.5)	25 (96.2)	43 (72.9)	58 (98.3)
Mean±SD	8.4±1.7	6.8±2.9	8.9±2.1	7.5±2.9	8.6±1.8$^{\varphi}$	7.2±2.9$^{\varphi}$
HDL (mmol/L)						
Desirable (>1.6)	32 (76.2)	31 (93.9)	13 (76.5)	24 (92.3)	45 (76.3)	55 (93.2)
Borderline (1.03-1.59)	3 (7.1)	1 (3.0)	1 (5.9)	2 (7.7)	4 (6.8)	3 (5.1)
Increased risk (<1.59)	7 (16.7)	1 (3.0)	3 (17.6)	0 (0.0)	10 (16.9)	1 (1.7)
Mean±SD	3.1±1.1	2.0±0.9	2.2±0.9$^{\varphi}$	3.7±1.6$^{\varphi}$	2.1±0.9$^{\varphi}$	3.3±1.3$^{\varphi}$
LDL (mmol/L)						
Desirable (<3.4)	14 (33.3)	0 (0.0)	5 (29.4)	0 (0.0)	19 (32.2)	0 (0.0)
Borderline (3.4-4.09)	6 (14.3)	0 (0.0)	1 (5.9)	2 (7.7)	7 (11.9)	2 (3.4)
Increased risk (>4.1)	22 (52.4)	33 (100.0)	11 (64.7)	24 (92.3)	33 (55.9)	57 (58.7)
Mean±SD	7.1±2.1	4.6±1.9	7.5±3.1$^{\varphi}$	4.2±1.9$^{\varphi}$	7.2±2.4$^{\varphi}$	4.1±1.9$^{\varphi}$

$^{\varphi}p<0.01$

levels; these lipoproteins may have prognostic significance for the development of CRA. Another study by Park *et al.* in the year 2000 found significant association between total cholesterol and risk for CRA, but no conclusive findings in relationship with HDL-C and LDL-C. In this case-control study, significant relationship was found between total cholesterol and LDL with risk for CRA, but there HDL was not found to influence the risk. Otani *et al.* (2006) suggested that a higher serum triglyceride level may be related to a larger number of adenomas. Adenoma development involving an elevated serum triglyceride level may be modified by smoking as higher prevalence of CRA was found in smokers.

Conclusion and recommendations: Our results support the notion that nutritional status and factors associated with it are of importance for the development of these pre-cancerous lesions. Identification of risk factors through this case-control study should be able to supplement the available data in order to develop an intervention package that focuses on multiple risk factors to reduce the chances for developing CRA or CRC.

The sample size for this study is rather small and the fact that it focuses on subjects in the Klang Valley may limit the extrapolation of these findings to the entire Malaysian population. Thus, a larger study and possibly a prospective cohort study which incorporates study subjects from various places in Malaysia may be a better option to identify if these risk factors are applicable to Malaysians in other parts of the country.

The possibility that the associations may be confounded or modified by other genetic or dietary factors could not be excluded. The cases and controls have not been matched by age, which may affect the results in our study. However, the controls were recruited from the same population as the adenoma cases. Further, our controls have been screened and found polyp free by colonoscopy and the risk of any of them having colon cancer at the time of inclusion is not very likely.

Risk factors which have potential to be modified such as smoking habit, drinking habit, lower intake of fruits and vegetables should be intervened. An intervention study focusing on behavioural change may be able to improve one's risk for colorectal adenomas, thus subsequently reducing his/her risk for developing colorectal cancer in the future.

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Effect of Chemical Preservatives on the Shelf Life of Bread at Various Temperatures

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Abstract: The study was conducted to evaluate the "effect of chemical preservatives on the shelf life of bread at various temperatures" at the Institute of Food Sciences and Technology, Sindh Agriculture University, Tandojam, in 2008. Two chemical preservatives i.e. calcium propionate (CP) and calcium lactate (CL) at (14±1), (22±1) and ambient temperature (32±2°C) were assessed for their effect on keeping quality of bread. The results conclude that effects of CP and CL at 14, 22 and (32±2°C) were equally significant at ($p<0.01$) for all chemical characteristics of bread. CP was more effective than CL such as moisture, protein, ash, TSS and carbohydrates were found 37.75 and 36, 9.24 and 8.75, 1.28 and 1.24, 5.10 and 4.71 and 47.75 and 47.01% with CP and CL respectively. Among the storage temperatures, 14°C demonstrate superior performance by increasing shelf life up to a maximum level i.e. 370 h/15.44 days followed by 22°C for 197 h/8.21 days and (32±2°C) for 123 h/5.12 days in decreasing order.

Key words: Bread, calcium propionate, calcium lactate

INTRODUCTION

Bread is the most important article of food. History reveals its use thousands of years even before the Christian era. Despite its importance as a food item, bread making on scientific lines has received due studies for quality and shelf life in the country. Bread is made from wheat flour or other cereals by addition of water, salt and ferment (Yeast). Wheat flour is best adopted for bread making, as it contains gluten in the right proportion to make the spongy loaf. Being deficient in fat, wheat bread is a preferred food. Bread has played a key role in the development of mankind and is one of the principal sources of nutrition. The history of bread is lengthy and largely obscure. Clearly, bread was being consumed long before recorded history. The quality of bread making is contingent upon availability of cereal flours to the consumer in an attractive, palatable and digestive form (Ammirati, 1999). People all over the world eat bread as it provides energy and protein. To make bread one has to bake dough that consists of flour or grain meal. The flour or grain meal is mixed with water or milk. The people in many Western countries mainly bake loaves or rolls made with wheat flour. However, some people prefer to eat crispy and thin sheets of bread called flat bread in many parts of the world. Flat bread is made from grains like barley, corn, oats, rice, rye, wheat, or flour made from combination of these grains. Flat bread does not have yeast or other leavening agents to make it rise. Some people make bread by hand. In commercial bakeries bread is made by machine (Hillers, 1993).

Making of bread has come down through the ages from the simplest methods to the more elaborate processes of the present day. It has been noted that bread prepared by adding baking powder has a very short shelf life. As it contains water after preparation which become the cause of fungal infestation. To extend shelf life of bread there are some chemicals which are being indiscriminately added to prolong the shelf life of bread. Bread should be stored with original package at room temperature and be used within 2-3 days. However, bread stored in the refrigerator will have a longer shelf-life due to delayed mold growth. Whole wheat flour may be stored in the refrigerator or freezer to retard rancidity of the natural oils (Trager and James, 1995). Shelf life extension of bread may be obtained using a single ingredient or process change or a combination of many alternative changes according to food legislation, ingredient availability and cost, consumer acceptance and social trends (Cauvain and Young, 2000). Deterioration of bread includes staling, moisture loss or gain and microbial spoilage. The most common source of microbial spoilage is mould growth (Pyler, 1988; Legan and Voisey, 1991). Viable vegetative moulds and mould spores are destroyed by the heat of the baking process and their subsequent thermal inactivation. However, post baking contamination occurs from the mould spores present in the atmosphere, during handling operations such as cooling, finishing and wrapping (Doerry, 1995; Yang, 1998). Besides mould, bacterial and yeast spoilage can also occur (Grundy, 1996; Pateras, 1999). Thus, it is evident that shelf life

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prolongation of bread is of great importance since it is related to the maintenance of fresh-keeping properties of bread and the productivity and profitability of a company.

Several methods of bread preservation are mentioned in the relevant literature including mould inhibitors (propionates, sorbates, dimethyl fumarate, acetates and ethanol), modified atmosphere packaging, pasteurization, infrared and ultraviolet irradiation, freezing etc. (Islam, 1982; Pyler, 1988; Matz, 1989a,b; Legan, 1993). Selection of appropriate ingredients and adjustment of their levels in a bread recipe is a powerful tool which leads to a significant inhibition of bread mould or microbial growth and therefore, extension of bread Mould-free Shelf Life (MFSL) can be achieved (Cauvain and Young, 2000; Doulia *et al*., 2000a). Therefore, the manipulation of the levels of ingredients used in a bread recipe affects the shelf life. Sucrose is a very important ingredient in bread recipes because it is very effective in binding moisture as well as acting as an anti-staling agent. Doerry (1995) reported that salt on the other hand has powerful water-binding properties because of its ionic structure. Even a relatively small quantity of salt could affect significantly the MFSL. However, there is a limit to the salt's quantity because of its strong effect on flavour, its negative effects on processing (e.g. changes in viscoelastic properties of gluten and inhibition of yeast in bread dough) and the social trend to unsalted or low-salted foods for potential health benefits. The amount of salt is self-limiting in the range of 1.5-2.5% of the total flour weight.

The use of preservatives in bread is common and extensive all over the globe because of their effectiveness in preventing or inhibiting microbial spoilage in general and mould growth in particular (Doerry, 1995). Preservatives do not significantly affect water activity and their action depends on the product's pH, product's composition, storage temperature and water activity (Davidson, 2002). Potassium sorbate and calcium propionate are among the principal mould inhibitors used in bread and their inhibitory action has been extensively studied (Legan, 1993; Grundy, 1996). Potassium sorbate is effective up to pH 6. At higher pH levels, its effectiveness decreases significantly (Sofos, 2000). Calcium propionate is the most commonly used chemical antimicrobial and it is ideal for yeast-leavened bakery foods as it is most effective at pH levels below 5.5. It is also very effective against spoilage caused by rope spores from bacteria *Bacillus subtilis*, surviving the baking process. The recommended level is within 0.19-0.32 g per 100 g flour. At higher application levels, calcium propionate imparts a distinct acid taste to bread (Doerry, 1995). Ethanol, a strong bactericide, has recently been used for its effective preservative action in bread (Matz, 1989a,b; Cauvain and Young, 2000). The addition of ethanol at levels 0.5% and 3.5% of loaf leads to a substantial extension of the shelf life of bread (Doulia *et al*., 2000b).

MATERIALS AND METHODS

The present study was carried out to investigate the effect two different types of chemical preservatives on the shelf life of bread at various temperatures. The types of chemicals calcium propionate (Merck Germany) and calcium lactate (Merck Germany) procured from the local market of Hyderabad were used. They were added as an ingredient to create wheat flour at the time of dough preparation and development. The study was carried out in Bakery Technology Laboratory of Institute of Food Sciences and Technology, Sindh Agriculture University, Tandojam during 2008. The details of treatments are given are as under:

- Preparation of dough with addition of Calcium propionate at the rate of 0.4 g kg^{-1} of flour
- Preparation of dough with calcium propionate at the rate of 0.8 g kg^{-1} of flour
- Preparation of dough with addition of calcium lactate at the rate of 0.4 g kg^{-1} of flour
- Preparation of dough with addition of calcium lactate at the rate of 0.8 g kg^{-1} of flour
- Preparation of dough without addition of any chemical preservative (control)

The separate batches of dough were prepared by adding two types of chemical preservatives and breads were baked on the same day to evaluate their effect on the quality and storage period. The Sensory parameters were evaluated by panel of judges by scoring quality on score card for each parameter. The following sensory parameters were recorded.

Determination of moisture: The moisture percentage of bread was determined according to the method of AOAC. The sterilized empty flat bottomed dish was weighed on balance machine and weight was recorded. Fifteen grams sample in dish was placed in an oven at 70°C for 24 h then dish was removed and cooled in a desicator and weight was recorded. Sample was placed again in oven at 70°C for another two hours. The moisture % was calculated according to the following formula:

$$\text{Moisture \%age} = \frac{\text{Weight of fresh sample} - \text{Weight of dry sample}}{\text{Weight of fresh sample}} \times 100$$

Determination of total ash: The Ash percentage was determined by using gravimetric techniques according to the method of AOAC (2000c).

Determination of protein: The protein percentage was determined by using method of British Standard

Table 1: Effect of different chemical preservative on the moisture, protein, ash, TSS, carbohydrate content of bread

Chemical preservatives	Moisture	Protein	Ash	TSS	Carbohydrates
Control	36.50[b]	7.42[e]	1.14[d]	4.46[c]	43.44[d]
Calcium propionate 0.4 g	33.25[d]	8.34[c]	1.14[d]	4.30[d]	45.40[c]
Calcium lactate 0.4 g	37.75[a]	8.19[d]	1.24[b]	4.35[e]	45.27[c]
Calcium propionate 0.8 g	36.00[b]	9.24[a]	1.18[c]	4.71[b]	47.01[b]
Calcium lactate 0.8 g	35.25[c]	8.75[b]	1.28[a]	5.10[a]	47.45[a]
SE	0.1670	0.0274	0.0071	0.0400	0.0640
LSD AT 1%	0.7215	0.1183	0.0306	0.1728	0.2766
LSD AT 5%	0.5147	0.0844	0.0218	0.1233	0.1973

Table 2: Effect of chemical preservatives on the cold storage (14±1°C) of bread (h/days)

	Storage (Hours/Days)				
Chemical preservatives	R1	R2	R3	R4	Mean
Control	264.00 (11.00 days)	270.00 (11.25 days)	274.00 (11.41 days)	268.00 (11.16 days)	269.00[e] (11.21 days)
Calcium propionate 0.4 g	336.00 (14.00 days)	330.00 (13.75 days)	345.00 (14.37 days)	342.00 (14.25 days)	338.00[c] (14.09 days)
Calcium lactate 0.4 g	312.00 (13 days)	318.00 (13.25 days)	320.00 (13.33 days)	325.00 (13.54 days)	318.00[d] (13.28 days)
Calcium propionate 0.8 g	360.00 (15.00 days)	368.00 (15.33 days)	372.00 (15.56 days)	381.00 (15.87 days)	370.00[a] (15.44 days)
Calcium lactate 0.8 g	359.00 (14.95 days)	363.00 (15.12 days)	367.00 (15.29 days)	15.41 days)	364.00[b] (15.19 days)

SE = 0.8597; LSD at 1% = 3.714; LSD at 5% = 2.649

Institution (BSI), 1990 prepared with two types of chemical preservatives. The small bread samples were digested using micro kejldhal digester in the presence of Catalyst (0.2 g) copper sulphate and 2 g sodium sulfate/potassium sulfate or catalyst tablets and sulfuric acid (30 ml). They were used as an oxidizing agent. The digested samples were diluted with distilled water (250 ml) and 5 ml portion from the diluted samples were mixed with NaOH (40%) using micro-Kjeldhal distillation unit. Where steam was distilled over 2% boric acid (5 ml) containing an indicator for 3 min.

The ammonia trapped in boric acid was determined by titrating with 0.1 N HCl. The nitrogen percentage was calculated using the following formula:

$$N\% = \frac{1.4(V1 - V2)\,x\,\text{normality})}{\text{Wt of sample}}$$

While protein percentage was estimated by conversion of nitrogen percentage to protein, assuming that all the nitrogen in bread was present as protein i.e

Protein % = N% x Conversion factor

Where:

Conversion factor = 100/N (N% in fruit products) as suggested by Trager and James (1995).

Determination of carbohydrates (Anthrone method): Carbohydrates were determined by the method of Bajaracharya (1999).

RESULTS AND DISCUSSION

The data collected on moisture content of bread prepared with different chemical preservatives indicated that highest moisture percentage of 37.75 was recorded in the bread prepared by CP 0.8 g concentration followed by the bread prepared under control treatment where percent moisture was recorded as 36.50. Whereas the lower moisture percentage was recorded as 33.25 in the bread prepared with CL at concentration of 0.4 g. The data showed high protein content with CP at 0.8 g concentration and was recorded 9.24 percent on an average. The bread prepared with CL at 0.8 g concentration ranked second in protein content of 8.73 percent. The lowest protein content of 7.42 was noted in bread prepared in control treatment. CP and CL at 0.4 g ranked 3rd and 4th in protein content of bread as values were recorded 8.34 and 8.19% respectively (Table 1-4). Results at Table 1-4 further indicate highest ash content of 1.28 in bread prepared with CL at 0.8 g. The bread prepared with CL 0.4 g concentration ranked second in ash content of 1.24. The lowest ash content of 1.14 was noted in bread prepared in control treatment. CP at 0.4 and 0.8 g ranked 3rd and 4th in ash content of bread, with values of 1.14 and 1.18 respectively. Besides, it was further observed that the bread prepared with CL at concentration of 0.8 g recorded higher values of 5.10 for Total Soluble Solids of bread as compared to other chemical preservatives. CP at 0.8 g concentration ranked second in total soluble solids of bread with scores of 4.71. CP and CL at 0.4g concentration resulted in lower scores of 4.30 and 4.35 respectively. The data showed highest carbohydrate content of 47.45 percent

Table 3: Effect of chemical preservatives on the cold storage (22±1°C) of bread (h/days)

	Storage (Hours/Days)				
Chemical preservatives	R1	R2	R3	R4	Mean
Control	160.00	167.00	172.00	166.00	166.25[d]
	(6.66 days)	(6.95 days)	(7.16 days)	(6.91 days)	(6.92 days)
Calcium propionate 0.4 g	192.00	188.00	186.00	190.00	189.00[c]
	(8.00 days)	(7.83 days)	(7.75 days)	(7.91 days)	(7.87 days)
Calcium lactate 0.4 g	187.00	189.00	188.00	186.00	187.50[b]
	(7.79 days)	(7.87 days)	(7.83 days)	(7.75 days)	(7.81 days)
Calcium propionate 0.8 g	200.00	194.00	198.00	196.00	197.00[a]
	(8.33 days)	(8.08 days)	(8.25 days)	(8.16 days)	(8.21 days)
Calcium lactate 0.8 g	196.00	194.00	190.00	197.00	194.25[ab]
	(8.16 days)	(8.08 days)	(7.91 days)	(8.20 days)	(8.09 days)

SE = 0.7781; LSD at 1% = 3.361; LSD at 5% = 2.397

Table 4: Effect of chemical preservatives at room temperature (h/days)

	Storage (Hours/Days)				
Chemical preservatives	R1	R2	R3	R4	Mean
Control	78.00	96.00	82.00	84.00	85.00[d]
	(3.25 days)	(4.00 days)	(3.41 days)	(3.50 days)	(3.54 days)
Calcium propionate 0.4 g	115.00	110.00	124.00	112.00	115.25[b]
	(4.79 days)	(4.58 days)	(5.16 days)	(4.66 days)	(4.80 days)
Calcium lactate 0.4 g	96.00	104.00	100.00	108.00	102.00[c]
	(4.00 days)	(4.33 days)	(4.16 days)	(4.50 days)	(4.25 days)
Calcium propionate 0.8 g	120.00	126.00	118.00	128.00	123.00[a]
	(5.00 days)	(5.25 days)	(4.91 days)	(5.33 days)	(5.12 days)
Calcium lactate 0.8 g	118.00	110.00	126.00	120.00	118.50[ab]
	(4.91 days)	(4.58 days)	(5.25 days)	(5.00 days)	(4.94 days)

SE = 1.436; LSD at 1% = 6.205; LSD at 5% = 4.426

in bread prepared with CL 0.8 g. The bread prepared with CP 0.8 g concentration ranked second in carbohydrate content i.e. 47.01 percent. The lowest carbohydrate content was recorded 43.44 percent in bread prepared as control. CP and CL at 0.4 g ranked 3rd and 4th in carbohydrate content of bread, where the recorded values were 45.40 and 45.27 respectively.

The data illustrates that the bread prepared with CP at 04. g, 0.8g and CL at 0.8 g concentration were not significantly different from each other but there was a significant difference in storage period of bread prepared with CL at concentration of 0.4 g and under control treatment. The longer storage period 370 h (15.41 days) was observed in bread prepared with CP at 0.8 g concentration, which was followed by 364 h (15.16) with CL 0.8 g. The lowest shelf life of 269 h (11.20) was recorded in bread prepared as control. The data further indicated that the bread prepared with CP at 0.8 g and CL at 0.8 g concentration were highly different from each other but there was less significant difference in CP at concentration of 0.4 g and calcium lactate 0.4 g. The longer shelf life of 197 h (8.20 days) was recorded in bread prepared with CP at 0.8 g concentration and the second shelf longer life 194.25 h (8.09 days) was recorded in bread prepared with CL 0.8 g. The lowest shelf life of 166.25 h (6.92 days) was recorded in bread prepared in bread labeled as control treatment. The data further elaborates that the bread prepared with CP at 0.8 g and CL at 0.8 g concentration recorded 117.02 h (4.87 days) and 116 h (4.83 days) respectively, as shelf life at the room temperature. Whereas the bread prepared with the addition of CP 0.4 g and CL 0.4, the shelf life observed was 123 h (5.12days) and 118.50 h (4.93 days) respectively. The lowest mean value 85 h (3.54 days) was recorded in control.

Conclusion: From the research, it may be concluded that fortification of CP and CL at 0.8 g under 14°C cold storage of bread showed better quality of bread with improved physico-chemical properties and extensive shelf life.

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Nutritional Evaluation of Bee Wax Residue Meal in the Diet of Lactating Goat

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Abstract: Nine West African dwarf goats were used to investigate the effect of bee wax residue meal on the lactation performance, feed intake and weight gain. Goats were fed a diet with or without bee wax residue meal in a completely randomized design model for a 156 day period. The three diets were A (control), B (1%) bee wax residue meal inclusion) C (3% bee wax residue meal inclusion). Water and feeding were given *ad libitum*. Supplementing the diet with bee wax residue meal increased the crude protein intake from 47 g^{-d} (diet A) to 133g^{-d} (Diet C). The dry matter intake was greater for diet C followed by diet B and C which are similar ($p>0.05$). Animal on diet C consumed the highest percentage of minerals. Milk yield was significantly increased ($p<0.05$) by bee wax supplementation [407g^{-d}] (A), [412g^{-d}] (B) and [446g^{-d}] (C). Supplementation also increased butter fat, protein, calcium and phosphorus contents. The potassium sodium and iron contents were significantly ($p<0.05$) highest in diet C followed closely by diets B and A (control) which are similar ($p>0.05$). It could be concluded that bee wax residue meal could be used to supplement lactating West African goats diet in the tropical environment.

Key words: West African dwarf goats, milk production, bee wax residue meal

INTRODUCTION

Bee wax residue is usually obtained after the honey has been extracted from the comb and wax has been removed as well as the shaft that is thrown away (Monmouth, 1986). The residue from wax rendering contains sufficient nutrient and could be used as poultry feed or tuned into good compost (Faruga *et al*., 1975) Bee wax was considered safe for human consumption and was approved as an ingredient in human food in the USA (USA, 1978). The wax has also been used as a separate agent in the confectionary industries (Ribot, 1960).

Several methods of rendering wax are possible and it may be adapted to numerous circumstances. Wax may be separated in solar wax melters (2) by boiling in water and later filter (3) by using steam or boiling water and special presses. If rain water or soft water is not available, hard water of high calcium content could be used with the addition of 0.1% vinegar (Crane, 1980). Wax should not be heated above 85°C else discolouration occurs. Excessive heating during rendering or further processing changes the wax structurally and alters the beneficial characteristics of many of its minor compounds as well as its aromatics and volatile compounds (Crane, 1980). Direct exposure of wax to hot steam results in partial saponification (Graham, 1992)

Bee wax is needed for the production of artificial combs as well as for musical instruments, coloured crayons and paints. It is vital ingredient in polishing and furniture wax, shoe polish, car wax and lubricants and metal polish. The thrust of this study was to evaluate the efficacy of bee wax residue meal on the performance characteristics of lactating West African dwarf goats.

MATERIALS AND METHODS

Study area: The experiment was conducted between September and October 2007 at the Animal Pavilion of the Department of Animal Production, University of Ilorin, Nigeria.

Preparation of bee wax residue meal: The processing was done at the Teaching and Research Farm, University of Ilorin, Nigeria using the hot water extraction method thus: The wax was obtained by breaking the comb into pieces and soaked in water for 24 h. The comb was then tied in a bligated sack and boiled in a container fill of water while the wax floats on the surface. It was left overnight to cool and a round cake solid of bee wax was formed afterwards.

The waste was collected and dried in the oven at about 60°C later milled and mixed with other ingredients (Table 2).

Experimental diets and management of the animals: Three experimental diets (A, B and C) were prepared with diet A being the Control while bee wax residue meal was included at one and three percentages in diets B and C respectively.

Eighteen West African dwarf goats used for the experiment were kept in a well cleaned, disinfected and well ventilated pens for 180 days (i.e. 150 days

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pregnancy and 30 days milk collection period). The animals were fed and water *ad-libitum* throughout the experimental period.

Milk collection: Milk collection started 5 days after the kids were allowed to take the colostrums. The animal was hand milked for about 5-7 min daily by expressing the milk from the udder of the animal into a clean bowl. The milk was immediately taken to the laboratory for weighing to record the daily milk yield.

Analysis: The chemical composition of the milk was determined by the method of AOAC (1990) while the fat was determined by using Gerber's method.

All data collected were subjected to analysis of variance of a completely randomized design model (Steel and Torrie, 1960) while means were separated using the method of Duncan (1955) multiple range test.

RESULTS AND DISCUSSION

Table 1 showed the proximate composition of the bee wax residue used in this study. The crude protein content (23.07%) of the bee wax residue was higher than the crude protein of most grasses and leguminous crop. Additionally, the bee wax residue meal was higher in dry matter and ether extract. The higher ether extract could be an added advantage in the fat content of the milk.

The proximate composition of the experimental diets (Table 2) revealed increasing percentage of most of the parameters studied in diets B and C compared to the control (diet A). The crude protein content increased by 1.4 or 2.6 times in diets B and C than the control (diet A). Compared with the control (diet A), the animals showed a marked consumption of dry matter, crude protein, ether extract, calcium, phosphorus, potassium and iron in diet A (Control). A moderate consumption of the aforementioned parameters was recorded for animal on diet B (1% bee wax residue inclusion) and the least for the control (diet A).

Contrarily, poor crude fibre intake was recorded for diet A. The increase crude protein content of the diet agreed with the work of Adegbola (1974) that intake of crude protein increased with the level of dietary protein due to the attempt of the animal to satisfy their protein requirement.

The highest milk yield recorded for diet C could be due probably to the highest crude protein and fat intake of the diet. This confirmed the assertion of Broster *et al.* (1969) that good nutrition improves milk production. A progressive increase ($p<0.05$) in the fat content as well as an increase in the protein content of the diet were exhibited as the levels of the protein and fat of the milk were enhanced. The increase of the protein and fat in the milk produced by goats on diets B and C must be regarded as a direct effect of feeding and the positive

Table 1: Composition of the experimental diets

	Experimental diets		
Ingredients (%)	Diet A	Diet B	Diet C
Cassava waste	54.00	53.00	52.00
Soybean meal	2.00	2.00	2.00
SBDG	23.00	23.00	23.00
Rice husk	20.00	20.00	20.00
Bee wax residue			
Meal	0.00	1.00	2.00
Salt	1.00	1.00	1.00

Table 2: Proximate composition of bee wax residue meal and the experimental diets

		Experimental diets		
Parameters (%)	Bee wax residue meal	Control Diet A	Diet B	Diet C
Dry matter	83.44	96.29	94.6	98.5
Crude Protein	23.07	9.39	13.49	24.16
Crude fibre	2.4	23.86	19.21	11.04
Ether extract	42.19	8.43	8.77	8.66
Mineral content (g/kg)				
Calcium	0.14	0.22	0.22	0.25
Phosphorous	1.14	0.14	0.43	0.14
Potassium	0.83	0.73	0.74	0.71
Sodium	0.55	0.49	0.49	0.48
Iron	1.06	0.47	0.4	0.52

Table 3: Nutrient intake of the experimental diets

Parameters (%)	Control Diet A	Diet B	Diet C	±SEM
Dry matter	504.27^{a}	504.53^{a}	551.28^{b}	8.62*
Crude protein	47.35^{a}	68.06^{b}	132.82^{c}	1.42*
Ether extract	41.89^{a}	44.25^{a}	47.75^{b}	0.81*
Crude fibre	120.47^{c}	96.93^{b}	60.89^{a}	1.60*
Mineral content (g/kg)				
Calcium	1.09^{a}	1.22^{b}	1.38^{c}	0.04*
Sodium	2.47^{a}	2.47^{a}	2.65^{b}	0.06*
Potassium	3.68^{a}	3.71^{a}	3.92^{b}	0.06*
Phosphorus	0.70^{a}	2.17^{c}	0.77^{b}	0.02
Iron	2.37^{b}	2.02^{a}	2.87^{c}	0.04*

Means along the rows with similar super scripts are not significant at $p<0.05$

Table 4: Milk quality and quantity of the experimental diets

	Experimental diets			
Parameters	Control Diet A	Diet B	Diet C	±SEM
Milk yield (g/d)	407.33^{a}	412.24^{b}	446.84^{c}	0.08*
Total solids (%)	16.10^{b}	14.33^{a}	16.91^{c}	o.81*
Solids not Fat	13.09^{c}	11.04^{b}	11.12^{a}	0.58*
Fat (%)	3.13^{a}	3.60^{b}	6.20^{c}	0.25*
Protein (%)	2.54^{a}	3.36^{a}	4.86^{b}	0.73*
Lactose (%)	4.44^{a}	3.72^{b}	3.10^{c}	0.03*
Mineral content (g/kg)				
Calcium	0.25^{a}	0.28^{b}	0.31^{c}	0.02*
Phosphorus	0.02^{a}	0.06^{b}	0.14^{c}	0.01*
Potassium	0.47^{a}	0.48^{a}	0.51^{b}	0.03*
Sodium	0.49^{a}	0.50^{a}	0.66^{b}	0.05*
Iron	0.19^{a}	0.17^{a}	1.31^{b}	0.02

Means along the rows with similar superscripts are not significant at $p>0.05$

effect of bee wax residues meal based diets in enhancing these two vital components for dairy industry since milk is purchased based on its fat and protein contents (Cerbulis and Farrel, 1975). The high fat content of milk reported herein will make the milk to command high premium level in the market (Maxine, 1988).

In addition, a positive effect of bee wax residue meal on the mineral content was noted (Calcium, phosphorus, potassium, sodium and iron). This could be due probably to increasing amount being utilized by the animals.

The moderate lactose content reported for animal on diets B and C might be ascribed to the small amount of dietary starch received by the animals on these diets. The assertion agreed with the reports of Succi and Sandrucci (1995) and Rotuno *et al.* (1998). Such moderate lactose content makes possible the milk to be consumed by many people that are lactose intolerance (Alm, 1982).

Conclusion and Implications:

- Bee wax residue meal based diets brought about significant variations in the fat and crude protein contents of the milk of West African dwarf goats
- A progressive increase in dry matter intake, crude protein intake, ether extract intake and considerable increase in the mineral intake were exhibited as the levels of bee wax residues was increased.
- The increased milk yield with high levels of milk protein and fat may be regarded as an index of the nutritional characteristics of the diets. In addition, there was no detrimental effect of the diet on the experimental animals.
- Therefore, these results indicate that the addition of bee wax residues meal in goat diet is effective in altering the composition of goat milk.

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Evaluation of Microbial Quality of Goat Meat at Local Market of Tando Jam

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Abstract: The research consist of the goat meat to investigate the relationship between goat meat in different age groups, group A (≤7 m), group B (8-10 m) and group C (≥11 m). The level of contamination of goat meat processed by butchers under local marketing conditions was investigated during 2008-9. A total of 21 goat meat samples were collected equally from three age groups each containing 7 samples Aerobic plate count, Coliform and yeasts and moulds counts enumerated from meat of group A ($3.8 \times 10^5 \pm 2.3 \times 10^4$, $1.8 \times 10^5 \pm 1.0 \times 10^4$ and $1.5 \times 10^3 \pm 2.2 \times 10^2$ cfug^{-1}, respectively) were not significantly different ($p>0.05$) from goat meat of group B ($3.3 \times 10^5 \pm 4.1 \times 10^4$, $1.7 \times 10^5 \pm 5.9 \times 10^4$, $1.4 \times 10^3 \pm 2.9 \times 10^2$ cfug^{-1}, respectively) and group C ($3.6 \times 10^5 \pm 2.4 \times 10^4$, $1.6 \times 10^5 \pm 1.7 \times 10^4$ and $1.5 \times 10^3 \pm 3.1 \times 10^2$ cfug^{-1}, respectively).The results conclude the meat of goat slaughtered in advanced age may have an extensive advantage to reduce qualitative and quantitative losses of end products; the fact of unhygienic and poor sanitary condition under which the goat meat was handled sold at local meat shops/stalls.

Key words: Goat meat, aerobic plate count, coliform counts, yeasts and molds count

INTRODUCTION

Meat is an important edible postmortem component originating from the live animals that are used as food by human. These animals include domesticated cow, buffalo, sheep, goat, camels and some wild animals i.e. dear, hog and rabbit. In addition poultry have become a major meat producing species, while various game animals and birds provide a substantial amount of meat particularly in localized areas. Fish and other sea foods have also important part of human diet since earliest time. However, cow, buffalo, sheep and goat are the main sources of red meat in Asia. Goat meat is without a doubt one of the staple red meat in human diet. Indeed goat meat is acceptable throughout the world but cultural and social tradition and economic condition often influence consumer preferences.

Goat is the animal of developing countries where more than 95% of goat population are reared indicating their economics importance and adaptation in the different agro-ecological zones of Asia and Africa (Chowdhury and Mutalib, 2003). The goat meat is popular in the Middle East, Africa and South Asia including Pakistan. The perception of consumers in the Western world is not in favor of goat meat; however, in Pakistan the meat consumption pattern is entirely different to those in developed countries, where majority of Pakistani consumers prefer goat meat. There is also a worldwide tendency for rapid increase in demand for goat meat (Stankov *et al*., 2002). Goat meat has an immense market potential, as it can become an ideal choice for health conscious consumers (Johnson *et al*., 1995; Carlucci *et al*., 1998). In recent time market of meat have been adapting to different requirements of contemporary consumers, insisting of lean and easily digestible meat of high quality and good test (Lesiak *et al*., 1997). Goat meat market and geographical pattern of consumption in sub-tropical and tropical developing countries are different. Goat meat for longer occupied a special place in the diet for variety of reason including test preference, prestige, religion, tradition and availability, in almost all the communities of the country with the nutritional aspect (Dahnda, 2001). Pakistan is the second largest goat producing country in South Asia region having 56.7 million goats contributing about 578, 000 tons of mutton (Anonymous, 2008-09). Goat production in Pakistan has expanded considerably over recent decades as a result population densities have also increased.

Limited studies on carcass microbiological quality of goat meat has appeared in literature (Babiker *et al*., 1990; Mahgoub and Lodge, 1996; Babji *et al*., 2000) and no studies have been reported so far on the same aspects of goat meat particularly in Sindh. Therefore keeping in view the importance of the subject, this study is designed to evaluate the selected microbial attributes of goat meat available in local market of Tando Jam.

MATERIALS AND METHODS

Collection of meat samples: A total of 21 goat meat samples were randomly collected from local meat market of Tando Jam. All the samples were grouped

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according to the age at slaughter as per butcher's information and accredited with A (≤7 m, age), B (8-10 m, age) and C (≥11 m, age) codes. Whereas boneless meat samples for microbiological analysis were collected in sterilized screw caped bottles and brought under refrigeration in ice box. All the samples were brought to Laboratory of department of Animal products Technology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tando Jam, for further analysis.

Preparation of test samples: Minced meat sample (10 g) was reconstituted aseptically with 90 ml of 0.1% peptone water (Oxoid England) in a laboratory blender (AOAC, 1990).

Enumeration of total viable count (Colony count technique at 30°C): Total viable counts were enumerated according to the method of (IDF, 1991). Pre prepared test sample (1 ml) of 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6} and/or 10^{-7} dilutions was transferred into sterile petri dishes in duplicate through sterile graduate pipette and/or dispensing pipette (1000 µl) with sterile plastic tips and warm (45±1°C) sterile plate count agar medium (15 ml) was mixed with inoculum. The mixture was allowed to solidify and incubated (30°C) for 72±2 h. Parallel to that, control plates were also prepared using similar medium (15 ml) to check the sterility. The dishes containing more than 30 and/or fewer than 300 colonies were selected and counted using colony counter. The result was calculated using following formula:

$$N = \frac{\sum c}{(n_1 + 0.1 \times n_2)\, d}$$

$\sum c$: Sum of colonies counted on all the dishes retained.
n_1 : Number of dishes retained in the first dilution.
n_2 : Number of dishes retained in the second dilution.
d : Dilution factor corresponding to the first dilution.

Enumeration of Coliform counts (Colony count technique at 37°C): Coliform counts were enumerated according to the method of British Standards Institution (BSI, 1993). Pre prepared test sample (1 ml) of 10^{-1}, 10^{-2}, 10^{-3} 10^{-4} and/or 10^{-5} dilution was transferred into sterile petri dishes through dispensing pipette (1000 µl) with sterile plastic tips and warm (45±1°C) sterile violet red bile agar medium (15 ml) was mixed with inoculum. The mixture was allowed to solidify and incubated (37°C) for 24±2 h. Parallel to that control plates were also prepared using similar medium (15 ml) to cheek its sterility. The dishes containing more than 10 and/or fewer than 200 colonies were selected and counted using colony counter. The result was calculated using formula as mentioned in section 3.11.

Enumeration of yeasts and moulds counts (Colony count technique at 25°C): Yeasts and moulds count were enumerated according to the method of IDF (1990). Pre prepared test sample (1 ml) of 10^{-1}, 10^{-2} and/or 10^{-3} dilution was transferred into sterile petri dishes through dispensing pipette (1000 µl) with sterile plastic tips and warm (45±1°C) sterile potato dextrose agar medium (15 ml) was mixed with inoculum. The mixture was allowed to solidify and incubated (25°C) for 5 days. Parallel to that control plates were also prepared using medium (15 ml) to cheek the sterility. The dishes containing more than 10 and/or fewer than 150 colonies were selected and counted using colony counter. The result was calculated using formula as mentioned in section 3.11.

Statistical analysis: Statistical analysis was performed using the computer programme i.e. Student Edition of Statistics (Sxw), version 8.1 (Copy right 2005, Analytical Software, USA).

RESULTS

Aerobic plate count: Goat meat samples of different age groups were analyzed and the results are summarized in Fig. 1. The mean concentration of Aerobic Plate Count (APC) detected from meat of group A, group B and group C goat were $3.8 \times 10^5 \pm 2.3 \times 10^4$, $3.3 \times 10^5 \pm 4.1 \times 10^4$ and $3.6 \times 10^5 \pm 2.4 \times 10^4$ cfug^{-1} (colony forming unit per gram), respectively and ranged between 2.8×10^5 to 4.6×10^5, 1.5×10^5 to 4.8×10^5 and 2.8×10^5 to 4.7×10^5 cfug^{-1} respectively. There are no significant differences ($p>0.05$) in aerobic plate counts detected from goat meat samples among different age groups of goat meat.

Coliform count: Coliform counts in goat meat of different age groups (group A, group B and group C), were examined and results are presented in Fig. 2. The coliform count varied from 1.5×10^5 to 2.3×10^5 cfug^{-1} (average, $1.8 \times 10^5 \pm 1.0 \times 10^4$ cfug^{-1}) in group A goat meat, while 1.6×10^5 to 2.1×10^5 cfug^{-1} (average, $1.7 \times 10^5 \pm 5.9 \times 10^4$ cfug^{-1}) in group B goat meat and 1.4×10^5 to 2.7×10^5 cfug^{-1} (average, $1.6 \times 10^5 \pm 1.7 \times 10^4$ cfug^{-1}) in group C goat meat. The overall mean of coliform count in goat meat computed in range between 1.4×10^5 and 2.7×10^5 cfug^{-1} (mean, $1.7 \times 10^5 \pm 6.9 \times 10^3$ cfug^{-1}). Further more the result shows that there is no significant ($p>0.05$) variation in the means of Coliform count found in the meat of different age groups of goat meat.

Yeasts and molds count: Three different age groups of goat meat were analyzed for yeasts and molds count, and results are presented in Fig. 3. The average yeasts and molds count varied between 1.0×10^3 to 2.6×10^3 cfug^{-1} (average $1.5 \times 10^3 \pm 2.2 \times 10^2$ cfug^{-1}) in group A goat

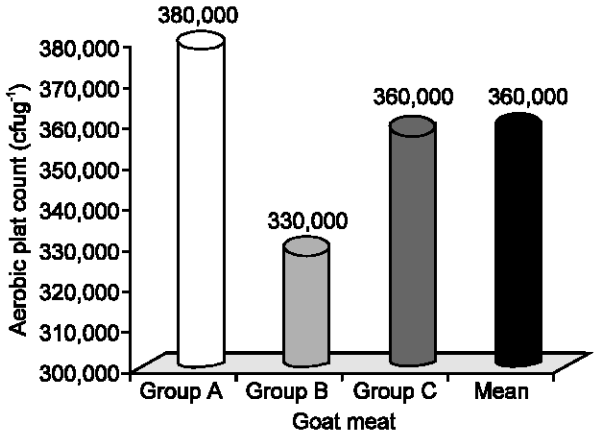

Fig. 1: Aerobic plate count (cfug^{-1}) of different age groups of goat meat

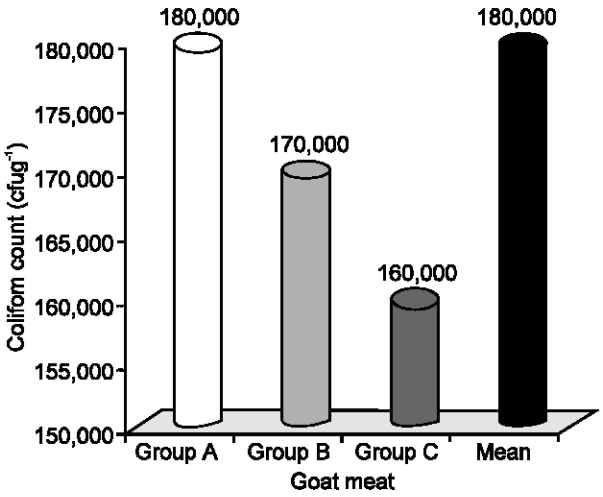

Fig. 2: Coliform count (cfug^{-1}) of different age groups of goat meat

meat, while ranged between 4.0 x 10^2 to 2.5 x 10^3 cfug^{-1}, (mean 1.4 x 10^3±2.9 x 10^2 cfug^{-1}) in group B goat meat and in between 7.0 x 10^2 to 3.0 x 10^3 cfug^{-1}, (averaged 1.5 x 10^3±3.1 x 10^2 cfug^{-1}) in group C goat meat. The overall yeasts and molds count in goat meat ranged between 4.0 x 10^3 to 3.0 x 10^3 cfug^{-1} (mean 1.5 x 10^3±1.5 x 10^2 cfug^{-1}). Furthermore statistical analysis showed no significant differences ($p>0.05$) in yeasts and molds counts in the means of meat from different age groups of goat meat.

DISCUSSION

There were no significant differences in aerobic plate count in goat meat samples in three groups ($p>0.05$). The higher aerobic plate count enumerated from goat meat 3.6 x 10^5±1.7 x 10^4 cfug^{-1}, suggested that an unusual amount of contamination and/or growth of

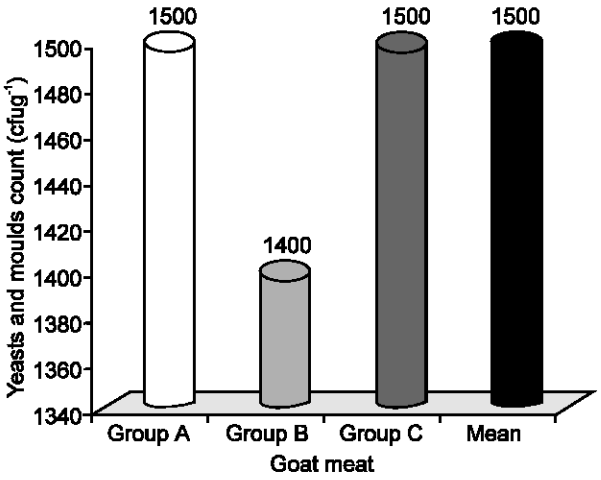

Fig. 3: Yeasts and molds (cfug^{-1}) of different age groups of goat meat

natural floral occurred during marketing; The finding of Vanderline *et al.* (1998) were similar to those of the present study. Higher numbers of bacteria could be transmitted from the fleece of goat to the carcass surfaces during hide removal (Bell *et al.*, 1993). The area of highest contamination was those sites where cuts were made through the skin (Bell and Hathaway, 1996). Over all study revealed that the level of contamination in the traditional meat shops was significantly higher compared to the reported from developed countries. The finding of present study reflected the hygienic status of meat production in the developing world (Bhandare *et al.*, 2007). According to Pace (1975) and Solberg *et al.* (1986) that bacterial count exceeding 10^5/g^{-1} in delicatessen food products are indicative of dangerous contamination.

Coliform count was observed from goat meat revealed no significant relation ($p>0.5$) with an increasing slaughter age. However, the concentration of Coliform count enumerated from goat meat (1.7 x 10^5±6.9 x10^3 cfug^{-1}) is very higher which is assumed to be an indicator of fecal contamination. It is likely that the observed incidence of fecal bacteria is due to problem associated with removal of the fleece and its coming into contact with the surface of carcass (Ozlem-Erdogrul, 2005). Chaubey *et al.* (2004) enumerated the Coliform the majority of the meat samples and being suggested that raw meat and meat products should be handled under strict hygienic condition and stored in cool places to avoid contamination and safeguard the health of consumers. According to Pace (1975) and Solberg *et al.* (1986) that Coliform count higher than 10^2/g in delicatessen food products are indicative of dangerous contamination.

The count of yeasts and molds observed in goat meat averaged 1.5 x 10^3±1.5 x10^2 cfug^{-1}. There were no

significant differences within different age groups of goat meat in term of yeasts and molds count ($p>0.05$). The value displayed a similarity to the illustrative values of (Ozlem-Erdogrul, 2005).

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Optimization of Extrusion on Blend Flour Composed of Corn, Millet and Soybean

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Abstract: Formulations made of blends of 44% corn flour, 36% millet flour and 20% soybean flour were performed using DPSv 11.50 software. The blends were extruded in Twin screw extruder and orthogonal array L_9 (3^4) was used to evaluate the optimized extrusion conditions. The explanatory variables used were temperature (for the feeding, mixing, cooking and die zones), Rolling speed, feeding speed and moisture content of the samples. The response variables were bulk density, WAI, WSI, pasting properties, thermal analysis, swelling power and the color of the extrudates. The better factors and levels showed that the temperature 80, 110, 140 and 170°C, rolling speed of 110 rpm, feeding speed of 37 g/min and moisture content varies from 25-30% are the best for the extrusion of that formulation.

Key words: Optimization, extrusion, corn, millet and soybean

INTRODUCTION

Cereals are staples food worldwide. To produce nutritious products, cereals are usually fortified with lysine or pulse proteins. Legumes are an important source of food protein and other nutrients (Thakur and Saxena, 2000). Soybeans are widely recognized by medical and health professionals for their health benefits. Soybeans protein has been found to reduce the risk of coronary heart disease when consumed as part of a diet low in saturated fat and cholesterol (Tripathi and Misra, 2005). So blend flour composed of corn, millet and soybean can give a product which has a high-energy value and proteins with high biological value.

Extrusion cooking of cereals is a very important process in food industry, since it regards a wide range of products such as snack-foods, baby-foods, breakfast cereals, noodle, pasta and cereals based blends. Extruders minimize the operating costs and higher productivity than other cooking process, combining energy efficiency and versatility (Ficarella *et al*., 2004).

Guy (2001) found that the ability of extruders to blend diverse ingredients in novel foods can also be exploited in the developing functional foods market such as soybean which is relatively unpalatable alone can be incorporated into new food items.

The purpose of using DPSv11.5 software was to find the formulation of corn, millet and soybean flours that would result in optimized nutritional qualities in protein, fat, fiber and Iron with minimized cost.

The purpose of this study was to optimize the extrusion cooking parameter in order to obtain a product with good physico-chemical properties. An L_9 (3^4) orthogonal array was selected for experimental layout and nine experiments was carried out.

MATERIALS AND METHODS

Yellow maize, millet and soybeans grains were purchased from the local market, sorted, washed and dried overnight in oven (DHG-9140A, Shanghai Sanfa Scientific Instruments co., LTD., China) at 40°C and milled by using the WK-800 high-speed miller (Shangdong mechanism co., LTD., China) and the flour obtained was passed through a 80 mesh sieve to get a homogenized flour.

Chemical composition of raw materials: Samples of corn, millet and soybean flours were analyzed for protein, fat, fiber, ash, moisture and carbohydrates according to AOAC (1984). Minerals content were analyzed using Atomic Absorption Spectrophotometer and vitamins content by using agilent 1100.

Formulation: The objective was to make a formulation which has a low cost and contain at least the minimum quantity of four nutrients protein 17%, fat 3%, dietary fiber 5% and Iron 3.5 μg.

Variables:

X_1 = Amount of corn in 1 kg of mixture
X_2 = Amount of millet in 1 kg of mixture
X_3 = Amount of soybean in 1 kg of mixture
Where $X_1 >= 0$, $X_2 >= 0$ and $X_3 >= 0$

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Constraints

Nutrients constraints:

$12x_1+15x_2+45x_3>17$(nutrient A)
$3.5x_1+2.5x_2+19x_3>3$(Nutrient B)
$19.3x_1+7x_2+20x_3>5$(Nutrient C)
$2.7x_1+3.9x_2+4.42x_3>3.5$(nutrient D)

Balancing constraint:

$$X_1 + X_2 + X_3 = 1$$

Objective: Presumably to minimize cost, i.e

$$\text{Minimize } 7x_1 + 12x_2 + 13.2x_3$$

DPS (Data Processing System) v11.50 software was used to find out the formulation to be done, where regression analysis was used at $p<0.05$.

Extrusion process: The formulation was extruded using PTW 24/25D laboratory co-rotating fully-intermeshing Twin screw extruder (thermo Haake Polylab System Rheomex, Germany) with 4 zones (feeding zone, mixing zone, cooking zone and die zone) and a 5 mm die was used. An orthogonal array L_9 (3^4) experimental design was used to carry out the extrusion (Jurkovic *et al*., 2006; Wu *et al*., 2008; Bolboaca and Jantschi, 2007) and the variables used were temperature (attributed to feeding, mixing, cooking and die zones), rolling speed, feeding speed and moisture content of the samples. According to orthogonal test, nine products resulted from the 9 runs. These extrudates were dried in an air dryer oven (DHG-9140A, Shanghai Sanfa Scientific Instruments co., LTD., China) at 105°C for 15 min, milled. The Flour obtained was passed through 80 mesh sieve to get homogenized extruded flour and stored into plastic bags at room temperature for further analysis.

Chemical composition of the extrudates: Protein, fat, fiber, ash, moisture and carbohydrates of the ground extrudates were analyzed according to the official methods of AOAC (1984). Mineral contents were analyzed using Atomic Absorption Spectrophotometer and Vitamins B1, B2, B6, B12, Folic acid; Niacin, Vitamin E and A were determined using Agilent 1100 Liquid chromatography. Energy conversion factors were used in calculating the calorific value of the nutrients:

Color: The color of the grounded extruded products was determined by using the color meter (DATA PROCESSOR DP-400 for chroma meter, KONICA MINOLTA SENSING, INC) where values in lightness (L*), redness (a*) and yellowness (b*) were recorded. Three measurements were taken for each sample and their means reported.

Bulk density: The bulk density of the extrudates was determined using a 25 ml graduated cylinder by packing gently and tapping on the bench. The volume of the flour was recorded and the weight of the flour was weighed using an electronic analytical balance (FA1104, Shanghai Balance Instrument Factory, China). The bulk density was calculated as the weight of flour divided by the volume and it was recorded as grams per cubic centimeter (g/cm^3). Three measurements were performed for each sample.

Water Absorption Index (WAI) and Water Solubility Index (WSI): Water Absorption Index (WAI) and Water Solubility Index (WSI) were measured with a slight modification of the method used by Anderson *et al*. (1969). 1 g of the ground extrudates was suspended in 20 ml of distilled water in a tared 45 ml centrifuge tube. The slurry was shaken with a glass rod and put in water bath at 30°C for 30 min then centrifuge (Himac CR21GII, High-speed refrigerated centrifuge, Hitachi koki co., LTD, Japan) at 3000 rpm for 15 min. The supernatant was decanted into an evaporator dish of known weight. The WAI was calculated from the weight of the remaining gel and expressed as grams of gel per grams of solid. The WSI expressed as gram of solids per gram of original solids, was calculated from the weight of dry solids recovered by evaporating the supernatant overnight at 105°C.

WAI = Weight of sediment/weight of dry solids

WSI = (wt of dissolved solids in supernant/wt of dry sample solids in the original sample) *100.

Pasting properties: The pasting properties of extrudates starches were determined using Rapid Visco Analyser (RVA tec master, Newport scientific Pvt., LTD., Australia) according to the method reported by Zaidul *et al*. (2007) and Kim *et al*. (2005) and Fred *et al*. (2003)with some modifications. 4 g of extrudates flour was added to 25 ml of distilled water. The heating and cooling cycles were programmed in the following manner. The sample was held at 50°C for 1 min, heated to 95°C in 3.42 min, held at 95°C for 2.7 min, cooled to 50°C in 3.88 min and finally held at 50°C for 2 min. The total time for analysis was 13 min.

Swelling power: The swelling power (by weight) of starch was measured using a modified method from the one reported by Tester and Marrison (1994). 0.5 g of extrudate was dispersed in 15 ml of distilled water. The suspension was heated at 90°C in a water bath for 30 min with vigorous shaking very 5 min. The starch gel was then centrifuged at 3000 rpm for 15 min. The weight of sediment was used to calculate the swelling power. Swelling power was calculated as follow:

$$\text{Swelling power} = \frac{\text{Mass of swollen sample (g)}}{\text{Initial mass (g)}}$$

Thermal analysis: Thermal analysis of extruded products proteins was done according to the method reported by Leblanc *et al.* (2008) and Zaidul *et al.* (2007) with some modifications. The thermal behavior of the proteins from extrudate was examined with a Perkin Elmer Model PYRIS 1- DSC Differential Scanning Calorimeter. 10 mg of extrudates was weighed in silver pan and an empty pan was used as blank. Measurements were carried out under Nitrogen ambience (100/ml) with a heating rate of 10°C/min by a scanning temperature range from 20-100°C. The gelatinization on set temperature (T_0), peak temperature (T_p) and enthalpy (delta H) were recorded.

Statistical analysis: The determination of color attributes (L*, a* and b*) was carried out in triplicate and the values were averaged. Data was assessed by the Analysis of Variance (ANOVA) (Snedecor and Cochran, 1987). Duncan Multiple Range Test was used to separate means. Significance was accepted at $p<0.05$.

RESULTS AND DISCUSSION

Formulation: The results obtained after using DPS V11.50 are shown in Table 3 and 4. 1 kg of the formulation should contain: 440 g of corn, 360 g of millet, 200 g of soybean and it will cost 10RMB.

Equation:

Y = 11.86069983-4.854160070*x15.632241114*x3+ 6.956906615*x3*x3+7.050481863*x1*x3+7.167 660759*x2*x3

Chemical composition: The proximate nutrient composition of the nine extruded products is presented in Table 7. The results indicated that the minimum quantity of nutrients based on which the formulation was done in Table 2 were achieved for dietary fiber and fat (for the 2nd, 6th, 8th and 9th products). Therefore, the nutrients obtained meet the requirements for FAO/WHO standards (1991) and FAO/WHO (1971).

Bulk density: The bulk density of extrudates is important in relation to their ability to float or sink when poured into water and their packaging requirement. The bulk density of extruded products varies from 0.8974 up to 1.0782 (see Table 8). There is an increase in bulk density as the temperature increases; this is due to the liquefaction of sugar via melting during extrusion process (Meuser and Wiedmann, 1989). As it is shown in Table 9, the optimized factor levels for density were in the combination $A_3D_2B_2C_2$ - which means [T: 80, 110, 140 and 170°C, Feeding speed of 3 (37 g/min), Rolling speed 130 rpm and Moisture content varies from 20-25%]. These extrusion conditions were not done but they are slight similar to the last three experiments we did (7th, 8th and 9th experiments/runs). These conditions can also be suggested for the bulk density.

Table 1: Chemical composition of raw materials

Raw materials	Corn	Millet	Soybean
Protein	12	15	45
Fat	3.5	2.5	19
Ash	2.8	0.81	4.77
Moisture	9.7	4.39	5.2
Fiber	19.3	7	20
Carbohydrates	48.9	70.3	6.03
Zn (µg/ml)	0.965	1.14	1.21
Fe (µg/ml)	2.7	3.9	4.42
Na (µg/ml)	2.80	1.15	5.50
K (µg/ml)	136	110	553
Mg (µg/ml)	120	35.5	140
Ca (µg/ml)	135	10.7	178
P (µg/ml)	0.728	0.022	0.72
Vit A (µg/ml)	2.1	9.575	11.3
Vit E (µg/ml)	444.6	58.484	-
Vit B1(µg/ml)	1.6	0.770	6.6
Vit B2 (µg/ml)	8.7	9.258	5.6
Niacin (µg/ml)	5.7	0.976	7.3
Folic acid (µg/ml)	10	0.568	3
Vit B6 (µg/ml)	7.8	140.757	35.9
Vit B12 (µg/ml)	11.6	6.131	3.2

Table 2: Nutrients values and cost of the raw materials

Raw materials	Protein (A)	Fat (B)	Dietary fiber (C)	Fe (D)	Cost/ 500 g
Corn (g/100 g)	12%	3.5%	19.3%	2.7 µg	7 RMB
Millet	15%	2.5%	7%	3.9 µg	12 RMB
Soybean	45%	19%	20%	4.42 µg	13.2 RMB

Table 3: Formulation made

Raw materials	Corn	Millet	Soybean
g	440	360	200
%	44	36	20
Cost	10 RMB/1 kg		

Table 4: Each factor combination at lowest index

Y	x1	x2	x3
10	0.4400	0.3600	0.2000

Table 5: Variables and their levels under extrusion process

		Levels		
Symbol	Variables	1	2	3
A	Temperature (T)	40, 70, 100, 130°C	60, 90, 120, 150°C	80, 110, 140, 170°C
B	Rolling Speed (RS)	110	130	150
C	Moisture Content (M.C)	15-20%	20-25%	25-30%
D	Feeding Speed (FS)	2 (20 g/min)	3 (37 g/min)	4 (55.4/min)

Table 6: Experimental layout using an L_9 (3^4) orthogonal array

Experiment number	Variables and levels A	B	C	D
1	1	1	1	1
2	1	2	2	2
3	1	3	3	3
4	2	1	2	3
5	2	2	3	1
6	2	3	1	2
7	3	1	3	2
8	3	2	1	3
9	3	3	2	1

Water Absorption Index (WAI) and Water Solubility Index (WSI): Water absorption index of the extrudate varied from 3.63 and 6.04 g/g and the products with high WAI were those with the highest moisture content before extrusion (25-30%). As shown on Table 10, the best factors' combination for WAI is $C_3A_3B_1D_2$ which means MC: 25-30%, T: 80-170°C, RS: 130 rpm and FS: 3 (37 g/min). These extrusion conditions were fulfilled in the 7th treatment.

In general, the WSI increased as the temperature increased and it varies from 9.1-16.95%. This may be due the inactivation of antiphysiological factors contained in the soybean or may be due to the damage of starch content at high temperature. The best factors and combination for WSI was the treatment $C_1A_1D_1B_2$ (Table 11); this means Moisture content varies from 15-20%, temperature of 40, 70, 100 and 130°C, Feeding speed 2 (20 g/min) and Rolling speed of 130 rpm. These extrusion conditions were not done but they are almost similar to the 1st experiment ($A_1B_1C_1D_1$), the only difference is the rolling speed which is 110 rpm. So these conditions are also recommended for WSI.

Color: Color is an important characteristic of extruded foods. Color changes can give information about the extent of browning reactions such as caramelization, maillard reaction, degree of cooking and pigment degradation during the extrusion process (Ilo and Berghofer, 1999). The lightness (L*) is an indication of the brightness. The lightness value of the products ranges from 79.70-84.73. The Table 12 shows that there are no significant different between the 2nd, 3rd, 4th, 6th and 8th products and there are significant difference between them and others products for ($p<0.005$). The color parameter a*, indicative of the redness of sample varied from 1.62-4.84 and there is a significant difference between all the products for $p<0.05$ and in general, the products cooked with high temperature have the highest values. Dark color is also developed during caramelizarion of sugar from the maillard reaction that why redness (a*) value is high as the temperature increases. The yellowness value (b*) of extruded products varies from 21.87-26.07. There was a significant different between the yellowness value of almost all the product for $p<0.005$. The change in yellowness during extrusion cooking was most induced by the effects of nonenzymatic browning and pigment destruction reactions. All these differences could have been due to the shear forces generated during extrusion which accelerated the chemical reactions between amino acids and reducing sugars (maillards reaction) that take place during extrusion (Guy, 2001) and to the different temperature cooking, rolling speed and feeding speed conditions during extrusion. The combination $A_3B_1C_3D_2$ (Moisture content varies from 25-30%, Temperature 80, 110, 140 and 170°C, Rolling speed of 130 rpm and Feeding speed 37 g/min) yielded the lowest lightness value (L* = 79.70), the highest redness value (a*= 4.84) and the 2nd highest value for brightness (b* = 25.04).

Table 7: Chemical composition of extrudates

	1	2	3	4	5	6	7	8	9
Protein %	15.22	15.35	15.13	15.33	15.46	15.92	15.89	16.43	15.88
Fat %	2.78	3.72	2.32	2.8	2.7	3.6	2.64	4.58	3.16
Moisture %	10.57	10.49	10.37	10.40	9.13	7.15	8.63	6.06	6.67
Ash %	1.72	1.80	1.84	1.76	1.97	2.04	1.86	1.84	1.94
Fiber %	13.8	14.3	12.7	12	13.6	14.9	15.4	13.6	14.5
*Carbohydrates %	55.91	54.34	57.67	58.34	57.14	56.39	55.58	57.49	57.85
Zn (µg/ml)	1.03	0.922	1.11	0.873	0.989	0.949	0.977	0.950	0.974
Fe (µg/ml)	1.20	1.64	1.70	1.80	1.94	1.47	1.69	1.81	1.60
Na (µg/ml)	2.55	2.20	2.02	1.79	1.99	1.67	1.95	1.63	2.65
K (µg/ml)	278	285	284	285	282	302	305	307	296
Ca (µg/ml)	30.1	32.6	31.1	28.2	30.8	32.7	31.6	33.5	33.9
Mg (µg/ml)	38.1	46.1	45.5	40.9	44.1	48.1	46.8	50.5	46.1
P (µg/ml)	0.033	0.032	0.031	0.034	0.032	0.031	0.032	0.034	0.030
Vit A (µg/ml)	1.63	2.26	1.33	1.27	4.70	1.30	2.54	2.59	1.22
Vit E (µg/ml)	100.78	83.29	37.79	33.96	70.15	55.65	65.99	70.92	44.19
Vit B1 (µg/ml)	0.68	0.26	0.31	7.35	4.25	0.70	1.17	1.95	1.32
Vit B2 (µg/ml)	11.09	11.15	18.16	11.32	0.16	10.55	11.22	10.04	10.63
Niacin (µg/ml)	4.86	0.35	5.40	3.32	3.30	3.95	3.97	4.86	4.28
Folic acid (µg/ml)	2.63	1.49	2.31	1.18	1.10	1.27	1.56	3.26	1.39
Vit B6 (µg/ml)	226.82	172.68	247.64	168.16	150.61	192.60	208.46	210.31	196.10
Vit B12 (µg/ml)	6.35	6.71	11.46	6.59	6.44	5.85	8.80	5.68	6.27

*Carbohydrates by difference

Table 8: results for orthogonal test

Experiment number	Density	WAI	WSI	Swelling power
1	0.9191	3.63	16.95	5.80
2	0.9810	3.95	14.1	5.77
3	0.8974	4.78	11.45	5.53
4	1.0146	4.53	13.25	6.51
5	1.0296	5.16	12.15	5.83
6	1.0598	4.03	15.3	5.42
7	1.0564	6.04	9.1	13.79
8	1.0782	4.52	13.35	5.87
9	1.0681	4.65	12.25	9.09

Table 9: Bulk density results

	A	B	C	D
K_1	0.9325	0.9967	1.0190	1.0056
K_2	1.0347	1.0296	1.0212	1.0324
K_3	1.0676	1.0084	0.9945	0.9967
R-value	0.1351	0.0329	0.0267	0.0357

Where A is temperature variable, B is rolling speed, C is moisture content and D is feeding speed.
K_1 is means of combination of all 1 in the same column
K_2 is means of combination of all 2 in the same column
K_3 is means of combination of all 3 in the same column and
R-value is the highest K minus the lowest K in the same column ($K_{maximum}$-$K_{minimum}$)

Table 10: WAI results

	A	B	C	D
K_1	4.12	4.73	4.06	4.48
K_2	4.57	4.54	4.38	4.67
K_3	5.07	4.49	5.33	4.61
R-value	0.95	0.24	1.27	0.19

Table 11: WSI results

	A	B	C	D
K_1	14.17	13.1	15.2	13.78
K_2	13.57	13.2	13.2	12.83
K_3	11.57	13	10.9	12.68
R-value	2.6	0.2	4.3	1.1

Table 12: Color attributes results

	1	2	3
L*	80.33C	84.63A	84.10A
a*	2.4E	1.67F	1.62F
b*	26.07A	23.26E	24.48BC
	4	5	6
L*	84.68A	82.27B	84.73A
a*	1.69F	3.39C	2.20E
b*	23.19E	23.87D	22.46F
	7	8	9
L*	79.70C	84.04A	80.62C
a*	4.84A	2.68D	4.60B
b*	25.04B	21.87G	23.93CD

Swelling power: Swelling power of the extrudates varied from 5.42-13.79. The Table 13 showed that the best combination in factors levels for a good swelling power is $A_3B_1C_3D_2$ (Moisture content: 25-30%, Temperature: 80, 110, 140 and 170°C, Rolling speed: 130 rpm and Feeding speed: 37 g/min). In general, the products cooked with the highest swelling power, these may be due to starch gelatinization and degradation during extrusion process.

Table 13: Orthogonal results for swelling power

	A	B	C	D
K_1	5.7	8.7	5.70	6.91
K_2	5.92	5.82	7.12	8.33
K_3	9.58	6.68	8.38	5.97
R-value	3.88	2.88	2.68	2.36

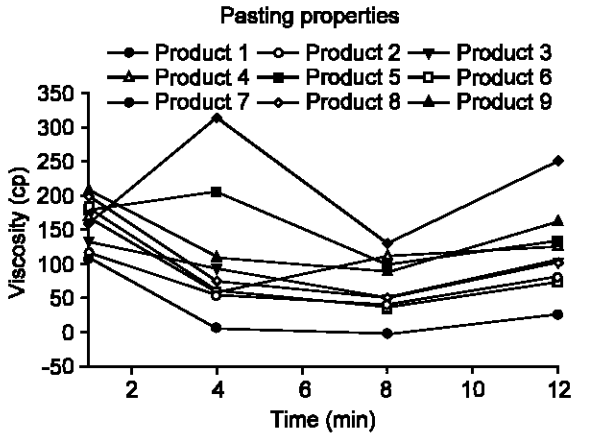

Fig. 1: Pasting properties

Pasting properties: The results in pasting properties of extrudates are shown in Table 13 and the Fig. 1. As it is shown on this figure, the viscosity increases as the temperature increases for the products cooked with a high temperature (from 80-170°C). These may be attributed to the starch gelatinization during extrusion and the Table 13 shows that the product cooked with these condition $A_3B_1C_3D_2$ has the highest peak, trough, breakdown, final viscosity and setback 46.17, 10.58, 35.58, 22.83 and 12.25 cp respectively.

Thermal analysis: Thermal properties of the extrudates starch are shown in Table 15. T_0 of extrudates were ranged from 43.99-96.17°C and the high temperature was found with starches derived from the 7th product (MC: 25-30%, T: 80-170°C, RS: 130 rpm and FS: 37 g/min)while the lowest was observed from the 2nd product (temperature 40, 70, 100 and 130°C, rolling speed: 110 rpm, feeding speed: 37 g/min and moisture content: 20-25%). Gelatinized extrudates starches were ranged from 51.27-99.17°C for T_p and 58.09-99.92 for T_e. In general, it has been found that the products cooked at low temperature had a low T_0, T_p and T_e while those cooked at high temperature had a high T_0, T_p and T_e and the 7th product has the highest values (MC: 25-30%, T: 80-170°C, RS: 130 rpm and FS: 37 g/min). These may be due to the increase of temperature, shear and pressure during extrusion which increased the rate of gelatinization. Guy (2001) and O'Connor (1987) reported that the complete gelatinization of starch is necessary in human nutrition for a good digestibility and this can be done by using a high temperature in cooking starches.

Table 14: Pasting characteristics of extrudates

	Peak viscosity	Trough	Breakdown	Final viscosity	Set back	Peak time	Pasting temperature
1	1.67	-0.33	2	2.25	2.58	1.53	46.45
2	7.83	3.17	4.67	6.75	3.58	1.6	46.6
3	13.83	4.17	9.67	9	4.83	1.07	46.85
4	14.58	3.08	11.5	10.5	7.42	1.4	46.9
5	24.5	6.5	20.08	11.5	5	2.53	47.4
6	16.5	2.75	13.75	6.33	3.58	1.4	47.3
7	46.17	10.58	35.58	22.83	12.25	1.67	47
8	16.17	3.83	12.33	8.67	4.83	1.93	46.95
9	23.17	5.75	17.33	14.08	8.33	2.2	46.75

Table 15: Results of thermal analysis

	T_0	T_p	T_e	Delta H	Peak height	Area
1	45.50	52.52	59.88	0.9184	0.1849	6.796
2	43.99	51.27	58.09	0.7600	0.1754	6.300
3	45.70	53.27	60.83	1.0508	0.2505	9.194
4	91.08	95.66	96.16	0.2825	0.1416	1.661
5	47.39	54.04	59.52	0.1584	0.0321	0.968
6	86.67	87.15	87.15	2.0006 e-4	0.0019	0.003
7	96.17	99.17	99.92	0.0287	0.0225	0.207
8	86.64	87.39	87.64	9.5095 e-4	0.0086	0.008
9	90.15	90.90	90.90	8.8323e-4	0.0029	0.008

Where t_0: temperature onset, Tp: peak temperature, Te: end temperature

Conclusion: According to the physico-chemicals results of the extruded products; It has been found that the product cooked with these conditions (Moisture content: 25-30%, Temperature: 80, 110, 140 and 170°C, Rolling speed: 130 rpm and Feeding speed: 37 g/min) are the best. So extrusion of the formulation done should be cooked in these conditions.

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Performance and Digestibility in Nubian Goats Fed Steam Treated Sorghum Stover

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Abstract: The effects of steam under pressure treatment on sorghum stover (Tabat variety) Dry Matter Intake (DMI), water consumption, body weight changes and digestibility were studied in the Gezira, Sudan. Sorghum stover was steam treated at 4 kg/cm^2 for 15 min (ST_1S) or 30 min (ST_2S) or not treated (US) as the control and fed to twelve female Nubian goats (four foreach treatment). Dry matter intakes (g/d, g/kg LW and g/kgW$^{0.75}$) were decreased with steam treatment with no significant differences among treatments. It was (55.81, 54.00 and 54.01) g/kgW$^{0.75}$ for US, ST_1S and ST_2S, respectively. Weekly weight loss (kg) was decreased by steam treatments and was 0.097, 0.062 and 0.0 kg/week for US, ST_1S and ST_2S, respectively. Water intake (g/d) was increased by steam treatment at 15 min (3405) and decreased at 30 min treatment (2948). Steam treatments decreased sorghum stover apparent digestibilities, except for CF and EE at 30 min.

Key words: Sorghum stover, digestibility, steam treatment, Nubian goats

INTRODUCTION

Nutrition is one of the main constraints for animal production in the Sudan, since most animals are reared in traditional systems based on natural pastures with seasonal migrations for feed and water (Elhag, 1984). The seasonal pattern of rainfall is associated with seasonal variations in available feeds and their nutritive value which are diminishing in the dry season with serious impacts on animals' health and performance.

Straws are important feed resources to fill the gap between animals' nutrient requirements and pasture yields (National Strategy, 2002). Straws are inferior in chemical composition with high fibres and low CP (Sundstol and Owen, 1984) limiting dry matter intake and performance when used untreated (Preston, 1986). The major constraint in using straws is their low digestibility due to the lignin component of the cell wall and its association with other cell wall carbohydrates (Kerley *et al*., 1987).

Many procedures are employed to upgrade the nutritive value of straws and to enhance Dry Matter Intake (DMI) using physical, chemical, biological methods and their combinations. Steam under pressure is conducted to upgrade the nutritive value and utilization of straws (He *et al*., 1989; Viola *et al*., 2008) and it increased DMD of maize cobs, bagasse and pith with no effect on *in vitro* DMD of rice straw (Abaza *et al*., 1981). Microbial growth in the rumen is generally limited by energy rather than N supply (Russell *et al*., 1992). Steam processing of low quality roughages has been recommended to increase energy availability (Bender *et al*., 1968). There is no information on the effects of steam treatment of sorghum stover on the digestibility and performance of Nubian goats in the Sudan. Consequently, this project was launched to investigate the effects of steam on the nutritive value of straw.

MATERIALS AND METHODS

Source of steam: Steam under pressure for treating stover was produced using boiler unit. The treatment was conducted at the laboratory of the Faculty of Engineering and Technology, University of Gezira, Wad Medani. The unit for steam production was manufactured by Falton in England. It was operated by diesel and electricity. The unit consists of a boiler, pipes and a steel container where the steam is injected.

Chopped sorghum stover (96% DM) was placed in the container and was steamed at required pressure (4 kg/cm^2) for different times (15 or 30 min). The time was calculated after reaching the desired pressure and the pressure was controlled by opening the valves. When the planed pressure and time were reached a tank valve was suddenly opened and the pressure was released and then the lid of the container was opened. Treated stover was removed manually from the container.

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Preparation of the experimental diets: In this experiment chopped sorghum stover (Tabat variety) was divided into three portions. The first part was not treated (control diet). The second was steam treated under pressure 4 kg/cm^2 for 15 min. The third part was treated with 4 kg/cm^2 steam for 30 min. The treatment was by direct steam injection in the boiler unit described before. The treated stover was spread on plastic sheets, sun-dried for 3 days and stored in plastic bags under room temperature.

Animals: Twelve female Nubian goats about 1.5-2 years old and with an average BW of 26.63±2.10 kg were used in this experiment. They were ranked according to body weight and randomly allocated to the three treatments with four animals for each treatment. The animals' treated against ecto (Amitraz) and endo (Albendazole) parasites, ear tagged and housed in individual pens (1 m x 1.5 m).

Feeding: The three rations were offered *ad libitum* in two equal meals at 9:00 am and 4:00 pm. The control group was offered Untreated Sorghum Stover (US). The second group was offered sorghum stover steam - treated for 15 min (ST_1S).The third group was given sorghum stover steam-treated for 30 min (ST_2S). A concentrate mixture was offered to all groups (235 g/head/day) in a separate feeder once daily before the roughage. The composition of the concentrates mixture is shown in Table 1. The experiment lasted 9 weeks with the first week as an adaptation period.

Table 1: The composition of the concentrate mixture offered to Nubian goats fed Steam treated sorghum stover

Ingredients (As fed)	%
Sorghum grain	48
Wheat bran	35
Groundnut cake	7
Molass	6.5
Limestone	1.5
Common salt	1.0
Mineral/vitamin mix	1.0
CP	15.5
ME (MJ/kg DM)	11.17

Parameters studied: The parameters studied were Dry Matter Intake (DM1), water consumption, body weight changes and digestibility.

Voluntary feed intake: The daily DM1 for each goat was calculated by the difference of the feed offered and the refusals on dry matter bases.

Water intake: The daily water intake for each animal in all groups was recorded daily for the last 7 days.

Body weight changes: The body weight of each goat was recorded at the beginning of the experiment. They were weighed weekly before the morning meal throughout the experiment.

Digestibility: A digestibility trial was conducted with the same animals at the last week of the experiment.

Dry matter intake: The offered feeds and their refusals were weighed daily for each animal to determine daily intake. Samples of feeds offered and refusals were collected for proximate analysis. Daily DMI for each animal was calculated.

Faecal output: The animals were fitted with nylon bags for faecal collection and were emptied daily. Faeces collected for each animal were weighed and representative 10% samples were taken for DM determination. Daily DM faecal output for each animal was calculated. The remaining faecal samples were separately stored for proximate analysis.

Statistical analysis: Results were analyzed by ANOVA for completely Randomized design using the general linear model procedure (SAS, 1997).Mean comparison was carried out by Scheffes test with an alpha level of 0.05.

RESULTS

Table 2 shows effects of steam treatment of sorghum stover on DMI, water intake and body weight changes in Nubian goats in the Gezira, Sudan.

Dry matter intakes (g/d, g/kg LW and g/kg $W^{0.75}$) were decreased by steam treatment for 15 and 30 min with no significant differences ($p>0.05$) among treatments. It was also decreased when expressed as percentages of live weight and metabolic body weight.

Water intakes (g/d, g/kg LW and g/kg $W^{0.75}$) were increased by steam treatment for 15 min and were decreased for 30 min which were lower than the untreated sorghum stover. Similar trends were observed when water intake was expressed as percentages of body weight and metabolic weight. Water intake expressed as part of DMI was increased by steam treatment for 15 min and decreased for 30 min which was lower than untreated stover. Weekly weight loss (kg) was decreased by steam treatments.

Table 3 shows *in vivo* digestibility of sorghum stover treated with steam under pressure in Nubian goats in the Gezira, Sudan.

Steam treatments generally decreased DM, OM and CP apparent digestibilities, but not significantly ($p<0.05$). Steam treatment for 15 min had the least DM, OM and CP digestibilities. Ether extract and CF digestibilities were increased with 30 min steam treatment and decreased by 15 min treatment. However, these effects were only significant for CF.

Table 2: The effects of steam under pressure treatments of sorghum stover (Tabat variety) on DMI, water intake and body weight changes in Nubian goats in the Gezira, Sudan

Treatment	US	ST_1S	ST_2S	SE	CV (%)
DMI					
g/d	647	640	627	8.02	4.59
g/kg LW	24.67	23.70	23.88	0.52	1.94
g/kgW^{75}	55.81	54.00	54.01	0.93	3.40
%LW	2.47	2.28	2.39		
% W^{75}	5.58	5.40	5.40		
Water intake					
g/d	3285	3405	2948	111.37	11.31
g/kg LW	125.34	125.91	111.90	4.38	15.04
g/kgW^{75}	283.54	287.04	253.31	9.43	31.61
%LW	12.53	12.59	11.19		
%W^{75}	28.35	38.27	25.33		
Kg/kg DMI	5.08	5.32	4.68		
LW loss (kg/wk)	0.097	0.062	0.00	0.056	19.79

US = Untreated Sorghum Straw. ST_1S = Steam-Treated Sorghum Stover, (4 kg/cm^2 for 15 min). ST_2S = Steam-Treated Sorghum Straw (4 kg/cm^2 for 30 min). SE = Standard Error of Mean. Means in same row with different superscripts are significantly ($p<0.05$) different

Table 3: *In vivo* digestibility (%) of steam under pressure treated sorghum stover in Nubian goats in the Gezira, Sudan

Treatments	DM	OM	CP	EE	CF
US	54.12	54.80	64.96	$62.71^{b,a}$	32.51^{a}
ST_1S	43.32	45.56	59.98	50.41^{b}	14.27^{b}
ST_2S	51.78	50.16	62.98	73.52^{a}	37.58^{a}
SE	2.323	2.253	1.641	3.754	3.669
CV (%)	14.28	14.84	9.34	15.06	28.42

US = Untreated Sorghum Straw; ST_1S = Steam Treated Sorghum Straw (4 kg/cm^2 for 15 min); ST_2S = Steam Treated Sorghum Straw (4 kg/cm^2 for 30 min); Means in the same column with different superscripts are significantly ($p<0.05$) different, SE = Standard Error of Mean

DISCUSSION

Dry matter intake: The dry matter intake of stover in this experiment (2.28-2.47% of the live body weight) was within the minimum range (2.1-6.3%) for goats (Devendra and Mcleroy, 1982). The low intake was mainly due to the fibre content and gutfil (Van Soest, 1982). Steam treatments had slightly, but not significantly depressed DMI. However, many workers (Rangnekar *et al*., 1982; Awadelkerim and Osman, 1993; Hou *et al*., 1997) reported improvements in DM1 for steam treatments. The variations in response could be due to differences in treatment conditions affecting chemical reactions and formation of browning compounds and the extent of nutrient solublization and losses. Many factors were reported affecting Maillard reaction products including temperature, duration, moisture content and type of substrates (Van Soest, 1982; Tipson and Horton, 1988). The duration of steam treatment had no significant effects on DMI indicating that it is better to treat for 15 min to reduce costs.

Water intake: Water intake of untreated sorghum stover in this study (3.3 L/day) was close to that reported by Awadelkariem and Osman (1993) for wheat straw (3.4 L/kg). Water intake was increased by steam treatment for 15 min and decreased after that, but not significantly. The increased water intake with steam treatment was similar to the findings of Rangnekar *et al*. (1982) and Awadelkerim and Osman (1993). Water intake was close for all feeds when expressed as L/Kg DMI and this was mainly due to non significant differences on DMI for steam treatment.

Weight loss: The higher weight loss when goats fed sorghum stover was expected due to the low N and higher CF depressing rumen fermentation and DMI. This is supported by the findings of Preston (1995) who suggested strategies for improving the soluble nutrients for rumen microbes. The reduced weight loss with steam treatment indicated the beneficial effects of the treatment and the response would have been better if effluents were added. The thirty min treatment prevented the weight loss although DMI was the least among treatments suggesting an improvement in nutritive value. The thirty min treatment significantly improved EE, CP and ash and significantly decreased CF and NFE indicating improved chemical composition and could be due to increased delignification. The 30 min treatment had also improved CF and EE digestibilities.

***In vivo* digestibility:** The least digestibility of sorghum stover steam-treated for 15 min was supported with the findings of Awadelkerim and Osman (1993) where more than 30% of steam-treated wheat straw at 125°C for 15 min had adversely affected OM, DM and CP digestibilities.

Dry matter, OM and CP digestibilities for 30 min steam treatment were close to the untreated straw. However, many workers reported improvements in apparent digestibility for steam treatment (Rangnekar *et al*., 1982; He, *et al*., 1989 and Hou *et al*., 1997). Furthermore, high steam pressure (30 kg/cm^2) had increased *in vitro* digestibility by 20% (Gugloz *et al*., 1971) and improved in vivo digestibility of straw by 25% at 1980c for 2-5 min (Viola *et al*., 2008). This could be because the pressure used in our experiment was not sufficient to exert marked effects. This agrees with the findings of Castro (1994) where mild steam treatment was ineffective in solubilizing hemicellulose. Steam treatment had decreased CP digestibility and could be due to browning reaction which is indigestible (Zahedifar, 1996). The improved EE and CF digestibilities at the higher duration were in line with the findings of Pearce (1982). This could be because steam treatment reduced lignin and cellulose, solubilized nutrients and increased reducing sugars.

The decreased digestibility at 15 min for all nutrients was mainly due to the lower pressure and duration and browning effect. Higher pressure and longer durations are required for optimum effects.

Conclusion: Tabat steam treatment (4 kg/cm^2 for 15 or 30 min) decreased DMI but it increased water consumption. Steam treatment of Tabat (4 kg/cm^2) not significantly decreased apparent digestibility except for EE and CF which were increased at 30 min. The increased in apparent digestibility for CF and prevention in weight losses at 30 min treatment indicating that the long duration of treatment had a better effect on fibre fraction.

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Nutritional Status of Mothers and Children in Pakistan as Compared to Other Neighbouring South Asian Countries

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Abstract: Malnutrition of women and children is one of the major problems in Pakistan. The prevalence of Protein Energy Malnutrition (PEM) in children under five year is 51%. It is one of the contributory factors of high infant and child under 5, morbidity and mortality in the country. This problem needs to be addressed properly in Pakistan for which the study should be undertaken urgently. According to state of world children 2000 UNICEF report, Pakistan position has been revealed worst in child under 5, mortality and infant mortality rate as compared to other neighbouring South Asian countries. The mortality rate was 136/1000 live births, in under five children in Pakistan. Similarly the mortality rate was 12 and 87/1000 in Sri Lanka and Maldives respectively during the same period. Infant mortality under one year was also on high side, it was 95 as compared to 38 in China during 1998. As compared to other south Asian countries the position in demographic and socio-economic indicators is hardly satisfactory, inspite of the fact that Pakistan is fully equipped with natural resources. There is need for sincere efforts for the development of socio-economic indicators.

Key words: Malnutrition, infant nutrition, socio-economic indicators

INTRODUCTION

Malnutrition is a silent emergency but the crisis seems real and its persistence has profound and frightening implications for children society and the future of humankind. Three quarters of the children who die worldwide have causes related to malnutrition. In some parts of the world, notably Latin America and East Asia, there have been dramatic gains in reducing child malnutrition. But overall, the absolute number of malnourished children worldwide has grown (UNICEF, 1998). Half of the South Asia's children are malnourished. South Asia has a long way to go to meet the U.N millennium development goal target of reducing maternal and infant mortality by 66-75% by the year 2015. In Africa, one of every three children is underweight and in several countries of the continent, the nutritional status of children is worsening. Malnourished children are much more likely to die as a result of a common childhood disease than those who are adequately nourished. Malnutrition lowers the body's ability to resist infection by undermining the functioning of the main immune-response mechanism. This leads to longer, more severe and more frequent episodes of illness.

Of the nearly 12 million children under five who die each year in developing countries mainly from preventable causes, the deaths of over 6 million, or 55%, are either directly or indirectly attributable to malnutrition. Some 2.2 million children die from diarrhoeal dehydration as a result of persistent diarrhea that is often aggravated by malnutrition.

According to the national figures given by the state of world's children by UNICEF (2004), adult literacy rate in male is 57% but father's literacy status must be lower than this actual figure-1. Prevalence of stunning and wasting is 32.5% and 16.5% respectively in rural areas of Pakistan which were higher as compared to urban areas (Pakistan Medical Research Council, 1990-94), this may be attributed to limited access and utilization of health services. 35% of rural areas have access to health, whereas 90% of urban areas have this facility (UNICEF, 1996).

Ahmed and Akram (1984) in Pakistan Medical Research Journal elaborated nutritional risk factors in 2947 children. The percentage of children with nominal weight for age was worked out to be 52%, whereas 48% were malnourished. The risk factors were large family, late weaning, low literacy of parents specially fathers literacy status needs to be uplifted and low family income. These were found to affect the nutritional status of the children significantly and caused malnutrition in them (Bhutta *et al*., 2004).

Situation analysis: Malnutrition of women and children is one of the major health problems in Pakistan. The prevalence of Protein Energy Malnutrition (PEM) in

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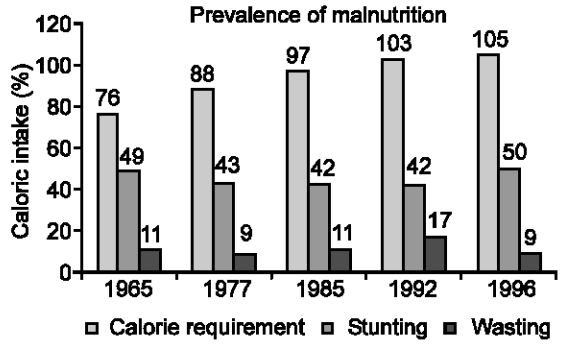

Fig. 1: Nutrition trends in Pakistan (Children under 5)

children under five years is 51% (Fatima Barmal, 2000). It is one of the contributory factors of high infant and child under 5, morbidity and mortality, in the country.

In accordance to the status of world children 2000 UNICEF (1998), Pakistan position has been revealed worst in children under 5, mortality and infant mortality rate as compared to other neighbouring countries. According to 1998 information published by UNICEF in the year 2000 under five mortality rate was 136/1000 live births in Pakistan. It is surprisingly observed that in Sri Lanka and Maldives under five mortality rates stood at only 19 and 87/1000 live births respectively, during the same period. Infant mortality under one year was also on the high side, it was 95 as compared to 38 in China during 1998.

Population of Pakistan is ranked at 41 serial numbers in Demographic indicators. Population has been upsurged at the rate of 2.7% highest in the South Asian countries, fertility rate is 5.0 marked highest after Afghanistan in the region. Detail position is depicted in Table 1, 2 and 3.

Trends of malnutrition: Although several sequential studies have highlighted the importance of malnutrition, few have recognized that this is virtually resistant to change despite numerous intervention programs. Figure 1 vividly illustrates the fact that despite improving adequate per capita energy intake the rates of wasting (low weight for age) and stunting (low height for age) among children are unchanged for over 3 decades (Bhutta, 2001). In addition to this widespread malnutrition, rates of maternal malnutrition are also high and almost 25-30% of all newborn infants are born with low birth weight i.e. weighing under 2.5 kg at birth. This large number of new born infants is not only at much greater risk of complications and mortality after birth, but are also destined to remain malnourished throughout infancy and childhood, with higher risk of long term complications.

We approach the 21st century with the burden of adding over 1 million such infants to our population every year, with limited potential for physical development, education performance and lowered life expectancy due to premature onset of chronic diseases (Bhutta, 2000). It is not surprising that women in Pakistan are likely to be malnourished largely due to their under privileged position in the society (Women's Health Project (Pakistan), 1997; Mahbub-ul-Haq, 2000). Furthermore maternal malnutrition leads to childhood malnutrition and malnourished mothers give birth to low birth weight infants. Those who survive become undernourished children and adolescence with early marriages and with lack of contraceptive practices, these young adolescents become pregnant themselves putting an additional strain on their already deprived bodies. Because the lack of knowledge regarding the increased requirements of pregnancy and inability to provide for these additional demands, the nutritional status of these women deteriorates further. These malnourished women give birth to further low birth weight babies leading to a viscous cycle of persistent malnutrition (Winkvist *et al.*, 1992; Planning Division, 1976-77). This relationship may be understood by looking at this life course approach, illustrating that this may be the Vicious Cycle

Table 1: Basic indicators

Name of country	<5 MR	Under 5 M.R. 1960	Under 5 M.R. 1998	I.M.R. (Under-1) 1960	I.M.R. (Under-1) 1998	T.P. 1998	ANB 1998	AND 1998	G.N.P 1997*	LEB 1993	TALR 1995	PSER 90-96	% of share of HI 90-96 L 40%	% of share of HI 90-96 H 20%
Afghanistan	4	360	257	215	165	21354	1113	286	250x	46	32	49	-	-
Bangladesh	48	247	106	151	79	124774	3468	368	360	58	38	69	23	38
Bhutan	41	300	116	175	84	2004	75	9	430	61	42	25	-	-
China	79	209	47	140	38	1255698	20134	946	860	70	80	120	15	48
India	49	236	105	144	69	982223	24671	2590	370	63	50	101	22	39
Nepal	51	297	100	199	72	22847	779	78	220	58	36	110	19	45
Pakistan	33	226	136	139	95	148166	5306	722	500	64	39	74	21	40
Sri Lanka	137	133	19	83	17	18455	327	6	800	73	90	109	22	39
Maldives	56	300	87	180	62	271	9	1	1180	65	95	125	-	-

<5 MR = Under 5 mortality rank | Under 5 M.R. = Under 5 mortality rate | I.M.R. (Under-1) = Infant mortality rate (Under-1)
T.P. = Total population (Thousands) | ANB = Annual number of births (Thousands) | AND = Annual number of (under 5) deaths
* = G.N.P per capital US $ | LEB = Life expectancy at birth (years) | TALR = Total adult literacy rate
PSER = Primary school enrolment ratio (Gross) | HI = House hold income | L 40% = Lowest 40% | H 20% = Highest 20%
- = Data not available. x = Indicates data that refer to years or periods other than those specified in the column heading, Differ from the standard definition, or refer to only part of a country. * = Data refer to the most recent years available during the period specified in the column heading

Table 2: Demo graphic indicators

Name of country	<MR	Population thousands 1998 Under 18	Under 5	PAGR (%) 70-90	90-98	LE 1970	1998	TFR 1998	% of PU 1998	AAGRUP (%) 70-90	90-98
Afghanistan	4	10321	4014	0.4	4.6	37	46	6.8	20	2.9	6.2
Bangladesh	48	55857	14697	2.5	1.6	44	58	3.1	19	5.9	3.8
China	79	380453	98570	1.6	1.0	61	70	1.8	31	3.8	3.4
India	49	395791	115615	2.1	1.8	49	63	3.1	27	3.4	2.2
Maldives	56	138	42	2.9	2.8	50	65	5.3	27	6.3	3.1
Nepal	51	11068	3449	2.5	2.5	42	58	4.4	11	6.6	4.5
Pakistan	41	71952	23470	3.0	2.7	49	64	5.0	34	4.2	3.6
Sri Lanka	137	6233	1590	1.5	1.0	65	73	2.1	23	1.3	1.9

<MR = Under Mortality Rank; PAGR = Population Annual Growth Rate (%); LE = Life Expectancy;
TFR = Total Fertility Rate; PU = Population Urbanized AAGRUP = Average Annual Growth Rate of Urban Population (%)
- = Data not available. x = Indicates data that refer to years or periods other than those specified in the column heading, Differ from the standard definition, or refer to only part of a country. *Data refer to the most recent years available during the period specified in the column heading

Table 3: Nutrition

Country name	< 5 M.R.	% of ILBW 90-97*	% of children 1990-99 who are EBF 0-3 Months	BFCF 6-9 Months	SBF 20-23 Months	% of <5 1990-98 suffering from Under weight MS	S	Wasting MS	SMS	Vit. A SCR 6-59 Months 1998	% of HCIS 92-98
Afghanistan	4	20	25	-	-	48	-	25	52	-	-
Bangladesh	48	50	52	69	90	56	21	18	55	95	78
Bhutan	41	-	-	-	-	38x	-	4x	56x	87	82
China	79	9	64	-	-	16	-	-	34	-	83
India	49	33	51	31	67	53	21	18	52	25	70
Maldives	56	13	8	-	-	43	10	17	27	-	-
Nepal	51	-	83	63	88	47	16	11	48	90	93
Pakistan	33	25	16	31	56	38	13	-	-	-	19
Sri Lanka	137	25	24	60	66	34	-	14	18	-	47

< 5 MR = Under 5 mortality rank; ILBW = Infant with Low Birth Weight; EBF = Exclusively Breast Feed
BFCF = Breast Feed with Complementary Food SBF = Still Breast Feeding <5 = Under 5
MS = Moderate and Severe S = Severe SMS = Stunting Moderate and Severe
SCR = Supplementation Coverage Rate HCIS = House-holds Consuming iodized Salt
- = Data not available. x = Indicates data that refer to years or periods other than those specified in the column heading, Differ from the standard definition, or refer to only part of a country. *Data refer to the most recent years available during the period specified in the column heading

that feeds persistent malnutrition (Fig. 2) (ACC/SCN, 2000). Existing data in Pakistan clearly supports the fact that maternal and childhood malnutrition is inexorably related (Khan and Bhutta, 2001).

Risk factors and determinate of malnutrition: That the problem of malnutrition is widespread in Pakistan a country that has been self sufficient in food production and availability is puzzling to many. It is important to recognize the importance of proximal or underlying determinants of malnutrition, as without addressing them, meaningful and sustainable change can be made difficult (Fig. 3).

Poverty does play a very important contributory role in malnutrition, as indicated by variable food consumption patterns of families of different income group (Planning Division, 1976-77; Khan and Bhutta, 2001; Final Report, 1998). Efforts to reduce under nutrition morbidity and mortality depends on reducing poverty and raising living standard by improving the quality of homes and by increasing access to clean drinking water and sanitation (World Health Organization, 2005).

Until recently in Pakistan, the trends in increasing poverty levels raised burden of debt, drought stagnant agriculture growth as well as natural calamities, these were also contributory factors and caused to malnutrition. Several successive governments have failed to recognize the importance of nutrition on the health and development of the populace and nutrition has thus remained the key element missing from the widely discredited social action program.

In addition to well designed intervention we need a mass campaign for public awareness by realizing the importance and impact of malnutrition towards nation health. Implementations of these interventions go some way towards fulfilling people's basic rights.

Any nutrition intervention should be part of community based intervention also targeting some of the underlying determinants of malnutrition such as household food security, culturally acceptable decision making for promotion of health and nutrition. These interventions must be firmly grounded in the principles of equity, community participation and ownership while retaining Scientific Validity. These interventions are particularly important during the period from birth to age three years,

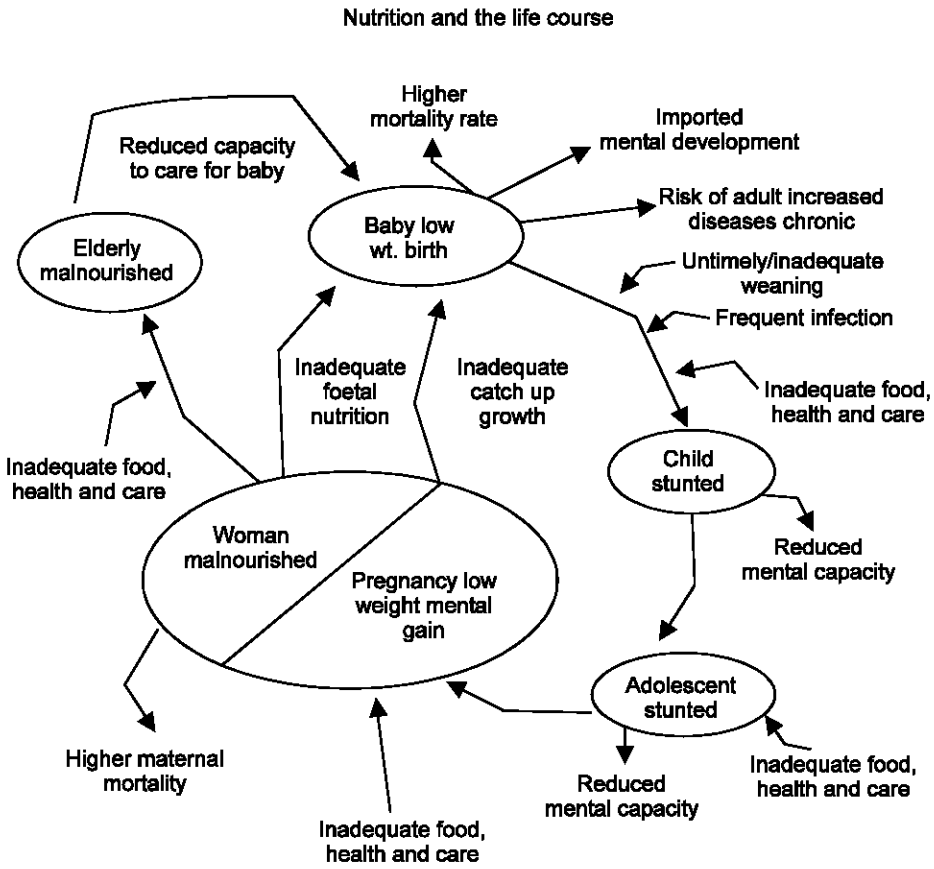

Fig. 2: Nutrition and the life course

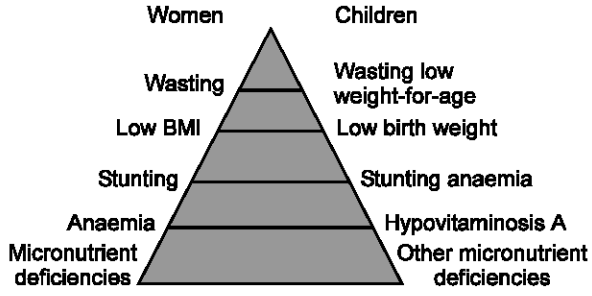

Fig. 3: What constitutes malnutrition?

the critical time in which growth failure and malnutrition occur (Shrimpton *et al*., 2001).

A national program addressing the needs of women and infant through a community based participating program and that is not owned by the community. It should be noted that no country in the world has achieved its economic and educational development targets with a sickly malnourished population. Pakistan cannot afford to ignore this any longer.

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