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Iron Status of Malaria Patients in Douala - Cameroon

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Abstract: In Africa, anaemia associated with malaria infection is a major cause of childhood morbidity and mortality. Problem of severe anemia linked to malaria is increasing as antimalarial drugs resistant parasites are widespread throughout Africa. In Cameroon, malaria turns out to be the major disease with the higher number of annual deaths, especially among children under fives and pregnant women. To assess the iron status among malarial patients in Douala where malaria is endemic, 163 malarial subjects (aged 0 to 60 years) and 98 uninfected volunteer subjects (aged between 0 - 65 years) were screened for this study. Iron status was evaluated using three biochemical (Serum Iron: SI, Total Iron Binding Capacity: TIBC and Transferrin Saturation: TS) and five haematological (Haemoglobin: HGB, Haematocrit: HTC, Mean Cell Volume: MCV, Mean Cell Haemoglobin: MCH and Mean Corpuscular Haemoglobin Concentration: MCHC) parameters. It was observed that 41.7% and 63.20% of malaria patients were serum iron and haemoglobin deficient respectively. Moreover, the rates of SI, TS, HGB, HTC, MCV and MCH were significantly lower in malarial than controls ($P<0.01$). However, the TIBC rates were significantly higher among the malarial in comparison with the uninfected subjects ($P<0.01$). This rate decreased with age while HGB, HTC and MCV percentages increased with age. Parasitic density is higher in patients aged between 0 - 3 years than those between 4 - 25 years and between 26 - 60 years of age. We noticed significant ($P<0.01$) increase of SI with moderate parasitemia. Significant correlations ($P<0.001$) were observed among malarial. Malaria negatively affects iron status, but we need further research on iron metabolism for the better comprehension of the mechanism by which *Plasmodium falciparum* interact with iron status.

Key words: Iron, malaria, parasitaemia, plasmodium falciparum, ferritin, transferrin

Introduction

Iron is an essential micronutrient necessary for the transportation of respiratory gases via haemoglobin in the red blood cells. Iron also intervenes in the constitution of enzymatic systems such as catalases, peroxydases and cytochromes that play an essential role in cellular respiratory mechanisms, in mitochondrial respiratory channel (Heberg and Galan, 1991). Iron has three levels of distribution in human body: "Functional" iron in haemoglobin, tissues and various haeminic enzymes; "Store" iron as ferritin and haemosiderin and "Circulating" iron bound to transferrin in the plasma.

Aneamia is a major and pressing problem around the world. Recent WHO statistics indicate a worldwide prevalence of about 30% with higher figures in developing countries. Many causes of anaemia have been identified: Nutritional deficiency due to lack of bioavailable dietary iron or vitamin folate, parasitic infections such as hookworm or malaria.

Iron deficiency anaemia is the main nutritional deficiency in the world. According to the WHO estimations, more than 700 millions people are affected by iron deficiency anaemia (INACG, 1986). The vulnerable groups are: Children, women of reproductive age and pregnant women (Crawley, 2004). Iron status of human is affected by the quality of the diet, the physiological and pathological status. Many infections among others: Schistosomiasis, ankylostomiasis and malaria (Olsen *et al*., 2000) are able to modify iron status. These modifications can cause iron deficiency or iron excess. The consequences of iron deficiency anaemia ranging from effects on energy metabolism and immune function to effects on cognitive and motor development (Walter, 1993).

Malaria is a disease caused by protozoa of the genus *Plasmodium*; it is a serious health problem in tropical and subtropical areas. World wide, more than 400 millions people are affected by malaria, with about 200 millions in Sub - saharan Africa. In Cameroon, malaria is a public health priority (Breman *et al*., 2004).

It would be important to investigate the effect of malaria on the iron status and to establish the relation between intravascular haemolysis and iron circulation, since red blood cells are the target for infection by *P. falciparum*.

Materials and Methods

This was a prospective study, carried out in Laquintinie Hospital of Douala - Cameroon; a littoral town situated along the river Wouri, in a transitional zone between the forest and savannah. The climate is the hot humid

equatorial climate which favors the development of anopheles mosquitoes, a vector for the transmission of malaria parasite. 163 volunteers' of malaria patients (78 males and 85 females) aged 0 to 60 years and 98 controls (42 males and 56 females) aged 0 to 65 years were enrol after informed consent.

Thick and thin blood films prepared from a finger prick with Giemsa stained and examined by light microscopy under oil - immersion objective, at 100X magnification. Parasitaemic (asexual or sexual) in thick films was estimated by counting asexual or sexual parasites relative to 1000 leukocytes. From this figure, the parasite density was calculated assuming a leukocytes count of 8000 $\mu 1^{-1}$ of blood.

Further, blood (1 - 2 ml) was collected by venipuncture from the arm and stored in sample tubes (one EDTA tube and one dry tube) and properly labelled. The tubes were then stored and transported to the laboratory where the dry tube was centrifuged at 4500 rpm for 5 min.

The serum obtained from it was used for biochemical analysis: Serum iron (SI), Total iron binding capacity (TIBC), Transferin saturation (TS). The EDTA tubes was used for Haematological analysis: Haemoglobin (HGB), Haematocrit (HTC), Mean cell volume (MCV), Mean cell haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) using an Automatic Counter of blood cells (ABX). The study was approved by the Ethical Committee of Clinical Study (ECCS) at the University of Douala.

Statistical analysis was done using Pearsons's correlation coefficient. Student's t - test and the analysis of variances (ANOVA) with Statistica and SPSS 6.1 software.

Results

An overview of Table 1 shows that: age and parasitaemia varies from 0 to 60 years and from 1 to 12% respectively, whilst controls, aged between 0 to 65 years, were distinguished by the presence of no positive thin or thick blood smear during malaria diagnosis. The analysis of biochemical and haematological parameters using Student t-test shows significant decrease (at 1%) of SI, TS, HGB, HTC, MCV and MCH in malaria patients; but the TIBC is significantly high in malaria patients. As far as MCHC is concerned, no significant variation was observed between malaria patients and controls.

Frequencies distributions of parameters studied (SI, TS, HGB and HTC) of 163 malaria patients and 98 controls are presented in Table 2. In the population of patients, 41.71% and 63.19% have low values in SI and HGB respectively. More than half of these patients were between 0 - 3 years of age with 20.85% and 32.50% of deficiencies for SI and HGB respectively. Whereas in the controls population, 5.10% and 23.46 % of subject were respectively deficient on SI and HGB.

Table 3 presents the results by parasitaemia intervals. We notice that patients with smooth parasitaemia (< 1%) have an SI mean of 53.59±2.69µg/dl. The SI mean (81.68±6.16 µg/dl) is significantly high ($P<0.01$) with moderate parasitaemia (1 - 5%); But we notice a significant decrease ($P<0.01$) in SI (57.35±8.53 µg/dl) with high parasitaemia (>5%). Concerning the TIBC, the mean is proportional to parasitaemia ($P=0.01$). But HGB, HTC and MCV values decreased with high parasites densities.

In Table 4, the results by age intervals in malaria patients are presented. The TIBC is significantly higher in patients aged between 0 - 3 years than in those between 4 - 25 and 26 - 60 years ($P<0.01$). However, we noticed a significant variation at 1% of HGB, HTC and MCV between age intervals; the mean in infants are then less high than those in adults.

The analysis of results by sex at 5% shows that no significant difference between males and females concerning the parameters of our interest in our patients. In Table 5 we noticed the high number of correlations between parameters analysed.

Discussion

After a general analysis of the results, we have observed that SI, TIBC, TS, HGB, HTC and MCV, are highly affected by malaria ($P<0.01$), whereas MHCH does not shows significant variations at 5%. These differences observed between the two groups of individuals (malaria patients and controls) could be essentially due to clinical manifestations of malaria: Fever, pulse acceleration, sweating and shivering (Lariviére *et al.*, 1987); physiological phenomena, that upset iron metabolism. Moreover, during an infection such as malaria, minerals are redistributed from circulation to tissues and cause a reduction of minerals in the circulation (Keusch, 1998). All these explain why SI, TS, HGB, TC and MCV are significantly lows in malaria patients than in controls. The increase of TIBC proves that stored iron is exhausted (INACG, 1986). This rise also shows liberation of iron in the plasma, an increase in transferin synthesis and of transferin activity.

The rise of serum iron with moderate parasitaemia is due to iron from haemolysis, because we have also noticed a reduction of HGB. Moreover, during certain diseases, SI increases after haemolysis (Olsen *et al.*, 2000). HGB is a constituent of red blood cells and the destruction of red blood cells lead to the release of iron into the plasma. Therefore, the rise of SI is not for a long time because significant reduction of HGB brings significant decrease of SI. Further, clinical manifestations of malaria and the non respect of RDA (Recommended Dietary Allowance) can affect iron bioavailability and it metabolism (Caulfield *et al.*, 2004). HGB, HTC, MCV are lows in the age interval of 0 - 3 years compared to those of 4-25 and 26-60 years (Table 4). These differences could be explained by the parasitaemia, higher in the 0 - 3 years old patients than

Table 1: Means and ranges of results in malaria patients and controls

	Groups			
Parameters	Malaria patient (n=163)	Controls (n=98)	F	P
Age (years)	16.25±1.27[a]	22.05±1.99	12.4267	<0.01**
	0-60[b]	0 - 65		
Parasiteamia (%)	2.96±0.23	-	49.5049	/
	1-12			
[SI] (µg/dl)	59.73±2.49	83.68±3.50	32.3222	<0.01**
	20.09-196.45	35.05-199.27		
[TIBC] (µg/dl)	305.49±9.24	257.51±7.98	12.7415	<0.01**
	85.24-773.28	120.12-482.43		
TS(%)	20.93±0.73	32.89±0.83	109.2634	<0.01**
	5.02-39.73	19.16-64.58		
[HGB] (g/dl)	10.07±0.13	12.00±0.13	89.4905	<0.01**
	5.2-13.90	10.00-15.10		
HTC (%)	30.25±0.43	35.42±0.37	66.2080	<0.01**
	16.50-44.50	26.20-44.10		
MCV (μm^3)	76.52±0.48	83.40±0.467	90.6304	<0.01**
	54.00-90.00	78.00-94.00		
MCH (pg)	25.86±0.24	27.79±0.24	28.4648	<0.01**
	14.40-32.00	22.5-34.60		
MCHC (g/dl)	32.97±0.14	33.18±0.20	0.7039	0.40 NS
	26.60-38.20	29.70-37.50		

[a]:Mean±SE, [b]: ranges, SI: Serum Iron, TIBC : Total Iron Binding Capacity, HGB: Haemoglobin, HTC: Haematocrit, TS: Transferrin Saturation, MCV: Mean Cell Volume, MCHC: Mean Corpuscular Haemoglobin Concentration, MCH: Mean Cell Haemoglobin,
**:Significant at 1%, NS: Not Significant. N: Number of subjectL.

Table 2: Frequencies distribution of parameters analyzed according to age levels

	Patients				Controls
Parameters	0-3 years n=70	4-25 years n=59	26-60 n=34	Total n=163	n=98
SI<45µg/dl or TIBC<16% (low)	34[a] (20.85%)[b]	21 (12.88%)	13 (7.97%)	68 (41.71%)	5 (5.10%)
SI ≥ 45 and ≤ 160 µg/dl or TIBC ≥ 16 and ≤ 35%:(normal)	32 (19.63%)	36 (22.08%)	19 (11.65%)	87 (53.37%)	78 (79.59%)
SI>160µg/dl or TIBC>35%: (high)	4 (2.45%)	2 (1.22%)	2 (1.22%)	8 (4.90%)	15 (15.30%)
HGB<11g/dl or HTC<33% (low)	53 (32.50%)	32 (19.63%)	18 (11.04%)	103 (63.19%)	23 (23.46%)
HGB ≥ 11 and ≤ 15g/dl or HTC ≥ 33 and ≤ 44% (normal)	17 (10.12%)	27 (16.59%)	16 (9.81%)	60 (36.80%)	73 (74.48%)
HGB>15g/dl or HTC>44% (high)	0 (0.00%)	0 (0.00%)	0 (0.00%)	0 (0.00%)	2 (1.02%)

[a]: number of subjects. [b]: frequencies

Table 3: Results obtained by ranges of parasitaemia

	Ranges of parasitaemia			
Parameters	Smooth: <1% N=104	Moderated: 1-5% N=32	High: >5% N=27	P (ANOVA)
Age (years)	18.37±1.69[a]	8.80±2.35	3.90±1.06	<0.01**
	0-60[b]	0 - 60	0-22	
Parasiteamia (%)	0.426±0.022	2.659±0.256	7.929±0.436	<0.01**
	0.1-0.9	1-5	5-12	
[SI] (µg/dl)	53.59±2.69	81.68±6.16	57.35±8.53	<0.01**
	20.58-125.29	38.76-160	20.09-196.45	
[TIBC] (µg/dl)	291.70±11.71	298.55±17.10	366.82±23.05	0.01*
	88.26-773.28	104.33-500.20	80.24-696.27	
TS(%)	19.83±0.76	28.01±1.63	16.76±2.09	<0.01**
	7.86-39.39	10.91-39.73	5.02-36.04	
[HGB] (g/dl)	10.28±0.16	10.59±0.21	8.61±0.33	<0.01**
	5.20-13.90	8.20-12.30	5.50-11.90	
HTC (%)	31.02±0.55	31.19±0.67	26.16±1.04	<0.01**
	17.30-44.50	25.00-38.20	16.50-36.50	
MCV (μm^3)	77.31±0.63	75.46±0.89	74.74±0.88	0.08 NS
	54.00-90.00	60.00-83.00	65.00-86.00	
MCH (pg)	26.17±0.33	25.51±0.44	25.07±0.42	0.19 NS
	14.40-32.00	19.20-29.90	22.10-30.40	
MCHC (g/dl)	32.82±0.20	33.17±0.22	33.32±0.31	0.38 NS
	26.60-38.20	30.50-35.30	29.70-36.20	

NS: Not significant *:Significant at 5% **:Significant at 1%, [a]:mean ± SE. [b]Ranges.

Table 4: Results by age intervals in malaria patients

	Age intervals (Years)			
Parameters	0-3 [N =70]	4-25 [N = 59]	26-60 [N =34]	P (ANOVA)
Parasiteamia (%)	3.365±0.432[a]	1.483±0.293	0.602±0.108	<0.01**
	0.1-12[b]	0.1-9.2	0.1-3.5	
[SI] (µg/dl)	61.18±4.11	58.58±4.17	58.73±4.54	0.88NS
	20.09-196.45	20.69-159.02	20.58-125.29	
[TIBC] (µg/dl)	335.38±12.45	294.26±16.13	262.79±20.74	<0.01**
	128.79-696.27	80.24-773.28	90.00-502.62	
TS(%)	19.29±1.18	21.40±1.22	23.47±1.30	0.09NS
	5.02-39.73	7.50-39.16	9.21-39.39	
[HGB] (g/dl)	9.47±0.21	10.42±0.15	10.68±0.37	<0.01**
	5.20-12.60	6.80-12.60	5.20-13.90	
HTC (%)	28.21±0.60	31.49±0.56	32.29±1.20	<0.01**
	16.50-40.20	23.50-42.00	17.30-44.50	
MCV (μm^3)	73.95±0.60	77.30±0.74	80.47±1.15	<0.01**
	58.00-84.00	55.00-90.00	54.00-90.00	

NS: Not significant *: Significant at 5% **: Significant at 1%. [a]: mean±SE [b]: Ranges.

Table 5: Correlations observed among subjects

Para meters	FS	CTF	CST	HGB	HTC	VGM	TGMH	CCMH	Para
A-Infected subjects									
CTF	0.4172"***[b]	/							
CST	0.6310***	-0.3776***	/						
HGB	0.3442***	-0.1456[NS]	0.4536***	/					
HTC	0.2717***	-0.1067[NS]	0.3774***	0.8673***	/				
VGM	0.2033**	-0.1504[NS]	0.3053***	0.5139***	0.4856***	/			
TGMH	0.1046[NS]	-0.2009**	0.2447**	0.4767***	0.3447***	0.7916***	/		
CCMH	-0.0404[NS]	-0.1939*	0.0895[NS]	0.1142[NS]	-0.1055[NS]	0.1943*	0.4847***	/	
Para	0.0949[NS]	0.2454**	-0.0984[NS]	-0.3759***	-0.3711***	-0.1550*	-0.0960[NS]	-0.1551*	/
Age	-0.0238[NS]	-0.2568***	0.2010**	0.2520***	0.2915***	0.4099***	0.2756***	-0.1486[NS]	-0.3231***
B-Uninfected subjects									
CTF	0.7937"***	/							
CST	0.5523***	0.0170[NS]	/						
HGB	0.3018**	0.1626[NS]	0.3906***	/					
HTC	0.4557***	0.2462*	0.4401***	0.7635***	/				
VGM	0.2913**	0.1547[NS]	0.2900**	0.4267***	0.4460***	/			
TGMH	0.0958[NS]	0.0618[NS]	0.0545[NS]	0.3104**	0.1083[NS]	0.4885***	/		
CCMH	-0.1078[NS]	0.0435[NS]	-0.0160[NS]	0.2616**	-0.0807[NS]	0.2638**	0.6194***	/	
Age	0.1605[NS]	0.1179[NS]	0.0821[NS]	-0.1776[NS]	0.0304[NS]	0.1607[NS]	0.1316[NS]	0.0137[NS]	

[a] = Coefficient de corrélation de PEARSON, [b]= Valeur de P. NS = Non significatif, * = Significatif au seuil de5%, ** = Significatif au seuil de 1%. *** = Significatif au seuil de 1% 0.

in others. Parasitic densities of malaria in the tropical and equatorial zones are higher in infants, but decrease with age (Crawley, 2004), because of acquired immunity and consolidation of immune system in adults whereas this system seems weak in infants. Among infected subjects, positive correlations (P<0.001; r = 0.63; P<0.001, r = 0.86) was observed between SI and TS, HGB and HTC respectively. But a negative correlation between age and parasitemia (P<0.001, r<-0.3) was also observed.

We have also noticed that 63.19% of malaria patients have low levels of haemoglobin, a trend toward anaemia. That is in line with WHO data which consider HGB as a true maker of iron deficiency anaemia and that infant are more affected than adults (Mungala *et al.*, 2004).

Our results suggest that malaria negatively affects the iron status in human. This is in accordance with the result obtained by Oppenheimer *et al.* (1986a, 1986b). In fact they have reported a carefully controlled clinical trial of the effects on morbidity of Fe supplementation in a chronically Fe-deficient population. Because of the degree of Fe deficiency they hypothesized that Fe would have a beneficial effect on infection morbidity; on the contrary they found that the children who were given Fe had an increased morbidity; in particular, they had a higher prevalence of malaria and respiratory infection with undefined organisms. Some authors (Murray *et al.*, 1975, 1978, 1980, Keusch and Farthing, 1986) have provided evidence from studies in East Africa that there is an increased risk of malaria and tuberculosis when Fe supplements are given to humans. These reports have been supported by research on animals (Keusch and Farthing, 1986).

Thus, several studies provide strong evidence that Fe deficiency can protect against particular infections and Fe supplementation may increase the risk of these infections. However further investigations taking into

account iron metabolism in *P. falciparum* are necessary. Because, much of the confusion and argument that surrounds the relationship between Fe stains and infection stems from a failure to appreciate the heterogeneity of infections, the different strategies adopted by organisms, the relative importance of the various host defences, the different ways in which the host is compromised and the consequences of changes in Fe metabolism on both host and parasite need to be investigated.

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Evaluation of Moisture, Total Cyanide and Fiber Contents of Garri Produced from Cassava (*Manihot utilissima*) Varieties Obtained from Awassa in Southern Ethiopia

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Abstract: Cassava *(Manihot esculent crantz)* as one of the most important root crops in Sub-Saharan Africa plays a vital role in the diet of many African countries especially the grass root people, being the major source of daily carbohydrate intake. Traditionally processed staple foods from cassava are available in Western and some Eastern African countries, such as garri, foofoo, boiled cassava meal, etc. Several varieties of *M. Utilissima* obtained from Awassa, Ethiopia, were processed, fermented and converted into garri. The moisture, total cyanide and fibre contents of the processed garri were analyzed. The results showed that the moisture, total cyanide and fiber contents varied from 26.12-40.02 %, 1.51-2.81 mg HCN/100 g and 1.80-2.40% respectively. The largest reduction in cyanide content (41%) between the third and fourth day fermentation was found in the MM 96/5280 variety which had an increase of 32% fiber content when compared with other varieties. These results compared favorably with similar garri products obtained from Ghana and Nigeria. The ease of preparation and potential nutritional value resulting from fermentation, the low moisture, cyanide and improved fiber contents found, indicate the suitability of the garri products as nutritional food items. The Kello44/72 and MM96/5280 varieties with the lowest cyanide and comparable fiber contents are most suitable. In order to enrich the nutritional contents of the garri product, fortification with leguminous plants or fish is recommended so as to make it more suitable as an alternative food product to injera in Ethiopia.

Key words: Cassava, fermentation, garri, total cyanide, fiber

Introduction

Cassava is one of the few most important root crops known and used in many countries of Africa, Latin America and some Asian countries. Though it has its origin in South America, cassava has become an indigenous crop in the tropics having been widely grown and used. It has played vital roles in the diets of many African countries as a major source of low cost carbohydrate (O'Hair, 1990). Cassava contains significant amount of iron, phosphorus, calcium, and is relatively rich in vitamin C. In many parts of Western and Central Africa, cassava is being processed into a number of traditional staple foods. Dried tubers of cassava can be milled into flour. Maize may be added during the milling process to add protein to the flour. The flour can be used for baking breads. Typically, cassava flour may be used as partial substitute for wheat flour in making bread. Bread made wholly from cassava has been marketed in the U.S.A. to meet the needs of people with allergies to wheat flour (O'Hair, 1995).

Garri, one of the staple food products from cassava is obtained by fermentation and subsequent frying; a process similar to the production of *injera* (Ethiopia staple food) from teff. Garri can be fortified with legume like soybean to offer a nutritionally rich meal.

The major factor that limits the use of cassava as food is the toxicity of hydrogen cyanide (HCN) which occurs as a result of the hydrolysis of cyanogenic glucosides (Rickard, 1985). The cyanide present in cassava may be considered to be of two types: Bound cyanide present as the cyanogenic glucoside and free cyanide present as the cyanohydrin, that is, free hydrogen cyanide which is a gas above 26°C (under alkaline conditions) and as cyanide ion, CN^- (Bradbury and Holloway, 1988). The total cyanide which comprises both the bound and the free cyanide indicate the potential of the cyanogenic glucoside in the root tubers or leaves. Cyanide is widely distributed in nature and is normal constituent of blood, usually at low concentration, <12μ mol 1^{-1} (Solomonson, 1981).

Using improperly processed cassava can increase the cyanide content in the human body and eventually cause goiter, cretinism, paralysis and neurological disorders (Delange and Ahluwalia, 1983) as cited in Bradbury *et al.* (1991). There is a great concern about the levels of cyanide in many varieties of cassava products including garri. There is equally good evidence that some fibre depleted diets cause pathological effects (Umoh *et al.*, 1984). These effects are manifested not only in the gastro - intestinal tract but other anatomical structures

such as the arteries, lower limb veins and gall bladder. Cyanide in cassava has been linked with tropical amploipia (blindness that is common in West Africa) and tropical ataxic neuropathy (TAN) (Umoh *et al.*, 1984). The effects of storage and cooking practices on the total cyanide content of two cultivars have been investigated. The highest total cyanide reduction was obtained when the tubers were soaked in water, sun-dried and baked (Taye and Biratu, 1999). The cyanide and fibre contents of different garri samples obtained from various sources in Nigeria have been determined by (NIS, 1988; Ukpabi and Ndimele 1990; Nwokoro *et al.*, 2005). The fibre content in garri and feeds generally has been regarded as being of no nutritive value, but its importance in recent times has been appreciated both clinically and in animal husbandry. Through the various processing operations which involve peeling, washing, grating, fermentation, drying/dewatering, milling/ pulvering and frying/roasting, the level of the hydrogen cyanide content has been significantly reduced if not completely eliminated (Ihekoronye and Ngoddy 1985; O'Hair, 1990; Massaquoi *et al.*, 1990). Recently, Malu *et al.* (2007) reported a 36% reduction in cyanide content between the third and fourth day fermentation with a minimal increase in the fiber content of processed garri from Nigeria.

Cassava has been grown and used as food for about a century in different regions of Ethiopia. In the Southern Ethiopia, particularly in Amaro-kello area (Gedeo Zone), cassava is almost used as a staple food. In Wolaita area (North Omo Zone), cassava roots are widely consumed after washing and boiling or in the form of bread and "*injera*" after mixing its flour with that of some cereal crops such as maize (Zea mays), sorghum (sorghum bicolor), or Tef (*Eragrostis tef*) (Taye, 1994). Several local and identified cultivars which vary in their morphology, agronomic characters and cyanogenic glucosides content are cultivated in the Southern and South Western regions of Ethiopia (Taye, 1993).

This paper reports on the levels of moisture, total cyanide and fiber present in garri products from several varieties of cassava obtained from Awassa, Ethiopia, in order to determine both their nutritional contents and suitability as an alternative staple food product to injera in Ethiopia.

Materials and Methods

Seven varieties of cassava (*Manihot utilissima*) tubers namely Kello 44/72, Quelle 104/72, MM96/7151, MM 96/5280, MM96/1871, MM96/3868 and Amarokello (local red) were obtained from Awassa Agricultural Research Centre (AARC) Awassa, Ethiopia. A sample of garri product was obtained from Uyo, Nigeria and another sample produced in Ghana was obtained from Shoa supermarket in Addis Ababa, Ethiopia. Grater, sifter, turning stick and bailing dish were fabricated using locally obtainable materials. *Injera* baking pot which served as the frying pot and plastic containers were obtained from the local market in Awassa town. Sticks used for the wooden pressing machine were also locally procured.

Sample preparation: 5kg each of fresh tubers from the seven varieties were peeled, washed with water, grated, packed in cotton cloth bags and fermented using the wooden pressing machine. Each sample was fermented for 3 and 4 days respectively. The fermented and semi-dry cassava pulp was pulverized and fried to produce garri.

Analysis: Total cyanide content of the garri samples was obtained through hydrolysis and the isolate determined by the method of A.O.A.C. (1975). The fiber content was determined by acid digestion as percentage of dry matter (%DM), while the moisture content was determined using the method of A.O.A.C. (1975).

Statistical analysis: The data on moisture, cyanide and fiber contents for the seven garri varieties and the Nigerian and Ghanaian products were subjected to Chi-square analysis. Significance was accepted at 5% probability level. Values were reported as the mean ± SD for five determinations.

Results

The results of moisture, cyanide and fiber contents of garri produced from seven varieties of cassava (*Manihot utillissima*) after 3 days of fermentation are presented in Table 1. There were significant differences ($p < 0.05$) in moisture and cyanide contents among the various garri products. Moisture contents were higher in MM96/3868, MM96/1871 and MM96/5280 and lower in the other varieties. The total cyanide in MM96/3868 and Quelle 104/72 were also higher than the other varieties with the lowest content found in Kello44/72. The fiber contents in all the samples were similar. Table 2 shows the results of the studies on the 4 days fermented garri. There were significant differences ($p < 0.05$) in the moisture, cyanide and fiber contents of the various garri products. Moisture contents were higher in MM96/5280, MM96/1871 and MM96/7151 and lower in the other varieties but all the varieties were lower in moisture content when compared with the varieties obtained from Nigeria and Ghana (produced after 4 days fermentation). The total cyanide content was higher in Quelle104/72 and MM96/3868 and lower in other varieties including the Nigerian variety with the lowest value found in Kello44/72 and MM96/5280. But the highest cyanide content compared with that obtained for the variety from Ghana. The fiber contents were however similar in all the varieties averaging 2.26% DM among the Awassa varieties but comparable

Table 1: Moisture, cyanide and fiber contents of garri produced after 3 days fermentation

Cassava variety	Moisture content (%)	Cyanide content (mgHCN/100g)	Fiber content (% DM)
Kello 44/72	26.21±0.01	1.89±0.02	1.70±0.01
Quelle 104/72	26.90±0.02	2.80±0.02	1.75±0.01
MM96/7151	28.71±0.01	2.70±0.02	1.76±0.01
MM96/5280	30.02±0.02	2.59±0.03	1.78±0.01
MM96/1871	30.44±0.02	2.59±0.02	1.74±0.02
MM96/3868	30.61±0.04	2.81±0.01	1.80±0.01
Amarokello	26.12±0.13	2.69±0.01	1.79±0.02

Values are mean ±SD for five determinations.

Table 2: Moisture, Cyanide and Fiber Contents of Garri produced after 4 days Fermentation

Cassava variety	Moisture content (%)	Cyanide content (mgHCN/100g)	Fiber content (%DM)
Kello 44/72	25.09±0.26	1.51±0.13	1.98±0.01
Quelle 104/72	25.18±0.15	2.70±0.12	2.02±0.02
MM96/7151	27.20±0.07	2.05±0.03	2.30±0.04
MM96/5280	28.13±0.04	1.51±0.03	2.35±0.08
MM96/1871	27.48±0.14	2.26±0.17	2.40±0.05
MM96/3868	25.58±0.38	2.70±0.01	2.40±0.04
Amarokello	24.90±0.12	2.53±0.01	2.37±0.02
Nigeria	29.47±0.20	2.05±0.02	2.35±0.07
Ghana	29.48±0.16	2.76±0.03	2.30±0.05

Values are mean ± SD for five determinations.

to the samples obtained from Nigeria and Ghana, with the lowest value found in Kello44/72 variety. The percentage reductions in moisture and cyanide contents of the processed garri between the third and the fourth day fermentation are presented in Fig. 1.

Discussion

As shown in Table 1, Kello 44/72, Quelle 104/72 and Amarokello (local red) showed similar moisture contents which averaged 26.41% but much lower than the 29.95% average obtained for MM96/7151, MM96/5280, MM96/1871 and MM96/3868. The cyanide content (1.89 mgHCN/100g) in Kello 44/72 was lower than the average of 2.6 mgHCN/100g observed in the other samples. The results of 4 days fermentation presented in Table 2 showed that, Kello 44/72, Quelle 104/72, MM96/3868 and Amarokello (local red) had similar moisture contents but these were lower than that of MM96/7151, MM96/5280 and MM96/1871. From the results, there are significant reductions in the cyanide contents of the four-day fermented garri in the range of 3.6-41.7% and the moisture contents in the range of 4-16% (Fig. 1). Corresponding increases in the levels of fiber which ranged from 15-37% were observed for all the samples with the lowest in Kello44/72 (15%) and the highest in MM96/1871 (37%). In comparison with 29.47 and 29.48% moisture, 2.05 and 2.76 mgHCN/100g cyanide and 2.35 and 2.30 (%DM) fiber contents obtained from garri samples produced in Nigeria and Ghana respectively, the cyanide content in Kello44/72 and MM96/5280 were significantly lower, while the

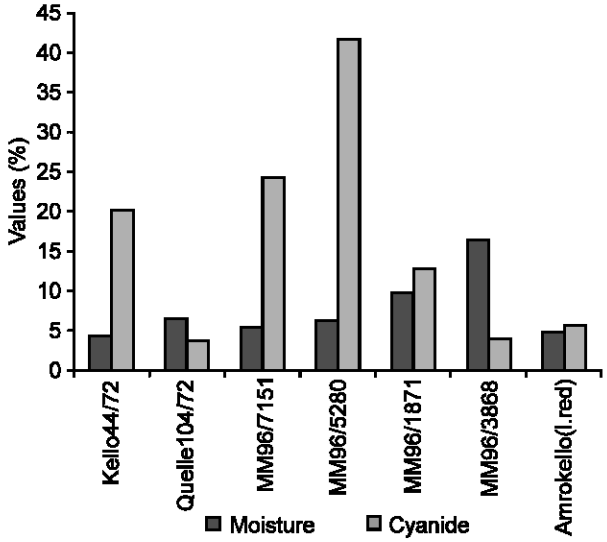

Fig. 1: Percentage reduction in moisture and cyanide contents of garri between 3 and 4 days fermentation.

moisture and fiber contents were similar. Generally, the cyanide range of 1.51 to 2.81 mgHCN/100g obtained from the three and four days fermentation experiments are within the reported values of 0.84 and 2.12 mgHCN/100g by Nwokoro *et al.* (2005) and 0.00 and 3.20 mgHCN/100g by Hahn (1983). The results are also within the 2-3 mgHCN/100g regarded as acceptable level of cyanide in garri (IITA, 1989). The fiber contents also agree with the crude fiber range of 0.5 to 3.0 (%DM) reported by Ukpabi and Ndimele (1990), for commercially available garri although the Nigerian Industrial Standard, NIS (1988) had recommended that garri should have crude fiber of less than 2.0%. The importance of fiber clinically has been reported by Umoh *et al.* (1984) which stated that fiber depleted diets cause pathological effects which manifest in the gastro-intestinal tracts as well as other anatomical structures such as the arteries, lower limb veins and gall bladder, suggesting therefore that there is need for minimum obtainable level of fiber in diets.

As cyanide is very poisonous because it binds cytochrome oxidase and stops its action in the electron transport chain, which is a key energy conversion process in the body, excess cyanide content in cassava products could have deleterious effects on the consumer. Non-fatal amounts of cyanide cause acute intoxication with symptoms of dizziness, headache, stomach pains, vomiting and diarrhea (CCDN, 2006). As has been shown in the studies, the extension of fermentation period from 3 to 4 days had the advantage of greater reduction in cyanide through further breakdown of cyanogenic glucosides in the cassava pulp as well as leaching out of HCN along with the

cassava fluid. The 4-41% reduction in cyanide content in the 4-day fermentation product (Fig.1), is in accordance with the FAO report that longer fermentation period reduces the content of free hydrocyanic acid, as well as the moisture content (FAO, 1981). Odoemelam (2005) also reported that, the longer the fermentation period, the less the residual cyanide content in the final garri product. However, the variation in the cyanide concentration of the individual garri samples is attributable to differences in the cassava cultivars as reported by O'Brien *et al.* (1992). Asegbeloyin and Onyimonyi (2007) also reported that the crude protein content of garri product obtained by fermentation was higher (2.65%) than that obtained (2.03%) without fermentation. In view of the low protein content and lack of essential amino acids of most cassava products, methods of upgrading the protein content of cassava and reducing the anti-nutrient content such as cyanide, phytate and tannin have been developed (Odetokun *et al.*, 1998). Fortification of garri with proteinous plants such as soybeans and melon has been reported Oshodi (1988). Recently, Ugwu and Odo (2008) reported that protein content in Soy-garri ranged from 5.15 to 6.17% as against protein content of 0.7 to 1.2% in normal garri.

From the results, the garri produced from the various cassava species obtained from Awassa, with their low moisture and cyanide contents along with considerable amount of fiber indicate their suitability as nutritional food products. The Kello44/72 and MM96/5280 are more suitable in view of their considerably low moisture and cyanide contents. The garri products can however be fortified to enrich their nutritional value with leguminous plants such as soybean, melon or fish in order to make them more suitable and attractive as an alternative staple food to injera particularly in Ethiopia.

Conclusion: Garri produced from Awassa cassava tubers compared favorably with similar garri products obtained from Ghana and Nigeria. The low moisture, cyanide and increased fiber contents indicate their suitability as nutritional food product. The Kello44/72 and MM96/5280 varieties with the lowest cyanide and comparable fiber contents are most suitable. For improved nutritional value, fortification with such proteinous plants as legumes (soya bean and melon) or fish is recommended.

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Microflora and Pathogen Bacteria (*Salmonella*, *Klebsiella*, *Yersinia*, *Pseudomonas*, *Aeromonas*, *Escherichia coli*, *Staphylococcus aureus*) In Urfa Cheese (A Traditional White-Brined Turkish Cheese)

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Abstract: Urfa cheese is normally produced from ovine or bovine milk or appropriate mixture of these two especially in Urfa. Urfa cheeses are being manufactured, as regional, by the use of raw milk or scalded milk that has been heated up to 30-35°C. This cheese use to be consumed in the region, without waiting the completion of its maturation period. Gastroenteritis case number in Urfa is considerably high as compared to the other regions. For these reasons 11 different Urfa regional cheeses have been purchased within their brine concentration. They were studied during 0-7th, 30th and 60th day as regard to micro flora and pathogen microorganisms. At the end of micro flora research of Urfa cheeses, *Escherichia, Salmonella, Enterobacter, Klebsiella, Pseudomonas, Yersinia, Aeromonas, Hafnia, Serratia, Morgenella* and also *Cedecea, Citrobacter, Hafnia* have been detected. Besides, *Staphylococcus* (*S. aureus)* has been determined therein. *Steptococcus, Lactococcus, Lactobacillus, Pediococcus, Leuconostoc* were identified as well. In our research we have detected that the variety of micro organism are quite a lot in Urfa cheeses. We identified various pathogen bacteria also. It has recommended that the region's people not consume fresh Urfa cheese.

Key words: Urfa cheese, microflora, bacteria enumeration, *Staphylococcus aureus, Escherichia coli, Yersinia enterocolitica, Aeromonas, Salmonella typhi*

Introduction

The use of pasteurized milk and milk products is not a health risk for human. After pasteurization milk products can be contaminated by pathogen microorganisms. In Turkey milk products do not manufactured on an industrial scale, some as still being produced traditionally at a small diary level. These kinds of products every time carry risks for pathogen microorganisms. Cheese is an important integral part of diet consumed in Turkey. Urfa cheese is a traditional white cheese produced in mainly South-east of Turkey. It is normally produced from ovine or bovine milk or appropriate mixture of these two. In the traditional method of manufacture of Urfa cheese, microbiological safety was provided by keeping cheese in a very dense brine solution. Sometimes manufacturers scald the fresh cheese blocks in boiling whey for about 2-3 min. However scalding is not satisfactory enough to provide microbial safety.

Materials and Methods

Cheese: Eleven different Urfa cheeses were purchased from 11 different markets in Urfa on May 2002. They were transported to the Ankara Gazi University, Biology Faculty laboratory under 4°C for microbiological analyses. Isolations and identifications were carried out in Gazi University Biology Faculty, microbiology laboratory.

There isn't any Standard cheese-making method of Urfa cheese but the traditional cheese-making procedure is outlined in Fig 1.

Microbiological analyses

Cheese processing and sampling: The preparation of samples was determined accordance to Turkish Standards TS 591 (Anonymous, 591, 1989). Samples were taken from cheese pieces after brining and at days 0-7, 30 and 60 of storage. Sampling for microbiological examinations was done according to White *et al*. (1993). Representative 10 g. samples of the cheese were taken aseptically using an ethanol-sterilized cheese borer. One percent (wt/v) peptone water was used for the preparation of the decimal dilutions. The solid samples (10 g.) were blended with 90 ml. of peptone water using a stomacher 10^{-1} of dilution and 0.1 ml. of cheese brine were poured onto duplicate plates.

Enumeration and assessment of total alive mesophyll bacteria: To utilize total alive mesophyll bacteria counts and isolation, the cheese samples of which dilution has

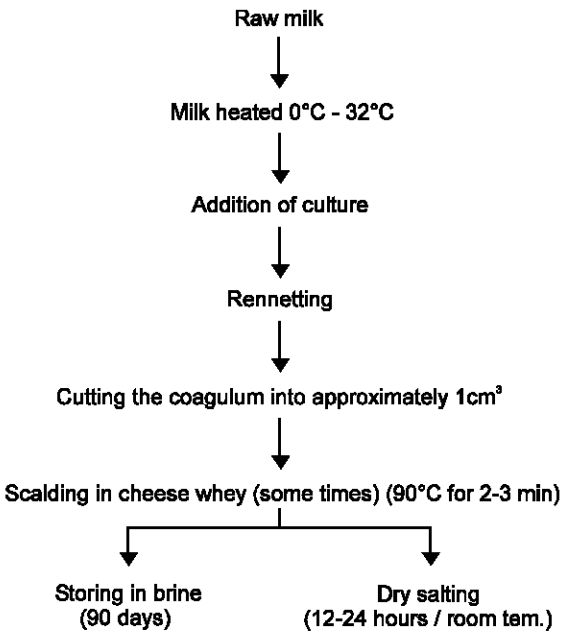

Fig 1: Flow diagram of Urfa cheese-making.

been prepared plated on Plate Count Agar (PCA). Plates were incubated at 30°C for a period of 48 hours. Afterward, the total microorganism count in 1 gram and 1 ml. sample was separately calculated by taking the colonies at the plates and the dilution rates into account. (Anonymous, 7725,1989).

Enumeration and assessment of total alive coliforms: To utilize total alive coli form bacteria counts and isolation, the cheese samples of which dilution has been prepared plated on Eosin Methylene Blue agar (EMB: Difco, Detroit, Michigan), Brillant green agar (BG: Difco, Detroit, Michigan) and Mc Conkey (Difco, Detroit, Michigan) agar .Plates were incubated at 37°C for a period of 48 hours. For identification of Gram negative colonies they were examined for mannitole, sucrose, glucose, lactose fermentation, indole production lisine and ornithine decarboxylase, aesculine hydrolysis. They were screened using triple sugar iron agar (TSI: Oxoid, Basingstoke, Hampshire, UK), Simmons citrate agar (Merck, Darmstadt, Germany) and urea agar. Biochemical characterization of the strains was performed with bioMerieux API 20 E (Marcy-l'Etoile, France). Afterward, the total microorganism count in 1 gram and 1 ml. sample was separately calculated by taking the colonies at the plates and the dilution rates into account. (Anonymous, 7725, 1989).

Enumeration and assessment of *Salmonella* and *Shigella:* To utilize total alive coli form bacteria counts and isolation, the cheese samples of which dilution has been prepared plated on *Salmonella* and Shigella Agar

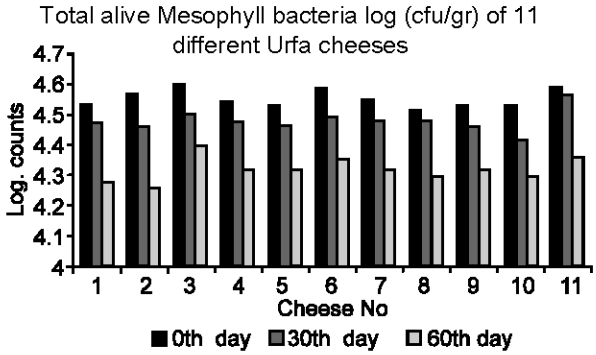

Fig. 2: Total alive mesophyll bacteria count (cfu/gr) of 11 different cheese samples.

(SS: Oxoid, Basingstoke, Hampshire, UK). Plates were incubated at 37°C for a period of 24 hours. Typical colonies were subjected to tests which include the lactose, mannitole, raffinose, sucrose, xylose, indole, urea, citrate, H_2S, arjinine, ornithine, lisine and aesculine. Biochemical characterization of the strains was performed with bio Merieux API 20 E (Marcy-l' Etoile, France).

Enumeration and assessment of lactic acid bacteria (LAB): To utilize total alive coli form bacteria counts and isolation, the cheese samples of which dilution has been prepared plated on MRS (Difco, Detroit, Michigan) Plates were incubated at 30°C for a period of 48 hours. The LAB strains were identified following the criteria of Sharpe *et al.* (1966), for isolates of *Lactococci*, the criteria of Kandler and Weiss (1986) for Lactobacilli and the criteria of Garvie (1986) for Leuconostocs, as described by Centeno *et al.*, (1996). Biochemical characterization of the strains was performed with bioMerieux API 50 CHL (Marcy-l' Etoile, France). Afterward, the total microorganism count in 1 gram and 1 ml. sample was separately calculated by taking the colonies at the plates and the dilution rates into account.

Enumeration and assessment of *Staphylococcus aureus:* To utilize *S. aureus* isolation, the cheese samples of which dilution has been prepared plated on Baired Parker Agar BP (Oxoid, Basingstroke and Hampshire, UK). The plates were incubated at 25°C for a period of 48-72 hours. Afterward, the total micro organism count in 1 gram and 1 milliliter sample was separately calculated by taking the colonies at the plates and the dilution rates into account. (Anonymous, 6582, 1989) First of all, Gram painting was applied on the colonies which have been grown on the plates and then they were subjected to catalase, DNase production, mannitol fermentation and coagulase tests.

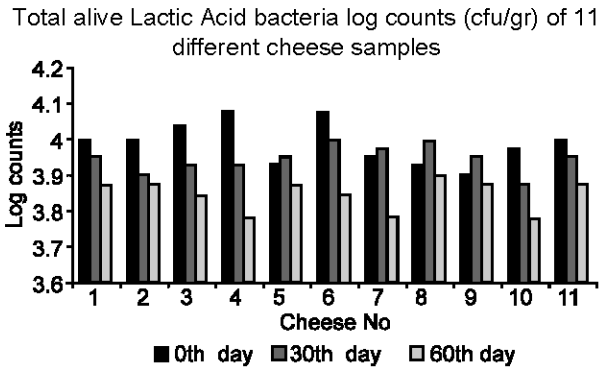

Fig. 3: Total alive lactic acid bacteria log. count (cfu/gr) of 11 different cheese samples.

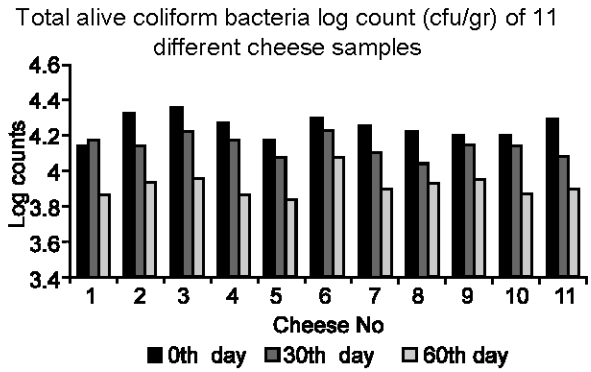

Fig. 4: Total alive coliform bacteria count (cfu/gr) of 11 different cheese samples

Results and Discussion

In our research, 11 different Urfa regional cheeses have been purchased within their brine concentration. It is concentrated on these cheese samples thereof, as regard to micro flora and pathogen micro organisms, during 0-7th, 30th and 60th days.

Bacteria counts in 11 different cheese samples: Total alive mesophyll bacterium count (cfu/gr) in 11 different cheese samples is shown in Fig. 2.

As it is shown in Figure 2 in 2nd, 3rd, 6th and 11th Urfa cheese serials, the Total alive mesophyll bacteria counts are very high in 0-7th and 30th days. But in these Urfa cheese serials a sudden decrease in 60th day was observed. The fresh cheeses and the cheeses of 30th day, on all regions' cheeses, the mesophyll live bacterium count has been observed as around 3.2×10^4 cfu/gr. Because all regions' cheeses are the cheeses that have been made from unscalded or scalded milk heated up to 30, 35°C. But also the total alive mesophyll bacterium counts in all 11 serials have been reduced. In 0-7, 30th. and 60th. days, total alive mesophyll bacterium count of 2nd, 3rd. 6th and 11th cheese samples are 2.8×10^4 cfu/gr, 3.2×10^4 cfu/gr, 3.1×10^4 cfu/gr and 3.2×10^4 cfu/gr respectively.

In Fig. 3, total lactic acid bacterium count (cfu/gr.) in 11 different Urfa cheeses is given.

Total lactic acid bacterium count (cfu/gr.) in 11 different regions' cheeses was enumerated as minimum as $9,8 \times 10^{-3}$ in 0-7th day. In 30th day, an increase in total lactic acid bacterium counts has been determined of the 7th, 8th and 9th serial Urfa cheeses. In 60 th day, the average of total lactic acid bacterium counts is $7,4 \times 10^{-3}$ cfu/gr.

The total coliform bacteria count (cfu/gr.) of 11 different Urfa cheeses is given in Fig. 4.

Although, when it is looked to the coliform bacteria counts of 11 different serial Urfa cheeses, the Total coliform bacteria counts were found very high in 2st, 3nd, 6rd, 9th and 11th serial cheeses.

Lactic acid bacterium isolation and their identifications: In our research, 80 lactic acid bacteria have been isolated from 11 different serial Urfa cheese samples. The varieties and species numbers and their per cents are given in Fig. 5.

The maximum lactic acid bacterium in 11 different variety Urfa cheeses is *Enterococcus faecalis* with a ratio of 33%. The next higher one, *Enterococcus faecium*, with a ratio of 13 % has been isolated.

Gram negative and *Staphyllococcus* isolation and its identification: In the cheese samples that were used in the research, Gram negative bacteria identifications had been made. In Fig. 6, the Gram negative bacteria percent values that were identified from 11 different Urfa cheeses.

In Urfa cheeses, *Salmonella* (*S. choleraesuis*, *S. typhi*) has been isolated from 2nd, 3rd and 6th cheese samples. During 60 days while *S. choleraesuis* has been observed in 2nd cheese sample, it was monitored in 3rd cheese sample for 30 days. *S. typhi* which was available in the 1st cheese sample has also been isolated for a period of 60 day. It is thought that the same bacterium is contaminant due to it was unable to be isolated from the cheese of same sample.

While *Morganella morganii* has been isolated in 2nd. and 3rd. Urfa cheese samples up to 30 days, but in the meantime it was not isolated from the brines.

Pseudomonas is the bacterium species established mostly in the Urfa cheeses. It was detected in 9 out of 11 Urfa cheeses. (81,8%). *P. fluorescence* was specified in the samples of 1st, 3th, 5th, 6th, 7th, 8th 9th and 11th cheese samples. While it survived for 60 days in 1st, 2th, 3th, 5th, 6th, 7th and 11th cheese samples it remained viable only 30 days in 8th and 9th. *P. maltophilia* has also been found in 3nd, 8rd, 9th, 10th Urfa cheese experiments.

Klebsiella is determined in the 8 Urfa cheese sample. *K. pneumoniae* has been isolated along 60 and 30 days

The distribution of total lactic acid bacterium in 11

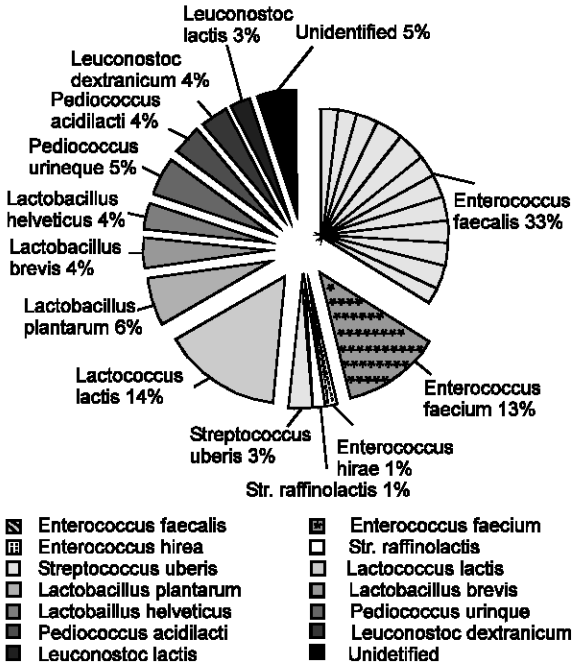

Fig. 5: The distribution of total lactic acid bacterium in 11 different Urfa cheese.

different Urfa cheese from 1st, 3rd and 8th cheese samples respectively. *K. oxytoca* survived for 60 days in the 2nd, 5th, 6th cheese samples. *K. oxytoca* survived 30th day in the 2nd sample and 60th days in the others. *K. ozane* has been determined in the cheese samples of 9th and 11th.

3 Different species of *Citrobacters* are determined in the specimen of eleven Urfa cheeses. *C. freundii* has been isolated along 60 days, from 1st and 11th cheese samples. In one of the 2nd ad 6th cheese samples, *C. youngae* survived 60th day *C. diversus* was isolated for 30 days in the cheese samples of 4th and 7th and for 60 days in the 5th cheese sample.

Yersina was established in 4 Urfa cheeses. In the 1st cheese sample, *Y. mortareti* stayed alive up to 0-7th day. *Y. intermedia* was detected in the 6th cheese sample throughout of 60 days and in 10th one for 30 days. As regards to 11th cheese sample, *Y. enterocolitica* was able to be isolated up to 60 days.

Aeromonas salmonicida was isolated for 30 days in the cheese samples of 9th and 11th as well as in the brines thereof. On the other hand, *Aeromonos sobria* sustained its viability up to 30th day in the 7th cheese sample.

Serratia liguefaciens was isolated for 60 days in the samples of 1st, 2th, 4th, 5 th, 6th and 11 th cheeses as well as the brines.

At the end of micro flora research of Urfa cheeses, *Escherichia, Enterobacter, Cedecea, Citrobacter,* Persentages of Gram negatif bacteria in 11 different Urfa cheeses *Klebsiella, Pseudomonas, Yersinia,*

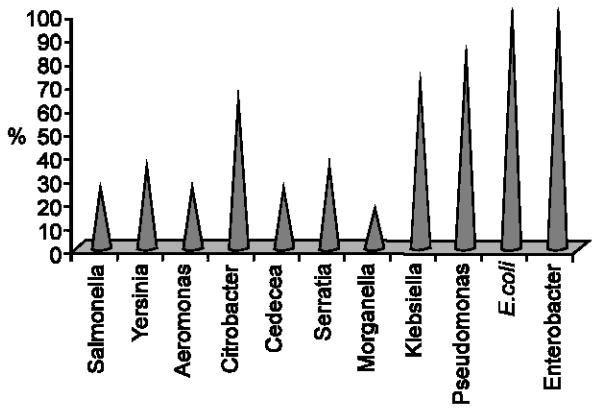

Fig. 6: The percentages of Gram negatif bacterium isolated from 11 different Urfa cheeses.

Aeromonas, Serratia and *Morgenalla* have been detected. Besides, *Staphylococcus* has been determined therein. *Steptococcus, Lactocossus, Lactobacillus, Pediococcus*, *Leuconostoc* were identified as well.

Average total alive mesophyll count of eleven different Urfa cheeses between 0-7th days is 3,5 x 10^4 cfu/gr. The average of 30th day is 3,1 x 10^4 cfu/gr. The average of 60th day is 2,0 x 10^4 cfu/gr. Total mean coliform bacteria count is, between 0-7th days, 1,9 x 10^4 cfu/gr. The average of 30th day is 1,3 x 10^4 cfu /gr while the average of 60th day is 7,6 x 10^3 cfu/gr. Especially in 4 (2nd, 3rd, 6th and 11th) out of the all Urfa cheese experiments, total mesophyll count and total alive coli form bacteria counts during 0-7th, 30th and 60th days are pretty high. In the direction of our studies, the previous researches that have been applied on the Urfa cheeses experiments sold in the local market indicates that *E. coli, S. aureus*, coli form bacteria counts being specified in these cheeses are extremely high from the limits given in the International Standards. (9; 10; 11). Average 0-7th days of total yeast and mold count in our experiments is 1,7 x 10^3 cfu/gr, while the average of 30th day is 3,2x10^3 cfu/gr. In addition the average of 60th day is found as 2,7 x 10^3 cfu /gr. The average of total lactic acid bacterium count during 0-7th days is 7,0 x 10^3 cfu/gr., the average of 30th day is 8,9 x 10^3 cfu/gr and the average of 60th day is found as 5,5 x 10^3 cfu/gr.

E. coli, among the Gram negative bacteria, that have been isolated from the Urfa cheese experiments in this study has been detected in all Urfa cheese samples.

In accordance with various researches, *Pseudomonas* are the contaminant bacteria in the raw milk flora. (Muir *et al.*, 1979; El - Bassiony *et al.*, 1985). Urfa cheese is produced from raw milk or the milk heated to 30-35°C. In our study, *Pseudomonas*, among the Urfa cheese experiments, is the most extensive bacterium, after *E. coli. Pseudomonas* has been proliferated at a ratio of 81.8% at 11 Urfa cheese experiments and *P. fluorescens* was determined in the 1st, 3th, 5th, 6th, 7th,

8th 9th and 11th cheese samples. While it survived throughout 60 days in 1st 3th and 11th cheese experiments, it has been capable of living up to 30 days in the other cheese samples. *P. maltophilia* has also been found in 3nd, 8rd, 9th, 10th Urfa cheese experiments. It was unable to survive after 0-7th day in the 8th cheese sample; it stayed alive up to 60th days in the 3th, 9th, 10th cheese experiments. Apart from importance of *Pseudomonas* in the sense of the public health, it also adds bitterness to cheese (Francoise *et al*., 2004).

In the research, *Salmonella* (*S. choleraesuis* and *S. typhi* at the rate of 18.1% and 9.09% respectively) at the rate of 27.2% exists among the bacterium species proliferated in Urfa cheeses. Both specie of *Salmonella*, gastroenteritis were effective. *S. choleraesuis* was observed during 60 days in the 2nd cheese sample and 30 days in the 3rd Cheese sample. *S. typhi*, that was available in 1st cheese experiment, isolated from cheese for 60 days. While *S. choleraesuis* having isolated from the brine of 2nd cheese sample throughout 60 days, *Salmonella* has been isolated up to 30th days in the brines of other samples. In the other countries, *Salmonella* has caused, by dependant to cheese, to a great deal of epidemic diseases. (Bergdoll, 1989; Zottola and Smith, 1991). Some researchers have been mentioned about "Salmonellosis" events originated from cheese at the Urfa and its proximities. (Ozer *et al*., 2003).

In our study, 8 out of 11 Urfa cheese experiments (72, 7%) *Klebsiella* (*K. pneumoniae*, *K. ozaneae*, *K. ornithinolytica*, *K. oxytoca* at rates of 27.2%, 27.2%, 9.09% and 27.2% respectively) has been isolated. *K. pneumonia* was able to survive up to 30th -60th day in some Urfa cheeses. *K. oxytoca* stayed alive for 60 days in 5th and 6th cheese experiments. *K. ozanea* was determined in 5th and 6th samples for 60 days. *K. ozaneae* has been found in the 9th and 11th cheese samples brines for 60 days. *K. ornithinolytica* survived 30 days in 2nd. cheese sample.

Yersinia (*Y. intermedia*, *Y. enterokolitica* and *Y. mortareti* at rates 18.1%, 9.09%, 9.09% respectively) has been obtained from 4/11 (36.3%) of our samples. *Y. mortareti* could be stayed alive in the 1st cheese sample up to 0-7th day. *Y. intermedia* has been detected for 60 days in the 6th cheese sample and 30 days in 10th and 11th ones. *Yersinia* species either might be available in pasteurized milk owing to insufficient pasteurization process, mixing of raw milk into pasteurized milk or contamination of pasteurized milk with *Y. enterokolitica* afterward with the other *Yersinia* species or also it is available in the cheese due to being decontaminated during the production of cheese. (Hamama *et al*., 1992; Ozer *et al*., 2004).

Likewise, in another study that has been made on Urfa cheeses, *Y. enterocolitica* at a rate of 1% (wt/v) was added to the milk during the production process of Urfa cheese, which was manufactured by using pasteurized milk and survival period thereof has been investigated. The half of them, after the production of the cheeses, has been subjected to the scalding process for 2-3 min. whiles the other half not. *Y. enterocolitica* was able to be survived maximum 30th day in the both 2 species of cheeses rested in the brines having salt concentration with 15% and 17, 5% salt. (Freitas *et al*., 1993).

In our research, the availability of *Aeromonas* as percentage in 11 different Urfa cheese samples is 27.2% (3/11). (18.1% *A. salmonicida* and 9, 09% *A. sobria*). *Aeromonas* were able to be remained alive up to 30th day, in the cheeses. *Aeromonas* is a pathogen causes a lot of diseases in human and animals. It infects by means of water and various foods and resulting the diseases together with gastroenteritis, indicating very different symptoms. (Araujo *et al*., 2002).

At our study, *S. aureus* was determined in 3 / 11 cheeses. *S. aureus* is a micro organism being available very often in raw milk and in the vicinity of manufacturing plant at the cheese industry. It has tolerance against salt and has a wide growth spectrum. Having insufficient amount of acidity in the cheese increases proliferation of *S. aureus* on one hand and causes proliferation of enterotoxin on the other hand. (Bergdoll, 1989). In a similar study made in Brazil, 45 white cheese samples that were offered to market for consumption, have been purchased and the evidence of *S. aureus* and enteropathogen bacterium was investigated. *E. coli, S. aureus* and *Aeromonas* were found at the rates of 97, 7%, 77.7% and 17.7% respectively in the said 45 white cheeses (Araujo *et al*., 2002).

At our research, in all of the Urfa cheeses, *Enterobacter* was found. The following amounts were identified therein; *E. intermedium* (18.1%), *E. nimipressuralis* (18.1%), *E. cancerogenus* (45.5%) and *E. gergovia* (18.1%).

At our study, within 11 cheese samples, 33% *Enterococcus faecalis* and 13% *Enterococcus faecium* were found in the lactic acid bacterium identifications. In a similar study being made by Aleksieva (1983) *E. faecalis* and *E. faecium* rates were found as 22% and 33% respectively.

Parallel to our study, a study has been made in Spain in regard to Roncal and Idiazabal cheeses those are being produced from raw ovine milk. These two different cheese samples were investigated, subsequent to a maturation period of 120 days, as microbiologically. No conspicuous difference, in the both cheeses, was observed from the micro organism species (Arizcun *et al*., 1977).

In the Urfa cheeses those have been manufactured from raw milk or the milk, which has not been treated by sufficient pasteurization processes, pathogen bacteria with gastroenteritis agent were detected. During 30-60

days isolated Gram negative bacteria were observed. Urfa cheeses are being manufactured, as regional, by the use of raw milk or scalded milk that has been heated up to 30-35°C. Therefore the number and variety of micro organism are quite a lot. This cheese use to be consumed in the region, without waiting the completion of its maturation period. Gastroenteritis case number in Urfa is considerably high as compared to the other regions. In conclusion it has recommended that the region's people not consume fresh Urfa cheese.

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The Effects of Heat Treated Lima Beans (*Phaseolus lunatus*) on Plasma Lipids in Hypercholesterolemic Rats

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Abstract: The effects of legume consumption on a dietary induced model of hypercholesterol in rats have been determined. Grower's mash from Bendel Feed and Flour Mill (BFFM) Ewu, Nigeria, was fed to two groups of rats: the test and control groups for 30 days. To induce hypercholesterolemia in the test group, 25% Coconut oil and 1% cholesterol was included in the diet of the test group. The hypercholesterolemic rats were divided into two subgroups. The first group was fed only with heat treated Lima beans and the second group with the grower's mash mixed with 0.1% Saponin. This was done for another 30 days. Biochemical analysis was carried out on blood samples of the rats. The results show that there was a significant ($p<0.05$) reduction in the amount of serum lipids in rats fed the lima beans Legume Diet (LD) and Saponin Diet (SD) when compared to the control (CD) and Hypercholestrolemic Diet (HD). The consumption of lima beans could be recommended to also lower cholesterol and promote cardiovascular health due to the presence of saponin in the legume.

Key words: Lima beans (*Phaseolus lunatus*), Saponins, Hypercholesterolemia, Health benefit, Thermal processing

Introduction

Grain legumes are a major source of cheap proteins for humans in West Africa. In Nigeria, Cowpeas and soybeans are the most widely consumed legume seeds. Lima beans, Pigeon peas, African yam beans and jackbeans are popular legumes consumed in the Esan Community of Edo State, Nigeria (Aletor and Aladetimi, 1989; Edem *et al*., 1990). Chemical composition of these grain legumes were evaluated and shown to contain high quantities of proteins, amino acids and minerals (Apata and Ologhobo, 1994a,b). Despite the rich composition of nutrients these legumes are under-utilized because of their hard-to-cook defect, which lead to long cooking periods which require the use of scarce, expensive fuels (Uzogara and Ofuya, 1992).

Saponins are steriodal or triterpenoid glycosides which occur primarily in legumes (Oakenfull, 1981). They could be beneficial or deleterious (Shi *et al*., 2006; Koratkar and Rao, 1997). Several authors (Singh *et al*., 2002; Koulshon *et al*., 2005; Han *et al*., 2002; Brown *et al*., 1999), have reported the significance of plant sterols and fiber in lowering blood cholesterol level in rats and humans. Their high intake has been associated with reducing the risk of developing diabetes, hypertension, cancer and hypercholesterolemia (Gardner *et al*., 2005; Anderson, 2005). Messina (1999) and Erdman (2000), reported that the mechanisms by which legume seed constituents lower plasma lipid could be by LDL receptor up regulation, inhibitory effects on intestinal cholesterol absorption, increased fecal acid bile excretion, bile acid synthesis from cholesterol and cholesterol loss from the body.

The present study was designed to examine the cholesterol lowering ability of lima beans in diet induced hypercholesterolemic rats.

Materials and Methods

Preparation of experimental diets: Legume seed samples of Lima beans (*Phaseolus lunatus*) were purchased in October 2005, from Uromi Market, Uromi, Edo State, Nigeria. The whole seeds that were free from injury and insect invasion were sorted out and washed in distilled water.

The legume seeds were added to boiling distilled water (1:5w/v) at 100°C and cooked on an electric hot plate for 3 h. (Ikatherm HCT, Ika Staufen, Germany). The cooked seeds were drained to remove the cook water and dried at 70°C in an oven (Gallenkamp, UK) for 16 h. The dried seeds were cooled in a dessicator, milled to powder in a warring blender. The bean flour was stored in an air-tight container at-10°C until used as feed for the rats.

The other Diet types were prepared by weighing out the different constituents as presented in Table 1. Fresh diets were prepared daily and utilized as feed for the animals. Stale remnants were discarded after weighing.

Animals experiment: Twenty four male albino rats (3-4 months old) weighing about 200g-250g were obtained from an animal breeder in Benin City. They were marked and randomly assigned to two groups, control rats (n = 6) and experimental rats (n = 18). This was done such

Table 1: Composition of Experimental Diets

Dietary Component (g)	CD	HD	LD	SD
Growers mash (Bendel feed and Flour Mills BFFM Limited, Ewu)	99.0	73.0	-	98.9
Coconut oil	-	25.0	-	-
Cholesterol	-	1.0	-	-
Lima bean flour	-	-	99.0	-
Saponin-soyasapogenin	-	-	-	0.1
*Mineral and vitamin mix (OPIMIX PREMIX)	1.0	1.0	1.0	1.0
Total Composition	100g	100g	100g	100g

*Mineral and vitamin mix, CD: Control Diet, HD: Hypercholesterolemic Diet, LD Legume Diet, SD: Saponin Diet

(OPIMIX PREMIX)			
Vitamin A	8,000,000IU	Copper	5gm
Vitamin D	1,600,000IU	Iron	20gm
Vitamin E	5,000IU	Iodine	1.2gm
Vitamin K	2,000mg	Selenium	200mg
ThiamineB_1	1,500mg	Cobalt	200mg
Riboflavin B_2	4,000mg	Cholin chloride	200gm
Pyridoxine-B_6	1,500mg	Anti oxidant	125gm
Niacin	15,000mg	Manganese	80gm
Vitamin B_{12}	10mg	Zinc	50gm
Pathothenic acid	5,000mg	Biotin	20mg
Folic acid	5,000mg		

The constituents are reported, as the composition stated on the feed bag

that the differences in average weight per group did not exceed 2 grams. The rats were housed in wire cages kept in the animal house of the Medical Biochemistry Department, School of Basic Medical Science, College of Medicine, University of Benin. The animals in the two groups were acclimatized on Growers mash (Bendel Feed and Flour Mills (BFFM) Limited, Ewu, Nigeria) for 2 weeks, prior to the study. Food and water was given *ad libitum*.

Control rats (n = 6) were fed throughout the experimental period with grower's mash (Bendel Feed and Flour Mills (BFFM) Limited, Ewu, Nigeria). This is the Control Diet (CD). Experimental rats (n = 18) received grower's mash enriched with 25% coconut oil and 1% cholesterol. After 30 days, blood samples were collected from the rats and serum lipids assayed for total cholesterol, HDL-cholesterol, LDL-cholesterol, VLDL-cholesterol and triacylglycerol. Total cholesterol was found to be significantly elevated to 60mg/dl. The rats were assigned to three new subgroups. Group one (n = 6) continued to receive the high fat diet (HD). The group two (n = 6) were fed on the diet containing Lima Beans (LD) only. The group three (n = 6) were fed on grower's mash mixed with 0.1% soyasapogenin (Merck) in the diet (SD). 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).

Collection and analysis of blood samples: Baseline blood samples were collected from the tail region of the rats in all groups, before the feeding of the different diets commenced.

After 30 days of feeding the different experimental and control diets, the animals were fasted for 18h and were anesthetized with pentobarbital (60mg/Kg body weight). Insertion was made into the heart region for collection of blood samples with the use of a needle and heparinized syringe. The blood samples were collected into labeled bijou bottles containing heparin as anticoagulant and centrifuged immediately (3,000×g for 10 min), to obtain the serum. The serum samples were stored in the biofreezer at-10°C until analyzed for, total serum cholesterol (TC), High Density Lipoproteins (HDL), Low Density Lipoproteins (LDL) and triaclyglycerols (TAG) (Anderson *et al.*, 1971) using commercial kits (Boehringer Mannheim).

Statistical analysis: Data were expressed as mean±Standard error of the mean (SEM) for each group of rabbits. Comparison between the control and experimental set of data was analyzed by the ANOVA and p values <0.05 were indicative of significance. The statistical analyses were done with INSTAT statistical package.

Results and Discussion

Table 1 shows the Composition of Experimental Diets fed to the rats.

The weight gain, feed and water intake, feed efficiency and dry fecal output of the rats in the control and experimental groups are presented in Table 2.

Statistical analysis showed that there was no significant (p = 0.05) decrease in weight gain, feed and water intake and feed efficiency in the experimental diet when compared with the control diet.

Table 3-5 shows the Concentration of the serum lipids in Experimental and control rats. fed the different diet; Control Diet (CD), Hypercholesterolemic Diet (HD) Saponin Diet (SD) and Legume Diet (LD). Statistical analysis showed that there were significant (p<0.05) differences between the basal and final values of the serum lipids in all the groups fed the different experimental diets, except the control diet which showed that there were no significant (p<0.05) differences between the basal and final values of the lipid profile concentrations.

Table 2: Weight Gain, Feed Intake, Water Intake, Feed Efficiency and Dry Fecal Output of Rats in the Control and Experimental Groups

Group	Control diet	Experimental diet
Weight gain (g/rat)	35±8.0[a]	25±6.0[a]
Feed intake (g/rat/day)	5.8	4.5
Water intake(ml/rat/day)	10.50±2.0[a]	9.80±2.50[a]
Feed efficiency (g/body weight/g feed)	6.03	5.5
Dry fecal output (g/rabbit/day)	2.24±1.14[a]	2.25±1.02[a]

Values are mean (gm) ± SEM of six rats; Means of the same row followed by different letters differ significantly (p<0.05)

Table 3: Effect of changes in serum lipids in rats fed the Control Diet (CD)

Serum lipids (mg/dl)	Basal values	Final values
Total Cholesterol	21.5±1.5[a]	22.3±0.87[a]
HDL-Cholesterol	8.7±2.2[a]	8.6±1.6[a]
LDL-Cholesterol	4.1±1.2[a]	5.2±1.0[a]
VLDL-Cholesterol	15.8±2.3[a]	14.9±1.8[a]
Triacylglycerol	79.4±1.2[a]	82.01±3.0[a]

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

Table 4: Effect of changes in serum lipids in rats fed the Hypercholesterolemic Diet (HD)

Serum lipids (mg/dl)	Basal values	Final values
Total Cholesterol	20.3±1.20[a]	60.0±2.0[b]
HDL-Cholesterol	9.2±0.87[a]	36.3±1.2[b]
LDL-Cholesterol	4.1±0.6[a]	20.1±0.7[b]
VLDL-Cholesterol	18.5±1.9[a]	49.8±2.3[b]
Triacylg;ycerol	82.7±3.1[a]	218.5±3.9[b]

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

Table 5: Effect of changes in serum lipids in rats fed the Saponin Diet. (SD)

Serum lipids (mg/dl)	Basal values	Final values
Total Cholesterol	60.0±2.0[b]	39.6±3.7[c]
HDL-Cholesterol	34.0±0.5[b]	23.0±1.7[c]
LDL-Cholesterol	16.0±1.1[b]	10.4±0.5[c]
VLDL-Cholesterol	44.67±1.3[b]	36.0±1.5[c]
Triacylg;ycerol	215.67±3.4[b]	134.0±2.3[c]

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

Table 6: Effect of changes in serum lipids in rats fed the Legume Diet (LD)

Serum lipids (mg/dl)	Basal values	Final values
Total Cholesterol	62.0±1.56[a]	39.6±3.7[b]
HDL-Cholesterol	36.3±1.2[a]	30.0±1.7[b]
LDL-Cholesterol	20.4±0.7[a]	14.0±0.5[b]
VLDL-Cholesterol	49.8±2.3[a]	26.0±1.5[b]
Triacylg;ycerol	218.5±3.9[a]	104.0±2.3[b]

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

Table 4 represents the basal and final values of serum lipids of the rats fed the hypercholesterolmic diet. The hypercholesterolemic diet led to a significant increase (p<0.05), in the levels of the serum lipids. This was diet induced by the addition of 25% coconut oil and 1% cholesterol to the growers mash used for the hypercholesterolmic diet.

Table 7: Mean Concentrations of Serum lipids on rats fed the control, hypercholesterolemic and Saponin Diet

Serum lipids (mg/dl)	CD	HD	SD	LD
Total Cholesterol	21.5±1.5[a]	60.0±2.0[b]	39.6±3.7[c]	39.6±3.7[c]
HDL-Cholesterol	8.7±2.2[a]	36.3±1.2[b]	23.0±1.7[c]	30.0±1.7[c]
LDL-Cholesterol	4.1±1.2[a]	20.1±0.7[b]	10.4±0.5[c]	14.0±0.5[c]
VLDL-Cholesterol	15.8±2.3[a]	49.8±2.3[b]	36.0±1.5[c]	26.0±1.5[c]
Triacylg;ycerol	79.4±1.2[a]	218.5±3.9[b]	134.0±2.3[c]	104.0±2.3[d]

Values are mean±SEM of six rats; Means of the same row followed by different letters differ significantly (p<0.05). CD: Control Diet, HD: Hypercholesterolemic Diet, LD Legume Diet, SD: Saponin Diet

The changes in serum lipids as affected by the saponin diet and legume diet are represented in Table 5 and 6 respectively. The values of the serum lipids which includes, total cholesterol, HDL-Cholesterol, LDL-Cholesterol, VLDL and Triacylglycerol, were significantly (p = 0.05) reduced in the rats fed the saponin and legume diet.

A significant reduction in serum lipids has been observed in experimental rats, when heat-treated legumes, were fed to hypercholesterolemic rats (Zulet and Martinez, 1995; Singh *et al.*, 2002). The saponin content of raw and processed lima beans has been determined and found to be 1.26g/Kg for the raw and 732.3g/Kg for the heat treated samples respectively (Oboh *et al.*, 1998; Oboh and Osagie, 2003).

Saponins form strong insoluble complexes with cholesterol and bile making them unavailable for absorption (Oakenfull and sidhu, 1990). This mixture is then removed from the body through the normal elimination process. Increased bile acid excretion may cause compensatory increase in bile acid synthesis from cholesterol in the liver. As the body needs more cholesterol for bile acid production used for digestion, the liver removes cholesterol from the bloodstream and thus lowers serum cholesterol. These findings confirm the role of saponins in lowering blood cholesterol as suggested by Oakenfull and sidhu (1984). The saponins in lima beans can be extracted and used in the synthesis of drugs which can lower blood cholesterol. This will provide a good market for the use of lima beans in the manufacture of cholesterol lowering drugs.

Table 7 Represents a summary of the mean concentration of serum lipids in rats, fed the control and Experimental Diets. Significant (p<0.05) differences were observed in rats fed the Control, Saponin and hypercholesterolemic diet. No significant difference (p<0.05) was observed between the saponin and legume diet for the serum lipids assayed except for the triacylglycerol values, where, the legume diet, had significantly (p<0.05) reduced the values, when compared to the saponin diet. It is notable that, the lima beans were cooked without dehulling. Oshodin and Adeladun (1993), reported that lima beans contain a fiber level of 4.3%. The fiber content of the legume could have a contributory role in the reduction of cholesterol

levels. The role of dietary fiber in the reduction of cholesterol has been reported (Glore *et al*., 1994). This study shows that the legume diet gave lower serum lipid values in dietary induced hypercholesterolemic rats. The presence of saponins and fiber in the heat treated legume is probably responsible for the cholesterol lowering ability of the legume.

Conclusion: The Hypocholesterolemic effect of lima beans could be due to the combined effect of the saponins and fiber present. This study shows that there is a distinct beneficial effect from the consumption of heat treated lima bean; therefore the use of lima beans is shown to likely diminish the risk of artherogenic dyslipidemia.

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Effects of High - Protein, Low - Carbohydrate and Fat, Nigerian - like Diet on Biochemical Indices in Rabbits

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Abstract: This study aimed to assess the effects of high protein, low carbohydrate and fat (HP/LCF) Nigerian-like diet. Twelve rabbits were randomly divided into two groups of six rabbits per group. The groups were; Group I, which was fed the control diet and the second group II, received the high protein, low carbohydrate and fat diet, containing 65% protein, 28% carbohydrate and 5% Palm oil. The diets were administered for 30 days. At the end of the feeding periods, biochemical analyses were done on the blood samples of rabbits. Results showed that the total protein, albumin and globulin were significantly ($P<0.05$) increased for the rabbits fed a HP/LCF diet. AST (Aspartate transaminase) and ALT (Alanine transaminase) levels increased significantly ($P<0.05$) while ALP (Alkaline phosphatase) decreased significantly ($P<0.05$). The value obtained for serum electrolyte concentrations show a significant ($P<0.05$) increase in sodium and chloride ions when compared with control. Although, potassium increased significantly, the increase was lower than that observed for sodium and chloride ions. Bicarbonate ions showed significant increase ($P<0.05$). Urea values showed significant ($P<0.05$) increased levels. However, Creatinine levels did not show a significant ($P<0.05$) increase. Total cholesterol levels were significantly ($P<0.05$) reduced. The lipoprotein fractions showed significant ($P<0.05$) elevated HDL levels. Triacylglycerol levels and LDL-Cholesterol levels showed no significant change when compared with controls. From the results, the HP/LCF Nigerian diet promotes low total cholesterol levels while increasing the protective HDL-Cholesterol levels. The LDL-Cholesterol and triacylglycerol levels did not change significantly. The increased serum urea levels, AST and ALT levels are indicative of dysfunctional liver and kidney.

Key words: High-protein low carbohydrate, fat diet, serum lipid, serum enzymes, electrolytes

Introduction

The typical Nigerian diet consists of low protein and high carbohydrate levels. However, in recent times, the Atkins Diet in which carbohydrate is restricted but protein is increased, leads to weight loss (Anderson *et al*., 2000), has been proposed to help people maintain desirable body weight (Jeor *et al*., 2001) and induce feeding-suppressive effects (Bensaid *et al*., 2002). The increased incidence of overweight and obesity in the middle class in Nigeria has made this diet popular as a strategy to achieve efficient and long term weight loss.

The middle class Nigerian diet is characterized by elevated intake of red meat and saturated fat. Protein cannot be stored, they must be produced adequately in diet and therefore excess is unnecessary, as it is metabolized to energy.

The positive effects of replacing dietary protein for carbohydrate includes, improvement in the cardiovascular disease risk profile and insulin sensitivity in type 2 diabetes (Parker *et al*., 2002) lowering of white adipose tissue and reduced lipogenesis.(Pichon *et al*., 2006). Johnston *et al*. (2002), reported increases in total energy expenditure by means of increased thermogenesis. This is associated with protein digestion.

Despite the benefits of the high protein diet, there are concerns about the safety of the diet. The diet is linked to detrimental renal and hepatic function (Hammond and Janes, 1998, Morens *et al*., 2000), increase of urinary calcium excretion and a possible bone resorption (Kerstetter *et al*., 1999, Licata *et al*., 1981), an enhancement of oxidative stress (Petzke *et al*., 2000) and a modification of detoxification enzymes (Rao, 1996).

A suitable diet should promote good health and satisfy the needs of the individuals. The present study aims to examine the effect of consuming a high-protein, low-carbohydrate and fat Nigerian-like diet on biochemical indices in rabbits.

Materials and Methods

Animals and management: 3 months old New Zealand white rabbits (Initial mean weight 1.75Kg) 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 12hr-light and 12hr dark cycle and the temperature was uniform. The animals were acclimatized on growers mash (Bendel feed and Flour Mills (BFFM) Limited, Ewu, Nigeria) for two weeks. Prior

Table 1: Composition of Experimental Diets

Dietary Component	Diet type: Control	Diet type: HP/LCF
Garri	60.00g	28.40g
Fish	15.40g	65.00g
*Mineral and vitamin mix (*OPIMIX PREMIX)	1.00g	1.00g
Palm oil	18.00g	5.00g
Methionine	0.60g	0.60g

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

*OPIMIX PREMIX

Vitamin A	8,000,000IU	Copper	5.0gm
Vitamin D	1,600,000IU	Iron	20.0gm
Vitamin E	5,000IU	Iodine	1.2gm
Vitamin K	2,000mg	Selenium	200.0mg
ThiamineB_1	1,500mg	Cobalt	200.0mg
Riboflavin B_2	4,000mg	Cholin chloride	200.0gm
Pyridoxine-B_6	1,500mg	Anti oxidant	125.0gm
Niacin	15,000mg	Manganese	80.0gm
Vitamin B_{12}	10mg	Zinc	50.0gm
Pathothenic acid	5,000mg	Biotin	20.0mg
Folic acid	5,000mg		

to the study, food and water was given *ad libitum*. The rabbits were divided into two groups of six rabbits per group, according to body weight similar after the adaptation period. One group was fed the control diet and the second group was fed the High protein, low fat and carbohydrate (HP/LCF) diet for a total period of 30 days.

The composition of both diets is shown in Table 1. Fresh feed was provided on daily basis while stale remnants were discarded after weighing. On the average each rabbit received about 150g/feed/day. Clean drinking water was provided *ad lib*. 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 18hr and baseline blood samples were drawn from the rabbit ear veins using 21-gauge syringes. At the end of the feeding period, the rabbits were anesthetized with pentobarbital (60mg/Kg body weight). Insertion was made into the heart region for collection with the use of a needle and syringe. The blood samples were collected into labeled bijou bottles and were allowed to clot at room temperature for 45 minutes before being centrifuged (3,000 x g for 10 minutes), to obtain the serum. The serum samples were stored in the biofreezer at -10^{0}C until analyzed. Duplicate serum samples for each animal group were analyzed for Total proteins (Brown, 1976), albumin, globulins (Baertl *et al.*, 1974) electrolytes (Na^+, K^+, HCO_3^-, Cl^-) (Kinsley and Schaffert, 1953), lipid (Anderson, 1971), Alkaline phosphatase (ALP), Aspartate transaminase (AST), Alanine transaminase (ALT) (Moss *et al.*, 1971) urea and creatinine (Carr, 1959) were measured using commercial kits (Boehringer Mannheim).

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

Group	Control diet	Experimental diet
Weight gain (g/rabbit)	650±8.0^a	150±6.0^b
Feed intake (g/rabbit/day)	53.8	66.5
Water intake(ml/rabbit/day)	20.50±2.0^a	14.50±7.9^b
Feed efficiency (g/body weight/g feed)	12.08	2.25
Dry fecal output (g/rabbit/day)	5.24±1.14^a	3.60±1.3^b

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

Statistical analysis: Data were expressed as mean ± Standard error of the mean (SEM) for each group of rabbits. Comparison between the control and experimental set of data was analyzed by the student's t-test and P values < 0.05 were indicative of significance. The statistical analyses were done with INSTAT statistical package.

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 analysis showed that there was a significant decrease in weight gain, the mean body weights were 650±8.0g with animals fed the control diet and 350±9.0g with animals fed the experimental diet. Feed and water intake and Dry fecal output in the experimental diet were also significantly reduced when compared with the control diet.

The values observed for total protein, albumin and globulin are given in Table 3. The values obtained for the control samples, is total proteins (1.28±0.41mg/dl), albumin (0.26±0.15mg/dl) and globulin (1.75±0.52mg/dl).These values were significantly (P <0.05) elevated in rabbits fed the experimental diet.

The values obtained for AST, ALT, ALP is shown in Table 4. The AST and ALT levels significantly (P<0.05) increased while ALP levels significantly (P<0.05) decreased, when compared to the values obtained from rabbits fed the experimental diet.

Table 5 shows the level of electrolytes, creatinine and urea in rabbits. The rabbits fed the HP/LCF diet had a significant increase (P<0.05) in sodium and chloride ions content when compared with the control.

Potassium and bicarbonate ions increased significantly content when compared with the control (P<0.05).The creatinine did not change significantly (P<0.05) while serum urea concentration were 34.9±0.86mg/dl after feeding with the high protein diet, this value increased

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

Parameters	Control diet	Experimental diet
Total Protein	1.28±0.41[a]	7.46±0.31[b]
Albumin	0.26±0.15[a]	5.03±0.57[b]
Globulin	1.75±0.52[a]	2.86±1.18[b]

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 of rabbits on the control and experimental diet

Parameters	Control diet	Experimental diet
AST(IU/dl)	2.90±0.07[a]	17.5±0.86[b]
ALT(IU/dl)	7.65±0.47[a]	95.6±0.68[b]
ALP(IU/dl)	32.5±16.48[a]	8.9±0.48[b]

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

significantly (P<0.05) when compared with control diet. Table 6 shows the values of serum lipids of rabbits fed the control and experimental diet. Serum cholesterol levels were significantly (P<0.05) reduced. The lipoprotein fractions were also significantly altered. HDL-Cholesterol levels were significantly elevated (P<0.05) to 22.67±3.54mg/dl when compared with the control value of 6.5±0.96mg/dl. LDL-cholesterol and triacylglycerol concentration were not significantly altered (P<0.05) in the experimental groups when compared with the control diet.

Discussion

The aim of this study was to determine the consequences of a long term high protein and low carbohydrate and fat Nigerian-like diet in Rabbits. The results show that, the high protein diet increased food intake. There was also, a significant reduction in body weight. This finding is in agreement with the previous studies in rats fed a high protein diet for 6 months. (Lacroix *et al.*, 2004). The source of protein consumed could affect the results obtained for the experimental diet. The source of the protein is fish. He *et al.* (2004), reported that increase intake of fish as a protein source had beneficial effects. Compared with red meat, the white meat from fish contains similar amount of protein and has substantially less saturated fat and cholesterol (Hu, 2005).

The mean concentration of total proteins, albumin and globulins of rabbits were significantly elevated. This showed that the high protein diet favored protein synthesis of new cells and tissues. The high albumin values, is a predictor of future development of clinical renal disease.

The liver enzymes AST and ALT were elevated significantly, in animals fed the high protein diet. The roles of the liver and kidney in amino acid and nitrogen

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

Parameters	Control diet	Experimental diet
Potassium(mM/L)	0.47±0.11[a]	4.23±0.112[b]
Sodium(mM/L)	17.0±2.55[a]	147.3±0.89[b]
Bicarbonate(mM/L)	3.67±0.58[a]	15.34±0.37[a]
Chloride(mM/L)	12.00±0.58[a]	110.0±1.14[b]
Creatinine(mg/dl)	0.46±0.06[a]	0.66±1.71[a]
Urea(mg/dl)	8.33±3.69[a]	34.9±0.86[b]

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 on rabbits fed the control and experimental diet

Parameters	Control diet	Experimental diet
Total Cholesterol(mg/dl)	50.18±7.75[a]	40.16±0.87[b]
HDL-Cholesterol(mg/dl)	6.5±0.96[a]	22.67±3.54[b]
LDL-Cholesterol(mg/dl)	32.18±7.22[a]	32.50±5.23[a]
Triacylgl;ycerol(mg/dl)	10.5±7.59[a]	11.6±4.18[a]

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

metabolism had led to the suspicions of a high protein diet having potential deleterious effects on the structure and function of the organs. (Jean *et al.*, 2001). The high protein diet led to a significantly increase in serum urea levels, this observation in the experimental animals could be due to the inability of the liver to mobilize urea, leading to a build up. A similar observation has been reported by Jenkins *et al.* (2001).

Serum creatinine increased slightly, but not significantly, this suggests that fish protein had no major adverse effects on renal function in the short term. The effects of the long term are unknown. Street (2001), reported no dysfunction in the kidney of healthy individuals consuming an *ad lib* high protein diet. This study is in agreement with the observation.

Creatinine remains significantly unchanged. This is an indicator of normal kidney function. However, the significantly increased levels of the serum electrolytes namely sodium, potassium and chloride ions, could be a pointer to a reduction in renal functioning capacity, due to the inability of the kidney to regulate the levels of these electrolytes. Sodium ion and chloride ion is distributed principally in the extracellular fluid and so associated with its retention.

Wachman and Bernstein (1968) reported dietary protein (animal based) results in acidification of urine. In order to buffer the urine and maintain acid-base homeostasis, bicarbonate is increased. This was observed in this study. The production of endogenous acids may result in the mobilization of calcium from the skeleton resulting in a net calciuria. This results in reduced bone mineral density and increasing the risk of fractures/ osteoporosis

In this present study, the significantly reduced ALP levels observed could be due to the irreversible inhibition of

ALP activity by high urea concentrations (Fleisher *et al.,* 1977). Bone isoenzyme is most susceptible to urea inhibition. This could be linked to the risk of bone loss or osteoporosis. Although, the literature has conflicting reports as to the effect of high protein diet on bone density (Reddy *et al.,* 2002, Bowen *et al.,* 2004). The recent review by (Bonjour, 2005) reported that there is no convincing published data showing that, a high protein diet, consumed for a prolonged period of time under strictly controlled dietary conditions could lead to osteoporosis.

The high fish protein diet resulted in a significantly reduced total cholesterol levels while, HDL increased significantly. Scott *et al.* (1991), reported that a predominantly fish based diet lead to a 40% reduction of plasma lipoproteins-an independent cardiovascular risk factor.

The significantly higher HDL-Cholesterol concentration is also protective. Hu *et al.* (1999a), reported that high protein intake reduces the risk of ischemic heart disease.

This is in agreement with studies performed in humans (Farmsworth *et al.,* 2003; Layman *et al.,* 2003). There was a stabilization of Triacylglycerol and LDL-Cholesterol. This may reduce cardiovascular disease risk. The high protein diet was associated with a reduced risk of cardiovascular disease (Hu *et al.,* 1999b)

Conclusion: The increased substitution of fish for carbohydrate may have health benefits which includes weight control and prevention of cardiovascular disease. Although, Kidney function is not affected, there are indications that declining function may be possible. The potential negative side effects of increased protein, in this study, showed elevated liver enzymes and the apparent inability to mobilize urea which could lead to a build up of intermediary metabolites which could be toxic, indicating liver dysfunction.

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Some Physical Properties of Tabarzeh Apricot Kernel

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Abstract: Physical properties of apricot kernel are necessary for the design of equipments for processing, transportation, sorting and separating. In this paper the physical properties of apricot kernel have been evaluated as a function of moisture content varying from 3.19% to 17.46% (w.b.). With increasing in moisture content, kernel length, width, thickness, Geometric mean diameter and surface area increased; the sphericity varying from 59.79 % to 62.21%; mass, thousand grain mass, volume and true density increase from 0.380 to 0.448 (gr), 381.6 to 447.9 (gr), 0.442 to 0.463 (cm^3) and 882.588 to 983.383 (kg/m^3) respectively; The porosity and bulk density decreased from 52.68% to 51.33% and 471.6 to 406.8 (kg/m^3) respectively; the coefficient of static friction on all surfaces increased as the moisture content increased; and the rupture strength in weakest direction (through length) decrease from 23.443 to 16.620 (N).

Key words: Apricot kernel, apricot fruit, seeds, moisture content

Introduction

Apricot (*Prunus armenia L.*) has an important place in human nutrition and apricot fruits can be used as fresh, dried or processed fruit. Stone-fruit crops, including apricot are temperate fruits which are grown in climates with well-differentiated seasons. Mechanisms against the impact of low winter temperatures and frost damage have been developed by species growing under these conditions. Dormancy and freezing tolerance are the main mechanisms developed against these difficulties and, although they could be independent (Irving and Lamphear, 1967), freezing tolerance cannot be developed adequately without growth cessation (Fuchigami *et al*., 1971), which marks the onset of dormancy. As known, the fruit of apricot is not only consumed fresh but also used to produce dried apricot, frozen apricot, jam, jelly, marmalade, pulp, juice, nectar, extrusion products etc. Moreover, apricot kernels are used in the production of oils, benzaldehyde, cosmetics, active carbon and aroma perfume (Yildiz, 1994). Apricot is rich in minerals such as potassium and vitamins such as β - carotene. β-carotene, which is the pioneer substance of mineral A, is necessary for epithelia tissues covering our bodies and organs, eye-health, bone and teeth development and working of endocrine glades. Moreover, vitamin A plays important role in reproduction and growing functions of our bodies, in increasing body resistance against infections. Iran is the second apricot producer in the world with 275580 ton production and 8.2% share (FAO, 2005). In Iran, the most widely produced types are Tabarzeh, Kardi Damavandi, Nakhjavan and Sonnati. Turkey, Iran, Italy, Pakistan and France are the principal apricot countries. Trees are also grown in Spain, Japan, Syrian Arab Republic and Algeria. Iran has exported more than 680 tones to different countries in 2005 (FAO, 2005). The trees of these types of apricot are high, strong and grow rapidly and have wide and shallow branches. They bear fruits every year in fertile and irrigated soils. The distance between trees is approximately 10 m, average fruit weight ranges between 20 and 60 g, dried substance percentage in fruit is 18-28%, pH value is between 4.0 and 5.0 and their color are yellow. Their harvesting phase is between the last of June and the beginning of July. The first three of these varieties are evaluated as dry products; the other three are evaluated as fresh products. The agriculture of apricot needs extensive labor and energy. In Iran, apricot fruits are harvested at about 77% moisture level (ASB, 2005). Apricot pits are also separated into shells and kernels in the regional conglomerates which have washing, sorting and breaking and separation units. The resulting shells are generally used as fuel. The physical properties of apricot are important for the design of equipments for harvesting and post-harvesting technology transporting, storing, cleaning, separating, sorting, sizing, packaging and processing it into different food. Since currently used systems have been generally designed without taking these criteria into consideration, the resulting designs lead to inadequate applications. These results in a reduction in work efficiency, an increase in product loss. Therefore, determination and consideration of these criteria have an important role in designing of these equipments.

Many studies have reported on the physical properties of kernels and seeds such as Dutta *et al*. (1988) for gram, Gupta and Das (1997) for almond nut and kernel, Ogut (1998) for white Lupin, Aydin (2002) for Hazel nuts, Kaleemullah and Gunasekar (2002) for arecanut kernels, Gezer *et al*. (2002) for apricot pit and its kernel, Sahoo and Srivastava (2002) for okra seed, Konak *et al*. (2002) for chickpea seeds, Aydin (2003) for Almond nut

Fig. 1: Tabarzeh apricot kernel.

Fig. 2: The device for determining coefficient of static friction

and kernel, Baryeh and Mangope (2003) for pigeon pea, Kashaninejad *et al.* (2005) for pistachio nuts and kernels, Karababa (2006) for popcorn kernels, Razavi *et al.* (2007) for pistachio nuts and their kernels. As it can be found from literature review, there was no published paper about the physical properties of Iranian apricot kernel, who studied some of their physical properties have been evaluated as a physical of moisture content. As it can be found from literature review, there was no published paper about the physical properties of Iranian apricot kernel, who studied some of their physical properties have been evaluated as a physical of moisture content.

It is clear that investigating on physical and mechanical properties of apricot kernel is very essential and practical for its process. Then for achieving this aims, some important physical properties of apricot such as axial dimensions, thousand grain mass, true and bulk density, porosity, spherisity, coefficient of static friction and rupture strength on 4 level of moisture were determined.

Materials and Methods

Apricot of Tabarzeh variety (Fig.1) used for this study was collected from the orchard located in Salmas village in west Azarbayjan, Iran in August 2007. Broken pits and foreign matters such as dust, dirt, stones and chaff were removed from 7 kg apricot pit then 4 kg apricot kernel was obtained. Apricot kernels were cleaned by exposing them to air screen cleaner for foreign matters. All products were kept in the room temperature for two days. Moisture content was immediately measured on arrival. The kernels were divided into four batches in order to obtain four moisture levels for the experiments. For obtain the desired water contents of apricot kernel, the moisture of kernel samples were measured at six day intervals after apricots harvesting. The first water content of kernel was 17.46%, then after 6 days the water content of apricot kernels had fallen down to 11.69%, 4.49% and 3.19% (w.b.), respectively. Moisture contents of the kernels were determined by using a standard method (USDA, 1970).

One hundred apricots randomly selected and its kernel parameters including length (L) in mm, width (W) in mm, thickness (T) in mm, weight (g) in kg, volume (V) in cm^3, true density (Td) in kg/m^3, geometrical mean diameter (Dg) in mm, sphericity (ϕ) factor and surface area (S) in mm^2 measured. Geometrical dimensions and mass of kernels, measured by micrometer and digital balance with accuracy 0.01mm and 0.001g respectively. By use of three dimensions, geometrical mean diameter, sphericity factor and surface area (S1 and S2) obtained from fallow equations (Mohsenin, 1970; Jain and Bal, 1997).

$$D_g = (LWT)^{0.333} \quad (1)$$

$$\Phi = (Dg/L)*100 \quad (2)$$

$$S1 = \pi\_Dg^2 \quad (3)$$

$$S2 = \frac{\pi BL^2}{2L - B} \quad \text{Where } B = (WT =)^{0.5} \quad (4)$$

In order to determining thousand grain mass (M_{1000}) in g, randomly 100 seed selected and weighted. True density (Td) in kg/m^3 and volume (V) determined by use of displacement in liquid method. We use toluene instead of water as liquid, because it is more advantages. As we know toluenes have less surface tension and degeneration. (Mohsenin, 1970; Ogut, 1998). The bulk density (Bd) is in kg/m^3 the ratio of the mass sample of the kernels to its total volume. It was determined by filling a 1000 ml container with kernels from a height of about 15 cm, striking the top level and then weighing the contents (Desphande *et al.,* 1993). Also porosity (P) calculated by follow equation (Thompson and Isaacs, 1967; Mohsenin, 1970).

$$P = \frac{1-Bd}{Td} \quad (5)$$

Coefficient of static friction (μ) of kernels on four surface including wood, fiberglass, glass and galvanize seet were determined. In order to determining coefficient of static friction, we put products on the surface with

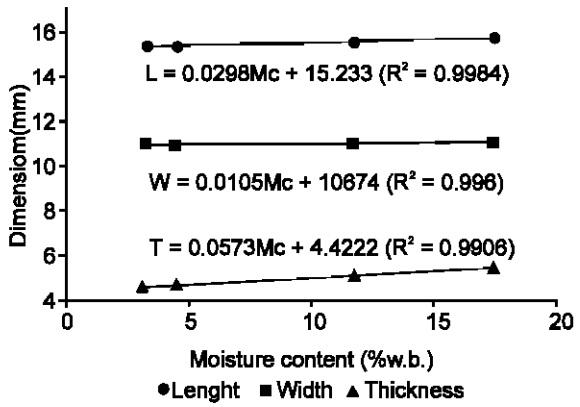

Fig. 3: Effect of moisture content on symmetrical dimensions

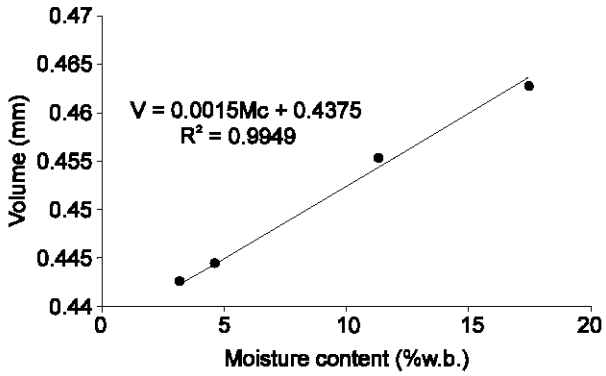

Fig. 4: Effect of moisture content on grain volume of apricot kernel

changeable slip (Fig.2). When product started to motion, tangent of slip angle show the coefficient of friction (Baryeh, 2001).

Rapture strength for apricot kernel determined from forces acting on the three dimensions length, width and thickness with speed of load 50mm/min (Gezer *et al.*, 2002). Method of test was putting shelled apricot on desired dimension and selecting speed of loading and after that applying force till product fractured. On the monitor of device shows graph of force- displacement.

Results and Discussion

Physical properties mean comparison by Duncan's multiple range tests: Average physical properties of apricot kernel in different moisture content was compared by Duncan's multiple rang test. Comparing average data by Duncan method related to dimension, mass, volume and density are shown in Table 1. Results show that with increasing moisture content of apricot kernels, its physical properties including geometrical dimensions, spherically, surface area, volume, mass, true density and coefficient of static friction on varying surfaces such as wood, glass, galvanized sheet and fiberglass sheet will be increase and bulk density were decrease.

Investigating effect of moisture content on physical properties of apricot

Dimensions and Geometrical mean diameter: Effect of moisture content on geometry dimension, such as length, width and thickness of apricot kernels, was shown on Fig. 3. It is clear that there is much correlation between them. Reason for these phenomena is cellules inflation and penetration water in the porous area. Also by increasing humidity content, Geometrical mean diameter was increased. The positive linear relationship of dimension and geometric mean diameter with moisture content were also observed by other research workers such as Gezer *et al.* (2002) and Kashaninejad *et al.* (2005) for apricot kernel and pistachio nut, respectively.

Sphericity: Spherically is a measure to determining shape of seed which can describe Figure of it. In the present study, change in spherically percentage with multi moisture content, were measured. Results show that spherically at 3.19%, 4.49%, 11.68%, 17.64% moisture content were 59.79 %, 59.74%, 61.04% and 62.21% respectively. As it is clear, spherically increase with increasing in moisture content, but moisture between 3.19% till 4.49% were exception. Maybe their reasons were related to proportion change in width and thickness to change in length. Gezer *et al.* (2002) and Desphande *et al.* (1993) have found an increasing relationship between sphericity and moisture content in their experiments with apricot kernel and soyabean, respectively.

Volume: Trend of change in volume of seed with multi moisture content, were shown on below (Fig. 4). As it is obvious, volume increase with increasing moisture content and its relation is as below:

$$V = 0.0015Mc + 0.4375 \quad (R^2 = 0.9949) \qquad (6)$$

Similar results have been reported by Desphande *et al.* (1993) for soybean, Ogut (1998) for white lupin, Gezer *et al.* (2002) for apricot kernel and Karababa (2006) for popcorn kernels.

True density: As shown on the Figure 5, true density of apricot kernels at multi moisture content was between 882.59 till 983.38 kg/m^3. Following equation can be used to for determining relation between density and moisture content. Desphande *et al.* (1993) also observed the linear increase in kernel density with increase in grain moisture in the range 8.7-25% db for JS-7244 soybean. Aydin (2003) reported effect of moisture content on true density of almond nut and kernel showed an increase

Table 1: Physical properties mean comparison in different moisture content by Duncan's multiple range

Moisture content*	3.19%	4.49%	11.69%	17.46%
Length (mm)	15.33^{a}(1.247)	15.37^{a}(1.242)	15.57^{a}(1.211)	15.76^{a}(1.194)**
Width (mm)	10.91^{a}(0.916)	10.92^{a}(0.918)	10.99^{a}(0.885)	11.06^{a}(0.877)
Thickness (mm)	4.64^{c}(0.809)	4.66^{c}(0.810)	5.05^{b}(0.718)	5.45^{a}(0.712)
Geometrical mean diameter (mm)	9.138^{c}(0.738)	9.155^{c}(0.740)	9.479^{b}(0.685)	9.785^{a}(0.686)
Spherically (%)	59.791^{b}(4.513)	59.74^{b}(4.479)	61.04^{ab}(3.729)	62.21^{a}(3.385)
Surface area 1 (mm2)	264.93^{a}(42.451)	283.62^{a}(40.743)	297.59^{a}(55.747)	302.17^{a}(60.211)
Surface area 2 (mm2)	199.98^{b}(34.326)	224.29^{ab}(35.516)	239.79^{a}(34.235)	255.33^{a}(35.285)
Volume (cm3)	0.442^{a}(0.108)	0.444^{a}(0.109)	0.456^{a}(0.108)	0.463^{a}(0.109)
Mass (gr)	0.380^{b}(0.103)	0.383^{b}(0.105)	0.417^{ab}(0.101)	0.448^{a}(0.103)
True density (kg/m3)	882.588^{b}(174.68)	885.570^{b}(178.03)	934.054^{ab}(167.20)	983.383^{a}(165.58)
Bulk density (kg/m3)	471.6^{c}(8.303)	440.8^{b}(8.478)	412.2^{a}(8.285)	406.8^{a}(0.011)
Coefficient of static friction on:				
wood	0.3679^{a}(0.121)	0.3685^{a}(0.093)	0.3772^{a}(0.015)	0.3838^{a}(0.051)
glass	0.1768^{c}(0.011)	0.1781^{a}(0.028)	0.1793^{bc}(0.101)	0.2065^{ab}(0.039)
galvanize sheet	0.2141^{a}(0.061)	0.2145^{a}(0.034)	0.2150^{a}(0.015)	0.2216^{a}(0.074)
fiberglass sheet	0.2356^{a}(0.119)	0.2363^{a}(0.093)	0.2369^{a}(0.034)	0.2400^{a}(0.016)

tests (at 5% level). *Above result was for average 100 apricot kernels. **Superscript letters indicate that means with the same letters designation in a column are not significantly different at P = 0.05.

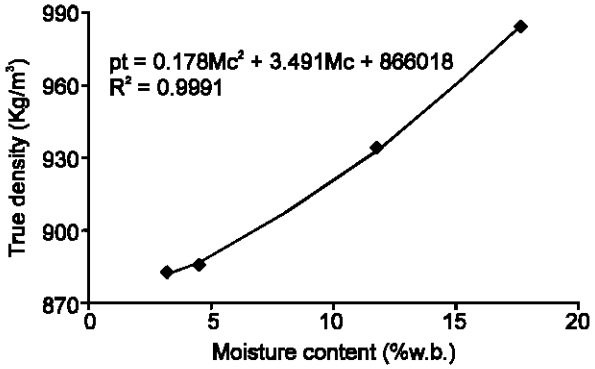

Fig. 5: Effect of moisture content on actual density of apricot kernel.

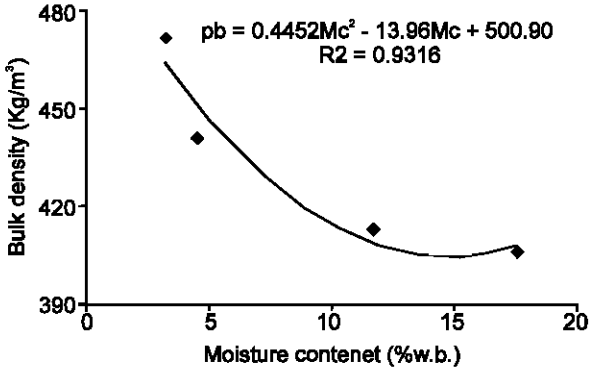

Fig. 6: Effect of moisture content on bulk density.

with moisture content. The negative linear relationship was also observed by Desphande *et al.* (1993) for soy bean, Sahoo and Srivastava (2002) for okra seed, Konak *et al.* (2002) for chickpea seeds and Kaleemullah and Gunasekar (2002) for arecanut kernels, Karababa (2006) for popcorn kernels and Razavi *et al.* (2007) for pistachio nuts and their kernels.

$\rho t = 0.1787Mc^2 + 3.4911M + 868.18$ (R^2 = 09991) (7)

Bulk density: According to Fig. 6, bulk density of apricot kernels at multi moisture content were 407 to 472 Kg/m^3 which its relation was as below:

$\rho b = 0.4452Mc^2 - 13.096Mc + 500.92$ (R^2 = 0.9316) (8)

The negative relationship of bulk density with moisture content was also observed by Aydin (2003) and Gupta and Das (1997) for almond nut and kernel and sunflower seeds, respectively. The relationship between bulk density and moisture content was statistically significant ($p < 0:05$). In contrast, the negative linear relationship of bulk density with moisture content was observed by various research workers (Konak *et al.*, 2002; Nimkar and Chattopadhyay, 2001). Razavi *et al.* (2007) reported that there is a linear increase in bulk density of pistachio nut and its kernel with an increase in moisture content.

Surface area: In this study, effect of moisture content on surface area of apricot kernels investigated. According to Fig. 7, results show that equation S1 with moisture content 3.19%, 4.49% , 11.69% and 17.64% surface area were 264.93, 283.62, 297.59 and 302.17 mm^2 and with equation S2 it was 199.98, 224.29, 239.79 and 255.33 mm2 respectively.

$S1 = 2.2619Mc + 266.25$ (R^2 = 0.8077) (9)

$S2 = 3.3219Mc + 199.26$ (R^2 = 0.8765) (10)

As it is clear equation S1 show more surface area than equation S2 which this difference related to change in

Table 2: Duncan comparing of mean rupture strength data of apricot kernels in multi moisture content

Moisture content	17.46%	11.69%	4.49%	3.19%
Through length	16.620[a](2.089)	18.331[a](1.814)	22.588[a](9.014)	23.443[a](5.937)
Through width	32.25[a](1.331)	35.923[ab](3.257)	38.870[ab](15.734)	46.420[b](11.124)
Through thickness	91.221[a](13.509)	115.64[ab](16.245)	130.550[b](27.761)	135.292[b](21.307)

Above result was for average 30 apricot kernels.

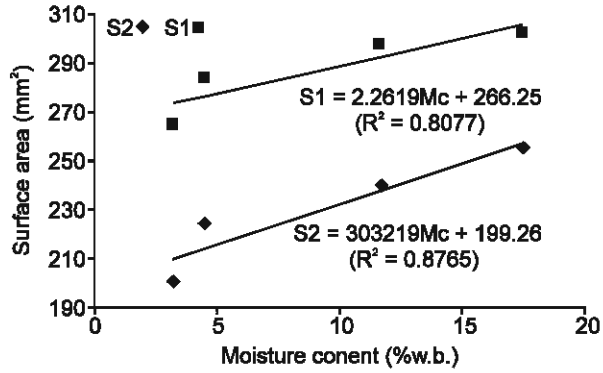

Fig. 7: Effect of moisture content on surface area.

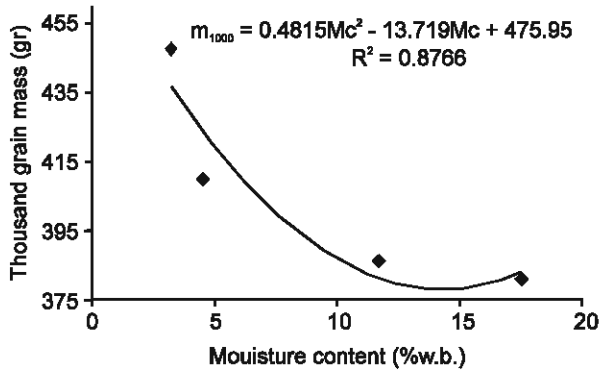

Fig. 8: Effect of moisture content on 1000 grain mass.

data apparatus act on the equations. Similar trends were reported for many other seeds (Mohsenin, 1970). Desphande *et al.* (1993) found that the surface area of soybean grain increased from 0.813 to 0.952 cm^2, when the moisture content was increased from 8.7% to 25% db.

Thousand grain mass: Effect of moisture content from 3.19% to 17.46% on weight of 1000 seed of apricot kernels, were shown on the Fig. 8. Thousand grain mass at this range of moisture content was between 381.6 to 447.9 g. Its equation was:

m_{1000}=0.4815Mc^2-13.719Mc+475.95 (R^2= 0.9458) (11)

Similar results have been reported by Desphande *et al.* (1993); Ogut (1998); Baryeh (2002); Baryeh and Mangope (2003) and Karababa (2006) for soybean, white lupin, millet, pigeon pea and popcorn kernels, respectively.

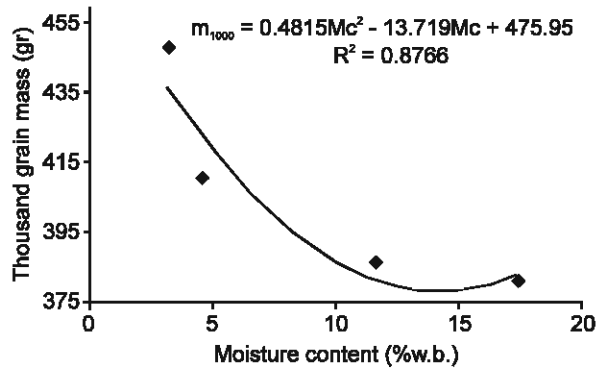

Fig. 9: Effect of moisture content on porosity of apricot kernel.

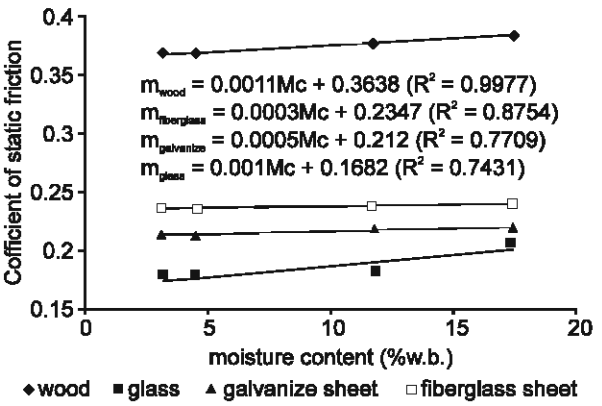

Fig. 10: Effect of moisture content on coefficient of static friction of apricot kernel.

of apricot kernel (with value 51.33% to 52.68%) given in the below equation. Following Fig. 9 shows trend of this variation.

Porosity: Reverse relation between porosity and moisture content

P = -0.0853Mc + 52.789 (R^2 = 0.9463) (12)

Other researchers were reported for gram (Dutta *et al.*, 1988), sunflower seeds (Gupta and Das, 1997), white lupin (Ogut, 1998) hazel nuts (Aydin, 2002) chickpea seeds (Konak *et al.*, 2002), arecanut kernels (Kaleemullah and Gunasekar, 2002), okra seeds (Sahoo and Srivastava, 2002) and pigeon pea (Baryeh and Mangope, 2003).

Coefficient of static friction of kernels on 4 surfaces (wood, glass, galvanized sheet and fiberglass sheet).
Fig. 10 shows relations between coefficient of static friction on 4 surfaces (wood, glass, galvanized steel and fiberglass). As it is clear, with increasing moisture content, this coefficient increased for all surfaces but with different levels. Relation between moisture content and coefficient of static friction of apricot kernels on different surface given as bellow:

$$\mu_{galvanize} = 0.0005Mc + 0.212 \ (R^2 = 0.771) \quad (13)$$

$$\mu_{wood} = 0.0011Mc + 0.3638 \ (R^2 = 0.997) \quad (14)$$

$$\mu_{glass} = 0.0018Mc + 0.1682 \ (R^2 = 0.744) \quad (15)$$

$$\mu_{fiberglass} = 0.0003Mc + 0.2347 \ (R^2 = 0.875) \quad (16)$$

Gezer *et al.* (2002) stated that as the moisture content increased so the coefficient of static friction increased.

Comparing mechanical properties of apricot kernels by Duncan methods: Comparing average data of rupture strength at three dimension i.e. length, width and thickness by Duncan method given on the Table 2. Results showed that by increasing moisture content of apricot kernels, fracture forces decreases in all three dimensions.

Rapture strength: Following Table 2 shown variations between fracture forces and moisture content for apricot kernels. As it is clear, maximum force was for fracturing shelling apricot at thickness direction. Relation between fracture forces and moisture content presented bellow.

$$F_L = 0.0234Mc^2 - 0.9Mc + 25.32 \ (R^2 = 0.9371) \quad (17)$$

$$F_W = 0.046Mc^2 - 1.8196Mc + 50.22 \ (R^2 = 0.9397) \quad (18)$$

$$F_T = -0.1261Mc^2 - 0.2289Mc + 134.14 \ (R^2 = 0.9733) \quad (19)$$

The study of Gezer *et al.* (2002) supported this result.

Conclusion:

1. The symmetrical dimensions and weight of apricot kernel increased depending on moisture content. This situation stems from water absorption of kernel.
2. measured sphericity from equation 6 and 7 and Geometrical mean diameter value of apricot kernel increased with increasing moisture content.
3. In apricot kernels, 1000 grain weight, grain volume, grain density, and surface area increased with moisture content.
4. The bulk density value decreased with moisture content in apricot kernels. There was a negative relationship between them.
5. Porosity value decreased with increase moisture content. It was maybe due to decreasing sphericity in lesser moistures content.
6. In apricot kernel, the angle of static friction was found to be higher on a wood than on a fiberglass sheet, galvanize sheet and glass, respectively. The value of angle of static friction on all surfaces increased with increase moisture content.
7. Rupture strength decrease when the moisture content increased. That is a negative relationship between them. The force applied through length was found to be lesser and through thickness was found to be highest in apricot kernel.

Nomenclature

L	Length	V	Volume
W	Width	Td	True density
T	Thickness	Bd	Bulk density
Dg	Geometrical mean diameter	M1000	Thousand grain mass
ϕ	Sphericity	p	Porosity
S	Surface area	μ	coefficient of static friction
M	Mass	F	Rapture strength
Mc	Moisture content		

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Effect of Postharvest Storage Techniques on the Nutritional Properties of Benin Indigenous Okra *Abelmoschus esculentus* (L) Moench

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Abstract: In Nigeria, okra *Abelmoschus esculentus* are packed and stored in polypropylene bag when moved from outlying villages to the city market. The study aims at assessing other storage method for Benin indigenous okra other than polypropylene bag with respect to nutrients, antinutrients and antioxidants. In this study fresh harvested Benin okra were harvested and divided into three parts. One part was stored in 100% RH at the temperature of 10°C±2°C, another at the temperature of 10°C±2°C and the last part in polypropylene bag. The nutrients, antinutrients (Phytate and Saponin) antioxidants (Vitamin C and Total Phenol) and the Viscosity were subsequently determined. The result of the study indicates that the nutrient, antinutrient and antioxidant content reduces significantly ($p>0.05$) in the three storage methods: Moisture (88.73-84.62)%, fibre (10.63-7.22)%, protein (14.87-12.84)%, fat (9.67-7.96)%, phytate (3.84-1.18)%, saponin (0.612-0.284)%, vitamin C (46.28-14.39) mg/100g, total phenol (0.095-0.059)% and the viscosity (58.16-53.42)cp. The method of storage of 100%RH recorded the least percentage loss in moisture, fibre antioxidant and viscosity content of the okra while the least % loss was recorded for protein and fat in the polypropylene bag method. The highest loss of the antinutrient was recorded at the storage method of temperature of 10°C ±2°C. On the average the storage method of 100%RH at temperature of 10°C ±2°C shows to be better method than the others.

Key words: Nutrients, anti-nutrient, antioxidant, benin okra, relative humidity

Introduction

Okra *Abelmoschus esculentus* (L) moench is a tall annual dicotyledonous plant related to cotton and thought to be of African origin. It is still found growing wild along the river Nile in Egypt as well as Ethiopia (Kochhar, 1986) French colonialist carried okra to the new world soon after 1700. Now it is a widely grown vegetable crop in the tropics and sub tropics and also in the warmer temperate areas (Kochhar, 1986).

Young pods may be harvested 60-180 days from sowing about 5-10 days after flowering depending on the cultivar grown. Successional harvesting of young pods is generally recommended. The pods are harvested by detaching using a slight twist to break the stalk (Tindall, 1986).

The fresh and green tender fruits are used as vegetable. Tender and edible fruit is easily cut by the kitchen knife and set into mucilaginous consistency after cooking (Sowumi and Chukwudebe, 1979).

Okra mucilage has medicinal applications; when used as a plasma replacement or 'blood-volume expander'. The mucilage of Okra not only binds cholesterol but the bile acid carrying toxins dumped into it by the filtering liver. It also has industrial applications; when added as size to glaze paper and used in confectionary (Siemonsma and Kouame, 2004; Kochhar 1986; Shalau, 2002).

In Nigeria fresh okra is preferred to dried Okra by the Majority of people and as such consumption is highest in the raining season when production is highest. The site of production of these okra are always very far to the market and where they are been consumed, therefore post-harvest deterioration of fresh okra result in loss of produce due to the poor storage and transport conditions employed by farmers in bringing the produce from the farms in outlying villages to the city markets.

In Nigerian Okra are packed and stored in a bag called polypropylene bag when moved from the outlying villages to the city markets which may be up to 520km, and it may get to the city market after 48hours to 72hours due to transportation problem.

The aim of this study is to exploit other method that can be use in storing Benin indigenous Okra for utilization and transportation other than polypropylene bag with respect to nutrients, antinutrients and antioxidants.

Materials and Methods

Fresh Benin Okra was harvested from a pilot farm in Benin City Nigeria. The chemicals were analytical grade while the water used in the analysis was glass distilled. The freshly harvested okra was randomly divided into three parts. One part was stored at the relative humidity of 100% at the refrigerated temperature of 10°C±2°C prepared by putting 100ml of distilled water in a

Table 1: Nutrient composition of Benin Okra in %

Parameters	Day	Polypropylene bag	100% R.H	10°C ±2°C
Moistur	0	88.73±0.044a	88.73±0.044a	88.73±0.044a
Content	2	88.06±0.035b	88.61±0.044a	88.01±0.035b
	4	86.42±0.044c	88.53±0.035a	87.53±0.044b
	6	85.76±0.044c	88.27±0.044a	87.00±0.035b
	8	85.00±0.044c	88.06±0.035a	86.81±0.044b
	10	84.62±0.044c	87.82±0.044a	86.03±0.044b
	%Loss	4.63	1.03	3.04
Crude	0	10.63±0.015a	10.63±0.015a	10.63±0.015a
Fiber	2	10.41±0.021b	10.60±0.053a	8.76±0.017c
	4	10.04±0.053bc	10.56±0.047a	8.52±0.017c
	6	9.82±0.026b	10.41±0.017a	8.21±0.044c
	8	9.61±0.017bc	10.26±0.040a	7.68±0.017bc
	10	9.02±0.035c	10.17±0.017a	7.22±0.036b
	%Loss	15.13	4.32	32.05
Crude	0	14.87±0.010a	14.87±0.010a	14.87±0.010a
Protein	2	14.36±0.027ab	13.94±0.026b	13.89±0.026c
	4	14.14±0.026a	13.88±0.028c	13.74±0.026c
	6	13.88±0.028a	13.62±0.026b	13.43±0.021c
	8	13.74±0.026a	13.17±0.045c	13.06±0.026c
	10	13.04±0.026a	12.84±0.026a	12.84±0.033a
	%Loss	12.26	13.6	13.6
Fat	0	9.67±0.023a	9.67±0.023a	9.67±0.023a
	2	9.28±0.044a	9.21±0.035bc	9.17±0.017cd
	4	9.21±0.036a	9.18±0.017a	9.05±0.015b
	6	9.08±0.017a	9.05±0.017a	8.86±0.017c
	8	8.91±0.026a	8.26±0.026c	8.33±0.017bc
	10	8.52±0.036ab	8.06±0.026b	7.96±0.017b
	%Loss	11.89	16.65	17.68

Value represent mean of triplicate. Values with the same alphabet along the same row are not significantly different (p> 0. 05)

dessiccator and covered for 72 hours (Wiston and Bate,1960) another part at the refrigerated temperature of 10°C±2°C while the last in polypropylene bag.

Sample analysis: Freshly harvested Benin Okra was analyzed. Stored samples of Benin Okra were analyzed on alternate days with regard to the nutrient, antinutrient and antioxidant content. The Okra were sliced and dried in the oven at 45°C to a constant weight and analyzed as follows:

The nutrient composition, (moisture, fat and crude fibre) of the fresh and stored Benin Okra were determined using the standard AOAC (1990) method and the protein content was determined using the micro-kjeldhal method (Nx6.25). The phytate content was determined by the method of Maga, 1982. which depend on the ability of standard ferric chloride to precipitate phytate in dilute Hcl extract of the Okra.

The Saponin was determined using the spectro-photometric method of Brunner, 1984. in which the mixture of okra and Isobutyl alcohol (2g in 250ml isobutyl l alcohol) was filtered into 20ml 40% saturated solution of magnesium carbonate. The mixture is filtered to get a colorless solution, 2ml of 5% iron (iii) chloride is added to 1m of the colorless solution and made up to 50ml mark with distilled water and the absorbance was measured after 30minuties at 380nm.

The viscosity was measured using the ostwald viscometer as described by AOAC (1990).

The vitamin C content of the Benin Okra were determined by AOAC (1990) method, described thus 5g of the sample was extracted by 100ml H_2O, 25ml of 20% glacial acetic acid was added to 10ml of the sample extract and titrated against standardized 2,6 dichloroindophenol (0.05g / 100ml) solution.

The total phenol was determined by mixing 0.2ml phenolic extract (0.2g of the Okra extracted by 20ml 70% Acetone) with 0.8ml folin – ciocalteu reagent and 2ml of 7.5% sodium carbonate. The mixture was diluted to 7ml distilled water and the absorbance was measured after 2hours at 765nm, the result was calculated as gallic acid equivalent. (Iqbal *et al.*, 2004).

Statistical analysis: Data Collected were subjected to the analysis of variance (SAS, 2002). Mean separation were done where there is significant differences using Duncan multiple range test procedure as described in the SAS soft ware. Significance was accepted at $P \leq 0.05$.

Results and Discussion

Juvenile products such as Okra or lettuce are harvested when they are activity growing and often contain 85% or more of water and they have little protection against water loss (Kays, 1991).

Therefore appropriate storage can minimize moisture loss, slow respiration rate and inhibit the development

Table 2: Vitamin C content in mg/100g and Total phenol content in % of Benin Okra

Parameters	Day	Polypropylene bag	100%R.H	10°C ±2°C
Vitamin C	0	46.28±0.026a	46.28±0.026a	46.28±0.026a
Content	2	18.36±0.035c	22.54±0.017a	19.72±0.052b
	4	17.28±0.026c	21.46±0.035a	18.36±0.035b
	6	16.48±0.026c	20.54±0.010a	17.74±0.026b
	8	15.26±0.026c	19.72±0.026a	17.13±0.010b
	10	14.39±0.035c	19.04±0.035a	16.58±0.017b
	%Loss	63.78	54.48	59.4
Total Phenol	0	0.095±0.004a	0.095±0.004a	0.095±0.004a
	2	0.082±0.003ab	0.086±0.003a	0.085±0.003a
	4	0.076±0.003c	0.084±0.003a	0.081±0.003ab
	6	0.068±0.003bc	0.081±0.003a	0.078±0.003a
	8	0.062±0.003bc	0.079±0.003a	0.076±0.003a
	10	0.059±0.003b	0.073±0.002a	0.072±0.003a
	%Loss	37.87	23.14	24.19

Value represent mean of triplicate. Values with the same alphabet along the same row are not significantly different (p > 0. 05)

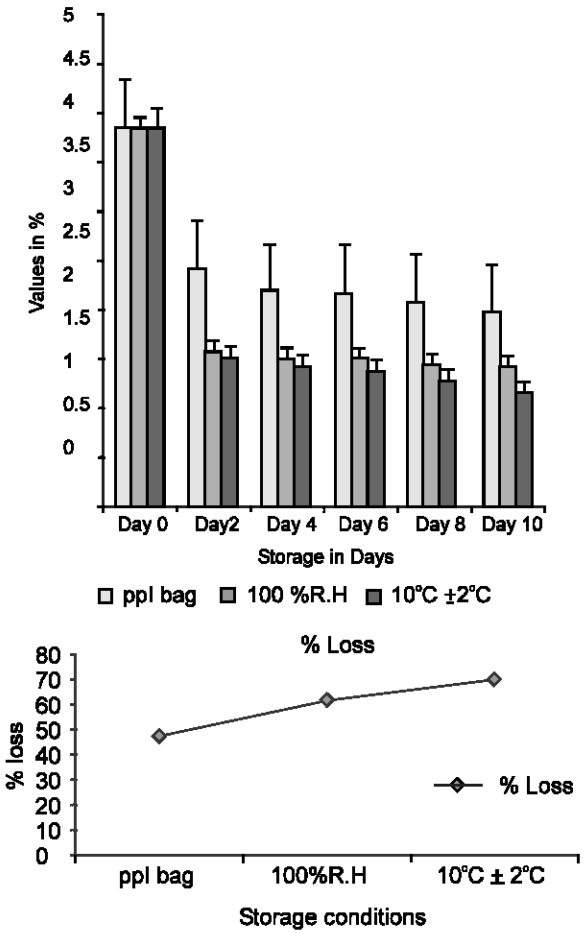

Fig. 1: Viscosity Content of Benin Okra in Cp and its percentage loss in storage

of microorganism. Commodities vary widely in their requirement and in their tolerance of non-optimal conditions (Anonymous, 1979, Munoz-Delgado, 1979). It show from Table 1 that the moisture content of Benin Okra decrease significantly (P>0.05) as the day of storage increases from day 0 to day 10 in the storage methods. (88.73 - 84.62%) the losses in moisture content of the Okra during Storage agrees with the findings of Gupta and Mukherjee (1982) in the storage of Okra waxed with morphactin reported moisture loss when they were stored for 7days.

The rate of loss was least when okra was stored at 100% R.H and recorded the lowest percentage (1.03%) followed by temperature of 10°C (3.04%) while polypropylene bag recorded the highest loss (4.63%).

The lowest percentage loss in 100% R.H at 10°C ±2°C may be as a result of a decrease in the metabolic water which are not been release since they have been arrested by the cold temperature and the high relative humidity might have not allowed for moisture migration from the fruit to its immediate environment which has been already saturated.

Dietary fibres are constituents of many fruits and vegetables, though dietary fibre cannot be digested by man but provides roughage that aids digestion (Eva, 1983). The fibre were decreasing significantly (P>0.05) as the storage days increase. The least rate of reduction and the least percentage loss were recorded at the relative humidity of 100% at 10°C ±2°C (4.32%). This decrease in crude fibre does not conform to the findings of Ketiku (1973) that crude fibre of banana fruit increase as the storage period increases. The reduction in fibre may be as a result of the conversion of the fibre which is cellulose to carbohydrate and used during respiration and at the relative humidity of 100% at 10°C ±2°C all respiratory and metabolic activities were retarded.

Protein are extremely important components of living cells in that they regulate metabolism and in some produce represent storage form of carbon and nitrogen (Kays, 1991)

The crude protein of Benin Okra were decreasing significantly (P>0.05) when stored using these storage methods over a period of 10days. This reduction in protein conform with the findings of Agbor-Egbe and Rickard (1990) that the crude protein content of aroid

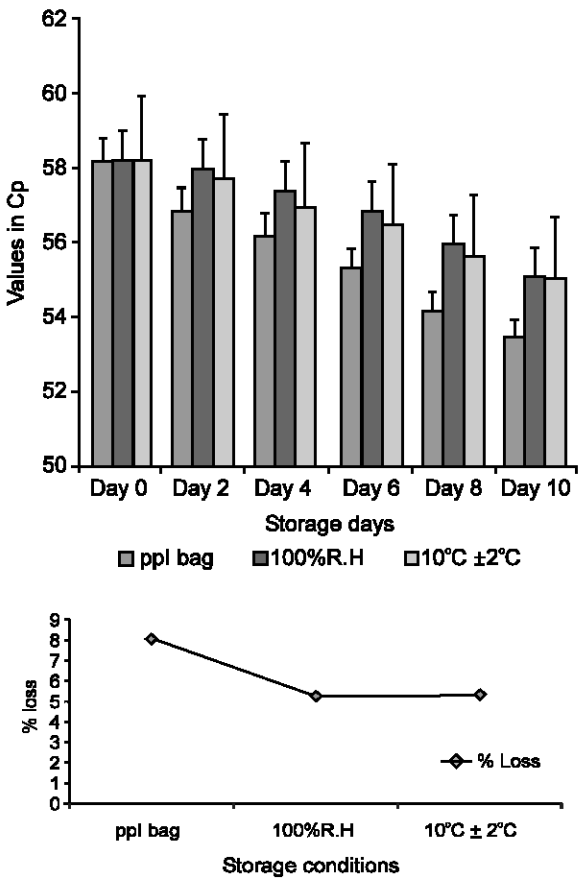

Fig. 2: Phytic acid Content of Benin Okra in % and its percentage loss in storage.

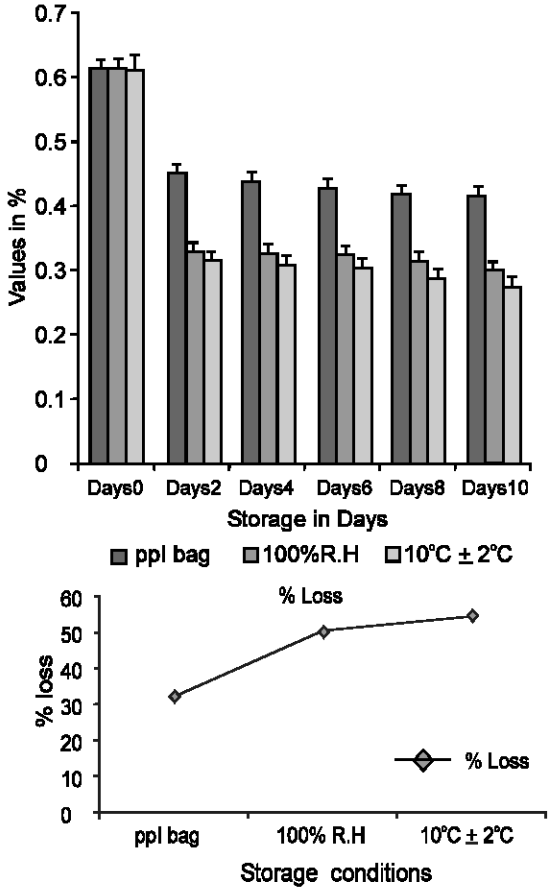

Fig. 3: Saponin Content of Benin Okra in % and its percentage loss in storage.

stored for 14days decrease as the storage period increase. The least rate of decease and percentage loss was recorded at the storage method of polypropylene bag (12.26%). This decrease in protein may be attributed to the physiological and metabolic activities within the cells of the Okra pod and at the same time due to proteolysis which is the breakdown of protein.

The fat content of this Benin Okra decrease in storage significant ($P>0.05$) as the storage period increases. The storage method of polypropylene bag recorded the least percesntage loss of fat (11.89%) the percentage loss of fat in Okra stored in 100% R.H and in temperature of 10°C±2 ℃ are close (16.65% and 17.68%). The reduction in the fat content can be as a result of the recycling of the carbon stored as triacylglycereols in lipids through the action of the enzyme lipase.

The viscosity of Benin Okra as shown in Fig. 1 shows that the viscosity is decreasing as the storage day's increase. Relative humidity of 100% and temperature of 10°C±2°C recorded the following percentage loss of 5.30% and 5.35% respectively while polypropylene bag has 8.05% loss. The reduction in viscosity could be attributed to the utilization of carbohydrate during metabolic process in the stored Okra because mucilage is a polysaccharide which is carbohydrate.

The complexing of phytic acid with nutritionally essential minerals are suggested as responsible for the antinutritional activity. Phytic acid interferes with Ca, Fe, Mg, and Zn absorption because of its ability to chelate divalent cationic minerals (Oboh, 2005).

The phytic acid of this Okra reduces in storage, fig. 2, the storage method that experience the highest loss of phytic acid is the temperature of 10°C ±2°C of 69.79% closely followed by the storage method of 100% R.H 61.72% the least is polypropylene bag 47.66%.This decrease in phytic acid agree with the report of Hernandez-Unzon and Ortega - Delgado (1989) that there was a decrease of 4% in phytic acid of stored common been seeds (*Phaseolus vulgarie* L).

Saponin are characterized by either bitter or astringent taste, foaming properties and their hemolytic effect on red blood cells .They are widely distributed in the plant kingdom (Agarwal and Rastogi 1974; Osagie 1998).

The saponin content of Benin okra in storage as shown in Fig. 2, shows that the saponin decreases significantly

as the storage period increases over a period of 10 days. The trend of decrease of saponin is closely related to that of phytate in that polypropylene bag recorded the least percentage loss 32.03%,the highest percentage loss took place in the storage method of temperature of 10°C ±2°C closely followed by relative humidity of 100% (50.00%).

Vitamin C contributes to the antioxidant properties of vegetables by protecting the membrane erythrocyte, maintaining the blood vessel flexibility and improving blood circulation in the arteries of smokers as well as flexibility the absorption of iron in the body (Oboh, 2005). As shown in Table 2, the vitamin C of the okra in storage reduces significantly ($p>0.05$) over the period of storage. The storage method of 100% R.H recorded the least percentage loss in vitamin C (58.85%), temperature of 10°C ±2°C recorded a percentage loss of 64.17% while the highest loss was recorded at the storage method of polypropylene bag (68.90%) . It has been reported by Albuquerque *et al.* (2005) that the Vitamin C content in water melon decreased with increasing time of storage. This decreased in vitamin C could be as a result of the activity of the enzyme ascorbate oxidase which convert vitamin C to dehydroascorbic acid in stored produce.

Phenols have antioxidant capacities that are much stronger than those of vitamin C and E (Amic *et al.,* 2003). The total phenol of Benin okra reduces in storage with the storage method of polypropylene bag recording the highest loss of 37.89% while the storage method of 100% R.H and temperature of 10°C ±2°C recorded 23.16% and 24.21% respectively. This decrease is in agreement with the finding of Ose *et al.* (1997) that the total phenol content of water convolvulus leaves decreases in storage. This reduction could be attributed to the fact that phenols are susceptible to oxidation by phenolase which convert them to quinones that are extremely reactive and therefore short lived.

Conclusion: In conclusion, it is shown that the storage method of 100%R.H at the temperature of 10°C ±2°C is the best method to store this Benin indigenous okra when transporting them from the outlying villages to the market and its final destination since it recorded the least percentage loss.

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Comparative Study of Quality Changes in Shea Butter Coated Pawpaw *Carica papaya* Fruit During Storage

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Abstract: The storage of shea butter coated pawpaw *carica papaya* fruit at different storage temperature was investigated as regard the nutrients, sugars and minerals. Freshly harvested just ripe fruit of pawpaw *carica papaya* was coated with shea butter and stored at room temperature (27°C±1°C), refrigeration temperature (10°C±1°C)and freezing temperature (-5°C±1°C) for 8 days. The proximate, sugar (glucose, fructose and galactose) and minerals (Ca, Mg, Fe and Zn) were subsequently determined. The result of the study shows that the moisture content increases significantly ($P \leq 0.05$) (85.25-95.25%) while other nutrients reduce significantly ($P \leq 0.05$) in storage, the sugar and the minerals content also reduce significantly ($P \leq 0.05$) in storage. The storage temperature of freezing temperature recorded the highest mineral content (Ca-113.33mg/100g, Mg- 53.33mg/100g, Fe- 2.33mg/100g and Zn- 7.00mg/100g) at the end of the storage period which was significantly ($P \leq 0.05$) higher than other storage temperatures. At the end of the storage period, there was no significant difference ($P \leq 0.05$) in the glucose and fructose content of the pawpaw at the different storage temperatures.

Key words: Nutrient, pawpaw, storage temperature, sugar, minerals

Introduction

Pawpaw *carica papaya* is a small tropical tree native to South America. It normally grows with a single and branched trunk which may reach 10m in height but is more commonly 4-5m tall. (Rice *et al*., 1987). The pawpaw plant is wide spread throughout tropical Africa. It belongs to the group (*Caricaceae*). It is a berry developing from syncarpous superior ovary with parietal placentation (Kochhar, 1986; Rice *et al*., 1987).

Fruit is harvested at the first sign of the yellowing if it is to be sent to distant markets, it may remain on the tree a day or two longer if intended for local markets (Rice *et al*., 1987).

Pawpaw fruit soften rapidly at room temperature after harvest and indicated a 2 - to - 3 day shelf life was to be expected (Archbold *et al*., 2003). If the fruit are not very quite ripe, they may be refrigerated for about two weeks and they ripened at room temperature for several days. Pawpaw is favored by the people of the tropic as breakfast and as ingredients in jellies, preservers or cooked in various ways (Oloyede, 2005). The juice makes a popular beverage; young leaves shoot and fruits are cooked as vegetables. Papain, the proteolytic enzymes has a wealth of industrial uses. It is used for meat tenderizers and chewing gum (Oloyede, 2005).

In Nigeria, pawpaw thrives in the southern part but the product is not yet in commercial quantities (Kochhar, 1986). Pawpaw fruit is one of the most nutritional and cheapest fruits grown and consumed in Nigeria. However in the average, fruits are increasingly becoming popular in the Nigerian diet, but the production of these crops remain low and inadequate (Baiyewu and Amusa, 2005). The flavour of pawpaw is very distinctive and needs to be preserved for the consumer (Archbold *et al*., 2003). In Nigeria, very little research has been done on the storage potential of pawpaw since they are not yet in commercial quantity though in some area in Ondo state of Nigeria pawpaw is been coated with palm oil and store on the shelf. It is therefore the aim of this study to determine the proximate, minerals and the sugar content of pawpaw and during storage at the room, refrigeration and freezing temperature.

Materials and Methods

The pawpaw used for this work was a freshly harvested just ripe pawpaw with slight appearance of yellow colour and shea butter obtained from "oja oba" market in Akure.

Sample preparation: The pawpaw was coated with the melted shea butter and divided into three lots. One was stored on the bench in the laboratory, another one was stored in the fridge and the last one was stored in the freezer.

Sample analysis: The nutrient composition, (moisture, crude fibre, ash and fat) of the pawpaw *carica papaya* were determined using the standard AOAC (1990) method, the protein content was determined using the micro-kjeldhal method (Nx6.25) and the carbohydrate was determined by difference. Sugars (glucose, fructose and galactose) were identified and quantified according to the method of Albuquerque (2005) by HPLC using a

Table 1: Nutrient content of shea butter coated pawpaw carica papaya in storage (%)

Nutrients	St.Temp.	Day 0	Day 2	Day 4	Day 6	Day 8
Dry	Rm T	14.74±0.74a(a)	12.85±0.03b(b)	10.95±0.03b(b)	8.94±0.02b(c)	4.74±0.03c(c)
matter	Rf T	14.74±0.74a(a)	14.12±0.07a(a)	13.43±0.02a(a)	12.05±0.03a(b)	10.26±0.04b(b)
	Fr T	14.74±0.74a(a)	14.60±0.02a(a)	13.95±0.03a(a)	12.71±0.95a(a)	11.09±0.05a(a)
Moisturecontent	Rm T	85.25±0.72a(a)	89.15±0.04b(b)	89.05±0.03b(b)	91.06±0.02b(b)	95.25±0.02c(c)
	Rf T	85.25±0.72a(a)	85.88±0.07a(a)	86.57±0.03a(a)	87.24±0.04b(a)	87.68±0.04b(a)
	Fr T	85.25±0.72a(a)	85.39±0.02a(a)	86.07±0.03a(a)	87.26±0.95b(a)	88.91±0.05c(b)
Carbohydrate	Rm T	7.85±0.35a(a)	5.76±0.16c(b)	6.76±0.11b(b)	5.58±0.18c(c)	2.02±0.23d(c)
	Rf T	7.85±0.35b(a)	8.09±0.14a(a)	8.29±0.13a(a)	6.20±0.22d(b)	7.87±0.16c(a)
	Fr T	7.85±0.35b(a)	8.14±0.16a(a)	8.21±0.18a(a)	7.66±0.20b(a)	6.79±0.21c(b)
Protein	Rm T	2.33±0.16a(a)	1.58±0.25b(b)	0.99±0.16c(b)	0.53±0.24d(b)	0.23±0.33e(b)
	Rf T	2.33±0.16a(a)	2.04±0.16a(a)	1.57±0.24b(a)	1.23±0.24b(a)	0.82±0.16c(a)
	Fr T	2.33±0.16a(a)	2.16±0.16a(a)	1.87±0.16b(a)	1.69±0.16b(a)	1.17±0.32b(a)
Ash	Rm T	1.95±0.02a(a)	1.80±0.03a(a)	1.58±0.03a(b)	1.46±0.02b(b)	1.24±0.01c(b)
	Rf T	1.95±0.02a(a)	1.82±0.04a(a)	1.74±0.02b(a)	1.62±0.03b(a)	1.54±0.03b(a)
	Fr T	1.95±0.02a(a)	1.67±0.05b(b)	1.74±0.07b(a)	1.62±0.03b(a)	1.54±0.03b(a)
Fiber	Rm T	2.02±0.02a(a)	1.55±0.04b(b)	1.41±0.04b(a)	1.21±0.03b(b)	1.14±0.01b(b)
	Rf T	2.02±0.02a(a)	1.76±0.03b(a)	1.59±0.02b(a)	1.51±0.02b(a)	1.32±0.02b(a)
	Fr T	2.02±0.02a(a)	1.91±0.01a(a)	1.66±0.02b(a)	1.45±0.02b(a)	1.33±0.02b(a)
Fat	Rm T	0.60±0.07a(a)	0.29±0.08b(a)	0.21±0.01b(a)	0.16±0.02b(a)	0.12±0.01b(a)
	Rf T	0.60±0.07a(a)	0.41±0.02a(a)	0.24±0.04a(a)	0.20±0.04a(a)	0.09±0.02b(a)
	Fr T	0.60±0.07a(a)	0.53±0.02a(a)	0.45±0.01b(a)	0.32±0.01b(a)	0.24±0.01b(a)

Rm T- Room temperature, Rf T- Refrigeration temperature and Fr T- Freezing temperature. Value represent mean of triplicate and standard deviation. Values with the same letter along the same Column are not significantly different ($p > 0.05$) while values with the same letter inside brackets along the row are not significantly different ($p > 0.05$).

waters R401 refractive index detector and a sugar-pack water column. The minerals were determined on aliquots of the solutions of the ash by established flame atomic absorption spectrophotometry method using atomic absorption spectrophotometer (model 372) (Perkin Elmer, 1982).

Analysis of data: Data Collected were subjected to the analysis of variance (SAS, 2002). Mean separation were done where there is significant differences using Duncan multiple range test procedure as described in the SAS soft ware. Significance was accepted at $P \leq 0.05$.

Results and Discussion

Fruits form integral part of African diet and are consumed as relishes and snacks. Fruits are found to be rich in vitamins, especially vitamin C minerals, fat and sugars (Achinewhu, 1983; Ogbonna, 1991). Despite this, fruits have not been given a pride of place in the diet of the Nigeria people. The main reason for the neglect is ignorance of the nutritive values of most of the fruits which abound in our environment (Umoh, 1998). Fruits are usually recommended for weight reducing formulas as well as for providing energy for convalescing patients who most often lack appetite during the period of ill-health (Umoh, 1998).

The result of the nutrient, minerals and sugar content of shea butter coated pawpaw in storage is highlighted as follows:

The result of proximate analysis of pawpaw *carica papaya* fruit as shown in Table 1 revealed that the fruit has 85.25% moisture. However, at the various storage temperature the moisture content increases significantly ($P \leq 0.05$) (85.25 - 95.25%) with the least increase at the storage temperature of refrigerator. The value reported in this work for just ripe pawpaw was lower to what was reported by Umoh (1995) for just ripe papaya 88.87% but higher than the value of ripe *dennetia tripatela* 82% (Udoessien and Ifon, 1984). It is worth noting that in all the storage temperatures observed the nutrient content of pawpaw reduces significantly ($P \leq 0.05$) as the days of storage increases but reduces non significantly ($P \leq 0.05$) except for carbohydrate with respect to each day of storage.

Carbohydrates contain mainly the elements carbon, hydrogen and oxygen; they are usually synthesized by green plants from water and carbondioxide with the aid of sunlight. They are a source of calories or energy (Enwere, 1998; Ihekoronye and Ngoddy, 1985). The carbohydrate content of pawpaw decreases (dry weight basis) significantly ($P \leq 0.05$) in the three storage temperatures at the end of the storage period (7.85-2.02%). The reduction in carbohydrate does not conform with the finding of Umoh (1995) who reported increase in the carbohydrate content of pawpaw as it ripen. The storage method of refrigeration temperature recorded the highest carbohydrate content in day 8 (7.87%) which was significantly higher ($P \leq 0.05$) than other storage temperatures. The decrease in carbohydrate could be attributed to the breaking down of the carbohydrate to sugars as the pawpaw ripens in storage.

Proteins are macromolecules containing one hundred to one thousand amino residue linked together by peptide bonds (Clucas, 1981). They are extremely important components of living cells in that they regulate metabolism, act as structural molecules and in some products represent storage forms of carbon and nitrogen (Kays, 1991).

Table 2: Mineral content of shea butter coated pawpaw carica papaya fruit in storage (%)

Minerals	St.Temp.	Day 0	Day 2	Day 4	Day 6	Day 8
Ca	Rm T	243.33±8.8a(a)	170.0±4.14b(b)	126.67±8.8c(c)	86.67±8.0d(b)	36.67±8.8e(c)
	Rf T	243.33±8.8a(a)	193.3±5.56a(a)	136.67±8.8b(b)	70.00±8.8c(b)	56.67±8.8d(b)
	Fr T	243.33±8.8a(a)	213.3±9.42a(a)	193.3±8.8a(a)	153.3±8.8b(a)	113.3±23.5c(a)
Mg	Rm T	160.0±21.2a(a)	103.3±9.42b(b)	73.33±9.42c(b)	43.3±8.85d(b)	16.67±23.5e(b)
	Rf T	160.0±21.2a(a)	123.3±9.42b(a)	76.67±8.8c(b)	56.67±8.85d(a)	20.00±4.14e(b)
	Fr T	160.0±21.2a(a)	133.3±9.42b(a)	103.33±9.5c(a)	76.67±9.42d(a)	53.33±9.42e(a)
Fe	Rm T	9.00±1.41a(a)	4.67±0.94b(b)	2.67±0.94b(c)	1.33±1.32c(b)	0.00±0.00d(b)
	Rf T	9.00±1.41a(a)	6.67±0.94b(a)	3.67±0.09c(b)	2.00±±1.41c(b)	0.00±0.00d(b)
	Fr T	9.00±1.41a(a)	6.67±0.94b(a)	5.67±0.94b(a)	4.00±1.41c(a)	2.33±0.94c(a)
Zn	Rm T	18.0±4.24a(a)	9.00±1.41b(c)	5.67±1.88c(c)	2.67±0.94d(c)	0.33±0.94e(b)
	Rf T	18.0±4.24a(a)	12.33±1.88b(a)	8.33±1.88c(b)	5.33±1.88d(b)	1.00±1.41e(b)
	Fr T	18.0±4.24a(a)	15.67±0.94b(a)	13.67±0.94b(a)	9.67±0.94c(a)	7.00±1.41c(a)

Rm T- Room temperature, Rf T- Refrigeration temperature and Fr T- Freezing temperature. Value represent mean of triplicate and standard deviation. Values with the same letter along the same Column are not significantly different (p> 0.05) while values with the same letter inside brackets along the row are not significantly different (p> 0.05).

The protein of pawpaw reduces significantly (P≤0.05) on dry weight basis as the days of storage increases in all the three storage temperatures (2.33-0.23%) though there was no significant (P≤0.05) difference in the three storage temperatures on daily basis. The protein content recorded for just ripe pawpaw was higher than what was observed for papaya by Umoh (1995) 0.41%, it was in the same range of the protein value of *terminalia* catappa fruits pulp 2.30% (Jeremiah, 1992) but was far lower to the protein value of the fruit of *tetrapleura tetraptera* (Essien *et al.*, 1994) and mango *magnifera indica* 18.3% (Ibiyemi *et al.*, 1990). The reduction in protein was in agreement with the findings of Umoh 1995 that protein content of papaya reduces as they ripen. Agbor - Egbe and Rickard (1990) also reported decrease in the crude protein of avoid stored for 14days. However, this decrease does not agree with the findings of Amusa *et al.* (2002) who reported increase in the protein content of breadfruit in storage. The decrease in the protein content could be attributed to proteolysis which is the breakdown of proteins and it begins fairly rapidly after harvest.

The ash content reduces significantly (P≤0.05) as the storage period increases (2.02-1.41%) though with respect to days in storage temperatures, there was no significant (P≤0.05) difference. Dietary fibres are constituent of many fruits and vegetables; although dietary fibres cannot be digested by man but have useful roles in providing roughage that aids digestion (Eva, 1983). The fibre reduces significantly (P≤0.05) as the storage period increases (1.95-1.24%) though with respect to days in storage temperatures, there was no significant (P≤0.05) difference. Umoh (1995) reported decrease in fibre of papaya as they ripen. This decrease is not in agreement with the report of Amusa *et al.* (2002) who reported increase in crude fibre of bread fruit in storage. This decrease in fibre could be due to the conversion of the fibre, a cellulose, to carbohydrate and used during respiration.

Fats and oils in the unrestricted sense are macromolecules that are highly soluble in organic

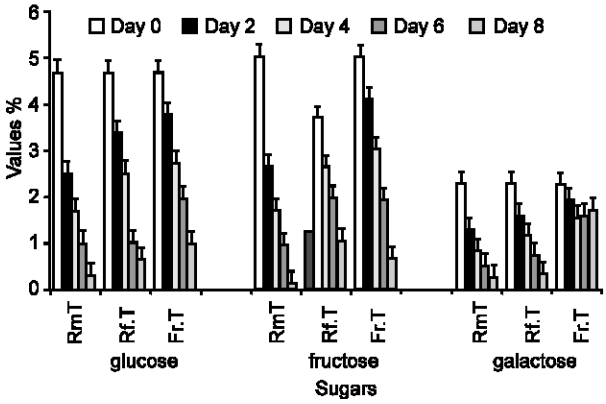

Fig. 1: Sugar content of shea butter coated pawpaw *carica papaya* in storage. Rm T- Room temperature, Rf T- Refrigeration temperature and Fr T- Freezing temperature.

solvents and only sparingly soluble in water (Davidson *et al.*, 1975). Fats and oils are generally called lipids; they provide a convenient and concentrated source of energy in the diet (Ononogbu, 1988). The fat content decreases significantly (P≤0.05) as the storage time increases (0.60-0.09%) but with respect to days in the different storage temperatures, there was no significant (P≤0.05) difference. the fat content obtained was higher than the value obtained by Umoh (1995) 0.46% for just ripe papaya but lower to the value recorded for Africa star apple pulp 15.1% (Edem *et al.*, 1984). The decrease in fat content of pawpaw could be attributed to the recycling of the carbon stored as tricyglycerols in lipids through the action of the enzyme lipase. At the end of the storage period, the freezing temperature recorded the highest value of fat 0.28% which was significantly (P≤0.05) higher than other storage temperatures.

Glucose and fructose are found abundantly in nature occurring free in some foodstuffs and combined with each other. Glucose is the sugar normally found in the blood, it therefore, has an important medical application as a substance that can be administered as a source of

carbohydrate for patients unable to take nourishment by mouth (Ihekoronye and Ngoddy, 1985). The result of the sugar content is shown in Fig. 1, the result revealed that the sugar of just ripe pawpaw to be glucose 4.64%, fructose 4.99% and galactose 2.26%. The sugar are lower to the values reported by Umoh (1995) for pineapple but higher than the values reported by Ladele *et al.* (1984) for unripe and just ripe plantain. However, the value of these sugars reduces significantly ($P \leq 0.05$) as the storage period increases glucose (4.64-0.29%), fructose (4.99-0.13%) and galactose (2.26-0.23%). The reduction in sugar could be attributed to natural degradation. In fact they become metabolically consumed in the respiratory chain due to phosphorylated equivalent synthesis (Albuquerque *et al.*, 2005). When the storage temperatures were analyzed with respect to days, it shows that there was a significant ($P \leq 0.05$) difference in the sugar content with the freezing temperature having the highest value of 1.12% glucose, 1.65% fructose, 1.71% galactose at the end of the storage period which was significantly ($P \leq 0.05$) higher than other storage temperature.

Minerals are classified into major and minor elements, with the major been calcium, potassium, phosphorus, magnesium, sodium, sulphur and iron, all are required in large quantities. (Davidson *et al.*, 1975). Mineral content of (Ca, Mg, Fe and Zn) of pawpaw fruits are shown in Table 2, the result shows that Ca (243.33mg/100g) and mg (160.00mg/100g) were high while Zn (18.00mg/100g) and Fe (9.00mg/100g) were low. The minerals were in the same range with the values reported by Jeremiah (1992) for *T.* catappa fruits pulp but higher than the values reported by Oloyede (2005) for unripe pawpaw. During the storage of pawpaw fruits, the fruits ripen and the colour changes from green to yellow with the formation of carotenoids which could require minerals, this could account for the reduction in the minerals as the storage period increases. However, when the storage temperatures were analyzed with respect to days, it shows that there was a significant ($P \leq 0.05$) difference in the mineral content with the freezing temperature having the highest value of 113.33mg/100g calcium, 53.33mg/100g magnesium, 2.33mg/100g iron and 7.00mg/100g zinc at the end of the storage period which was significantly ($P \leq 0.05$) higher than other storage temperature.

Conclusion: It is therefore advocated that pawpaw after coated should not be stored at the room temperature since this recorded the lowest values of nutrients, sugar and minerals which were significantly ($P \leq 0.05$) lower to other storage temperatures and it makes these nutrients, sugar and minerals not available to the consumer.

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Effects of Aqueous Extract of *Mangifera indica* L. (Mango) Stem Bark on Haematological Parameters of Normal Albino Rats

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Abstract: The effects of crude aqueous extract of *Mangifera indica* (Mango) stem bark on body weight and haematological parameters in normal albino rats were investigated. Albino rats of both sexes weighing between 75 g and 125 g were used. At least 14 mL of the test aqueous extract of the plant was administered to each rat in the group for a period of 14 days. Observations showed that the extract of the medicinal plant have some effects on the haematopoietic system manifested by a positive increase in the levels of PCV (haematocrit), erythrocyte, leukocyte, platelet counts and lymphocytes, while the haemoglobin (Hb) and neutrophil levels were decreased. The test plant also caused an increase in the weights of the rats. Therefore, it is not possible that its use can advance any adverse effects on haematological parameters.

Keys words: Aqueous extract, haematology, *Mangifera indica*, albino rats

Introduction

For thousands of years, people have looked to natural means of healing. Hebrew physicians in Bible times, used remedies such as oil, balsam, wine and poulticesi (Isa 1: 6; Jer46: 11; LK10: 34; 2Kings20: 7). In developing countries of the world, most of the people depend on herbal medical care (Ekpe *et al*., 1990). In most parts of Rivers State of Nigeria, traditional medicine has been claimed to be vital in preventing and curing various diseases, thereby playing an important role in the health services of the state especially among the low socio-economic class. These herbs are orally administered or can be applied onto the skin surface as ointment. Extracts of root, stem, bark and leaves of some medicinal plants have been shown to have activities against most dreaded pathogenic organisms like the bacteria, fungi etc (Bannerman *et al*., 1975; Madunagu *et al*., 1990; Khan *et al*., 1980; Singh and Pattak, 1994), while some others are cytotoxic (Russel *et al*., 1997; Prohp and Alaiya, 2003; Prohp and Maduemezia, 2004; Prohp *et al*., 2004; Prohp *et al*., 2006a; Prohp *et al*., 2006b). Also, some other still serve as liver tonic (Crescent bloo.com.1998).

The popularity of traditional medicine is due to the belief that some diseases only respond to traditional treatment (Bannerman *et al*., 1975). The claim that some plants have therapeutic action and are used for the treatment of myriads of pathological conditions has aroused my curiosity and choice of this commonly used Medicinal plant (*M. indica*) in the Niger Delta area of Nigeria (Ogoni area in particular), in attempt to cure diseases such as malaria, fever, dysentery, etc. There are many of these plants not yet identified and properly classified scientifically (Watts *et al*., 1997).

Mangifera indica L. (Mango) tree which is believed to be a native of Asia, is a member of the Anacardiaceae family (http:// 216. 239. 59. 104/ DAVMWSKLW24J: en. wikipedia.org/wiki/mango). It is grown widely in different parts of Africa, especially in the southern part of Nigeria. *Mangifera indica* is used medicinally to treat ailments such as asthma, cough, diarrhea, dysentery, leucorrhoea, jaundice, pains and malaria (Agoha, 1981; Madunagu *et al*., 1990). *Mangifera indica* contain alkaloids and glycosides which are of great importance pharmacologically (Madunagu *et al*., 1990; Ross and Brain, 1977). This conferred on *Mangifera indica*, its medicinal ability. Therefore this study is designed to investigate the possible effects of aqueous extract of the medicinal plant, under study on some haematological parameters of normal albino rats. This is with the view to properly classify the herb as safe for use, for curing diseases and devoid of some complications.

Materials and Methods

Collection of medicinal plant: *Mangifera indica* stem bark was obtained freshly from a farm in Nyogor Beeri (Ogoni), Rivers State of Nigeria, for preparation of the extract.

Experimental animals: The animals used in the study were albino rats (Sprague Dawley) weighing between 75 g and 125 g, of both sexes, obtained and maintained at the Animal House of the Department of Biochemistry, University of Port Harcourt. They were kept in rat cages and all rats were fed with grower's mash and allowed free access to clean fresh water in bottles *ad libitum* throughout the duration of the study.

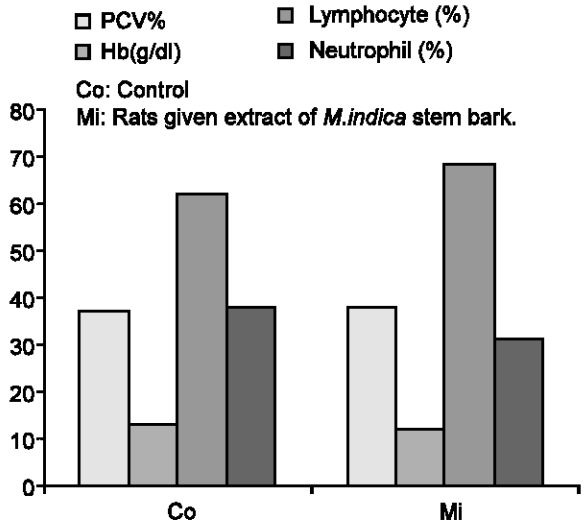

Fig. 1: Changes in PCV, Hb, and White cell differentials of rats given extract of *M. indica* stem bark

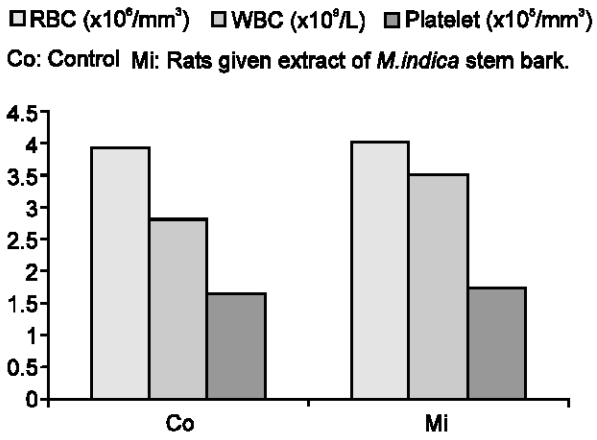

Fig. 2: Changes in RBC, total WBC and platelet counts of rats given extract of *M. indica* stem bark

Preparation and administration of the crude extract of the medicinal plant: The stem bark of *Mangifera indica* was cut into smaller pieces and dried in a Corsair heating oven (Ovl-350). The dried sample was pulverized using mortar and pestle. The resulting powder material was used in the extraction process. Extraction was carried out by the methods of Harboone (1972), Ekpe *et al.* (1990) and Uhegbu *et al.* (2005) using distilled water as the solvent. 20 g of powdered sample of the herb was extracted by soaking in 180 mL of distilled water in a beaker, stirred for about 6 minutes and left overnight. Thereafter, the solution was filtered using filter paper (Whatman No. A-1) to remove cellulose fibers and extract stored in a refrigerator at 4°C. 1 mL of the extract was administered daily to each of the designated rats using stomach canula for 14 days. The control group received clean water instead of the extract.

Phytochemical screening: The phytochemical analysis of aqueous extract of *Mangifera indica* has been reported to contain Tanins, Phlobatanins, Cardiac glycosides, Saponin and Polyphenol (Madunagu *et al.*, 1990).

Design of the study groups: Fourteen rats divided into two groups of seven rats per group were used in the study. Thus:

Group 1: control (given clean water)
Group II: Rats given extract of *M. indica*

Weight assessment: The weight of each rat was monitored daily as an index of the physical status of the animals over the period of study using compression spring balance (BAW-660-M).

Technique for obtaining blood: At the end of the experiments (14 days), each rat was anaesthetized with chloroform and sacrificed by cutting through the jugular vein. The blood pooled from each rat in a group was collected into heparinized bottles for hematological studies.

Determination of haematological parameters: Determination of Packed Cell Volume (PCV) was carried out using the Haematocrit method as described by Schalm *et al.* (1975), Dacie and Lewis (1991). Haemoglobin concentration was determined using the cyanomethaemoglobin method (Jain, 1986). The total White Blood Cells (WBC), White blood cell differentials, Red Blood Cell (RBC) and the platelet counts were estimated using the improved Neubauer counting chamber (Jain, 1986; Dacie and Lewis, 1991).

Results

The results of the study are presented in Fig. 1-3. Fig. 1 showed levels of haemoglobin (Hb) and PCV of rats given extract of *Mangifera indica* stem bark, as compared to that of the control. It showed a slight increase in PCV and a slight decrease in Hb. The value obtained for RBC (Fig. 2) also agreed with the reported trend for PCV above. The rats in group II that were given extract of *Mangifera indica* stem bark recorded an increase in the total WBC level in contrast to the control rats (group 1) that were given water. There was also an increase in the number of platelets for rats in group II as compared to those of the control group. The white cell differential counts (Fig. 1) showed an increase in Lymphocytes for the group II rats that were given extract of *Mangifera indica* stem bark. A reverse trend was seen in neutrophils.

Fig. 3 illustrates the pattern of weight changes among the rats in groups I and II. Seven animals (rats) making up the control group were not given extract of *M. indica*

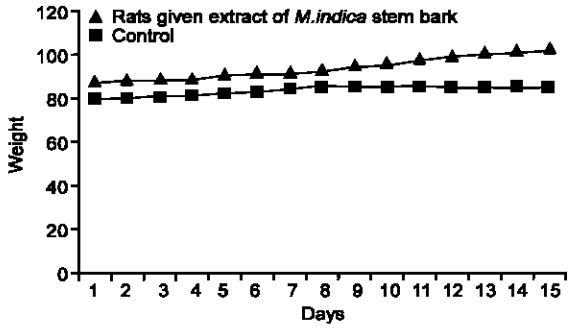

Fig. 3: Mean body weight of rats (g) given extract of *M. indica* stem bark (n = 7)

and they showed a weight gain of about 2.04% as compared to the weights of rats in group II given the extract with weight gain of about 5.75% at the end of the study period (14 days).

Discussion

As observed, the extract of *Mangifera indica* stem bark had some positive effect on the haemopoietic system of the test rats. This was manifested by an increase in red blood cell, packed cell volume (PCV or haematocrit), total white blood cell and platelet counts following administration of the medicinal plant extract to the rats. The percentage PCV increased slightly from 37% for the control rats to 38% for the group II rats which were given the plant extract. The raised haematocrit is an indication of haemoconcentration which may be due to increased RBC mass (http:// www. gpnotebook. co. uk/ medwebpage.cfm). Though, administration of the plant extract slightly decreased the level of haemoglobin from 13.0g/dl for the control to 12.0g/dl for the rats given the plant extract. The value obtained for RBC count (3.90×10^{6} cells) for the control animals increased slightly to 4.00×10^{6} cells for the rats given the medicinal plant extract. An increase of 3.5×210^{9}/L was recorded for WBC of group II rats as against WBC of the control rats with value of 2.8×10^{9}/L, while the value obtained for the platelet count of animals given the plant extract was 1.74×10^{5} cells as compared to the control animals with 1.65×10^{5} cells. The higher values of RBC and associated parameters are suggestive of polycythemia (American Diabetes Association, 2000). Therefore the extract of *Mangifera indica* may not have had any adverse effect on the bone marrow, kidney and haemoglobin metabolism, since the value of red blood cells are not greatly affected (Young and Maciejewski, 1997).

Also the value of Lymphocytes for the group II animals which were given the plant extract increased to 68% from 62% recorded for the control rats. This may go a long way to suggesting that extract of *Mangifera indica* stem bark must have influenced the defense mechanism of the test rats. So the continuous exposure of the body systems of animals to this medicinal products (herbs) may cause lymphocytosis, which may then account for the use of this plant for medicinal purposes (Keenwe and Bekalo, 1996).

There is a positive effect of *Mangifera indica* extract on the weight status of the test rats. The weights of rats given the plant extract remained higher (87g-102g) in contrast to those rats not given the plant extract, 80g-85g (that is, control group) at the end of the 14 days. This showed that the physical status of the rats were better. So the plant extract did not impose any acute fluid loss, proteolysis and lipolysis on the test rats and therefore the rats had no great loss in body weight (Alberti and Zimmet, 1998). Conclusively, extract of *Mangifera indica* stem bark possesses medicinal properties (Madunagu *et al.*, 1990). This suggests that it is not possible that its use can advance any adverse complications on haematological parameters of the body.

Acknowledgement

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Some Physical Properties of Apple

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Abstract: Several physical properties as physical characteristics, mechanical, hydrodynamic and nutritional properties of two apple varieties (Redspar and Delbarstival), were determined and compared using Duncan's multiple ranges test. Physical characteristics such as: average fruit length, width, thickness, the geometric, arithmetic and equivalent mean diameter, projected area, surface area, sphericity index, aspect ratio, fruit mass, volume, true density and moisture content, were determined for both varieties. The coefficient of static friction on plastic, plywood and galvanized iron, flesh firmness, failure stress, modulus of elasticity were found. The terminal velocity, coming up time, bouncy and drag forces, as hydrodynamic properties and total dry matter, total soluble solid, pH and titratable acidity, as nutritional properties, were determined. It was concluded that most of properties of two apple varieties was statistically different at the one percent probability level.

Key words: Apple (redspar, delbarstival), harvest and post-harvest processing, terminal velocity

Introduction

In spite of 2.66 million tons of Iranian annual apple production, exportation of that is low (Anonymous, 2005). Loss of postharvest operation is considered heavy. That may cause less exportation. Physical characteristics of agricultural products are the most important parameters to determine the proper standards of design of grading, conveying, processing and packaging systems (Tabatabaeefar and Rajabipour, 2005). Among these physical characteristics, mass, volume and projected area are the most important ones in determining sizing systems (Khodabandehloo, 1999). Quality differences in fruits can often be detected by differences in density. When fruits are transported hydraulically, the design fluid velocities are related to both density and shape.

Postharvest evaluation gives possibilities for delivering a high quality product and a basic understanding of apple texture is necessary for the development of technology for postharvest evaluations (Ioannides *et al*., 2007). Mechanical properties of the tissue determine the susceptibility to mechanical damage that can occur during harvest, transport and storage and that eventually leads to a profound reduction in commercial value (Oey *et al*., 2007). Mechanical properties such as failure stress and strain as well as modulus of elasticity can also be used to evaluate the behavior of the fruits mechanically under the static loading. Firmness or hardness is another important attribute of fruits and it is often used for fruit quality assessment (Vursavus *et al*., 2006).

Hydrodynamic properties are very important characters in hydraulic transport and handling as well as hydraulic sorting of agricultural products. The velocity of mixture to transport agricultural products depends on terminal velocity of those and characters of channel (Mohsenin, 1986). To provide basic data essential for development of equipment for sorting and sizing apples needed to determine several properties of apple such as: Fruit density and terminal velocity of that (Matthews *et al*., 1965; Dewey *et al*., 1966). Jordan and Clerk, 2004 reported that an approach to fruit sorting is to use the terminal velocity of fruit moving in a fluid that has a density above or below the fruit density. Fruit with different terminal velocities will reach different depths after flowing a fixed distance in a flume and may be separated by suitably placed dividers.

Information regarding chemical properties of fruit is crucial in processing it into different foods (Vursavus *et al*., 2006). Fruit weight and dry matter can be used in order to determine the best time to harvest fruits. Considering postharvest operations of apples, some mechanical and nutritional properties of those are more important in both machinery and equipment design and also in controlling the actual process procedure. Therefore, in the current study, researchers investigated the mentioned properties of apple fruits, by comparing the two apple varieties, newly grown in Iran and then establishing a convenient reference table for apple mechanization and processing.

Materials and Methods

Two apple, Malus domestica Borkh, L., cultivars namely, Redspar and Delbarstival, new-planted varieties in Iran were randomly hand-picked in 2007 summer season from orchard located in Horticultural Research Center, Department, Faculty of Agriculture, University of Tehran.

Fig. 1: Apparatus for measuring static coefficient of friction.

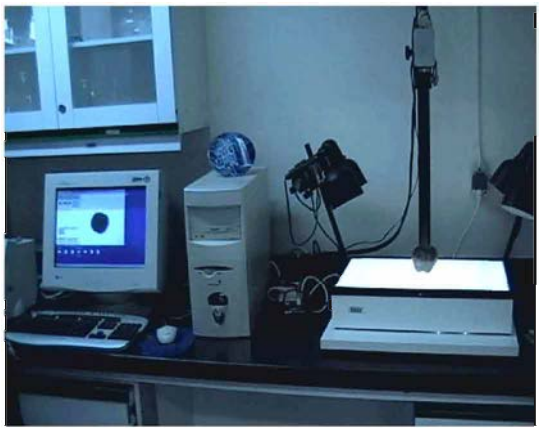

Fig. 2: Area Measurement System-Delta Tengland for measuring projected area of apples.

The two cultivars are also late season. Redspar is red-color variety but Delbarstival is bicolor variety. They are very sweet and delicious in taste.

The 50 fruits were randomly harvested and transferred to the laboratory in polyethylene bags to reduce water loss during transport. The initial moisture content of fruits was determined by using dry oven method (AOAC, 1990). The remaining material was kept in cold storage in 4°C until use. All of the analyses were carried out at a room temperature, 25°C, in the Biophysical laboratory and Biological laboratory of university of Tehran, Karaj, Iran.

To determine the average size of the fruits three linear dimensions namely as length, width and thickness were measured by using a digital caliber with sensitively of 0.01 mm and fruit mass was determined with a electronic balance of 0.1 g sensitively. The geometric, Dg, equivalent, Dp and arithmetic mean diameter, Da, in mm was calculated by considering Eq. (1), Eq. (2) and Eq. (3), respectively (Mohsenin, 1986).

$$D_g = (LDT)^{\frac{1}{3}} \quad (1)$$

$$D_p = \left[L \frac{(W+T)^2}{4} \right]^{\frac{1}{3}} \quad (2)$$

$$D_a = \frac{(L+W+T)}{3} \quad (3)$$

The sphericity (S_p) defined as the ratio of the surface area of the sphere having the same volume as that of fruit to the surface area of fruit, was determined using following formula (Mohsenin, 1986).

$$S_p = \frac{(LDT)^{\frac{1}{3}}}{L} \quad (4)$$

The surface area of the fruit was calculated by using following formula (Mohsenin, 1986).

$$S = \pi (d_g)^2 \quad (5)$$

The aspect ratio (Ra) was calculated by (Omobouwajo *et al.,* 1999).

$$R_a = \frac{W}{L} \quad (6)$$

Volume and fruit density were determined by the water displacement method (Mohsenin, 1986). Projected area with two major axis of the apple was determined from pictures of the fruits taken by Area Measurement System-Delta Tengland, Fig. 2. Packing coefficient was defined by the ratio of the volume of fruit packed to the total and calculated by the following formula (Topuz *et al.,* 2004).

$$\lambda = \frac{V}{V_0} \quad (7)$$

where V is true bulk of fruits and V_0 is bulk of the box. Mechanical properties of apples were evaluated using 20 cylindrical specimens of each variety, taken in radial direction with diameter as 14 mm and height as 18 mm and then Universal Testing Machine (Santam, MRT-5), as shown in Fig. 3. This machine has three main components, which are a stable forced and moving platform, a driving unit (A C electric motor, electronic variator and reduction unit) and a data acquisition (load cell, PC card and software) system (Vursavus and Ozguven, 2004). The machine was equipped with a load cell of 500 N at a compressive rate of 25 mm/min. Failure stress and strain of apples are expressed in terms of the change in compression force and compact area and deformation and initial length, respectively as (Vursavus *et al.,* 2006):

$$s_f = \frac{\Delta F}{\Delta A} \quad (8)$$

$$e_f = \frac{d}{l_i} \quad (9)$$

where σ_f, ΔF, ΔA, ε_f, d and l_i are designated as failure stress, failure force, cross section, failure strain, diameter and specimen length of fruits. Modulus of

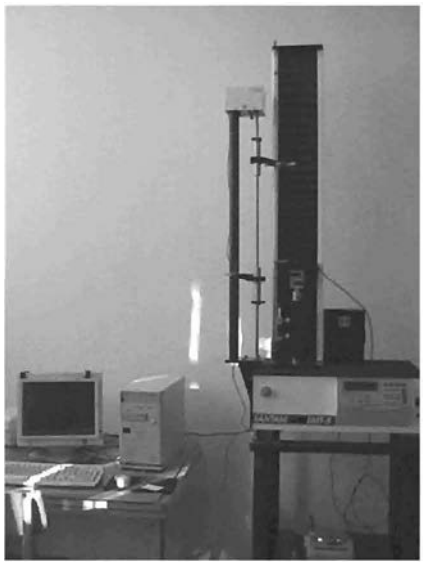

Fig.3. A Universal Testing Machine (Santam, MRT-5) for measuring mechanical properties of apples.

elasticity value (MPa) was calculated as the slope of the line from the origin (0:0) to 50% of failure point and failure energy was also considered as the total area in failure point (Mohsenin, 1986). Firmness was then calculated as the failure force dividing to the failure deformation. The coefficient of static friction was determined with respect to different surfaces: Plywood, compacted plastic and galvanized iron. A hollow metal cube (Fig. 1) open at both ends was filled with the fruits placed on adjustable titling surface such that the metal cube did not touches the surface. Then the surface was raised gradually until the filled cube just started to slide down (Razavi and Milani, 2006).

To determine some hydrodynamic properties of apples, a glued Plexiglas column was constructed, height = 1200 mm and cross-section = 400 × 400 mm, shown in Fig. 4. This column was optimal, fruit diameter approximately 20% of tank diameter (Vanoni, 1975). The column was filled with tap water to a height of about 1100 mm. Each fruit was placed in the bottom of column and any bubbles appearing on them were removed by rubbing. Fruit were then positioned flat (i.e., with their largest two dimensions oriented horizontally) in the bottom of column. A digital camera, JVC with 25 frames per second, recorded the moving of fruits from releasing point to the top of water column, simultaneously. Each fruit was tested three or four times. Video to Frame software were used to change video film to pictures and subsequently to calculate Coming up times and terminal velocities of fruits by knowing the fact that each picture takes 0.04 s.

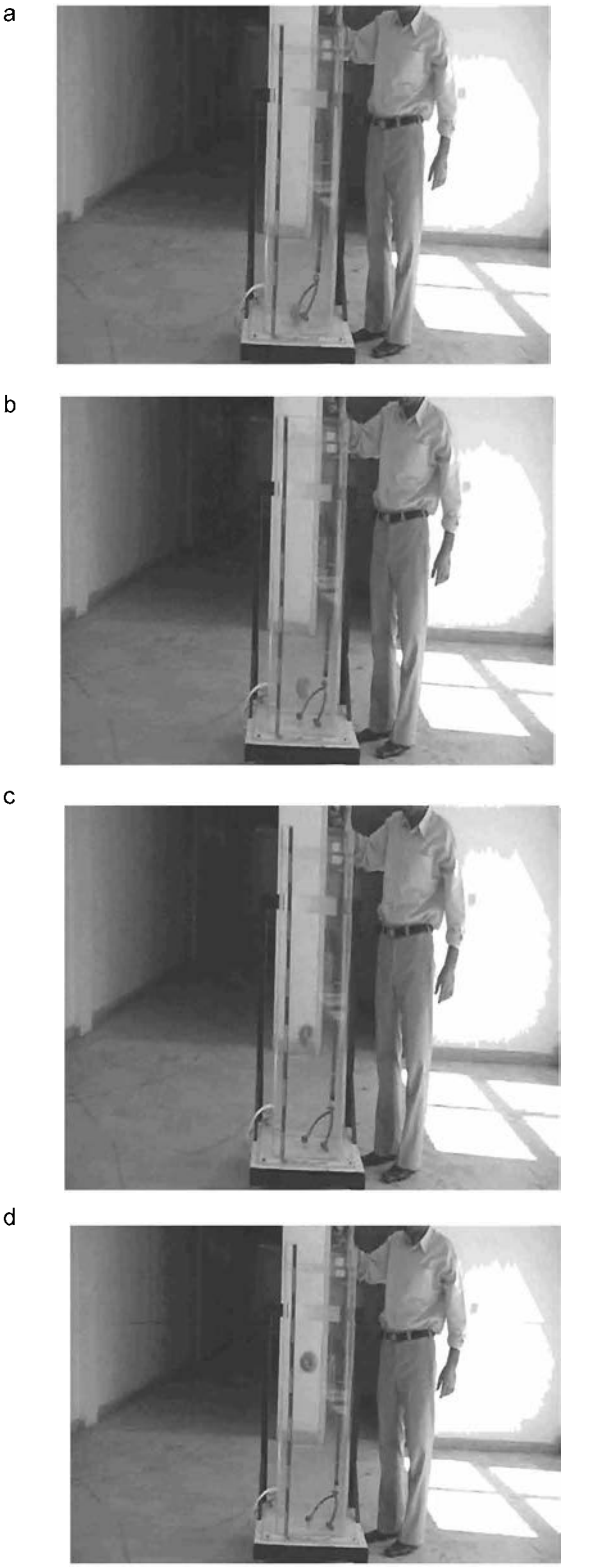

Fig. 4: The position of apple in water column a: at the rest, b: after 0.5 s, c: after 1 s and d: after 1.5 s.

Bouncy (F_b) and drag (F_d) are forces for and against moving of fruits in water defined by following formula, respectively:

$$F_d = C_d A_p \frac{p_f v^2}{2} \quad (10)$$

Where C_d, drag coefficient, is a function of fruit velocity and can be modeled well at low velocity using Stokes' law (Crowe *et al.*, 2001). Thus:

$$C_d = \frac{24}{N_R} \quad \text{For } N_R < 1 \quad (11)$$

$$N_R = \frac{Vd}{U} \quad (12)$$

$$F_b = p_f V g \quad (13)$$

where N_R is Reynolds number, v is the dynamic viscosity of water, g is gravity force, V is the velocity, d is the diameter, p_f is the true density and is the volume of fruit. The nutritional composition of the apple fruit juices were studied as explained following: Total dry matter was determined according to AOAC (1990). The total titratable acidity was determined by titration with sodium hydroxide (0.1 N) and expressed as a % of malic acid. The pH value was measured using a Macroprocessor pH meter (iHANNA pH211, Made in Italy). Total soluble solids (TSS) were measured as °Brix a Neerveld 14-B22550, GETI (Belgium) refractometer.

All data were statistically analyzed using the analysis of variance (ANOVA) test and means were compared using Duncan's multiple ranges test.

Results and Discussion

A summary of the physical characteristic, mechanical, hydrodynamic and nutritional properties of Redspar and Delbarstival cultivars is shown in Table 1. The moisture contents were 82.80 and 81.16% for Redspar and Delbarstival apples, respectively. According to this results of the dimensional Properties of two apple cultivars; the mean fruit length was 75.28 mm, fruit width was 84.12 mm and thickness was 80.64 mm for Redspar variety, whereas these values were 58.31 mm and 67.17 mm 65.04mm for Delbarstival variety. Tabatabaeefar and Rajabipour, 2005 studied on two different common commercial export varieties of Iranian grown apples (Red Delicious and Golden Delicious) from four different regions. They concluded 73, 70 and 67 mm as the mean fruit length, width and thickness for these varieties. The geometric, D_g, equivalent, D_p and arithmetic mean diameter, D_a, of Redspar and Delbarstival apples resulted in different means as 79.90, 80.01, 79.92 mm and 63.38, 63.51, 63.38 mm, respectively. The surface area and projected area of the apple varieties were found to be statistically different. When the fruit mass in this study was compared with previous studies, the mean mass of the Redspar (229.65 kg) fruits was greater than that of the mixed varieties of Red Delicious and Golden Delicious, 165 kg (Tabatabaeefar and Rajabipour, 2005). The true density of Redspar and Delbarstival cultivars varied from 837.68 to 827.91 kg/m^3. The packaging coefficient and volume were 0.62, 0.53 and 275.15 cm^3 and 143.19 cm^3 for Redspar and Delbarstival varieties, against Topuz *et al.*, 2004, the packing coefficient increased with decreased fruit volume. This result is due to extended volume values for Redspar variety (138.5 cm^3 - 424.2 cm^3) in other hand the small fruits filled the vacancy among big fruits. In spite of significant differences between all parameters of two varieties, aspect ratio and sphericity were not.

As seen in Table 1, all the failure properties such as stress, strain and energy and modulus of elasticity were found to have statistically significant difference at the 1% probability level. The mean values of the failure stress and strain for the Redspar variety were 0.43 MPa and 0.20 mm/mm, respectively. This values were greater than those of Delbarstival variety that were 0.24 MPa and 0.15 mm/mm, respectively. Similar study was undertaken and reported by Masoudi, Tabatabaeefar, Borghei and Shahbake (2004) for Red Delicious (0.13 MPa and 0.07 mm/mm), Golden Delicious (0.28 MPa and 0.13 mm/mm) and Grani Smith (0.34 MPa and 0.11 mm/mm). Failure energy values of variety resulted in different means 127.59 and 51.06 N.mm. Also, the Redspar had more module of elasticity (2.53 MPa) than that of Delbarstival (1.77MPa) and according to Masoudi *et al.* (2004), Red Delicious (1.53) and Golden Delicious (1.92 MPa) but less than that of the Grani Smith (2.84 MPa). The firmness parameter for each apple variety was found to have different means as 18.55 N/mm for Redspar and 14.15 N/mm for Delbarstival. The coefficient of static friction for Redspar and Delbarstival fruits was determined on the compacted plastic, plywood and galvanized iron. These coefficient values varied from 0.28 to 0.31 and 0.34 to 0.44, respectively. On the plywood surface, the coefficient of static friction of the Redspar and Delbarstival was not very different (0.31 and 0.34, respectively). This value for the Redspar fruits was found to be 0.31 on the galvanized iron that was less than that of Delbarstival as 0.37. The coefficient of static friction of the Redspar fruits, with a mean of 0.28 was significantly smaller than that of the Delbarstival. Considering above information on Redspar and Delbarstival apples, can be concluded that Redspar variety is more endurable than Delbarstival variety under static loads. The depth of boxes includes Redspar apples are more than that of Delbarstival apples, according to Sitkei, 1986, damage increases with increasing in depth of packaging boxes.

Terminal velocity of Redspar and Delbarstival cultivars was found to be 0.47 and 0.42 m/s. The similar

Table 1: Several physical characteristics and mechanical, hydrodynamic and nutritional properties of two apple varieties

	Redspar			Delbarstival			
Property	Max	Min	Mean	Max	Min	Mean	Significant level
L	88.03	57.13	74.78±8.2	66.66	50.98	58.31±4.09	**
W	101.27	66.98	83.80±7.57	77.13	50.55	67±4.37	**
T	95.61	62.6	80.37±7.05	72.35	58.85	65.04±3.77	**
D_g	94.15	62.94	79.54±7.32	70.57	57.01	63.38±3.89	**
D_p	94.17	62.96	79.55±7.32	70.57	57.01	63.39±3.89	**
D_a	94.36	63.1	79.65±7.30	70.64	57.11	63.51±3.89	**
S_p	1.13	0.99	1.07±0.04	1.14	1.02	1.09±0.03	**
S	278.33	124.4	200.29±35.42	156.39	102.05	126.59±15.54	**
A_p	83.16	36.63	59.73±10.84	49.15	30.86	38.95±5.04	**
R^a	1.24	0.99	1.12±0.06	1.23	1.07	1.15±0.04	**
M_f	347	119.1	228.72±54.34	159.6	87.6	118.43±20.97	**
ρ_t	882.88	811.49	837±34.29	868.74	795.02	827.91±13.45	ns
M	85.21	81.1	82.80±1.17	86.4	79.55	81.84±2.37	**
λ	0.65	0.6	0.62±0.03	0.55	0.52	0.53±0.01	**
V_t	0.76	0.33	0.47±0.07	0.49	0.35	0.42±0.04	**
T_c	3.2	1.6	2.33±0.32	2.88	2.24	2.52±0.16	**
F_d	0.76	0.18	0.46±0.14	0.36	0.16	0.24±0.05	**
F_b	4.16	1.36	2.69±0.67	1.92	1.04	1.40±0.26	**
Φ_p	0.31	0.31	0.31±0.00	0.33	0.35	0.34±0.01	**
Φ_g	0.3	0.32	0.31±0.00	0.41	0.34	0.37±0.04	ns
Φ_c	0.27	0.29	0.28±0.00	0.47	0.41	0.44±0.03	**
σ_f	0.55	0.33	0.43±0.05	0.36	0.16	0.24±0.06	**
δ_f	0.29	0.16	0.20±0.03	0.19	0.1	0.15±0.03	**
E	3.69	1.87	2.53±0.47	4.44	0.98	1.77±0.79	**
E_f	192.47	85.29	127.59±26	80.25	25.4	51.06±17.2	**
F_i	23.94	11.18	18.55±3.5	22.83	8.41	14.15±3.80	**
D_m	18.9	14.75	17.2±1.17	20.45	13.6	18.6±2.37	**
PH	4.3	3.74	3.91±0.14	3.93	3.46	3.61±0.16	**
T_c	0.034	0.017	0.025±0.01	0.05	0.03	0.041±0.01	**
TSS	13.2	8.4	10.73±1.5	15.1	9.5	12.54±2.23	ns

** Significant (1% level) ns: Nonsignificant

researches were conducted by Matthews *et al.*, 1965; Dewey *et al.*, 1966. They concluded 0.61 and 0.53 m/s as coming up terminal velocity, 74.68 and 72.14 mm as geometric mean diameter, 760 and 820 kg/m^3 as true density of Jonathan and Grani Smith apple cultivars. In comparison terminal velocity of these cultivars, with considering other characters, can be concluded that terminal velocity increased with decreasing of true density and increasing of geometric mean diameter. For Delbarstival and Redspar cultivars the effective factor on terminal velocity was geometric mean diameter, because of little deference in true density (varied from 827.91 to 837kg/m^3) compare with deference in geometric mean diameter (varied from 79.54 to 63.38 mm). As seen in Table 1 Redspar and Delbarstival cultivars had 2.33 and 2.52 s as coming up time. Logically, would be concluded that with decreasing terminal velocity, the coming up time of apples increased. Finally, the drag and bouncy force were 2.69 N and 0.46 N for Redspar variety and 1.40 N and 0.24 N for Delbarstival variety, respectively.

The values of all the chemical properties of apple juices were statistically different with respect to the varieties. Also, the cultivar of Redspar has the smaller dry matter (17.2 %) than Delbarstival (18.12 %). In the case of the TSS, as shown in Table 1, there were non significant differences between the studied cultivars. For Delbarstival variety, the average value of the TSS was 12.54 whereas 10.73 obtained for Redspar variety, but Ragni and Berardinelli, 2001, reported this value as 14.3, 13.7, 14.3 and 12.9 for Golden Delicious, Stark Delicious, Grani Smith and Rome Beauty, respectively. The juice of Redspar cultivar also represented the higher ratio of pH, 3.91, compared with Delbarstival, 3.61. Eventually, titrable acidity value found for Delbarstival variety in this experiment was 0.034 that was higher than 0.021 for Redspar variety.

Conclusion: Some engineering properties of Redspar and Delbarstival varieties which may be useful in designing much of the equipment used for harvest and post-harvest processing were studied in this paper. Authors concluded that all studied properties of two apple varieties were found to be statistically different at the probability level (1%), except for true density, static coefficient of friction on galvanized iron surface and TSS, This paper concludes with information on engineering properties of Redspar and Delbarstival varieties which may be useful in designing much of the equipment used for postharvest processing.

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Notations: L = length, mm, Φ_g = on galvanized iron surface, W = width, mm,Φ_c = on compressed plastic surface, T = thickness, mm, Φ_p = on plywood surface, D_g, = geometric mean diameter, mm, σ_f = Failure stress, kPa, D_p = equivalent diameter, mm, δ_f = Failure strain, mm/mm, D_a = arithmetic diameter, mm, E = Elasticity module, kPa, S_p = sphericity, %, E_f = Failure energy, kPa, S = surface area, mm^2, F_i = Firmness, N/mm, A_p = Projected area, cm^2, V_t = Terminal velocity, m/s, M_f = Mass of fruit, g, C_r = Coming up time, s, V = volume, mm^3, F_d = Drag force, N, ρ_t = true density, kgm^{-3}, F_b = Bouncy force, N, M = moisture content, % , D_m = Dry matter, %, λ = Packing coefficient, T_c = Titratable acidity, Φ = static coefficient of friction, TSS, Total soluble solid °Brix.

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Investigation and Biological Activity of Some Nonpolar Iron Amino Acid Chelates

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Abstract: The nonpolar Fe (III) phenylalanine and glycine chelates have been synthesized and characterized by using different tools, in particular; elemental analysis, molar conductance, infrared and electron paramagnetic resonance spectra. The elemental analysis showed the formation of 1:3 [M: L] ratio. The molar conductance measurements revealed a non-electrolytic nature. The infrared spectral data displayed the complexation behavior which takes place through the nitrogen and oxygen atoms of the present amino acids. The electron paramagnetic resonance data showed the existence of an octahedral geometry structure. Thirty-eight adult rats divided into four groups; group I: included 10 rats received 0.5 mL physiological saline, group II: included 9 rats received 0.5 mg ferrous fumarate/kg body weight, group III: included 9 rats received 0.5 mg Iron phenylalanine chelate/kg body weight/day, group IV: included 10 rats received 0.5 mg Iron glycine chelate/kg body weight/day. All rats were killed then blood, serum samples and liver homogenate were obtained, in blood were measured Hb and HCT.

Key words: Nonpolar amino acids, $FeCl_3.6H_2O$, hemoglobin, kidney and liver functions

Introduction

Iron deficiency continues to be one of the most prevalent nutritional deficiencies in the world. For physiological reasons, the most commonly affected groups are infants, children, adolescents and women of childbearing age (DeMayer and Adiels, 1985). A simplest way to improve Iron status of individual suffering from Iron deficiency occurs by supplementation with Iron tablets. Pharmaceutical Iron supplementation is generally indicated in adults when hemoglobin concentration fall below 12g/dl (Schumann, 1998). Fortification of food with Iron is the best way of preventing Iron deficiency, most difficult technical hurdle is finding the adequate combination of Iron fortification compound and food vehicle and the selection of an appropriate Iron compound (Olivares *et al*., 1997). Harvey *et al*. (1998) were reported that oral Iron supplements in the form of ferrous salt (ferrous sulfate), are more likely to cause gastrointestinal irritation. In order to enhance Iron bioavailability and avoid its side effects, chelating Iron with amino acid have been employed, since amino acids are absorbed well from intestinal lumen by specific active transport mechanisms. The amino acid compounds are bidentate ligands and used as chelating ligands to form many transition metal complexes with the most periodic table elements (Hawary *et al*., 1975). Three chelates of Fe (III) ion with alanine, histidine and aspartic acid were prepared and investigated by using different tools; in terms of elemental analysis, infrared and electronic spectra. An octahedral geometry was proposed for these chelate (Abdel-Gayoum *et al*., 2001).

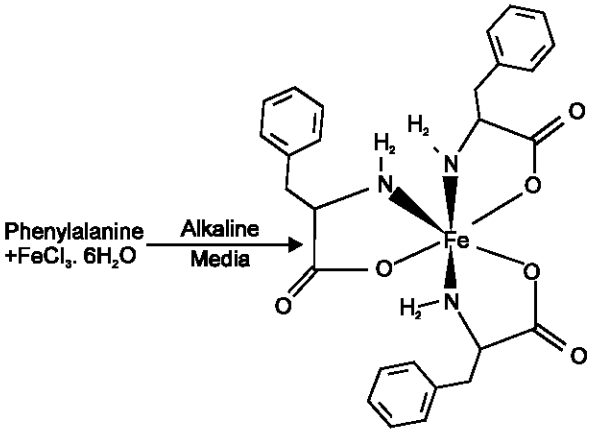
The present paper aims to synthesis two chelates of Fe (III) with nonpolar amino acids (phenylalanine and glycine) and to characterize their geometrical structures and to elucidate whether Iron supplements in the form of ferric amino acid chelates would increase the bioavailability of the Iron. Meanwhile, delivered ferric Iron would be as effective as the ferrous Iron in improving the hematology and serum Iron status.

Materials and Methods

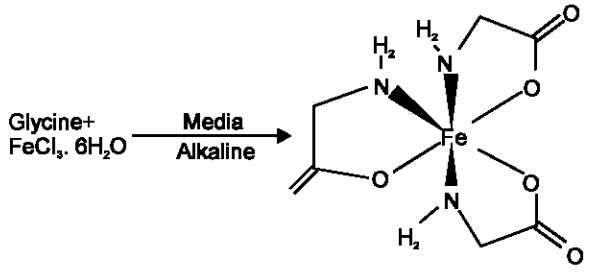
Solvents and reagents: All chemicals used in this study were laboratory pure, including Phenylalanine, glycine, Iron (III) chloride hex hydrate ($FeCl_3.6H_2O$), dimethylsulphoxide (DMSO) and alkaline medium and double distilled water, ferrous fumarate tablets were purchased from drug store (limassol-Cyprus-Europe).

Synthesis of chelates

Synthesis of Iron (III) phenylalanine chelate: The chelate under investigation was synthesized by mixing $25cm^3$ of an aqueous solution of the phenylalanine (0.01mole; 1.65g) with (0.01mo1; 2.71g), Iron (III) salt in same solvent and the obtained mixture was reflexed for 2hrs. Few drops of alkaline solution were added to adjust the pH at which the solid chelate was separated. The formed chelate was filtrated, collected and washed several times with hot double distilled water until the filtrate becomes colorless. The chelate was dried in a desiccator over anhydrous $CaCl_2$ under vacuum. The yields were estimated to be about 70% and the purity of the complex was confirmed by the elemental analysis and TLC technique.



Synthesis of Fe (III) glycine chelate: The synthesis of this chelate was performed by mixing an aqueous solution (25 mL distilled H_2O) of glycine (0.01mol; 0.75g) with Fe (III) salt (0.01mo1; 2.71g) in same amount of solvent, then adjust the pH to desired ratio. The obtained mixture was reflexed for 2hrs until the chelate isolated. The precipitate was separated by filtration and washed several times with hot double distilled water. The resulted chelate was dried in a desiccator over anhydrous $CaCl_2$ and the analyses were confirmed by the elemental analysis and TLC technique.



Physical measurements: The elemental analyses (C, H, N) of the chelate was carried out by 2400 elemental analyzer. The molar conductance was carried out in DMSO solvent using conductivity meter model CMD 650 digital. The infrared spectra were obtained by KBr disc on using IFS-25DPUS/IR spectrometer (Bruker) 1998Y. The electron paramagnetic resonance spectra of the chelates were carried out using EMX ESR spectrometer (Bruker) 1998Y. All the mentioned analyses were done at Micro-Analytical Center, Giza, Egypt.

Animals: Thirty eight female adult Sprague Dawley rats weighing 158-261g obtained from the Central Animal House of Garyounis University, Benghazi, Libya, were used in this study. The animals were housed in stainless steel cages with a free supply of diet and water and maintained under constant 12hrs light and dark cycle in an environmental temperature of 20-30°C, they were divided into 4 groups:

Group I: Included 10 rats received 0.5 mL physiological saline.
Group II: Included 9 rats received 0.5mg ferrous fumarate/kg body weight.
Group III: Included 9 rats received 0.5mg Fe (III) phenylalanine chelate/kg body weight/day.
Group IV: Included 10 rats received 0.5 mg Fe (III) glycine chelate/kg body weight/day.

*All Iron forms were dissolved in 0.5 mL of normal saline and given by gastric intubations for 10 consecutive day.

Sampling: All rats were killed by decapitation and fasting blood sample was taken using sterile syringe and transferred into two tubes: one tube contains heparinzen for Hb, pack cell volume and other tube was for separation of serum. In serum samples, Iron concentration, TIBC concentration, % transferrin saturation, liver function tests (ALT, AST, Total protein, albumin and total bilirubin), kidney function tests (Urea and Creatinine) we measured and liver homogenate were done for liver ferritin.

Statistical analysis: The statistical analysis was done by using student's t-test. A $p<0.05$ value was considered statistically significant. All values are presented as Mean±SD.

Results

The condensation of ferric chloride hexahydrate with amino acids was done in double distilled water. All the results are shown in Table (1-6). In serum we measured Iron concentration, total Iron binding capacity, % transferrin saturation, total bilirubin, total protein, creatinine, urea, albumin, alanine transaminase (ALT) activity, aspartate transaminase (AST) activity, in liver homogenate were measured liver ferritin. Serum Iron (µg/dl) concentration was a significantly increased in ferric phenylalanine chelate compared with each of control group ($p<0.001$), ferric glycine chelate ($p_1<0.002$) and ferric glycine chelate ($p_2<0.001$). Hemoglobin concentration (g/dl) significant increases ($p<0.010$) in ferrous fumarate and ferric phenylalanine chelate ($p<0.001$) compared with control group and also in ferric phenylalanine ($p<0.025$) compared with ferric glycine chelate. Serum TIBC (µg/dl) concentration significantly increased ($p<0.014$) in ferric glycine chelate group, but decreased ($p<0.001$) in ferric phenylalanine chelate compared with controls. Liver ferritin (ng/gm tissue) concentration was significantly increased in ferrous fumarate ($p<0.001$), ferric phenylalanine chelate ($p<0.001$) and ferric glycine ($p<0.002$) compared with controls, but there were significant decrease in ferric glycine chelate ($p_1<0.002$) compared with ferrous fumarate and there were significant increase ($p_2<0.001$) in ferric phenylalanine chelate compared with ferric

Table 1: Elemental analysis and molar conductance of chelates

Chelates	Molecular weight	% C		% H		% N		Λ_m
		Calculate	Found	Calculate	Found	Calculate	Found	
[Fe (Phe)$_3$]	548.04	58.63	59.17	4.31	5.52	7.07	7.67	2.16
[Fe (Gly)$_3$	277.63	25.57	25.96	4.82	4.36	14.22	15.14	3.00

Λ_m = molar conductivity (ohm^{-1} cm^2 mol^{-1})

Table 2: Infrared and electron paramagnetic resonance of chelates

Ligand/chelates	IR (cm^{-1})						
	υ C = O	υ COO$^-$	υ NH_3^+	υ NH_2	υM-N	υ M-O	g_{eff}
Phenylalanine (Phe)	1628	1562	3150	-	-	-	-
[Fe (Phe)$_3$]	1605	1496	-	3300	586	463	2.007
Glycine (Gly)	1504	1585	3166	-	-	-	-
[Fe (Gly)$_3$]	1612	1612	-	3400	594	421	2.004

IR = Infrared. g_{eff} = E.P.R.

Table 3: Mean values±significant of initial and final body weight of control and studied groups

Group Parameter	Controls No. (10)	Ferrous fumarate (9)	Ferric glycine chelate (10)	Ferric phenylalanine chelate (9)
Initial body weight (g)	237±18.18	184±2.86	157.30±20.97	217.22±1.92
Final body	214±19.6	203.56±12.62	185.00±26.93	238.78±9.24
weight (g)	p<N.S	p<0.001	p<0.019	p<0.000

P: Initial body weight versus final body weight, Signifiant p>0.05., NS. No Significant

Table 4: Mean values±S.D of serum urea (mg/dl), serum creatinine mg/dl) in controls and studied groups

Group Parameter	Controls No. (10)	Ferrous fumarate (9)	Ferric glycine chelate (10)	Ferric phenylalanine chelate (9)
serum	18.20±6.11	35.11±8.92	22.90±6.82	30.44±3.84
Urea		p<0.001	p. N.S	p<0.001
(mg/dl)			p1<0.004	p1. N.S
				p2<0.010
serum	0.31±0.088	0.24±0.073	0.30±0.08	0.40±0.18
Creatinine		p. N.S	p. N.S	p. N.S
(mg/dl)			p1. N.S	p1<0.029
				p2. N.S

P. Versus controls. P1. Versus ferrous fumarate, P2. Versus glycine, NS. No Significant

glycine chelate. % transferrin saturation there were significant increases in ferric phenylalanine chelate compared with controls (p<0.001) ferrous fumarate (p_1<0.001) and ferric glycine chelate (p_2<0.001). The results of the present study also demonstrated that the Iron amino acid chelate have no harmful effects on the liver or kidney as indicated by liver and kidney function tests, there were no serious side effects from the supplementation of the Iron amino acid chelates. We concluded that, in the inorganic form, which has been the form of choice for supplementation and fortification ferrous salt demonstrated restricted bioavailability with some side effects on liver. Modification on the inorganic forms of Iron gains some improvement in absorption. Iron amino acid chelates may be the best form for supplement and fortification of Iron into human diets.

Discussion

Microanalysis and molar conductance: The elemental analysis results of the prepared chelates (Table 1) revealed the stoichiometric of measurements 1:3 [M:L] ratio. The isolated chelates are air stable and insoluble in most common organic solvents, but partially soluble in dimethylsulphoxide (DMSO). The molar conductance measurements of the chelates indicate a non-electrolyte nature (Raman *et al*., 2001).

Infrared spectra: The infrared spectral data of the two chelates under investigation displayed two bands at 1605 and 1612 cm^{-1} due to C = O of COOH group and these bands changed on comparing with their original position in the free ligands (1628 and 1504 cm^{-1}). That indicating the involvement of C = O group in chelation with Fe (III) ion (Condrate and Nakamoto, 1965). Where the NH_2 group of the amino acids was effected by the chelation with the same metal ion (Table 2) and the appearance of new bands which are not seen in the spectra of the free ligands attributed to υ (M-N) and υ (M-O) vibrations respectively supporting the participation of nitrogen and oxygen atoms in chelation with Fe (III) ion (Faniran *et al*., 1976).

Electron paramagnetic resonance spectra: The electron paramagnetic resonance spectral data of these chelates exhibited g_{eff} values (Table 2) corresponding to the presence of an octahedral structures (Aml Jarbou, 2006).

Table 5: Mean values±S.D of Hb (g/dl), RBC/106/µl), HCT%, serum iron (µs/dl), serum total iron binding capacity (TIBC) (µs/dl), liver ferritin µ tissues) and % transferring saturation in controls and studied groups

Group Parameters	Controls (10)	Ferrous fumarate (9)	Ferric glycine chelate (10)	Ferric phenylalanine chelate (9)
Hb	11.76±0.70	12.94±1.05	12.64±1.19	13.74±0.66
(g/dl)	0	p<0.010	p. N.S	p<0.001
			p1. N.S	p1. N.S
				p2<0.025
RBC	6.99±0.49	7.08±0.44	6.65±0.58	7.37±0.48
(10^6/µl)		p. N.S	p. N.S	p : N.S, p1 : N.S
			p1.N.S	p2<0.010
HCT	40.27±2.29	40.40±2.51	39.45±4.21	42.03±2.02
(%)		p. N.S	p. N.S	p. N.S, p1. N.S
			p1. N.S	p2. N.S
serum	196.93±25.13	214.78±39.03	203.00±52.55	323.11±81.75
Iron		p. N.S	p. N.S	p<0.001
(µg/dl)			p1. N.S	p1<0.002
				p2<0.001
serum	368.20±57.61	378.78±62.74	455.40±82.95	103.78±27.88
TIBC		p. N.S	p<0.014	p<0.001
(µg/dl)			p1<0.038	p1<0.001
				p2<0.001
liver	2.70±0.55	4.35±0.64	3.46±0.38	7.39±0.64
ferritin		p<0.001	p< 0.002	p<0.001
(ng/gm tissue)			p1<0.002	p1<0.001
				p2<0.001
% transferrin	54.90±12.01	57.78±14.08	45.70±13.47	332.78±125.21
saturation		p. N.S	p. N.S	p<0.001
			p1. N.S	p1<0.001
				p2<0.001

P. Versus controls., P1. Versus ferrous fumarate, P2. Versus glycine, NS No Significant

Table 6: Mean values±S.D values of serum got (U/L), G, PT (U/L), ALP (U/L), total protein g/dl, albumin (g/dl) and total bilirubin (mg/dl) in controls and studied groups

Group Parameters	Controls (10)	Ferrous fumarate (9)	Ferric glycine chelate (10)	Ferric phenylalanine chelate (9)
Serum	80.40±36.28	169.44±26.01	116.40±29.05	101.56±15.75
AST		p<0.001	p<0.025	p. N.S
(U/L)			p1<0.001	p1<0.001
				P2. N.S
Serum	25.90±12.00	72.89±18.43	14.60±5.32	18.89±6.15
ALT		p<0.001	p<0.014	p. N.S
(U/L)			p1<0.001	p1<0.001
				p2. N.S
Serum	113.50±50.36	191.22±64.04	132.70±36.45	109.33±38.53
ALP		p<0.009	p. N.S	p. N.S
(U/L)			p1<0.024	p1<0.005
				p2. N.S
Serum	7.060±0.4835	6.59±0.831	6.40±0.73	6.98±1.161
Total protein		P. N.S	p<0.029	p. N.S, p1. N.S
(g/dl)			p1. N.S	p2. N.S
Serum	2.660±0.51	2.36±0.347	2.21±0.42	2.97±0.68
albumin		p. N.S	p<0.045	p. N.S
(g/dl)			p1. N.S	p1<0.028
				p2<0.009
Serum	0.15±0.05	0.19±0.13	0.21±0.13	0.34±0.19
Total Bilirubin		p. N.S	p. N.S	p<0.006
(mg/dl)			p1. N.S	p1. N.S, p2. N.S

P. Versus controls., P1. Versus ferrous fumarate, P2. Versus glycine, NS No Significant

Biochemical data: In the present study, Iron supplementation (0.5 mg kg^{-1} body weight) in the form of ferrous fumarate, ferric phenylalanine and ferric glycine chelates was done. Serum Iron was increased significantly (p<0.001) in ferric phenylalanine chelate group (64.07%) in comparison with control group, but increased non significantly in ferrous fumarate (9.06%) and ferric glycine chelate (3.08%). Puurcell and Kotz (1980) explained why more Iron can be absorbed from the gastro-intestinal tract into the mucosal cell as a

chelate than as a salt, first is that in the amino acid chelated form, the sequestered atom of Iron has no polarity (neutral). The amino acid chelated Iron is not as reactive with food a ingredient which leaves more of the Iron available for absorption. So that, there is a protective effect of the amino acid moiety both chelating and protecting Iron from inhibitors of the diet and keeping it soluble and available for incorporation into the cell (Mazariegos *et al*., 2004). Second, Iron amino acid chelate enters into fewer absorptive inhibiting reactions in the gut. Third, absorption of the Iron amino acid chelate did not inhibit by the presence of other minerals in the diet. There was a significant increase in the mean hemoglobin level in ferrous fumarate (10.03%) and ferric phenylalanine chelate (16.84%) groups compared to controls but non significant increase ($p>0.05$) was found in glycine chelate group. The mean of hemoglobin level in ferric phenylalanine increased but insignificantly as compared to ferrous fumarate. These results demonstrated that, the mean of hemoglobin concentration was significantly higher in fed Iron amino acid chelates especially ferric phenylalanine chelate but there was no statistically difference in the packed cell volume. These results concluded that most of the absorbed Iron from the chelate incorporated into (16.84%) increase in hemoglobin level as regard phenylalanine chelate rather than being stored in the liver. Fomon *et al*. (1988) reported that (90%) of the absorbed Iron is used for hemoglobin and is a valid method for comparing the absorption and bioavailability of different chemical forms of Iron within the same individual. Faiweather-Tait *et al*. (1992) noted that the amount of Iron absorbed from the chelate source must have been far greater than was reflected in the increased hemoglobin in order to meet the anabolic needs of the rapidly growing animal. This was confirmed in our study that showed significant increases in ferrous fumarate ($p<0.001$) group, ferric glycine chelate ($p<0.019$) group, ferric phenylalanine chelate ($p<0.001$) group in comparison of final body weights with initial body weights and non significantly changed ($p>0.05$) in controls. The greater bioavailability of the Iron amino acid chelate allows for the rapid incorporation of Iron into hemoglobin first, followed by a quicker repletion of tissue Iron stores. This was seen in our study in which there was a significant increases ($p<0.001$) in mean liver ferritin in all rats supplemented with ferrous salt compared to controls and also in rats supplemented with both Iron amino acid chelates compared to ferrous fumarate group (Faiweather-Tait *et al*., 1992).

Our results demonstrated that, the greater bioavailability of the Iron amino acid chelate allows for satisfying hemoglobin requirements and a more rapid repletion of tissue Iron stores, so more Iron was stored from ingesting Iron amino acid chelate than equivalent amounts of Iron from salts. The increasing in mean liver ferritin could be attributed to the daily Iron supplement in the present study causing surplus of Iron in the blood and uptake by the liver. This was confirmed by the % transferrin saturation which was significantly increased in ferric phenylalanine chelate ($p<0.001$) as compared to control. Increased % saturation of transferrin indicates the presence of an adaptive mechanism of the body to absorb Iron when it is greatly needed as indicated by the significantly increase of Iron as result of absorption after oral doses. Our findings confirmed that the Iron phenylalanine chelate is indeed a highly bioavailability form of Iron amino acid chelate because it was found to be statistically effective in raising hemoglobin level, serum Iron and % transferrin saturation as compared to ferrous fumarate. This research study showed that Iron from Iron phenylalanine chelate is preferentially absorbed over Iron from ferrous fumarate. Pizarro *et al*. (2002) suggested that these differences could be explained on the basis of physical and chemical characteristics that determine different Iron bioavailabilities and so we suggested that the increase in absorption of Iron amino acid chelate was probably due to the chemical structure of this compound, which partially prevents Iron inhibitors interaction. The results of the present study also demonstrated that the Iron amino acid chelate preferentially phenylalanine had no harmful effects on the liver or kidney as indicated by liver and kidney function tests, there were no serious side effects from the supplementation of the Iron amino acid chelates. This suggested that in people with normal Iron levels, there is a little potential danger of overloading or subsequent toxicity when consuming foods fortified with nutritionally appropriate amounts Iron amino acid chelate. This result was in agreement with the study of Jeppsen (2001).

Conclusion: We concluded that, in the inorganic form, the choice for supplementation and fortification ferrous salt demonstrated restricted bioavailability with some side effects on liver. Modifications on the inorganic forms of Iron gain some improvement in absorption. Iron amino acid chelate may be the best form for supplement and fortification of Iron into human diets. Increasing in absorption of Iron shown by increasing of the amount of hemoglobin, so the amino acid chelated with Iron is suitable for hemoglobin repletion in Iron deficiency anemia and allowed daily lower doses of Iron than would be expected.

Recommendations: We recommended that the use of Iron amino acid chelate as phenylalanine source of Iron in food intended for the general of population, food supplements and food for particular nutritional uses including foods intended for infants and young children.

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Evaluation of Antimicrobial Properties of Some Medicinal Plants for Fresh Cassava Roots Preservation

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Abstract: The inhibitory effects of water, petroleum ether and ethanol extracts of three medicinal plants were investigated on the growth of *Botryodiplodia theobromae, Aspergillus flavus, Fusarium solani, Rhizopus sp., Mucor* and *Erwinia spp* isolated from rotted cassava roots on potato dextrose agar. The ethanol extracts of *garlic* and *Landolphia owerrience* gave a wider spectrum of activity against the pathogens compared to that of petroleum ether and water. The combination of the extracts of *Garlic* (*Alium sativum*) and *Garcinia kola* demonstrated a remarkable inhibition of the pathogens after 16 days in storage with 2% rot, while that of *Garlic* and *Landolphia owerrience* roots exhibited little or no activity unlike the individual extracts. The potency shown by the extracts in overcoming the colonies of the tested pathogens recommends their use as protectants rather than eradicants.

Key words: Medicinal plants, cassava root rot, pathogens, biological control

Introduction

Fresh cassava roots (*Manihot esculenta Crantz*), unlike those of most other roots or tuber crops, are highly perishable under ambient conditions. Primary and secondary deterioration are known to occur. Physiological changes begin within one day and it is enzymatically mediated with loss of root quality in just three days. Roots also become subject to fungal invasion, further exacerbating deterioration (Bertram, 1990). Postharvest decay of cassava is a complex matter involving a number of different species of fungi and bacteria. Primary deterioration is a physiological change characterized by an internal root discoloration called vascular streaking. The onset of primary deterioration and the rate at which it progresses, the intensity, pattern and distribution of the discoloration varies between cultivars and roots of the same plant. Some varieties deteriorate so fast that they become inedible 24 h after harvest (Booth *et al*., 1976), while others have been reported to show no sign of discoloration for 7 to 11 days at room temperature. Secondary deterioration is induced by micro-organisms that cause rotting.

Garlic, Landolphia owerrience roots and *Garcinia kola* possesses varying degrees of antimicrobial properties. *Garlic* is acclaimed to have wide range of medical applications. Okeke *et al*. (2001) reported that the extracts of *Landolphia owerrience* roots demonstrated a wide spectrum of antimicrobial activities while Iwu *et al*. (1999), reported that *Garcinia kola* has both antiparasitic and antimicrobial properties. It can also be used as a purgative. The objective of this study is to assess the antimicrobial properties of locally and readily available medicinal plants with a view to exploiting them for control of postharvest rot of cassava roots.

Materials and Methods

Sample collection: Wholesome and rotting cassava roots were harvested from NRCRI, Umudike cassava farms.

Isolation of the pathogens: Two freshly rotted pieces from surface sterilized cassava root were aseptically inoculated on potato dextrose agar (PDA) plates. It was replicated three times. They were incubated at room temperature (28°C). Observations were made daily for emergence of colonies for six days. Identification was done and compared with the illustrated genera of imperfect fungi by Barnette and Hunter (1972) and the class work of fungi by Dade and Gunnel (1969).

Establishment of pathogenicity: Thirty wholesome cassava roots were washed under running water and surface sterilized with 70% ethanol. Two groups of ten roots each were inoculated with the isolated organisms at concentration of 4.3×10^9 spores/mL at the rate of 10mL of suspension per root according to Okigbo and Ikediugwu, 2000. The remaining ten roots were spray-inoculated with the equivalent volume of blank sterile distilled water. The three groups were covered separately with sterile transparent polyethylene sheets and incubated on a raised platform at room temperature for five days. The cassava roots were examined on daily basis for the occurrence of rot and for the

determination of the root surface mycoflora according to Harley and Waid (1955).
Re-isolation of the inoculated pathogens was done and were subsequently identified and compared with the illustrated genera of imperfect fungi by Barnett and Hunter (1972).

Preparation of extracts: A 100g amount of the pulverized *Garcinia kola*, *Garlic* and *Landolphia owerrience* roots were continuously extracted with petroleum ether, water and ethanol in a soxhlet extractor for 2½ h. The solvents were subsequently distilled off in a rotatory evaporator. The extracts were poured into weighed flasks and further dried in a desiccating chamber to a constant weight. The dried extracts were exposed to ultra violet (UV) rays for 24 h. and checked for sterility by streaking on nutrient agar plates.

Determination of minimum inhibitory concentration (mic): The mic for the extracts were determined by two test methods: a modified agar-well diffusion method (Okeke *et al*., 2001) and a modified macro-broth dilution technique (Ibrahim *et al*., 1997). In the agar-well diffusion technique, a two-fold serial dilution of the extracts were prepared by first reconstituting in 20% dimethylsulphoxide (DMSO). They were diluted in sterile distilled water to achieve a decreasing concentration range of 50mgmL^{-1} to 8.021mgmL$^{-1.}$ A 100µL volume of each dilution was introduced in triplicate wells into potato dextrose agar (PDA) plates already seeded with the standardized inoculum (5×10^5) of the test fungal cells. The test plates were incubated at 28^{o}C for 72 h. The least concentration of each extract showing a clear zone of inhibition was taken as the mic.
In the macro-broth dilution technique, a two fold serial dilution of the reconstituted extracts were prepared in potato dextrose broth (PDB). Each dilution was seeded in triplicates with 100µL of the standardized suspension of the test fungal strain to achieve a final concentration of 5×105cfu/mL. The culture tubes were incubated at 28^{o}C for 72 h. The tubes were subsequently plated on solid PDA plates and incubated for 72 h at 28^{o}C. The least concentration showing zero growth on the PDA plates was taken as the mic.

Test for combined activity of the extracts: A total of three different combinations of the extracts were tested against the susceptible pathogens at a 50mgmL^{-1} concentration. The combinations were: *Landolphia owerrience* roots and *Garcinia kola*, *Landolphia owerrience* roots and *Garlic* and *Landolphia owerrience* roots, *Garcinia kola* and *Garlic*. The agar well diffusion method was used and the inhibition zone diameter measured to the nearest millimeter.

Treatment of cassava roots with plant extracts: Fresh and wholesome cassava roots were grouped into nine and replicated three times. The first three groups were sprayed separately with the individual extracts at a 50mgmL^{-1} concentration. The three different combinations of the extracts were also separately sprayed on the second three groups of cassava roots with the same concentration. They were inoculated with the four susceptible pathogens at a concentration of 4.3×10^9 spores/mL at the rate of 10mL of suspension per cassava root according to Okigbo and Ikediugwu (2000). The remaining three groups were sprayed with the equivalent volume of blank sterile distilled water before spraying with the same concentration of the pathogens. The application method was also reversed by inoculating the pathogens first on the cassava roots before spraying with the extracts.

Results

Biodeterioration of cassava roots occurs in most cases after harvest. Micro-organisms isolated from the rotten cassava roots plated on potato dextrose agar (PDA) includes: *Botryodiplodia theobromae*, *Aspergillus flavus, Fusarium solani, Rhizopus sp*., *Mucor* and *Erwinia spp*. Pathogenicity test revealed that *B. theobromae*, *Rhizopus sp*., *Aspergillus flavus* and *Fusarium solani* were mostly implicated in cassava root rot under storage.
All the extracts reduced to a varying degree the radial growths of the pathogens (Table 1). The spectrum of activity against the pathogens by the extracts was in the following decreasing order: *Garlic*, *Landolphia owerrience* and *Garcinia kola*. The percentage rot ranged from 1% to 25% (Table 2). The combinations of *Garlic* and *Landolphia owerrience*, *Garcinia kola* and *Landolphia owerrience* exhibited little or no antimicrobial properties. However, the combination of *Garlic* and *Garcinia kola* gave the best result with only 2% rot after 16 days of storage.

Discussion

Deterioration of cassava roots is due to microbial attack, physiological changes and mechanical injuries. *B. theobromae*, *Rhizopus sp*, *Aspergillus flavus* and *Fusarium solani* were found to be the major causal organisms. Interestingly, the results obtained in the inhibition of the extracts against the postharvest pathogens of cassava roots, suggests that the extracts were bioactive (Table 1). The ethanol extracts of *Garlic* and *Landolphia owerrience* gave a wider spectrum of activity against the pathogens compared to that of petroleum ether and water. The effectiveness of ethanol extracts in inhibiting the biomass and the mycelial growth of the pathogens may be ascribed to the soluble nature of the antimicrobial properties of the test plant materials. Ebi and Ofoefule (1997) and Okeke *et al*. (2001) have reported the presence of the following phytochemical compounds in selected medicinal plants: glycoside, saponin, tannin, flavonoids, terpenoids and alkaloids.

Table 1: Percentage growth inhibition

	Ethanol extract (in days)				Water extract (in days)				Petroleum ether extract (in days)			
Extracts	6.0	8.0	12.0	14.0	6.0	8.0	12.0	14.0	6.0	8.0	12.0	14.0
Garlic	100.0	100.0	84.3	65.6	12.6	10.2	10.2	8.3	56.0	52.5	41.6	33.4
Landolphia owerrience	100.0	100.0	78.7	61.3	36.2	21.1	18.4	12.4	48.3	42.6	30.8	28.6
Garcinia kola	53.4	38.2	33.4	20.6	26.1	20.5	10.1	8.4	42.6	36.5	28.4	17.6
Control Ethanol	0.0	0.0	0.0	-	-	-	-	-	-	-	-	-
Water	-	-	-	-	0.0	0.0	0.0	0.0	-	-	-	-
Petroleum Ether	-	-	-	-	-	-	-	-	0.00	0.00	0.00	0.00

Note: Data are averages of three replications

Table 2: Incidence of rot during storage of cassava roots treated with Plant extracts

	% Rot during storage for a period of 6-16 days					
Treatment	6	8	10	12	14	16 (days)
Uninoculated (Control)	2.5	5	12	16	20	24
Ethanol	1	2	8	13	16	21
Petroleum ether	2	4	10	14	20	23
Water	3	5	13	17	21	25
Garlic	0	0	0	0	0	8
Landolphia owerrience	0	0	0	0	0	12
Garcinia kola	0	0	12	12	14	16
Garlic+Garcinia Kola	0	0	0	0	0	2
Garlic+Landolphia owerrience	2	5	8	15	18	20
Garcinia Kola+Landolphia owerrience	3	6	12	17	21	24

Note: Data are averages of three replications

Combination of Garlic and Garcinia Kola demonstrated a remarkable inhibition of the pathogens after the 16 days (2%) rot (Table 2). The combinations of *Garlic* and *Landolphia owerrience* roots, *Garcinia kola* and *Landolphia owerrience* roots exhibited little or no activity unlike the individual extracts. This could be as a result of the antagonistic properties of *Landolphia owerrience* roots. This finding is in line with the earlier report of Okeke *et al*. (2001). The contribution of the phytochemical properties of the extracts as a means of biocontrol is indicated not only by the *in vitro* inhibition of spore germination of all the tested pathogens, but also by the potency of the extracts against the colonies of the pathogens of the cassava roots.

The observed drastic suppression of the pathogens by the plant extracts suggest strongly that they are potent and are therefore suitable for postharvest control of cassava root rot. The efficiency shown by the extracts in overcoming the colonies of the tested pathogens recommends their use as protectants rather than eradicants. The overall strategy of bio-control fits in well with the current concerns of sustainable agriculture, whereby renewable resources are used, with less impact on the environment as a whole.

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Detection of ß - Lactam Antibiotic Residues in Market Milk

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Abstract: The present study was conducted to evaluate the extent of ß - lactam antibiotics residues in unprocessed market milk during the year 2006. Milk samples were randomly collected from Hyderabad city, Latifabad and Qasimabad. Microbial screening test (*Bacillus subtilus* Field Disc Assay) and High Performance Liquid Chromatography (HPLC) methods were used to detect, identify and quantify the ß-lactam residues in milk. A total of 137 milk samples were screened. Among these 63.50% were negative and 36.50% positive for ß - lactam antibiotics residues. The zones size of positive samples appeared between 5.0 and 15.0mm (mean 8.91±0.36mm). Residues level quantified between 0.4 to 400 µg/L for Penicillin G, between 1.0 to 190µg/L for Amoxicillin, between 0.5 to 141µg/L for Ampicillin and between 2.1 to 122µg/L (40.74± 10.59µg/L) for unknown antibiotics. The residues of Penicillin G (mean 59.53µg/L) in unprocessed milk was 14.9 and 11.9 fold, Amoxicillin (mean 36.11µg/L) 9.03 and 3.61 fold, Ampicillin (mean 46.91µg/L) 11.73 and 4.69 fold higher than (MRL's) standards of EU (4µg/L) and FDA (5 and 10µg/L), respectively.

Key words: Milk, Beta lactam, antibiotics, Penicillin residues

Introduction

Antibiotics are vital medicines considered as the ultimate strategy to treat human infections. Their effectiveness is however, threatened by extensive and inappropriate use of these, not only in medicine but also in agriculture. In veterinary practice, antibiotics are utilized at therapeutic levels primarily to treat diseases and to prevent infection. They are also used at sub-therapeutic levels to increase feed efficiency, promote growth and prevent diseases.

The frequent use of antibiotics may result in drug residues that can be found at different concentration levels in products from animal origin, such as milk or meat. Presence of drugs or antibiotics residues in food above the maximum level recognized world wide by various public authorities is illegal (Kempe and Verachtert, 2000). Consumers want to be confident that their food supply is free of contamination by herbicides, pesticides, drugs or antibiotics due to the fact that they may cause potential health hazards, for example allergic reaction, carcinogenicity and promotion of the spread of bacterial resistance to antibiotics used in human medicines. Approximately 5-10 percent of the population is hypersensitive to Penicillin at a concentration as low as 1 ppb or other antibiotics and suffers allergic reactions (skin rashes, hives, asthma, anaphylactic shock). Beside this, antibiotics may interfere with the manufacture of several dairy products. Concentration of 1 ppb delays starter activity during butter and yoghurt making. Antibiotics also decrease the acid and flavor production associated with butter manufacture and they reduce the curdling of milk and cause improper ripening of cheese (Jones, 1999). The USA Food and Drug Administration (FDA) have recently identified approximately 80 drugs which are likely residues in animal-derived human food (Ghidini *et al*., 2002).

ß - lactam is the oldest group of antibiotics which are frequently used for the treatment of sick animals in Pakistan. Milk of such animals is used without any safety measures, causing problems in dairy industries as well as in human health. However, the molecules belonging to the group of ß - lactam have the lowest tolerance in the EU between all the antimicrobials. Consequently the EU regulations 2377/90 set the maximum residues limit (MRL) for some ß - lactam antibiotics in milk, for example, penicillin G 4 µg/L, Ampicillin 4 µg/L, Oxacillin 30 µg/L, Amoxicillin 4 µg/L, Dicloxacillin 30 µg/L, Cephalexin 100 µg/L and Cephairin 60 µg/L (Ghidini *et al*., 2002). Since, no work has been reported on antibiotic residues on market milk in Pakistan. Thus, present study has been planned to evaluate the level of ß - lactam antibiotics in market milk.

Materials and Methods

Screening of milk samples for the presence of ß - lactam antibiotic residues: A total of 137 unprocessed market milk samples from Hyderabad city, Latifabad and Qasimabad were collected and brought to the laboratory of Department of Dairy Technology, Sindh Agriculture University Tandojam for detection, identification and quantification of ß - lactam antibiotic residues. Milk samples were screened for the presence of ß - lactam antibiotic residues using *Bacillus subtilis* Qualitative Field Disc Assay as described by Association of Official Analytical Chemists (AOAC, 2000). The blank disc of filter papers (Whatman 1, 12mm) were completely dipped into milk sample using forcep and placed on the surface of agar medium containing the sensitive test

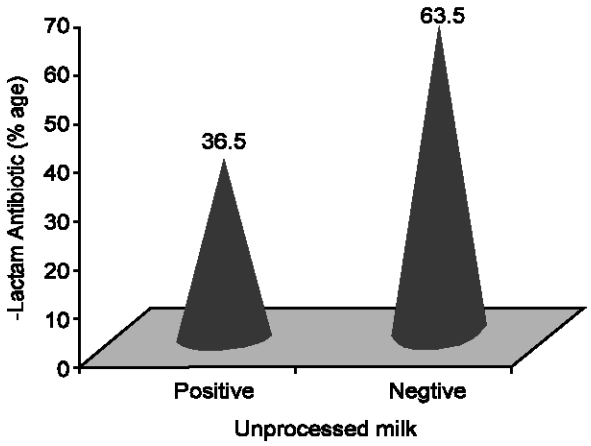


Fig. 1: Screening of unprocessed market milk samples (%age) for ß - lactam antibiotic residues.

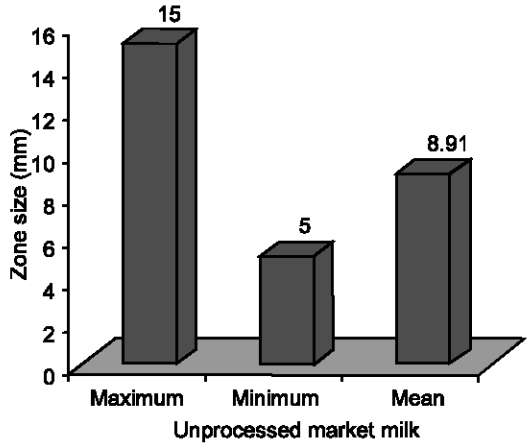


Fig. 2: Diameter of inhibitory zones (mm) determined by microbiological assay of unprocessed market milk.

organism (*Bacillus subtilis*). The plates were incubated at 35°C for 24h. The positive results (the presence of ß - lactam antibiotic residues) were manifested by formation of transparent zones around disc. Parallel to that antimicrobial susceptibility standards test discs of Ampicillin, Penicillin G and Amoxicillin (Oxoid) were processed for comparison purpose.

Quantification of ß - lactam antibiotics residues: ß-lactam antibiotic residues of market milk were quantified by the method as described by Ghidni *et al.* (2002). A High Performance Liquid Chromatography (HPLC) system (Hitachi) was used, which equipped with an autosampler (Model, L-2200), a UV detector (Model, L-2400), a pump (Model, L-2130) isocratic, Column Oven (Model, L-2300) and C18 (ODS-3) column. The column effluent was monitored at a detector wave length of 365nm.

Table 1: HPLC analysis of unprocessed market milk samples (+ve in screened test)

Category of antibiotic residues	Positive over 50 samples (No.)	%age
ß-Lactam Residues		
Penicillin G	32	64
Amoxicillin	28	56
Ampicillin	24	48
Unknown	15	30

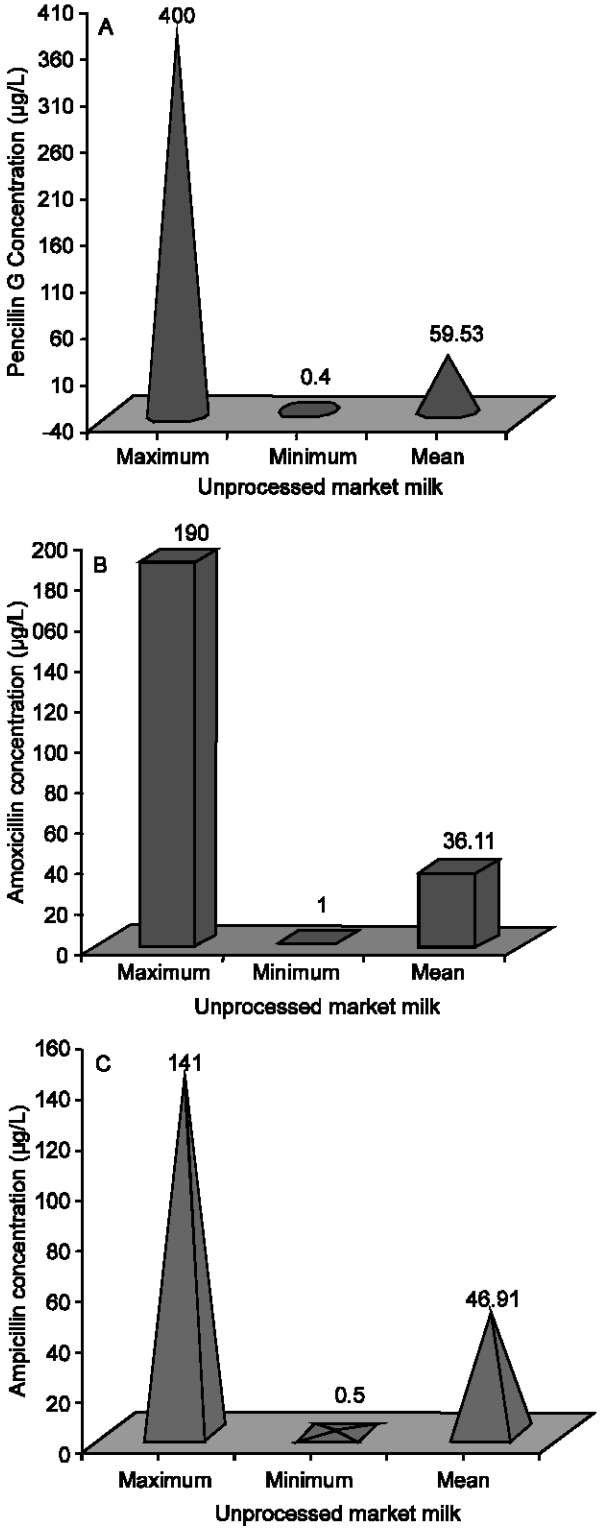
Preparation of sample: Milk sample (5 ml) was taken in 10 ml sterilized pyrex screw cap centrifuge tubes and vortex mixed with 10% aqueous solution of acetic acid (400 µl). The acidified milk was then centrifuged at 3500 rpm for 10 minutes at 4°C in the Backman centrifuge machine. The clear supernatant phase was taken by a disposable syringe, avoiding taking upper fat layer and filtered through a 0.45 µm nylon filter (13 mm diameter) with the back pressure of pump. The filtered extract was put into 2 ml sterilized vials and injected (5µl) into HPLC system. Each sample was prepared in duplicate.

Preparation of standard: Stock standard solutions at a concentration of 100µg/ml of Ampicillin (Sigma), Penicillin G (Sigma) and Amoxicillin (Sigma) were prepared individually in deionized water, previously filtered in 0.45µm filter paper. Mixed standard working solution containing Ampicillin, Amoxicillin and Penicillin G were prepared from the stock solution at concentrations level of 0.2 µg/ml, 0.4 µg/ml, 0.6 µg/ml, 0.8 µg/ml, 1.6 µg/ml, 2.0 µg/ml, 2.4µg/ml, 3.0µg/ml and 4.0µg/ml. The solutions were stored at freezing temperature till use (not more than one month).

Quantification: The quantification of analysis was carried out by injecting standard solution, blank sample and spiked samples. For each ß - lactam analyzed, the factor of response consisting of the ratio between the height of analyte peak and height of internal standard was verified.

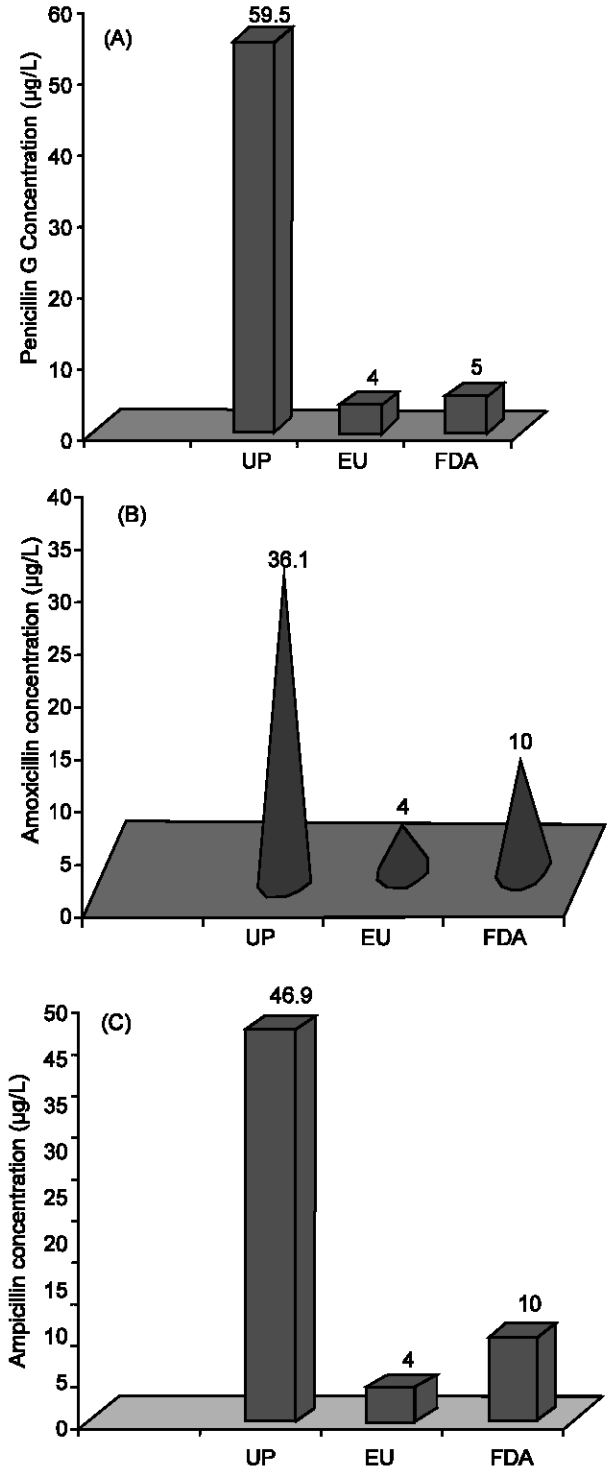
Results and Discussion

Screening of milk samples for ß - lactam antibiotic residues: Raw (unprocessed) milk samples (137) were screened for ß - lactam antibiotic residues through *Bacillus subtilis* Quantitative Field Disc Assay. Majority of samples 87 (63.50%) were negative for ß - lactam antibiotic i.e. no visible transparent zone around disc. While 50 (36.50%) of samples was positive with a zone size ranging from 5.0 to 15.0 mm (mean 8.91±0.37 mm). The present residues limit indicates that milk sold at the vicinity of Hyderabad city, Latifabad and Qasimabad was expected to be at 36.50% risk level for the presence of ß - lactam residues. However, Arora and Chhabra (2004) also reported the prevalence (23.80%) of relatively similar residues in milk samples over the 105 samples analyzed with similar technique at India. While relatively



SE (±) = 17.97, 9.46, 8.39, respectively for A, B and C.

Fig. 3: Penicillin G, Amoxicillin and Ampicillin antibiotics concentration (µg/L) in market milk samples determined by HPLC method.



UP = Unprocessed, EU = European Union, FDA = Food and drug Administration, U.S.

Fig. 4: Penicillin, Amoxicillin and Ampicillin residues in unprocessed market milk v/s Maximum Acceptable Residual Level (MRL standard).

within the similar range to those was reported by Jevinova *et al.* (2003) i.e. 4 to 20mm up to 96 hour after administration of antibiotic to cows suffering from mastitis.

HPLC analysis of market milk samples (+ve in screened test): Milk samples (50 +ve) in screened test were confirmed for ß - lactam antibiotic residues through High Performance Liquid Chromatography (HPLC method) and results are shown in Table 1. The quantification of analyses was limited to Penicillin G, Amoxicillin and Ampicillin due to unavailability of other ß - lactam antibiotic standards at the time of study period. Other peaks appeared were quantified as unknown inhibitors. The result reveals that over the 50 samples analyzed, 32 (64%) were contaminated with Penicillin G residues, 28 (56%) with Amoxicillin, 24 (48%) with Ampicillin and 15 (30%) with unknown inhibitors.

Penicillin G residues in raw market milk ranged between 0.4 to 400µg/L and averaged 59.53±17.91µg/L (Fig. 3A). The results of present study are remarkably lower than maximum residues level of Penicillin G (upto 6240±550µg/L) reported in bovine raw milk (Ghidini *et al.*, 2003). However, the residues are not agreed with the result of maximum residual limits (MRL's) of the European Union regulations (Ghidini *et al.*, 2002) i.e. 4µg/L, Food and Drug Administration (U.S. FDA), (Holstage *et al.*, 2002) i.e. 5µg/L or Junqueira and Brito (2006) i.e. 3.0µg/L. Penicillin residues in market milk were14.9 fold higher than European Union regulation and 11.9 fold than FDA regulations (Fig. 4A).

Amoxicillin residues in market milk varied between 1.0 and 190µg/L and averaged 36.11±9.46µg/L (Fig. 3B). The mean of the Amoxicillin in the present study is within the range of reported values (8.5±1 to 53.7±2.3µg/L) of Cozzani (2005). However, the result of the present study is not in line with the MRL's standards (Ghidini *et al.*, 2002; Holstage *et al.*, 2002) i.e. 4.0 and 10.0µg/L, respectively. Amoxicillin residues level in unprocessed market milk (36.1µg/L) was 9.03 fold higher compared to values recommended by European Union and 3.61 fold to values of FDA regulations (Fig. 4B).

Ampicillin residues level in the market milk samples ranged between 0.5 to 141.0µg/L and averaged 46.91±8.30µg/L (Fig. 3C). The mean value in the present study is 11.72 fold higher than MRL's standards i.e. 4.0µg/L as reported by Ghidini *et al.* (2002) and Holstage *et al.* (2002) i.e. 10µg/L (Fig. 4C).

Conclusion: Among 137 market milk samples (unprocessed) screened, 50 (36.50%) concluded to be contaminated with ß - lactam antibiotic residues. Penicillin residues in market milk samples were dominant followed by Amoxicillin, Ampicillin and unknown antibiotics. Remarkably higher level of Penicillin G, Amoxicillin and Ampicillin residues revealed in raw milk contrast to Maximum Residual Limits (EU and FDA standard). Improper use of antibiotics could be incurred in the vicinity of Hyderabad city, Latifabad and Qasimabad.

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The Effect of Lyophilized Goat Testes Meal as First Feed on the Growth of '*Wesafu*': An Ecotype Cichlid of Epe-Lagoon, in Lagos State, Nigeria

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Abstract: A study was conducted to determine the possible sex inversion effect of lyophilized goat testes meal on hatchlings of '*Wesafu*'. Eighty-five fish and a control group were treated with the meal for forty days in an indoor facility and subsequently transferred into outdoor cages and monitored for growth, the result of manual sex ratio was not conclusive and gonad examination for sex ratio determination has been suspended until fish are big enough. Some mortalities (35%) were recorded during the treatment periods while the control group was intact (100%). The chemical composition of goat testes meal revealed a useful 47% protein and 1.23% oil and a good complement of mineral elements. After the initial 25day exposure of fish to GTM, there was no noticeable length difference (2.9cm) as compared to the control group (3.5cm). The lengths were also similar at 6.03cm and 6.07cm for GTM and the control group respectively after 25 days in outdoor green water culture. There were some non statistical differences in both weight and length of fish in the two groups with the test group clearly showing improvements in terms of length and weight indicating the influence of male hormone (testosterone in GTM) in maleness of the test group.

Key words: Lyophilize, goat testis meal, first feed, sex inversion, phenotypic male

Introduction

It still remains worrisome and sad that Nigeria with immense water resource and expertise does not have a thriving *Tilapia* industry. Also sad is the fact the academia seem to have given up on the *Tilapia* as a commercial culture species in Nigeria. The tilapias are well suited for our environment and low level of technological development. The ease of hypophysation of the African Mud Catfish and the inability of institutions to develop suitable commercial and improved strain of tilapias that will grow to table size in good time are few of the problems militating against a viable *Tilapia* industry in Nigeria.

The problem of precocious sexual maturity and unwanted reproduction has long been accepted as a major constraint to further development and expansion of *Tilapia* culture in Nigeria. The ease of reproduction, actually, represents the principal problem in the optimization of yield in*Tilapia* culture. Energy is diverted from growth, into the behavioral and physiological interactions between the sexes and into the production of eggs. Furthermore, unwanted reproduction leads to excessive recruitment (overpopulation), particularly in ponds, resulting in competition for available food and space resources.

Different techniques including manual sexing (Guerrero, 1982), stock manipulation (Swingle, 1960), polyculture of *Tilapia* with predatory fish (Lovshen, 1975), Hybridization (Hickling, 1960) prevention of breeding in cages and monosex culture (Shell, 1968) have been adapted to culture systems for *Tilapia*.

All-male tilapias have been produced by hybridization or by direct sex reversal (feeding of androgen hormones to sexually sex undifferentiated fry). Sex reversal is more successful and has been adopted in many commercial hatcheries but has yet to be widely adopted in developing countries.

When natural androgens are used the results have been variable. Haylor and Pascual (1991) prepared a diet for *O. niloticus*, which was 57% ram testes. They examined 27 fish and found 23 males and 4 intersex fish. Phelps *et al.* (1996) obtained a 65% male population using a diet, half of which was freeze dried bull testes. This present method used complete freeze-dried sample as food and immersion medium of androgen.

The objectives of this research were to determine; the efficacy of goat testes meal in *Tilapia* sex reversal, secondly, to also determine the utilization of the Goat testes as a feed source in comparison to commercial starter diet (coppers), using weight and length relationship.

Materials and Methods

Experimental Setup: The study was carried out at a private farm, located at Abia village in Badagry Local Government Area of Lagos State. One hundred and seventy five newly hatched ecotype Cichlid of Epe Lagoon "*Wesafu*" were collected from the buccal cavity of the brooding parent (female fish) on the 2nd of Aug 2005. The fish were divided into two-eighty five frys treatments in rectangular glass troughs of (42X24X6) cm. The study was conducted indoor to prevent the proliferation of algae and additional source of food during the initial 40 day treatment with GTM.

Table 1: Proximate and Mineral Composition of Goat testes

% Protein	% Fat	% Ash	% Fiber	Moisture Content
47.33	1.23	1.02	0.582	10.33
% Calcium (Ca)	% Magnesium (Mg)	% Potassium (k)	ppm Sodium (Na)	ppm Manganese (Mn)
1.74	0.29	2.972	6.11	0.67
Ppm Iron (Fe)	ppm Zinc (Zn)	ppm Copper (Cu)		
0.34	0.27	0.13		

NFE 29.54%

Table 2: Chemical Composition of Control Commercial (Coppers) Starter Fish Feed

% Protein	% Fat	% Ash	% Fiber	Vitamin C
56	15.5	9.0	0.4	300mg/kg
% Calcium (Ca)	% Phosphorus	% Lysine	Vitamin A	Vitamin D
18	1.5	3.7	22500lu (IE) /kg	2500lu (IE)/kg
Vitamin E	Copper ($CuSO_4$)	Selenium		
200mg/kg	500mg/kg	0.4mg/kg		

Source: ISO-9001 Certified Coppers label

Table 3: Days and Length Relationship of Sample Treatments

Days of treatment with Goat testes and their corresponding average length		Days of treatment with Coppers and their corresponding average length	
0 day	0.9	0 day	0.9
7 days	2.0	7 days	2.4
14 days	2.1	14 days	2.5
21 days	2.7	21 days	2.9
40 days	2.9	40 days	3.5

*Number of survival at the end of the 40 days of treatment was 55 (65%) for GTM while Coppers starter diet group had no mortality

Table 4: Length of Samples after 25 days of Feeding in Outdoor Tanks

	Goat Testes Meal	Coppers Starter Diet
Wk.1	6.5	6.1
Wk.2	5.8	6.3
Wk.3	5.5	6.1
Wk.4	6.4	5.9
Wk.5	6.2	6.0
Wk.6	5.8	6.0
Wk.7	6.1	6.0
Wk.8	6.0	5.8
Wk.9	6.0	6.3
Wk.10	6.0	6.2
Av.	6.03	6.07

Sample Collection and Diet preparation: Fresh goat testes were obtained from restaurants in the Lagos State University, Ojo. The fresh testes were immediately taken to the Fisheries laboratory where the Individual testis were skinned, sliced and completely homogenized without dilution.

The homogenized samples were then lyophilized in the Biochemistry laboratory after freezing the samples for a minimum of 24 hours. The extract was completely lyophilized within 48 hours and maintained the creamy colour of the homogenized sample. The resultant crumbs were used to feed the *Tilapia* frys exclusively for forty days.

Feeding Regime: The fries were fed ad libitum, a minimum of six times daily with the extract for forty days,

Table 5: Average Weight Range of the Sample Test and Control per Week

Week	Test Diet	Control
1	45.20	40.40
2	48.50	46.40
3	64.30	48.25
4	67.65	52.15
5	72.05	54.15
6	73.55	57.60
7	75.50	63.75
8	77.25	65.85
9	81.35	69.70
10	82.80	67.55
11	83.80	60.60
12	77.75	66.80
13	92.45	73.95
Total Average	72.47	59.01

and after the six weeks of indoor feeding; they are transferred into cages in the outdoor tank where they were fed with a commercial catfish feed [Coppers (0.5-0.8) mm].

Chemical Analysis: The proximate composition of the goat testes meal was determined at IITA (international institute of tropical agriculture) Ibadan, Oyo state. The sample meal was analyzed for moisture, crude protein, crude fiber, lipid, ash content and also for mineral composition; calcium, iron magnesium. The determination was carried out using standard methods of the association of official analytical chemist (AOAC, 1990).

Results

Table 1 and 2 show the chemical compositions of the goat testis meal (GTM) and the control (coppers) starter diet respectively. Table 3 shows the day-length relationships of the test and control diets in indoor facility while Table 4 shows the day-length relationships of the two groups in outdoor facility post indoor treatment.

The average body weight and total length of the specimen of *Wasafu* pick at random from the tank per week were calculated and presentation (Table 5 and 6).

Table 6: Average Total Length of Test and Control Sample

Wk1	Wk2	W3	Wk4	Wk5	Wk6	Wk7	Wk8	Wk9	Wk10	Wk11	Wk12
Sample	6.56	7.21	7.11	7.27	7.41	7.52	7.73	7.81	18.02	8.01	7.80
Control	6.51	6.61	6.85	6.79	6.81	7.05	7.47	7.14	7.27	7.35	7.80

Table 5 shows the growth responses of frys earlier fed with/without goat test meal for forty days and later raised on commercial diets for 13 weeks while Table 6 shows the average total length per week for the sample test and the control.

Discussion

The goat testes meal exposure (GTM) period was completed and the nursery-rearing phase initiated during the first week of September. Nursery rearing and gonadal sex determination was completed December 2005.

After the 40-d Goat testes treatment period, fry total length ranged from 2.5-2.9cm for the group fed with goat testes meal, and 3.0-3.5 cm for the control group, which were fed with coppers starter diet (Table 3).

Fry survival in the treatment sample group was 65%, while in the control group (group fed with coppers) it was 100%, that is, no mortality was recorded. With the 70% survival rate it shows that with Goat testes meal a desirable level of survival can be achieved in the indoor culture, contrary, to the opinion of Popma (1987) who reported a survival of 40% and concluded that higher level of survival might not be visible in the indoor culture. Table 1 and 2 show the Chemical Composition of the trial diets.

Also, after the 40 days treatment, it was clear that a better performance was recorded for the group fed with coppers. This should be expected, because of the lower protein composition of the goat testes meal and the coppers in Table 2 and Table 3 respectively, it seem that the coppers is of a better quality as starter diet. For fish to grow, in their early stages of development they require a lot of crude protein, which must be readily available. Coppen starter diet had 56% in terms of it crude proteins content while the goat testes meal was 47.33% CP. However to be able to use GTM as starter meal, some level of supplementation is required to boost its composition. Supplemented GTM would hold a lot of promise for the hatchery business with the difficulty being experienced with live fish foods in Nigeria.

Data collected after 25 days of feeding on similar diet in outdoor tanks indicated some level of success in achieving phenotypic maleness of the fish. The fish fed with the goat testes meal (GTM) grew faster, almost equal in length with the control group fed with coppers starter diet. This could be an indication of an all male fish emanating from the GTM starter group.

This is in agreement with the findings of Haylor and Pascual who fed ram testis meal and found it to support fish growth and phenotypic male in fish fed diets rich in ram testis meal for forty days.

The same group of fish were further fed indoors on commercial diet for additional 13 weeks. Table 5 and 6 show the weight and length relationships of the control and test feed trial and affirmed the earlier position of influence of male hormone in GTM in the outcome of the study. Lyophilized goat testes meal therefore hold lots of promise in Nigerias' aquaculture industry, however some quantification of the level of testosterone in the goat meal need be established.

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Characterization of *Lactococcus* Phages from Dahi Whey

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Abstract: For the production of yogurt and related products, the local and the commercial yogurt manufacturers of Pakistan are still, by and large using an undefined, unclassified mixture of lactic acid bacteria together with a large number of contaminated strains. Phages isolated in our part of world show different characteristics in terms of phage morphology, lytic patterns and burst size etc, compared to phages isolated elsewhere in the world cause serious problems to fermentation process and ultimate economic losses to dairy industry. In order to develop indigenous resistant starter cultures phages were isolated from samples of Dahi whey isolated phages were characterized on the basis of host range, latent period and burst size. Five virulent phages KT-1, KT-2, KT-3, KT-4 and KT-5 were isolated from twelve samples of whey using *Lactococcus lactis* strains as host. These phages caused lysis and ultimately retarded acid production of host strains. Three phages i.e. KT-1, KT-2 and KT-3 have shown multiple host specificity. Phages KT-1 and KT-2 have same latent period of 15 minutes but exhibited a slightly different rise period that is 20 minutes and 25 minutes respectively, the burst size of 90 and 65 phages per cell were detected for KT-1 and KT-2 respectively. KT-4 showed burst size of 80 phages per infected cell, whereas KT-5 showed burst size of 90 phages per cell. The results reveal that bacteriophages from this region are different from phages isolated in other parts of world.

Key words: *Lactococcus*, bacteriophages, Whey, yogurt

Introduction

Lactococcus bacteria have found their use in the manufacture of several fermented dairy products in which they are responsible for the acidity and characteristic flavor. Sufficient lactic acid formation by lactic acid bacteria during the manufacture of fermented dairy products is of utmost importance. Factors contributing to a slow starter are composition of milk, contaminating microorganisms, changes in fermentation behavior and most commonly encountered bacteriophages attack (Klaenhammer, 1984). The first to attribute the cause of starter failure to bacterial cell dissolving activities of bacteriophages. Since then failure due to phages have been observed throughout the world (Whithead and Cox, 1985).

Elaborate studies on various aspects of phage including morphological and structural characterization, host phage interaction, effect on lactic fermentation and product quality and genetic studies have been carried out in order to combat phage attacks in dairy industry (Klaenhammer, 1984). However, such a detailed study on the phages isolated from our indigenous dairy products such as Dahi, Lassi and Whey, has not been attempted despite its tremendous significance. The present work is concerned with the isolation and characterization of *Lactococcus* phages specifically from Dahi whey as it provides an excellent medium for the propagation of bacteriophages. The prime objective of this work was to isolate and characterize *Lactococcus* bacteriophages. The with respect to their of host range, latent period and burst size and to study the effect of these bacteriophages on lysis and ultimate reduction in acid formation.

Materials and Methods

Bacterial strains and media: Bacterial strains used in this study were isolated and characterized in the Department of Food Technology, University of Arid Agriculture Rawalpindi, Pakistan. The M-17 media was used in this study for the growth and maintenance of bacterial strains and M-17 broth was also used for the phage propagation. Phage suspensions that were used regularly in this research were kept refrigerated at 4°C in M-17 broth.

Whey samples: Twelve samples of whey were collected from different localities of Rawalpindi city. Samples were collected randomly in sterilized bottles. Samples were cooled on ice immediately after collection for transportation and brought to the laboratory for further analysis.

Isolation of bacteriophages: Isolation of bacteriophages was performed according to methods described by Jarvis (1989). First whey was centrifuged at 3000g for 20 minutes and supernatant was collected and filtered through 0.45 µm sterilized filter and filtrate was kept at 4°C for further isolation of bacteriophages. Aliquots (0.1

mL) of an overnight broth culture of bacterial host *Lactococcus lactis* were placed in sterilized test tubes. One drop (0.05 mL) of sterilized one molar calcium chloride (1M $CaCl_2$) was added to each tube followed by the addition of 0.1 mL of test filtrate.

After incubation at 37°C for 15 minutes, then top soft agar M-17 (2.5 mL per tube) was added into the tube. Tube contents were mixed by swirling gently and then immediately poured on the surface of set bottom (M-17) agar plates. Plates were incubated at 37°C and observed periodically for the appearance of plaques from three hours onwards.

Two well isolated plaques were separated with the help of sterile toothpick into a 0.5 mL broth M-17 chloroform was added 0.2 percent (V/V). After overtaxing for 60 seconds, the tubes were kept at 4°C overnight.

Large scale phage production: Phage stocks were prepared by addition of 0.5 mL of phage lysate and 0.1 mL of 1M $CaCl_2$ to 100 mL broth cultures in M-17 (Absorbance 0.5 at 650 nm) at 37°C, containing 1 percent host bacterium. During incubation at 37°C, lysis occurred after two hours and completed within three to four hours which was indicated by the optical density which reaches at 0.16 then lysate was centrifuged at 3000g for 20 minutes. Supernatant was collected and chloroform was added at the rate of 0.2 percent (v/v). After vortexing, the tubes were kept at 4°C for further examinations.

Growth and culture maintenance methods: The purity of cultures was tested according to the method proposed by Harrigan and McCance (1976). Cultures were inoculated in tubes with 10 mL of reconstituted sterile skimmed milk, using 15% inoculum and then frozen at 0°C before incubation for cell growth. Incubating at 30°C until milk coagulation made reactivation.

Test for bacteriophage presence: This test was carried out according to method of Terzaghi and Sandine (1975). 0.1 mL of cultures was placed in test tube containing 2.5 mL of M-17 broth. 0.5 mL of 1 M $CaCl_2$ was added to the test tubes. After slight agitation, the material was poured into Petri dishes with a solidified layer of M-17 agar. After solidification of the second layer, a drop of ultra purified whey was placed on its surface to test the presence of bacteriophages. Plates were then incubated at 30°C for 18 hours. Plaques of bacteriophages were observed where they caused the lysis of bacterial strains.

Isolation and storage of bacteriophages: Storage of bacteriophages was carried out according to the method of Terzaghi and Sandine (1975). Plaques were carefully removed from plates and transferred to the test tubes containing 0.1 mL of host culture and 0.05 mL of 1 M $CaCl_2$. Test tubes were then incubated at 30°C for six hours when cell lysis was observed. The lysis was indicated by the relative clearness of broth when compared with the controls. The controls were more turbid due to the presence of bacterial growth. Those tubes where lysis was observed were filtered through 0.45 µm membrane filter and stored at temperatures between 2°C and 5°C.

Loss in acid production: This test was carried out in reconstituted skimmed milk as described by Anderson and Meanwell (1942). For each bacterial strain under study, two controls and five test tubes were prepared with 10 mL of milk each. In control, 2% cultures were added. (Cultures were first incubated for 18 hours in reconstituted milk). Test tubes received 2% of cultures and 1 mL of filtered whey. All tubes were incubated in water bath at 30°C for two hours and then at 35°C for four hours. After that, acidity was determined by titration with 0.1 N NaOH using phenolphthalein indicator.

Addition of bacteriophages to commercial starters: This test was conducted by using the reconstituted skimmed milk at 11%. In this case each bacterial strain had one control and two test tubes for each fermentation hour. Test tubes received 1% of reactivated cultures and 0.5% of a mixture of all bacteriophages isolated in the samples. The controls were containing only starter cultures. All tubes were incubated at 30°C for 8 hours and a sample was taken every hour and acidity was determined by titration with 0.1N NaOH.

Isolation and sensitivity of cultures isolated from commercial starters: For this test reactivated commercial starters were used. Starters were successively diluted up to 10^{-9} in 0.1% peptone water.1 mL of each solution was plated in APT agar (APT broth agar). After overlaying with same medium, petri dishes were incubated at 30°C for 48 hours. Then isolated colonies were inoculated in milk until coagulation of milk. After coagulation, cultures were transferred to M-17 broth and incubated at 30°C for 18 hours. Cultures were then tested with all those bacteriophages, which were isolated in this study. All the cultures were tested using method of Hull (1977) to evaluate the host sensibility of bacteriophages.

Host range determination: Host ranges of isolated bacteriophages were determined by spot test with cross-reactions on all *lactococcal* host strains as described by Moineau *et al.* (1994). In this test, 2.5 mL of M-17 agar was mixed with 0.1 mL of actively growing *lactococcal* culture. After gentle mixing, the mixture was

Table 1: Bacteriophage presence in whey samples against *Lactococcus strains*

Whey Samples	*Lactococcus lactis* Starins				
	C1	C2	C3	P1	P2
A	-	-	-	-	-
B	+	+	-	-	-
C	+	+	-	-	+
D	-	-	-	-	-
E	-	+	-	+	-
F	-	-	-	-	-
G	-	-	-	+	-
H	-	-	-	-	-
I	-	-	-	-	+
J	-	-	-	-	-
K	-	-	-	-	-
L	-	-	-	-	-

+Lysis observed, _No lysis observed

poured onto the M-17 agar plate. After solidification, 20 µL of phage lysates were spotted on the lawns. Plates were incubated upright at 30°C for 24 hours and then examined for lysis.

Burst size and latent period: The percentage of adsorption, minimum latent period and average burst size was determined by the method of Loof *et al.* (1983).

Percentage of adsorption: Percentage of adsorption of phages to bacterial host was determined by using the method of Loof *et al.* (1983). The adsorption was allowed for 10 minutes and supernatants were filtered through 0.2 µm sterilized filters to separate unadsorbed phages and bacteria. Experiments were replicated thrice and mean adsorption values were determined from all the trials.

Burst size and latent period determination: Burst size and latent period were determined through following test. All host strains were grown to an optical density of 1.2 (600nm). Phages were added to hosts at multiplicities of infection ranging from 0.002 to 0.04. After the adsorption period, cells were centrifuged, resuspended in M-17 and diluted to 10^2 to 10^3 infected bacteria per mL. Suspensions were incubated at 30°C. Samples taken over time of 0 to 75 minutes were immediately titrated for phage determinations.

One step growth curves were constructed to determine the latent periods and burst sizes. Burst sizes were determined by dividing the mean plaque count of sample following the rise period by the mean plaque count of samples from the latent period. Mean values were determined by pooling means from three times replicated experiments for each phage.

Results and Discussion

This study was designed to isolate and characterize those bacteriophages, which are present in our environment and are causing serious damage to starter cultures and ultimate losses to dairy industry. Dahi whey provides very conducive medium for the *Lactococcus* bacteriophages. Twelve samples of whey were collected from different localities of Rawalpindi to check out the incidence of *Lactococcus* bacteriophages. These samples were then subjected to further analysis. First of all these samples were tested for presence of bacteriophages against *Lactococcus* strains.

Test for bacteriophage presence: Five strains of *Lactococcus lactis* were used in this study. Twelve samples were tested against all these strains to check the incidence of Lactococcus bacteriophages in those whey samples. Whey samples were centrifuged at 3000g for 20 minutes and then filtered through 0.45 µm sterilized filters. These samples were then assessed for the presence of bacteriophages. All twelve samples were spotted neatly on the lawns of five strains of *Lactococcus lactis*. It was observed that only five, out of twelve tested samples, showed presence of bacteriophages. The bacteriophages were found present in five samples of whey. These samples were B, C, E, G and I and phages from these samples were designated as Phages KT-1, KT-2, KT-3, KT-4 and KT-5, respectively in Table 1.

A variable size of plaque formation occurred. Large size plaques were selected for further characterization of phages. It was further observed from the table that the lytic activity of these bacteriophages was different against different strains. Phage KT-1 showed lytic activity against two strains of *Lactococcus lactis* and caused complete lysis. Whereas phage KT-2 caused lysis of three strains i.e.C1, C_2 and P_2, respectively.

On the other hand, phage KT-3 showed lytic activity against two strains. These strains were C2 and P1. Strain P1 was also the only strain that was lysed by the Phage KT-4. Phage KT-5 was able to attack only one strain that is P2.No bacteriophage was able to cause lysis of strain C3 (Tale 1). The possible reason for this lytic pattern may be difference of receptive sides on host, cell wall composition as well as the concentration of Ca++ ions that facilitates the adsorption of phage to host cell.

Loss in acid production: The addition of bacteriophages in any culture causes the lysis of culture which ultimately leads to loss in acidification ability of lactic acid bacteria. The normal acidification rate of milk is retarded in presence of bacteriophages. Loss of acid production of *Lactococcus* cultures was determined by using skimmed milk that was reconstituted at 11%. Selected five strains of *Lactococcus lactis*were used to determine the presence of bacteriophages in whey samples. For each tested strain, five test tubes were prepared to check their acidification ability in presence of

Table 2: Comparison of rate of acid production among control and phage containing samples incubated at 35°C for 6 hours

	Treatments					
Strains	Control	KT1	KT2	KT3	KT4	KT5
C1	0.573LM	0.31OP	0.29OPQ	0.59L	0.63JKL	0.61KL
C2	0.82I	0.25PQ	0.32OP	0.42N	0.67JK	0.69J
C3	1.41CDE	1.15CDE	0.98G	1.07EF	0.90H	1.11DE
PI	1.43A	1.2BC	1.093EF	0.51M	0.42DE	1.12DE
P2	1.26B	1.03FG	0.22Q	0.98G	1.19BCD	0.36NO

*Means having same letters are statistically non significant at 5% alpha, *DMR test at LSD value 0.07304 and SD 0.02582, *Values are mean of three replications

Table 3: Addition of Bacteriophages to commercial starter cultures

Commercial starter	Treatments							
cultures	1 hr	2hr	3 hr	4hr	5hr	6hr	7hr	8hr
BD1	0.42^	0.55YZ[\]	0.63VWXY	0.74TUV	0.92OPOR	1.08JKLM	1.19GHIJK	1.31DEFG
BD2	0.46\]^	0.6WXYZ[	1.033LMNO	0.82ORST	1.04LMN	1.12IJKL	1.23FGHI	1.41BCDE
BD3	0.51YZ[\]^	0.59TUVW	0.71PORS	0.900POTST	1.10ILKL	1.93GHIJ	1.307DEFG	1.517ABC
BD4	0.43]^	0.54YZ[\]^	0.63VWXY	0.93NOPO	1.09KLM	1.21GHI	1.4CDE	1.52AB
O1	0.45]^	0.49[\]^	0.59WXYZ	0.98MNOP	1.18HIJK	1.31DEFG	1.42BCD	1.59A
O2	0.52YZ[\]^	0.62VWXYZ	0.71TUVW	1.02LMNO	1.21GHI	1.41BCDE	1.503ABC	1.6A
O3	0.51YZ[\]^	0.63VWXY	0.79STU	0.63VYXY	0.50Z[\]^	0.50]^	0.49[\]^	0.49[\]^
O4	0.49[\]^	0.58XYZ[\	0.76TU	0.78TU	1.02LMNO	1.123IJKL	1.2GHI	1.30DEFGH
O5	0.54YZ[\]^	0.69UVWX	0.81RST	0.95NOP	1.2GHIJ	1.29EFGH	1.33DEF	1.42BCD
D1	0.60WXYZ	0.70YUVWX	0.82ORST	0.71TUVW	0.55YZ[\]	0.50Z[\]^	0.48[\]^	0.44]^
B	0.51YZ[\]^	0.61WXYZ	0.81RSTU	0.97MNOP	1.19GHIJK	1.21GHI	1.307DEF	1.420BCD

*Means having same letters are statistically non significant at 5% alpha, *DMR test at LSD value 0.1019 and Standard Deviation 0.365, *Values are mean of three replications

bacteriophages. The acidity was determined after a period of 6hrs of incubation at 35°C using 0.1N NaOH with the help of phenolphthalein indicator (Table 2).

These results further confirm the results obtained from test of loss in acid production, All those phages which were isolated from whey samples confirmed their presence by attacking the same strains and causing their lysis. These results indicate that rate of acid production in control tubes was steady. Whereas those strains which were susceptible to specific bacteriophages, their rate of acid production declined with the passage of time. It was observed that lytic activity started approximately after one hour of infection in the host strain which caused the decrease in acidity and after four hours culture was almost completely lysed. This may be attributed to the repression of host protein synthesis. These results show that in the presence of bacteriophages, there was a considerable reduction in the acidification rate. Reduction in the production of lactic acid above 10% indicates the presence of bacteriophages. These findings correlate with the findings of Anderson and Meanwell (1982).

Addition of bacteriophages to commercial starters: Once the bacteriophages were isolated from whey samples and their effect on *Lactococcus* strains was established, further studies were conducted to evaluate the effects of these bacteriophages on commercial starters. For this purpose ten samples of commercial starters were selected. Skimmed milk reconstituted at 11% was used in this test. In test tubes, 1% of commercial starter and 0.5% of mixture of all isolated bacteriophages was mixed. Acidity was determined using 0.1N NaOH with phenolphthalein indicator after an interval of one hour (Table 3).

These results show that most commercial starters were not sensitive to inoculated bacteriophages except O3 and D1 starters. According to our studies, results after 8 hours were qualitatively identical to those obtained after 6 hours. Quantitative difference was higher after 8 hours of incubation. It indicates that incubation for 8 hours generated more sensitive results than 6 hours. Although some authors have suggested that incubation for shorter periods (6hours) was more adequate to evaluate the starter behavior in presence of bacteriophages (Stadhouders and Leenders, 1984).

Mechanism of phage resistance that have been found in *Lactococci* include super infection immunity by prophage, reduction in adsorption efficiency, restriction-modification systems and abortive infection. Several of these mechanisms are plasmid encoded (Sanders, 1988). Investigations of the resistance mechanisms the starter strains are currently underway. Strains that demonstrate powerful mechanisms of phage resistance may become the prime candidates for use in other starter systems. Additionally, if these mechanisms are plasmid-linked, they may be useful for the genetic construction of new phage resistant strains (Daly and Fitzgerald, 1987).

Table 4: Sensitivity of strains isolated from starter culture BD1 against all isolated bacteriophages

Isolated Strains	KT1	KT2	KT3	KT4	KT5
C10	-	-	+	+	-
C11	-	-	+	+	-
P11	-	+	+	+	-
P21	-	-	+	-	+
P31	-	-	+	-	-
C20	-	+	+	+	-
C21	-	-	+	-	-

Table 5: Sensitivity of strains isolated from starter culture BD2 against all isolated Bacteriophages

Isolated Strains	KT1	KT2	KT3	KT4	KT5
C10	-	-	+	+	+
P21	-	-	+	-	-
P31	-	+	+	+	-
C20	-	-	-	+	+
T3	-	-	+	+	-

Table 6: Sensitivity of strains isolated from starter culture BD3 against all isolated Bacteriophages

Isolated Strains	KT1	KT2	KT3	KT4	KT5
M17-1	-	-	+	-	-
M17-2	-	+	+	-	-
P10	-	-	+	-	-
P11	-	-	+	+	+
C11	-	-	+	-	-
T3	-	-	+	+	-

Table 7: Sensitivity of strains isolated from starter culture O3 against all isolated Bacteriophages

Isolated Strains	KT1	KT2	KT3	KT4	KT5
M17-1	-	-	+	+	-
M17-4	-	-	+	+	-
C7	-	-	+	-	-
P31	-	+	+	-	-

Table 8: Sensitivity of strains isolated from starter culture O4 against all isolated Bacteriophages

Isolated Strains	KT1	KT2	KT3	KT4	KT5
P31	-	-	+	+	-

Table 9: Host specificity of Lactococcus bacteriophages

	Lactococcus Phages				
Starter Cultures	KT1	KT2	KT3	KT4	KT5
C1	+	+	-	-	-
C2	+	+	+	-	-
C3	-	-	-	-	-
P1	-	-	+	+	-
P2	-	+	-	-	+

Sensitivity of commercial starter isolated cultures: Since culture rotation is one of the methods used in industrial process to control bacteriophage attack so it is very important to determine the sensitivity level of starters bacteria to the bacteriophages found in industry. Keeping in view, the importance of this work, strains were isolated from commercial starters BD1, BD2, BD3, O3 and O4. There was a wide variation in sensitivity among isolated strains (Table 4). No starter isolate was sensitive to phage KT-1. On the other hand, almost every isolate (except for isolate 3 from starter BD2) was sensitive to phage KT-3. The absence of infective phages in certain isolates may be attributed to the non utilization of such cultures in industry. These findings correlate the findings of Hemming *et al.* (1968). Also if the tested starters were only recently used in the plant, there was no sufficient time for the multiplication of bacteriophages to be experimentally detected. The sensitivity of various strains isolated from starter cultures shown in tables given in following pages.

Host range studies: *Lactococcus* phages with multiple host range have been isolated by several workers (Jarvis and Meyer, 1986). Three out of five phages isolated in this study showed multiple host range (Table 9).

Prevalence of the phages capable of attacking more than one host is due to the commonly adopted practice of using multiple strain starters. Moreover, it is known that phages may adapt to the original non permissible host by a range mutation, which can alter its adsorption specificity or by host controlled modification.

Burst size and latent period: The infection cycle of bacteriophages were characterized by their one step growth kinetics. Phages KT-1 and KT-2 have same latent period of 15 minutes but exhibited a slightly different rise period that is 20 minutes and 25 minutes respectively. The burst size of 90 and 65 phages per cell were detected for KT-1 and KT-2 respectively (Fig. 1 and 2). On the other hand phages KT-3, KT-4 and KT-5 showed similar growth kinetics. The latent period for these three phages was calculated 25 minutes, their rise period was 30 minutes and however all these phages exhibited slightly different burst sizes. The burst size for phage KT-4 was calculated as 75 phages per cell.KT-4 showed burst size of 80 phages per infected cell. Whereas KT-5 exhibited burst size of 90 phages per cell (Fig. 3). The difference in latent period and burst size may be due to the fact that it is very difficult to obtain an exact correlation between colony forming units and number of cells in these microorganisms.

Composition of the medium strongly influences the growth kinetics of *Lactococcus* phages. A drastic decrease in the burst size was observed in complete medium in the presence of citrate (Cherry and Watson, 1994).

Shorter latent periods or more liberation on burst or both are characteristics of phage races that develop quickly and achieve high populations in cheese whey. These phages can cause complete failure of starter culture even when present in low concentration at the start of cheese making. The differences observed in burst sizes of the phages KT-3, KT-4 and KT-5 in spite of their identical latent period may be due to the disparity in the

individual eclipse phase of these phages. Composition of the medium strongly influences the growth kinetics of *Lactococcus* phages. The latent period, rise period and burst size of a *Lactococcus lactis* phage on a complete medium containing tryptone, yeast extract and glucose have been reported as 31 to 33 minutes, 15 to 18 minutes and 90 phage particles, respectively, whereas on a deficient medium that lacked tryptone, the latent period o phage growth increased 55 minutes, rise period decreased to 10 minutes and average burst size decreased to 24 phage particles. Also a drastic decrease in the burst size was observed in complete medium in the presence of sodium citrate (Cherry and Watson, 1994).

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Effect of Different Additives from Local Source on the Quality of Yoghurt

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Abstract: Yoghurt is the most popular fermented milk product in the most parts of world. It involves use of specific symbiotic culture of *L. bulgaricus* and *S. thermophilus*. Yoghurt consists of valuable nutrients as in milk but it seems to have more than milk. In the present study different food additives from local source are used as additives and the effect is estimated for possible influence on the quality of yoghurt. Yoghurt was prepared by using different stabilizers like carboxymethyl-cellulose (CMC), guar gum, gelatin, cornstarch and their combinations like CMC-gelatin, gelatin-cornstarch and CMC-cornstarch at different levels i.e. 0.1, 0.2, 0.3, 0.4 and 0.5% at 0, 7, 14 and 21 day of storage interval. Guar gum at 0.1% gives best result for low acidity and low pH where as total solid free fatty acid, acetaldehyde contents comes best with the cornstarch. Food additives have influence on pH, acidity, total solid and acetaldehyde contents. During the storage of 21 days with increasing amounts of food additives, there was an increase in acidity, free fatty acids, total solid and acetaldehyde contents but decrease in pH.

Key words: Yoghurt quality, stabilizers, thickeners, CMC, Gelatin

Introduction

Yogurt is fermented and coagulated milk product with a smooth texture having mildly sour taste and pleasant flavor. It is obtained from pasteurized or boiled milk by souring natural or other wise using lactic acid fermented bacteria. (Soomro *et al*., 2003). It is one of the oldest popular foods of the world because of its nutritional and therapeutic value in the human diet. (Zahoor *et al*., 2002). Typical plain yoghurt contained 3.5% fat 12.06% total solids, 3.60% protein 18.94% moisture, 0.76% ash and 4.2% lactose (Ather, 1986).

Yoghurt is characterized as a smooth, viscous, gel with specific taste of sharp acid and green apple flavor (Bodyfelt *et al*., 1988). Some yoghurts exhibit a heavy consistency that closely resemble custard of milk pudding. In contrast, others are purposely soft boiled and are essentially drinkable (Connolly *et al*., 1984). The moist important textural characteristics of yoghurt are firmness and other ability to retains water. The type of culture is an important factor affecting microstructure and the textural properties of yoghurt (Hussan *et al*., 1999).

Stabilizers and thickeners are important in several manufactured products such as chocolate, dressing milk drinks, ice cream and yoghurt. These substances prevent separation of various ingredients, increase the viscosity and inhibit the formation of large crystals. Substances used as stabilizers and thinkers include vegetable gums such as gum tragacanth and gum Arabic agar and pectin. Cellulose compound like methyl cellulose and CMC are also used (Awan, 1995).

Keeping in view the importance of the subject this study is designed to check the effect of different stabilizers on the yoghurt quality different concentration with combination also used to analyze their effects. Mains objectives are, to compare the effect of stabilizers on yogurt quality and to assess the yogurt chemically.

Materials and Methods

Preliminary preparation: Standardized milk and stabilizers (carboxymethyl-cellulose (CMC), guar gum, gelatin and cornstarch) were collected from local market. These stabilizers were used in concentration of 0.1, 0.2, 0.3, 0.4 and 0.5% either individually or in combinations. Additions of stabilizers were made in each sample of milk at room temperature and were blended.

Preparation and storage of yoghurt: After addition of stabilizers milk were pasteurized at73°C for 15 min. milk was cooled at 42°C till and starter culture was added at the rate of 2% and transferred to polytheyne cups. The culture milk was incubated at 42°C till the desired body texture with 0.8%v acidity and pH 4.25 was obtained. The yoghurt was cooled downed to 6°C.

Product analysis: Acidity, PH, total solid, free fatty acids and acetaldehyde contents was measured by methods as described in No. 967.16, 981.12, 941.08, 940.28 and 906.02 respectively of AOAC (1990).

Statistical analysis: The data obtained was analyzed by tow factor factorial design according to Steel and Torrie (1980).

Results and Discussion

Yoghurt samples of different stabilizer concentrations were chemically analyzed for acidity, pH, total solids free

Table 1: Effect of different additives during storage periods (6°C) on acidity of yoghurt

Stabilizers	Treatments	0 days	7 days	14 days	21 days
CMC	T1 (0.1)	0.86t-x*	0.92q-u	1.14i-n	1.42bc
	T2 (0.2)	0.86t-x	0.92q-u	1.15i-n	1.45ab
	T3 (0.3)	0.87s-x	0.93q-u	1.14i-n	1.4bcd
	T4 (0.4)	0.84 t-y	0.90q-u	1.16h-m	1.41bc
	T5 (0.5)	0.86t-x	0.91q-u	1.13i-n	1.37b-g
Guar Gum	T6 (0.1)	0.69z	0.89r-v	1.02m-r	1.24f-k
	T7 (0.2)	0.79u-z	0.91q-u	1.04m-r	1.32b-g
	T8 (0.3)	0.74w-z	0.93q-u	1.08m-p	1.33b-g
	T9 (0.4)	0.74w-z	0.90q-u	1.09i-o	1.25e-j
	T10 (0.5)	0.72xyz	0.93q-u	1.07m-p	1.31b-g
Gelatin	T11 (0.1)	0.82t-z	0.91q-u	1.10k-n	1.31b-g
	T12(0.2)	0.83t-y	0.93q-u	1.11j-n	1.34b-g
	T13 (0.3)	0.83t-y	0.93q-u	1.15i-n	1.38b-f
	T14 (0.4)	0.83t-y	0.92q-u	1.13i-n	1.32b-g
	T15 (0.5)	0.86t-x	0.95p-t	1.15i-n	1.4bcd
Cornstarch	T16 (0.1)	0.83t-y	0.90q-u	1.07m-p	1.23g-l
	T17 (0.2)	0.83t-y	0.92q-u	1.08m-p	1.29c-h
	T18 (0.3)	0.84t-y	0.90q-u	1.08m-p	1.3c-g
	T19 (0.4)	0.83t-y	0.92q-u	1.08m-p	1.29c-h
	T20 (0.5)	0.89r-v	0.95q-u	1.01n-s	1.29c-h
Gelatin/	T21 (0.1)	0.89 r-v	0.94q-t	1.12j-n	1.32b-g
Corn starch	T22 (0.2)	0.83t-y	0.90q-u	1.08m-p	1.25e-j
	T23 (0.3)	0.89r-v	0.96o-t	1.14i-n	1.29ch
	T24 (0.4)	0.89r-v	0.95p-t	1.13i-n	1.27d-l
	T25 (0.5)	0.86t-x	0.96o-t	1.12j-n	1.27d-l
Gelatin/CMC	T27 (0.1)	0.79u-z	0.90q-u	1.15i-n	1.3c-g
	T28 (0.2)	0.75v-z	0.91q-u	1.15i-n	1.32b-g
	T29 (0.3)	0.71yz	0.93q-u	1.13i-n	1.24f-k
	T30 (0.4)	0.69z	0.93q-u	1.15i-n	1.30c-g
	T31 (0.5)	0.77xyz	0.95p-t	1.14i-n	1.33b-g
CMC/	T31 (0.1)	0.86t-x	0.90q-u	1.11j-n	1.37b-g
Corn starch	T32 (0.2)	0.86t-x	0.912q-u	1.14i-n	1.42bc
	T33 (0.3)	0.86t-x	0.92q-u	1.15i-n	1.45ab
	T34 (0.4)	0.84t-y	0.90q-u	1.14i-n	1.39b-d
	T35 (0.5)	0.86t-x	0.93q-u	1.14i-n	1.4bed
Control	T36	0.90q-u	1.02m-r	1.23g-l	1.56a

*All values are results of three replication. *Mean sharing the same letter do not differ significantly. *LSD at 0.05 Alpha for treatment, interval and interaction is 0.5682, 0.01894 and 0.1136 respectively.

Table 2: Effect of different additives during storage periods (6°C) on pH of yoghurt

Stabilizers	Treatments	0 days	7 days	14 days	21 days
CMC	T1 (0.1)	4.15a-h	4.09d-l	3.87l-r	3.65wxy
	T2 (0.2)	4.16a-g	4.11c-l	3.85l-s	3.62xy
	T3 (0.3)	4.13b-l	4..04hij	3.88l-r	3.68u-y
	T4 (0.4)	4.14a-h	4.1c-l	3.85l-s	3.8o-t
	T5 (0.5)	4.2abcd	4.1c-l	3.9l-p	3.83m-s
Guar Gum	T6 (0.1)	4.25a	4.1c-l	3.95jkl	3.8o-t
	T7 (0.2)	4.25ab	4.09d-l	3.91imno	3.8o-t
	T8 (0.3)	4.19a-e	4.06fgh	3.89l-q	3.78q-u
	T9 (0.4)	4.19a-e	4.06fghi	3.9l-p	3.8o-t
	T10 (0.5)	4.12abc	4.07ghi	3.91lmno	3.82n-s
Gelatin	T11 (0.1)	4.14a-h	4.09 d-l	3.94klm	3.62xy
	T12 (0.2)	4.13b-l	4.08e-l	3.92klmn	3.86i-s
	T13 (0.3)	4.15b-l	4.1c-l	3.89l-q	3.75s-w
	T14 (0.4)	4.13b-l	4.07fghi	3.91l-q	3.68u-y
	T15 (0.5)	4.12b-l	4.06fghi	3.92l-q	3.62o-t
Cornstarch	T16 (0.1)	4.16a-g	4.1c-l	3.92l-p	3.82n-s
	T17 (0.2)	4.15a-h	4.07fghi	3.89l-p	3.79p-t
	T18 (0.3)	4.11a-g	4.07fghi	3.89l-q	3.87l-r
	T 19 (0.4)	4.16a-g	4.06fghi	3.90l-p	3.87l-r
	T20 (0.5)	4.17a-f	4.1c-l	3.90l-p	3.83o-t
Gelatin/	T21 (0.1)	4.11c-l	4.07fgh	3.89l-q	3.71t-x
Corn starch	T22 (0.2)	4.16a-g	4.1c-l	3.86klmn	3.7t-y
	T23 (0.3)	4.14a-h	4.08e-l	3.89klmn	3.6y
	T24 (0.4)	4.16a-h	4.08e-l	3.93klmn	3.68u-y
	T25 (0.5)	4.16a-g	4.06fhgi	3.92l-q	3.68u-y
Gelatin/CMC	T27 (0.1)	4.12b-h	4.09dd-l	3.89l-q	3.65wxy
	T28 (0.2)	4.15a-h	4.05ghi	3.93klim	3.75s-w
	T29 (0.3)	4.12b-l	4.06fghi	3.92klmn	3.77r-v
	T30 (0.4)	4.13b-l	4.07fghi	3.93klmn	3.75s-w
	T31 (0.5)	4.13b-l	4.07d-i	3.89l-q	3.62xy
CMC/	T31 (0.1)	4.15a-h	4.08e-l	3.89l-q	3.66wxy
Corn starch	T32 (0.2)	4.17a-f	4.09d-i	3.86l-s	3.63xy
	T33 (0.3)	4.14a-h	4.02ijk	3.89l-s	367wxy
	T34 (0.4)	4.21abc	4.06fghi	3.89l-q	3.75s-w
	T35 (0.5)	4.20abcd	4.06fghi	3.87l-r	3.84l-s
Control	T36	4.14a-h	3.95jki	3.83m-s	3.8o-t

*All values are results of three replication. *Mean sharing the same letter do not differ significantly. *LSD at 0.05 Alpha for treatment, interval and interaction is 0.04401, 0.01467 and 0.08802, respectively45.

fatty acids and acetaldehyde content. The data obtained was subjected to statically analysis. The results obtained for each determination are described individually as under.

Total titratable acidity: The data on the effect of different doses of CMC, guar gum, gelatin, corn starch and their combinations like corn starch/gelatin, gelatin/CMC and CMC/ cornstarch at level (T1 to T36) of 0.1, 0.2, 0.3, 0.4 and 0.5% on the acidity of yoghurt during storage period are presented in Table 1. The results show that maximum acidity (1.56) was obtained from the yoghurt with at control (T36) after 21 days of storage followed by CMC (1.45) at 0.2% (T2) after 21 days of storage and CMC/gelatin at 0.3% (T33) after 21 days of storage respectively. The lowest values for acidity was found in all treatment on 0 days that increase from incubation period till end of storage, so maximum acidity was found during 21 days storage interval. The statistical analysis showed that effect of different amount of food additives and effect of storage period was highly significantly differ for acidity of yoghurt while interaction is non significant. Results obtained confirmed the finding of Georgala *et al.* (1995) who observed similar changes in the acidity of yoghurt during storage.

PH: The data for the effect of storage on pH under different stabilizer treatments are presented in the Table 3. The results showed that pH decreased throughout the storage interval. The maximum pH value was found with T6 followed by T7 and T5. The lowest value for pH was found with T23. The pH at 0 day interval was the highest in all treatment and it gradually decreased throughout the storage interval. The statistical analysis showed that effect of different amount of food additives and effect of storage period was highly significantly differ for acidity of yoghurt while interaction is non significant. The comparison of four stabilizers and their combination showed that the final of yoghurt manufactured with guar gum stabilizers was higher than the others which was related to better quality yoghurt. It is evident from the results that the pH decreased throughout the storage

Table 3: Effect of different additives during storage periods (6°C) on Total solid of yoghurt

Stabilizers	Treatments	0 days	7 days	14 days	21 days
CMC	T1 (0.1)	13.12kl	13.14jkl	13.25hij	13.3ghi
	T2 (0.2)	13.3ghi	13.32gh	13.34gh	13.5f
	T3 (0.3)	13.52f	13.54f	13.56f	13.7de
	T4 (0.4)	13.72de	13.74de	13.76d	13.9c
	T5 (0.5)	14.9a	14.92a	14.94a	14.95a
Guar Gum	T6 (0.1)	13.11kl	13.12ki	13.13jkl	13.14jkl
	T7 (0.2)	13.3ghi	13.32gh	13.33gh	13.35gh
	T8 (0.3)	13.5f	13.51 f	13.52f	13.54fde
	T9 (0.4)	13.7de	13.71de	13.73d	13.74d
	T10 (0.5)	14.9.a	14.92a	14.95a	14.98a
Gelatin	T11 (0.1)	13.1kl	13.12ki	13.13jkl	13.15jkl
	T12 (0.2)	13.3ghi	13.32gh	13.33gh	13.35gh
	T13 (0.3)	13.5f	13.51f	13.51f	13.52f
	T14 (0.4)	13.7de	13.71de	13.73d	13.77d
	T15 (0.5)	14.9a	14.92a	14.92a	14.96a
Cornstarch	T16 (0.1)	13.1kl	13.14jkl	13.14jkl	13.16jkl
	T17 (0.2)	13.3ghi	13.34gh	13.34gh	13.38gh
	T18 (0.3)	13.5f	13.56f	13.52f	13.6ef
	T 19 (0.4)	13.7de	13.74de	13.73d	13.77d
	T20 (0.5)	14.9a	14.72ba	14.93a	14.96a
Gelatin/	T21 (0.1)	13.12kl	13.12ki	13.15jkl	13.18jkl
Corn starch	T22 (0.2)	13.3ghi	13.32gh	13.36gh	13.35gh
	T23 (0.3)	13.52f	13.51f	13.58f	13.56fde
	T24 (0.4)	13.7de	13.71de	13.76d	13.77d
	T25 (0.5)	14.9a	14.92a	14.74a	14.96a
Gelatin/CMC	T27 (0.1)	13.11kl	13.12ki	13.15jkl	13.25hij
	T28 (0.2)	13.3ghi	13.32gh	13.36gh	13.36gh
	T29 (0.3)	13.5f	13.51f	13.58f	13.56f
	T30 (0.4)	13.7de	13.71de	13.76d	13.76d
	T31 (0.5)	14.9.a	14.92a	14.74a	14.95a
CMC/	T31 (0.1)	13.1kl	13.12ki	13.13jkl	13.16jkl
Corn starch	T32 (0.2)	13.3ghi	13.32gh	13.33gh	13.38gh
	T33 (0.3)	13.5f	13.51f	13.51f	13.6ef
	T34 (0.4)	13.7de	13.71de	13.73d	13.77d
	T35 (0.5)	14.9a	14.92a	14.94a	14.96a
Control	T36	12.9m	13.05l	13.1kl	13.35gh

*All values are results of three replication. *Mean sharing the same letter do not differ significantly. *LSD at 0.05 Alpha for treatment, interval and interaction is 0.05082, 0.0.1694 and 0.1016, respectively.

Table 4: Effect of different additives during storage periods (6°C) on Free Fatty acid of yoghurt

Stabilizers	Treatments	0 days	7 days	14 days	21 days
CMC	T1 (0.1)	0.98q	0.72[	1.1z	1.4y
	T2 (0.2)	1.1z	1.13z	1.2z	1.5wxy
	T3 (0.3)	1.15z	1.19z	1.22Z	1.6zw
	T4 (0.4)	1.45xv	2imn	2.15ijk	2.25hi
	T5 (0.5)	1.55vwx	1.6vw	1.8q-u	2.24defg
Guar Gum	T6 (0.1)	0.99q	1.85opq	1.25z	1.4y
	T7 (0.2)	1.19z	1.2z	1.59vwx	1.6vw
	T8 (0.3)	1.55vwx	1.6vw	1.89m-q	2.24hi
	T9 (0.4)	1.6vw	1.86nopq	2mnl	2.5bcde
	T10 (0.5)	1.68tuv	1.9m-q	2.1jkl	2.58bc
Gelatin	T11 (0.1)	1.14z	2.4efg	2.48bcde	2.52bcde
	T12 (0.2)	1.16z	2.45cdef	2.5bcde	2.56bcd
	T13 (0.3)	1.17z	2.32fgh	2.41efg	2.59bc
	T14 (0.4)	1.12z	1.98l-p	2.2hij	2.45cdef
	T15 (0.5)	1.98lmnop	2.02klm	2.49bcde	2.62b
Cornstarch	T16 (0.1)	1.61vw	1.68tuv	1.7r-v	2.45cdef
	T17 (0.2)	1.63vw	1.69stuv	1.84prq	2.6bc
	T18 (0.3)	1.7r-v	2.25hi	2.57bcd	2.78a
	T 19 (0.4)	1.81qrst	2.31fgh	2.57bcd	2.8a
	T20 (0.5)	1.84pqr	2.32fgh	2.59bc	2.82a
Gelatin/	T21 (0.1)	1.58vwx	1.65vw	1.99lmno	2.3gh
Corn starch	T22 (0.2)	1.62vw	1.68tuv	2.07fg	2.41efg
	T23 (0.3)	1.66uv	1.83qrs	2.26c	2.5bc
	T24 (0.4)	1.71m	1.99hi	2.45a	2.68a
	T25 (0.5)	1.82l	2.11ef	2.59a	2.68/a
Gelatin/CMC	T27 (0.1)	1.01q	1.56n	1.99hi	2.2d
	T28 (0.2)	1.45n	1.58n	2.07fg	2.36ab
	T29 (0.3)	1.78l	1.87jkl	2.26c	2.58a
	T30 (0.4)	1.91jik	1.99hi	2.45a	2.68a
	T31 (0.5)	1.99hi	1.86jkl	2.59a	2.76a
CMC/	T31 (0.1)	1.03q	1.23q	1.67m	1.99hi
Corn starch	T32 (0.2)	1.56n	1.64m	1.99hi	2.1de
	T33 (0.3)	1.6m	1.89jkl	2.15de	2.46a
	T34 (0.4)	1.62vw	1.9ijk	2.2d	2.54a
	T35 (0.5)	1.67m	1.99hi	2.4a	2.72a
Control	T36	0.99q	1.32fgh	1.56bcd	1.67zw

*All values are results of three replication. *Mean sharing the same letter do not differ significantly. *LSD at 0.05 Alpha for treatment, interval and interaction is 0.06224, 0.02075 and 0.1245, respectively.

period. The reason for decrease in the pH was increase in acidity during storage. The results obtained are similar to the findings of Radke and Sandine (1986); Moon *et al.* (1993) who reported decrease in the pH valve of the yoghurt during storage.

Total solid: The data regarding the total solid of all treatments during storage are shown in the Table 3 .The results showed that total solids increased throughout the storage interval. The maximum total solids were found with T10 followed by T35 and T5. The lowest value for acidity was found with T23. The total solid at 0 day interval was the highest in all treatment and it gradually decreased throughout the storage interval. The statistical analysis showed that effect of different amount of food additives and effect of storage period was highly significantly differ for total solid of yoghurt while interaction is non significant. The average increase in the total solids was lower in the case of control. The initial means values for total solid in case of CMC treated samples ranged from 13.12 to 14.90%. These values increased from 13.30 to 14.95% in the final observation. In the guar gum the initial mean values were ranged from 13.11 to 14.90%, while the final observation indicated an increase in total solid from 13.14 to 14.98%. In the case of combination treatment of gelatin and corn starch, the initial mean value ranged between 13.12 to 14.71 where as the final values ranged between 13.18 to 14.78%.

The comparison of four stabilizers and their combination shows that final total solid of yoghurt manufactured with guar gum T10 was high than the other, which was related to better quality. The results re in agreement with the finding of Salji *et al.*(1985) who reported a gradual increase in the total solids o the yoghurt during storage.

Free fatty acid: The effect of different stabilizers and their combination at storage intervals on the free fatty acids of the yoghurt is presented in Table 4. The results showed that free fatty acid increased throughout the storage interval. The maximum free fatty acid was found with T20 followed by T19 and T18. The lowest value for acidity

Table 5: Effect of different additives during storage periods (6°C) on Acetaldehyde of yoghurt

Stabilizers	Treatments	0 days	7 days	14 days	21 days
CMC	T1 (0.1)	10.25r	12.5edf	13d	12.07b
	T2 (0.2)	10.32q	12.36efgh	13d	12.28a
	T3 (0.3)	10.5q	10.9wxyz	11.5opqrs	11.60ef
	T4 (0.4)	10.25r	10.9wxyz	11.25q-u	11.10lmn
	T5 (0.5)	11 u-y	11.8mn	12.33hijk	12.13b
Guar Gum	T6 (0.1)	10.5q	11u-y	11.25q-u	11.19kl
	T7 (0.2)	9.25s	10.2r	10.9wxyz	10.32r
	T8 (0.3)	10.s	10.48r	10.75yz	10.65q
	T9 (0.4)	10.25s	10.9wxyz	11.45opqr	11.04mno
	T10 (0.5)	10.5q	10.9wxyz	11.5opq	11.13lm
Gelatin	T11 (0.1)	10.2r	10.99u-y	11.58nop	11.10lmn
	T12 (0.2)	10r	10.5q	11.35prs1	10.81p
	T13 (0.3)	10.25r	10.85xyz	1.36o-s	10.97o
	T14 (0.4)	10.2q	10.8xyz	11.56o-s	11.09lmno
	T15 (0.5)	10.25q	11u-y	11.35pqrs	11.091mno
Cornstarch	T16 (0.1)	8.76t	9.2t	10.2r	9.74s
	T17 (0.2)	10.5q	1093 wxyz	11.56nop	11.32ij
	T18 (0.3)	10.76yz	11.04 t-x	11.87 im	11.51fgh
	T 19 (0.4)	10.76yz	11.35 pqrst	12.08 ijkl	11.67de
	T20 (0.5)	10.63q	12.04jklm	12.89d	12.34s
Gelatin/ Corn starch	T21 (0.1)	9.76s	10.68z	11.45opqr	10.99no
	T22 (0.2)	10.33r	10.06 t-x	11.79 mn	11.30jk
	T23 (0.3)	10.34r	11.13 s-w	11.8 mn	11.41hij
	T24 (0.4)	10.36r	11.2 r-v	11.99 klm	11.53fg
	T25 (0.5)	10.4r	11.01 t-y	11.78 mn	11.40hij
Gelatin/CMC	T27 (0.1)	10.01s	10.67 z	11.45 opqr	11.03mno
	T28 (0.2)	10.32r	10.87 wxyz	11.56 nop	11.19k
	T29 (0.3)	10.39r	11.u-y	11.78 mn	11.43ghi
	T30 (0.4)	10.41r	11.35 pqrs	12.24 hijk	11.75cdl
	T31 (0.5)	10.42r	11.37 opqrst	12.28 ghij	11.79c
CMC/ Corn starch	T31 (0.1)	10.33r	10.96 wxyz	11.39 o-s	11.17l
	T32 (0.2)	10.34r	11.01 t-y	11.89 lm	11.42ghi
	T33 (0.3)	10.44r	11.21 r-v	12.35 efgh	11.82c
	T34 (0.4)	10.11s	10.77 yz	11.26 qust	10.98ij
	T35 (0.5)	10.45r	10.99 no	11.56 fg	11.34ij
Control	T36	8.22t	9.01t	9.45s	9.17t

*All values are results of three replications. *Mean sharing the same letter do not differ significantly. *LSD at 0.05 alpha for treatment, interval and interaction is 0.1078, 0.3593 and 0.2156, respectively.

was found with T1. The free fatty acid 0 day interval was the highest in all treatment and it gradually decreased throughout the storage interval. The statistical analysis showed that effect of different amount of food additives and effect of storage period was highly significantly differ for total solid of yoghurt while interaction is non significant. Results shows that free fatty acid increased gradually in all treatments, results support he finding the Beshkova *et al*. (1988) who reported that free fatty acid contents depend on the fat level further more microbial activity play important roles. Georgala *et al*. (1995) found that the chemical composition of the milk and processing conditions.

Acetaldehyde contents: The effect of different stabilizers and their combination at storage intervals on the acetaldehyde contents of the yoghurt is presented in Table 5 The statistical analysis showed that effect of different amount of food additives and effect of storage period was highly significantly differ for total solid of yoghurt while interaction is non significant. The results showed that total solids increased throughout the storage interval. The maximum acetaldehyde contents were found with T20 followed by T2 and T5. The lowest value for acidity was found with T36. The acetaldehyde contents at 0 day interval was the highest in all treatment and it gradually decreased throughout the storage interval. The results showed that acetaldehyde contents gradually increased in all treatments with the increased storage period. The results agree the finding of Wilkins *et al*. (1986) who reported that threonine is converted by the enzymes threonine aldolase to produced acetaldehyde contents. Results also support the finding the Veduamuthu (1991), who concluded that the optimum range for acetaldehyde contents in the yoghurt is 10-15 ppm.

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Performance of Weaner Rabbits Fed Graded Levels of Yam and Sweet Potato Peel Meal in Place of Maize-Based Diet

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Abstract: Performance of weaner rabbits fed graded levels of yam and sweet potato peel meal in place of maize based diet was investigated using 20 weaner rabbits of average weight of 389 ± 55g. They were randomly allotted to 5 dietary treatment groups having 2 replicates with 2 rabbits per replicate in a completely randomized design. Diet I was maize based and served as control diet. The test ingredients replaced maize at 20%, 30 % 40% and 50 % in diets 2, 3, 4 and 5 respectively. The yam and sweet potato peel meals were combined in ratio 3:2.Each diet was offered *ad libitum* for a period of 56 days. The proximate composition and gross energy (G E) of the test ingredients are as follows: DM (89.74, 89.61), crude protein (6.34, 11.1) ether extract (1.3, 1.3), crude fibre (0.36, 7.20) ash (4.58, 10.17), nitrogen free extract (70.39, 77.13), and gross energy (3.21, 2.98). The anti-nutritional factors of the test ingredients are as follows: Tannin (0.60, 0.22 %), saponin (0.90, 0.67%), trypsin inhibitors (0.00, 0.00) and phytate (0.94, 0.74 %). There was no significant difference ($P>0.05$) for all the growth parameters considered except for feed intake. The values significantly ($P<0.05$) increased as the quantity of the test ingredients increased. The feed conversion ratio values (7.9900,7.2900, 7.4150, 6.5600 and 7.6550) numerically favoured diet 4. Carcass characteristics values showed significant difference for percentage dressed weight and drumstick only. The percentage dressed weight for all the treatment groups fell within the normal range of dressing percentage of rabbits. The drumstick values (4.7400, 5.0000, 7.4100, 7.6900 and 7.1400) favoured diet 4 among others. The weight of organs showed no significant difference among treatment groups except for the heart, values of which did not follow any specific pattern that could be attributed to the effect of the test ingredients. Biochemical values showed no significant difference except for the value of total protein; this and other biochemical parameters (total protein, urea, creatinine and alkaline phosphatase) fall within the normal range of biochemical indices for rabbits. Gross margin values (N780.6000, N1070.7350, N1061.0100, N1260.1430, N1148.0800) favoured diet 4 among others. Judging from growth performance, carcass characteristics, organ weights, biochemical indices and economics of the diet, diet 4 is recommended.

Key words: Performance, weaner rabbits, graded levels, yam and sweet potato peel meal, maize-based diet

Introduction

The third world countries, including Nigeria, are afflicted with malnutrition, especially with regards to inadequate intake of various nutrients, such as protein and calories (Oluyemi and Ologhobo, 1998). Animal protein is obtained from various animals. One of such animals is the rabbit which is richer in protein (20.8%) and lower in fat (10.2%) than other meat species (Baymen, 1984).The rabbit's rapid rate of reproduction, with short gestation period of 28-32 days, has made its production a wise choice for Nigerians as a means of alleviating protein food shortages. The major hindrance to the production of these animals to satisfy the required protein needs of most Nigerians has been attributed to high cost of production of which feed cost is highly significant. Feed accounts for about 70-80% of the total cost of animal production (Akinmutimi , 2001). This has been attributed to escalating prices of conventional feed ingredients especially the energy sources such as maize, sorghum etc. (Akinmutimi, 2006). This has brought about the quest to search for alternative feedstuffs (Ijaiya and Awonusi, 2001).

Yam peels and sweet potato peels have been identified as alternative feedstuffs that can form major sources of energy in livestock and poultry feeds (Adeyemo and Borire, 2002). Sweet potato peel for example contains as much as 3.4 calories per gram (Jansen, 1989). The peels constitute about 10% of the yam or sweet potato tuber (Ijaiya and Awonusi, 2005). However, information on the combined use of these peels meal in rabbit feed, to replace maize based diet is yet to be reported. This forms the objective of the study.

Materials and Methods

Environment of Study: The study was carried out at the Michael Okpara University of Agriculture, Umudike. Umudike bears the co-ordinates of 5° 28' North and 7° 31' East and lies on altitude of 122m above sea level. Its annual rainfall is about 2177m, with relative humidity of about 72% and temperature range of 12-36°C. Umudike is within the tropical rain forest zone. Umudike is a town in Umuahia of Abia State of Nigeria.

Table 1: Ingredient Composition of Experimental Diets

Ingredient	D1 (0%)	D2 (20%)	D3 (30%)	D4 (40%)	D5 (50%)
Maize	50.00	40.00	35.00	30.00	25.00
Yam peel	-	6.00	9.00	12.00	15.00
Sweet potatoes peel	-	4.00	6.00	8.00	10.00
Soyabean meal	16.00	16.00	16.00	16.00	16.00
Palm kernel	20.00	20.00	20.00	20.00	20.00
Fish meal	1.00	1.00	1.00	1.00	1.00
Wheat offal	9.50	9.50	9.50	9.50	9.50
Bone meal	3.00	3.00	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25	0.25
Vitamin premix	0.25	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00	100.00
Crude protein	16.90	16.94	16.96	16.98	17.00
ME (Kcal/kg)	2788.00	2737.20	2711.97	2686.63	2661.28

To provide the following/kg feed: Vitamin A 5,000,000 IU, Vitamin D3 1,000,000 IU, Vitamin E 16.000mg, Vitamin K3 800mg, Vitamin B1 1,200mg Vatamin B2 22,000mg, Niacin 22,000mg Calcium pentothenate 4,600mg Vitamin B6 2,000mg ,Vitamin B12 10mg, Manganese 948,000mg, Folic acid 400mg, Biotin 32mg, Chlorine chloride 200,000mg, Iron 40,000mg, Zinc 32,00mg Copper 3,400mg, Iodine 600mg Cobalt 120mg ,Selenium 48mg, Anti-oxidant 48,000mg.

Table 2: Proximate composition of experimental diets and the test ingredients

Constituents (%)	D1	D2	D3	D4	D5	Sweet potato peel meal	Yam peel meal
Dry matter	89.74	89.62	89.90	89.64	89.81	89.74	89.61
Crude protein	15.84	16.79	17.15	16.97	17.28	6.34	11.10
Ether-extract	3.51	3.57	3.63	3.71	3.73	1.30	1.30
Crude fiber	6.61	6.70	6.83	6.99	7.11	0.36	7.20
Nitrogen free extract	56.66	55.34	54.95	54.55	54.18	70.39	77.13
Ash	7.12	7.22	7.34	7.39	7.51	4.58	10.17
Gross Energy (Kcal/g)	3.03	3.03	3.04	3.05	3.06	3.21	2.98

Table 3: Anti-nutritional factors in yam and sweet potato peel meals

Factors (%)	Yam peel	Sweet potato peel
Tannin	0.6	0.220
Saponin	0.9	0.665
Oxalate	1.26	1.040
Phytate	0.94	0.740
Trypsin inhibitor (TIU)	0.00	0.000

Experimental animals and management: Twenty weaner rabbits of mixed sexes and breeds were used for the experiment. The weaner rabbits were of average weight of 389 ± 55g. The rabbits were randomly allotted to five dietary treatment groups. Each treatment had two replicates with two animals per replicate. Feed and water were given *ad-libitum*. The feeding trial lasted for 56 days.

Processing of the test feedstuff: The peels were collected within 12 hours of disposal from fast food restaurants within the metropolis and were sun dread, milled and kept for feed formulation and analysis.

Experimental diets: There were five treatment diets with diet 1 serving as control diet. While the test ingredients replaced maize at 20, 30, 40 and 50% in diets 2, 3, 4 and 5 respectively. The yam and sweet potato peel meals were combined in the ratio of 3:2.

Chemical and statistical analysis: The test ingredients and diets were analyzed for proximate composition and gross energy according to A.O.A.C (1990). The test ingredients were analyzed for anti-nutritional factor such as tannin, saponin, oxalate, phytate, trypsin inhibitor, using the method of A.O.A.C. (1990). The experiment was carried out in complete randomized design. The data was subjected to analysis of variance (ANOVA) as described by Steel and Torrie (1980), Separation of significantly different means was carried out using Duncan's Multiple Range Test as described by Duncan (1955).

Parameter evaluated

Growth performance: Data were collected on the initial and final weights of the animals, quantity of feed given and refusal, during the experiment.

The values obtained were used to calculate;

Daily weight gain per rabbit/day (g)

$$\frac{\text{Final live weight} - \text{Initial weight}}{\text{Number of rabbits X 56 days}}$$

Feed intake /rabbit /day (g)

$$\frac{\text{Quantity of feed given} - \text{left over}}{\text{Number of rabbits X 56 days}}$$

$$\text{Feed conversion ratio} = \frac{\text{Quantity of feed consumed}}{\text{Weight gain}}$$

Table 4: The mean values for growth performance of weaner rabbits fed experimental diets

Parameters	D1	D2	D3	D4	D5	SEM
Initial weight (g)	275.00	455.000	400.000	430.000	418.750	135.0660
Final weight (g)	781.25	1025.000	1018.750	1190.625	1067.500	182.5225
Weight gain/rabbit/day(g)	9.04	10.175	10.870	13.585	11.940	1.7108
Feed intake/rabbit/day(g)	65.93^{d}	74.220^{c}	80.080^{b}	87.890^{a}	89.125^{a}	1.1766
Feed conversion ratio	7.99	7.290	7.415	6.560	7.660	1.2202

Gross margin/profitability of the diets were determined using Sonaiya *et al.* (1986) method.

Carcass assessment: The carcass quality was examined using Ojewola and Longe (1999) method. This involved the selection of two rabbits from each treatment that were closest to the treatment mean weight of the replicate. The selected rabbits were starved overnight and thereafter bled by severing the jugular vein.

The skin was removed by flaying; the head and feet cut off. Each rabbit was eviscerated, the internal organs collected weighed separately and was expressed as a percentage of dressed weight. The fore arms were removed by cutting interiorly, severing at the humero-scapular joint, the cut being made close to the body line. Lateral cuts were made, to separate the thighs, drumsticks, back cuts, shoulders from each carcass. These were weighed and expressed as percentage of dressed weight.

Biochemical indices/ serum chemistry: Serum chemistry samples were collected into sample bottles without anticoagulants.

Serum protein, urea etc were analyzed as described by Morbert (1979).

Results and Discussion

The proximate composition for the experimental diets and the test ingredients is as shown in Table 2.

The determined values were closely related to the calculated values, and they all fall within the nutrient requirement of rabbits especially the crude protein and the energy values. The determined crude protein values for yam and sweet potato peel is in line with earlier reports of Akinmutimi *et al.* (2006) who reported 11.21% for yam peel, and Ajala (2001) who reported 6.37 for sweet potato peel.

The gross energy values of 2.985 and 3.21kcal/g respectively for sweet potato peels and yam peels reveals their potential as alternative energy sources for maize in rabbit production.

Table 3 reveals the anti-nutritional factors both in yam and sweet potato peel meals. The presence of these anti-nutritional factors confirms the reports of earlier workers, Udoessien and Ifon (1990); Osagie (1998) who reported in similar vein.

Table 4 shows the mean values for growth performance of weaner rabbits fed experimental diets.

There was no significant (P<0.05) difference for all the parameters evaluated with the exception of feed intake per rabbit per day. The feed intake values, increased significantly (P<0.05) as the quantity of the test ingredients increased in the diets. This significant (P<0.05) increase in feed in take could be due to lower metabolizable energy value of the test diets and hence increase in feed intake to meet the energy requirement of the animals (Enwenro and Udedibe 1998; Akinmutimi *et al.*, 2006).

The feed conversion ratio favoured diet 4 numerically, although they are statistically similar. This makes diet 4 a better diet than all, since lower value of feed conversion ratio, shows superiority of the diet (Ogbonna *et al.*, 2002).

There was no significant (P<0.05) difference for all the parameters measured expect for percent dressed weight and drumstick values.

Although there was significant (P<0.05) difference among the diets, they all fall within the normal range of dressing percentage of rabbit (50-57%) as was reported by Aduku and Olukosi (1990). This implies that the treatments had good edible parts (Akinmutimi, 2004).

The drumstick value favoured diet 4 followed by diet 3 and then control diet being the least. This makes diet 4 a more economic diet, drumstick being a prime part (Akinmutimi, 2004). This shows the superiority of Diet 4 to others.

Table 6 shows mean weights of internal organs expressed as percentage dressed weight.

There was no significant (P>0.05) difference for all the parameters considered expect for the heart. The heart did not follow a specific pattern that could be attributed to the effect of the diet. This implies that any of the diets could be selected. It is believed that, if there is any major effect of anti-nutritional factors, organs like liver and kidney should be significantly (P<0.05) affected being the major detoxification organs (Ukachukwu, 2000; Akinmutimi, 2004). This also confirms the earlier statement that the anti-nutritional factors in the test ingredients are within a tolerable level.

Table 7 shows the mean biochemical values of weaner rabbits fed control and experimental diets.

There was no significant (P<0.05) difference for all the parameters considered with the exception of total protein. All the parameters considered fall within the normal range of biochemical indices for rabbits (Van Praag, 2004). This also confirms that the anti-nutritional factors in the test ingredients are still within a safe level as earlier observed.

Table 5: Mean weight of carcass characteristics expressed as percentage dressed weight

Parts	D1	D2	D3	D4	D5	SEM
Drumstick	4.740^{e}	5.00^{d}	7.41^{b}	7.6900^{a}	7.140^{c}	0.9447
Forearm	2.275	1.84	2.09	1.9400	2.550	0.7103
Thigh	18.205	18.75	18.52	26.2800	20.190	4.4310
Shoulder	9.105	9.00	10.91	10.4600	11.125	1.2890
Back cut	33.330	32.50	33.33	34.6200	30.770	1.1180
Breast cut	3.325	4.00	3.70	3.9075	3.850	0.4213
% dressed weight	51.720^{e}	55.56^{b}	56.25^{a}	52.0000^{d}	53.850^{c}	0.0224

Table 6: Mean weight of internal organs expressed as percentage dressed weight

Parts	D1	D2	D3	D4	D5	SEM
Spleen	0.1100	0.0700	0.1300	0.0800	0.4000	0.0000
Lung	1.3600	1.1250	1.2000	1.3700	1.0800	0.1304
Kidney	1.7300	1.5950	1.8400	1.5150	1.7800	0.2012
Heart	0.5800^{b}	0.4000^{e}	0.5100^{c}	0.4450^{d}	0.7200^{a}	0.0000
Liver	4.9850	4.6300	4.9900	5.3050	6.5050	0.8479

Table 7: Mean biochemical values of weaner rabbits fed experimental diets

Parameters	D1	D2	D3	D4	D5	SEM
Total protein	50.0^{b}	56.0^{b}	56.0^{a}	57.0^{a}	54.5^{b}	0.671
Urea	34.5	40.0	40.0	36.0	40.0	3.585
Creatinine	0.5	0.5	0.5	0.5	0.6	0.045
Alkaline phosphatase	58.0	70.0	64.5	65.0	59.0	4.249

Table 8: Mean values of the economics of experimental diets

Parameters	D1	D2	D3	D4	D5	SEM
Cost /kg feed /N	42.9750^{a}	38.3300^{b}	36.0100^{c}	34.2500^{d}	31.3800^{e}	0.0000
Cost of feed consumed (N)	158.6470^{ab}	159.2635^{ab}	161.4900^{ab}	168.5725^{a}	156.9850^{b}	2.6317
Revenue (N)	937.5000	1230.0000	1222.5000	1428.7500	1305.0000	219.0270
Gross margin (N)	780.6000	1070.7350	1061.0100	1260.1430	1148.0800	218.9810

The economics of the diets is as shown in Table 8. There were significant (P<0.05) differences for cost /kg of feed and cost of feed consumed. Cost per kg of feed favoured diet 5. This may be due to the prices of the test ingredients. The cost of feed consumed also favoured Diet 5. This may be due to effect of the cost of kg of feed. Although the gross margin values did not differ significantly (P<0.05), numerically it favoured Diet 4 (40% inclusion of yam and sweet potato peels). This may be the product of moderate good weight gain and good revenue (Akinmutimi, 2004)

Conclusion: Judging from growth performance, carcass characteristics, organ weights, biochemical values and economics of the diet, diet 4 (30 % replacement of maize) is recommended

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Response of Weaner Rabbits Fed Graded Levels of Sweet Potato Meal in Place of Maize-Based Diet

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Abstract: Performance of weaner rabbits fed graded levels of sweet potato peel meal in place of maize-based diet was investigated. 24 weaner rabbits aged between 5-7 weeks and of equal live weight were randomly allocated to 4 dietary treatments. Each treatment having 3 replicates of 2 rabbits per replicate in a completely randomized design (CRD). Diet 1 was maize based, which served as control and the test feedstuff (sweet potato peels) quantitatively replaced 5 (11.11%) 10 (22.22%) and 15 (33.33%) of maize meal in diets 2, 3 and 4 respectively. Each diet was offered ad libitum for a period of 56 days. The proximate composition of the test ingredient is as follows: Dry matter - 89.74%, Crude protein 6.34%, Ether extract 1.37% C.F 0.36%, Ash 4.58%, NFE 87.42 and Gross energy 3.214. The anti nutritional factors of the test ingredient are as follows: Saponin 0.67%, Oxalate 1.08%, Tannin 0.22% phytate 0.74% and Trypsin inhibitor 0.00. There was significant ($P<0.05$) difference for growth parameters considered among the diets. Diet 2 had the highest weight gain of 10.4267 as against 8.8367, 9.5300 and 8.2700 for diets 1, 3 and 4 respectively, and the least feed conversion ratio of 7.4733 as opposed to 8.8100, 9.0467 and 10.9867 for diets 1, 3 and 4 respectively. There were significant ($P<0.05$) differences for the values obtained for cut parts. The result favoured diets 1 and 2 when compared to others. However, diet 2 had the highest values for the prime parts (thigh, drumstick, shoulder, breast cut and back cut). The values of the organs showed significant ($P<0.05$) difference with the exception of lung and spleen. For haematological and serum chemistry (Hb, PCV, RBC, WBC, lymphocytes, neutrophil, eosine, MCV, MCHC, MCH, creatinine and alkaline phosphates), values obtained fall within the normal range of haematological and biochemical indices for rabbits. For gross margin, diet 2 had the highest value (N227.4600) when compared with the control diet (N147.5800), diet 3 (N178.4500) and diet 4 (N174.1000) making diet 2 a choice diet. Judging from growth performance, carcass characteristics, organ weights, haematological and biochemical values and economics of the diets, diet 2 is recommended.

Key words: Weaner rabbits, sweet potato, peel meal, maize-based diet

Introduction

Rabbits are found in many different continents and climatic zones and it has been estimated by the World's Statistics at 709 million, about 50 and 30 percentage of this figure are found in Africa and Nigeria respectively (Lukefahr, 1990).

Rabbits are unique animals. They serve as a flexible financial reserve for rural population and as well play other socio-cultural roles in the customs and traditions of many Nigerian Societies. The prolific nature of rabbits coupled with its short gestation period and generation interval, makes it the animal of choice for multiplication and a short way of increasing animal protein intake (Akinmutimi *et al*., 2006).

Feed is one of the major problems of intensive rabbit production in Nigeria due to the competition between man, animal and industries for conventional feed materials. This has led to the escalating cost of conventional feed ingredients and has made feed cost to account for about 70% of total cost of production (Akinmutimi, 2004). This problem has been the prime stimulants for the continuous search for alternative feedstuffs that can meet the nutritional requirements of micro-livestock, reduce the cost of feed and animal production (Olorede *et al*., 2002).

Such a feedstuff should be one that has very low human food preference and of low industrial usage (Olorede *et al*., 2002).

The answer may lie in the use of farm or agro by-products such as sweet potato peels.

Sweet potato is a staple food in Nigeria, it ranks fourth in production and importance after, cassava, yam and cocoyam (Ikwelle *et al*., 2003). The production level of sweet potato was 2.5 metric tonnes in the year 2004 (FAO, 2005) which some fractional part constitute the peels. Tewe (1997) projected that the crop residue from sweet potato peels will be 4.72 metric tones, as of the year 2000. The peels are good sources of quality plant carbohydrate. It contains about 6.3% crude protein and metabolizable energy of about 3411kcal/kg (Oyenuga, 1968; Jansen, 1989).

The objective for this study is to determine optimal

Table 1: Experimental diets

Ingredients	T1 0%	T2 11.11%	T3 22.22%	T4 33.33%
Maize	45.00	40.00	35.00	30.00
Sweet potato peels	0.00	5.00	10.00	15.00
Soybean meal	17.00	17.00	17.00	17.00
PKC	20.00	20.00	20.00	20.00
Wheat offal	13.50	13.50	13.50	13.50
Fish meal	1.00	1.00	1.00	1.00
Bone ash	3.00	3.00	3.00	3.00
Salt	0.25	0.25	0.25	0.25
VMP	0.25	0.25	0.25	0.25
Total	100.00	100.00	100.00	100.00
Calculate Analysis				
Crude protein	17.58	17.45	17.31	17.18
ME(Kcal/kg)	2717.75	2716.60	2715.45	2714.30

1 Kg of premix contains vitamins A (5,000,000 I.u), vitamins D3 (1,000,000 i.u), vitamins E (16,000mg), vitamins K3 (800mg), vitamins B1 (1,200mg), vitamins B2 (22,000mg), Niacin (22,000mg), Calcium pentothenate (4,600mg), Vitamins B6 (2,000 mg), vitamin B12 (10mg), Folic acid (400mg), Biotin (32mg), Choline chloride (200,000mg), Manganese (948,000mg), Iron (40,000mg), Cobalt (120mg), Zinc (32,000mg), Copper (3,400mg), Iodine (600mg), Selenium (48mg), Anti-oxidant (48,000mg).

dietary level of inclusion of raw sweet potato peel meal as a substitute for maize in weaner rabbits diet.

Materials and Methods

Environment of study: The study was carried out at Michael Okpara University of Agriculture Umudike Teaching and Research Livestock Farm. The area is located within the tropical rainforest zone. It is characterized by an annual rainfall of sabout 2177mm high relative humidity of above 72% at the rainy season, monthly temperature of about 17 to 36°C with the highest average range of 22-30°C at the month of March. The environment lies at the latitude of 5° 28 North and 7° 31' East, altitude of 122m above sea level.

Housing: The experimental rabbits were housed in hutches located inside the rabbitory building. Each hutch had a feed and water trough for concentrates and water respectively. The building is made of asbestos roofing and long windows this is to facilitate proper ventilation and proper dissipation of heat as fast as possible.

The animals and their management: A total of twenty-four (24) rabbits of mixed breeds, aged between 5-7 weeks and of equal live weight were selected for this trial. The rabbits were housed in individual cages for one week for adaptation. After the adaptation period, the rabbits were randomly divided into four (4) treatment groups. Each treatment group had three (3) replicates with two (2) rabbits per replicate. The rabbits were fed *ad- libitum* for 56 days. Feed intake and weight gain were determined for this period.

Test feedstuff and diets: The sweet potato peels were collected from the surrounding environment. It was sun dried and milled before it was used for chemical analysis and formulation of diets. Four diets were formulated and fed *ad-libitum* to the 24 rabbits. All the diets contained soybean meal, palm kernel cake and fishmeal as major ingredients. Sweet potato peels meal was used to replace maize meal at (0%), 5 (11.11%), 10 (22.22%) and 15% (33.33%) in diets 1, 2, 3 and 4 respectively.

Parameters evaluated

Growth performance: Data were collected on initial and final weights of the animals, feed given, the left over and the number of animals that died during the experiment in each replicate. The values obtained were used to obtain the following parameters

$$\text{Feed Intake} = \frac{\text{Quantity of feed given} - \text{Left over (g)}}{\text{number of rabbits X 56 days}}$$

$$\text{Daily weight gain/rabbit} = \frac{\text{Final weight} - \text{Initial weight}}{\text{number of rabbits X 56 days}}$$

$$\text{Feed conversion ratio} = \frac{\text{Quantity of feed consumed}}{\text{Weight gain}}$$

$$\% \text{ mortality} = \frac{\text{Number died}}{\text{number purchased}} \times \frac{100}{1}$$

Gross margin: This reveals the profitability of the diets. Gross margin was calculated using the method of Sonaiya *et al.* (1986).

Evaluation of carcass quality / organ weight: Evaluation of carcass quality was carried out as described by Akinmutimi *et al.* (2006). This involves the random selection of two rabbits from each treatment group. The selected rabbits were fasted over night to clear the gut before slaughtering. The prominent body parts such as the thigh, drumstick, forearm, shoulder, breast cut and back cut were separately weighed. The internal organs such as the lungs, heart, spleen, kidney and the liver were also weighed and both expressed as percent of the dressed weight.

$$\frac{\text{Organ/cut-part weight}}{\text{Dressed weight}} \times \frac{100}{1}$$

Haematology and biochemical indices: Two rabbits per treatment were selected, starved overnight and slaughtered by severing the jugular vein for blood collection. Blood samples were collected separately from each animal .into bottles containing EDTA for haematological parameters (Hb, PCV, WBC and RBC) and universal bottles without anticoagulant for biochemical indices. These samples were analyzed for haematological parameters according to Dacie and Lewis (1991) and for biochemical studies according to Morbert (1979).

Table 2: Proximate Composition of the Diets And Test Ingredient

	T1 (0%)	T2 (11.11%)	T3 (22.22%)	T4 (33.33%)	Test ingredient
Dry matter	89.630	89.57	89.420	89.620	89.740
Crude protein	16.790	17.06	17.190	17.190	6.340
Ether extract	3.510	3.68	3.760	3.800	1.300
Crude fiber	5.550	5.68	5.750	5.860	0.360
Ash	6.610	6.81	6.950	6.990	4.580
Nitrogen free extract	57.170	56.34	55.770	55.780	77.160
Gross energy	3.049	3.056	3.076	3.081	3.214

Table 3: Percentage anti-nutritional factors in sweet potato peel meal

Anti-nutritional factors	Percentage
Saponin	0.67
Oxalate	1.08
Tanin	0.22
Phytate	0.74
Trypsin inhibitor	0.00

Chemical and statistical analysis: The experiment was carried out in a completely randomized design (CRD). The data was subjected to analysis of variance (ANOVA) as described by Steel and Torrie (1980), separation of significantly different means was carried out using Duncan's Multiple Range Test as described by Duncan (1955).

The four treatment diets were analyzed for proximate composition and gross energy according to (AOAC, 1990). The test ingredient (sweet potato peels) were also analyzed to determine the levels of anti-nutritional factors such as oxalate, tannin, saponin and trypsin inhibitor at the Institute of Agricultural Research and Training (IAR & T) Ibadan according to AOAC (1990).

Results and Discussion

Table 2 shows the proximate composition of experimental diets and the test ingredient. The value of crude protein determined for the diets fall within the range of the nutrient requirements for weaner rabbits (Akinmutimi, 2006). They were closely related to the calculated ones. The crude protein value (6.34%) obtained for the test ingredient is in line with the earlier reporter, Ajala (2001). The energy value of 3.21kcal/g makes it a potential substitute for maize.

Table 3 shows the anti-nutritional factors present in sweet potato peel meal. This confirms the report of earlier researcher who reported presence of these anti-nutritional factors in sweet potato peel meal (Eka, 1977; Osagie, 1998).

The growth performance of weaner rabbits fed sweet potato peel meal in place of maize- based diet is as represented in Table 4.

There was significant ($P<0.05$) difference for all the parameters considered.

The final live weight observed shows that diet 2 was significantly ($P<0.05$) higher than diets 1, 3 and 4. Although diets 1, 3 and 4 were statistically similar but differ numerically with diet 1 having the least value. This implies that even at 15% dietary level of inclusion the effect of anti-nutritional factors is within a tolerable level (Ologbobo *et al.*, 1993; Akinmutimi, 2004).

There was progressive increase in the value of feed intake as the quantity of the test ingredient increased in the diet. It became significant ($p<0.05$) from the control diet at 15% dietary level of inclusion (diet 4). This may be due to slight lower energy value of the diets containing the test ingredient and hence increase in feed intake to meet their energy requirement (Akinmutimi, 2004; Akinmutimi *et al.*, 2006).

For the weight gain, the control diet was statistically similar to the test diets. Diet 2 differed significantly from diet 4. The downward trend observed from diet 2 to diet 4 could be attributed to the effect of anti-nutritional factors becoming more pronounced as the quantity of the test ingredient increased.

Saponin an anti-nutritional factor in the test ingredient (Table 3) for example has been reported to cause decrease in daily weight gain through binding to the cell of the small intestine thereby affecting the absorption of nutrients across the intestinal wall (Olumu, 1995; Akinmutimi, 2004).

The feed conversion ratio values observed shows that the control diet is not significantly ($P<0.05$) different from the test diets but the slightly higher numerical values obtained for diets 3 and 4 could be attributed to poor nutrient utilization as a result of increased effect of anti-nutrients as the quantity of the test ingredient increased in the diet.

The 0% mortality confirms that the effect of anti-nutrient is within a tolerable level. The growth performance result showed that diet 2 is the choice diet judging from good feed intake, better weight gain and having the least value for feed conversion ratio when compared with other diets.

Table 5 shows the carcass characteristics of weaner rabbits fed sweet potato peel meal in place of maize-based diet.

There were significant ($P<0.05$) differences for all the parameters considered. The result favoured diets 1 and 2 when compared to others. Considering the prime parts such as thigh, drumstick, shoulder, breast-cut and back-cut, both statistically and numerically diet 2 is more favoured.

The result of organ weight of weaner rabbits fed sweet potato peel meal in place of maize-based diet is as

Table 4: The growth performance of weaner rabbits fed sweet potato peel meal in place of for maize-based diet

Parameters	T1	T2	T3	T4	SEM
Initial weight(g)	880.0000	885.0000	832.6667	837.6667	54.0352
Final weight(g)	1175.0000^{b}	1487.5000^{a}	1306.2333^{b}	1325.0000^{b}	45.5364
Weight gain/rabbit/day(g)	8.8367a^{b}	10.4267^{a}	9.5300^{ab}	8.2700^{b}	0.5670
Feed intake/rabbit/day(g)	75.9400$_{b}$	77.9167^{b}	83.0700^{b}	90.8467^{a}	2.3462
Feed conversion ratio(g)	8.8100^{ab}	7.4733^{b}	9.0467^{ab}	10.9867^{a}	0.7650
Percent mortality (%)	0.0000	0.0000	0.0000	0.0000	0.0000

Table 5: Carcass Characteristics Of Weaner Rabbits Fed Sweet Potato Peel Meal in place of Maize- Based Diet

Parameters	T1	T2	T3	T4	SEM
Thigh	29.9600^{ab}	33.7000^{a}	29.9933^{ab}	29.5600^{b}	1.1410
Drumstick	7.2767^{ab}	8.3300^{a}	6.8400^{ab}	4.8933^{b}	0.7966
Forarm	3.8000^{a}	3.4800^{a}	2.2533^{b}	0.9033^{c}	0.3595
Shoulder	29.9600^{a}	29.6200^{a}	19.9333^{b}	20.4300^{b}	0.6562
Breast cut	6.7867^{a}	6.7533^{a}	4.8800^{b}	4.2200^{c}	0.1913
Back cut	18.4667^{b}	26.3833^{a}	23.3133^{ab}	18.6900^{b}	1.7776
Life weight	1112.5000^{ab}	1375.000^{a}	1025.0000^{b}	1362.5000^{a}	97.2271
Dressing percentage	60.7400^{a}	57.4700^{ab}	55.0400^{b}	54.8000^{b}	1.2629

Table 6: Organ weight of weaner rabbits fed sweet potato peel meal in place of maize-based diet

Organs	T1	T2	T3	T4	SEM
Lung	1.0500	1.2200	1.3100	1.35	17.3257
Liver	4.5133^{c}	4.5100^{c}	4.9700^{b}	5.2600^{a}	0.0244
Kidney	1.4067^{c}	1.4933^{b}	1.2933^{d}	1.5800^{a}	0.0173
Heart	0.5100^{b}	0.4000^{c}	0.6300^{a}	0.3837^{c}	0.0000
Spleen	0.3433	0.2633	0.2433	0.2733	0.0447

Table 7: The heamatological values and some biochemical indices for weaner rabbits fed sweet potato peel meal in place of maize-based diet

Parameters	T1	T2	T3	T4	SEM
Hb (g/dl)	10.5000^{NS}	10.5500^{NS}	9.2000^{NS}	8.5500^{NS}	0.5912
PCV (%)	33.0000^{b}	38.0000^{a}	27.5000^{c}	25.5000^{d}	0.2039
RBC (mm^2)	5.4867^{a}	4.7000^{ab}	4.4000^{b}	4.1000^{b}	0.3026
WBC	4.1500^{b}	5.0500^{b}	9.2500^{a}	11.4000^{a}	1.0258
Neut (%)	66.0000^{a}	57.5000^{ab}	62.0000^{ab}	44.1667^{c}	2.4971
Lymph (%)	23.0000^{bc}	38.0000^{b}	32.0000^{ab}	48.5000^{a}	2.5860
Eosine (%)	11.0000^{a}	4.5000^{b}	6.0000^{b}	4.0000^{b}	1.0508
MCV(µm3)	58.7100^{b}	72.2200^{ab}	66.6600^{ab}	109.8200^{a}	13.5954
MCH (g/dl)	19.5233^{b}	23.8800^{ab}	20.9600^{b}	36.7833^{a}	4.5866
MCHC (%)	33.3133^{ab}	32.9800^{b}	33.4533^{a}	33.5300^{a}	0.1195
Creatinine (mg/dl)	0.5000^{b}	0.5000^{b}	0.6000^{b}	1.2500^{a}	0.1612
Alkaline phosphorus	63.0000^{c}	58.0000^{d}	66.0000^{b}	88.0000^{a}	0.1048

Table 8: The economics of the diets

	T1	T2	T3	T4	SEM
Cost/kg of feed	32.14720	32.98800	31.8288	30.6696	0.0000
Cost of feed consumed	144.92000	143.79000	147.8000	155.9000	4.4524
Revenue	292.5000^{b}	371.2500^{a}	326.2500^{b}	330.0000^{b}	11.5131
Gross margin	147.5800^{b}	227.4600^{a}	178.4500^{b}	174.1000^{b}	14.8751

revealed in Table 6. There were significant ($p<0.05$) differences for all parameters considered except for lung and spleen.

The values of kidney and heart did not follow any specific pattern that could be attributed to the effect of test ingredient. The value of the liver became significantly ($P<0.05$) different for diets 3 and 4 when compared with control diet. This could be attributed to the effect of anti-nutritional factors present in the test diet. Liver being a major detoxification organ and hence increasing in weight as a result of increased activity to detoxify the anti-nutritional factors (Akinmutimi, 2004; Akinmutimi *et al.*, 2006).

The above result strengthened the choice of diet 2.

The haematological values and some biochemical indices for weaner rabbits fed sweet potato peel meal in place of maize-based diet is as presented in Table 7, there were significant difference ($P<0.05$) in all the parameter considered, although, they all fell within the normal range of heamatological values for rabbits as

reported by Van Praag (2004). This implies that the anti-nutritional factors present in test diets is still within a tolerable level.

The creatinine value shows that diet 1, 2 and 3 are statistically similar to one another but differ significantly from diet 4. Despite this, they all fall within the normal range of biochemical indices as established by Vaan Praag (2004). This still implies a tolerable level on the anti-nutritional factors present in the test ingredient and hence the animals are not living on their body reserve (Akinmutimi, 2004; Adeyemo *et al*., 2000).

The alkaline phosphatase values for all the diet did not follow a specific pattern that can be attributed to the effect of the test ingredient, although they all fall within the normal range as established by Vann Praag (2004). This confirms a tolerable effect of the anti-nutritional factors present in the test ingredient.

The economics of the diet is as shown in Table 8.The cost per kilogram of feed and cost of feed consumed did not differ from one another for the diets. The values for revenue and gross margin showed significant ($P<0.05$) difference for the diets. Diet 2 had values that were significantly ($p<0.05$) higher than other diets making it a most profitable diet among the diet and hence a choice diet.

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Glycemic Indices and Glycemic Load of Some Nigerian Foods

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Abstract: The concept of glycemic index (GI) lists food items by virtue of their influence on postprandial glucose. Though the glycemic index of common food items has been determined, the GI of the popularly processed and commonly consumed foods in Nigeria is not known. This study determined the GI of ten processed Nigerian foods and revealed their similarity in the release of glucose on consumption. The food items tested were made from yam tubers, cassava tubers and local cereals. These foods were served to human volunteers in several processed forms which resulted in viscous pastes. The GI results are presented and it is recommended that these processed foods should be discouraged in the regular dietary plan of people with chronic diseases such as coronary heart diseases, diabetes and cancer.

Key words: Glycemic index, postprandial glucose, processed Nigerian foods, diabetes mellitus and pastes

Introduction

The concept of glycemic index (GI) was proposed by Jenkins and colleagues to characterize the rate of carbohydrate absorption after a meal (Jenkins *et al.*, 1981). GI is defined as the area under the glucose response curve after consumption of 50g carbohydrate from a test food divided by the area under the curve after consumption of 50g carbohydrate from a control food, either white bread or glucose. (Wolever *et al.*, 1991).

Over the past three decades, the GI of over 800 foods has been determined worldwide and more foods are being tested on weekly basis. The latest update in 2005 has 1300 entries derived from published and unpublished verified sources (Foster-Powell *et al.*, 2002). However, only limited information is available on African traditional foods. Many factors together, including carbohydrate type, fiber, protein, fat, food form and method of preparation, determine the GI of a particular food (Bjorck *et al.*, 1994, Welch *et al.*, 1987, Wolever *et al.*, 1991). High GI foods elicit, calorie for calorie, higher insulin levels and c-peptide excretion than low GI foods (Haber *et al.*, 1997; Jenkins *et al.*, 1987; Wolever and Bolognesi, 1996). The reductions in dietary GI may also lower the risks for various conditions associated with hyperinsulinemia, such as diabetes mellitus (Salmeron *et al.*, 1997) and cardiovascular disease.

There is need for more research into the GI of our locally consumed foods in order to produce data that can effectively enable use of GI along with other dietary recommendations in the treatment, management and prevention of diseases. There are many proven benefits of using the GI in nutrition. These include: (i) decreased risk of cardiovascular disease; (ii) better diabetes management and (iii) more successful body weight management. Inspired in part by a hope to learn to predict better, the GI of variants of foods of known GI value, several groups have studied associations between GI and defined components in groups of foods (Jenkins *et al.*, 1981; Wolever, 1990; Hollenbeck and Coulston, 1991; Nishimune *et al.*, 1991). Apparently, GI values reflect, mainly, how promptly and rapidly glucose enters the blood after food ingestion. In Nigeria, the adult population eats foods made from yam tubers (*Dioscorea* spp.), plantain (*Musa* spp.), cassava (*Manihot* spp.) and locally grown cereals. The dry powdered forms of these plant storage organs are reconstituted in hot water to form solid pastes which are swallowed with soup. The effects of processing these food items into diet pastes on the GI have not been determined.

Materials and Methods

Experimental design: Fifty healthy human beings were offered in a single meal, one of the ten food samples. Blood samples were collected before feeding and during the 180 min after the meal. Blood glucose was determined. The integrated areas under the postprandial glucose response curves were calculated.

Subjects: Fifty subjects aged between 16 and 40 years (23 male and 27 female) were selected from students and staff of the University of Benin, Benin City, Nigeria. They were clinically normal, non-smokers and non-diabetic. The subjects were appraised verbally and they gave their informed consent.

Preparation of experimental diets: The dry powdered food samples were purchased from Edaiken Market in Benin City, Nigeria. The food samples were powdered maize, rice, millet, wheat, sorghum, yam and cassava. These were each sieved to pass through a 100-mesh filter and then reconstituted into solid pastes in hot water by a trained cook to ensure consistency (Table 1). The pastes obtained were as follows:

Table 1: Processing and Preparation of the Diet Pastes (Okoh, 1998)

Agricultural Form	Pre-Processing	Paste Preparation
1. Cassava tuber (*Manihot utilisima*): Eba/Garri Cassava Starch	Tuber was homogenized in water. The starch was allowed to settle, filtered out and dried at 28°C.	Dry powdered starch was reconstituted in hot water with addition of small quantity of palm oil.
2. Cassava tuber (*Manihot utilisima*):	Tuber was grated and dried then fried in shallow heated pots.	Dry powder was added to boiling water to form a paste.
3. Yam tuber (*Dioscorea rotundata*): *Amala*	Fresh tuber was sliced into thin pieces. and sun-dried for 7 days. Dried slices were milled to powder	Dry powder was added to boiling water and stirred until a solid paste was . obtained
4. Maize (*Zea mays*): *Agidi*	The dry grains were soaked in water and fermented for about 3 days. The fermented grains were milled and sieved to remove pericarp and bran fractions. The starch fraction was dried slowly.	Dry powder was added to boiling water and stirred until a semi-solid paste resulted. Paste hardened further on cooling.
5. Maize seeds (*Zea mays*): *Tuwo Masara*	Clean, dry grains were moistened with water and milled. The hulls were removed by aspiration while the endosperm and germs were removed by passing through a sieve leaving the maize grits.	Dry powder was added to boiling water and stirred until a solid paste resulted.
6. Millet grains (*Pennisetum typhoides*): *Tuwo Gero*	The grain was pounded in a wooden mortar. The bran was winnowed off. The separated grain was then pounded into flour.	Dry flour was added to boiling water and stirred until a solid paste resulted
7. Sorghum seeds (*Sorghum bicolor* L. Moench): *Tuwo Dawa*	The moist grain was pounded with a wooden pestle in a mortar until most of the pericarp was removed. The bran fraction was removed by winnowing. The dehulled grain was again pounded to make flour.	Dry flour was added to boiling water and stirred until a solid paste was obtained.
8. Rice (*Oryza sativa*): *Tuwo Shinkafa*	Polished rice was pounded and fltered through a sieve.	Dry flour was added to boiling water and stirred until a solid paste was obtained.
9. Wheat (*Triticum aestivum*): *Semovita*	Wheat grains were cleaned, conditioned and milled into flour.	Flour was added to boiling water and stirred until thick and consistent paste cooked for additional 1-2 minutes.
10. Wheat (*Triticum aestivum*): *Semolina*	Wheat grains were cleaned, conditioned and milled into flour.	Flour was added to boiling water and stirred until thick and consistent paste was obtained. Paste was cooked for additional 1-2 minutes.

1. Cassava: Starch
2. Cassava: *Eba Garri*
3. Yam: *Amala*
4. Maize: *Agidi*
5. Maize: *Tuwo Masara*
6. Millet: *Tuwo Gero*
7. Sorghum: *Tuwo Dawa*
8. Rice: *Tuwo Shinkafa*
9. Wheat: Semovita
10. Wheat: Semolina

Preliminary trials were carried out using local foods prepared in a similar manner from plantain (*elubo*), yam (pounded yam), cassava (*lafun*) and fermented cassava (*akpu*). The processed pastes were analyzed for proximate composition of moisture, ash, crude fat, crude fibre and protein (AOAC, 1983). Carbohydrate was determined by difference. 50g of available carbohydrate for each test food sample was calculated from the results of the proximate analysis and the measured portion of the food was served to the subjects.

Determination of blood glucose: All subjects for the investigation fasted overnight. Their blood samples were collected through finger prick using a hypodermic needle or lancets. Each blood sample was placed on a test strip which was inserted into a calibrated glucometer. (Accu-Check/One touch) which gave direct readings after 45 seconds based on glucose oxidase assay method. The determination of glucose level was done at intervals i.e. 0 (fasting level), 30mins, 60mins, 120mins and 180mins.

Glycemic index calculation and statistics: Changes in blood glucose concentration were calculated separately for each post meal period by using the blood concentration before meal (time 0) as a baseline. Postprandial responses were compared for maximum increase and incremental area under the glucose curves for each food. The integrated area under the postprandial glucose curve was calculated by the trapezoidal method (Wolever *et al.*, 1987). Area increments under the curves for a given food were determined for the 3 hour period after the meal. The

Table 2: Proximate Analysis of Ten Processed Nigerian Foods (in Dry Weight Percent)

Food Components	Moisture (g% Fresh Weight)	Ash (g%)	Crude Protein (g%)	Crude Lipid (g%)	Crude Fibre (g%)	Carbohy-drate (g%)
Starch (cassava)	75.10±0.10	1.06±0.06	2.46±0.62	1.55±0.35	2.25±1.75	92.68±0.05
Eba (cassava)	72.00±1.0	1.10±0.07	4.50±0.35	0.58±0.4	2.15±0.12	86.45±0.27
Amala (yam)	65.50±0.50	1.65±0.05	4.47±0.19	0.25±0.05	0.75±0.25	92.88±0.13
Agidi (maize)	84.80±0.24	2.90±0.10	6.82±0.27	0.35±0.05	1.75±1.2	88.18±0.12
Tuwo Masara (Maize)	74.50±0.5	1.63±0.01	9.94±1.91	0.65±0.25	1.7±0.25	86.03±0.03
Tuwo Gero (Millet)	65.00±0.05	1.58±0.01	9.51±0.23	0.45±0.05	1.03±0.03	87.43±0.06
Tuwo Dawa (Sorghum)	60.30±0.25	1.58±0.01	9.28±0.43	0.40±0.05	1.25±0.25	87.49±0.02
Tuwo Shinkafa (Rice)	73.50±1.50	1.65±0.03	9.23±0.43	0.90±0.20	1.0±0.50	87.22±0.12
Semovita (wheat) + 10% corn supplement	44.70±0.10	2.10±0.60	10.63±0.46	1.00±0.30	1.25±0.25	85.02±0.18
Semolina (wheat)	54.90±0.10	2.48±0.08	10.73±0.41	1.50±0.05	1.26±0.38	84.03±0.14

Values are expressed as mean±SEM (n = 3 determinations).

Table 3: Available Carbohydrate in Processed Foods (Serving Size)

Samples	Serving Size (Processed) (g)
Starch (Cassava)	162.15
Eba (Cassava)	206.50
Amala (Plantain)	156.25
Agidi (Maize)	373.13
Tuwo Masara (Maize)	158.00
Tuwo Gero (Millet)	163.40
Tuwo Dawa (Sorghum)	144.00
Tuwo Shinkafa (Rice)	160.90
Semovita (Wheat) + 10% Corn supplement	106.70
Semolina (Wheat)	131.90

relative glycemic index of each food was calculated as percent of the mean of individual areas under the glucose response curves. (Wolever *et al*., 1987) The increase in glucose response area was analysed statistically using one way ANOVA and Scheffe's test (Allison *et al*., 1995).

Results

The results of the proximate analysis of the test food samples are shown in Table 2. The proximate analysis on the processed food from wheat, sorghum, rice and maize showed low lipid contents compared to the analysis of the unprocessed seeds (Ekpenyong, 1973; Okoh, 1998). The cereal flours had higher crude protein content than the tuber flours. From previous studies yam and cassava tubers were naturally low in fat (Osagie and Opute, 1981; Bradbury and Holloway, 1988). Thus, all the processed powders used in making the experimental pastes can be regarded as having low fat content. The two test samples made from cassava tuber (starch) and (*eba*) differed significantly in crude protein content. Semolina and semovita are wheat products and their proximate composition was similar.

The serving size for each meal was calculated from the carbohydrate content (Table 3). The glucose concentration attained after consumption of the test foods and glucose (reference food) are graphically displayed in Fig. 1 - 10. The Glycemic Index and

Table 4: Glycemic Index and Glycemic Load of the Processed Food

Food Samples	Glycemic Index	Glycemic Load
Starch (Cassava)	98.60±2.68	49.30±3.5
Eba (Cassava)	82.25±0.05	41.13±3.3
Amala (Yam)	84.35±2.68	42.18±4.2
Agidi (Maize)	92.30±0.05	46.15±3.1
Tuwo Masara (Maize)	86.80±0.5	43.40±1.5
Tuwo Gero (Millet)	93.60±2.25	46.80±3.4
Tuwo Dawa (Sorghum)	85.30±1.05	42.65±3.2
Tuwo Shinkafa (Rice)	95.30±1.25	47.65±2.2
Semovita (Wheat) + 10% Corn supplement	95.80±0.28	47.90±2.5
Semolina (Wheat)	95.28 ± 0.04	47.64±1.5

Values are mean ± SEM (n = 3 determinations)

Glycemic Load of the food samples were calculated (Table 4). All the test samples are high Glycemic Index foods. Cassava starch gave the highest GI value followed by semovita. In two hours, these foods deliver as much glucose as the free sugar (control) to the blood system. In the absence of adequate insulin delivery, these foods would certainly overwhelm the sugar metabolic system. They are thus not considered suitable or adequate meals for type II diabetics.

Discussion

Before plant foods are consumed by man, they are generally processed. The processing methods include cooking, (i.e. boiling, roasting, frying, steaming, baking, autoclaving), drying, mashing, grinding into flour and fermentation. In this study, the test foods were basically dried, ground into flour, sieved and then reconstituted to paste with hot water. Thus the particle sizes were reduced, fine and the starch was retrograded (gelatinized) to a variable extent. These treatments might have led to their having high glycemic indices (Ludwig, 2003; Bjorck and Elmstahl, 2005). This is similar to reports that increased processing and starch retrogradation can affect GI (Foster-Powell *et al*., 2005). Processing the seeds removes the fiber-rich outer bran and the vitamin and mineral rich inner germ leaving

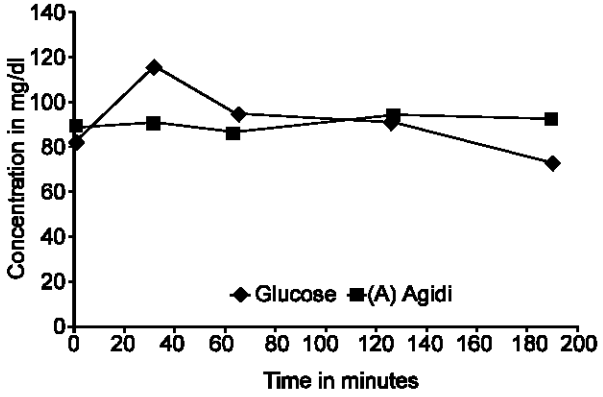

Fig. 1: Graphical representation showing the glucose response area of test food A (*Agidi*) and reference food (Glucose D).

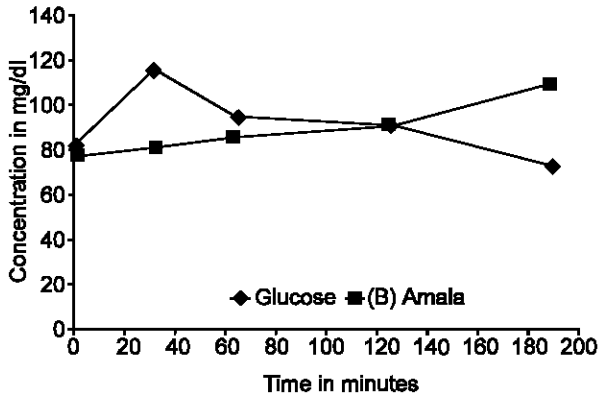

Fig. 2: Graphical representation showing the glucose response area of test food B (*Amala*) and reference food (Glucose D).

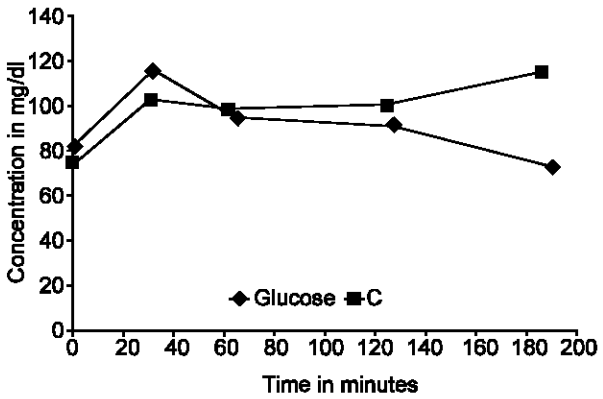

Fig. 3: Graphical representation showing the glucose response area of test food C (Starch) and reference food (Glucose D).

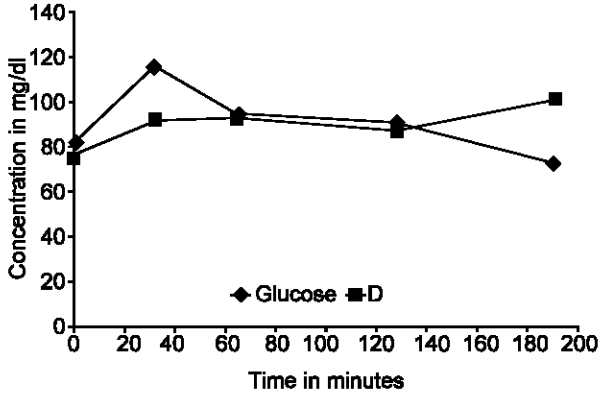

Fig. 4: Graphical representation showing the glucose response area of test food D (semovita) and reference food (Glucose D).

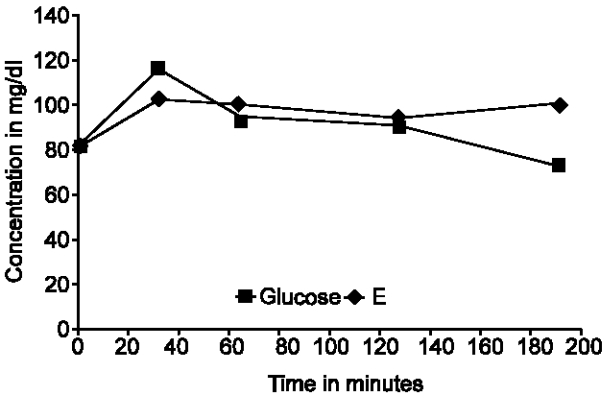

Fig. 5: Graphical representation showing the glucose response area of test food E (Semolina) and reference food (Glucose D).

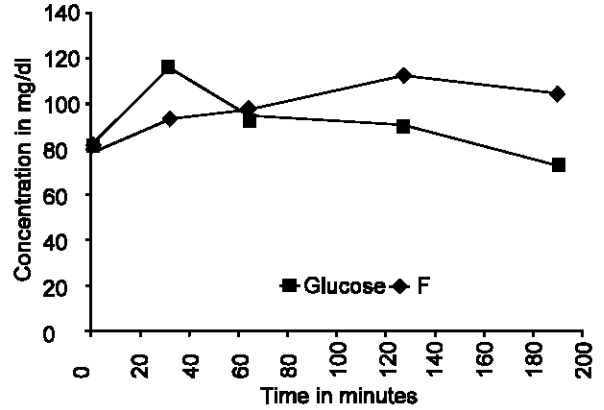

Fig. 6: Graphical representation showing the glucose response area of test food F (*Eba*) and reference food (Glucose D).

endosperm. This treatment caused reduction in particle size and faster gelatinization of starch, thereby increasing the GI. Our study agrees with the finding in Kenya where similarly processed maize flour

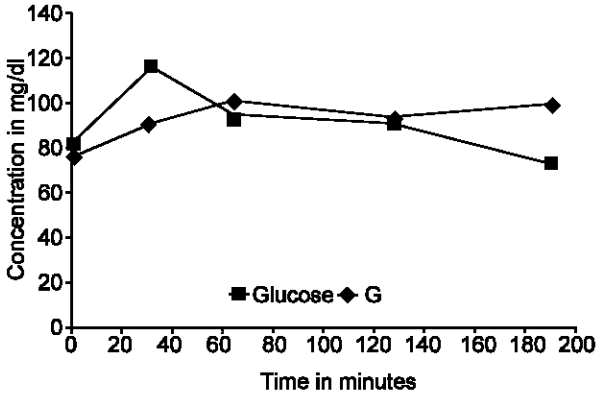

Fig. 7: Graphical representation showing the glucose response area of test food G (*Tuwo* Rice) and reference food (Glucose D).

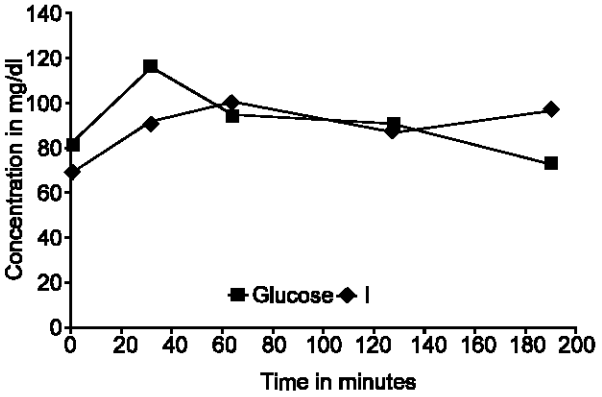

Fig. 9: Graphical representation showing the glucose response area of test food I (*Tuwo* maize) and reference food (Glucose D).

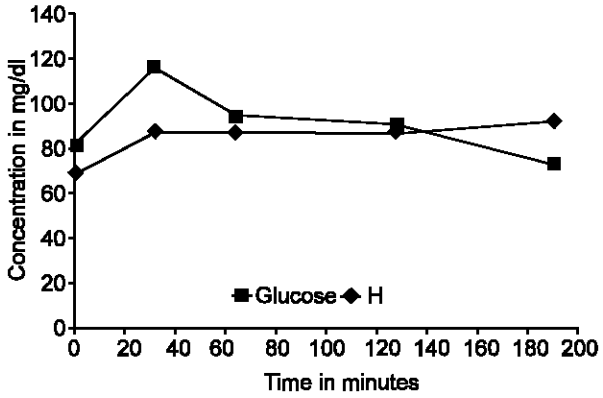

Fig. 8: Graphical representation showing the glucose response area of test food H (*Tuwo* Millet) and reference food (Glucose D).

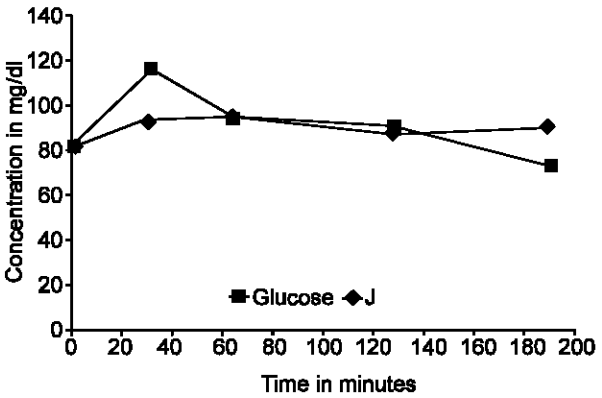

Fig. 10: Graphical representation showing the glucose response area of test food J (*Tuwo* rice) and reference food (Glucose D).

and millet flour made into gruel had high GI (Foster-Powell *et al*., 2002).

The test foods were swallowed without chewing. Chewing normally reduces the particle size of foods and facilitates mixture with salivary amylase, hence reducing digestion time of carbohydrates. Despite the direct swallowing of these test food pastes, they resulted in the same level of blood glucose as the reference sample, within two hours. This is in agreement with the fact that different food products with similar quality and type of carbohydrate form show different glycemic response. (Thorsdottir *et al*., 2005). Since these test foods were reconstituted in hot water, the nature of starch retrogradation or the production of resistant starch may be similar. It is desirable that modern food processing techniques be modified so as to reduce preparation time while at the same time preserving slow digestion properties.

The health implications of the high GI of the processed foods are that they could cause a fast and short - lived rise in blood sugar, with the result that one is lacking in energy and hungry within a short time, thus the desire to eat will arise. If this pattern is repeated, there is the likelihood of gaining weight as a result of constantly eating. The overall effects are that the individual will gain weight i.e. obesity might result. It could trigger diabetes in individuals that are prone to the disease, or worsen the management of the disease (Gilberston *et al*., 2001). Type II diabetes which is associated with insulin insensitivity may also result in elevated blood sugar levels and increased insulin demand; thus overburdening the ability of the pancreas to produce insulin. Reports by workers like Salmeron *et al*. (1997) have indicated a positive correlation between high GI and risk of type II diabetes. Again, the consumption of the processed foods under reference in these studies might have serious health implications in such diseases like the heart diseases via insulin resistant syndrome called metabolic syndrome X (Ludwig, 2003). Additionally, high blood sugar levels have been

associated with increased blood pressure, blood clot formation and reduced endothelial dependent blood flow (Ludwig, 2003).

In recent years, the GI has been transformed by its popularizers from a potentially useful tool in planning diets for diabetic patients to a key player for the prevention of diabetes, dyslipidemia, cardiovascular disease and even certain cancers in the general population. The debate concerns whether such a transformation is justified. That is, whether it is wise and reasonable to set as a public health policy for the entire population the avoidance of certain foods because of their high GI. To explore this question, one needs to examine the supporting data, their quantity and quality, their relation to causation and the possible presence of confounders.

There are 2 theories about how high - GI foods increase food intake. The first is that it is a result of the elevation in glucose and the second, more commonly expressed recently, is that it is the result of a high insulin response. This high insulin response has been related to several phenomena including increased food intake leading to obesity (Roberts, 2000), hyperinsulinaemia leading to insulin resistance (Frost *et al*., 1998), cell exhaustion leading to type 2 diabetes (Salmeron *et al*., 1997), dyslipidemia leading to coronary heart disease (CHD) (Liu *et al*., 2001) and unknown factors leading to certain kinds of cancers.

The foods tested in this study were selected to represent the nutritional variability that adult Nigerians consume. Many of them suffer from chronic diseases such as coronary heart diseases, obesity and diabetes. Direct relationship of these diseases to consumption of high GI foods will require further enlarged and long-term studies. There is also need for more research into the GI of our locally consumed foods in order to produce data that can effectively enable use of GI alongside other dietary recommendations in the management and prevention of diseases. In conclusion, this study could assist food manufacturers and processors to develop a greater range of low-GI processed foods from African farm produce. The findings have obvious importance in formulating rational dietary and therapeutic goals for diabetic patients and others with clinical conditions necessitating carbohydrate restriction.

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Mineral Composition, Quality and Physico-chemical Parameters of the Local Tallow of Pakistan

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Abstract: Tallow samples were obtained form the local slaughterhouses and were evaluated for iodine value, saponification value, anisidine value, acid value, peroxide value, FFA, ash content and cholesterol content. The results were compared with that of Codex standard for the edible animal fats. The acid value of tallow was 1.009-1.99 mg KOH/g. The saponification value (195-197 mg KOH/g) indicate that the tallow contain high molecular weight fatty acids. The iodine values (48.66-49.15) suggest a low degree of unsaturation compared to palm oil. The FFA, AnV and POV were 0.507-1.00 (as oleic acid %by wt.), 6.33- 9.57 and 3-6.5 (meq / kg) respectively. The cholesterol and %ash content were in the range of 1.00-1.10 (mg/g) and 0.183-0.57, respectively. In minerals, the level of Na is 10.037-18.952 ppm, K (2.216-2.821 ppm), Ca (1.123-1.752 ppm), Cu (0.001 ppm), Fe (0.430-0.493 ppm), Pb (1.147-1.153 ppm), Mg (0.412-0.473 ppm), Cd (0.070-0.071 ppm), Zn (0.028-0.055 ppm) and Cr (0.896-0.982 ppm).

Key words: Animal tallow, quality and physico-chemical parameters, minerals

Introduction

Tallow plays an important role in a balanced diet and in the manufacture of food products contributing to texture and palatability. It is a valuable source of concentrated energy and essential fatty acids needed for growth and development (Brooke, 1985; Zeb and Ali, 2008). A number of characteristics of tallow are of importance to determine the quality of food and feed. A low percentage of free fatty acids (FFA) in combination with a low peroxide value (POV) can elucidate as a fresh product. The peroxide value is a size for the oxidation of fatty acids. In addition, moisture and insoluble impurities (II) should be as low as possible in animal fats. Typically freshly melted edible animal fats have the following commercial specification; FFA < 0.50%, POV < 4%, moisture < 0.20% and II < 0.02% (typical value < 0.01%, not detectable) (Woodgate and van der Veen, 2004). Tallow is statistically one of the most important animal fats, which is extracted from animal tissues containing fat by a variety of processes called rendering or melting. Tallow is used in a broad range of applications from shortenings, frying fats and margarine to animal and pet feeds and in the manufacture of oleo-chemical products for a very wide variety of applications including food, feed, cosmetics, medicinal and pharmaceutical products (The European Oleochemicals and Allied Products Group, 2003). Traditionally, rendering was a simple heating process that allows melted tallow to be separated from animal tissues so that it could be used for a variety of purposes. Now-a-days, there is a sector for rendering that process only the large deposits of discrete adipose tissues that can be obtained from animals declared fit for human consumption. These renders (known more specifically as fat melters) produce high-quality tallow that is considered to be safe for human consumption, if the adipose tissues has been sourced from animals declared fit for human consumption (Taylor and Woodgate, 2003).

Moreover, Food composition data is important in nutritional planning and provides data for epidemiological studies (Bruce and Bergstrom, 1983). Trace metals composition of foods is of interest because of their essential or toxic nature (Onianwa *et al*., 1999). The presence of small amounts of trace metals in oils and fats is known to produce deleterious effects on quality. The strongest and most notable pro-oxidants are copper and iron, which produce a noticeable oxidative effect at concentrations as low as 0.005 and 0.03 ppm respectively (Marfec and Bulinski, 1997; Persmers and Toregrand, 1971). Micronutrients play very important roles in different metabolic processes and their excess or deficiency may disturb normal biochemical function of the body (Akhter *et al*., 2002). In Pakistan, tallow is mostly rendered at the slaughterhouse using the conventional methods that has been used for the centuries. This rendered fat is then used widely for the edible and frying purposes especially in frying kebab, which is one of the mostly available traditional fast food of the Pakistan. There is no information on the mineral composition, quality parameters and physicochemical characteristics of the tallow rendered at the local slaughterhouses. The aim of this study was to evaluate the tallow rendered at the local slaughterhouses for the mineral and physicochemical parameters and compare it with the international quality standards.

Materials and Methods

Two different samples of the tallow were collected from the local slaughterhouses in Chakdara, NWFP,

Pakistan. Sample selections were made on the basis of difference in color of the fats. The tallow with pale yellow color was named "Tallow A", while the tallow with off-white color was named "Tallow B". All analytical reagents used for analysis were either from Merck (Darmstadt, Germany) or Sigma Aldrich (Buchs, Switzerland).

Physico-chemical parameters: Iodine value, Acid Value, % free fatty acids (%FFA), Anisidine value (An-V), peroxide and saponification values of the local tallow were determined using standard AOCS methods (1997). The cholesterol content was determined by using UV-visible spectrophotometer as described by Sabir *et al.*, (2003). Moisture content (%) was determined by using methods as described by the IUPAC (1979). Ash value was determined by using muffle furnace. For this purpose organic matter was destroyed by heating completely charred tallow sample at 600°C for 2 hrs in muffle furnace and present ash was determined as:

$$\text{Ash value (\%)} = \frac{m_2 - m_1 \times 100}{m_3 - m_1}$$

Where,
m_1= weight of the china dish
m_2= weight of the china dish + Tallow
m_3= weight of the china dish + ash

Mineral Composition: The mineral composition (Na^+ and K^+) was determined with the help of flame photometer (Jenway PFP7) by the method describe recently (Khan and Zeb, 2007). Heavy metals like Ca, Cu, Fe, Pb, Mg, Cd, Zn and Cr 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 (Hanlon, 1992).
All the parameters were determined at least in triplicate and the results were presented in mean ± standard deviation (SD).

Results and Discussion

Moisture and volatile matter: The moisture and volatile matter at 105°C is 0.223-0.226 percent and according to the Codex Standards, 1999 the maximum level of volatile matter at 105°C is 0.3% (CODEX-STAN-211, 1999). Nawaz *et al.* (2003) has also reported 0.34 percent moisture level in the tallow, which is in agreement with our results.

Acid value and % free fatty acid (FFA): Acid Value was 1.009-1.99, while the FFA expressed as % oleic acids was 0.507-1.00. The increase in FFA could be attributed to oxidation and hydrolysis, which produces FFAs (Peeled *et al.*, 1975; Abdel-Aal and Karara, 1986).

Table 1: Physicochemical parameters of the local tallow

Parameter	Tallow A	Tallow B
Ash value (%)	0.57±0.021	0.183±0.014
%Moisture	0.266±0.001	0.233±0.007
POV(meq active oxygen/ kg)	3±0.5	6.5±0.2
FFA (as oleic acid %by wt.)	0.507±0.056	1.00±0.052
Acid Value(mg KOH/g fat)	1.009±0.112	1.99±0.078
An-V	9.57±0.05	6.33±0.51
Cholesterol (mg/g)	1.1±0.2	1.00±0.7
Saponifiation Value(mg KOH/g fat)	195±3.54	197±4.47
Iodine Value(Wijs)	48.66±2.66	49.15±2.1

*Mean±standard deviation.

According to the regulations of the CODEX standard for named animal fats (CODEX-STAN-211, 1999), edible Tallow should have their maximum acid values 2.5 mg KOH/g of fat = FFA max 1.25 %.

Anisidine value (AnV): Anisidine value is one of the methods used to quantify secondary oxidation products of oil by measuring the aldehydes, principally 2, 4-dienals (Augustine and Chong, 1986). Anisidine value measures 2-alkenals, hydroperoxide decomposition products that can be used to determine how much per-oxidized material has already broken down (Brewer *et al.*, 1999). The anisidine value ranges from 6.33 to 9.57 as given in table 1. The samples of the tallow have the An-V less than 10 which reflect its good quality.

Peroxide value (POV): The POV of the samples is 3-6.5 meq/kg. According to the regulations of the CODEX standard for named animal fats (CODEX-STAN 211 - 1999), edible tallow should have their POV up to 10 meq/kg fat. The results of the tallow A and B are in agreement with this notion.

Saponification value: The saponification value for tallow A and B was 195±3.54 and 197±4.47 respectively. The saponification value is directly related to the mean molecular mass (Yanty *et al.*, 2008). The saponification values are less than that of palm oil. The low saponification value indicates that the tallow have larger molecular weight than the common oils. This may be due to the presence of higher saturated fatty acids. The low saponification value suggests that the oils can be used for candle and soap production and as chemical feed stocks for lubricants (Agatemor, 2006). The saponification value of the tallow is in agreement to the Codex (1999). The saponification value of the tallow (195-197 mg KOH/g) was also similar for those reported in the literature for cottonseed oil (189-198 mg KOH/g), but lower than those for coconut oil (248-265mg KOH/g) (Codex Alimentarius Commission, 1982).

Iodine value: The iodine value for the tallow A, B was 48.66±2.66 and 49.15 respectively. The iodine value is the measure of the degree of unsaturation of fat (Yanty *et al.*, 2008). The iodine value is also an index for

Table 2: Mineral composition of the Tallow A and B (all the concentrations are shown in ppm)

Element	Tallow A (ppm)	Tallow B (ppm)
Sodium	18.952±1.325	10.037±1.213
Potassium	2.216±0.397	2.821±0.351
Calcium	1.123±0.664	1.752±0.0024
Copper	0.001±0.0006	0.001±0.0001
Iron	0.493±0.0120	0.430±0.0096
Lead	1.153±0.0194	1.147±0.0152
Magnesium	0.412±0.0051	0.473±0.0055
Cadmium	0.070±0.0003	0.071±0.0004
Zinc	0.028±0.0016	0.055±0.0021
Chromium	0.982±0.0223	0.896±0.0138

*Mean±standard deviation

assessing the ability of oil to become rancid (Eka, 1980; Amoo *et al.*, 2004). The tallow has a low iodine value compared with palm oil (Which is 56.10 according to Agatemor, 2006). The low iodine value indicates that the tallow has a low content of unsaturated fatty acids relative to palm oil. The Wijis iodine value is also in accordance to the Codex (1999), which shows its good quality.

Cholesterol content (mg/g): The cholesterol content of the tallow ranges from 1.1±0.2 to 1.00±0.7 (mg/g). These results are in agreement with Sabir *et al.* (2003) who determined the cholesterol in beef tallow to be 1 mg/g. Among the samples of butter and Haleeb Desi ghee contained the high amount of cholesterol, while milk fat, beef tallow and fish fat were low in cholesterol content. Beef fat is poor source of cholesterol but it contains Myristic acid, which leads to cholesterol in blood (Sabir *et al.*, 2003).

Ash value (%): The % ash (0.183-0.57) value of the tallow rendered at the local slaughterhouses is <1%, which indicates that it is best for the production of the biodiesel. Using animal fat as fuel could help to solve the problem of waste disposal (Wiltsee, 1998).

Mineral composition: The results regarding elemental composition is shown in Table 2. The analysis of the mineral composition showed the level of Na (10.037-18.952 ppm), K (2.216-2.821 ppm), Ca (1.123-1.752 ppm), Cu (0.001 ppm), Fe (0.430-0.493 ppm), Pb (1.147-1.153 ppm), Mg (0.412-0.473 ppm), Cd (0.070-0.071 ppm), Zn (0.028-0.055 ppm) and Cr (0.896- 0.982 ppm). The CODEX standard (CODEX-STAN 211 - 1999) shows the maximum permissible concentration for Lead (0.1mg/kg), Iron (1.5 mg/kg) and copper (0.4 mg/kg). The table clearly shows that the concentration of copper and Iron is below the maximum permissible concentration while that of the Lead is very high as compared with the codex standards. The contamination of pro-oxidants and toxic metals in such products may be kept to a minimum level, with proper treatment and handling of oils and fats e.g., pretreatment with phosphoric acid and effective purification during refining and bleaching, citric acid treatment after deodorization and fine filtration after post treatments etc. (Anwar *et al.*, 2004). High levels of metals in the controlled tallow may be due in part to poor operating and maintenance conditions in our industries.

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