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Antioxidant, Antimicrobial and Antitopoisomerase Screening of the Stem Bark Extracts of *Ardisia compressa*

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Abstract: The *in vitro* antioxidant, antimicrobial and antitopoisomerase activities of crude Methanolic (Me) and Hexane (He) extracts of the stem bark of *Ardisia compressa* (AC) were investigated. Free radical-scavenging activity against 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was evaluated, acid ascorbic was used as reference standard. Antimicrobial activity was determined using the agar diffusion method; the bacteria *K. pneumoniae*, *E. coli*, *S. epidermidis* and the fungus *C. albicans*. Controls employed were chloranphenicol (30 µg) and nystatin (100 units). Topoisomerase inhibition was determined by a clone-forming assay, which uses yeast (*S. cerevisiae*) strains as a model. Controls included dimethyl sulfoxide (1.66%); campothecin (50 µg/ml), etoposide (100 µg/ml) and sobuzoxane (150 µg/ml). Ascorbic acid was a substantially more powerful antioxidant than the extracts from the stem bark of AC. The Antiradical Efficiency (AE) of ascorbic acid was 249-fold that for He and 8.3-fold that for Me. Only the growth of *Klebsiella pneumoniae* was inhibited by Me (MIC = 1.875 mg/ml) and He (MIC = 0.9375 mg/ml), while the growth of the other strains were not inhibited. He extract not showed antitopoisomerase activity. Me extract showed antitopoisomerase activity (-15.3%). Open column chromatography of the methanolic extract was conducted and fourteen fractions were collected and tested. Fractions VI (-21.55%), X (-42.49%), XI (-97.68%), XII (-52.34%) and XIII (-35.97%) showed antitopoisomerase I activity and fraction XIV (-79%) antitopoisomerase II (poison) activity. These results suggest that Me extract of the stem bark of *A. compressa* could be promising in its potential usefulness for treatment of cancer and deserves further investigation.

Key words: *Ardisia compressa*, bioactivity, natural products, chemoprevention

INTRODUCTION

The potential for using herbal tea components as anticancer botanicals and functional foods is promising. Tea is one of the most popular beverages consumed worldwide and several reports have attributed to tea chemopreventive and therapeutic properties (Mann *et al*., 2009; Adhami and Mukhtar, 2007; Siddiqui *et al*., 2007). In this regard, a newly investigated tea is ardisia extracted from the leaves of *Ardisia compressa* (AC), a plant of the Myrsinaceae family found in tropical and sub-tropical regions. There are approximately 500 species of Ardisia throughout the world (Kobayashi and De Mejia, 2005) and a few species such as *A. japonica* (Nikolovska-Coleska *et al*., 2004) and *A. compressa* (Ramirez-Mares *et al*., 1999) have been used for cancer treatment in indigenous medicine. While *A. japonica* is used in traditional Chinese medicine for treatment of pancreatic cancer (Nikolovska-Coleska *et al*., 2004), *A. compressa* is used in some Latin America regions, where its leaf-extracts are consumed in the form of herbal tea for the treatment of various liver conditions including liver cancer (Ramirez-Mares *et al*., 1999). However, data from well-controlled clinical studies are lacking and the medicinal claims are only substantiated by testimonial reports. Nevertheless, various alkylphenols have been isolated from ardisia species and some of them have anticancer properties (Chitra *et al*., 1994; Kang *et al*., 2001; Sumino *et al*., 2002). For example ardisin, an alkylphenol found in AC, has been reported to possess antioxidant and anti tumor activities in experimental animals (Gonzalez De Mejia *et al*., 2002). The antioxidant properties of ardisin were discovered when preincubation of hepatocytes with ardisin, in comparison to Epigallocatechin Gallate (EGCG), resulted in improvement in glutathione content combined with significant reductions in glutathione peroxidase activity and malondialdehyde formation (Ramirez-Mares and Gonzalez De Mejia, 2003). Additionally, ardisin also showed potent catalytic inhibition of topoisomerases I and II (Ramirez-Mares *et al*., 2004) and the administration of AC tea completely prevented tumor formation in Wistar rats exposed to diethylnitrosamine and acetylaminofluorene (Gonzalez De Mejia *et al*., 2004). Moreover, AC tea was cytotoxic to HT-29 and Caco-2 cells (Gonzalez De Mejia *et al*., 2006). However, there is no data that supports the claims made about the benefits for health of the *A. compressa* stem bark.

Several assays have been developed to evaluate the ability of a compound to modulate biochemical events

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presumed to be mechanistically linked to carcinogenesis (Shureiqi *et al*., 2000). Examples of such assays include: (a) topoisomerase inhibitors, which constitute a class of agents that inhibit carcinogenesis via their antiproliferative or cell-differentiating action and are considered an attractive targeting strategy in both chemotherapy and chemoprevention (Cho *et al*., 2000); (b) antimicrobial activity, allows the identification of novel agents capable of interfere with a specific molecular target, that may avoid the shortcomings of conventional chemotherapy because certain antimicrobials exhibit selective cytotoxicity against a broad spectrum of human cancer cells (Schweizer 2009); (c) antioxidant activity, potent scavengers of Reactive Oxygen Species (ROS) may serve as a possible preventive intervention for free radical-mediated diseases such as cancer (Ralph *et al*., 2010). The aim of this work was to investigate the *in vitro* antioxidant, antitopoisomerase and antimicrobial activities of crude methanolic and hexanic extracts of the stem bark of *A. compressa*, as part of the exploration for new and novel bioactive compounds.

MATERIALS AND METHODS

Biological material: *Saccharomyces cerevisiae* mutant cells JN362a, JN394, JN394 t_1, JN394t_{2-4} and JN394t_{2-5}; were kindly provided by Dr. John Nitiss of St. Jude Children's Research Hospital, Memphis, Tennessee. *Ardisia compressa* stem bark was collected from the Pacific Coast of Mexico (State of Michoacan). Standard microorganisms were purchased from American Type Culture Collection (ATCC, Manassas, VA): Gram negative bacteria; *Klebsiella pneumoniae (*ATCC 13883), *Escherichia coli (*ATCC 35218), Gram positive bacteria; *Staphylococcus epidermidis (*ATCC 12228) and the fungus *Candida albicans* (ATCC 14053).

Chemicals: Peptone bacto, yeast extract, agar bacto, Mueller Hinton (MH) agar, MH broth, trypticase soy agar, trypticase soy broth, sabouraud dextrose agar, sabouraud dextrose broth and dextrose were purchase from Difco (Sparks, MD). Methanol (HPLC grade), methanol (spectrophotometric grade), Hexane (HPLC grade), Camptothecin (CPT), Etoposide (ETP), Sobuzoxane (SBZ), dimethyl sulfoxide (DMSO-Hybri-Max), adenine hemisulfate salt, chloranphenicol, nystatin, ascorbic acid and 2,2-Diphenyl-1-picrylhydrazyl (DPPH) were obtained from Sigma Chemical (St. Louis, MO). C_{18} (Li Chroprep RP-18, 40-63 μm) was from Merck (Darmstadt, Germany).

Collection of plant material: Fresh stem bark of *A. compressa* collected on the Pacific coast of Mexico (Michoacan State) was first air-dried without exposure to sunlight; the dry material was chopped into pieces and kept in large plastic bags and stored in a cool and dry place.

Preparation of extracts: 475 g of powdered bark of *A. compressa* were soaked in 2000 ml of methanol and hexane for two weeks respectively, at room temperature (25°C). The extracts were filtered and the filtrates were concentrated to dryness using a rotary evaporator at 30°C at reduced pressure, to form Methanolic (Me) and Hexanic (He) extract respectively. The dried and powdered crude extracts (Me = 6.65 g and He = 3.2 g) were kept at -20°C and protected from light and moisture in a glass container sealed with parafilm. The extracts were dissolved in DMSO prior to use in all assays.

Fractionation of the methanol extract: Open Column Chromatography (OPC) using a column (300 mm x 20 mm I.D.) packed with C_{18} (reversed-phase) were conducted to isolate the fractions responsible for antitopoisomerase activity of the methanolic extract of the stem bark of AC. A combination of MeOH and H_2O, were used to perform the separation of 1 g of Me. The analysis was started with 200 ml of MeOH/H_2O: 50%/50%, then 200 ml of MeOH/H_2O: 70%/30%, after 200 ml of MeOH/H_2O: 90%/10% and finally 200 ml of pure MeOH. Fourteen fractions were collected and passed through a 0.22 μm filter and evaporated until dried using a rotary evaporator and a freeze dryer. The dried solid fractions were protected from light and kept at -20°C. The inhibition of topoisomerase activity was then measured in the fractions using the antitopoisomerase yeast assay.

Antioxidant activity of the extracts: The antioxidant activity was evaluated in terms of radical-scavenging ability of Me and He extracts using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical assay (Brand-Williams *et al*., 1995). A methanolic (spectrophotometric grade) solution (50 μl) of the herbal extract (Me or He) at five different concentrations was added to 1.95 ml of DPPH● solution (7.6×10^{-5} M in methanol). The decrease in the absorbance at 515 nm was determined using a uv/vis spectrophotometer (Beckman DU-530) until the reaction reached the steady state in the dark (Siddhuraju and Becker, 2003).

The DPPH● concentration in the reaction medium was calculated from the following calibration curve, determined by lineal regression:

$$A_{515nm} = 0.009\ [DPPH\bullet]_T - 0.007$$

Where $[DPPH\bullet]_T$ was expressed as μM, $r^2 = 0.998$

The percentage of remaining DPPH● (% $DPPH\bullet_{REM}$) was calculated as follows:

$$\%\ DPPH\bullet_{REM} = [DPPH\bullet]_T / [DPPH\bullet]_{T=0}$$

Where $[DPPH\bullet]_T$ was the concentration of DPPH● at the time of steady state and $[DPPH\bullet]_{T=0}$ was the

concentration of DPPH● at zero time. The % $DPPH●_{REM}$ against the standard concentration was plotted to obtain the amount of antioxidant necessary to decrease by 50% the initial DPPH● concentration (EC_{50}). The time needed to reach the steady state to EC_{50} concentration (T_{EC50}) was determined. Antiradical Efficiency (AE) was also calculated ($AE = 1/EC_{50}\ T_{EC50}$). Ascorbic acid was used as a reference standard. All experimental were carried out in triplicate. The activity of each extract was expressed as percentage of that achieved for the reference standard.

Antibacterial and antifungal screening: Antibacterial and antifungal assays were carried out of the Me and He extracts against *Klebsiella pneumoniae (*ATCC 13883), *Escherichia coli (*ATCC 35218), *Staphylococcus epidermidis (*ATCC 12228) and the fungus *Candida albicans* (ATCC 14053). The antibacterial and antifungal activities were determined using the agar diffusion method. The bacterial strains were placed in plates of trypticase soy agar and the fungus in plates of sabouraud dextrose agar. After 24 h incubation at 37°C (bacteria) and 30°C (fungus), four or five colonies were inoculated in 4 ml of Mueller-Hinton broth or sabouraud dextrose broth and incubated for 2 h at 37°C and 35°C, respectively. These inocula were adjusted to the 0.5 MacFarland standard (0.048 M $BaCl_2$ 0.5 ml + 0.18 M H_2SO_4 99.5 ml).

For susceptibility testing, each 150 µl of adjusted bacterial or fungal suspension was spread on the sterile medium (trypticase soy agar or sabouraud dextrose agar) using sterile cotton swabs. The positive controls employed were chloranphenicol (30 µg) in the antibacterial and nystatin (100 units) in the antifungal assays. Application of the samples and controls (25 µl) was done directly in the solid medium. The application point was marked on the lower surface of the Petri dish. The preparations were left to diffuse. Subsequently the plates were incubated at 37°C for 24 h in the case of the bacteria; while the fungus was cultured at 30°C for 48 h. Plates were prepared using the same procedures without extract or antibiotic, but with DMSO (25 µl) were equally set as negative control. After incubation, the growth inhibition rings were quantified by measuring the diameter for the zone of inhibition in millimeters from the lower surface of the plates. All assays were carried out in triplicate.

Determination of minimal inhibitory concentration (MIC): The method of Greenwood (1989) was used to determine the MIC of the extracts. 50 µl of extract (Me or He) was mixed with 950 µl of sterile water to get an X concentration. Eight sterile test tubes were arranged in a test tube rack and 1 ml of sterile water was pipetted into each test tube. Thereafter, there was a two fold serial dilution of the extract to obtain 1 ml of each one of the following concentrations: X/2, X/4, X/8, X/16, X/32, X/64, X/128 and X/256. The test organism (1 ml = 5 x 10^5 CFU/ml) was pipetted into each of the test tubes containing the extract, the final concentrations were: X/4, X/8, X/16, X/32, X/64, X/128, X/256 and X/512. Finally the tubes were incubated at 37°C for 24 h in the case of the bacteria, while the fungus was cultured at 30°C for 48 h. The MIC was recorded as the least concentration of extract that completely inhibited the growth of the test organism.

Yeast antitopoisomerase assay: The antitopoisomerase activity was assessed using mutants *Saccharomyces cerevisiae* JN362a, JN394, JN394 t_1, $JN394t_{2-4}$ and $JN394t_{2-5}$ strains (Nitiss and Nitiss, 2001). Briefly, yeast cells were grown in YPDA media at 30°C (25°C for $JN394t_{2-4}$ and $JN394t_{2-5}$) for 18 h in a shaking incubator. The logarithmically growing cells were then counted using a hematocytometer and adjusted to a concentration of 2 x 10^6 cells/ml media. Yeast cells (6 x 10^6 cells) were incubated at 30 or 25°C for 24 h in the shaking incubator, in the presence of the Me or He that were dissolved in 50 µl DMSO. DMSO (1.66%) was used as negative control, while CPT (50 µg/ml) a topoisomerase I inhibitor, ETP (100 µg/ml) a topoisomerase II poison and SBZ (150 µg/ml) a topoisomerase II inhibitor were the positive controls. Treated cells from each mixture were then duplicate plated to petri dishes containing 1.75% Agar Bacto solidified YPDA as media. Cells were incubated at growth temperature of 30°C or 25°C for 48 h. The antitopoisomerase activity was then measured by comparing the number of counted colonies in each plate to that of the negative control plate (DMSO).

RESULTS AND DISCUSSION

Evaluation of the antioxidant activity: The antioxidant activity of plant extracts which containing various kinds of compounds are due to their abilities to be donors of hydrogen atoms or electrons and to capture free radicals (Wu *et al*., 2010). DPPH, a stable free radical with purple color, changes into a stable yellow compound on reacting with an antioxidant. The extent of the reaction depends on the hydrogen-donating ability of the antioxidant. The concentration of the antioxidant needed to decrease the initial DPPH concentration by 50% (EC_{50}) is a parameter widely used to measure antioxidant activity. Another parameter was defined as antiradical efficiency ($AE = 1/EC_{50}\ T_{EC50}$) where T_{EC50} is the time needed to reach the steady state to EC_{50} concentration. The lower the EC_{50} or T_{EC50}, the higher is the antioxidant activity (Brand-Williams *et al*., 1995). The Table 1 shows the free radical-scavenging activity of the Me and He extracts from the stem bark of AC. The antioxidant activity of the extracts was expressed as percentage of efficiency by comparing AE of the

Table 1: Estimation of free radical-scavenging activity of the stem bark of *Ardisia compressa* extracts

Extracts and control	EC_{50} g/kg $_{DPPH}$	T_{EC50} min	AE x 10^{-3}	% of efficiency
Me	473±10	2.83±0.2	0.747	12.1
He	2882±15	14.00±0.3	0.0248	0.402
Ascorbic acid	103±2	1.57±0.1	6.183	100

Results are means±SD (n = 3)

extracts with AE of ascorbic acid. From the results it was appeared that the methanol extract has the strongest DPPH radical-scavenging activity (EC_{50} = 473. g/Kg_{DPPH}) whereas the hexane extract gave the lowest (EC_{50} = 2882 g/Kg_{DPPH}). Table 1 also shows that the lower the EC_{50}, the shorter was the reaction time (T_{AC50}) and the higher the Antiradical Efficiency (AE). Inspection of Table 1 showed that ascorbic acid was a substantially more powerful antioxidant than the extracts from the stem bark of AC. The AE of ascorbic acid was 249-fold that for He and 8.3-fold that for Me. The classification order of AE for the tested antioxidants was: ascorbic acid > Me > He. Due to the diversity and complexity of the natural mixtures of antioxidants compounds in the different plant extracts, it is rather difficult to characterize every compound and assess or compare their antioxidant activities. Antioxidant activity of the extracts may also be attributable to synergistic interactions. Because of these the 12.1% of efficiency of Me compared to ascorbic acid (100%) do not deserve to fractionate the extract to look for the responsible compounds of the antioxidant effect.

Antimicrobial activity and MIC: In order to improve the screening of natural products with antimicrobial activity, we applied the extracts directly to the agar medium, because when the paper (cellulose) disc is used, the free hydroxyl groups present on each glucose residues becomes the surface of the disc hydrophilic (Burgess *et al*., 1999). Consequently the polar natural products would be expected to adsorb to the surface of the disc and not diffuse into the agar medium.

The results of antibacterial activity of the extracts against *Klebsiella pneumoniae, Escherichia coli* and *Staphylococcus epidermidis* showed that the methanolic and hexanic extracts only inhibited the growth of *Klebsiella pneumoniae* while the growth of the other strains were not inhibited (Table 2). The hexane extract (inhibition zone 13 mm) was found to be more effective than the methanol extract (12 mm) against *Klebsiella pneumoniae* at the same concentration tested (10 mg/ml). Both extracts showed low antifungal activity with inhibition zones ranging between 1 and 3 mm for *Candida albicans*. Contrary to the results of Nostro *et al.* (2000), the antibacterial activity of the Me and He extracts was more pronounced against Gram-negative bacteria than against Gram-positive bacteria. Gram-negative is expected to be impermeable to lipophilic solutes due to have an outer phospholipidic membrane carrying the structural lipopolysaccharide components. Gram-positive bacteria have an outer peptidoglycane layer that is not an effective permeability barrier, thus these bacteria are more susceptible to antibacterial compounds (Nikaido and Vaara, 1985). The antibacterial activity not only depends of the kind of microorganism and its morphology, but also of other factors such as: (a) diffusion capacity of substances present in the extracts (number, size, polarity and shape of particles) in the agar medium, (b) antimicrobial activity of diffused compounds, (c) density of inoculation, (d) growth and metabolic activity of bacteria in the medium and (e) pH of substrates in plates. This suggests that the antibacterial activity that can be seen in the agar medium, not necessarily is due to the morphological differences between bacteria. These could be the reasons why our results were different than those from Nostro *et al.* (2000). Moreover, in support of our explanation, polar (Me extract) and no-polar (He extract) compounds were effective against *Klebsiella pneumoniae*.

The MIC illustrates (Table 3) a decreasing inhibitory effect of the bark extracts and chloranphenicol as the concentration decreases. This suggests that antimicrobial activity of a compound is a concentration dependent and that the antimicrobial activity is a function of the bioactive ingredient reaching a microorganism. The minimum inhibitory concentration of Me and He against *Klebsiella pneumoniae* were 1.875 and 0.9375 mg/ml respectively (Table 3). These concentrations are remarkable for a crude extract and indicate that when further isolation and purification of the extracts is carried out, increased activity would be obtained or a possible synergism of antimicrobial activity among the components of the extract would be demonstrated. It is important to point out that there is a clear dependence of inhibition zone sizes on antibiotic concentration and the MIC value determined by the broth dilution assay. The hexane extract resulted more effective (inhibition zone 13 mm, MIC = 0.9375 mg/ml) than methanol extract ((inhibition zone 12 mm, MIC = 1.875 mg/ml. The agar diffusion method do not expose the test bacteria to the full volume of extract solution and are less sensitive to the size of the inoculum and in the broth dilution assay, bacterial susceptibility depends on the ratio of extract solution concentration to inoculum size. Nevertheless, there was a good agreement between the results of both methods.

Table 2: Zone of inhibition (mm) produced by extracts of the stem bark of *Ardisia compressa* and standard antimicrobial agents

Organism	Gram	Me (10 mg/ml)	He (10 mg/ml)	Chloranphenicol (1.2 mg/ml)	Nystatin (30 units)
Escherichia coli	(-)	0	0	12±0	---
Staphylococcus epidermidis	(+)	0	0	24.8±0.8	---
Klebsiella pneumoniae	(-)	12±0	13±0	20±0.5	---
Candida albicans	---	2.5±0.5	1.5±0.5	---	25±0.7

Results are means±SD (n = 3)

Table 3: Minimal inhibitory concentration of the extracts of the stem bark of *Ardisia compressa* and chloranphenicol against *Klebsiella pneumoniae*

	mg/ml							
Extracts	3.75	1.875	0.9375	0.46875	0.23437	0.11718	0.05859	0.02929
Me	-	-	+	+	+	+	+	+
He	-	-	-	+	+	+	+	+
	µg/ml							
Standard	36	18	9	4.5	2.25	1.125	0.5625	0.28125
Chloranphenicol	-	-	+	+	+	+	+	+

-: Susceptible; +: Resistance

Table 4: Percent[a] of inhibition of Me and He extracts treatments on the survival of JN394 and JN362a strains

Extract or control	Concentration (µg/ml)	JN394	JN362a
Me	21,500	-15.3±4.5	+17.5±6.2
He	21,000	- 33.0±5.7	-16.9±4.3
CPT (TOP 1 poison)	50	- 99.0±0.4	0

DMSO (1.66%) was used as a control and all the results were referred to this value. [a]Values are means±SD of triplicate determinations

Table 5: Percent[a] of inhibition of methanol extract fractions on the survival of JN394 and JN362a strains

Fraction or control	Concentration (µg/ml)	JN394	JN362a
I	190	-39.10±2.4	-49.79±3.9
II	480	+19.00±5.3	ND
III	10	-86.85±4.1	-52.09±7.2
IV	60	-42.05±5.2	-72.31±5.4
V	1160	-88.12±3.4	-89.10±4.4
VI	680	-21.55±6.3	+180.61±8.9
VII	1120	+12.75±3.3	ND
VIII	45	+36.24±4.7	ND
IX	22	+22.15±5.2	ND
X	16	-42.49±3.3	+104.59±5.8
XI	30	-97.68±2.3	-97.20±2.1
XII	480	-52.34±4.4	+66.84±4.6
XIII	300	-35.97±3.9	+87.76±5.0
XIV	230	-12.23±6.5	+80.58±4.5
CPT (TOP 1 poison)	50	-99.0±0.4	0

DMSO (1.66%) was used as a control and all the results were referred to this value. [a]Values are means±SD of triplicate determinations. ND: Not Determined

Antitopoisomerase activity: The concentration of the extracts used in this assay was based on the solubility factor of each solid extract in DMSO. As shown in Table 4, the strain JN394 was hypersensitive to CPT (99.0%), which is a Topo I poison. Me and He showed 15.3 and 33% inhibition, respectively. The strain JN394 is DNA repair-deficient and drug-permeable (carry *ise2* and *rad52* mutations) (Nitiss and Wang, 1988). These mutations increase the sensitivity of these cells to drugs. The yeast JN362a, a DNA repair-proficient strain (Nitiss and Wang, 1988), was no affected by Me (+17.5%) or CPT (0%), but was inhibited by He (-16.9%). These results mean that the methanol extract has compounds with antitopoisomerase activity. In order to find the fraction or fractions responsible for the antitopoisomerase activity, a guide-fractionation was conducted using an open column packed with C_{18} and a combination of MeOH and H_2O. Fourteen fractions were collected and tested in the strains JN394 and JN362a (Table 5). Fractions I (-39.10%, 190 µg/ml), III (-86.85%, 10 µg/ml), IV (-42.05%, 60 µg/ml), V (-88.12%, 1160 µg/ml), VI (-21.55%, 680 µg/ml), X(-42.49%, 16 µg/ml), XI (-97.68%, 30 µg/ml), XII (-52.34%, 480 µg/ml), XIII (-35.97%, 300 µg/ml) and XIV (-12.23%, 230 µg/ml), showed inhibition against the JN394 strain. The rest of the fractions (II, VII, VIII and IX) not inhibited the growth of this strain and for this reason they were not tested against the yeast strain JN362a. The yeast JN362a was affected by fractions I (-49.79%), III (-52.09%), IV (-72.31%), V (-89.10%) and XI (-97.20). The fractions VI, X, XII, XIII and XIV no inhibited the growth of JN362a, these are the fractions with antitopoisomerase activity.

The strain JN394$t_{.1}$ is isogenic to JN394 and contains a disrupted top1 gene (Nitiss and Wang, 1988); the absence of the gene resulted in diminished cytotoxicity of antitopoisomerase I drugs. Table 6 shows that CPT failed to reduce the growth of these mutant cells and also the fractions VI, X, XII and XIII were incapable of affect the growth of the cells with the top1 mutation. This means that Topo I is the target of these fractions. In

Table 6: Percent[a] of inhibition of methanol extract fractions with antitopoisomerase activity on the survival of JN394t_{-1}, JN394t_{2-4} and JN394t_{2-5} strains

Fraction or control	Concentration (µg/ml)	JN394t_{-1}	JN394t_{2-5} 25°C	JN394t_{2-4} 25°C	JN394t_{2-4} 30°C
VI	680	0	ND	ND	ND
X	16	0	ND	ND	ND
XII	480	0	ND	ND	ND
XIII	300	0	ND	ND	ND
XIV	230	-99.0±0.3	+145±10.2	-79±5.2	-15±3.6
CPT (TOP 1 poison)	50	0	-99±0.4	-80±4.6	-99±0.3
ETP (TOP 2 poison)	100	-99±0.4	+71±6.3	-99±0.4	-80±3.9
SBZ (TOP 2 catalytic)	150	-99±0.2	-17±4.4	+213±7.8	-99±0.4

DMSO (1.66%) was used as a control and all the results were referred to this value.
[a]Values are means±SD of triplicate determinations. ND: Not Determined

contrast, no resistance was observed when top1 cells were treated with fraction XIV (-99%) or the antitopoisomerase II drugs ETP and SBZ (-99%). Sensitivity in the top1 cells was higher than in the parent cells (JN394). This finding provided evidence that Topo I was not the cellular target of fraction XIV.

The strain JN394t_{2-5} carries a top2 allele that is resistant to multiple classes of topoisomerase II poisons at its permissive temperature (25°C) (Jannatipour *et al*., 1993). Table 6 shows that cells with the top2-5 mutation are able to growth in ETP (+71%) or in fraction XIV (+145%). The sensitivity of the top2-5 strain to CPT (-99%) and SBZ (-17%) is also shown in Table 6. The strain has essentially the same sensitivity to CPT as JN394 (*rad52 top2*$^+$ cells), indicating that the observed resistance is specific to antitopoisomerase II agents.

Eliminating the possibility that Topo I is the target of fraction XIV, two additional possibilities exist for the physiological mechanism of cytotoxicity. The first is that Topo II is the primary target responsible for killing cells by trapping the enzyme-mediated DNA cleavage. The second is that the cytotoxicity is correlated with the ability to block the overall catalytic activity of the enzyme. The possibilities described above can be scrutinized by utilizing a yeast strain JN394t_{2-4}. This yeast strain expresses the temperature-sensitive top2-4 mutant in place of the wild type top2 gene (Nitiss and Wang, 1988). The top2-4 protein shows wild type activity at 25°C, while its activity is reduced to about 5-10% of the wild type at the semi-permissive temperature of 30°C. Therefore, if the fraction XIV functions as a Topo II "poison", a reduction in enzyme activity should greatly diminish the induced cell death. Conversely, if the cytotoxicity is correlated with the ability to impair the catalytic function of the enzyme, cells with decreased levels of Topo II activity should become hypersensitive. ETP was a potent toxic agent toward JN394t_{2-4} yeast cells at 25°C (Table 6). A smaller value was obtained when the cytotoxicity of ETP was examined at 30°C. The sensitivity to CPT at 30°C (99%) was greater than at 25°C (80%) (Table 6). The fraction XIV displayed weak cytotoxicity (-15 %) toward JN394t_{2-4} yeast at 30°C. Top 2-4 cells were hypersensitive (-79%) at the permissive temperature (25°C). The increased toxicity toward cells that contain increased levels of Topo II activity strongly suggests that trapping the enzyme-mediated DNA cleavage is the primary physiological target of fraction XIV.

In summary, the results of the present study indicate that compounds extracted in the methanolic fraction of the stem bark of *Ardisia compressa* possess potential chemopreventive activity, but further work is required in order to isolate the phytochemicals responsible for the antibacterial and antitopoisomerase activities.

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REFERENCES

Adhami, V.M. and H. Mukhtar, 2007. Anti-oxidants from green tea and pomegranate for chemoprevention of prostate cancer. Mol. Biotechnol., 37: 52-57.

Brand-Williams, W., M.E. Cuvelier and C. Berset, 1995. Use of a free radical method to evaluate antioxidant activity. Lebensm. Wiss. Technol., 28: 25-30.

Burgess, J.G., E.M. Jordan, M. Bregu, A. Mearns-Spragg and K.G. Boyd, 1999. Microbial antagonism: A neglected avenue of natural products research. J. Biotechnol., 70: 27-32.

Chitra, M., E. Sukumar, V. Suja and C.S. Devi, 1994. Antitumor, antiinflammatory and analgesic property of Embelin, a plant product. Chemotherapy, 40: 109-113.

Cho, K.H., J.M. Pezzuto, J.L. Bolton, V.E. Steele, G.J. Kelloff, S.K. Lee and A. Constantinou, 2000. Selection of cancer chemopreventive agents based on inhibition of topoisomerase II activity. Eur. J. Cancer, 36: 2146-2156.

Gonzalez De Mejia, E., M.V. Ramirez-Mares and M.G. Nair, 2002. Topoisomerase I and II enzyme inhibitory aqueous extract of *Ardisia compressa* and ardisin protect against benomyl oxidation of hepatocytes. J. Agric. Food Chem., 50: 7714-7719.

Gonzalez De Mejia, E., M.V. Ramirez-Mares, E. Arce-Popoca, M. Wallig and S. Villa-Trevino, 2004. Inhibition of liver carcinogenesis in Wistar rats by consumption of an aqueous extract from leaves of *Ardisia compressa*. Food Chem. Toxicol., 42: 509-516.

Gonzalez De Mejia, E., S. Chandra, M.V. Ramirez-Mares and W. Wang, 2006. Catalytic inhibition of human DNA topoisomerase by phenolic compounds in *Ardisia compressa* extracts and their effect on human colon cancer cells. Food Chem. Toxicol., 44: 1191-1203.

Greenwood, D., 1989. Antibiotic Sensitivity Testing. In: Antimicrobial Chemotherapy (Greenwood, D., Ed.), Oxford University press, New York, pp: 91-100.

Jannatipour, M., Y.-X. Liu and J.L. Nitiss, 1993. The *top 2-5* mutant of yeast topoisomerase II encodes an enzyme resistant to etoposide and amsacrine. J. Biol. Chem., 268: 18586-18592.

Kang, Y.H., W.H. Kim, M.K. Park and B.H. Han, 2001. Antimetastatic and antitumor effects of benzoquinonoid AC7-1 from *Ardisia crispa*. Int. J. Cancer, 93: 736-740.

Kobayashi, H. and E. De Mejia, 2005. The genus Ardisia: A novel source of health-promoting compounds and phytopharmaceuticals. J. Ethnopharmacol., 96: 347-354.

Mann, C.D., C.P. Neal, G. Garcea, M.M. Manson, A.R. Dennison and D.P. Berry, 2009. Phytochemicals as potential chemopreventive and chemotherapeutic agents in hepatocarcinogenesis. Eur. J. Cancer Prev., 18: 13-25.

Nikaido, H. and M. Vaara, 1985. Molecular basis of bacterial outer membrane permeability. Microbiol. Rev., 1: 1-32.

Nikolovska-Coleska, Z., L. Xu, Z. Hu, Y. Tomita, P. Li, P. P. Roller, W. Renxiao, X. Fang, R. Guo, M. Zhang, M. E. Lippman, D. Yang and S. Wang, 2004. Discovery of embelin as a cell-permeable, small molecular weight inhibitor of XIAP through structure-based computational screening of traditional herbal medicine three-dimensional structure database. J. Med. Chem., 47: 2430-2440.

Nitiss, J.L. and J.C. Wang, 1988. DNA topoisomerase-targeting antitumor drugs can be studied in yeast. Proc. Natl. Acad. Sci. USA, 85: 7501-7505.

Nitiss, J.L. and K.C. Nitiss, 2001. Yeast systems for demonstrating the targets of antitopoisomerase II agents. Methods Mol. Biol., 95: 315-327.

Nostro, A., M.P. Germano, V. D´Angelo, A. Marino and M. A. Cannatelli, 2000. Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Appl. Microbiol., 30: 379-384.

Ralph, S.J., S. Rodríguez-Enríquez, J. Neuzil, E. Saavedra and R. Moreno-Sánchez, 2010. The causes of cancer revisited: Mitochondrial malignancy and ROS-induced oncogenic transformation - Why mitochondria are targets for cancer therapy. Mol. Aspects Med., (in press).

Ramirez-Mares, M.V., S. Fatell, S. Villa-Trevino and E.G. De Mejia, 1999. Protection of extract from leaves of *Ardisia compressa* against benomyl-induced cytotoxicity and genotoxicity in cultured rat hepatocytes. Toxicol. *In Vitro*, 13: 889-896.

Ramirez-Mares, M.V. and E.G. De Mejia, 2003. Comparative study of the antioxidant effect of ardisin and epigallocatechin gallate in rat hepatocytes exposed to benomyl and 1-nitropyrene. Food Chem. Toxicol., 41: 1527-1535.

Ramirez-Mares, M.V., S. Chandra and E.G. De Mejia, 2004. *In vitro* chemopreventive activity of Camellia sinensis, Ilex paraguariensis and *Ardisia compressa* tea extracts and selected polyphenols. Mutat. Res., 554: 53-65.

Schweizer, F., 2009. Cationic amphiphilic peptides with cancer-selective toxicity. Eur. J. Pharmacol., 625: 1-3.

Shureiqi, I., P. Reddy and D.E. Brenner, 2000. Chemoprevention: General perspective. Crit. Rev. Oncol. Hematol., 33: 157-167.

Siddhuraju, P. and K. Becker, 2003. Antioxidant properties of various solvent extracts of total phenolic constituents from three different agroclimatic origins of drumstick tree (*Moringa oleifera* Lam.) leaves. J. Agric. Food Chem., 51: 2144-2155.

Siddiqui, I.A., M. Saleem, V.M. Adhami, M. Asim and H. Mukhtar, 2007. Tea beverage in chemoprevention and chemotherapy of prostate cancer. Acta Pharmacol. Sin., 28: 1392-1408.

Sumino, M., T. Sekine, N. Ruangrungsi, K. Igarashi and F. Ikegami, 2002. Ardisiphenols and other antioxidant principles from the fruits of *Ardisia colorata*. Chem. Pharm. Bull., 50: 1484-1487.

Wu, N., Y. Zu, Y. Fu, Y. Kong, J. Zhao, X. Li, J. Li, M. Wink and T. Efferth, 2010. Antioxidant activities and xanthine oxidase inhibitory effects of extracts and main polyphenolic compounds obtained from *Geranium sibiricum* L. J. Agric. Food Chem. (in press).

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Utilization of Groundnut Milk in Manufacturing Spread Cheese

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Abstract: The utilization of groundnut milk in manufacturing the spread cheese in Sudan was investigated. Groundnut milk was prepared from grinded groundnut seeds. Four samples of spread cheese were prepared from groundnut milk with different levels of skim milk powder 0, 5, 10 and 15. The prepared spread cheese samples were stored for 6 months at 30±2°C. Analyses of chemical composition were carried on prepared spread cheese samples and the analyses were carried out at intervals 0, 1, 2, 3, 4, 5 and 6 months during storage period. The chemical analyses of spread cheese samples at zero time processing were for total solids 35.79, 37.91, 39.59 and 41.49%, the protein content 12.82, 14.35, 15.98 and 17.56%, the fat content 14.98, 14.99, 14.99 and 15.0%, the ash content 4.16, 4.18, 4.21 and 4.23% for samples A, B, C and D respectively. The pH was affected by the levels of skim milk powder and storage period. The levels of skim milk powder significantly ($p \leq 0.05$) affected the pH value of the spread cheese. Sample D (15% skim milk powder) recorded the lowest (5.14) and sample A (0% skim milk powder) the highest (5.60). Sample B (5% skim milk powder) and sample C (10% skim milk powder) were at an intermediate position (5.56 and 5.45), respectively. Storage period significantly ($p \leq 0.05$) affected the pH value of spread cheese the highest value (5.44) at the beginning of the storage period, while the lowest value (3.91) at the end of the storage period. The titratable acidity was affected by the levels of skim milk powder and storage period. The levels of skim milk powder significantly ($p \leq 0.05$) affected the titratable acidity of the spread cheese. Sample D (15% skim milk powder) was the highest, while sample A (0% skim milk powder) was the lowest. Sample B (5% skim milk powder) and sample C (10% skim milk powder) occupy an intermediate position. Storage period significantly ($p \leq 0.05$) affected the titratable acidity of spread cheese. The highest titratable acidity (2.09%) obtained at the end of storage period and the lowest (0.64%) at the beginning of the storage period. The best score of appearance, texture, flavour and overall acceptability obtained by sample B (5% skim milk powder), compared with others samples. Three months is found to be quite satisfactory to attain good quality spread cheese.

Key words: Groundnut milk, spread cheese, storage, skim milk powder

INTRODUCTION

Groundnut (*Arachis hypogaea* L.) is a major source of edible oil and protein meal and therefore considered to be highly valuable in human and animal nutrition (Nwokalo, 1996). Since groundnut has a potential role to play in combating malnutrition, the present low level in its consumption, especially in the developing countries, should be increased. It is, therefore, necessary to adequately research into the possibility of groundnut processing into other useful and edible products. Fermentation of groundnut milk may serve as one such effort than can increase the protein availability and consumption (Roberts-Sunny *et al*., 2004).

Spread cheese means cheese which has been subjected to a process of melting and mixing with milk products other than the cheese, with or without the addition of emulsifying salts. Spread cheese has currently increased in the Sudan, although originally, it is a main meal supplement in countries where such a food has not been customarily used (particularly the Asian countries). Most spreadable cheese products are made from dairy products derived from cow milk patents (Tamime *et al*., 1999).

This group of cheese products differ from natural cheeses in that, they are made directly from milk, but rather from various ingredients such as natural cheese, skim milk, water, butter oil, casein caseinate other dairy ingredients, vegetable oil, and from vegetable proteins and minor ingredients. The two main categories are namely pasteurized processed cheese products and substitute or imitation products (Patrick *et al*., 2000).

Due to the high cost of milk in Sudan, which ultimately increases the cost of milk products, other alternatives are currently being sought in order to process nutritionally acceptable fermented dairy products, such as groundnut milk.

No attempt has been made in the Sudan to study the manufacture of spread cheese. The objectives of this

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work are to prepare the milk from groundnut and to study their effect on quality of spread cheese as affected by level of skim milk powder during storage period.

MATERIALS AND METHODS

Groundnut seeds, skim milk powder and salt were obtained from the local market. Bulk culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*) were obtained from the Blue Nile Dairy Product Company (Capo). Rennet (chr. Hansen's, Denmark) were obtained from the local market.

Preparation of groundnut milk: Groundnut milk was prepared from kernels previously soaked in 1% sodium bicarbonate solution for 16-18 h, drained, washed with tap water, grinded and steeped for 4-5 h in tap water (100 g of groundnut mixed with 750 ml tap water). The mixture was then filtrated by cheese cloth, to obtain the groundnut milk.

Preparation of spread cheese samples: The prepared groundnut milk were heated to 70°C for 10 min and then cooled to 45°C. Skim milk powder(0, 5, 10 and 15%) and 10% of starter culture were added to the milk. 0.5 gm; rennet powder added to the mix and incubated at 45°C for 6 h, after that the mix was filtrated thoroughly cheese cloth. The curd mix was cooked to 70°C for 30 min with addition of 5% whey and mixed. The spread cheese was packed in glass container and stored at 30±2°C.

Chemical analysis: The content of moisture, protein, fat, ash, pH value and titratable acidity were carried out according to AOAC (1995).

Sensory evaluation: The sensory evaluation was performed by 10 untrained panelists using scoring procedure according to Drake *et al.* (2003).

Statistical analysis: The statistical analysis was carried out by computer using SAS pogramme 1997.

RESULTS AND DISCUSSION

Groundnut milk: The results of chemical properties of groundnut milk were of total solids (9.39%), pH-value (6.7), titratable acidity (0.10%), protein content (3.64%) fat content (2.95%) and ash content (0.85%). These results fairly agree with that of Hinds *et al.* (1997) who concluded that, the groundnut milk contained 11.8% total solids, 2.0% fat and 3.71% protein. Roberts-Sunny (2007) stated 2.8% fat content while, Graham (1970) found 3.48% protein content in groundnut milk. Vargas *et al.* (1998) reported that, the physic-chemical composition of Argentinean groundnut milk, to have pH-value, total solids, protein, lipids and ash contents to be 7.12, 9.10, 2.97, 4.24 and 0.2%, respectively. Variation in the results could be due to the origin of groundnut and to the processing condition (Lee and Beuchat, 1992).

Chemical properties of spread cheese: Physicochemical properties of spread cheese made by groundnut milk at zero time are presented in (Table 1). The total solid of spread cheese made from groundnut milk at zero time was affected by the levels of skim milk powder. The level of skim milk powder significantly ($p \leq 0.05$) affected the total solids of the spread cheese. Sample D (15% skim milk powder) recorded the highest content of total solids (41.49%) compared with sample A (0% skim milk powder), that gave the lowest content (35.79%). Samples B and C show an intermediate position. Salem *et al.* (1987) found that the total solids of processed spread cheese ranged from 56.02-61.12%. Gouda and El-Shibiny (1987) reported that, the total solids of processed spread cheese spread using ultrafiltered dried skim milk ranged from 35.40-41.31%. Abdel-Baky *et al.* (1987) stated that, the total solids of processed spread cheese manufacture from ras cheese ranged from 44.15-45.00%. Tamime *et al.* (1999) found the total solids of processed spread cheese ranged from 34.2-44.4%. Abd-Rabo *et al.* (2004) reported that, the total solids content of spread cheese ranged from 51.67-54.79%.

The protein content of spread cheese made from groundnut milk at zero time was affected by the levels of skim milk powder. The levels of skim milk powder significantly ($p \leq 0.05$) affected the protein content of the spread cheese (Table 1). Sample D (15% skim milk powder) was the highest (17.56%) and sample A (0% skim milk powder) was the lowest (12.82%). Sample B (5% skim milk powder) and sample C (10% skim milk powder) showed an in intermediate position (14.35%, 15.98%, respectively). Tamime *et al.* (1999) found that, the protein content of processed spread cheese ranged from 11.6-14.4%. Salem *et al.* (1987) reported that the protein content of processed spread cheese were increased with increasing the ratio of whey powder. Kebray *et al.* (1998); Abd-EL-Salam *et al.* (1996); Khader *et al.* (1997) and Mahfouz *et al.* (1986) reported that the protein content of processed spread cheese ranged from 16.2-20.09%. Gouda and El-Shibiny (1987) concluded that the protein content of processed spread cheese increased with increasing the ratio of skim milk powder. Salem *et al.* (1987) reported that the protein contents of processed spread cheese were increased with increasing the ratio of whey powder. Kebray *et al.* (2001) found positive correlation between the protein content of spread cheese and the rate of whey powder.

The fat of spread cheese retained from different samples was nearly the same (Table 1). Salem *et al.* (1987) found that the fat content of low fat processed cheese varied from 9.0-12.0%. Kebray *et al.* (1998) concluded that, the fat content of low fat processed spread cheese ranged from 11.2-19.6%. Tamime *et al.* (1999) reported that, the fat content of processed cheese varied from 12.0-22.8%. Kebray *et al.* (2001) found that the fat content of low fat processed spread cheese ranged from 14.3-15.0%.

Table 1: Chemical composition* of spread cheese made by groundnut milk at zero time

	Samples			
Parameter	A	B	C	D
Total solids (%)	35.79±0.18^{d}	37.91±0.13^{c}	39.59±0.16^{b}	41.49±0.14^{a}
Protein content (%)	12.82±0.09^{d}	14.35±0.05^{c}	15.98±0.07^{b}	17.56±0.06^{a}
Fat content (%)	14.98±0.07^{a}	14.99±0.03^{a}	14.99±0.02^{a}	15.00±0.01^{a}
Ash content (%)	4.16±0.02^{ab}	4.18±0.06^{ab}	4.21±0.01^{a}	4.23±0.04^{a}

*Mean±S.D having different superscript letter in rows differ significantly ($p \leq 0.05$)
A = Spread cheese prepared from groundnut milk and 0% skim milk powder
B = Spread cheese prepared from groundnut milk and of 5% skim milk powder
C = Spread cheese prepared from groundnut milk and 10% skim milk powder
D = Spread cheese prepared from groundnut milk and 15% skim milk powder

Table 2: Effect of level of skim milk powder on pH-value* and titratable acidity* (as % lactic acid) of spread cheese

	Level of skim milk powder			
Quality attribute	0%	5%	10%	15%
pH-value	4.76^{a}±0.11	4.72^{ab}±0.03	4.61^{b}±0.01	4.40^{bc}±0.07
Titratable acidity	1.34^{c}±0.02	1.35^{c}±0.05	1.40^{b}±0.03	1.60^{a}±0.01

*Mean±SD having different superscript letters in rows are significantly different ($p \leq 0.05$)

The level of skim milk powder significantly ($p \leq 0.05$) affected the ash content of the spread cheese (Table 1). Sample D (15% skim milk powder) was the highest (4.32%). Sample A (0% skim milk powder) was the lowest (4.16%) with sample B (5% skim milk powder) and sample C (10% skim milk powder) occupy an intermediate position (4.18,4.21%, respectively). Tamime *et al.* (1999) found that ash content of processed spread cheese ranged from 3.0-4.0%.

The levels of skim milk powder significantly ($p \leq 0.05$) affected the pH-value of the spread cheese (Table 1). Sample D (15% skim milk powder) was the lowest (5.14) and sample A (0% skim milk powder) was the highest (5.60). Sample B (5% skim milk powder) and sample C (10% skim milk powder) occurred at an intermediate position (5.56 and 5.45), respectively.

Storage period significantly ($p \leq 0.05$) affected the pH-value of spread cheese (Table 3 and Fig. 1); the highest value (5.44) was obtained at the beginning of the storage period, while the lowest value (3.91) was obtained at the end of the storage period. Abdel-Baky *et al.* (1987) found that, the pH-value of processed spread cheese during storage at 4-5°C and at 20-25°C were 5.60 and 5.50, respectively. Salem *et al.* (1987) reported that, the pH-value of local-low fat processed cheese ranged from 5.95-5.70. Kebray *et al.* (1998) stated that, the pH-value of spread cheese decreased slightly as storage period progressed. Tamime *et al.* (1999) stated that, the pH-value of processed cheese analogues during storage at 5°C ranged from 5.91-5.85. Bisht and Jha (1999) found that the pH-value of spread cheese from acidified milk curd stored at 5°C was 5.32. Abd Rabo *et al.* (2004) concluded that, the pH-value of full-fat processed spread cheese ranged from 5.63-5.75.

Table 2 show the titratable acidity (as % lactic acid) as affected by the level of skim milk powder of spread cheese samples made from groundnut milk. The levels of skim milk powder significantly ($p \leq 0.05$) affected the

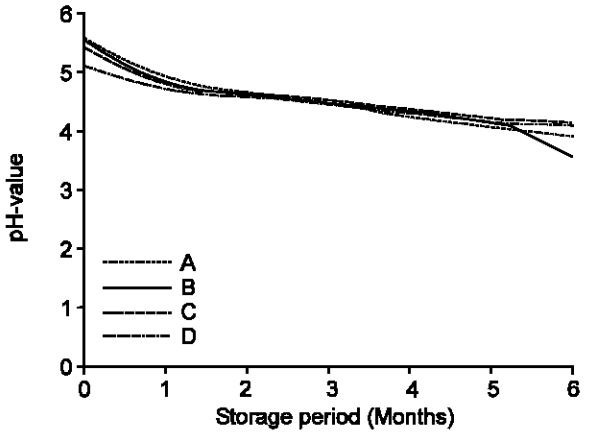

Fig. 1: Effect of storage period on pH-value of spread cheese

titratable acidity of the spread cheese. Sample D (15% skim milk powder) was the highest, while sample A (0% skim milk powder) was the lowest, with sample B (5% skim milk powder) and sample C (10% skim milk powder) being in an intermediate position.

Storage period significantly ($p \leq 0.05$) affected the titratable acidity of spread cheese (Table 3 and Fig. 2), the highest acidity (2.09%) was obtained at the end of storage period; the lowest acidity (0.64%) at the beginning of the storage period. Salem *et al.* (1987) reported that, the titratable acidity of low fat locally processed cheese increased with increasing the amount of denatured whey protein. Bisht and Jha (1999) found (0.38%) titratable acidity of spread cheese.

Organoleptic quality of spread cheese: The organoleptic quality of spread cheese made by groundnut milk was found to be affected by the levels of skim milk and storage period.

Table 3: Effect of storage period on pH-value* and titratable acidity (as % lactic acid)* of spread cheese

	Storage period (months)						
Quality attribute	0	1	2	3	4	5	6
pH-value	5.44^{a}±0.04	5.18^{b}±0.02	4.95^{c}±0.01	4.69^{d}±0.03	4.43^{e}±0.05	4.17^{f}±0.07	3.91^{g}±0.06
Titratable acidity	0.64^{g}±0.02	0.89^{f}±0.03	1.13^{e}±0.08	1.38^{d}±0.09	1.62^{c}±0.01	1.84^{b}±0.04	2.08^{a}±0.07

*Mean±SD having different superscript letters in rows are significantly different ($p \leq 0.05$)

Table 4: Effect of level of skim milk powder on organoleptic quality* of spread cheese

	Level of skim milk powder			
Quality attribute	0%	5%	10%	15%
Appearance	4.37^{c}±0.02	4.57^{a}±0.04	4.52^{b}±0.07	4.50^{b}±0.09
Texture	4.22^{c}±0.01	4.53^{a}±0.08	4.45^{b}±0.06	4.41^{b}±0.02
Flavour	4.30^{b}±0.07	4.47^{a}±0.02	4.46^{a}±0.05	4.45^{a}±0.03
Overall acceptability	4.29^{d}±0.06	4.53^{a}±0.01	4.47^{b}±0.04	4.36^{c}±0.03

*Mean±SD having different superscript letters in rows are significantly different ($p \leq 0.05$)

Results in Table 4 showed the effect of level of skim milk powder on organoleptic quality of spread cheese. B sample containing 5% skim milk powder significantly ($p \leq 0.05$) secured the best appearance (4.57) while other samples of spread cheese made with 10% and 15% skim milk powder scored 4.52 and 4.50 respectively. The worst appearance was recorded by spread cheese made with 0% skim milk powder (4.37).

Storage period significantly ($p \leq 0.05$) affected the appearance of the spread cheese (Table 5 and Fig. 3), the best score (4.63) was obtained at the 3rd month and the worst (4.30) at beginning of the storage period. Abdel -Baky *et al.* (1987) stated that, the processed spread cheese made from ras cheese revealed better flavour and appearance during storage at both refrigerator and at room temperatures. Kebray *et al.* (1998) reported that, the scores of organoleptic properties of spread cheese made from different blends decreased as storage period progressed except the scores of colour.

Results in Table 4 show the texture score of the spread cheese. Sample B (5% skim milk powder) significantly ($p \leq 0.05$) secured the best texture (4.53) followed by sample C made with 10% skim milk powder (4.45) and sample made with 15% skim milk powder (4.40). The worst texture (4.22) was recorded by sample A made with 0% skim milk powder.

Storage period significantly ($p \leq 0.05$) affected the texture of the spread cheese (Table 5 and Fig. 4), the best score (4.56) was obtained at the 3rd month, the worst texture (4.24) was obtained at the end of the storage period.

Results in Table 4 show the flavour score of the spread cheese. Samples B, C and D (5, 10 and 15% skim milk powder) significantly ($p \leq 0.05$) secured the best flavour (4.47, 4.47 and 4.36, respectively), the worst flavour (4.3) was recorded by sample A made with 0% skim milk powder. Storage period significantly ($p \leq 0.05$) affected the flavour of the spread cheese (Table 5 and Fig. 5), the best score (4.58) was obtained at the 3rd month and the worst score (4.26) was obtained at the end of the storage period. Abdel-Baky *et al.* (1987) stated that, the processed spread cheese made from ras cheese revealed better flavour and appearance during storage at both refrigerator and at room temperatures.

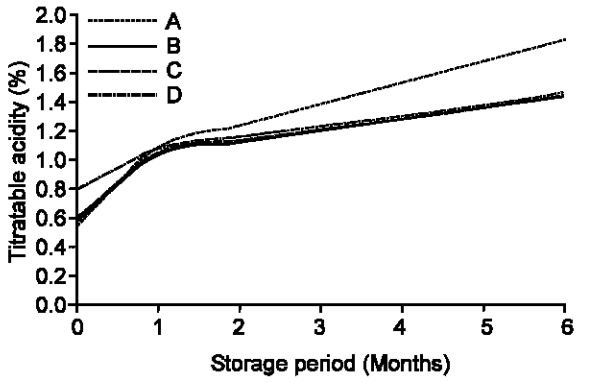

Fig. 2: Effect of storage period on titratable acidity of spread cheese

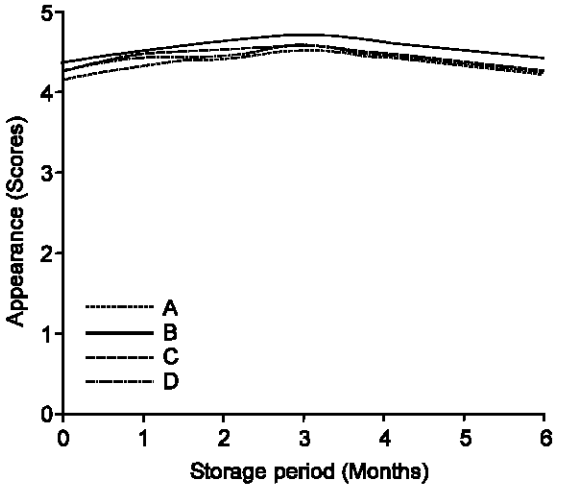

Fig. 3: Effect of storage period on appearance of spread cheese

Table 5: Effect of storage period on organoleptic quality* of spread cheese

	Storage period (months)						
Quality attribute	0	1	2	3	4	5	6
Appearance	4.30^{a}±0.02	4.46^{c}±0.08	4.53^{b}±0.03	4.63^{a}±0.05	4.51^{b}±0.04	4.41^{d}±0.01	4.44^{cd}±0.06
Texture	4.30^{d}±0.07	4.40^{c}±0.08	4.50^{b}±0.09	4.56^{a}±0.06	4.56^{a}±0.07	4.34^{cd}±0.02	4.24^{e}±0.01
Flavour	4.30^{e}±0.11	4.40^{c}±0.02	4.46^{b}±0.01	4.58^{a}±0.05	4.46^{b}±0.06	4.36^{d}±0.12	4.26^{e}±0.09
Overall acceptability	4.31^{cd}±0.06	4.40^{c}±0.09	4.51^{a}±0.08	4.59^{ab}±0.03	4.56^{b}±0.04	4.38^{d}±0.03	4.28^{e}±0.01

*Mean±SD having different superscript letters in rows are significantly different ($p \leq 0.05$)

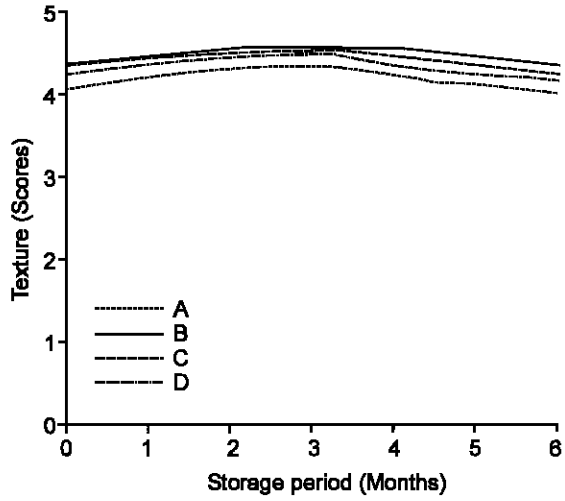

Fig. 4: Effect of storage period on texture of spread cheese

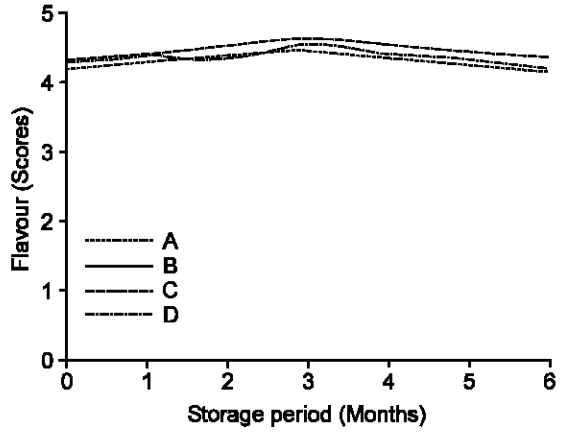

Fig. 5: Effect of storage period on flavour of spread cheese

Results in Table 4 show the overall acceptability of the spread cheese. Sample B (5% skim milk powder) significantly ($p \leq 0.05$) secured the best (4.53) acceptability and the worst (4.29) was recorded by sample A made with 0% skim milk powder.

Storage period significantly ($p \leq 0.05$) affected the acceptability of the spread cheese (Table 5 and Fig. 6), the best score (4.59) was obtained at the 3rd month and the worst value (4.28) was obtained at the end of the storage period. Salem *et al.* (1987) found that, the processed cheese prepared from milk fortified with whey protein gave a good and higher score than that of the control. Abdel-Baky *et al.* (1987) stated that, the processed spread cheese made from ras cheese revealed better flavour and appearance during storage at both refrigerator and room temperatures.

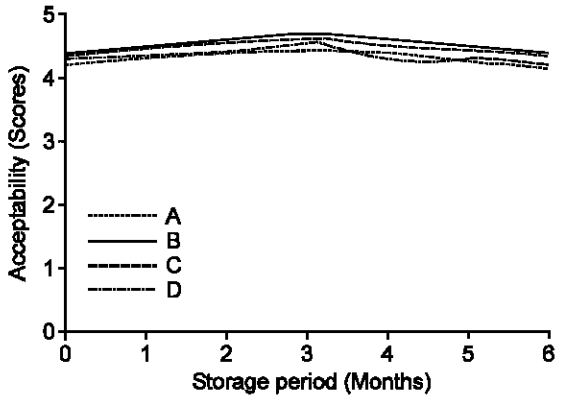

Fig. 6: Effect of storage period on acceptability of spread cheese

REFERENCES

Abdel-Baky, A.A., A.A. El-Neshawy and S.M. Farahat, 1987. The use of ras cheese made by direct acidification in the manufacture of processed cheese spread. Egyptian J. Dairy Sci., 15: 273-285.

Abd-El-Salam, M.H., A.F. Al-Khamy, G.A. El-Garawany, A. Hamed and A. Khader, 1996. Composition and rheological properties of processed cheese spread as affected by the level of added whey protein concentrates and emulsifying salt. Egyptian J. Dairy Sci., 24: 309-322.

Abd-Rabo, M.A., H.R. Fawzia, M.A. El-Asser and M. Mahassen, 2004. Characterization of low-fat proceeds cheddar cheese spreads prepared from mixture of buffaloes and cow's milk. The 9th Egyptian Conf. for Dairy Sci., Tech. "Milk and Dairy products for a Healthy Future", Dokki, Cairo, Egypt.

AOAC, 1995. Method of the Analysis of Official Analyst Chemists 15th Edn. Washington.

Bisht, R.S. and Y.K. Jha, 1999. Storage stability of cheese spread from acidified milk curd. J. Food Sci. Technol., 36: 15-18.

Drake, M.A., Y.K.R. Karagul-Yuccer, C.V. Civille and P.S. Tong, 2003. Determination ingredients. J. Sensory Stud., 18: 199-216.

Gouda, E. and S. El-Shibiny, 1987. The use of ultra-filtered skim milk in the manufacture of processed cheese spread. Egyptian J. Dairy Sci., 15: 255-262.

Graham, D.M., 1970. Industrial view of imitation milk products. J. Dairy Sci., 53: 103-105.

Hinds, M.J., L.R. Beuchart and M.S. Chinnan, 1997. Effects of homogenization pressure and stabilizers on some physical characteristics of a beverage prepared from partially defatted, roasted peanuts. J. Plant Hum. Nutr. (Formerly Qualitas Plantarum), 50: 269-277.

Kebray, K.M.K., A.M. Ahmed and R.M. Badawi, 1998. Impact of fat replacers on properties of low fat processed cheese spread. Proc. 8th Egyptian Conf. Dairy Sci. Tech., 383-401.

Kebray, K.M.K., S.A. Hussein and R.M. Badawi, 2001. The use of whey protein in flavored low-fat processed cheese spread. Proc. 8th Egyptian Conf. Dairy Sci. and Technol., 369-381.

Khader, A., A. Hamed, A.F. Al-Khamy, G.A. El-Garawany and M.M. Abd El-Salam, 1997. Chemical composition and some properties of market processed cheese. Egyptian J. Dairy Sci., 25: 269-279.

Lee, C. and L.R. Beuchat, 1992. Chemical, physical and sensory characteristics of peanut milk as affected by processing conditions. J. Food Sci., 57: 401-405.

Mahfouz, M.B., H.F. El-Dien, S. El-Shibiny, H.F. Maggag and M.N. Mahgoub, 1986. Chemical and microbiological quality of market processed cheese. Egyptian J. Dairy Sci., 14: 33-41.

Nwokalo, E., 1996. Peanut (*Arachis lypogaea* L.) In: Nwokolo E, Mart, J.S. (Eds.). Legumes and oil seeds in nutrition. Chapman and Hall, London, P49.

Patrick, F., P.G. Timothy, T.M. Cogan and L.H. Paul, 2000. Fundamental of Cheese Science. Aspen Publishers, Inc. Gaithersburg, Maryland.

Roberts-Sunny, E.O., 2007. An evaluation of the nutritional and physicochemical properties of candies from imitation milks. University of Kassel-Witzenhausen and University of Göffingen. Conf. on Int. Agric. Research for Devel.

Roberts-Sunny, E.O., E.T. Ofunda and B.T. Iwakun, 2004. An evaluation of some quality parameters of laboratory-prepared fermented groundnut milk. J. Eur. Food Res. Technol., 218: 452-455.

Salem, S.A., A.E. Salem and E. Gouda, 1987. Improvement of chemical, rheological and organoleptic properties for local-low fat processed cheese. Egyptian J. Dairy Sci., 15: 263-271.

SAS, 1997. SAS/STAT User's Guide, Version 6.03 edition, Cary, NC: SAS Institute Inc., pp: 1028.

Tamime, A.Y., D.D. Muir, M.E. Dhenana and A.M. Dawood, 1999. Processed cheese analogues incorporating fat-substitutes. I. Composition, microbiological and falvour changes during storage at 5^{o}C. Lebensm-Wiss.u.-Technol., 32: 41-49.

Vargas, G., N.R. Grasso, A.G. Lópen and A.C. Guzmán, 1998. Chemical and physical characteristics of Argentinean groundnut milk. International a rachis Newsletter.

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Optimization of Ultrasonic Extraction of Polysaccharides from Chinese Malted Sorghum Using Response Surface Methodology

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Abstract: Ultrasonic technology was applied for polysaccharides extraction from the Chinese malted sorghum and Response Surface Methodology (RSM) was used to optimize the effects of processing parameters on polysaccharides yields. Three independent variables were ultrasonic power (X_1), extraction time (X_2) and ratio of water to raw material (X_3), respectively. The statistical analysis indicated that three variables and the quadratic of X_1 and X_2 had significant effects on the yields and followed by the significant interaction effects between the variables of X_2 and X_3 ($p<0.05$). A mathematical model with high determination coefficient was gained and could be employed to optimize polysaccharides extraction. The optimal extraction conditions of polysaccharides were determined as follows: Ultrasonic power 600 W, extraction time 4 min, ratio of water to raw material 30 ml/g. Under these conditions, the experimental yield of polysaccharides was 17.08±0.33%, which was agreed closely with the predicted value 17.06%.

Key words: Response surface methodology, malted sorghum, optimization, polysaccharides extraction

INTRODUCTION

As more than 500 million people in the developing countries depend on sorghum as the main staple food, relevant scientific information generated for this crop can certainly play a key role in food development (Mutisya *et al*., 2009). Recently, there has been increased interest in sorghum as a gluten-free cereal to substitute the gluten-rich cereals in the diet of people suffering from celiac disease. Polysaccharides and lignin have been considered as the main functional compositions and great attentions have been paid for their great health effects (Yang *et al*., 2008a), such as promoting blood metabolism, soothe nerves, relieve insomnia, etc. (Yang *et al*., 2008b; Yang *et al*., 2009). Polysaccharides from plant, epiphyte and animals extracts are an interesting source of additives for several industries, in particular food and drug industry (Forabosco *et al*., 2006). They play important roles in the growth and development of living organisms and have been widely studied in recent years due to their unique biological, chemical and physical properties (Schepetkin and Quinn, 2006). Whereas, there have been only a few reports on Chinese malted sorghum polysaccharides and few on its functional effects. One of the reasons is the lack of high efficient extraction technology of polysaccharides from Chinese malted sorghum pulp. Hot-water technology is the main extraction method of Chinese malted sorghum polysaccharides in recent research, which is a classical extraction of polysaccharides. It usually requires long extraction time, high temperature and extraction efficiency is low (Li *et al*., 2007). Therefore, it's essential and desirable to find an economical and high efficient extraction method of polysaccharides from Chinese malted sorghum.

Ultrasonic treatment has been employed for preparing polysaccharides from different plant materials in recent years and showed the great extraction efficiency (Hromadkova *et al*., 1999; Hromadkova and Ebringerova, 2003; Hofmann *et al*., 2006; Hemwimon *et al*., 2007; Wang *et al*., 2009). This great extraction efficiency by ultrasonic treatment is mainly attributed to its mechanical effects, which greatly facilitate mass transfer between immiscible phases through a super agitation (Vinatoru *et al*., 1997) and the most important mechanical effects of ultrasonic treatments are microjetting and microstreaming (Tsochatzidis *et al*., 2001; Velickovic *et al*., 2006). Response Surface Methodology (RSM) is an affective statistical technique for optimizing complex processes. The main advantage of RSM is the reduced number of experimental trials needed to evaluate multiple parameters and their interactions. Therefore, it is less laborious and time-consuming than other approaches required to optimize a process (Giovanni, 1983). It is wide used in optimizing the extraction process variables, such as polysaccharides, anthocyanins, vitamin E, phenolic compounds and protein from varied materials (Cacace and Mazza, 2003; Ge *et al*., 2002; Chandrika and Fereidoon, 2005; Lee *et al*., 2005; Li and Fu, 2005; Liyana-Pathirana and Shahidi, 2005a; Qiao *et al*., 2009).

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Box-Behnken Design (BBD), one of RSM, only have three levels and need fewer experiments. It's more efficient and easier to arrange and interpret experiments in comparison with others and widely used by many researches (Box and Behnken, 1960; Ferreira *et al*., 2007).

In this study, the main objective was to optimize ultrasonic technology conditions for the extraction of polysaccharides from Chinese malted sorghum. RSM was designed to systemic analyze the effects of extraction parameters on the yields of polysaccharides from Chinese malted sorghum and their interactions.

MATERIALS AND METHODS

Experimental materials and chemicals: Sorghum [*Sorghum bicolor* (L.) Moench] was grown in Shandong, a coastal province East of China and known to have average January temperature of 0°C and July 28°C. The average annual rainfall is about 500 mm, most of which falls in the summer. Red sorghum was obtained from 2007 and 2008 harvest. The length/breadth ratio of sorghum kernel was 1.12/1.23 and the density (g/L) was 691.40. The average weight of 1000 kernels was 26.80 g.

All the chemicals used were of analytical grade and purchased from Sinopharm Chemicals Reagent Company (SCRC), Shanghai, China.

Soaking, malting and preparation of sorghum flour: After removing chaff and unviable grain, sorghum grains (1000 g) were thoroughly cleaned by washing with tap water and then soaked in wooden ash extract. The grains were soaked for 24 h at 30°C with the soaking water being changed at 6 h interval. After soaking, the grains were spread on jute bags and covered with the same material in a secluded and dark area. Malting was allowed to proceed for different time intervals (3, 5 and 6 days) and the temperature of malting kernels was 25°C. The growth was terminated by kilning in a forced air oven at 40°C for 24 h. The withered rootless were gently brushed off and dried grain were milled using a bench-top attrition mill (Dade, DFT-600, 25000 rpm, Zhejiang Linda Mechanic Co., Ltd., China). The resultant flour was sieved into a particle size of 70-mesh. The flour was then packaged in a low density polyethylene bag and was stored using plastic containers with lids in a refrigerator at 4°C for later analysis.

Extraction of polysaccharides from Chinese malted sorghum with ultrasonic treatment: The process of polysaccharides extraction from Chinese malted sorghum by ultrasonic treatment was performed in an ultrasonic cell disintegrator (JBT/C-YCL400T, Xinzhi Biotechnology and Science Inc., Lingbo, Henan Province, China). Two grams of Chinese malted sorghum powders were extracted with distilled water in a 100-ml beaker, then the beaker was held in the ultrasonic cell disintegrator and exposed to extract for different time at varied ultrasonic power. Ice bathing was used to ensure the temperature of solution was below 50°C in the whole extraction processing.

Isolation and determination yield of Chinese malted sorghum polysaccharides: After the extraction with ultrasonic treatment, the extracted slurry was centrifuged at 10000 rpm/min for 20 min to collect the supernatant, and the insoluble residue was treated again for 2-3 times as mentioned above. The supernatant was incorporated and concentrated to one-fifth of initial volume using a rotary evaporator (SENCO Technology and Science Inc., Shanghai, China) at 55°C under vacuum. The resulting solution was mixed with four volumes of dehydrated ethanol (ethanol final concentration, 80%) and kept overnight at 4°C. Then the solution was centrifuged at 10000 rpm/min for 20 min, washed six times with dehydrated ethanol and the precipitate was collected as crude extract. The extract was air-dried at 50°C until its weight was constant and then was weighted with a balance (AY 120, SHIMADZU, Japan). The percentage polysaccharides yield (%) was calculated as follows:

$$\text{Yield } (\%) = \frac{\text{Weight of dried crude extractraction (g)}}{\text{Weight of malted sorghum powder (g)}} \times 100 \quad (1)$$

Experimental design: A three level, three variable Box-Behnken Factorial Design (BBD) (Design Expert software, Version 6.0.5, Stat-Ease Inc., Minneapolis, MN) was applied to determine the best combination of extraction variables for the yields of Chinese malted sorghum polysaccharides. Three extraction variables considered for this research were X_1 (ultrasonic power), X_2 (extraction time) and X_3 (ratio of water to raw material) (Li *et al*., 2007) and the proper range of three variables were determined on the basis of single-factor experiment for the polysaccharides production (Table 1). Table 1 listed the whole design consisted of 17 experimental points, five replicates (treatment 13-17) at the centre of the design were used to allow for estimation of a pure error sum of squares. The triplicates were performed at all design points in randomized order. Experimental data were fitted to a quadratic polynomial model and regression coefficients obtained. The non-linear computer generated quadratic model used in the response surface was as follows:

$$Y = \beta_0 + \sum_{i=0}^{4}\beta_i X_i + \sum_{i=0}^{4}\beta_{ii} X_i^2 + \sum_{i=0}^{4}\sum_{j=0}^{4}\beta_{ij} X_i X_j \quad (2)$$

Where Y is the measured response associated with each factor lever combination; β_0 is an intercept; β_i is

Table 1: Box-Behnken experimental design with the independent variables

	Valuables levels			Polysaccharides yield (Y) (%)	
Run	X_1	X_2	X_3	Experimental	Predicted
1	-1(500)	-1(3.5)	0(30)	11.01±0.32	10.88
2	1(700)	-1(3.5)	0(30)	16.15±0.26	16.23
3	-1(500)	1(4.5)	0(30)	11.89±0.61	11.83
4	1(700)	1(4.5)	0(30)	16.70±0.11	16.83
5	-1(500)	0(4.0)	-1(25)	11.78±0.08	11.92
6	1(700)	0(4.0)	-1(25)	17.26±0.32	17.21
7	-1(500)	0(4.0)	1(35)	11.66±0.24	11.72
8	1(700)	0(4.0)	1(35)	16.90±0.43	16.76
9	0(600)	-1(3.5)	-1(25)	16.12±0.56	16.11
10	0(600)	1(4.5)	-1(25)	17.48±0.16	17.42
11	0(600)	-1(3.5)	1(35)	16.26±0.22	16.33
12	0(600)	1(4.5)	1(35)	16.54±0.09	16.56
13	0(600)	0(4.0)	0(30)	17.36±0.24	17.06
14	0(600)	0(4.0)	0(30)	17.03±0.93	17.06
15	0(600)	0(4.0)	0(30)	16.97±1.67	17.06
16	0(600)	0(4.0)	0(30)	17.08±0.33	17.06
17	0(600)	0(4.0)	0(30)	16.88±0.41	17.06

regression coefficients computed from the observed experimental values of Y and X_i is the coded levels of independent variables. The terms X_i, X_j and X_i^2 represent the interaction and quadratic terms, respectively.

Statistical analyses: Data were expressed as means Standard Errors (SE) of three replicated determinations. The responses obtained from each set of experimental design (Table 1) were subjected to multiple non-linear regressions using the Design Expert software (Version 6.0.5, Stat-Ease Inc., Minneapolis, MN). The quality of the fit of the polynomial model equation was expressed by the coefficient of determination R^2 and the significance of the regression coefficient were checked by F-test and p-value.

RESULTS AND DISCUSSION

Fitting the model: A regression analysis (Table 2) was carried out to fit mathematical models to the experimental data aiming at an optimal region for the responses studied. Predicted response Y for the yield of Chinese malted sorghum polysaccharides could be expressed by the following second order polynomial equation in terms of coded values:

$$Y = 17.06 + 2.59X_1 + 0.39X_2 - 0.16X_3 - 2.66X_1^2 - 0.46X_2^2 + 2.5.10^{-3}X_3^2 - 0.088X_1X_2 - 0.060X_1X_3 - 0.27X_2X_3 \quad (3)$$

Where Y is the yield of Chinese malted sorghum polysaccharides (g) and X_1, X_2 and X_3 are the coded variables for ultrasonic power, extraction time and the ratio of water to the raw material, respectively.

In general, exploration and optimization of a fitted response surface may produce poor or misleading results, unless the model exhibits a good fit, which makes checking of the model adequacy essential (Liyana-Pathirana and Shahidi, 2005b). The F-ratio in this table is the ratio of the mean square error to the pure error obtained from the replicates at the design centre. The significance of the F-value depends on the number of Degrees of Freedom (DF) in the model, and is shown in the p-value column (95% confidence level). Thus, the effects lower than 0.05 in this column are significant (Cai *et al*., 2008; Qiao *et al*., 2009).

Table 2 listed the Analysis of Variance (ANOVA) for the fitted quadratic polynomial model of extraction of Chinese malted sorghum polysaccharides.

F-test suggested that model had a very high model F-value (F = 302.64) and a very low p-value ($p<0.0001$); indicating this model was highly significant. The lack of fit measures the failure of the model to represent the data in the experimental domain at points which are not included in the regression. As showed in Table 2, F-value and p-value of the lack of fit were 0.91 and 0.5103, respectively, which implied it was not significant relative to the pure error and indicated that the model equation was adequate for predicting the Chinese malted sorghum polysaccharides under any combination of values of the variables. R^2 adj (adjusted determination coefficient) is the correlation measure for testing the goodness-of-fit of the regression equation. Higher it is the better degree of correlation between the observed and predicted values (Ravikumar *et al*., 2006). The value of R^2 adj for Eq. (3) was 0.994, which was reasonably close to 1 and implied that only less 1.0% of the total variations were not explained by model.

Meanwhile, it also confirmed that the model was highly significant and indicated a high degree of correlation between the observed and predicted data. Coefficient of Variation (CV) indicates the degree of precision with which the experiments are compared. A relatively low value of CV (1.14) in Table 2, which showed a better precision and reliability of the experiments carried out.

Table 2: Analysis of variance for the fitted quadratic polynomial model of extraction of polysaccharides

Source	SS	DF	MS	F-value	Prob>F
Model	86.6861	9	9.6300	302.6419	<0.0001
Residual	0.2227	7	$31.8257x10^{-3}$		
Lack of fit	$90.524 x10^{-3}$	3	$30.17467x10^{-3}$	0.91	0.5103
Pure error	$132.256 x10^{-3}$	4	$33.0640x10^{-3}$		
Cor Total	86.9089	16			
	R^2 = 0.997	R^2adj = 994	CV = 1.14		

SS: Sum of Squares; DF: Degree of Freedom; MS: Mean Square

Table 3: Estimated regression model of relationship between response variables (yield of Chinese malted sorghum polysaccharides) and independent variables (X_1, X_2, X_3)

Variables	DF	SS	MS	F-Value	p-Value
X_1	1	53.4578	53.4578	1679.7046	<0.0001
X_2	1	1.1889	1.1889	37.3560	0.0005
X_3	1	0.2060	0.2060	6.4753	0.0384
X_1X_1	1	29.8480	29.8480	937.8588	<0.0001
X_2X_2	1	0.8890	0.8890	27.9337	0.0011
X_3X_3	1	$2.6315x10^{-5}$	$2.6315x10^{-5}$	$8.2687x10^{-4}$	0.9779
X_1X_2	1	0.0309	0.0309	0.9733	0.3567
X_1X_3	1	0.0144	0.0144	0.4524	0.5227
X_2X_3	1	0.2981	0.2981	9.3671	0.0183

SS: Sum of Squares; DF: Degree of Freedom; MS: Mean Square

The significance of each coefficient was determined using p-value in Table 3. The p-value is used as a tool to check the significance of each coefficient and the interaction strength between each independent variable. The corresponding variables would be more significant at greater F-value and smaller p-value (Atkinson and Donev, 1992). The data in the Table 3 indicated that all the independent variables (X_1, X_2, X_3) and two quadratic terms (X_{12} and X_{22}) significantly affected the yield of Chinese malted sorghum polysaccharides and there was significant interaction between extraction time (X_2) and ratio of water to raw material (X_3). Meanwhile, the ultrasonic power (X_2) was the major factor affecting the yield of polysaccharides.

Analysis of response surface: The 3D response surface and 2D contour plots are the graphical representations of regression equation. They provide a method to visualize the relationship between responses and experimental levels of each variable and the type of interactions between two test variables. The shapes of the contour plots, circular or elliptical, indicate whether the mutual interactions between the variables are significant or not. Circular contour plot indicates that the interactions between the corresponding variables are negligible, while elliptical contour plot indicates that the interactions between the corresponding variables are significant.

The relationship between independent and dependent variables was illustrated in tri-dimensional representation of the response surfaces and two-dimensional contour plots generated by the model for yield of polysaccharides (Fig. 1-3), two variables were depicted in one tri-dimensional surface plots while the other variable kept at level zero. It is clear that the yield of

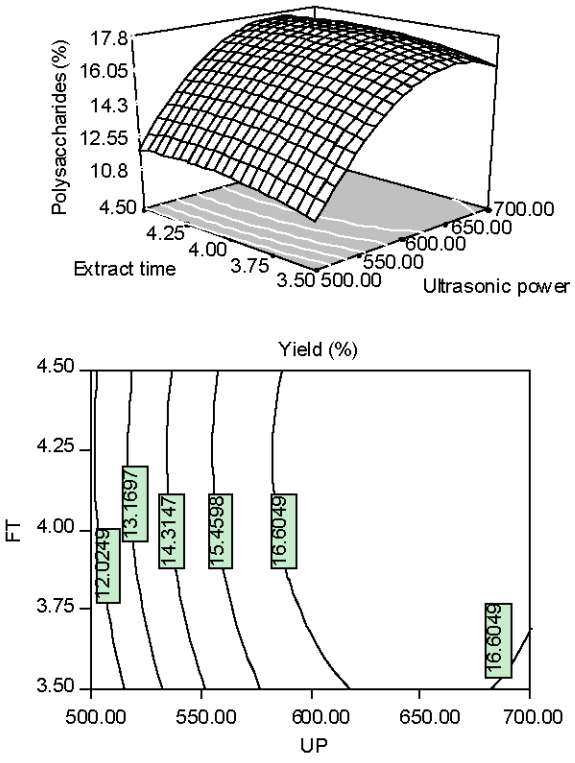

Fig. 1: Response surface plot and contour plot of Ultrasonic Power (UP) and Extraction Time (ET) and their mutual interactions on the yield of Chinese malted sorghum polysaccharides

polysaccharides was sensitive to minor alterations of the test variables (ultrasonic power, extraction time and ratio of water to raw material).

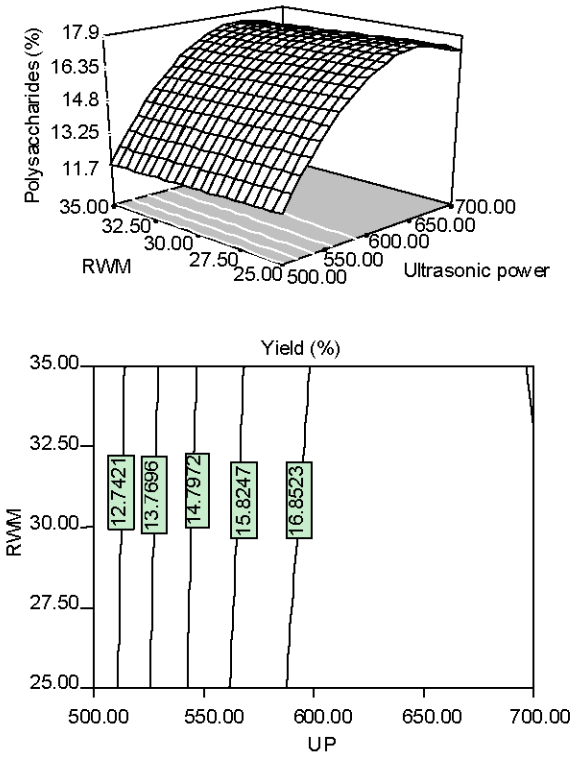

Fig. 2: Response surface plot and contour plot of Ultrasonic Power (UP) and the Ratio of Water to Material (RWM) and their mutual interactions on the yield of Chinese malted sorghum polysaccharides

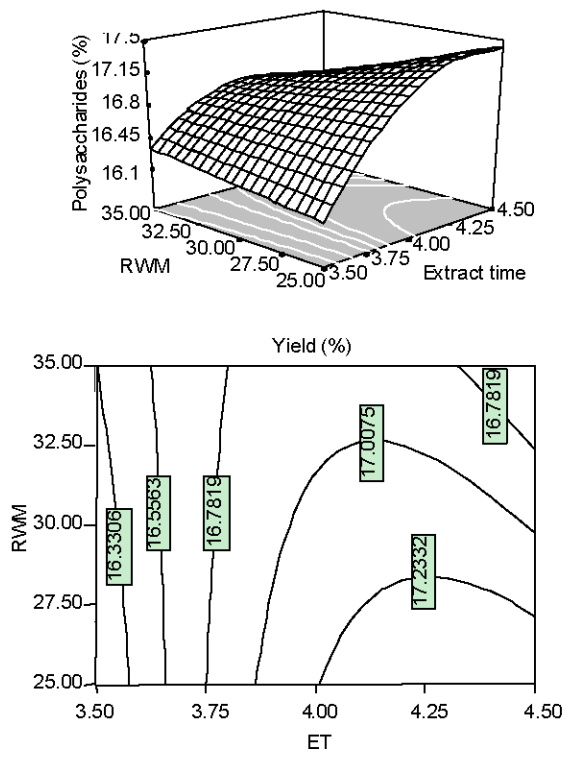

Fig. 3: Response surface plot and contour plot of Extraction Time (ET) and the Ratio of Water to Material (RWM) and their mutual interactions on the yield of Chinese malted sorghum polysaccharides

The interaction relationships of ultrasonic power (X_1) with the extraction time (X_2) and radio of water to material (X_3) on the yield of polysaccharides were shown in Fig. 1 and 2, respectively and indicated that these three variables had all significant effect on the yield of Chinese malted sorghum polysaccharides. As shown in Fig. 1 and 2, the ultrasonic power (X_1) and extraction time (X_2) had positive impact on the polysaccharides production, while the yield changed slightly when the ratio of water to material (X_3) was in the range of 25-35 ml/g. Yield of polysaccharides rapid enhanced with the increasing of ultrasonic power (X_1) and reached to the peak value at 600 W. With the farther increasing of ultrasonic power (X_1), the yield went to slight decrease. Longer extraction time (X_2) had positive effects on the yield extraction and had a critical value at 4 min when at a constant ultrasonic power (600 W). This suggested more yield was resulted at higher ultrasonic power, longer extraction time and lower ratio of water to material.

It was considered higher extraction efficiency of polysaccharides at higher ultrasonic power due to the increase in the number of cavitations bubbles formed and enhance mass transfer rates.

However, less yield was resulted at farther increasing ultrasonic power. The same result was gained by Li *et al.* (2007) and was considered for that a part of polysaccharides could be more depolymerize into some free sugars.

It was shown that the interactions between the ultrasonic power and other two extraction variables did not impact the yield of polysaccharides significantly (Table 3, Fig. 1 and 2), in spit of the ultrasonic power was the major factor affecting the yield of polysaccharides. This observation was in agreement with previous investigation. Li *et al.* (2007) researched the optimization of the ultrasonically assisted extraction of polysaccharides from Zizyphus jujuba cv. Jinsixiaozao by RSM and analyzed the effects of interactions of extraction variables on the yield of polysaccharides. They also indicated that the interactions between ultrasonic power and extraction time and ultrasonic power and ratio of water to material caused no significant effect on the extraction yield, while ultrasonic power had significant effect. Fig. 3 showed the response surface plot at various extraction times (X_2) and ratio of water to material (X_3). The response curves demonstrated that higher yield at longer extraction time. The response curves

were comparatively smooth at lower extraction time, indicating the less effect on the increasing of the yields extraction when ratio of water to material changed in the range from 25-35 ml/g. However, the yield decreased with the farther enhancing of ratio of water to material at longer extraction time. This result indicated that extraction time (X_2) had a different extent of influence on extraction yield in different ratio of water to materials (X_3), and significant interactions were existed between extraction time (X_2) and ratio of water to raw material (X_3). Higher yields of polysaccharides were resulted at longer extraction time and lower ratio of water to material in the experimental range.

As shown in Fig. 3 and Table 3, the interactions of extraction time and ratio of water to materials had significant effect on the extraction yields, which was the same with other research results (Rodrigues *et al*., 2008; Wang *et al*., 2009). This conclusion was inconsistent with the observation obtained by Li *et al*. (2007), who reported that this interaction caused no significant effect on the extraction yields at a constant ultrasonic power. This contradiction was possibly due to the large difference in parameters of sonic power. In this study, the sonic power (>500 W) were far greater than that (60 W) in Li *et al*. (2007).

Confirmative tests: The suitability of the model equation for predicting the optimum response value was tested using the recommended optimum conditions. Optimum values of independent variables (Ultrasonic power of 600 W, extraction time of 4 min and ratio of water to material 30 ml/g.) were incorporated into the regression equation. A mean value of 17.064±0.181% (N = 5) was gained and was in agreement with the predicted value significantly ($p>0.05$) obtained from real experiments which demonstrated the validation of the RSM model. The results of analysis confirmed that the response model was adequate for reflecting the expected optimization and the model of Eq. (3) was satisfactory and accurate.

Conclusion: Ultrasonic technology was performed for the polysaccharides extraction from Chinese malted sorghum in order to increase the yield extraction. The experimental value of polysaccharides yield varied from 11.01-17.48% of malted sorghum. Based on the single-factor experiments, Response Surface Methodology (RSM) was used to estimate and optimize the experimental variables-ultrasonic power, extraction time and ratio of water to raw material. All the independent variables, quadratic of ultrasonic power and extraction time had high significant effects on the response values, followed by the significant interaction effects between the extraction time and ratio of water to material. A high correlation of the quadratic polynomial mathematical model was gained and could be great employed to optimize polysaccharides extraction from Chinese malted sorghum by ultrasonic technology. The optimal extraction conditions for the polysaccharides were determined as follows: Ultrasonic power 600 W, extraction time 4 min, ratio of water to material 30 ml/g. Under these conditions, the experimental yield of polysaccharides was 17.08±0.33%, which was agreed closely with the predicted yield value.

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REFERENCES

Atkinson, G.L. and A.N. Donev, 1992. Optimum Experimental Design. Oxford: Clarendon Press.

Box, G.E.P. and D.W. Behnken, 1960. Some new three level designs for the study of quantitative variables. Technometrics, 2: 455-475.

Cacace, J.E. and G. Mazza, 2003. Optimization of extraction of anthocyanins from black currants with aqueous ethanol. J. Food Sci., 68: 240-248.

Cai, W.R., X.H. Gu and J. Tang, 2008. Extraction, purification and characterization of the polysaccharides from Opuntia milpa alta. Carbohyd. Polym., 71: 403-410.

Chandrika, L.P. and S. Fereidoon, 2005. Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chem., 93: 47-56.

Ferreira, S.L.C., R.E. Bruns, H.S. Ferreira, G.D. Matos, J.M. David and G.C. Brand, 2007. Box–Behnken design: An alternative for the optimization of analytical methods. Analytica Chimica Acta, 597: 179-186.

Forabosco, A., G. Bruno, L. Sparapano, G. Liut, D. Marino and F. Delben, 2006. Pullulans produced by strains of Cryphonectria parasitica-I. Production and characterisation of the exopolysaccharides. Carbohyd. Polym., 63: 535-544.

Ge, Y., Y. Ni, H. Yan, Y. Chen and T. Cai, 2002. Optimization of the supercritical fluid extraction of natural vitamin E from wheat germ using response surface methodology. J. Food Sci., 67: 239-243.

Giovanni, M., 1983. Response surface methodology and product optimization. Food Technol., 45: 3741.

Hemwimon, S., P. Pavasant and A. Shotipruk, 2007. Microwave-assisted extraction of antioxidative anthraquinones from roots of Morinda citrifolia. Sep. Purif. Technol., 5444: 5540.

Hofmann, R., T. Kappler and C. Posten, 2006. Pilot-scale press electrofiltration of biopolymers. Sep. Purif. Technol., 51: 303-309.

Hromadkova, Z. and A. Ebringerova, 2003. Ultrasonic extraction of plant materials-Investigation of hemicellulose release from buckwheat hulls. Ultrason. Sonochem., 10: 127-133.

Hromadkova, Z., A. Ebringerova and P. Valachovic, 1999. Comparison of classical and ultrasound-assisted extraction of polysaccharides from *Salvia officinalis* L. Ultrason. Sonochem., 5163: 168.

Lee, G.D., J.O. Kim and J.H. Kwon, 2005. Optimum conditions for the extraction of effective substances from the stem of Opuntia fiscus-indica. Food Sci. Biotechnol., 14: 190-195.

Li, J.W., S.D. Ding and X.L. Ding, 2007. Optimization of the ultrasonically assisted extraction of polysaccharides from *Zizyphus jujuba* cv. Jinsixiaozao. J. Food Eng., 80: 176-183.

Li, Q.H. and C.L. Fu, 2005. Application of response surface methodology for extraction optimization of germinant pumpkin seeds protein. Food Chem., 92: 701-706.

Liyana-Pathirana, C.M. and F. Shahidi, 2005a. Antioxidant activity of commercial soft and hard wheat (*Triticum aestivium* L.) as affected by gastric pH conditions. J. Agric. Food Chem., 53: 2433-2440.

Liyana-Pathirana, C.M. and F. Shahidi, 2005b. Optimization of extraction of phenolic compounds from wheat using response surface methodology. Food Chem., 93: 47-56.

Mutisya, J., C. Sun, S. Rosenquist, Y. Baguma and C. Jansson, 2009. Diurnal oscillation of SBE expression in sorghum endosperm. J. Plant Physiol., 166: 428-434.

Qiao, D.L., C.L. Kea, B. Hua, J.G. Luo, H. Ye and Y. Sun, 2009. Antioxidant activities of polysaccharides from Hyriopsis cumingii. Carbohyd. Polym., 78: 199-204.

Ravikumar, K., S. Ramalingam, S. Krishnan and K. Balu, 2006. Application of response surface methodology to optimize the process variables for reactive red and acid brown dye removal using a novel absorbent. Dyes Pigments, 70: 18-26.

Rodrigues, S., G.A.S. Pinto and F.A.N. Fernandes, 2008. Optimization of ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder by response surface methodology. Ultrason. Sonochem., 15: 95-100.

Schepetkin, I.A. and M.T. Quinn, 2006. Botanical polysaccharide: Macrophage immunomodulation and therapeutic potential. Int. Immunopharmacol., 6: 317-333.

Tsochatzidis, N.A., P. Guiraud, A.M. Wilhelm and H. Delmas, 2001. Determination of velocity, size and concentration of ultrasonic cavitation bubbles by the phase-Doppler technique. Chem. Eng. Sci., 56: 1831-1840.

Velickovic, D.T., D.M. Milenovic, M.S. Ristic and V.B. Veljkovic, 2006. Kinetics of ultrasonic extraction of extractive substances from garden (*Salvia officinalis* L.) and glutinous (*Salvia glutinosa* L.) sage. Ultrason. Sonochem., 13: 150-156.

Vinatoru, M., M. Toma, O. Radu, P.I. Filip, D. Lazurca and T.J. Mason, 1997. The use of ultrasound for the extraction of bioactive principles from plant materials. Ultrason. Sonochem., 4: 135-139.

Wang, Y.J., Z. Cheng, J.W. Mao, M.G. Fan and X.Q. Wu, 2009. Optimization of ultrasonic-assisted extraction process of Poria cocos polysaccharides by response surface methodology. Carbohyd. Polym., 77: 713-717.

Yang, B., M.M. Zhao, J. Shi, N. Yang and Y.M. Jiang, 2008a. Effect of ultrasonic treatment on the recovery and DPPH radical scavenging activity of polysaccharides from longan fruit pericarp. Food Chem., 106: 685-690.

Yang, C.X., N. He, X.P. Ling, M.L. Ye, C.X. Zhang and W.Y. Shao, 2008b. The isolation and characterization of polysaccharides from longan pulp. Sep. Purif. Technol., 63: 226-230.

Yang, B., Y.M. Jiang, R. Wang, M.M. Zhao and J. Sun, 2009. Ultra-high pressure treatment effects on polysaccharides and lignins of longan fruit pericarp. Food Chem., 112: 428-431.

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Potential Techniques of Interactive Packaging in Cardboard Carton and Their Effect on Overall Quality Characteristics Such as Sugars, Colour, Texture, Taste and Flavour of Chaunsa White Variety of Mango at Ambient Temperature During Storage

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Abstract: Potential techniques of Interactive Packaging in Cardboard Carton (IPCC) had significant effect ($p<0.05$) effect on overall quality characteristics such as Reducing Sugar (RS), Total Inert Sugar (TIS), Sucrose (SUC), Total Sugar (TS), Sugar Acid Ratio (SAR), Skin Colour (SKC), Flesh Colour (FLC), Texture (TEX), Taste (TAS) and Flavour (FLA) of Chaunsa white variety of Mango at ambient temperature (28-33°C and 56.7-69.7% relative humidity) during storage. It was determined that using of potential techniques of coating emulsions having fungicide, ethylene absorbent and anti-ripening agent for (IPCC) technology showed higher contents of RS ranged from (10.14-11.58%), lower TIS (20.05-24.58%, except carton having 29.58%), SUC (9.19-18.34%), TS (19.57-22.53%) and SAR (22.63-86.27) with an average mean of 10.73%, 24.12%, 13.68%, 24.75% and 46.10 respectively. Whereas, the higher scores of organoleptic characteristics such as SKC score ranged from (4.09-5.01), FLC (4.01-5.02), TEX (4.48-5.27), TAS (3.81-5.20) and FLA (4.05-4.69) with an average mean of 4.53, 4.47, 5.03, 4.48 and 4.38 score respectively was observed in IPCC system up to end of storage. On the other hand the control sample (T1) comparatively had lower RS (9.66%), higher TIS (28.51%) except carton with highest percent (29.58%), highest SUC (19.83%), TS (29.38%), SAR (103.6), SKC score (5.82), FLC (4.55), TEX (4.98), TAS (4.67) and FLA score (4.81) respectively at earlier stage of storage. The present studies show that IPCC system having with other protective chemicals had a vital role in delaying the ripening process of mango fruit and extended storage life up to 25 days with minimum quality loss as compared to Control sample due to its unattractive skin, brown pulp color and poor taste was unacceptable after 12 days of their storage and had greater compositional changes with maximum quality loss during storage.

Key words: Fruit, Chaunsa mango, interactive packaging, sugars, organoleptic, physico-chemical composition

INTRODUCTION

Potential techniques of Interactive packaging such as pre-cooling/ hot water treatments, use of antimicrobial agents, skin coatings prior to packaging, inducing or enhancing ripening agents and reducing or delaying ripening agents in the package are playing an important role in post harvest management of fruits and vegetables by delaying the ripening process and longer retention of quality pretreatments of fresh produce (John, 2008). Mango is subtropical and climacteric fruit that normally reach to maturity in 4-5 month depending on variety and environmental conditions, fruit takes 6-10 days to ripen under ambient temperature and become over-ripe and spoiled within 15 days. A series of biochemical changes such as degradation of chlorophyll, biosynthesis of carotenoids, anthocyanins, essential oils and flavour components, increase the activity of cell wall degrading enzymes are initiated by the autocatalytic production of ethylene and increase in respiration that causes physiological, biochemical and organoleptic changes results in characteristic color, taste, aroma with desirable softening (Tharanathan *et al*., 2006). In a recent study, it was reported that pre-treatments and polyethylene packaging had significant effect on overall chemical constituents such as sugars and organoleptic parameters and it was noted that the coated fruit packed in polyethylene had longer shelf life, minimum quality loss and slower increase in sugars (RS, TIS, SUC, TS) contents and lower score of colour, taste or higher texture and slower increase of flavour score of chaunsa due to slow process of ripening white variety of mango during storage (Rathore *et al*., 2009). In an other study fruit stored in wax- lined cartons sealed with chitosain films had a longer shelf life and retained a higher level of desirable quality attributes than fruits stored in wax-lined carton sealed with LDPE films or in

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perforated plastic boxes (Srinivasa *et al*., 2004). In India banana and mango treated with polysaccharide bases coating formulations of starch, Carboxymethyl cellulose, or chitosan, blended with a suitable lipid and wetting agent had retarded color development, lower acidity, greater firmness values, reduced in weight loss and CO_2 evolution. It was also noted that Chitosan based coating were much superior in prolonging the shelf life and quality of mango (Kittur *et al*., 2001). The waxes coating, oils and similar materials also reduce evaporation of water and exchange of respiratory gases (CO_2 and O_2) by adding natural resistance of skin and also directly improve the appearance of fruit. Koolpluksee *et al*. (1993) packaged Nam Dok Mai mango in Polyethylene (PE) or Polypropylene (PP) bags, perforated (8 pinholes/bag) or not perforated and with or without ethylene absorbent (EA) were stored at 10°C and 90-93% RH. Storage treatments reduced off-odours, off-flavours and Chilling Injury (CI) and delayed ripening compared with control fruits (stored in plastic buckets). Post harvest application of $CaCl_2$ indicated slightly delay in ripening, extended storage life by a week and lower rate of fresh weight loss in treated Haden mango, stored at 15°C with 90% RH (Zambrano and Manzano, 1995). Life of the fruit and maintenance of their quality through Modified Atmosphere Packaging (MAP) is possible, that depends on types and properties of packaging/fruit, concentration of gases (Srinivasa *et al*., 2002; Sarkar *et al*., 1997; Koolpluksee *et al*., 1993; Yantarasri *et al*., 1994), temperature and Storage treatments (Rana *et al*., 1992; Clarke *et al*., 1997), grading of fruit and size of fruit (Singh *et al*., 1989). The above-mentioned literature shows that coating, modified atmosphere, fungicide or ethylene absorbent had increased the storage life or quality of fruit during storage. Moreover, literature is silent about the potential techniques of interactive packaging in cardboard carton and their effect on overall quality characteristics such as reducing-non reducing and total sugars, sugar acid ratio, colour, texture, taste and flavour of chaunsa white variety of mango at ambient temperature during storage. Therefore, the aim of the present study was to evaluate the effect of potential techniques of Interactive Packaging in Cardboard Carton (IPCC) on overall chemical constituents such as sugars (RS, TIS, SUC, TS and SAR) and other organoleptic parameters like; color, texture, taste and flavour (SKC, FLC, TEX, TAS and FLA) of chaunsa white an important commercial variety of mango at ambient temperature during storage.

MATERIALS AND METHODS

Collection of sample, pretreatments and storage: Fresh arrival of un-ripened, matured, hard green and uniform size of Chaunsa white which is very important commercial variety of mango was purchased from wholesale fruit market at Islamabad and immediately transferred to post harvest laboratory of Department of Food Technology in University of Arid Agriculture Rawalpindi. After careful sorting, fruits in cotton bags were subjected to hot water treatment at 53°C for three minutes and immediately cooled by dipping in cold water at 20°C and were dried in air. Coatings were prepared according to the concentrations as described by Rathore *et al*. (2009). Fruit was graded according to their size and total 180 selected fruit were divided into 6 groups having 30 mangoes in each group respectively. These groups were under gone into following 6 treatments viz; Control (T1), Carton (T2), Wax-CMC having NaOCl coated fruit packed in Carton (T3), Wax-CMC Coated fruit with $KMnO_4$ package in Carton (T4), Wax-CMC Coating having 2,4,5-T in Carton (T5), H_3BO_3 and 2,4,5-T having oil treated fruit packed in Carton (T6) and then were stored at ambient temperature (28-33°C and 56.7-69.7% relative humidity) for a storage period of 30 days.

Physico-chemical and sensory evaluations: Physico-Chemical parameters such as reducing and non-reducing sugars were determined by Lane and Eynon method according to standard procedures as mentioned in AOAC (1990). The sensory evaluation of treated and untreated fruit were made by using hedonic 9 point scale for different characteristics such as peel color, flesh color, texture, taste and flavor by panel of trained Judges according to methods reported by Larmond (1977). The data obtained were statistically analyzed for Analysis of Variance (ANOVA) by using 2-Factorial Complete Randomized Design (CRD) and Duncan's Multiple Range Test (DMRT) was applied to compare the mean values obtained according to the method described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

It was investigated that Chaunsa white variety of mango uncoated fruit or coated fruit packed in carton had affected the overall quality characteristics such as sugars, colour, texture, taste and flavour of chaunsa white variety of mango at ambient temperature during storage.

Reducing sugar: The Table 1 presents that treatments and their interactions had highly significant effect on percent reducing sugar of mango during storage and the percent reducing sugar in coated fruit packaged in carton was 10.14-11.58% with an average means of 10.73% during 25 days of storage at ambient temperature compared to first day with very low percent reducing sugar (5.95 %). therefore, with the fluctuations showing an increasing trend of percent reducing sugar during storage. The increase in Reducing Sugar (RS) level could be attributed mainly due to breakdown of starch into water soluble sugars, sucrose and glucose

during ripening along with a proportional increase in RS level and further hydrolysis decreased the RS during storage. Srinivasa *et al.* (2002) and Kittur *et al.* (2001) have expressed similar views. It is evident from Table 1 that statistically there was a significant effect of all treatments on percent reducing sugar except T2 and T4 (10.14, 10.32) or T5 and T6 (10.44, 10.59) an insignificant difference in between treatments was noted, however these groups of treatments were significantly different from others treatments. The maximum percent reducing sugar was observed in T3 (11.58%) followed by T6 (10.59%), T5 (10.44 %), T4 (10.32 %) and T2 (10.14 %), respectively as compared to control T1 (9.67%) had lower percent reducing sugar than T3 (11.58%), however, the control showed lower content reducing sugar than other treatments during storage or at first day with minimum content reducing sugar (5.95%) at ambient temperature during storage. These results revealed that RS contents were higher in packaging either in polyethylene (T2), or the combination as poly-coat might be due to controlled or modified atmosphere showed better development of RS at later stage on 18th day as compared to control. It was also observed that addition of Wax-CMC coating having ethylene absorbent or antiripening agent in T5, T6 and T7 in polyethylene had lower RS contents as compared to packing in polyethylene or control. Furthermore, these treatment also reached to maximum RS at later stage on 18th day might be due to combination of coating with packing produced better environment that reduced metabolic process that caused a slow down in conversion of starch to simple sugars during storage. These results are correlated with the findings of Srinivasa *et al.* (2002). Kittur *et al.* (2001) also reported that mango and banana treated with polysaccharide-based coatings had lower reducing sugar contents than control at ambient temperature (27±2^{o}C and 65% RH, suggesting that the former synthesized reducing sugar at lower rate than control during storage.

Total invert sugar: The Table 1 indicates that treatments and their interactions had highly significant effect on percent invert sugar of late Chaunsa white mango at ambient temperature during storage. the percent total invert sugar in coated fruit packaged in carton was 20.05-29.58% with an average means of 24.12% in coated fruit packaged in polyethylene as compared to control having maximum total invert sugar percent (28.51%) after 25 days of storage at ambient temperature or at first day with very low total invert sugar percent (8.38%) at ambient temperature during storage. Therefore, with the fluctuations percent total invert sugar showing an increasing trend during storage. The increase in percent total invert sugar level could be attributed mainly due to breakdown of starch into water soluble sugars, sucrose, glucose and fructose during ripening as a result, a proportional increased in percent total invert sugar level and further hydrolysis decreased the percent total invert sugar during storage. It is obvious from Table 1 that statistically a significant difference of percent total invert sugar among all treatments was found during 18 days of their storage and the maximum percent total invert sugar of late Chaunsa white mango were observed in T2 (29.58%), followed by T4 (26.70%), T3 (25.14%) T6 (24.58%) and T5 (20.05%) as compared to control having maximum total invert sugar percent (28.51%) after 25 days of storage at ambient temperature or compared to first day having minimum total invert sugar percent (8.38%) in Chaunsa white mango. It was observed that ripening process was faster in control (T1); however the combination of poly-coat packaging had reduced the ripening process. The efficiency of polyethylene packaging was improved further, by coating the fruit with Wax-CMC having fungicide, ethylene absorbent or antiripening agent in T3, T4, T5 and T6 only packed in polyethylene might be due to reduction in metabolic activities by modified atmosphere produced by treatments during storage.

Sucrose: The Table 1 indicates that treatments and their interactions had highly significant effect on percent sucrose content of late Chaunsa white mango at ambient temperature during storage. the percent sucrose sugar in coated fruit packaged in poly-carton was 9.19-18.34% with an average means of 13.68% after 25 days of storage at ambient temperature as compared to control with maximum percent sucrose content T1 (19.83%) or at first day with very low percent sucrose content (2.26%) at ambient temperature during storage. The increase in percent sucrose sugar level could be attributed mainly due to breakdown of starch into water soluble sugars sucrose, glucose and fructose during ripening along with a proportional increase in percent sucrose sugar level and further hydrolysis decreased the percent sucrose sugar during storage. Similar results are reported by Kittur *et al.* (2001). It is obvious from Table 1 that statistically a significant difference of percent sucrose content among all treatments was found during storage. The maximum percent sucrose content of late Chaunsa white mango were observed in T2 (18.34%), followed by T4 (16.60%), T6 (15.99%), T3 (12.94%) and T5 (9.19%), however, these treatments maintained sucrose of mango at lower level as compared to control with maximum percent sucrose content T1 (19.83%) at ambient temperature during storage. These investigations show that control sample had higher sucrose content than polyethylene, however, the sucrose content was further reduced when fruit were packaged in poly-Wax-CMC polysaccharide based coatings having fungicide, ethylene absorbent or antiripening agent combinations in T3, T4, T5 and T6 respectively with minimum sucrose content might be

due to reduction in metabolic activities by a modified atmosphere produced by combinations of coating with these packaging materials. These results are in line with those of Kittur *et al.* (2001) who reported that polysaccharide-based coating of mango and banana had lower reducing sugar content at ambient temperature (27±2°C and 65% RH had lower reducing sugar content and TSS in polysaccharide-based coated fruits as compared to control.

Total sugar: The Table 1 reveals that treatments and their interactions had highly significant effect on total sugar percent of late Chaunsa white mango during storage and the percent total sugars in coated fruit packaged in carton were 19.57-28.53% with an average means of 24.75% in treated fruit packaged in polyethylene as compared to control with maximum total sugar percent T1 (29.38%) after 25 days of storage at ambient temperature or at first day with very low total sugar percent (8.21%). The increase in percent total sugar level could be attributed mainly due to breakdown of starch into simple sugars during ripening along with a proportional increase in percent total sugars level which was attributed to the increased activity of amylase and other enzymes resulting in gluconeogenesis and converted into sucrose, glucose and fructose during storage. Further hydrolysis decreased the level during storage. These results are correlated with the findings of Srinivasa *et al.* (2002); Kudachikar *et al.* (2001). It is obvious from Table 1 that statistically a significant difference of total sugar percent in all treatments except T4 (26.6%) and T6 (26.59%) an insignificant effect was found during 25 days of their storage. The maximum total sugar percent of late Chaunsa white mango were observed in T2 (28.53%), followed by T6 (26.59%), T4 (26.60%), T3 (24.34%) and T5 (19.57%) however, these treatments having comparatively lower percent sucrose content as compared to control with maximum total sugar percent T1 (29.38%) or at first day with very low total sugar percent (8.21%) at ambient temperature during storage. These results are correlated with the findings of (Srinivasa *et al.*, 2002) who reported that Alphonso mango treated with Carbendazim fungicide had an increasing trend of total sugar from 23.00-150 mg/g in control sample on 12th day and then spoiled, whereas, low-density polyethylene (100 gauge LDPE) top covered carton boxes fruit gave the maximum values on 16th day at ambient temperature 27±1°C at 65% RH respectively.

Sugar acid ratio: The Table 1 presents that treatments and their interactions had highly significant effect on sugar acid ratio of mango during storage. the sugar acid ratio in coated fruit packaged in carton was 22.63-86.27 with an average means of 46.10 in coat-carton combination as compared to control with maximum sugar acid ratio T1 (103.6 %) after 25 days of storage at ambient temperature or at first day with very low sugar acid ratio (8.21%). The increase in sugar acid ratio level could be attributed mainly due to breakdown of starch into water soluble sugars, sucrose and glucose during ripening along with a proportional increase in sugar acid ratio level and further hydrolysis decreased the sugar acid ratio during storage. It is obvious from Table 1 that statistically there was a significant difference of sugar acid ratio among all treatments except T3 and T6 (44.55%, 55.09%) or T4 and T6 (61.12%, 55.09%) during 25 days of their storage. The maximum sugar acid ratio of late Chaunsa white mango were observed in T2 (86.27%), followed by T4 (61.12%), T6 (55.09%), T3 (45.55%) and T5 (22.63%) however, these treatments having comparatively lower sugar acid ratio as compared to control with maximum sugar acid ratio T1 (103.6%) or at first day with very low sugar acid ratio (8.21%) at ambient temperature during storage. Manzano *et al.* (1997) observed similar decreasing trend in Hadden mango treated with waxes coating stored at different temperatures had significantly decreasing trend of TSS acid ratio from 170.24-37.50 during 20 days of their storage. TSS acid ratio value (98.74) was higher in ethylene treated as compared to no treated fruit. These results are coincide with those Ladaniya and Sonkar (1997) who observed a maximum retention of total soluble solids and acid ratio, flavor and shine in Nagpur mandrine (Citrus reticulata) in waxed treated fruit with 2000 ppm Carbendazim fungicide, packaged in ventilated corrugated fiber-board boxes lined with polyethylene during 21 days of storage at ambient temperature. The change in total soluble solids and acid ratio did not indicate any trend with respect to treatments, although the ratio declined slightly during storage. The least ratio (17.24) was found in mechanically waxed fruit packed without polyethylene. No effect on percent acidity and brix level indicates that respiration rate was unaffected in waxed treated fruit.

Skin color: The Table 2 illustrates that treatments and their interactions had highly significant effect on skin color score of mango during storage. the skin color score in coated fruit packaged in poly-carton was 4.09-5.01 with an average means of 4.53 in coat-carton combination as compared to controlled with maximum skin color score T1 (5.82) during 25 days of storage at ambient temperature or as compared to first day having minimum skin color score (3.00). The loss of green color was the most obvious change in mango, which was probably due to the physico-chemical changes by degradation of the chlorophyll structure and increased in carotenoid pigments during storage. The principal agents responsible for this degradation might be the oxidative system, pH change and enzymes like chlorophyllases (Wills *et al.*, 1982; Doreyappy-Gowda

and Huddar, 2001). It is obvious from Table 2 that statistically there was a significant effect of treatment on SKC score except T2 and T4 (4.80, 4.73) having an insignificant difference, however a significantly different SKC score among the treatments of one group to the treatments of other group was found during 25 days of their storage.

The maximum skin color score of late Chaunsa white mango were observed in T3 (5.01), followed by T2 (4.80), T4 (4.73), T5 (4.31) and T6 (4.09) as compared to control with maximum skin color score T1 (5.82) during 25 days of storage at ambient temperature. Similarities in skin color score among the treatments including control that might be due to the hot water treatment given to all fruit at initial stage raised the temperature that caused an increase in SKC for movement however, due to high temperature enzymes were inactivated, therefore control also retained a higher skin color up to 15th day. On the other hand Wax-CMC coated fruit had slow down the metabolism with higher skin color retention during storage as compared to control that might be due to variation in modified atmosphere created by different types of treatments delay in chlorophyll degradation and slower rate of synthesis of anthocynanine or carotenoids in modified atmosphere (Kittur *et al*., 2001; Carrillo-Lopez *et al*., 2000; Manzano *et al*., 1997). Ethylene absorbents or removers in poly-coat packaged had lower sensory score as compared to control might be due to the slower metabolic activities because of removal of produced ethylene, a catalyst for ripening and also had higher SKC retention might be due to increased CO_2 and reduced level of O_2 in MAP which reduce respiration rates and delay ripening, that is confirmed by other workers (Rodov *et al*., 1997; Srinivasa *et al*., 2002). Raje *et al*. (1997) in India also reported that the alphonso mangoes treated with different types of ethylene inhibitors including $KMnO_4$ showed higher sensory score at 16th day as compared to control on 8th day at 32-36^{o}C and RH of 70-75% and then spoiled. Rosa *et al*. (2001) in Isreal reported that $KMnO_4$ in MAP with polyethylene wrapped Tommy Atkin or Keitt cultivars of mangoes delayed ripening as expressed by less color development and ethylene absorption inhibited chlorophyll breakdown at 12 or 8^{o}C for 3 weeks plus 5 days shelf at 20^{o}C with out MAP. The main factors that retain mango quality in MAP are increased CO_2 levels and decreased O_2 levels which reduce respiration rates and delay ripening. The delay in ripening, degradation of chlorophyll and retention of green color for a longer period also depend on types of coating (Manzano *et al*., 1997; Kittur *et al*., 2001), hot water treatment before storage (Opara *et al*., 2000; Mortuza and Reza, 2001), coating concentrations and temperature during storage (Carrillo-Lopez *et al*., 2000; Makil and Sing, 2003). The results of the present studies show that increase in quality and availability of fruit with maximum skin color for a longer period in treated fruit is very encouraging. Coating of Chaunsa white packaged in only polyethylene (T2), coating having fungicide in Polyethylene (T3), coating with ethylene absorbent in polyethylene (T4), coating with antiripening agent in polyethylene (T5), coating with antiripening agent with oil and disinfectant in polyethylene (T6) had slower increase in color score due to slow ripening process may be very effective in delay ripening process with minimum skin color score during storage.

Flesh color: The Table 2 indicates that treatments and their interactions had highly significant effect on flesh color score of mango during storage. The skin color score in coated fruit packaged in poly-carton was 4.01-5.02 with an average means of 4.47 in poly-coating combinations as compared to control T1 (4.56%) with maximum flesh score during 25 days of storage at ambient temperature or at first day with minimum flesh color score (2.5). It was observed that in general the skin color score had increasing trend first and then significant decreased of skin color score during storage. During storage mangoes made the transition from green to yellow, which was due to degradation of chlorophyll indicating an increased acceptability for consumption. This change might be mediated through the action of enzyme chlorophyllase, enzymatic oxidation and or photo degradation. It is obvious from Table 2 that statistically there was a significant effect of all the treatments on flesh color score except T2 or T4 (4.70, 4.79) or T5 or T6 (4.01, 4.05) having an insignificant difference was observed during 25 days of their storage. The maximum flesh color score of late Chaunsa white mango were observed in T3 (5.02), followed y T4 (4.79), T2 (4.70), T6 (4.05) and T5 (4.01) and having comparatively lower flesh color score than controlled T1 (4.56 or at first day with minimum flesh color score (2.5) at ambient temperature during storage. Carrillo-Lopez *et al*. (2000) also stated that Haden mangoes coated with different concentrations of Semper fresh had higher retention of green color as compared to noncoated fruit at 13^{o}C during 32 days storage. Manzano *et al*. (1997) evaluated the effect of coatings on color development in Hadden mango and reported that mango treated with waxes coating had lower color values and retention of color also depend on types of coating is confirmed with our studies however, did not agree with that the control having higher color score than treated fruit might be lacked of hot water treatment in the research of others. Where as in our studies T2, T4 and T5 had no significant difference in FLC with control (T1) might be due to higher temperature of hot water treated fruit at initial stage caused an increase in carotenoids of flesh of fruit and later stage maintained carotenoids might be due to inactivation of enzymes. However after 12th day of storage due to over ripening control fruit could not

maintained flesh color as compared to T2, T3, T4 and T8 maintained higher FLC up to end of storage might be due to due to inactivation of enzymes and modified atmosphere slower process of changes in chlorophyll. These results are in line with Opara *et al.* (2000) who reported that hot water treatment at 52°C had higher pulp color score (4.5) as compared to control (3.4%) that was also depending on time of exposure and higher color was noted at 10 min than for 5 min of hot water treatment. Chaunsa white mango coated with WAX-CMC having some chemicals restricted the changes and formation of carotenoids were depressed might be due to the slow down of metabolic activities in T2, T3, T4 and T8 by the combined action of NaOCl, $KMnO_4$ and 2,4,5-T respectively. The chemical reactions are going side by side in both of SKC and FLC; however, comparatively higher score of FLC shows that changes were rapid in pulp than peel in these same treatments. The lower values of FLC in T3 and T8 show that $KMnO_4$ or 2,4,5-T with NaOCl that ripening process was slower in these treatments might be due to lower concentration of ethylene in surrounding atmosphere. These results are an agreement with those of Raje *et al.* (1997) in India reported that the alphonso mangoes treated with different types of ethylene inhibitors including $KMnO_4$ showed higher sensory score at 16th day as compared to control on 8th day at 32-36°C and RH of 70-75% and then spoiled.

Texture: The Table 2 reveals that treatments and their interactions had highly significant effect on TEX score of mango during storage. The TEX score in coated fruit packaged in poly-carton was 4.48-5.27 with an average means of 5.03 in coated fruit packaged in polyethylene combinations as compared to control with minimum TEX score T1 (4.98) during 25 days of storage at ambient temperature or at first day with very low TEX score (4.00) at first day with very low TEX score (4.00). Therefore, showing an increasing trend of texture in all treated fruits. The reduction of texture during storage that might be due to the breakdown of insoluble pectic substances to soluble forms and these pectin polymers became less tightly bound in the cell walls during ripening. The declining concentration of calcium might reduce calcium pectin interaction, allowing free release into flesh leading to reduce firmness as the fruit ripen, resulting in shriveling of over-ripe mango fruits, as discussed earlier. It is obvious from Table 1 that statistically there was a significant effect of treatment on TEX score in all treatments except T1, T3, T4 and T5 (4.98, 4.90, 4.97 and 4.99), an insignificant effect in between treatments was observed. However these treatments were significantly different to others during 25 days of their storage. The maximum TEX score of late Chaunsa white mango were observed in T6 (5.27), followed by T5 (4.99), T4 (4.97), T3 (4.90) and T2 (4.48) as compared to control with minimum TEX score T1 (4.98) or at first day with very low TEX score (4.00) at ambient temperature during storage. All of the treatments had delayed in the degradation of pectic substances and improved the TEX score of fruit, however the those treatments with WAX-CMC coating with $KMnO_4$ and 2,4,5-T in T4 and T5 respectively were more effective to retain higher texture of fruit at later stage on 25 days might be due to the difference in types of coating which caused a variation in increased CO_2 and decreased O_2 levels of modified atmosphere. These treatments reduced respiration rates and the activities of enzymes more effectively and lower metabolic activities or slower biochemical changes in modified as compared to $CaCl_2$ with the same chemicals in T5, T6 and T7 were over ripen with worse conditions of TEX. Moreover, the combination of $CaCl_2$ with WAX-CMC in T8 was more effective to retain higher TEX score up to end of storage. These investigations are correlated with Kittur *et al.* (2001) who recorded that banana and mango with carboxymethyle cellulose or other polysaccharide based coatings had higher firmness and delaying in the ripening at ambient temperature (27±2°C and 65% RH). Polysaccharide-based coating formulations had slower metabolism due to the reduced activities of pectic enzymes such as amylases, starch phosphorylase and α-1, 6 glucosidase and sucrose synthase responsible for alteration in cell wall structure and the degradation of starch into simple sugars and as result prolong storage life. These investigations are correlated with (Ladaniya and Sonkar, 1997) who reported that the Manually waxed Nagpur mandrine (Citrus reticulata) with 2000 ppm Carbendazim fungicide, packaged in ventilated corrugated fiber-board boxes lined with polyethylene, had maximum retention of natural freshness, firmness and shine in waxed treated fruit and decay was effectively controlled by fungicide during 21 days of storage at ambient temperature. The maintenance of the firmness and quality of fruit also depends on packaging material and MAP (Ladaniya and Sonkar, 1997; Rodov *et al*., 1997; Rosa *et al*., 2001), types or concentrations of coatings and ethylene absorbent used (Raje *et al*., 1997; Baldwin *et al*., 1999; Malik *et al*., 2003). Chitarra *et al.* (2001) also observed that firmness of Tommy Atkins mango was significantly affected by storage duration, salt concentration and storage temperature. The texture of the mango was decreased from 125.21-69.68 N when packaged in ventilated carton and stored at 10±1°C and 80-90% RH.

Taste: It is obvious Table 2 that treatments and their interactions had highly significant effect on TAS score of mango during storage period. The TAS score was increased in coated fruit packaged in poly-carton was 3.81-5.20 with an average means of 4.48 in coated fruit packaged in polyethylene combinations as compared to

control with minimum TAS score T1 (4.67) at ambient temperature during 25 days of storage at ambient temperature or at first day with very low on taste score (4.00). It is clear from Table 1 that statistically there was a significant effect of treatment on TAS score except T1 and T2 (4.67,4.60), or T5 and T6 (3.81, 3.94), an insignificant effect in between treatments, however these treatments were significantly different to one an others during 25 days of their storage. The maximum TAS score of late Chaunsa white mango were observed in T3 (5.20), followed by T4 (4.96), T2 (4.60), T6 (3.94) and T5 (3.81) however, having comparatively lower TAS score than control with minimum TAS score T1 (4.67) or at first day with very low on taste score (4.00) at ambient temperature during storage. The variation in the modified atmosphere due to different types of treatments might be caused of distinction in the improvement of TAS in treated fruit. The least TAS score in T2, T3, T4, T5 and T6 at 25th day showed that fruit were not fully ripened might be due to undesirable modified atmosphere, the biochemical or metabolic activities were slower that caused a delay in ripening process. Whereas, T1 having higher TAS score in Chaunsa mango might be due to comparatively more suitable environment in which starch was easily converted into simple sugars that enhanced the taste of ripened fruit during storage. These results are correlated with Kittur *et al.* (2001) who observed that maximum texture and taste depends on type of coating at ambient temperature (27±2^{o}C and 65% RH) and fruits having polysaccharide based coating formulations improved the sensory characteristics of banana and mango by maintaining dark green color, with glassy shining and moist-like appearance and were best even after 21 days of storage. Uncoated fruit on the other hand, blackened due to over ripening and fungal infection and exhibited a very soft, collapsed texture. Raje *et al.* (1997) who reported that taste score of Alphanso mangoes in India was affected by storage period and types of ethylene inhibitor used when stored at 32-36^{o}C and 70-75% RH. The lower sensory score (66.70) at first day of storage was increased to its maximum score (79.5) after 8 days in control samples and then spoiled as compared to the treated with ethylene adsorbent reached to the maximum taste score at later stage on 16th day of their storage. The ethysord treated fruit gave the highest score of 68.7, followed by oxidizer (68.2) and $KMnO_4$ treated (66.2) fruit after 16 days of storage. Thus retention of two dominant components of taste like sweetness due to sugar and sourness from organic acids in many fruits (Kays, 1991) depends on many factors like storage temperature (Opara *et al.*, 2000), types of packaging or MAP (Clarke *et al.*, 1997; Rodov *et al.*, 1997), hot water treatments for specific time (Nair *et al.*, 2001) and type of coating and its concentrations (Ladaniya and Sonkar, 1997; Malik *et al.*, 2003).

Flavor: The Table 2 indicates that treatments and their interactions had highly significant effect on flavor score of mango during storage. The flavor score in coated fruit packaged in poly-carton was 4.04-4.69 with an average means of 4.38 in coated fruit packaged in carton combinations as compared to control with maximum flavor score T1 (4.81) during 25 days of storage at ambient temperature or at first day with very low flavor score (3.00). These findings are generally correspond with Hayat *et al.* (2005) who reported that the organoleptic evaluation of Banky apple showed a decreasing trend in taste/flavor score from 9.00-4.48 with the passage of storage period and there was a significant superiority of treated fruit in the preservation of taste/flavor over control during storage at ambient temperature.

It is obvious from Table 2 that statistically there was a significant effect of all treatments on flavor score except T3 (4.59) and T4 (4.69) having an insignificant difference; however these treatments were significantly different to others during 25 days of their storage. The maximum flavor score of late Chaunsa white mango were observed in T4 (4.69), followed by T3 (4.59), T2 (4.50), T5 (4.19) and T6 (4.04) having comparatively lower flavor score as compared to control with maximum flavor score T1 (4.81) or at first day with very low flavor score (3.00) at ambient temperature during storage. The polyethylene with combination of coating, antiripening agent with or without disinfectant, are very effective to control on flavor score and delay ripening process. This difference in flavor retention might be due to different nature of treatment and variations in modified atmospheric conditions created by individual treatment. Similar findings by Ladaniya and Sonkar (1997) who reported that the combination of wax coating with 2000 ppm Carbendazim fungicide, packaged in ventilated Corrugated Fiber-Board Boxes (CFB) lined with polyethylene had maximum retention of natural freshness, firmness, total soluble solids and acid ratio, flavor and shine in treated fruit during 21 days of storage at ambient temperature. It was observed earlier that coatings improve the flavor of fruit that depends upon the type of coating and permeability of O_2. The polysaccharide based coatings had low permeability and anaerobic respiration caused an increased of ethanol and acetaldehyde content of fruit as a result reduction of flavor (Baldwin *et al.*, 1999), however, the higher CO_2, acteldehyde and ethanol levels may have contributed to a greater delay in ripening for NS fruit since these compounds are reported to have an effect on this process via retardation of ethylene synthesis. The retention of flavor also depend on concentrations of coating (Malik *et al.*, 2003) or MAP (Ladaniya and Sonkar, 1997; Rodov *et al.*, 1997) used etc.

Table 1: Effect of coating having fungicide, ethylene absorbent and antiripening agent packaged in cardboard carton on the physico-chemical composition of chaunsa white variety during storage

	Treatments						
	T1	T2	T3	T4	T5	T6	Overall effect of Carton-coat
Reducing sugar (%)	9.67ef	10.14d	11.58a	10.32cd	10.44bc	10.59b	10.73
Total invert sugar (%)	28.51b	29.58a	25.14d	26.70c	20.05n	24.58g	24.12
Sucrose	19.83a	18.34b	12.94h	16.60c	9.19n	15.99d	13.68
Total sugar (%)	29.38a	28.53b	24.34d	26.6c	19.57m	26.59c	24.75
Sugar acid ratio	103.6a	86.27b	45.55de	61.12c	22.63g	55.09cd	46.10

Mean values with different letters in same row are significantly different to each other at ($p<0.05$).

T1 = Control T2 = Carton T3 = Coat + NaOCl + Carton

T4 = Coat + $KMnO_4$ + Carton T5 = Coat+2,4,5-T + Carton T6 = Coat+2,4,5-T having oil + H_3BO_3-$CaCl_2$ + Carton

Table 2: Effect of coating having fungicide, ethylene absorbent and antiripening agent packaged in cardboard carton on the physico-chemical composition of chaunsa white variety during storage

	Treatments						
	T1	T2	T3	T4	T5	T6	Overall effect of Carton-coat
Skin colour	5.82a	4.80cd	5.01b	4.73de	4.31f	4.09g	4.53
Flesh colour	4.56c	4.70b	5.02a	4.79b	4.01e	4.05e	4.47
Texture	4.98ef	4.48g	4.90f	4.97ef	4.99ef	5.27c	5.03
Taste	4.67c	4.60c	5.20a	4.96b	3.81ef	3.94e	4.48
Flavour	4.81a	4.50cd	4.59bc	4.69ab	4.19e	4.04f	4.38

Mean values with different letters in same row are significantly different to each other at ($p<0.05$).

T1 = Control T2 = Carton T3 = Coat + NaOCl + Carton

T4 = Coat + $KMnO_4$ + Carton T5 = Coat+2,4,5-T + Carton T6 = Coat+2,4,5-T having oil + H_3BO_3-$CaCl_2$ + Carton

Conclusion: After a through study it is concluded that potential techniques of interactive packaging in cardboard carton had a significant effect on overall quality characteristics such as sugars, colour, texture, taste and flavour of chaunsa white variety of mango at ambient temperature during storage. The fruit under gone into different interactive potential techniques such Control (T1), Carton (T2), Wax-CMC having NaOCl coated fruit packed in Carton (T3), Wax-CMC Coated fruit with $KMnO_4$ package in Carton (T4), Wax-CMC Coating having 2,4,5-T in Carton (T5), H_3BO_3 and 2,4,5-T having oil treated fruit packed in Carton (T6) showed significant difference in quality characteristics among the treatments during storage. Moreover, treated fruit with a group of potential innovative techniques in cardboard carton were superior in quality than packaged in carton without treatments and due to slow process of ripening during storage treated fruit effectively maintained higher percent of sugars (RS, TIS, SUC and TS content) and higher retention of organoleptic characteristics up to the end of the storage. The present studies show that IPCC system having with other protective chemicals had a vital role in delaying the ripening process of mango fruit and extended storage life up to 25 days with minimum quality loss as compared to Control sample due to its unattractive skin, brown pulp color and poor taste was unacceptable after 12 days of their storage and had greater compositional changes with maximum quality loss during storage. Improving the present IPCC system by including polyethylene wrapping may increase further shelf life and therefore, further research studies are necessary for the development this system in near future.

REFERENCES

AOAC, 1990. Official Methods of Analysis. Association of Analytical Chemists. 15th Edn., Arlington, Virginia, 22201, USA.

Baldwin, E.A., J.K. Burns, W. Kazokas, J.K. Brecht, R.D. Hagenmaier, R.J. Bender and E. Pesis, 1999. Effect of two edible coatings with different permeability characteristics on mango (*Mangifera indica* L.) ripening during storage. Postharvest Biol. Technol., 17: 215-226.

Carrillo, L.A., F. Ramirez-Bustamante, J.B. Valdez-Torres, R. Rojas-Villegas and E.M. Yahia, 2000. Ripening and quality changes in mango fruit as affected by coating with an edible film. J. Food Qual., 23: 479-486.

Clarke, R., C.P. Moor and J.R. Gorny, 1997. The future in film technology, a tunable packaging system for fresh produce. Proceedings of the seventh international controlled atmosphere research conference (California, USA, 1997), pp: 68-75.

Doreyappy-Gowda, I.N.D. and A.G. Huddar, 2001. Studies on ripening changes in mango (*Mangifera indica* L.) fruits. J. Food Sci. Technol. Mysore, 38: 135-137.

Hayat, I., T. Masud and H.A. Rathore, 2005. Effect of coating and wrapping materials on the shelf life of apple (*Malus domestica* cv. Borkh). Int. J. Food Safety, 5: 24-34.

John, P.J., 2008. A handbook on post harvest management of fruits and vegetables. Daya Publishing House Delhi-110035, pp: 85-103.

Kays, S.J., 1991. Post harvest physiology of perishable plant products. Vas Nostrand Rein Hold Book, AVI Publishing Co., pp: 149-316.

Kittur, F.S., N. Saroja, Habibunnisa and R.N. Tharanathan, 2001. Polysaccharide-based composite coating formulations for shelf-life extension of fresh banana and mango. Eur. Food Res. Tech., 213: 306-311.

Koolpluksee, M., S. Ketsa and S. Subhadrabandhu, 1993. Effect of modified atmospheres on quality and chilling injury of Nam Dok Mai mango fruits. Kasetsart J. Natural Sci., 27: 115-124.

Kudachikar, V.B., S.G. Kulkarni, M.N.K. Prakash, M.S. Vasantha, B.A. Prasad and K.V.R. Ramana, 2001. Physico-chemical changes during maturity of mango (*Mangifera indica* L.) variety Neelum. J. Food Sci. Technol. Mysore, 38: 540-542.

Ladaniya, M.S. and R.K. Sonkar, 1997. Effect of curing, wax application and packing on quality of stored Nagpur mandarins. In. Agric. Sci., 67: 500-503.

Larmond, E., 1977. Laboratory Methods of Sensory Evaluation of Food Research Branch, Canada. Deptt. Agric., Publication, pp: 44.

Malik, A.U., Z. Sing and S.S. Dhaliwal, 2003. Exogenous application of putrescine affects mango fruit quality and shelf life. Acta Hort., 628: 121-127.

Manzano, J.E., Y. Perez and E. Rojas, 1997. Coating waxes on Haden mango fruits (*Mangifera indica* L.) cultivar for export. Acta Hort., 455: 738-746.

Mortuza, M.G. and M.M.A. Reza, 2001. Control of postharvest anthracnose and stem-end rot of mango fruits by hot water treatment. Bangladesh J. Agric. Res., 26: 543-548.

Nair, R., Z. Singh and S.C. Tan, 2001. Heat treatments affect development chilling injury, respiration, ethylene production and fruit quality of mango. Acta Hort., 553: 549-550.

Opara, L.U., H.X. Nguyen and Levanto, 2000. Postharvest technology for Vietnamese Buoi mango. Quality assurance in agricultureal produce. ACIAR Proceedings, 100: 633-638.

Raje, L., S. Sherlekar, K. Ramakrishnan, V.C. Malshe and G. Subbulakshmi, 1997. Post harvest preservation of mangoes by controlled chemical release agents and adsorbent. Acta Hort., 455: 622-628.

Rana, G.S., S. Kartar and K. Singh, 1992. Storage life of sweet orange fruits as influenced by fungicides, oil emulsions and packages practices. Crop Res., 5: 150-153.

Rathore, H.A., T. Masud, S. Sammi and E.H. Soomro, 2009. Effect of pre-treatments and polyethylene packaging on overall chemical constituents such as sugars and organoleptic parameters like colour, texture, taste and flavour of chaunsa white variety of mango during storage. Pak. J. Nutr., 8: 1292-1300.

Rodov, V., S. Fishman, R.D. Asuncion, J. Peretz and S.B. Yehoshua, 1997. Modified Atmosphere Packaging (MAP) of Tommy Atkins mango in perforated film. Acta Hort., 455: 654-661.

Rosa, B., D. Aharoni, O. Feygenberg, N. Aharoni, A. Keynannd and E. Pesis, 2001. Effect of modified atmosphere packaging on mango ripening. Acta Hort., 553: 607-610.

Sarkar, H.N., A.M. Hasan and P.K. Chattopadhyay, 1997. Influence on polyethylene packing on the post harvest storage behaviour of banana fruit. J. Hort., 10: 31-39.

Singh, B.P., S.K. Kalra and D.K. Tandon, 1989. Effect of size grading on the storage of mangoes. In. J. Hort., 46: 154-160.

Srinivasa, P.C., N.S. Susheelamma, R. Ravi and R.N. Tharanathan, 2004. Quality of mango fruit during storage: Effect of synthetic and eco-friendly films. J. Sci. Food Agric., 84: 818-824.

Srinivasa, P.C., R. Baskaran, M.N. Ramesh, K.V.H. Prashanth and R.N. Tharanathan, 2002. Storage studies of mango packed using biodegradable chitosan film. Eur. Food Res. Technol., 215: 504-508.

Steel, R.G.D. and J.H. Torrie, 1980. Principles and Procedures of Statistics. Mc Graw Hill Book Co. Inc. New York, pp: 134-145.

Tharanathan, R.N., H.M. Yashoda and T.N. Prabha, 2006. Mango (*Mangifera indica* L.) The King of Fruits-An Overview. Food Rev. Int., 22: 95-123.

Wills, R.B., T.H. Lee, D. Graham, W.B. Glasson and E.G. Hall, 1982. Post harvest: An Introduction to the Physiology and Handling of Fruits and Vegetables. AVI Publishing Co., Westport, pp: 34-35.

Yantarasri, T., J. Uthaibutra, J. Sornsrivichai, W. Kumpuan, V. Sardsud and N. Kana-Thum, 1994. Modified atmosphere packing by perforated polymeric film and its effect on physical properties of mango fruit. ACIAR Proceedings, 50: 438-440.

Zambrano, J. and J. Manzano, 1995. Effect of postharvest calcium application on mango ripening and storage. Fruits (Paris), 50: 145-152.

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An Exploratory Study of Weight-Loss Practices of Gymnasts in Rivers State, Nigeria

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Abstract: A cross-sectional survey design was used to explore the weight-loss practices among gymnasts in Rivers State, Nigeria. Data were collected from eighteen gymnasts using 11-item structured questionnaire. The generated data were analyzed using percentage and chi-square statistics. Results showed that majority of the gymnasts decreased their consumption of calories (77.78%), increased their expenditure of calories through exercise (88.89%), restrict food (94.44%), over-exercise (83.33%) and engage in voluntary fluid reduction or dehydration (83.33%) to lose weight. Among the findings was that the gymnasts' gender, age and years of sporting experience had significant influence on increase expenditure of calories through exercise, food restriction, use of drugs, use of nutritional supplements and use of steam baths or saunas for weight-loss. Among the recommendations are that nutritionist/dieticians should be employed and integrated into the gymnastics team to provide and guide the gymnasts on how to effectively use healthy and supervised nutrition to lose weight. The gymnasts should be educated on the health and performance effects of engaging in harmful weight-loss practices through seminars. Stakeholders should be sensitized on the weight-loss practices. This will enable them to discourage any gymnast planning to adopt unhealthy weight-loss measures.

Key words: Gymnasts, weight-loss, age, gender, years of sporting experience

INTRODUCTION

Weight-control is an issue for athletes in weight-restricted sports (Spelke, 1997). Athletes in weight-sensitive sports show great concern over their weight. Weight-sensitive sports, according to Perriello (2001) involve either a requirement to reach a specific weight or a perception that an advantage exists if the athlete performs at a lower weight. He further stated that gymnastics is among the sports often cited for having a high incidence of athletes engaging in unhealthy nutrition and weight loss practices. Sports that require athletes to meet weight requirements are interesting to explore because weight gains as little as one pound can prevent athletes from competing (Spelke, 1997). One of the weight-sensitive sports is gymnastics.

Gymnastics is a sport whereby people with smaller and slimmer bodies seem to have advantage. One of the areas of concern to athletes especially gymnasts is how to lose weight. Gymnasts have weight-control issues (Ryan, 1995; Ubbes, 1991). Gymnasts always think of how to compete within their weight class. Reginald *et al.* (2005) observed that gymnastics emphasizes thinness, leanness and/or competing at the lowest possible weight. The emphasis on thinness and appearance, according to Zaggelidis *et al.* (2005), encourages the gymnasts to closely focus on their weight and caloric intake. Smiley *et al.* (2008) noted that in sports in which the uniforms are relatively revealing, the human body is often highlighted. The body hugged and fitted gymnastics attire especially the leotard or unitard sometimes worn with tights reveals the body physique of gymnasts. Because of this, gymnasts are often self-conscious of their body physique, thereby necessitating every efforts of having a pleasant body physique. Gymnasts are often struggling with losing weight rather than gaining weight.

There are a variety of weight-control behaviours that may cause weight loss (Spelke, 1997). Athletes report engaging in weight control behaviours to increase performance or to successfully compete in their sport (Davis, 1992; Sykora *et al.*, 1993). The weight-control behaviours athletes employ are not always appropriate or healthy (Round-Table, 1985). Studies (Brownell and Rodin, 1992; Ashley, 1996; Perriello, 2001) have shown that in an attempt to lose weight and body fat, some athletes resort to unhealthy weight-control practices.

Among highly competitive athletes, unhealthy eating behaviours are rampant (Dummer *et al.*, 1987a; Ryan, 1995). Unhealthy attempts at weight-control include disordered eating (Rosen *et al.*, 1986; Black and Burckes-Miller, 1988). Dummer *et al.* (1987a) reported that athletes engage in behaviours such as taking pills,

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diuretics and laxatives to control their weight. In attempts to lose weight, Ubbes (1991) reported that majority of the gymnasts skipped breakfast or lunch daily. Also, Loosli *et al.* (1986) found that over 40% of gymnasts consumed diets that provided less than two-thirds of the daily recommendation for calcium, vitamin B6, iron and zinc. Harris and Greco (1990) reported that 61% of their sample was trying to lose weight, while Rosen and Hough (1988) found that all of the gymnasts in their study were dieting. Petri and Stoever (1993) reported the frequent use of pathogenic weight control methods by gymnasts.

In a study conducted in United States by French *et al.* (1999), majority of the adults increase exercise; decrease fat intake; reduce food amount and reduce calories to lose weight. Use of steroids among student-athletes is well documented (Chng and Moore, 1990). Spelke (1997) noted that unhealthy eating behaviours and illegal drugs are often employed in an effort to achieve a perceived ideal weight for a given sport. The weight-loss practices include; voluntary dehydration (Gisolfi and Duchman, 1992; Reginald *et al*., 2005), food restriction and over-exercising (Johnson, 1994) and vomiting, using rubber suits, steam baths or saunas, using anorexic drugs, laxatives, diuretics, diet pills, nutritional supplements (Reginald *et al*., 2005). Again decrease in energy or calories intake; increase in calories expenditure and increase fluid intake (Perriello, 2001; Reginald *et al*., 2005) are also weight-loss practices.

Athletes may practice weight-control methods during the sports season only or year-round (Reginald *et al*., 2005). Most athletes who want to either lose or gain weight are motivated by a desire for improved appearance, better performance, or perceived competitive advantage (Perriello, 2001) and Rivers state gymnasts cannot be exempted. Gymnasts are most likely to vigorously seek to lose weight during pre-competition and competition periods.

Gymnasts are often encouraged by their coaches to watch their weight. In effort to watch the weight, gymnasts resort to different weight-loss practices. Considering the health and performance effects of some of the weight-loss practices, there is need to establish the weight-loss practices adopted by the gymnasts for possible intervention if need be. From literature review, studies have so much been carried out on female gymnasts (Rosen *et al*., 1986; Rosen and Hough, 1988; Harris and Greco, 1990; Petri and Stoever, 1993). A study involving both male and female gymnasts to the best knowledge of the researcher has not been conducted in developing countries like Nigeria and Rivers state in particular. Hence, the purpose of the study was to explore the weight-loss practices of Rivers state gymnasts and the influence of demographic factors (gender, age and years of sporting experience) on the weight-loss practices of the gymnasts. The study postulates that gender, age and years of sporting experience may not have significant influence on the weight-loss practices of the gymnasts.

MATERIALS AND METHODS

A cross-sectional survey design was employed for the study. The population for the study comprised all the gymnasts in Rivers state. Eighteen gymnasts constituted the sample for the study. The instrument for data collection was 11-item structured questionnaire. The questionnaire was validated by three experts in the fields of Nutrition, Human Kinetics and Health Education and Medicine. In order to establish the reliability of the instrument, split-half method was used after administering ten copies to Imo state gymnasts. The instrument with Pearson product moment correlation in conjunction with Spearman-Brown prophecy statistic yielded a co-efficient of 0.79. Eighteen copies of questionnaire were administered to the gymnasts with the help of the gymnastics coach and captain after their practice session. All the copies of the questionnaire were returned the next day, thereby giving 100% return rate. Percentage and chi-square statistics were used to analyze the data.

RESULTS

Weight-loss practices: Table 1 shows that 14 (77.78%) gymnasts decrease their consumption of calories and 16 representing 88.89% of the gymnasts increase their expenditure of calories through exercise to lose weight. Only 2 (11.11%) of the participants use drugs and 4 (22.22%) use nutritional supplements to lose weight. Data in the Table 1 show that 17 (94.44%) participants restrict themselves from food, 15 (83.33%) over-exercise and voluntarily reduce fluid intake, respectively, to lose weight. On the other hand, none (0.00%) of the gymnasts use steam baths or sauna to lose weight.

Table 1: Weight-loss practices of the gymnasts

Practices	f	%
Decrease consumption of calories	14	77.78
Increase expenditure of calories through exercise	16	88.89
Food restriction	17	94.44
Over-exercising	15	83.33
Use of drugs	2	11.11
Use of nutritional supplements	4	22.22
Voluntary fluid reduction or dehydration	15	83.33
Use of steam baths or sauna	0	0.00

Influence of gender on weight-loss practices: Table 2 showed the differences in the proportion of the weight-loss practices of male and female gymnasts. It was apparent that the gymnasts decreased their consumption of calories (73.33% males; 100.00% females); restrict food (93.33% males; 100.00%

Table 2: Proportion of gymnasts who engaged in weight-loss practices based on gender

Practices	Male (n = 15) %	Female (n = 3) %	chi square cal.	chi square crit.
Decrease consumption of calories	73.33	100.00	1.87	3.841
Increase expenditure of calories through exercise	93.33	66.67	4.80*	3.841
Food restriction	93.33	100.00	5.47*	3.841
Over-exercising	86.67	66.67	3.33	3.841
Use of drugs	6.67	33.33	4.80*	3.841
Use of nutritional supplements	6.67	100.00	5.47*	3.841
Voluntary fluid reduction or dehydration	80.00	100.00	2.80	3.841
Use of steam baths or sauna	0.00	0.00	7.20*	3.841
Cluster %	55.00	70.83		

df = 1; p<0.05; *significant; cal. means calculated; crit. means critical

females); use of nutritional supplements (6.67% males; 100.00% females) and voluntary fluid reduction (80.00% males; 100.00% females). Above all, the Table showed that greater proportion of female gymnasts (70.83%) than male gymnasts (55.00%) engaged in weight-loss practices. It was found that gender had significant influence on gymnasts' increase expenditure of calories through exercise (chi square cal. 4.80 > chi square crit. 3.841, p<0.05, df 1), food restriction (chi square cal. 5.47 > chi square crit. 3.841, p<0.05, df 1), use of drugs (chi square cal. 4.80 > chi square crit. 3.841, p<0.05, df 1), use of nutritional supplements (chi square cal. 5.47 > chi square crit. 3.841, p<0.05, df 1) and use of steam baths or saunas (chi square cal. 7.20 > chi square crit. 3.841, p<0.05, df 1) to lose weight. On the other hand, gender had significant influence on gymnasts' decrease consumption of calories (chi square cal. 1.87 < chi square crit. 3.841, p<0.05, df 1), over-exercise (chi square cal. 3.33 < chi square crit. 3.841, p<0.05, df 1) and voluntary fluid reduction or dehydration (chi square cal. 2.80 < chi square crit. 3.841, p<0.05, df 1) to lose weight.

Influence of age on weight-loss practices: Data in Table 3 revealed the differences that exist in the proportion of gymnasts aged less than 25 years and those who are 25 years and above in their weight-loss practices. Prominent among them are decreased in consumption of calories (71.43% gymnasts < 25 years; 100.00% gymnasts ≥ 25 years); food restriction (92.86% gymnasts < 25 years; 100.00% gymnasts ≥ 25 years); increase expenditure of calories through exercises (85.71% gymnasts < 25 years; 100.00% gymnasts ≥ 25 years), and voluntary fluid reduction (78.57% gymnasts < 25 years; 100.00% gymnasts ≥ 25 years). In addition, the table revealed that gymnasts' age had significant influence on the increase expenditure of calories through exercise (chi square cal. 4.02 > chi square crit. 3.841, p<0.05, df 1), food restriction (chi square cal. 5.45 > chi square crit. 3.841, p<0.05, df 1), over-exercising (chi square cal. 4.45 > chi square crit. 3.841, p<0.05, df 1), use of drugs (chi square cal. 6.16 > chi square crit. 3.841, p<0.05, df 1), use of nutritional supplements (chi square cal. 4.45 > chi square crit. 3.841, p<0.05, df 1) and use of steam baths or saunas (chi square cal. 7.16 > chi square crit. 3.841, p<0.05, df 1) to lose weight. It could be seen from the table that gymnasts' age had no significant influence on their decrease consumption of calories (chi square cal. 2.02 < chi square crit. 3.841, p<0.05, df 1) and voluntary fluid reduction or dehydration (chi square cal. 2.88 > chi square crit. 3.841, p<0.05, df 1) to lose weight.

Influence of years of sporting experience on weight-loss practices: Differences in proportions are found in the weight-loss practices of gymnasts with less than 2 years and those with 2 years and above of sporting experience as could be seen in Table 4. Among the differences in the weight-loss practices are increased expenditure of calories through exercise (80.00% gymnasts < 2 years of sporting experience; 100.00% gymnasts ≥ 2 years of sporting experience); food restriction(90.00% gymnasts < 2 years of sporting experience; 100.00% gymnasts ≥ 2 years of sporting experience); over-exercising and voluntary fluid reduction (70.00% gymnasts < 2 years of sporting experience; 100.00% gymnasts ≥ 2 years of sporting experience). Data in the table showed that years of sporting experience had significant influence on gymnasts' increase expenditure of calories through exercise (chi square cal. 4.31 > chi square crit. 3.841, p<0.05, df 1), food restriction (chi square cal. 5.51 > chi square crit. 3.841, p<0.05, df 1), use of drugs (chi square cal. 4.61 > chi square crit. 3.841, p<0.05, df 1) and use of steam baths or saunas (chi square cal. 7.11 > chi square crit. 3.841, p<0.05, df 1) to lose weight. The study showed that years of sporting experience had significant influence on gymnasts' decrease consumption of calories (chi square cal. 2.01 < chi square crit. 3.841, p<0.05, df 1), over-exercise (chi square cal. 3.51 < chi square crit. 3.841, p<0.05, df 1), use of nutritional supplements (chi square cal. 2.51 < chi square crit. 3.841, p<0.05, df 1) and voluntary fluid reduction or dehydration (chi square cal. 3.15 < chi square crit. 3.841, p<0.05, df 1) to lose weight.

Table 3: Proportion of gymnasts who engaged in weight-loss practices based on age

Practices	Less than 25 years (n = 14) %	25 years and above (n = 4) %	chi square cal.	chi square crit.
Decrease consumption of calories	71.43	100.00	2.02	3.841
Increase expenditure of calories through exercise	85.71	100.00	4.02*	3.841
Food restriction	92.86	100.00	5.45*	3.841
Over-exercising	92.86	50.00	4.45*	3.841
Use of drugs	0.00	50.00	6.16*	3.841
Use of nutritional supplements	7.14	75.00	4.45*	3.841
Voluntary fluid reduction or dehydration	78.57	100.00	2.88	3.841
Use of steam baths or sauna	0.00	0.00	7.16*	3.841
Cluster %	41.96	71.88		

df = 1; p<0.05; *significant; cal. means calculated; crit. means critical

Table 4: Proportion of gymnasts who engaged in weight-loss practices based on years of sporting experience

Practices	Less than 2 years (n = 10) %	2 years and Above (n = 8) %	chi square cal.	chi square crit.
Decrease consumption of calories	70.00	87.50	2.01	3.841
Increase expenditure of calories through exercise	80.00	100.00	4.31*	3.841
Food restriction	90.00	100.00	5.51*	3.841
Over-exercising	70.00	100.00	3.51	3.841
Use of drugs	0.00	25.00	4.61*	3.841
Use of nutritional supplements	10.00	37.50	2.51	3.841
Voluntary fluid reduction or dehydration	70.00	100.00	3.15	3.841
Use of steam baths or sauna	0.00	0.00	7.11*	3.841
Cluster %	48.75	68.75		

df = 1; p<0.05; *significant; cal. means calculated; crit. means critical

DISCUSSION

It was found that majority of the gymnasts decreased their consumption of calories, increased their expenditure of calories through exercise, restrict food, over-exercise and engage in voluntary fluid reduction or dehydration to lose weight. It was not surprising that these gymnasts used both healthful and harmful measures to lose weight provided they have expected physical appearance and high performance and competitive advantage are achieved. To the gymnasts the harmful means may appear to be faster than the healthful means to lose weight. The finding that gymnasts restrict food to lose weight was in agreement with Ubbes (1991) who found that 66% of the gymnasts she surveyed skipped breakfast and lunch daily. Supporting the finding, French *et al*. (1999) reported that majority of the adults increase exercise, decrease fat intake, reduce food amount and reduce calories to lose weight. It is interesting that none (0.00%) of the gymnasts use steam baths or sauna to lose weight.

Higher proportion of males as could be seen in Table 2 increased their expenditure of calories through exercise and over-exercise than females to lose weight. This is supported by Weiss *et al*. (2006) who reported that among the adults who tried to lose weight, men were significantly more likely than women to exercise. On the other hand, more females than males engage in use of drugs, nutritional supplements, voluntary fluid reduction, and decrease in calories consumption to lose weight. Men were significantly less likely than women to use a liquid diet formula, join a weight-loss program, take diet pills prescribed by a doctor, take other pills, medicines, herbs or supplements not needing a prescription and drink a lot of water (Weiss *et al*., 2006). This was supported by Spelke's (1997) finding that females are losing weight with greater frequency. The finding corroborates with Weiss *et al*. (2006) finding that women had a higher prevalence of trying to lose weight. The finding was expected because females are more dissatisfied with their weight, body image and always perceive themselves to be overweight. As a result of this, they always engaged more in weight-loss practices. Gender had significant influence on majority of the weight-loss practices of the gymnasts. The finding was in agreement with Spelke's (1997) result that there was a significant relationship between the weight-control behaviours of student athletes and sex of the athletes.

The finding that gymnasts who are 25 years and above (71.88%) engaged in weight-loss practices than those aged less than 25 years (41.96%) showed difference. The finding was in corroboration with O'Dea and Caputi (2001) who reported that there were age differences in adolescents' weight control practices, but the finding of this study in relation to age and weight-loss was not surprising. This is because as the gymnasts' age

increases at adolescent and early adulthood, the bones and muscles increase in size thereby contributing to proportionate increase in weight. These gymnasts who are 25 years and above with much weight are more likely to be bothered on losing weight than those who are less than less than 25 years. Age had significant influence on majority of the weight-loss practices of the gymnasts. The finding was in disagreement with Neumark-Sztainer *et al.* (2002) report that weight control/disordered eating behaviours were not associated with age. The finding was also not in agreement with Knoke and Barrette-Connor's (2003) finding that there was no significant interaction between weight loss and age in men or women.

Gymnasts with 2 years and above of sporting experience (68.75%) engaged in weight-loss practices than their counterparts with less than 2 years of sporting experience (48.75%). Gymnasts with 2 years and above of sporting experience are likely to be much older and as such may have much weight compared to those with less than 2 years of sporting experience. Some of the gymnasts with 2 and above as their years of sporting experience may have made some records which they will like to maintain and seeing weight-loss as a means, they will not hesitate. Years of sporting experience had significant influence on majority of the weight-loss practices of the gymnasts.

Conclusion and recommendations: Gymnasts in order to meet the weight requirement of their sport, have improved performance, body image and competitive advantage over their opponents, engaged in both healthful and harmful weight-loss practices. The harmful weight-loss practices have been established to have deleterious effects on the health and performance of athletes including gymnasts. Hence, there is need for intervention aimed at preventing the gymnasts from engaging in harmful weight-loss practices. Based on this, the following recommendations are made.

- Gymnasts should be educated on the health and performance effects of engaging in harmful practices of losing weight through seminars. The healthy weight-loss strategies especially healthy nutrition and exercises should be emphasized.
- The weight of the gymnasts should be monitored, checked and examined regularly by the coaches, sports director, with the help of medical and paramedical (nutritionists/dieticians) personnel to know the rate at which a gymnast is losing weight and possibly detect those using harmful means to lose weight.
- The mass media should assist in disseminating information on health implications of harmful weight-loss practices.
- Weight management education should be integrated into the school curriculum. The healthy weight management strategies should be emphasized in the class.
- Gymnasts' parents/guardians, coaches, sports director, peers, friends, siblings and other stakeholders should be sensitized on the weight-loss practices. This will enable them to discourage any gymnast planning to adopt unhealthy weight-loss measures.

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REFERENCES

Ashley, C.D., J.F. Smith, J.B. Robinson and M.T. Richardson, 1996. Disordered eating in female collegiate athletes and collegiate females in an advanced program of study: A preliminary investigation. Int. J. Sports Nutr., 6: 391-401.

Black, D.R. and M.E. Burckes-Miller, 1988. Male and female college athletes: Use of anorexia nervosa and bulimia nervosa weight loss method. Res. Q Exerc. and Sport, 59: 252-256.

Brownell, K.D. and J. Rodin, 1992. Prevalence of eating disorders in athletes. In K. D. Brownell, and J. H. Wilmore (Eds.), Eating, body weight and performance in athletes: Disorders of modern society (pp: 128 - 145). Philadelphia: Lea & Febiger.

Chng, C.L. and A. Moore, 1990. A study of steroid use among athletes: Knowledge, attitude and use. Health Edu., 21: 12-17.

Davis, C., 1992. Body image, dieting behaviours and personality factors: A study of high-performance female athletes. The Int. J. Sports Psychol., 23: 179-192.

Dummer, G.M., L.W. Rosen, W.W. Heusner, P.J. Roberts and J.E. Counsilman, 1987a. Pathogenic weight-control behaviors of young competitive swimmers. Phys. Sportsmed, 15: 75-84.

French, S.A., R.W. Jeffery and D. Murray, 1999. Is dieting good for you?: Prevalence, duration and associated weight and behaviour changes for specific weight loss strategies over four years in US adults. Int. J. Obes., 23: 320-327.

Gisolfi, C.V. and S.M. Duchman, 1992. Guidelines for optimal replacement beverages for different athletic events. Med. Sci. Sports Exerc, 24: 679-687.

Harris, M.B. and D. Greco, 1990. Weight control and weight concern in competitive female gymnasts. J. Sport Exerc Psychol., 12: 427-433.

Johnson, M.D., 1994. Disordered eating in active athletic women. Clin. Sports Med., 13: 355-369.

Knoke, J.D., and E. Barrette-Connor, 2003. Weight loss: A determinant of hip bone loss in older men and women. Am. J. Epidemiol., 158: 1132-1138.

Loosli, A.R., J. Benson, D.M. Gillien and K. Bourdet, 1986. Nutrition habits and knowledge in competitive adolescent female gymnasts. Phys. Sportsmed, 14: 118-130.

Neumark-Sztainer, D., J. Patterson, A. Mellin, D.M. Ackard, J. Utter and J. Sockalosky, 2002. Weight control practices and disordered eating behaviors among adolescent females and males with type 1 diabetes. Diabetes Care, 25: 1289-1296.

O'Dea, J.A. and P. Caputi, 2001. Association between socioeconomic status, weight, age and gender and the body image and weight control practice of 6-to-19-year-old children and adolescents. Health Edu. Res., 6: 521-532.

Perriello, V.A., 2001. Aiming for healthy weight in wrestlers and other athletes. Contemp. Pediatr, 18: 55-74.

Petri, T.A. and S. Stoever, 1993. The incidence of bulimia nervosa and pathogenic weight control behaviors in female collegiate gymnasts. Research Quarterly for Exercise and Sport, 64: 238-241.

Reginald, L., T. David, S. Joel, G. Jorge, J.M. Thomas, E.R. Frederick and G.R. Stephen, 2005. Promotion of healthy weight-control practices in young athletes. Pediatrics, 116: 1557-1564.

Rosen, L.W. and D.O. Hough, 1988. Pathogenic weight-control behaviors of female college gymnasts. Phys. Sportsmed, 16: 141-144.

Rosen, L.W., D.B. McKeag, D.O. Hough and V. Curley, 1986. Pathogenic weight-control behavior in female athletes. Phys. Sportsmed, 14: 79-83.

Round-Table, A., 1985. Eating disorders in young athletes. Phys. Sportsmed, 13: 89-104.

Ryan, J., 1995. Little girls in pretty boxes: The making and breaking of elite gymnasts and figure skaters. New York: Doubleday.

Smiley, N., S.D. Aberedeen and J. Lim, 2008. Eating disorders among female college athletes. The Sport J., 11(3): Available from: http://www.thesportjournal.org/article/eating-disorders-among-female-college-athletes [Accessed 12 August 2009].

Spelke, A.E.M., 1997. Winning at all costs?: The weight-control behaviors of student athletes. MA Thesis: Virginia Polytechnic Institute and State University. Available from: http://scholar.lib.vt.edu/theses/public/etd-44371193973981/etd.pdf [Accessed 12 August 2009].

Sykora, C., C.M. Grilo, D.E. Wilfley and K.D. Brownell, 1993. Eating, weight and dieting disturbances in male and female lightweight and heavyweight rowers. Int. J. Eat Disord., 14: 203-211.

Ubbes, V.A., 1991. Relationship of self-concept, eating behavior and success of female collegiate gymnasts from big ten conference teams. In D.R. Black (Ed.), Eating Disorders among Athletes: Theory, Issues and Research (pp: 143-172). Reston, VA: American Alliance for Health, Physical Education, Recreation and Dance.

Weiss, E.C., D.A. Galuska, L.K. Khan and M.K. Serdula, 2006. Weight-control practices among U.S. adults, 2001-2002. Am. J. Prev. Med., 31: 18-24.

Zaggelidis, S., K. Martinidis, G. Zaggelidis and T. Mitropoulou, 2005. Nutritional supplements use in elite gymnasts. Physical Training, Available from: http://www.sportscience.ro/html/reviste_2005_44-2.html [Accessed 10 September 2009].

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Phytochemical Screening of Solvent Extracts from *Hyptis suaveolens LAM* for Fungal Growth Inhibition

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Abstract: *Hyptis suaveolens* was targeted on the basis of folkloric uses which suggest its toxicity to microbes, coupled with its importance as food to humans. The pulverized plant material was extracted with 96% ethanol and further partitioned using chloroform, distilled water, petroleum ether and methanol. Soluble solvent extracts of the plant were tested for phytochemicals which revealed the existence of alkaloids, flavonols, flavones, flavonones, terpenoids, tannins, aldehydes and ketones and the absence of steroids, saponins and anthraquinones. Antifungal screening exhibited growth inhibition in some instances which exceeded that of griseofulvin antibiotics. The presence of phytochemicals and activity against *Aspergillus niger*, *Candida albicans*, *Cryptococcus* and *Fursarium* species support ethno-medicinal uses of plant.

Key words: *Hyptis suaveolens*, soluble solvent extracts, phytochemicals, *Aspergillus niger*, *Candida albican*, *Cryptococcus* and *Fursarium* species, griseofulvin, fungal growth inhibition, zones of inhibition and toxic components

INTRODUCTION

A wide range of our recently used medicines had their roots directly or indirectly from plants. Some of these medicines are no longer synthesized in large quantities by competitors because they have shown toxicity to humans and other animals. This has made it possible for more investigations to be carried out on plants so as to enable us know the therapeutic status of newly discovered drugs of plant origin. In this respect, plant based research has made promising results in the fields of anticancer and anti-malarial therapies (De Smet, 1997).

Of the 250,000-500,000 species of existing plants on earth (Borris, 1996), only about 300 species are being used worldwide in the pharmaceutical, food, cosmetics and perfume industries (Robber and Speedie, 1996; Lee *et al*., 1997). A relatively small percentage (1-10 %) of these plants is consumed as food by both humans and other animal species, while more are used for medicinal purposes (Moerman, 1996).

Plant foods contain constituents such as flavonoids, saponins, tannins, phenolics, etc, which have been assessed for their anti-oxidant, anti-mutagenic, anti-carcinogenic and other biological effects (Krishnaswamy and Raghuramulu, 1998). Interestingly, natural product research guided by ethno-pharmacological knowledge has made substantial contributions to drug innovation by providing novel chemical structures and or mechanisms of action (De Smet, 1997).

According to a report by Walker, the medicinal properties of plants could be seen in their response to attacks from insect predators and disease organisms. This is achieved by the accumulation of phytochemicals at the sites of infection of plants, several of which are insecticidal, anti-bacterial, anti-fungal, etc (Walker, 1975; Ameen *et al*., 2005).

MATERIALS AND METHODS

The plant, *Hyptis suaveolens LAM* was randomly collected from the farmland on Navrongo Campus at University for Development Studies, Ghana. It was identified by Dr. Walters M. Kpikpi and Dr. Sarkey, both from Department of Applied Biology, Faculty of Applied Sciences. The entire plant was air-dried at room temperature for three weeks and later ground. The pathogenic fungi: *Aspergillus niger*, *Candida albican*, *Cryptococcus* and *Fursarium* species were collected from the Medical School of University of Legon, Ghana after characterization and identification using microbiological procedures of Cowan and Steel (1965). The solvents and other materials used in this study were of analytical grades bought from Timster laboratory Supplies Limited, Accra, Ghana. These include: 96% ethanol, chloroform, methanol, petroleum ether, distilled water, Dimethylsulphoxide (D.M.S.O), sabouaud dextrose agar, Petri dishes, Whatmann number 1. filter papers, etc.

Extraction procedure: Air-dried, ground plant sample (600 g) was percolated with 2,200 ml of 96% ethanol

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at room temperature for two weeks with intermittent shaking. The percolate was evaporated to dryness at room temperature and a crude extract (ethanol soluble) was obtained. 0.1 g of the crude extract was then transferred in to a vial and kept at the lower compartment of a refrigerator until required for use. The remainder of the crude extract was used in a fractionation process.

Fractionation of crude extract: The crude extract prepared as mentioned above was partitioned between chloroform and distilled water (120, 1:1), using a separating funnel. The chloroform and distilled water soluble fractions were separately evaporated at room temperature to residues. 0.1 g of the chloroform soluble residue and all the distilled water soluble residue were transferred in to two distinct vials and kept at the lower compartment of a refrigerator until required for use. The remainder of the chloroform soluble residue was also partitioned between methanol and petroleum ether (120, 1:1). These solvent soluble fractions were separated and concentrated as the chloroform and distilled water soluble fractions to their respective residues which were transferred in to distinct vials and kept as other residues until required for use.

Qualitative phytochemical evaluation: Phytochemical screening was conducted to determine the presence of natural products in the extracts obtained from the entire parts of *Hyptis suaveolens* LAM.

Alkaloids (Wakama test): 0.2 g of each extract was re-extracted with 1% HCl for 24 h. 2 ml portion of the filtrate was taken and tested for alkaloids by adding drops of Meyer's reagent. Alkaloids formed a picric yellow precipitate with the reagent.

Flavonoids (Willistatter test): To methanol solution of each extract, a piece of magnesium ribbon was added followed by drop-wise addition of concentrated HCl. Colors ranging from orange to red indicated flanones, red to crimson indicated flavonols and crimson to magenta indicated flavononones.

Terpenoids and steroids (Liebermann buchart test): A small quantity of each extract was dissolved in trichloromethane and a minimum amount of concentrated sulphuric acid was then added to its content. A blue or green color or a mixture of these two shades was taken as positive test for steroidal compounds, while red, pink or violet color indicates the presence of terpenoids.

Tannins: 0.2 g of each extract was re-extracted with ethanol. The solution obtained was later treated with 5% ferric chloride. A blue-black or blue-green appearance was taken as positive test for tannins.

Saponins: A small portion of each extract was added to 2 ml of distilled water and boiled for 3-5 min. The resultant mixture was filtered, allowed to cool with the filtrate shaken vigorously. Honey comb froth higher than the aqueous layer was taken as strongly positive for saponins. Froth as high as the aqueous layer was taken as moderate and lower than this as negative for the presence of saponins.

Anthraquinones (Bornstrager-test Kraus modified): About 0.5 g of each extract was boiled for a few minutes with 12 ml of 0.5 M potassium hydroxide and 2 ml of hydrogen peroxide (10 %). The mixture obtained was then cooled, filtered, acidified and extracted with a small quantity of ammonium hydroxide solution. A red color formed in the alkaline layer indicated the presence of anthraquinones.

Aldehydes: To 1 ml of freshly prepared Tollen's reagent in a tilted test tube rinsed with 3 Molar sodium hydroxide solution, one drop of aqueous solution of each extract was slowly added. A silver mirror on the surface of the test tube is a positive test for aldehydes.

Ketones: To 1 ml of aqueous solution of each extract in a test tube, a few drops of 3 molar sodium hydroxide solution was added, followed by a slow addition of 3 drops of iodine solution. The test tube was stopped and shaken vigorously. A positive test resulted from a brown color of the mixture disappearing and a yellow iodo-form solid precipitating out of solution.

Antifungal bioassay: The spreading method of Cruickshanks *et al.* (1980) and dose (agar) diffusion method were used.

Five days old cultures of *Aspergillus niger*, *Candida albicans*, *Cryptococcus* and *Fursarium* species to be tested were used. 0.1 ml solution of cultures were uniformly spread over the surface of sabouraud dextrose agar with the aid of a sterile inoculating loop. The solvent soluble extracts and griseofulvin antibiotics employed in the test were diluted to obtain different concentrations of 1,500, 1,000 and 500 µg /ml using Dimethylsluphoxide (D.M.S.O). 0.1 ml of various concentrations of the prepared extracts and griseofulvin antibiotics were used to fill holes bored by 5mm improvised cork borer in the inoculated agar. Three plates were made for each extract and organism-griseofulvin, standard drug. The plates were then incubated at 37°C for 24 h. Diameters of zones of inhibition were measured manually in millimeters for the created holes from which sample means were calculated.

RESULTS AND DISCUSSION

From Table 2 results, the crude ethanol extract of *Hyptis suaveolens LAM* did not reveal the presence of alkaloids, flavones, flavonols and flavonones, while subsequent fractions did. This can be explained by masking effect which often occurs when different phytochemicals form a mixture. In this effect, the presence of a particular phytochemical is not noticed because it is being masked or inhibited by other phytochemicals that make up the mixture. In search for phytochemicals from the plant, qualitative analyses of solvent soluble extracts/fractions revealed the presence of alkaloids, flavonoids, terpenoids, tannins, aldehydes and ketones, whereas steroids, saponins and anthraquinones were absent.

Results in Table 1 illustrate the growth inhibitory effect of *Hyptis suaveolens LAM* extracts/fractions and griseofulvin, standard antifungal drug on *Aspergillus niger*. At concentrations of 500, 1,000 and 1,500 µg/ml of the plant extracts/fractions and griseofulvin there was a uniform trend of increase in zones of inhibition. Of all the tested at 500, 1,000 and 1,500 µg/ml concentrations, the distilled water soluble fraction of the plant presented the highest growth inhibitory effect on the isolate. It recorded mean zones of inhibition of 7.5±0.10, 12.0±0.10 and 16.5±0.10. This was closely followed by the chloroform soluble fraction with mean zones of inhibition of 6.0±0.20, 11.0±0.27 and 15.0±0.10. Both the distilled water and chloroform soluble fractions of *Hyptis suaveolens LAM* showed higher growth inhibitory effects on *Aspergillus niger* than the antifungal drug. It is an indication that these soluble fractions contained more toxic components which inhibited the growth of the isolate.

Similarly, from Table 4 results it is observed that the plant extracts/fractions and griseofulvin antibiotics inhibited the growth of Candida albicans in an increasing trend at the concentrations of 500, 1,000 and 1,500 µg/ml. The most toxic soluble fraction of *Hyptis suaveolens LAM* to the isolate is the chloroform soluble fraction with mean zones of inhibition of 5.0±0.10, 10.50±0.10 and 12.50±0.10 in an increasing order at concentrations of 500, 1,000 and 1,500 µg/ml. The methanol soluble fraction of the plant showed the least growth inhibitory effect on Candida albicans just as it did on *Aspergillus niger* with mean zones of inhibition of 1.5±0.10, 3.5±0.20 and 5.50±0.15.

The results in Table 3 clearly revealed the resistance to *Hyptis suaveolens LAM* extracts/fractions and griseofulvin antibiotics by *Cryptococcus* species. There were no growth inhibitory effects for griseofulvin antibiotics, methanol and petroleum ether soluble fractions at the concentrations of 500, 1,000 and 1,500 µg/ml. It was only at 1,500 µg/ml concentration that the ethanol soluble extract, the distilled water and chloroform soluble fractions of the plant presented mean zones of inhibition of 5.5±0.10, 6.0±0.20 and 9.0±0.10 respectively. This is an indication that these three soluble fractions of *Hyptis suaveolens LAM* contain toxic components that inhibit the growth of *Cryptococcus* species.

Contrary to results in Table 5 and 6 results showed griseofulvin antibiotics and extracts/fractions of *Hyptis suaveolens LAM* to be toxic to Fursarium species with the methanol soluble fraction presenting the least growth inhibitory effect. At concentrations of 500, 1,000 and 1,500 µg/ml, the distilled water soluble fraction recorded the highest mean zones of inhibition of 12.50±0.10, 19±0.27 and 22.50±0.10 respectively. This is closely followed by the chloroform soluble fraction with mean zones of inhibition of 9.0±0.21, 11.05±0.10 and 18.0±0.10.

A comparison of results from Table 3, 4, 5 and 6 revealed that the soluble fraction of *Hyptis suaveolens LAM* with the highest mean zones of inhibition is alternating between the distilled water and chloroform soluble fractions. To some extend, these two soluble fractions are more active against the isolates than the antifungal drug and therefore contain ingredients which could serve as drugs.

Table 1: Texture, color and weight of solvent soluble extracts/fractions obtained from *Hyptis suaveolens LAM*

Extract/fraction	Texture	Color	Weight (g)
EtOH	Sticky	Dark brown	2.40
$CHCl_3$	Sticky	Dark brown	0.60
Distilled H_2O	Sticky	Dark brown	0.90
MeOH	Sticky	Dark brown	0.20
Petroleum ether	Sticky	Dark brown	0.60

Table 2: Phytochemical screening results of solvent soluble extracts/fractions from *Hyptis suaveolens LAM*

Phytochemicals	EtOH	Distilled H_2O	$CHCl_3$	MeOH	Petroleum ether
Alkaloids	-ve	+ve	-ve	+ve	+ve
Flavones	-ve	+ve	+ve	+ve	+ve
Flavonols	-ve	+ve	+ve	+ve	-ve
Flavonones	-ve	+ve	+ve	-ve	-ve
Terpenoids	+ve	+ve	+ve	+ve	+ve
Steroids	-ve	-ve	-ve	-ve	-ve
Tannins	+ve	+ve	+ve	+ve	+ve
Saponins	-ve	-ve	-ve	-ve	-ve
Anthraquinones	-ve	-ve	-ve	-ve	-ve
Aldehydes	+ve	+ve	+ve	+ve	+ve
Ketones	+ve	+ve	+ve	+ve	+ve

+ve means phytochemical is present; -ve means phytochemical is absent

Table 3: Growth inhibitory effect of *Hyptis suaveolens LAM* extracts/fractions and griseofulvin antibiotics on *Aspergillus niger*

Concentration	Zone of inhibition (mm)					
(µg/ml)	Griseofulvin	EtOH	$CHCl_3$	Distilled H_2O	MeOH	Petroleum ether
500	4.0±0.10	5.5 ±0.20	6.0±0.20	7.5±0.10	0.0±0.0	5.0±0.10
1,000	6.0±0.10	6.0 ±0.10	11±0.27	12.0 ±0.10	0.50±0.01	7.0±0.30
1,500	12.0±0.17	8.0±0.17	15±0.10	16.5 ±0.10	2.0 ±0.20	12.5±0.01

Table 4: Growth inhibitory effects of *Hyptis suaveolens LAM* extracts/fractions and griseofulvin antibiotics on *Candida albicans*

Concentration	Zones of inhibition (mm)					
(µg/ml)	Griseofulvin	EtOH	$CHCl_3$	Distilled H_2O	MeOH	Petroleum ether
500	4.5±0.10	4.0±0.20	5.0±0.10	4.0±0.10	1.5±0.10	5.0±0.20
1,000	10.0±0.20	10±0.10	10.5±0.10	8.0±0.10	3.5±0.20	8.5±0.10
1,500	12.0±0.10	10.5±0.29	12.5±0.10	11.5±0.10	5.5±0.15	11.0±0.10

Table 5: Growth inhibitory effects of *Hyptis suaveolens LAM* extracts/fractions and griseofulvin antibiotics on *Cryptococcus* species

Concentration	Zones of inhibition (mm)					
(µg/ml)	Griseofulvin	EtOH	$CHCl_3$	Distilled H_2O	MeOH	Petroleum ether
500	0	0	0	0	0	0
1,000	0	0	0	0	0	0
1,500	0	5.5±0.10	9.0±0.10	6.0±0.20	0	0

Table 6: Growth inhibitory effects of *Hyptis suaveolens LAM* extracts/fractions and griseofulvin antibiotics on *Fursarium* species

Concentration	Zones of inhibition (mm)					
(µg/ml)	Griseofulvin	EtOH	$CHCl_3$	Distilled H_2O	MeOH	Petroleum ether
500	5.5±0.10	8.0±0.10	9.0±0.21	12.50±0.10	0	9.0±0.20
1,000	7.0±0.17	12±0.10	11.05±0.1	19.0±0.27	3.0±0.10	12.0±0.10
1,500	10.0±0.10	13±0.17	18±0.10	22.5±0.10	6.0±0.10	14.0±0.10

Conclusion: The investigation was able to prove that *Hyptis suaveolens LAM* contained phytochemicals or agents which were effective against *Aspergillus niger*, *Candida albicans*, *Cryptococcus* and *Fursarium* species. It also went further to explain that bioactive agents of the plant were more effective in inhibiting the growth of isolates than griseofulvin, antifungal drug. These findings justify the ethno-medicinal uses of the plant and could be of interest to pharmaceutical companies.

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REFERENCES

Ameen, O.M., O.M. Fatope, L.A. Usman and S.A. Adebayo, 2005. Bioactive metabolites in improved cowpea seeds. Afr. J. Biotech., 4: 513-516.

Borris, R.P., 1996. Natural products research: perspectives from a major pharmaceutical company. J. Ethnopharmacol., 51: 29-38.

Cowan, S.T. and K.J. Steel, 1965. Manual for the identification of Medical Bacteria, 1965. Cambridge Univ. Press.

Cruickshanks, R., J.P. Oluguid, B.P. Mormoin and R.H.A. Swam, 1980. Medical Microbiology: A guide to laboratory diagnosis and control of infections, 12th edition volume I. Edinburgh: Churchill Livingstone, pp: 9-70, 132-143,157-234, 382-407.

De Smet, P.A., 1997. The role of plant-derived drugs and herbal medicines in healthcare. Drugs, 54: 801-804.

Krishnaswamy, K. and N. Raghuramulu, 1998. Bioacztive phytoczhemiczals with emphasis on dietary practices. In. J. Med Res., 108: 167-681.

Lee, O.S., H.H. Kang and S.H. Han, 1997. Oriental herbs in cosmetics. Cosmet Toiletries. Jan., 112: 57-64.

Moerman, D.E., 1996. An analysis of the food plants and drug plants of native North America. J. Ethnopharmacol., 52: 1-22.

Robber, J.M. and T.V. Speedie, 1996. Pharmacognosy, pharmacobiotechnology. Williams and Wilkins, Baltimore, pp: 1-14.

Walker, R.L., 1975. Functional aspects of phenolic compounds in plants. The biology of plant phenolics. 2nd Edn., Edward Arnold Ltd. London, pp: 23-32.

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Growth Inhibitory Effects of Solvent Extracts of Selected Plants on ß-Lactamase Producing Bacteria

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Abstract: Components of the stem-barks and stem of four different plants, reputed to be medicinal in Northern Nigeria in the treatment of genitourinary tract infections were extracted using 95% ethanol. Ethanol extracts obtained from parts of plants were partitioned using chloroform, distilled water, ethyl acetate, methanol and petroleum ether solvents of varying polarity indices in to fractions which were later screened together with saved ethanol extracts against ß-lactamase producing bacteria that have demonstrated some resistance to ß-lactam antibiotics. The screened extracts and fractions of both the stem-barks and stem of *Butyrospermum parkii*, *Kigelia pinnata* and *Maytenus senegalensis* inhibited the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumonia* which are causative agents of genitourinary tract infections in the paper disk-plate method employed in the investigation. This finding is in support of the ethno-medicinal uses of these plants. On a contrary, the ethanol extract of the stem of Anogeissus leiocarpus showed no growth inhibition on the five bacterial isolates.

Key words: *Anogeissus leiocarpus*, *Butyrospermum parkii*, *Kigelia pinnata*, *Maytenus senegalensis*, toxic component, growth inhibition, clinical isolates, folkloric or ethno-medicinal uses

INTRODUCTION

Plants have served as sources of drugs and pharmaceuticals for man and other animals from time immemorial. There are about half a million plants now growing on earth, many of which possess therapeutic and pharmaceutical properties (Sanberg and Bruhn, 1979). According to an earlier survey, about 25% of modern drugs and medicinal products are derived from plant secondary metabolites (Muller, 1973). Penicillin G or benzylpenicillin, a drug isolated from the mould Penicillium notatum has been active against ß-lactamase producing bacteria and it is recently being resisted by the enzyme ß-lactamase secreted by these bacteria. This has therefore posed the need for the search of antimicrobials with different activity profiles (Karaman *et al*., 2003). Currently, the biological activity and importance of secondary metabolites of some plants which support their folkloric uses are not fully or scientifically established, thus requiring more studies to be carried out. As a further development on this, *Anogeissus leiocarpus*, *Butyrospermum parkii*, *Kigelia pinnata* and *Maytenus senegalensis* were selected for investigation of their bioactive components which could be used to combat diseases (Richard, 1998; Ghazi *et al*., 1999).

The five clinical isolates used in this study are known to be disease causing agents and as earlier mentioned have proved resistance to benzylpenicillin. For instance, *Staphylococcus aureus* is often connected to cases of bacteraemia, septicaemia, endocarditis, osteomyelitis and furuncle etc., Pseudomonas aeruginosa and Proteus vulgaris for cystitis, pyelitis and urethritis, *Escherichia coli* for enteritis and diarrhea and Klebsiella species for Pneumonia (Komolafe and Adegoke, 2008).

MATERIALS AND METHODS

Plant materials: The stem-barks and stems of plants from four families were chosen on the basis of folkloric uses that suggest their toxicity to bacteria. Specimens were collected at random from Kaduna (Zaria), Bauchi and Kano States of Nigeria, identified by Baba Ali and authenticated by B.S. Aliyu both of the Biological Science Department, Bayero University Kano, Nigeria. The places and dates of collection of plant parts together with their voucher specimen numbers are shown in Table 1.

Extraction procedure: Air dried and ground parts of plant materials were extracted by percolation with 95% EtOH at room temperature for two weeks. 500 ml of EtOH was used in each instance to percolate *B. parkii* and *M. senegalensis*. 700 ml of the same solvent was used for the percolation of *K. pinnata*, whereas for *A. leiocarpus* 1000 ml of the solvent was used. In the end of the two weeks interval, percolates were evaporated to dryness on a rotary evaporator (R 110) at 40°C and EtOH extracts obtained were weighed and labeled F_{001}.

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Table 1: Families, names (scientific and local), parts, places and dates of collection and herbarium voucher numbers of plants under investigation

Plant name and family	Local name(s) in Hausa	Part(s) used	Place and date of collection	Voucher No.
Anogeissus leiocarpus DC (Combretaceae)	Marke	Stem-bark	Gwarzo town, Kano State. August, 2002	4/70
Butyrospermum parkii Kotschy (Sapotaceae)	Kadanya	Stem-bark	Zaria,Kaduna State. August, 2002	5/60
Kigelia pinnata Syn (Bignoniaceae)	Nonongiwa Raehana	Stem-bark	Yankari village 70 km South of Bauchi town, Bauchi State. August, 2002	80/193
Maytenus senegalensis Lam (Celastraceae)	Namijin Tsada	Stem	Zaria, Kaduna State. August, 2002	40/246

Portions of EtOH extracts prepared as mentioned above were transferred in to vials and kept for antibacterial tests while the remainders were partitioned between $CHCl_3$ and distilled H_2O solvents (200ml,1:1), with the aid of separating funnels. $CHCl_3$ soluble fractions, F_{002} and interface fractions between $CHCl_3$ and distilled H_2O solvents, F_{001*} (where applicable), were separately evaporated to dryness on a rotary evaporator at 40°C, whereas distilled H_2O soluble fractions, F_{003} were washed several times with EtOAc (100 ml), which yielded distilled H_2O soluble fractions, F_{003}, interface fractions between distilled H_2O and EtOAc solvents, F_{002*} (where applicable) and EtOAc soluble fractions, F_{004}. These soluble solvent fractions were separated and concentrated as the $CHCl_3$ soluble fractions and the interface fractions between $CHCl_3$ and distilled H_2O solvents earlier discussed. Portions of $CHCl_3$ residues obtained as mentioned earlier were transferred in to vials and kept for antibacterial tests while the remainders, were further partitioned between MeOH and petroleum ether solvents (200,1:1), to solvent fractions which were separated and concentrated as the $CHCl_3$ soluble fractions and the interface fractions between $CHCl_3$ and distilled H_2O solvents earlier discussed to give the MeOH (F_{005}), the interface (F_{003*}) and the petroleum ether (F_{006}) residues respectively. All residues obtained in the process were transferred in to distinct vials and kept at the lower compartment of a refrigerator until they were required for use.

Antimicrobial bioassay: Five bacterial strains, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumonia* which are the causative agents of most genitourinary tract infections were tested for growth sensitivity using the paper disk-plate method on ingredients from plants reputed to be of medicinal value in Northern Nigeria. All the organisms, ß-lactamase producing bacteria were clinical isolates obtained from the Department of Microbiology, Aminu Kano Teaching Hospital, Kano, Nigeria. A sterile inoculating loop was used to transfer a portion of the colony of each isolate on to trypton soya broth (T.S.B) that was incubated at room temperature for three days. 0.1 ml of the broth was diluted with 1 ml of distilled water in a ratio of 1:100 (Adoum *et al*., 1997).

A paper punch was used to prepare disks of about 6 mm diameter from Whatman number 1 filter paper. Batches of prepared disks were transferred in to a screw-cap bottle and sterilized in an oven at 140°C for 60 min.

The stock solution for the bioassay was prepared by dissolving 10 mg of each extract and fraction in 1 ml of Dimethylsulphoxide (DMSO) i.e. 10000 µg/ml. Concentrations of 5,000, 2,000 and 1,000 µg/ml of the plant extracts and fractions were prepared using DMSO and the stock solution already formed. 0.1 ml of each concentration of extracts and fractions was introduced in to labeled screw-cap bottles containing 10 disks. The prepared disks were then kept in a refrigerator until required for use.

Disk-diffusion method or paper disk-plate method was employed. Sabouroud's Dextrose Agar (S.D.A) plates were inoculated with standard test inocula by direct streaking. The prepared disks were then introduced on to the inoculated surfaces and the plates were incubated at room temperature for 48 h (Pelczar *et al*., 1993).

Cultures were examined for areas of no growth around the disks (zones of inhibition). Organisms sensitive to the test extracts were inhibited at a distance from the disks, whereas resistant strains grew up to the edge of the disks. In this method, disks impregnated only with D.M.S.O served as control disks (Cheesbrough, 2000). Diameters of zones of inhibition were measured in millimeters with a ruler and recorded as sample means as shown in Table 2, 3a, 3b, 4a, 4b, 5a and 5b.

RESULTS AND DISCUSSION

Results in Table 2 illustrate the ineffectiveness of the EtOH extract of the stem-bark of *Anogeissus leiocarpus* in inhibiting the growth of *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*. This extract showed no zones of inhibition at the tested concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml on the five clinical isolates and it is an indication that the components of the stem of *Anogeissus leiocarpus* are non-toxic to the bacteria employed in the investigation. Hence, the investigation does not support the folkloric or ethno-medicinal uses of the plant.

Of all the results presented in Table 3a, the $CHCl_3$, the $CHCl_3$-distilled H_2O interface and the EtOAc fractions of the stem-bark of Butyrospermum parkii recorded no inhibition of growth on *Staphylococcus aureus* at the tested concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml. This same trend of no growth inhibition was demonstrated by the EtOH extract, the $CHCl_3$ and the EtOAc fractions of the stem-bark of the plant at lower

Table 2: Zones of inhibition (mm) exhibited by EtOH extract of the stem-bark of *Anogeissus leiocarpus* on *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*

	Concentration (µg/ml)				
Bacterial isolate	1,000	2,000	5,000	10,000	Negative control
S. aureus	0	0	0	0	0
P. aeruginosa	0	0	0	0	0
E. coli	0	0	0	0	0
P. vulgaris	0	0	0	0	0
K. pneumoniae	0	0	0	0	0

concentrations of 1,000, 2,000 and 5,000 µg/ml but on *Pseudomonas aeruginosa*. On a contrary, these soluble solvent extracts or fractions of the stem-bark of *Butyrospermum parkii* exhibited growth inhibitory effects on this clinical isolate at the concentration of 10,000 µg/ml. The EtOH extract of the stem-bark recorded a mean zone of inhibition of 7±0.17 on *Pseudomonas aeruginosa*, whereas the $CHCl_3$ and the EtOAc fractions recorded mean zones of inhibition of 7.03±0.3 and 8±0.1 respectively. At the respective concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml, the EtOH soluble extract demonstrated an increasing trend of growth inhibition on *Staphylococcus aureus* with mean zones of inhibition of 1.4±0.1, 2±0.2, 5.5±0.2 and 7±0.2. A similar trend of increasing growth inhibition was exhibited by the distilled H_2O fraction of the stem-bark of the plant on both *Staphylococcus aureus* and *Pseudomonas aeruginosa* clinical isolates. The mean zones of inhibition recorded in an increasing order as the tested concentrations were 1.6±0.3, 2±0.2, 4±0.1 and 7±0.1 for *Staphylococcus aureus* and 2.2±0.1, 3.1±0.12, 5.3±0.17 and 7±0.17 for *Pseudomonas aeruginosa*.

The overall results in Table 3a revealed that the EtOH extract, the $CHCl_3$, the distilled H_2O and the EtOAc fractions of the stem-bark of *Butyrospermum parkii* contained toxic components which inhibited the growth of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. This research finding conforms with the folkloric or ethno-medicinal uses of the plant.

A comprehensive study of the results recorded in Table 3b for the growth inhibitory effect exhibited by extracts of the stem-bark of *Butyrospermum parkii* on *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae* revealed that the distilled H_2O and EtOAc fractions of the stem-bark of the plant were non-toxic to the three clinical isolates at the tested concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml. The $CHCl_3$ fraction, at the concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml was non-toxic to *Proteus vulgaris* and *Klebsiella pneumonia* and recorded no zones of inhibition. A similar trend of no zones of inhibition was exhibited by the $CHCl_3$-distilled H_2O interface fraction on *E. coli* and *K. pneumoniae* at these same concentrations. At concentrations of 1,000, 2,000 and 5,000 µg/ml, the EtOH extract of the stem-bark of *Butyrospermum parkii* showed no growth inhibitory effect on *E. coli*, *P. vulgaris* and *K. pneumoniaer*, whereas at 10,000 µg/ml concentration there were recorded growth inhibitions. The respective mean zones of inhibition recorded for *E. coli*, *P. vulgaris* and *K. pneumoniae* were 7±0.17, 7±0.27 and 7±0.10. In a similar manner as the EtOH extract, the $CHCl_3$ fraction did not exhibit growth inhibition on *E. coli* at lower concentrations of 1,000, 2,000 and 5,000 µg/ml, while at a higher concentration of 10,000 µg/ml there was a growth inhibition of mean value of 7±0.17. Also, there was no growth inhibition exhibited by the $CHCl_3$-distilled H_2O interface fraction on *P. vulgaris* at 1,000 and 2,000 µg/ml concentrations. It was only at 5,000 and 10,000 µg/ml concentrations that this fraction demonstrated growth inhibition with mean zones of 7±0.27 and 8±0.10 respectively.

From the results recorded in Table 3b, it could be seen that the EtOH extract, the $CHCl_3$ and the $CHCl_3$-distilled H_2O interface fractions showed the presence of components in the stem-bark of the plant which inhibited the growth of *E. coli*, *P. vulgaris* and *K. pneumoniae*. This research finding is in support of the folkloric or ethno-medicinal uses of *Butyrospermum parkii*.

Results in Table 4a revealed that at the concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml, the $CHCl_3$, the distilled H_2O, the distilled H_2O-EtOAc interface, the EtOAc, the MeOH and the petroleum ether fractions of the stem-bark of *Kigelia pinnata* exhibited no growth inhibitory effect on *S. aureus* and *P. aeruginosa*. All the negative controls presented a similar trend of no growth inhibitory effect on the two clinical isolates. It is the EtOH extract of the plant which demonstrated growth inhibition on *S. aureus* at concentrations of 5,000 and 10,000 µg/ml with mean zones of inhibition of 7±0.10 and 8±0.3.0 respectively. At lower concentrations of 1,000 and 2,000 µg/ml, this solvent extract showed no growth inhibitory effect on the isolate. Also, there was no growth inhibition observed for *P. aeruginosa* at 1,000 µg/ml concentration of the solvent extract, but at higher concentrations of 2,000, 5,000 and 10,000 µg/ml the respective mean zones of inhibition shown were 2±0.10, 4.5±0.10 and 7±0.20.

The results in Table 4a clearly indicate that the stem-bark of *K. pinnata* contained components which inhibited the growth of *S. aureus* and *P. aeruginosa* when combined as shown by the EtOH extract and these components when separated or partitioned did not

Table 3a: Zones of inhibition (mm) exhibited by extracts of the stem-bark of *Butyrospermum perkii* on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

	Staphylococcus aureus				*Pseudomonas aeruginosa*				
Solvent extract/	Concentration (µg/ml)				Concentration (µg/ml)				Negative
fraction	1,000	2,000	5,000	10,000	1,000	2,000	5,000	10,000	Control
EtOH	1.4±0.10	2±0.20	3.5±0.20	7±0.20	0	0	0	7±0.17	0
$CHCl_3$	0	0	0	0	0	0	0	7.03±0.3	0
$CHCl_3$-H_2O	0	0	0	0	0	0	0	0	0
DistilledH_2O	1.6±0.30	2±0.20	4±0.10	7±0.10	2.2±0.1	3.1±0.12	5.3±0.17	7±0.17	0
EtOAc	0	0	0	0	0	0	0	8±0.10	0

Table 3b: Zones of inhibition (mm) exhibited by extracts of the stem-bark of *Butyrospermum parkii* on *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*

	P. vulgaris				*E. coli*				*K. pneumonia*				
	Concentration (µg/ml)				Concentration (µg/ml)				Concentration (µg/ml)				
Solvent extract/fraction	1000	2000	5000	10000	1000	2000	5000	10000	1000	2000	5000	10000	Negative Control
EtOH	0	0	0	7±0.27	0	0	0	7±0.17	0	0	0	7±0.10	0
$CHCl_3$	0	0	0	0	0	0	0	7±0.17	0	0	0	0	0
$CHCl_3$-DistilledH_2O	0	0	7±0.27	8±0.10	0	0	0	0	0	0	0	0	0
Distilled H_2O	0	0	0	0	0	0	0	0	0	0	0	0	0
EtOAc	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4a: Zones of inhibition (mm) exhibited by extracts of the stem-bark of *Kigelia pinnata* on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

	S. aureus				*P. aeruginosa*				
	Concentration (µg/ml)				Concentration (µg/ml)				
Solvent extract/fraction	1,000	2,000	5,000	10,000	1,000	2,000	5,000	10,000	Negative Control
EtOH	0	0	7±0.10	8±0.30	0	2±0.10	4.5±0.10	7±0.20	0
$CHCl_3$	0	0	0	0	0	0	0	0	0
Distilled H_2O	0	0	0	0	0	0	0	0	0
H_2O-EtOAc	0	0	0	0	0	0	0	0	0
EtOAc	0	0	0	0	0	0	0	0	0
MeOH	0	0	0	0	0	0	0	0	0
Petroleum Ether	0	0	0	0	0	0	0	0	0

Table 4b: Zones of inhibition (mm) exhibited by extracts of the stem-bark of *Kigelia pinnata* on *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*

	P. vulgaris				*E. coli*				*K. pneumoniae*				
Solvent extract/	Concentration (µg/ml)				Concentration (µg/ml)				Concentration (µg/ml)				
fractions	1,000	2,000	5,000	10,000	1,000	2,000	5,000	10,000	1,000	2,000	5,000	10,000	Negative control
EtOH	2±0.30	3±0.10	5±0.20	7±0.10	1.5±0.10	2±0.10	3.4±0.10	7±0.20	0	2±0.10	5±0.20	8±0.30	0
$CHCl_3$	0	0	0	0	0	0	0	0	0	0	0	0	0
Distilled H_2O	0	0	0	0	0	0	0	0	0	0	0	0	0
Distilled H_2O-EtOAc	0	0	0	0	0	0	0	0	0	0	0	0	0
EtOAc	0	0	0	0	0	0	0	0	0	0	0	0	0
MeOH	0	0	0	0	0	0	0	0	0	0	0	0	0
Petroleum Ether	0	0	0	0	0	0	0	0	2.4±0.30	3±0.10	4.5±0.10	7±0.20	0

inhibit the growth of the two isolates as shown by the other solvent fractions of the plant.

Results shown in Table 4b clearly explain the growth inhibitory effect of extracts of the stem-bark of *Kigelia pinnata* on *E. coli*, *P. vulgaris* and *K. pneumoniae* at concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml. The EtOH extract, at these concentrations demonstrated growth inhibition with mean zones of inhibition of 2±0.30, 3±0.10, 5±0.20 and 7±0.10 on *P. vulgaris*. The trend of increasing growth inhibition as the concentration was also exhibited by this same extract on *E. coli*. The mean zones of inhibition exhibited were 1.5±0.10, 2±0.10, 3.4±0.10 and 7±0.20. For *K. vulgaris*, there was no inhibition of growth caused by the extract at 1,000 µg/ml concentration. A reverse of this occurred at higher concentrations of 2,000, 5,000 and 10,000 µg/ml with

Table 5a: Zones of inhibition (mm) exhibited by extracts of the stem of *Maytenus senegalensis* on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

	S. aureus				*P. aeruginosa*				
	Concentration (µg/ml)				Concentration (µg/ml)				
Solvent extract/fractions	1,000	2,000	5,000	10,000	1,000	2,000	5,000	10,000	Negative control
EtOH	0	0	0	0	0	0	0	7±0.17	0
$CHCl_3$	0	0	0	0	0	0	0	0	0
Distilled H_2O	0	0	0	0	0	0	0	0	0
Distilled H_2O-EtOAc	0	0	0	0	0	0	6±0.40	7±0.27	0
EtOAc	0	0	0	0	0	0	0	0	0

Table 5b: Zones of Inhibition (mm) exhibited by extracts of the stem of *Maytenus senegalensis* on *Escherichia coli*, *Proteus vulgaris* and *Klebsiella pneumoniae*

	P. vulgaris				*E. coli*				*K. pneumonia*				
Solvent extract/	Concentration (µg/ml)				Concentration (µg/ml)				Concentration (µg/ml)				
fraction	1,000	2,000	5,000	10,000	1,000	2,000	5,000	10,000	1,000	2,000	5,000	10,000	Negative control
EtOH	0	0	7±0.10	8±0.17	0	0	0	0	0	0	0	0	0
$CHCl_3$	0	0	0	0	0	0	0	0	0	0	0	0	0
Distilled H_2O	2±0.20	3±0.20	7±0.10	8±0.10	0	7±0.10	8±0.10	0	0	0	0	0	0
Distilled H_2O-EtOAc	0	0	6±0.10	7±0.3	0	5.5±0.42	7±0.44	8±0.52	0	0	0	0	0
EtOAc	0	0	0	0	0	0	0	0	0	0	0	0	0

mean zones of inhibition of 2±0.10, 5±0.20 and 8±0.30. Similarly, the $CHCl_3$, the distilled H_2O, the distilled H_2O-EtOAc interface, the EtOAc and the MeOH fractions of the stem-bark of the plant recorded no growth inhibition at all the tested concentrations on *E. coli*, *P. vulgaris* and *K. pneumoniae* just as they didn't on *S. aureus* and *P. aeruginosa* (Table 4a). Also, at the tested concentrations, the petroleum ether fraction demonstrated no growth inhibitory effect on *E. coli* and *P. vulgaris* just as it didn't on *S. aureus* and *P. aeruginosa* (Table 4a). It was only on *K. vulgaris* that this fraction exhibited growth inhibition with mean zones of inhibition of 2.4±0.30, 3±0.10, 4.5±0.10 and 7±0.20 at the respective concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml.

As drawn from the results given in Table 4b, it can be viewed that the EtOH extract and the petroleum ether fraction of the stem-bark of the plant contained components which are toxic that inhibited the growth of *E. coli*, *P. vulgaris* and *K. pneumoniae*. This finding serves as a back-up to the folkloric or ethno-medicinal uses of *Kigelia pinnata*.

For the results of mean zones of inhibition exhibited by extracts of the stem of Maytenus senegalensis on *S. aureus* and *P. aeruginosa* shown in Table 5a, it was only the EtOH extract and the distilled H_2O-EtOAc interface fraction that demonstrated growth inhibition on *P. aeruginosa*. The EtOH extract exhibited a mean zone of inhibition of 7±0.17 at 10,000 µg/ml concentration, while the distilled H_2O-EtOAc interface fraction presented mean zones of inhibition of 6±0.40 and 7±0.27 respectively at 5,000 and 10,000 µg/ml concentrations. For the $CHCl_3$, the distilled H_2O and the EtOAc fractions there were no growth inhibition recorded on both *S. aureus* and *P. aeruginosa* at the concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml. Also, the EtOH extract and the distilled H_2O-EtOAc interface fraction showed no growth inhibition on *S. aureus* at these concentrations. These extracts or fractions demonstrated no growth inhibition on *P. aeruginosa*. For the EtOH extract, there was no growth inhibition at 1,000, 2,000 and 5,000 µg/ml concentrations, whereas for the distilled H_2O-EtOAc interface fraction there was no growth inhibition only at 1,000 and 2,000 µg/ml concentrations.

From the results in Table 5a, it could be said that the EtOH extract and the distilled H_2O-EtOAc interface fraction of the stem of the plant contained components which are toxic that inhibited the growth of *P. aeruginosa*. This finding is in conformity with the folkloric or ethno-medicinal uses of *Maytenus senegalensis*.

A comprehensive analysis of the results in Table 5b revealed that the EtOH extract, the distilled H_2O and the distilled H_2O-EtOAc interface fractions of the stem of *Maytenus senegalensis* contained toxic components which inhibited the growth of *E. coli* and *P. vulgaris*, whereas the $CHCl_3$ and EtOAc fractions do not contain toxic components and did not inhibit the growth of the clinical isolates at the tested concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml. The EtOH extract inhibited the growth of *P. vulgaris* at 5,000 and 10,000 µg/ml concentrations with mean zones of inhibition of 7±0.10 and 8±0.17 respectively, whereas at lower concentrations of 1,000 and 2,000 µg/ml there were no growth inhibition. *E. coli* and *K. pneumoniae* experienced no growth inhibition from this extract at all the tested concentrations. The distilled H_2O fraction of the stem of *Maytenus senegalensis* demonstrated growth inhibition on *P. vulgaris* at the concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml with the respective mean zones of inhibition of 2±0.20, 3±0.20, 7±0.10 and 8±0.10. There were mean zones of inhibition of 7±0.10 and 8±0.10 exhibited by this fraction on *E. coli* at the

respective concentrations of 5,000 and 10,000 µg/ml, whereas at lower concentrations of 1,000 and 2,000 µg/ml there were no growth inhibition. Also, this fraction showed no growth inhibitory effect on *K. pneumoniae* at the concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml. For the distilled H_2O-EtOAc interface fraction of the stem of the plant, there were growth inhibitory effects on *P. vulgaris* and *E. coli*. It recorded mean zones of inhibition of 6±0.10 and 7±0.30 on *P. vulgaris* at the respective concentrations of 5,000 and 10,000 µg/ml, whereas at lower concentrations of 1,000 and 2,000 µg/ml there were no growth inhibition. Also, this fraction recorded mean zones of inhibition of 5.5±0.42, 7±0.44 and 8±0.52 on *E. coli* at the respective concentrations of 2,000, 5,000 and 10,000 µg/ml. At a lower concentration of 1,000 µg/ml there was no growth inhibition. This interface fraction demonstrated no growth inhibitory effect on *K. pneumoniae* at the concentrations of 1,000, 2,000, 5,000 and 10,000 µg/ml. The same results of no growth inhibition were recorded for the $CHCl_3$ and EtOAc fractions of the stem of the plant on the three clinical isolates just as for the same fractions on *S. aureus* and *P. aeruginosa* as shown in Table 5a.

The results recorded in Table 5b are indications that the EtOH extract, the distilled H_2O and the distilled H_2O-EtOAc interface fractions of the stem of the plant contained toxic components which inhibited the growth of *E. coli* and *P. vulgaris*. This research finding is in support of the ethno-medicinal uses of *Maytenus senegalensis*.

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REFERENCES

Adoum, O.A., N.T. Dabo and M.O. Fatope, 1997. Bioactivities of some savannah plants in the brine shrimp lethality test and invitro antimicrobial assay. Int. J. Pharmacog., 34: 334-337.

Cheesbrough, M., 2000. District laboratory practice in tropical countries (part 2). Cambridge Univ. Press, Cambridge, pp: 235-238.

Ghazi, H., M. Hirotsugu, N. Norio, H. Masao, K. Takuya, O. Toru, N. Nobuo and S. Kunitada, 1999. Inhibitory effect of Sudanese plant extracts on HIV-1 replication and HIV-1 protease. Phytother. Res., 13: 31-36.

Karaman, I., P. Sahin, M. Gulluce, H. Oguten, M. Songul and A. Adiguzed, 2003. Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J. Ethnopharmacol., 2837: 1-5.

Komolafe, A.O. and A.A. Adegoke, 2008. Incidence of bacterial septicaemia in Ile-Ife, Nigeria. Malaysian J. Microbiol., 4: 51-61.

Muller, L.P., 1973. Importance of secondary metabolites constituents as drugs. Phytother., 3: 354.

Pelczar, M.J. Jr., E.C. Chan and R. Nee, 1993. A textbook of microbiology. International edition. McGraw Hill Inc., New York, pp: 806-833.

Richard, J.P., 1998. Natural product isolation. Published by Hamana press, Tatawa, New Jersey, pp: 845-847.

Sanberg, F. and J.G. Bruhn, 1979. Sreeninig of plants for bioactive substances in Africa. African medicinal plants. O.A.U. Press, Nigeria, pp: 119.

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Protein and Amino Acid Compositions of *Sphenostylis stenocarpa*, *Sesamum indicum*, *Monodora myristica* and *Afzelia africana* Seeds from Nigeria

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Abstract: Flours produced from raw, blanched and boiled seeds of *Sphenostylis stenocarpa* (African yam bean), *Sesamum indicum* (Sesame seed), *Monodora myristica* (Calabash nutmeg) and *Afzelia africana (*African oak) were evaluated for total protein and amino acid compositions. The results show that the seeds of *S. indicum* had the highest total protein content (24.74±0.56%) relative to those of *S. stenocarpa* (18.90±0.35%), *M. myristica* (12.83±0.40%) and *A. africana* (9.56±0.12%). The amino acid contents followed the pattern 0.030±0.010 mol/l for *S. stenocarpa* >0.025±0.005 mol/l for *A. africana* >0.014±0.003 mol/l for *M. myristica* >0.010±0.002 mol/l for *S. indicum*. The seeds were found to be rich in amino acids especially the essential amino acids which relative to the non-essential amino acids had high percentages of 81, 80, 77 and 70% for *S. stenocarpa, M. myristica, S. indicum* and *A. Africana* respectively. Boiling significantly ($p<0.05$) reduced the protein and amino acid contents of all the seeds, while blanching only significantly ($p<0.05$) reduced the amino acid contents of *S. stenocarpa, S. indicum* and *A. Africana* seeds. The results suggest that the seeds are potentially important plant sources of proteins and amino acids especially the essential amino acids necessary for human and livestock nutrition.

Key words: African yam bean, sesame seed, calabash nutmeg, African oak, amino acid analyses

INTRODUCTION

Seeds have nutritive and calorific values which make them necessary in diets. Leguminous seeds are special good sources of plant proteins. For normal functioning the human body requires a sufficient amount of protein. The true value of a protein however depends upon its different amino acid composition especially its essential amino acid content. Furthermore, proteins apart from supplying these essential amino acids, also provide the nitrogen necessary for the synthesis of other macromolecules of life such as nucleic acids, while also being required for repair and maintenance of cells and tissues. Unfortunately, protein foods are in short supply, and thus not within the reach of low-income households who unfortunately form the bulk of the population in developing countries (Oshodi, 1992). In order to bridge this protein supply gap, scientists have focused their attention on lesser known protein-rich sources. This study is one of such attempts and is aimed at determining the protein and amino acid compositions of four lesser known but common legumes found in Nigeria.

Sphenostylis stenocarpa Hochst or African yam bean is locally called *Odudu, Ukpodudu* or *Okpodudu* in southern Nigeria. It is a leguminous crop of family *Leguminosae* grown in tropical regions of Africa, particularly in Cameroun, Cote d'Ivoire, Volta region of Ghana, Nigeria and Togo. This herbaceous climbing vine produces ellipsoid, rounded or truncated seeds, which show considerable variation in size and colour, varying from creamy-white or brownish-yellow to dark brown. Both the seeds and leaves of the plant are edible. The plant also produces tubers, which can be cooked and eaten. They are important sources of starch and protein (Edem *et al*., 1990; Asuzu, 1986).

Sesamum indicum (sesame seed or benni seed) of the family *Pedaliaceae* is locally called *Benisid*, *Kiero* and *Ewa ibejil* by the Igbos, Hausas and Yorubas of Nigeria respectively. It is an ancient oil seed, first recorded as a crop in Babylo and Assyria about 4000 years ago. However, it is presently grown in many parts of the world because of its nutritional, medicinal and traditional values. It is a flowering plant that bears small flat seeds that come in a variety of colours, from cream-white to charcoal black. Sesame seeds are unusually high in edible oil and are either consumed directly as a highly nutritious foodstuff or processed into various confectionery and bakery products. It is also used as medicine. The high methioine content of its oil may help to relieve symptoms of liver disease and aid in nitrogen balance maintenance in the body (Mba, 1980; Norman *et al*., 1995).

Monodora myristica, also known as calabash nutmeg and of the family *Annonaceae*, is a tropical evergreen tree native to Jamaica. It is locally called *Ehuru* or *Ehiri* in Southern Nigeria. It produces soft-ball sized edible seeds that have nutmeg-like flavour, with high protein composition and also having rich medicinal properties.

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The seeds and seed coats are used as a spice for seasoning food and as topical medication for wounds and headaches. The seed oils are used as condiments and carminative and to scent soaps and perfumes (Mayhem and Penny, 1988; Mba, 1980).

Afzelia africana (African mahogany or African oak) is a semi-deciduous plant in the *Fabaceae* family. It is locally called *Akparata* (Igbo), *Kawo* (Hausa) and *Apa* (Yoruba) in Nigeria. It is a large tree with very beautiful seeds having two coloursL: black with yellow/orange at the bottom. The seeds are edible and have high medicinal values. All parts of the plant are of immense traditional importance; its wood is used for carpentry, the sawdust for making and designing art work, its foliage for cattle forage, the pods containing rich ashes are used for making soap, while the leaves are used to enrich soil because of their rich nitrogen content (Acland, 1980).

MATERIALS AND METHODS

Collection and preparation of samples: Apparently healthy seeds of *S.* Stenocarpa, *S. indicum*, *M. myristica* and *A. Africana* were purchased from Umuahia Central Market, Abia State, Nigeria. They were authenticated at the Central Laboratory of National Root Crops Research Institute, Umudike, Nigeria.

The different seeds were divided into 3 portions each. One portion of each seed type was washed with distilled water, drained in a sieve and dried in an oven (Gallenkemp IH-100 model, UK) at 60°C to a constant weight. The dried seeds were mechanically ground into a powder and the powdered seed sample packed in a clean container and labeled 'Raw seed sample'.

The second portion of each seed type was washed with distilled water and blanched for 5 min. Then the water was drained off with a sieve and the blanched seeds dried to a constant weight in the oven at 60°C. They were later ground into powder and labeled 'Blanched seed sample'.

The remaining portion of each seed type was washed, boiled until the seeds became soft and the water was drained off. The boiled seeds were dried to a constant weight in an oven at 60°C, ground into powder and labeled 'Boiled seed sample'. These labeled samples were then stored in the refrigerator (about 4°C) until required for analyses (Ojiako and Igwe, 2007).

Determination of protein and amino acid composition: The protein content of the ground seed samples was determined by the standard Kjeldahl method (Pearson, 1976; James, 1995). The amino acid content of the samples was determined by alkaline titrimetric method of Ojiako and Akubugwo (1997).

The amino acid composition of the seed samples were determined by the use of an automatic amino acid analyzer (Technicon Sequential Multisample Analysa DNA0209, Ireland).

Statistical analysis: One-way ANOVA and Duncan's New Multiple Range Test were used to statistically analyze data generated in the study. Values for which $p<0.05$ were considered statistically significant (Sanders, 1990).

RESULTS

Percentage total protein content: The raw seeds of *S. indicum* had the highest total protein content (24.73±0.56%) compared to those of *S. stenocarpa* (18.90±0.35%), *M. myristica* (12.83±0.40%) and *A. africana* (9.56±0.12%) (Fig. 1). For all the seed samples, boiling significantly ($p<0.05$) reduced the protein content, while blanching did not.

Percentage amino acid content: Figure 2 shows the percentage amino acid content of the seeds. It shows that the amino acid content of the raw seeds varied thus: *S. stenocarpa* (0.031±0.010 mol/l) > *A. africana* (0.025±0.005 mol/l) > *M. myristica* (0.014±0.003 mol/l) > *S. indicum* (0.010±0.002 mol/l) (Fig. 2). Boiling significantly ($p<0.05$) reduced the amino acid contents of all the seeds giving percentage reductions of 68% for *A. africana*, 60% for *S. stenocarpa*, 38% for *S. indicum* and 14% for *M. myristica*, while blanching did not significantly ($p>0.05$) reduce the amino acid content in *M. myristica*.

Amino acid composition: The amino acid compositions of the raw, blanched and boiled seed samples of *S. stenocarpa* are shown in Fig. 3. Similarly, those of *S. indicum*, *A. africana* and *M. myristica* are shown in Fig. 4, 5 and 6 respectively. The figures show that arginine, glutamic acid, lysine, isoleucine and leucine were commonly present in all the seed types. Apart from these, Figure 3 also shows the presence of glycine, valine, methioine, threonine and phenylalanine in *S. stenocarpa* seed samples. Similarly, glycine, cysteine, methionine, tryptophan and phenylalanine were also found in *S. indicum* (Fig. 4). Figure 5 shows the presence of cysteine, alanine, valine, tryptophan and threonine in seeds of *A. Africana*, while alanine, valine, methionine, threonine and phenylalanine were found present in *M. myristica* seed samples (Fig. 6).

DISCUSSION

Plant sources, especially legumes are increasingly being used by thickly populated regions of the world to alleviate the problem of protein energy malnutrition. Yet the extent of interest in these plants, especially among the lesser known ones, is still very poor probably because their nutritional advantages have been inadequately highlighted. The results of this study show that the protein content of the seeds of *S. stenocarpa*, *S. indicum*, *M. myristica* and *A. africana* are comparable to those of *Cleome rutidosperma* (11.73%), *Lagenaria siceraria* (8.93%) and *Cucurbita maxima* (16.80%) (Ojiako and Igwe, 2007). However, their protein contents are slightly lower than those reported for common edible

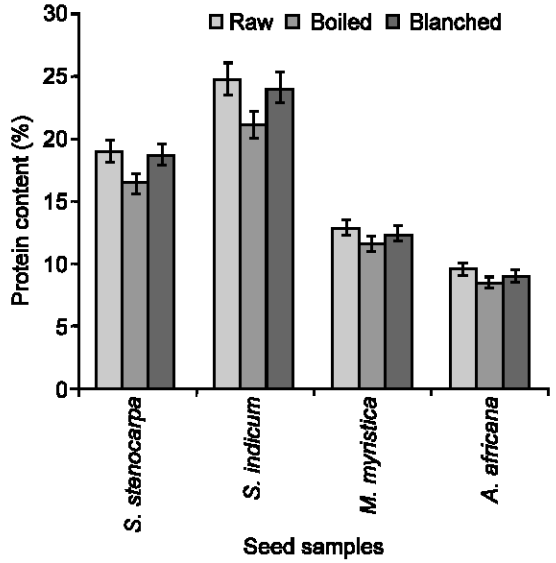

Fig. 1: Protein content of the raw, boiled and blanched seed samples

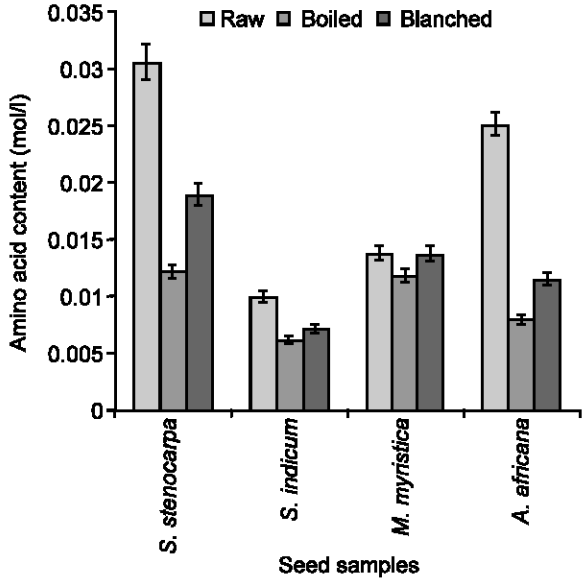

Fig. 2: Amino acid content of the raw, boiled and blanched seed samples

seeds such as 28.7% for fluted pumpkin (Fagbemi and Oshodi, 1991) and 36.2% for African oil bean (Odoemelam, 2005) seeds, but higher than that reported for snake tomato fruits (1.85%) (Ojiako and Igwe, 2008). An adult male of about 70 kg body weight requires 35 g of protein daily, therefore only about 166, 214, 303 and 411 g of *S. indicum, S. stenocarpa, M. myristica* or *A. Africana* seed respectively would be required to provide the minimum daily protein need. Each of these may be an equivalent of a plate of food typically consumed at a sitting by an average Nigerian adult. However, larger

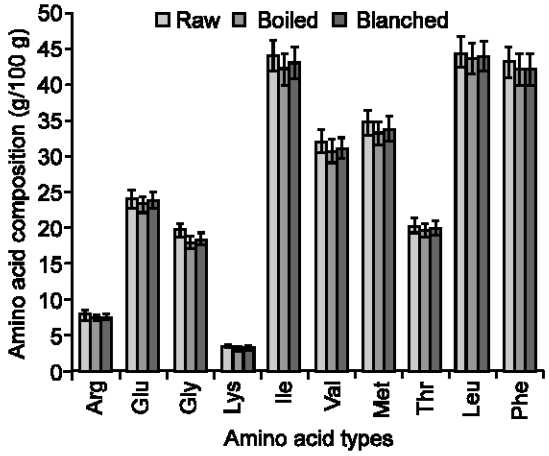

Fig. 3: Amino acid composition of raw, boiled and blanched seeds of *S. stenocarpa*

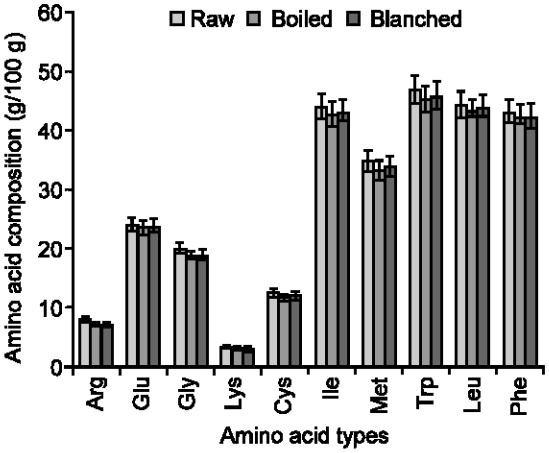

Fig. 4: Amino acid composition of raw, boiled and blanched seeds of *S. indicum*

amounts may be need to be consumed per day to meet the requirement, if allowance of 25% is made for indigestibility and the limiting sulphur amino acid content (Fagbemi and Oshodi, 1991).

The seeds studied, like other forms of seeds are known to contain antinutrients, which are reduced or eliminated by cooking (Egbe and Akinyele, 1990). Boiling and blanching, like other methods for processing of food reduce to minimal levels these antinutrients (Onwuliri *et al*., 2002). The results of the present study show that boiling significantly ($p<0.05$) reduced protein content of the seeds, while blanching did not. Similarly, boiling and blanching did significantly ($p<0.05$) reduce the amino acid contents of almost all the seeds except in *M. myristica* where blanching did affect the amino acid content. This shows that blanching may relatively be a better method of food processing in the preservation of protein and amino acid contents of edible seeds. This may be because of the longer heating involved in boiling

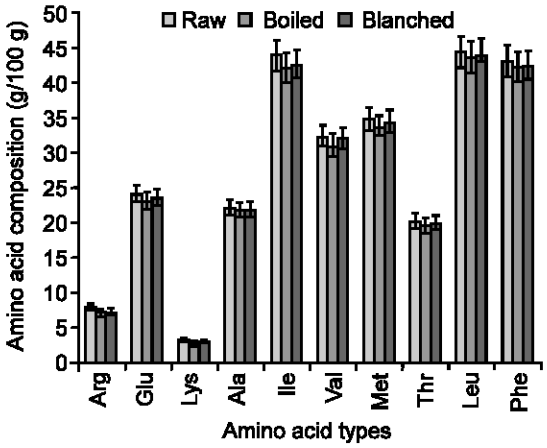

Fig. 5: Amino acid composition of raw, boiled and blanched seeds of *M. myristica*

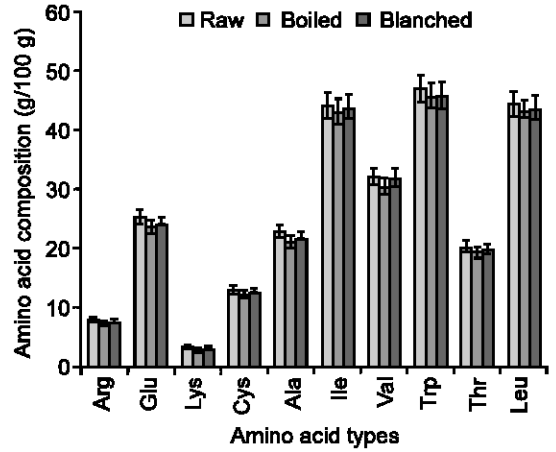

Fig. 6: Amino acid composition of raw, boiled and blanched seeds of *A. africana*

than blanching. However, there may be need to study the extent of reduction of antinutrients and availability of the proteins in the seeds after the processes of boiling and blanching.

A study of the amino acid distribution of the seeds show that they are rich sources of both essential and non-essential amino acids, with the essential amino acids being interestingly high at 81, 77, 70 and 80% for *S. stenocarpa*, *S. indicum*, *A. africana* and *M. myristica* respectively relative to the non-essential amino acid levels. The only sulphur-containing amino acid which could limit the seeds' protein availability to animals is methioine (Mba, 1980) and is present in all the seeds with the exception of *A. africana*.

Conclusion: The study has shown that the seeds of *S. stenocarpa*, *S. indicum*, *A. africana* and *M. myristica* are potentially important plant sources of proteins and amino acids, especially the essential amino acids needed by humans and livestock. The processes of boiling and blanching required for preparation of seeds to make them edible affected their protein and amino acid contents levels but did not eliminate them. Since processing eliminates or reduces anti-nutrient levels, it may be necessary to recommend that these seeds be incorporated into human and animal diets considering the observed nutritional composition, relative abundance of essential amino acids in them and the dire need to bridge the protein supply gap especially for the low-income and resource-constrained households in developing countries.

REFERENCES

Acland, J.D., 1980. East African Crops: An Introduction to the Production of Field and Plantation Crops in Kenya, Tanzania and Uganda. FAO/Longman Group Ltd, London.

Asuzu, I.U., 1986. Pharmacological Evaluation of the Forklore use of *Spenostylis stenocarpa*. J. Ethnopharm, 16: 263-267.

Edem, D.O., C.L. Amugo and O.U. Eka, 1990. Chemical Composition of African Yam Beans. J. Trop. Sci., 30: 59-63.

Egbe, I.A. and I.O. Akinyele, 1990. Effects of Cooking on the Anti-nutrtional Factors of Lima Beans (*Phaseolus lunatus)*. Food Chem., 35: 81S.

Fagbemi, T.N. and A.A. Oshodi, 1991. Chemical Composition and Functional Properties of Full Fat Fluted Pumpkin Seed Flour. Nig. Food J., 9: 26-32.

James, S.C., 1995. The Analytical Chemistry of Foods. Blackie, London, pp: 20-25.

Mayhem, S. and A. Penny, 1988. Tropical and Sub-tropical Foods. Macmillan Publisher Ltd., London, pp: 54.

Mba, A.U., 1980. Chemical Composition of Some Local Sources of Protein Food for Man. Nig. J. Nutr. Sci., 1: 142-147.

Norman, M.J., C.T. Pearson and P.G.E. Scale, 1995. Tropical Food Crops in the Environment. 2nd Edn., Cambridge University Press, UK.

Odoemelam, S.A., 2005. Proximate Composition and Selected Physico-chemical Properties of the Seeds of African Oil Bean (*Pentaclethra macrophylla)*. Pak. J. Nutr., 4: 382-383.

Ojiako, O.A. and E.I. Akubugwo, 1997. An Introductory Approach to Practical Biochemistry. CRC Publishers, Owerri, Nigeria, pp: 156-161.

Ojiako, O.A. and C.U. Igwe, 2007. Nutritional and Anti-nutritional Compositions of *Cleome rutidosperma*, *Lagenaria siceraria* and *Cucurbita maxima* seeds from Nigeria. J. Med Food, 10: 735-738.

Ojiako, O.A. and C.U. Igwe, 2008. The Nutritive and Hepatotoxic Properties of *Trichosanthes anguina* (Snake Tomato) Fruits from Nigeria. Pak. J. Nutr., 7: 85-89.

Onwuliri, V.A., I. Attah and J.O. Nwankwo, 2002. Anti-nutritional Factors, Essential and Non-essential Fatty Acids Composition of Ugba (*Pentaclethra macrophylla*) seeds at different stages of Processing and fermentation. J. Biol. Sci., 4: 671-675.

Oshodi, A.A., 1992. Proximate Composition, Nutritionally Valuable Minerals and Functional Properties of *Adenous breviflorus* benth seed flour and protein concentrate. Food Chem., 45: 79-83.

Pearson, D., 1976. The Chemical Analysis of Foods. 7th Edn., Churchill Livingstone, London, pp: 43-44.

Sanders, D.H., 1990. Statistics: A Fresh Approach. 4th Edn., McGraw Hill Inc., Singapore, pp: 380-385.

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Nutritional and Functional Properties of Some Promising Legumes Protein Isolates

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Abstract: Proteins are essential component of diet performing multifarious role in human body. Present project was an attempt to extract and characterize legumes protein isolates for their functional properties. Four different legumes i.e. cowpea, pigeon pea, peas and mungbean were evaluated for protein content, functional properties and their ability to improve nutritional quality of foods. Cowpea exhibited maximum protein content 27.88±1.95% followed by mungbean, peas and pigeon pea. As for as functional properties are concerned, cowpea protein isolates showed highest bulk density 0.71±0.05 g/cm^3 however, maximum protein solubility 82±4.97 was observed in pea protein isolates. Maximum water and oil absorption capacity 163±10.05, 168±11.72% was observed in mungbean and pigeon pea protein isolates, respectively. Likewise different legumes protein isolates showed significant results for emulsifying, foaming and gelling properties.

Key words: Nutritional profile, functional properties, protein isolates, legumes

INTRODUCTION

Protein malnutrition is one of the major nutritional problems in the developing world. The specific maladies like kwashiorkor and marasmus are prevalent in the children owing to protein deficiency, whereas in adults, results in poor health and reduced work capacity. Bridging the gap between increased food consumption and production is amongst the most challenging tasks round the globe especially in developing countries (Black *et al*., 2008). The existing problems of food security and malnutrition coupled with escalating population, uncertain crop yield and high cost of animal based food supplies have urged to identify and incorporate unconventional protein sources to enrich the traditional formulations (Awan, 2000). Generally there are two main sources of protein i.e. animal and plant; provision of adequate animal proteins is difficult due to high cost and changing consumer's attitudes towards animal based proteins. Consumers are more conscious in their food selection owing to growing awareness about nutritional dependent ailments. An alternative for improving protein intake of the people is to supplement the diet with plant proteins. For that reason, consumption of plant protein isolates with special reference to legumes is beneficial (Nunes *et al*., 2006; Iqbal *et al*., 2005). Legumes are inexpensive source of proteins with high nutritional profile and after cereals, important food source for humans (Vietmeyer, 1986; Doyle, 1994). Protein content in legume ranged from 17-40%, contrasting with that of cereals 7-13% and comparable to meat 18-25% (Genovese and Lajolo, 2001). Being a cheap source of protein for low income group of population, legumes are commonly used as a substitute for meat and play a significant role in alleviating the protein-energy malnutrition. In addition they are also a good source of complex carbohydrates, dietary fiber and contain significant amounts of vitamins and minerals (Morrow, 1991; Nielsen, 1991; Tharanathan and Mahadevamma, 2003). Protein isolates obtained from the legumes through isoelectrict precipitation have high percentage of protein contents, which make them potential protein sources for food industry applications and this potential usefulness will depend on their functional properties. Functional properties are the physical and chemical characteristics of the specific protein influencing its behavior in food system during processing, storage, cooking and consumption. The examples of functional properties include bulk density, protein solubility, water and oil absorption capacity, emulsifying and foaming properties. The factors that effect functional behavior of proteins in foods are their size, shape, amino acid composition and sequence, net charge, hydrophobicity, structure, molecular rigidity in response to external environment (pH, temperature, salt concentration) or interaction with other food constituents (Aluko and Yada, 1997). Proteins from legumes have gained immense importance in modern food design due to their nutritional value and favorable functional properties.

MATERIALS AND METHODS

Procurement of raw material: Cowpea, peas, pigeon pea and mungbean were purchased from the local market, while chemicals were obtained from Sigma-Aldrich (Sigma-Aldrich Tokyo, Japan). The legumes were cleaned, washed and dried to remove extraneous

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materials. The particle size of legumes was reduced to form fine flour through Cyclotec Mill.

Proximate analysis: The flour from each legume was analyzed for moisture, ash, crude protein, crude fat, crude fiber and NFE content according to their respective methods as described in AACC (2000).

Preparation of protein isolates: Legumes Flours were defatted by slurring the sample in an organic solvent (hexane) using soxhlet apparatus. After extraction, solvent was recovered through rotary evaporator. Protein isolates from the legumes flour were prepared by the method as described by Makri *et al.* (2005). The defatted flour was dispersed in distilled water (1/10); pH was adjusted to 9.5 with the aid of 1 N NaOH and shaked for 40 min at room temperature using a mechanical shaker. Following centrifugation at 4000 rpm for 20 min supernatant was collected. The residue was collected and dispersed in distilled water (1/5) and stirred. Following centrifugation at 4000 rpm for 20 min, the respective supernatant was collected and combined with the supernatant collected from the first centrifugation and the pH was adjusted to 4.5, the precipitated protein was recovered by centrifugation at 4000 rpm for 20 min, neutralized and freeze dried.

Functional properties

Bulk density: The bulk density of protein isolates of different legumes was determined as outlined by Okaka and Potter (1977). Ten grams of protein isolates were put into 100 ml graduated cylinder and was tapped several times on the laboratory bench till the isolate stopped settling and values were expressed as g/cm^3.

Protein solubility: The protein isolates (250 mg) were homogenized in 20 ml of 0.1 M NaCl at pH 7 for 1 h followed by centrifugation at 10,000 x g for 30 min. Nitrogen contents were determined in the soluble fractions and solubility was expressed as the percentage of total nitrogen of the original sample to that of soluble fraction (Morr *et al.*, 1985).

Water absorption: The sample (3 g) was mixed with distilled water (25 ml) and placed in pre-weighed centrifuge tubes. The tubes were stirred and centrifuged for 25 min at 3000 x g after 30 min interval. The supernatant was removed by 25 min drainage at 50°C, and protein isolate sample was re-weighed. Water absorption capacity was expressed as the number of grams of water absorbed per gram of sample (Sosulski *et al.*, 1976).

Oil absorption: The sample (0.5 g) was mixed with corn oil (6 ml) in preweighed centrifuge tubes. The tubes were stirred for one minute to get the complete dispersion of the sample in the oil. After 30 min holding time, the sample was centrifuged at 3000 x g for 25 min. The separated oil was then removed with a pipette and the tubes were inverted for 25 min to drain the oil prior to reweighing. The oil absorption capacity was expressed as grams of oil absorbed per gram of the sample (Sosulski *et al.*, 1976).

Emulsifying activity and stability: Protein isolate (3.5 g) was homogenized for 30 sec in 50 ml water using homogenizer at approximately 10,000 rpm. Corn oil (25 ml) was added and the mixture was homogenized again for 30 sec. The emulsion was divided into two equal volume aliquots and centrifuged at 1100 x g for 5 min; one aliquot was heated for 15 min at 85°C according to the procedure of Naczk *et al.* (1985). The ratio of the height of emulsion layer to the height of liquid layer was noted to calculate emulsion activity. The emulsion stability was expressed as the percentage of emulsifying activity remaining after heating (Naczk *et al.*, 1985).

Foaming capacity and stability: The capacity and stability of foams were determined by the method of Lin *et al.* (1974). A 50 ml of 3% (w/v) dispersions of protein isolate sample in distilled water were prepared and immediately transferred into graduated cylinder; volume was recorded before and after whipping. Foaming capacity was expressed as the percentage volume induced by whipping. The change in volume of foam after 60 min of standing at room temperature was recorded as foam stability.

Least gelation concentration (LGC): The LGC was determined by heating suspensions of protein isolates 2, 4, 6, 8, 10, 12, 14, 16, 18 and 20% (w/v) for 1 h in boiling water followed by swift cooling under cold running water. The tubes were further cooled at 4°C for 2 h. LGC is the concentration at which the sample did not slide along the test tube walls in inverted position (Sathe *et al.*, 1982).

Statistical analysis: Completely Randomized Design (CRD) was applied and results were analyzed through Analysis of Variance Technique (ANOVA) using Cohort version 6.1 (Costat-2003) to determine the level of significance (Steel *et al.*, 1997).

RESULTS AND DISCUSSION

The present project was designed to explore the nutritional and functional quality of legumes protein. The protein isolates were prepared by isoelectric precipitation and analyzed for functional properties. The investigated parameters and their respective results are discussed below.

Proximate composition: Proximate composition is important in determining the quality of raw materials and often the basis for establishing the nutritional value and

overall acceptance of the consumers. The values for moisture contents in cowpea (10.39±0.73%) and pigeon pea (11.07±0.50%) were significantly different from those for peas (9.05±0.55) and mungbean (8.30±0.51). Cowpea exhibited maximum protein content 27.88±1.95% that was at par with mungbean (25.90±1.60%) and both of these were significantly different from pigeon pea and peas. Fat contents ranged in different legume flour samples from 1.24±0.08% to 2.03±0.09%, and highest amount was noted in pigeon pea. Cowpea contained highest amount of crude fiber (9.58±0.67) and lowest was found in mungbean (4.61±0.28%). Results for ash content demonstrated significantly higher amount (4.36±0.27%) in mungbean followed by pigeon pea (3.96±0.18) and cowpea (3.84±0.27%), respectively. However, lowest was observed in peas (3.48±0.21%). The mean values for nitrogen free extract of peas, mungbean, pigeon pea and cowpea were 65.33±3.96, 63.89±3.94, 63.80±2.90 and 57.42±4.01%, respectively (Table 1). Findings of present study are corroborated with the research investigation by other scientists. Iqbal *et al.* (2006) reported that cowpea and green peas have moisture content of 9.4 and 7.8%, protein content 24.70 and 24.90%, fat contents 2.8 and 1.5% and ash contents 4.2 and 3.6%, respectively. Whilst these parameters for cowpea and peas were lowered than that reported by Mwasaru *et al.* (1999) and Costa *et al.* (2006). Even though fiber contents were comparable to that determined by Kabas *et al.* (2007); but difference observed in the NFE contents compared with those of Akubor (2003). In case of mungbean, Mubarak (2005) reported that values obtained for moisture, protein, fat, fiber, ash and NFE were 9.75, 26.37-27.50, 1.85, 4.63, 3.76 and 59.8-62.3%, respectively. Similar findings were observed by other scientists but with slight variations (Amarteifio and Moholo, 1998; Anwar *et al.*, 2007). In this regard, Oshodi *et al.* (1993) explicated that the pigeon pea has moisture, protein, fat, ash and NFE contents 5.2, 22.40, 2.6, 5.8-3.9% and 51.70%, respectively. Results are also in agreement with the findings of Eno-Obong and Carnovale (1992) and Amarteifio *et al.* (2002). Likewise, crude fiber contents ranged from 8.2-13.0% in pigeon pea (Amarteifio *et al.*, 2002).

Protein content of protein isolates: The proteins are polymer of amino acids and their relative proportion represents its quality that is dependent on the genetic makeup of legumes. The variations in protein contents are attributed to genetic makeup of legumes along with some environmental factors. Protein contents were significantly higher in cowpea (89.25±1.28%) followed by mungbean (85.46±1.52%) and pea protein isolates (83.61±1.49%). However, least protein contents were recorded in pigeon pea protein isolates i.e. 82.92±1.28% (Table 2). The variations in protein contents of different protein isolates could possibly be due to extent of soluble proteins present in raw materials. Previously, Shand *et al.* (2007); reported that pea protein isolates have 80.70% protein. Makri *et al.* (2005) determined 79.2% of protein contents in extracted isolates. Some scientists like Mwasaru *et al.* (2000) reported higher protein content in cowpea protein isolates i.e. 91.30%, however, their results supported our findings for other legumes too as they indicated that pigeon pea protein isolates contain 82.4%, while mungbean isolates have 81% of protein (Rahma *et al.*, 2000).

Table 1: Proximate composition of legumes

Legumes	Moisture (%)	Protein (%)	Fat (%)
Pigeon pea	11.07±0.50^{a}	22.01±1.00^{b}	2.03±0.09^{a}
Cowpea	10.39±0.73^{a}	27.88±1.95^{a}	1.27±0.09^{c}
Mungbean	8.30±0.51^{b}	25.90±1.60^{a}	1.24±0.08^{c}
Peas	9.05±0.55^{b}	22.95±1.39^{b}	1.41±0.09^{b}
Legumes	Ash (%)	Fiber (%)	NFE (%)
Pigeon pea	3.96±0.18^{b}	8.19±0.37^{b}	63.80±2.90^{a}
Cowpea	13.84±0.27^{bc}	9.58±0.67^{a}	57.42±4.0^{b}
Mungbean	4.36±0.27^{a}	4.61±0.28^{d}	63.89±3.94^{a}
Peas	3.48±0.2^{c}	6.83±0.41^{c}	65.33±3.96^{a}

Table 2: Means for Protein Content (PC)

Protein Isolates	PC (%)
PPPI	82.95±1.28^{c}
CPI	89.25±1.39^{a}
MBPI	85.46±1.52^{b}
PPI	83.61±1.49^{bc}

PPPI = Pigeon Pea Protein Isolates; CPI = Cowpea Protein Isolates; MBPI = Mungbean Protein Isolates; PPI = Pea Protein Isolates

Functional properties: The functional properties studied for cowpea, peas, pigeon pea and mungbean protein isolates are discussed below.

Bulk density: There existed significant variations in bulk density in different legumes protein isolates. The bulk densities were higher in cowpea and pea protein isolates i.e. 0.71±0.05 and 0.68±0.04 g/cm^{3}, respectively whilst pigeon pea and mungbean protein isolates behaved alike (Table 3). The defatting process results in porous texture of the defatted product that can be attributed for low bulk density would be an advantage in the formulation of complementary foods (Akpata and Akubor, 1999). Present results are supported by Akubor *et al.* (2003); the cowpea flour has bulk density 0.64 g/cm^{3}, while its isolates have bulk density of 0.82 g/cm^{3} as investigated by Ragab *et al.* (2004). Among the legume flours, peas and pigeon pea flours showed bulk density of 0.55 and 0.46 g/cm^{3}, respectively whereas, Kaur and Singh (2007) reported similar findings for chickpea and winged bean flours.

Protein solubility: Protein solubility is a useful indicator for the performance of protein isolates incorporated in the food systems and to determine the extent of protein

Table 3: Means for Bulk Density (BD), Protein Solubility (PS), Water and Oil Absorption Capacity (WAC&OAC) of protein isolates

Protein Isolates	BD (g/cm^3)	PS (%)	WAC (%)	OAC (%)
PPPI	0.53±0.02^b	68±3.09^{bc}	97±4.41^c	168±11.72^a
CPI	0.71±0.05^a	65±4.53^c	138±9.63^b	145±6.59^b
MBPI	0.55±0.03^b	72±4.44^b	163±10.05^a	113±6.84^c
PPI	0.68±0.04^a	82±4.97^a	152±9.20^a	140±8.63^b

denaturation because of heat or chemical treatment at different pH (Horax *et al*., 2004). Protein solubility of cowpea, peas, mungbean and pigeon pea at pH 7 were 65±4.53, 82±4.97, 72±4.44 and 68±3.09%, respectively (Table 3). The solubility of a protein is usually affected by its hydrophilicity or hydrophobic balance, depending on the amino acid composition, particularly at the protein surface (Moure *et al*., 2006). Higher solubility of pea protein isolates as compared to cowpea protein isolates may be due to the presence of low number of hydrophobic residues and the elevated charge. Results are comparable to the earlier findings of Sumner *et al*. (1980); they described 87% protein solubility for freeze dried pea protein isolates at neutral pH. Horax *et al*. (2004) reported the protein solubility of 80% for cowpea protein isolates at same pH. According to Mizubuti *et al*. (2000) pigeon pea protein solubility is more than 70%. Mwasaru *et al*. (2000) calculated 53.4 and 61.8% protein solubility for pigeon pea and cowpea, respectively. Afterwards, locust bean protein solubility was recorded upto 77% at pH 7 by Lawal (2004).

Water and oil absorption: Protein has both hydrophilic and hydrophobic properties thereby can interact with water and oil in foods. Results for water absorption revealed insignificant differences between mungbean (163±10.05%) and pea protein isolates (152±9.20%). Pigeon pea protein isolates showed lowest water absorption (97±4.41%) and highest oil absorption capacity (168±11.72%) whereas lowest oil absorption (113±6.84%) was observed in mungbean protein isolates (Table 3). Variation in water and oil absorption capacity of protein isolates may be due to different protein concentration, their degree of interaction with water and oil and possibly their conformational characteristics. The lower water absorption capacity of protein isolates is due to less availability of polar amino acids (Kuntz, 1971) and low fat absorption may be due to the presence of large proportion of hydrophilic groups and polar amino acids on the surface of the protein molecules (Sathe *et al*., 1982). Ragab *et al*. (2004) found that water and oil holding capacity for cowpea protein isolates are 220% and 110%, respectively. In case of pea protein isolates, Fernandez-Quintela *et al*. (1997); observed 170% water absorption capacity and 120% oil absorption capacity. According to El-Adawy (2000), mungbean had water and oil absorption capacity 200% and 135%, respectively and for pigeon pea 87% and 173% (Mizubuti *et al*., 2000). Also these findings are in line with those reported by others (Sefa-Dedeh and Yiadom- Farkye, 1988; Paredes-Lopez *et al*., 1991; Kaur and Singh, 2007).

Emulsifying activity and stability: Protein, being the surface active agents, can form and stabilize the emulsion by creating electrostatic repulsion on oil droplet surface (Makri *et al*., 2005). Maximum emulsifying activity was observed in pigeon pea protein isolates (49.50±3.00%) followed by cowpea (47.50±3.31%), peas (45.50±2.80%) and mungbean (41.10±1.87%) as indicated in Table 4. Significant differences occurred between legumes protein isolates regarding emulsion stability and mean values demonstrated higher stability (83.30±5.04%) in pigeon pea followed by 52.20±3.64 and 43.19±1.96% in cowpea and peas, respectively whilst, lowest 21.00±1.29% was observed in mungbean (Table 4). The results of the present study are in concordance with those reported earlier by Mwasaru *et al*. (2000); who calculated 48.16 and 54.90% emulsifying activity and stability for cowpea and 39.50 and 44.98% for pigeon pea protein isolates. According to Mizubuti *et al*. (2000) pigeon pea contained 97.97% emulsion stability. Ragab *et al*. (2004) reported the emulsifying activity (50%) and stability (82%) for cowpea protein isolates. Sumner *et al*. (1980) reported the emulsifying activity of freeze dried field pea protein isolates about 38%. Later, emulsion stability of 55.5% was observed by Fuhrmeister and Meuser (2003) for smooth pea protein isolates. The study conducted by El-Adawy (2000) showed that mungbean has emulsion activity about 65% and stability 18%. Afterwards, Lqari *et al*. (2002) reported the emulsion stability of 71% for lupin protein isolates.

Foaming capacity and stability: The foaming properties are used as indices of the whipping characteristics of protein isolates (Mwasaru *et al*., 1999). Maximum foaming capacity was observed in mungbean protein isolates (110±6.78%). However, that for cowpea and pigeon pea protein isolates exhibited no significant differences (Table 4). Whereas, means for different legumes demonstrated that peas showed significantly higher stability (79±4.78%) and lower (58±3.58%) was observed in mungbean protein isolates (Table 4). Low foaming capacity could be due to inadequate electrostatic repulsions, lesser solubility and hence excessive protein-protein interactions (Kinsella *et al*., 1985). Whereas, higher value for foaming stability

Table 4: Means for Emulsion Activity (EA) and Stability (ES), Foaming Capacity (FC) and Stability (FS) of protein isolates

Protein Isolates	EA (%)	ES (%)	FC (%)	FS (%)
PPPI	49.50±3.00[a]	83.30±5.04[a]	68±3.09[c]	71±3.23[b]
CPI	47.50±3.31[a]	52.20±3.64[b]	69±4.81[c]	65±4.53[b]
MBPI	41.10±1.87[b]	21.00±1.29[d]	110±6.78[a]	58±3.58[c]
PPI	45.50±2.80[a]	43.19±1.96[c]	78±4.72[b]	79±4.78[a]

Table 5: Least gelation concentration (LGC) of protein samples

Concentration (%)	PPPI	CPI	MBPI	PPI
2	(-)	(-)	(-)	(-)
4	(-)	(-)	(-)	(-)
6	(-)	(-)	(-)	(-)
8	(-)	(-)	(-)	(-)
10	(±)	(-)	(-)	(-)
12	(±)	(±)	(±)	(-)
14	(+)	(±)	(±)	(±)
16	(+)	(+)	(+)	(±)
18	(+)	(+)	(+)	(+)
20	(+)	(+)	(+)	(+)
LGC	14	16	16	18

indicates highly hydrated foams and decrease in foaming stability might be due to protein denaturation. Kaur and Singh (2007) observed decrease in foam volume with the passage of time for protein isolates of different chickpea cultivars. A similar trend has been reported for great northern bean proteins by Sathe and Solunkhe (1981) and for mucuna bean protein concentrates by Adebowale and Lawal (2003). The decrease in foam volume as a function of time was observed for all protein isolates. The results of the present investigation are in harmony with the finding of Mwasaru *et al.* (2000); they reported foaming capacity and stability for cowpea 35.30, 79.40% and 34.00, 77.80% for pigeon pea protein isolates, respectively. Ragab *et al.* (2004) reported foaming capacity of 65% for cowpea protein isolates. Pigeon pea had foaming capacity and stability 80, 102% as reported by Akintayo *et al.* (1999) and 44.70, 78.79% by Mizubuti *et al.* (2000), respectively. Foaming capacity 143% for freeze dried field pea protein isolates were determined by Sumner *et al.* (1980). Likewise, values for these traits in mungbean were 108 and 58% (El-Adawy, 2000). In a study conducted by Fernandez-Quintela *et al.* (1997), it was observed that pea protein isolate has 94% foaming stability.

Least gelation concentration: A qualitative parameter expresses the minimum protein concentration at which the gel does not slide along the test tube walls in inverted position (Moure *et al.*, 2006). The lower the least gelation concentration the better is the gelling ability of proteins (Akintayo *et al.*, 1999) because protein gels are aggregates of denatured molecules. Results showed that peas contained higher least gelation concentration (18%) followed by cowpea (16%) and mungbean (16%), whilst pigeon pea showed least gelation than others (14%) as obvious from Table 5. Fernandez-Quintela *et al.* (1997), reported 18% least gelation concentration for pea protein isolates; whereas 12% for cowpea protein isolates has been reported by Horax *et al.* (2004). Previously, Mwasaru *et al.* (2000) reported 14% LGC for pigeon pea and cowpea. Similarly, 16% least gelation concentration of cowpea protein isolates was observed by Onimawo and Akpojovwo (2006). According to Mizubuti *et al.* (2000); pigeon pea form gel at 12%. Circle and Smith (1972) reported that firm and resistant gels are formed from soy protein isolates at 16-17% concentrations.

Conclusion: In the developing countries there is an existing dilemma of protein energy malnutrition therefore some new indigenous sources must be exploited against the menace. Present investigation explicated the potential of various legumes for the preparation of protein isolates. Functional properties like water and oil absorption of protein isolates are suitable to be used further for the preparation of protein enriched products. Likewise bulk density, emulsion and foaming capacity and stability necessitate the use of such isolates in food system.

REFERENCES

AACC, 2000. Approved Methods of American Association of Cereal Chemists. American Association of Cereal Chemists, Inc., St. Paul, Minnesota.

Adebowale, K.O. and O.S. Lawal, 2003. Foaming, gelation and electrophoretic characteristics of mucuna bean (*Mucuna pruriens*) protein concentrates. Food Chem., 83: 237-246.

Akintayo, E.T., A.A. Oshodi and K.O. Esuoso, 1999. Effect of ionic strength and pH on the foaming and gelation of pigeon pea (*Cajanus cajan*) protein concentrates. Food Chem., 66: 51-56.

Akpata, M.I. and P.I. Akubor, 1999. Chemical composition and selected functional properties of sweet orange (*Citrus sinensis*) seed flour. Plant Foods Hum. Nutr., 54: 353-362.

Akubor, P.I., Flora, O. Adamolekun, C.A. Oba, H. Obari and I.O. Abudu, 2003. Chemical composition and functional properties of cowpea and plantain flour blends for cookie production. Plant Foods Hum. Nutr., 58: 1-9.

Aluko, R.E. and R.Y. Yada, 1997. Some physicochemical and functional properties of cowpea (*Vigna Unguiculata*) isoelectric protein isolate as a function of pH and salt concentration. Int. J. Food Sci. Nutr., 48: 31-39.

Amarteifio, J.O., D.C. Munthali, S.K. Karikari and T.K. Morake, 2002. The composition of pigeon pea (*Cajanus cajan* (L.) Millsp.) grown in Botswana. Plant Foods Hum. Nutr., 57: 173-177.

Amarteifio, J.O. and D. Moholo, 1998. The chemical composition of four legumes consumed in Botswana. J. Food Comp. Anal., 11: 329-332.

Anwar, F., S. Latif, R. Przybylski, B. Sultana and M. Ashraf, 2007. Chemical composition and antioxidant activity of seeds of different cultivars of mung bean. J. Food Sci., 72: 503-510.

Awan, J.A., 2000. Elements of Food and Nutrition. 2nd Edn., Uni-tech Communications, Faisalabad, Pakistan.

Black, R.E., L. Caulfield, Z.A. Bhutta and G. Cesar, 2008. Malnutrition kills directly, not indirectly. Victora, 371: 1749-1750.

Circle, S.J. and A.K. Smith, 1972. Functional properties of commercial edible soybean products. In: Symposium on seed proteins, Inglett, G.E. (Ed.). Westport, CT: Avi Publishing Co.

Costa, G.E.A., K.S. Queiroz-Monici, S.M.P.M. Reis and A.C. Oliveira, 2006. Chemical composition, dietary fibre and resistant starch contents of raw and cooked peas, common bean, chickpea and lentil. Food Chem., 94: 327-330.

Doyle, J.J., 1994. Phylogeny of the legume family: An approach to understanding the origins of nodulation. Annu. Rev. Ecol. Syst., 25: 325-349.

El-Adawy, T.A., 2000. Functional properties and nutritional quality of acetylated succinylated mung bean protein isolate. Food Chem., 70: 83-91.

Ene-Obong, H.N. and E. Carnovale, 1992. A comparison of the proximate, mineral andamino acid composition of some known and lesser known legumes in Nigeria. Food Chem., 43: 169-175.

Fernandez-Quintela, A., M.T. Macarulla, A.S. Del Barrio and J.A. Martinez, 1997. Composition and functional properties of protein isolates obtained from commercial legumes grown in northern Spain. Plant Foods Hum. Nutr., 51: 331-42.

Fuhrmeister, H. and F. Meuser, 2003. Impact of processing on functional properties of protein products from wrinkled pea. J. Food Eng., 56: 119-129.

Genovese, M.I. and F.M. Lajolo, 2001. Atividade inibito´ ria de tripsina do feija˜o (*Phaseolus vulgaris* L.): avaliaca crýtica dos metodos de determinac,a. Archivos. Latinoamericanos de Nutrica, 51: 386-394.

Horax, R., N.S. Hettiarachchy, P. Chen and M. Jalaluddin, 2004. Functional properties of protein isolate from cowpea (*Vigna unguiculata* L. Walp). J. Food Sci., 69: 119-121.

Iqbal, A., I.A. Khalil, N. Ateeq and M. Sayyar, 2006. Nutritional quality of important food legumes. Food Chem., 97: 331-335.

Iqbal, A., I.A. Khalil, N. Ateeq and M.S. Khan, 2005. Nutritional quality of important food legumes. Department of Agricultural Chemistry, Faculty of Nutrition Sciences, NWFP Agricultural University Peshawar, Pakistan Department of Plant Breeding and Genetics, NWFP Agricultural University Peshawar, Pakistan Bolan Medical College, Quetta, Pakistan.

Kabas, O., E. Yilmaz, A. Ozmerzi and I. Akinci, 2007. Some physical and nutritional properties of cowpea seed (*Vigna sinensis* L). J. Food Eng., 79: 1405-1409.

Kaur, M. and N. Singh, 2007. Characterization of protein isolates from different Indian chickpea (*Cicer arietinum* L.) cultivars. Food Chem., 102: 366-374.

Kinsella, J.E., S. Damodaran and B. German, 1985. Physicochemical and Functional Properties of Oilseed Proteins with Emphasis on Soy Proteins. In: New Protein Foods. Alschul, A.M. and H.L. Wilke (Eds.). Vol 5. New York, Academic press, pp: 107-179.

Kuntz, I.D., 1971. Hydration of macromolecules III. Hydration of polypeptides. J. Am. Chem. Soc., 93: 514-515.

Lawal, O.S., 2004. Functionality of African locust bean (Parkia biglobossa) protein isolate: Effects of pH, ionic strength and various protein concentrations. J. Cleaner Prod., 86: 345-355.

Lin, M.J.Y., E.S. Humbert and F.W. Sosulski, 1974. Certain functional properties of sunflower seed proteins. J. Food Sci., 39: 368-370.

Lqari, H., J. Vioque, J. Pedroche and F. Millan, 2002. Lupinus angustifolius protein isolates: Chemical composition, functional properties and protein characterization. Food Chem., 76: 349-356.

Makri, E., E. Papalamprou and G. Doxastakis, 2005. Study of functional properties of seed storage proteins from indigenous European legume crops (lupin, peas, broad bean) in admixture with polysaccharides. Food Hydrocolloids, 19: 583-594.

Mizubuti, I.Y., O.B. Júnior, L.W. De-Oliveira-Souza, R.S. Dos Santos Ferreira Da Silva and I. Elza Iouko, 2000. Functional properties of pigeon pea (*Cajanus cajan* (L.) Millsp) flour and protein concentrate. Archivos. Latinoamericanos Nutricion, 50: 274-280.

Morr, C.V., B. German, J.E. Kinsella, J.M. Regensten, J.P. Van Buren, A. Kilara, B.A. Lewis and M.E. Mangino, 1985. A collaborative study to develope a standardized food protein solubility procedure. J. Food Sci., 50: 1715-1718.

Morrow, B., 1991. The rebirth of legumes: Legume production, consumption and export are increasing as more people become aware of legumes nutritional benefits. Food Technol., 9: 96-121.

Moure, A., J. Sineiro, H. Dominguez and J.C. Parajo, 2006. Functionality of oilseed protein products. Food Res. Int., 39: 945-963.

Mubarak, A.E., 2005. Nutritional composition and antinutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes. Food Chem., 89: 489-495.

Mwasaru, M.A., K. Muhammad, J. Bakar, B. Yaakob and C. Man, 1999. Effects of isolation technique and conditions on the extractability, physicochemical and functional properties of pigeonpea (*Cajanus cajan*) and cowpea (*Vigna unguiculata*) protein isolates. I. Physicochemical properties. Food Chem., 67: 435-444.

Mwasaru, M.A., K. Muhammad, J. Bakar, B. Yaakob and C. Man, 2000. Influence of altered solvent environment on the functionality of pigeonpea (*Cajanus cajan*) and cowpea (*Vigna unguiculata*) protein isolates. Food Chem., 71: 157-165.

Naczk, M., L.L. Diosady and L.J. Rubin, 1985. Functional properties of canola meals produced by a two-phase solvent extraction system. J. Food Sci., 50: 1685-1689.

Nielsen, S.S., 1991. Digestibility of legume protein: Studies indicate that the digestibility of heated legume protein is affected by the presence of other seed components and the structure of the protein. Food Technol., 45: 112-114.

Nunes, M.C., A. Raymundo and I. Sousa, 2006. Rheological behaviour and microstructure of pea protein/k-carrageenan/starch gels with different setting conditions. Food Hydrocolloids, 20: 106-113.

Okaka, J.C. and N.N. Potter, 1977. Functional properties of cowpea-wheat flour blend in bread making. J. Food Sci., 42: 828-833.

Onimawo, I.A. and A.E. Akpojovwo, 2006. Toasting (Dry Heat) and nutrient composition, functional properties and antinutritional factors of pigeon pea (*Cajanus cajan*). J. Food Process. Pres., 30: 742-753.

Oshodi, A.A., O. Olaofe and G.M. Hall, 1993. Amino acid, fatty acids and mineral composition of pigeon pea (cajanus cajan). Int. J. Food Sci. Nutr., 43: 187-191.

Paredes-Lopez, O., C. Ordorica-Falomir and M.R. Olivares-Vazquez, 1991. Chickpea protein isolates: Physicochemical, functional and nutritional characterization. J. Food Sci., 56: 726-729.

Ragab, D.M., E.E. Babiker and A.H. Eltinay, 2004. Fractionation, solubility and functional properties of cowpea (*Vigna unguiculata*) proteins as affected by pH and/or salt concentration. Food Chem., 84: 207-212.

Rahma, E.H., S. Dudek, R. Mothes, E. Gornitz and K.D. Schwenke, 2000. Physicochemical characterisation of mung bean (*Phaseolus aureus*) protein isolates. J. Sci. Food Agric., 80: 477-483.

Sathe, S.K. and D.K. Salunkhe, 1981. Solubilization and electrophoretic characterization of great Nothern beans (*Phaseolus vulgaris* L) proteins. J. Food Sci., 46: 82-87.

Sathe, S.K., S.S. Deshpande and D.K. Salunkhe, 1982. Functional properties of winged bean (*Psophocarpus tetragonolobus* L. DC) proteins. J. Food Sci., 47: 503-509.

Sefa-Dedeh, S. and N.A. Yiadom-Farkye, 1988. Some functional characteristics of cowpea (*Vigna unguiculata*), bambara beans (*Voandzeia subterranean*) and their products. Can. Inst. Food Sci. Technol. J., 21: 266-270.

Shand, P.J., H. Ya, Z. Pietrasik and P.K.J.P.D. Wanasundara, 2007. Physicochemical and textural properties of heat-induced pea protein isolate gels. Food Chem., 102: 1119-1130.

Sosulski, F.W., E.S. Humbert, K. Bui and J.O. Jones, 1976. Functional properties of rapeseed flour concentrates and isolates. J. Food Sci., 41: 1348-1354.

Steel, R.G.D., J.H. Torrie and D. Dickey, 1997. Principles and Procedures of Statistics: A Biometrical Approach. 3rd Edn., McGraw Hill Book Co., Inc., New York.

Sumner, A.K., M.A. Nielsen and C.G. Youngs, 1980. Production and Evaluation of Pea Protein Isolates. Presented at the 40th Annual Meeting of the Institute of Food Technologists, New Orleans, La., June 8-11, 1980.

Tharanathan, R.N. and S. Mahadevamma, 2003. Grain legumes a boon to Human Nutrition. Trends Food Sci. Technol., 14: 507-518.

Vietmeyer, N.D., 1986. Lesser-known plants of potential use in agriculture and forestry. Sci., 232: 1379-1384.

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Constructing the Best Regression Model for Maiwa Variety

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Abstract: As difficult as it can be to determine the plant attribute that contributes most to better yield of cereal crop named Maiwa. We use multivariate regression model to determine the contribution of Plant height (X_1); Number of leaves (X_2); Number of tillers (X_3) and Leaf's area in square feet (X_4). Four multivariate regression models were developed by dropping each attribute. A data set collected from the Institute of Agricultural Research (IAR) Ahmadu Bello University, Samaru-Zaria was used for the analysis. Using each of the models to assess the contribution of each attribute, it was discovered that the Multivariate regression model that has the best fits of the data set, when covariates are dropped one after the other is $Y = 0.02371 - 0.003111X_2 + 0.001759X_3 - 0.002503X_4$. Thus, plant height ($X_1$) is an irrelevant plant attribute for the variety-Maiwa.

Key words: Regressor, predictor, goodness-of-fit, multivariate, yield, tiller

INTRODUCTION

The statistical technique that is used to establish the existence of linear relationship between the dependent variable and the independent variables is the Regression Analysis. If there is a single independent or predicator variable is referred to as simple linear regression, while if it involved more than one independent or predictor variables we have the case of Multivariate regression or multiple regression analysis. In many crops, especially arable crops, yield depend on some plants attributes such as plant height, number of leaves, stalk thickness, spacing variates etc. These plants attributes are referred to as the independent variables, covariates, predictors, or regressors; while the yield is the corresponding dependent variables or responses. Each of these regressors contributes to the variation in the yield of the variety.

Although the contribution varies from one crop to another, while in some crop it causes high variation in other the variation is insignificant.

According to Ogunremi (1970) the pod number or unit is an important independent variable that determines the yield in pod producing crops. Gilbert Tukers (1976) worked on sunflower and discovered that the number of heads of plant is very significant factor that determines the yield.

In fiber crops such as Kenaf, it is the plant height that determines the yield significant (Baker, 1970), while in cotton it is the number of bolls that determines the yield (Gardner and Tucker, 1976). Generally foods are produced by the leaves through photosynthesis and stored in the plant roots for plant consumption, while water required is transported through the stem from the roots to the leaves for food manufacturing. Therefore, leaves stem and roots are significant plant parts to plant attributes; hence, there is a relationship between these plant attributes and the yield.

If there is no correlation between two or more co varieties there is a possibility of having a good-fit, while if there is multicollinearity or relationship between these is the possibility of sources of variation. We shall in this paper considered the conditions of good fit in relation to the variables or attributes as proposed by Rizzi Laura (2008) and Gerald Keller and Brian Warrack (2003).

MATERIALS AND METHODS

Here we shall among other things distinguish between simple linear regression and multivariate regression model; furthermore discussed the assessing of model.

Regression analysis: A statistical technique that is used to establish the existence of linear relationship between the dependent variable and the independent variables is known as Regression analysis. It is also used to predict the value of one variable; this technique requires developing a Mathematical equation called Model.

According to Gerald Keller and Brian Warrack (2003): In developing a model it is necessary to known the nature of the relationship between dependent or response variables and each of the independent or predictor variables. This could be done by method of either Deterministic or Probabilistic models. The first model is not realistic because it does not assume the randomness of the variables involved or other external factors. The second includes the random component which measures the error of the deterministic component. The random component accounts for measureable and immeasurable variables that are not part of the model.

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The Regression analysis models: The regression analysis of probabilistic form is said to be simple linear regression equation or a first order linear model if the model is written as follows:

$$Y_{ij} = \beta_0 + \beta_1 x + \varepsilon_{ij} \quad (1)$$

Where Y_{ij} is the response, yield or dependent variable; x is the predictor, regressor or independent variable; β_0 is the intercept on the y-axis; β_1 is the slope or coefficient of regression; ε_i is the residual error.

The β_0 and β_1 are unknown parameters of the population but are estimated using the least square estimate method as stated:

$$(i)\,\beta_0 = y_{ij} - \beta_1 \bar{x}$$

and

$$\beta_1 = \frac{n\sum xy - (\sum x)(\sum y)}{n\sum x^2 - (\sum x)_2}$$

In multivariate regression model two or more variables are assumed to be linearly related if:

$$Y_{ij} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots + \beta_k x_k + \varepsilon_{ij} \quad (2)$$

Where Y_{ij} the response in i^{th} row, j^{th} Colum; x_i the i^{th} independent variable; β_i the i^{th} coefficient of regression; β_0 the intercept on the y-axis; ε_{ij} the i^{th} row, j^{th} column error of the term.

The least square method could also be used to estimate the population parameters β_i by minimizing the sum of squares of error and differentiating with respect to each population parameters. The (K+1) unknown parameters shall be obtained using any convenient Mathematical methods:

$$Y_{ij} = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \cdots + \beta_k x_k + \varepsilon_{ij} \quad (3)$$

$$\varepsilon_{ij} = Y_{ij} - \beta_0 - \beta_1 x_1 - \beta_2 x_2 - \beta_3 x_3 - \cdots - \beta_k x_k \quad (4)$$

$$\varepsilon_{ij}^2 = (Y_{ij} - \beta_0 - \beta_1 x_1 - \beta_2 x_2 - \beta_3 x_3 - \cdots - \beta_k x_k)^2 \quad (5)$$

$$\sum \varepsilon_{ij}^2 = \sum (Y_{ij} - \beta_0 - \beta_1 x_1 - \beta_2 x_2 - \beta_3 x_3 - \cdots - \beta_k x_k)^2 \quad (6)$$

$$\frac{d\sum \varepsilon_{ij}^2}{d\beta_0} = \frac{d\sum (Y_{ij} - \beta_0 - \beta_1 x_1 - \beta_2 x_2 - \beta_3 x_3 - \cdots - \beta_k x_k)^2}{d\beta_0} = 0$$

i.e

$$0 = -2\sum (Y_{ij} - \beta_0 - \beta_1 x_1 - \beta_2 x_2 - \beta_3 x_3 - \cdots - \beta_k x_k) \quad (7)$$

$$\frac{d\sum \varepsilon_{ij}^2}{d\beta_1} = \frac{d\sum (Y_{ij} - \beta_0 - \beta_1 x_1 - \beta_2 x_2 - \beta_3 x_3 - \cdots - \beta_k x_k)^2}{d\beta_1} = 0$$

$$0 = -2\sum x_{ik} (Y_{ij} - \beta_0 - \beta_1 x_1 - \beta_2 x_2 - \beta_3 x_3 - \cdots - \beta_k x_k) \quad (8)$$

Similarly, the matrix notation could be used in obtaining the estimate of the population parameter or coefficient of regression as shown below:

$$\underline{Y} = \underline{X}\beta + \underline{\varepsilon}$$

Where:

$$\underline{Y} = \begin{pmatrix} y_1 \\ y_2 \\ y_3 \\ . \\ y_k \end{pmatrix} \quad \underline{\beta} = \begin{pmatrix} \beta_0 \\ \beta_1 \\ \beta_2 \\ . \\ \beta_{kk} \end{pmatrix} \quad \underline{X} = \begin{pmatrix} x_{11}x_{12}x_{13}\ldots x_{1k} \\ x_{21}x_{22}x_{23}\ldots x_{2k} \\ x_{31}x_{32}x_{33}\ldots x_{3k} \\ \ldots\ldots\ldots\ldots \\ x_{p1}x_{p2}x_{p3}\cdots x_{pk} \end{pmatrix} \quad \underline{\varepsilon} = \begin{pmatrix} \varepsilon_1 \\ \varepsilon_2 \\ \varepsilon_3 \\ .. \\ \varepsilon_k \end{pmatrix}$$

and

$$\underline{\varepsilon} \sim iii\ N(0, \delta_i^2),\ E(\varepsilon) = 0,\ \mathrm{cov}(\varepsilon) = \begin{pmatrix} 1 & 0 & \ldots & \ldots & 0 \\ 0 & 1 & \ldots & .. & 0 \\ \ldots. & & \ldots & & .. \\ \ldots & \ldots & \ldots & \ldots\ldots & \ldots 1 \end{pmatrix} \delta^2$$

$\underline{X}$ is full rank of k+1, estimate of $\underline{\beta}$ is unbiased of β and $\hat{\delta}^2$ is biased estimate of δ^2 while the unbiased estimate of δ^2 is as follows:

$$E(\hat{\delta}^2) = \frac{\delta^2 (n-r)r}{r(n-r)} = \delta^2$$

According to Rizzi Laura (2008) the assumptions that guide the linear model are related to the distribution of Error matrix; Independent variables matrix and Unknown population parameters matrix.

If any of the assumptions is wrong, then there would be problems with the assumptions that relates to distribution errors; choice of regressors and the estimates of the parameters.

Rizzi Laura (2008) further stressed that if the ε_{ij} is not normally distributed, then the inference procedures shall only be valid asymptotically, if the variance of ε_{ij} terms are not constant or the same (homoscedasticity), then, the error are heteroscedasticity, which occurs in cross-section data and if the error terms are pair wise correlated, that is E (ε_i, ε_j) $\neq$ 0, I $\neq$ j this happens in time series data and the problem it causes is referred to autocorrelation.

Rizzi observed that a model is said to be a bad model if irrelevant independent variables are included; relevant independent variables excluded; incorrect functional form is used to fit the data; matrix of $\underline{X}$ has less than full column rank and the independent variables are correlation or correlated with the error term.

The last points cause multicollinearity and when there is a problem of multicollinearity, the coefficient of determination (R^2) is always high and the estimates of coefficient of regressions are always insignificant. There are always high correlation coefficients between the independent variables and high Variance Inflation Factors (VIF).

Multicollinearity can be remedied by removing one or more independent variables if they are observed to cause multicollinearity or increase the sample size or transform the equation model.

As a result of this, Rizzi Laura (2008) suggested a prediction criterion called Ameriya Prediction Criterium (PC). This is used to evaluate the goodness of fit of a model:

$$\text{Prediction criterium} = \frac{RSS[n+(k-1)]}{[n-(k-1)]} \qquad (9)$$

Where, RSS is the regression sum of square, n is the observations, (K-1) is the number of independent variables in the model.

Assessing the model: According to Gerald Killer and Brian Warrack (2003), Models are assessed using the following methods; Standard error of estimate; t-test of the slope, that is β_i; Coefficient of determination, that is R^2; F-ratio or p-value in ANOVA.

If the standard error is large then the error will also be large, which implies that the model is poorly fitted, but if the standard error is small this implies that some of the errors will be close to zero, this implies a good-fitted model.

The standard error is estimated using the relation:

$$\text{Standard error} = \sqrt{\frac{SSE}{n-2}}$$

Where SSE is the sum of square of error, n is the number of observations.

If the error is normally distributed the test statistics is student t- distributed with V = n-2 degrees of freedom. This is used to prove if there exists a linear relationship between two or more covariates or variables. Similarly a large value of F- indicates that a significant proportion of the variation in Y- is explained by the regression equation and that the model is valid, while a small value of F- implies that most of the variation in Y- is unexplained.

Data analysis: The experiment was conducted after the planting and successful germination of the seed and maturity stage of the plant. Sampled were examined and measure of twenty of the samples height of the plant (X_1), umber of leaves (X_2), number of tillers (X_3) and leaf's area in square feet (X_4). It should be noted that this attributes are indeed essential for the yield.

Data presentation: The data for this study is secondary data obtained from the Institute of Agricultural Research (IAR), A.B.U, Samaru, Zaria.

We developed four models dropping one plant attribute each for the subsequent Multivariate regression models.

Model 1: In this model all the four predictor variables were used and the model is

$$Yij = 0.01692 + 0.002595X_1 - 0.0003013X_2 - 0.001687X_3 - 0.002303X_4$$

From the equation, the intercept on Y-axis is 0.01692 and for every increase of one unit of the plant height there is an increase of about 0.002595 of the yield. But for every increase in the number of leaves of Maiwa variety there is a decrease of about 0.0003013 in the yield. There is an increase of about 0.001687 in the yield of Maiwa when the number of tillers is increased by one unit and a decreased of about 0.002303 was observed in the yield when the leaf square area is increased by one unit.

The coefficient of determination R^2 is 0.1087, this implies that about 11% of the variation in the Maiwa yield is explained by the regression model while the remaining percentages is unexplained due to environmental factors. This gives the p-value of 0.7658 which is greater than $\alpha = 0.05$, thus not significant and R^2 shows the data does not fit the model.

The p-values for all the independent variable are not significant , hence the t-test are also insignificant since the R^2

is less than 0.75, the variables are independent of one another. This implies that, there is no multicollinearity among the independent variables which is justified by the Variance Inflation Factors (VIF) for the coefficient which is less than 5.

Model 2: We further dropped leaves area (X_4) and obtained the model below:

$$Y_{ij} = 0.008794 + 0.005130X_1 - 0.0003071X_2 + 0.001505X_3$$

From the model, we deduced that for every unit increase in the plant height there is an increase of 0.005130 increases in the yield, but a decrease of 0.0003071 was observed for every increase of one unit in the leaves area. Similarly an increase of about 0.001505 was noted for every unit increase in the number of tiller.

The coefficient of determination R^2 obtained for the model was 8.75%, that implies about 9% of the total variation could be explained by the regression model, while the remaining 91% was due to error. The p-values are 0.6803 and then t-test of independent variable are insignificant. None of the independent variables makes

a significant contribution, since the R^2 is less than 0.75 there is no problem of multicollinearity. The correlation matrix shows that there a strong relationship between the number of leaves and the number of tillers, the variance inflation factors which is one of the litmus test for multicollinearity existence is less than 5 for the coefficient (Rizzi Laura, 2008). This justified the non-existence of multicollinearity; hence the data set fits the model best.

Considering when the number of tillers (X_3) is dropped in the regression, the following model Y_{ij} = 0.00342 + 0.006399x_1 - 0.000627x_2 - 0.001546x_4 from the result we have the coefficient of determination R^2 equals 0.0439 which is 4.39%, this shows that about 4.4% of the total variation was explained by the regression while, the remaining percentages was due to error. The p-value was also found to be 0.8636; both the p-value and the t-test for the coefficient were not significant.

Since the R^2 is less than 0.75 which shows that multicollinearity is not a problem. This is justified by the variance inflation factor value for the coefficient of the independent variables which is less than five. Hence there is no multicollinearity.

Model 3: When the number of leaves (X_2) was dropped in the regression, the effect of the leaves resulted in the following model:

$$Y_{ij} = 0.002021 + 0.005261X_1 + 0.000453X_3 - 0.002427X_4$$

The intercept on the y-axis shifted to 0.002021, while the R^2 equals 0.489. This value indicates that about 5% of the total variation was explained by the regression and that there is no multicollinearity; since R^2 is less than 0.75. In support of this claim, the variance inflation factor shows values that are less than five for each of the regressors, although the p-value and the t-test shows that there is no linear relationship between the yield and the regressors of the model, the standard error is large to have discarded the model and there is multicollinearity. It was observed that from the model that for every one unit increase in the plant height, number of tillers and the leaf 's square area, there are increase of 0.005261 and 0.00453 with a decrease of about 0.002427 in the yield respectively.

Model 4: When plant height (X1) was omitted in the regression, the following model was obtained:

$$Y_{ij} = 0.02371 - 0.0003111X_2 + 0.001759X_3 - 0.002503X_4$$

The intercept shifted to 0.02371 on the y-axis. This led to increase of about 0.001759 in the yield due to increase of one unit in the number of tillers. But an increase in one unit of the number of leaves led to decrease of 0.003111 of the yield. Similarly an increase of one unit of the square area of the leaf led to a decrease of about 0.002503 in the yield. The coefficient of determination R^2 obtained for the model was 0.1068. This shows that about 11% of the total variation was due to the regression; also for R^2 less than 0.75 and the VIF less than five for each of the coefficient of the independent variables, there is no multicollinearity. The p-value of about 0.6016 and the t-test showed that there is no linear relationship between the yield and the independent variables. Rizzi Laura (2008) further stressed that there three ways of verifying the presence of multicollinearity. High R^2 with insignificant estimated coefficient, if R^2 >0.75; High correlation coefficient between regressors; High Variance Inflation Factors (VIF).

Considering these three factors and the discussions so far on the variable Maiwa, it is clearly seen that there is no problem of multicollinearity.

We use Rizzi Laura Prediction Criterion (PC) as stated in equation (16) to investigate the goodness-of-fit of the models discussed above as follows:

The general model that contains all the independent variables used:

$$Yij = 0.01692 + 0.002595X_1 - 0.0003013X_2 + 0.001687X_3 - 0.002303X_4$$

RSS = 0.002942, R^2 = 0.1087, n = 20, k = 4 →k-1 = 3

$$PC_1 = \frac{0.002942(20+3)}{20-3} = \frac{0.002942 \times 23}{17} = 0.00398$$

When X_1, is excluded the resultant model:

$$Y_{ij} = 0.02371 - 0.0003111X_2 + 0.001759X_3 - 0.002503X_4$$

RSS = 0.002949, R^2 = 0.1068, n = 20, k = 3

$$PC_2 = \frac{0.002949(20+2)}{20-2} = \frac{0.002949 \times 22}{18} = 0.00360$$

When X_2, is excluded the resultant model:

$$Y_{ij} = 0.002021 + 0.005261X_1 + 0.000453X_3 - 0.002427X_4$$

RSS = 0.003140, R^2 = 0.0489, n = 20, k = 3

$$PC_3 = \frac{0.003140(20+2)}{20-2} = \frac{0.003140 \times 22}{18} = 0.00384$$

When X_3, is excluded the resultant model:

$$Y_{ij} = 0.00342 + 0.006399X_1 - 0.000627X_2 - 0.001546X_4 \quad 12$$

RSS = 0.003156, R^2 = 0.0439, n = 20, k = 3

$$PC_4 = \frac{0.003156(20+2)}{20-2} = \frac{0.003156 \times 22}{18} = 0.00386$$

When X_4, is excluded the resultant model:

$$Y_{ij} = 0.008794 + 0.005130X_1 - 0.0003071X_2 + 0.001505X_3$$

RSS = 0.003012, R^2 = 0.0875, n = 20, k = 3

$$PC_5 = \frac{0.003012(20+2)}{20-2} = \frac{0.003012 \times 22}{18} = 0.003681$$

It follows that $PC_2<PC_5<PC_3<PC_4<PC_1$, this shows that the best model was obtained when X_1 (Plant height) was excluded in the regression for it has the smallest PC of 0.00360. Hence, Y_{ij} = 0.002021 + 0.005261X_1 + 0.000453X_3 - 0.002427X_4 is the best model that fits the data set and has the best goodness- of -fit? Therefore plant height is an irrelevant plant attribute for this variety (Maiwa).

RESULTS AND DISCUSSION

The result of the Multivariate regression analysis model as extracted from Instat Graph statistic software output are presented as Appendix 1. Using all the require conditions to assess the goodness of fit of the model proposed by Rizzi Laura (2008) and Gerald Keller and Brian Warrack (2003) the best model was obtained when the plant height was dropped or excluded as an independent variable in the model. Thus,

$$PC_2<PC_5<PC_3<PC_4<PC_1$$

This explains that plant height is an irrelevant plant attributes of Maiwa variety. The best model therefore dependent on number of leaves, leaf 's square area and tillers in that order even though some of these attributes contributed insignificantly to the models goodness-of-fit.

Appendix 1:

Model 1 (MAIWA)

Multiple regression results: What equation fits the data the best?

[A:Y] = 0.01692 + 0.002595*[B:X1] -0.0003013*[C:X2] + 0.001687*[D:X3] -0.002303*[E:X4]

How good is the fit?

R^2 = 10.87%. This is the percent of the variance in A:Y explained by the model.

The P-value is 0.7658, considered not significant. The P-value answers this question:

If there were no linear relationship among the variables, what is the chance that R^2 would be that high (or higher) by chance.

Since P is high, the rest of the results will be of little interest.

Sum-of-squares [0.002942], SD of residuals [0.01401], R^2 [0.1087], Adjusted R^2 [0.1290], Multiple R [0.3297], F ---- 0.4573

Which variable(s) make a significant contribution?

Variable	t ratio	P-value	Significant?
(Constant)	0.4276	0.6750	No
B:X1	0.1781	0.8611	No
C:X2	1.003	0.3316	No
D:X3	1.044	0.3130	No
E:X4	0.5979	0.5588	No

Each P-value compares the full model with a simpler model omitting one variable. It tests the effect of one variable, after accounting for the effects of the others. Is multicollinearity a problem?

Variable	VIF	R^2 with other X
B:X1	1.15	0.1275
C:X2	2.58	0.6126
D:X3	2.68	0.6271
E:X4	1.21	0.1702

Each R^2 quantifies how well that X variable is predicted from the other X variables (ignoring Y). VIF is calculated from R^2.

All R^2 values are low (<0.75). The X variables are independent of each other. Multicollinearity is not a problem.

Model 2 (MAIWA)

Multiple regression results: What equation fits the data the best?

[A:Y] = 0.008794 + 0.005130*[B:X1] -0.0003071*[C:X2] + 0.001505*[D:X3]

How good is the fit?

R^2 = 8.75%. This is the percent of the variance in A:Y explained by the model.

The P-value is 0.6803, considered not significant. The P-value answers this question:

If there were no linear relationship among the variables, what is the chance that R^2 would be that high (or higher) by chance?

Since P is high, the rest of the results will be of little interest.

Sum-of-squares [0.003012], SD of residuals [0.01372], R^2[0.0875]' Adjusted R^2 [-0.0837], Multiple R [0.2957] F ------- 0.5111

Which variable(s) make a significant contribution?

Variable	t ratio	P-value	Significant?
(Constant)	0.2416	0.8122	No
B:X1	0.3755	0.7122	No
C:X2	1.044	0.3119	No
D:X3	0.9680	0.3474	No

Each P-value compares the full model with a simpler model omitting one variable. It tests the effect of one

variable, after accounting for the effects of the others. Is multicollinearity a problem?

Variable	VIF	R^2 with other X
B:X1	1.05	0.0469
C:X2	2.58	0.6122
D:X3	2.59	0.6134

Each R^2 quantifies how well that X variable is predicted from the other X variables (ignoring Y). VIF is calculated from R^2.

All R^2 values are low (<0.75). The X variables are independent of each other. Multicollinearity is not a problem.

Model 3 (MAIWA)

Multiple regression results: What equation fits the data the best?

[A:Y] = 0.003242 + 0.006399*[B:X1] -6.272E-05*[C:X2] -0.001546*[E:X4]

How good is the fit?

R^2 = 4.39%. This is the percent of the variance in A:Y explained by the model.

The P-value is 0.8636, considered not significant. The P value answers this question:

If there were no linear relationship among the variables, what is the chance that R^2 would be that high (or higher) by chance?

Since P is high, the rest of the results will be of little interest.

Sum-of-squares [0.003156]; SD of residuals [0.01404]; R^2 [0.0439]; Adjusted R^2 - [0.1353]; Multiple R [0.2096]; F ----- 0.2450

Which variable(s) make a significant contribution?

Variable	t ratio	P-value	Significant?
(Constant)	0.08661	0.9321	No
B:X1	0.4521	0.6573	No
C:X2	0.3211	0.7523	No
E:X4	0.4074	0.6891	No

Each P-value compares the full model with a simpler model omitting one variable. It tests the effect of one variable, after accounting for the effects of the others. Is multicollinearity a problem?

Variable	VIF	R^2 with other X
B:X1	1.07	0.0694
C:X2	1.09	0.0793
E:X4	1.16	0.1397

Each R^2 quantifies how well that X variable is predicted from the other X variables (ignoring Y). VIF is calculated from R^2.

All R^2 values are low (<0.75). The X variables are independent of each other. Multicollinearity is not a problem.

Model 4 (MAIWA)

Multiple regression results: What equation fits the data the best?

[A:Y] = 0.002021 + 0.005261*[B:X1] + 0.0004531*[D:X3]- 0.002427*[E:X4]

How good is the fit?

R^2 = 4.89%. This is the percent of the variance in A:Y explained by the model.

The P-value is 0.8432, considered not significant. The P-value answers this question:

If there were no linear relationship among the variables, what is the chance that R^2 would be that high (or higher) by chance?

Since P is high, the rest of the results will be of little interest.

Sum-of-squares [0.003140]; SD of residuals [0.01401]; R^2 [0.0489]; Adjusted R^2 - [0.1295]; Multiple R [0.2211]; F ----- 0.2740

Which variable(s) make a significant contribution?

Variable	t ratio	P-value	Significant?
(Constant)	0.05509	0.9567	No
B:X1	0.3670	0.7184	No
D:X3	0.4321	0.6714	No
E:X4	0.6302	0.5375	No

Each P-value compares the full model with a simpler model omitting one variable. It tests the effect of one variable, after accounting for the effects of the others. Is multicollinearity a problem?

Variable	VIF	R^2 with other X
B:X1	1.11	0.0975
D:X3	1.13	0.1139
E:X4	1.20	0.1694

Each R^2 quantifies how well that X variable is predicted from the other X variables (ignoring Y). VIF is calculated from R^2.

All R^2 values are low (<0.75). The X variables are independent of each other. Multicollinearity is not a problem.

Model 5 (MAIWA)

Multiple regression results: What equation fits the data the best?

[A:Y] = 0.02371 -0.0003111*[C:X2] + 0.001759*[D:X3] - 0.002503*[E:X4]

How good is the fit?

R^2 = 10.68%. This is the percent of the variance in A:Y explained by the model.

The P-value is 0.6016, considered not significant. The P-value answers this question:

If there were no linear relationship among the variables, what is the chance that R^2 would be that high (or higher) by chance?

Since P is high, the rest of the results will be of little interest.

Sum-of-squares [0.002949]; SD of residuals [0.01358]; R^2 [0.1068]; Adjusted R^2 - [0.0607]; Multiple R [0.3268]; F ------- 0.6378

Which variable(s) make a significant contribution?

Variable	t ratio	P-value	Significant?
(Constant)	2.356	0.0316	Yes
C:X2	1.087	0.2932	No
D:X3	1.160	0.2631	No
E:X4	0.7006	0.4936	No

Each P-value compares the full model with a simpler model omitting one variable. It tests the effect of one variable, after accounting for the effects of the others. Is multicollinearity a problem?

Variable	VIF	R^2 with other X
C:X2	2.50	0.5992
D:X3	2.51	0.6022
E:X4	1.10	0.0935

Each R^2 quantifies how well that X variable is predicted from the other X variables (ignoring Y). VIF is calculated from R^2.

All R^2 values are low (<0.75). The X variables are independent of each other Multicollinearity is not a problem.

REFERENCES

Baker, E.F.I., 1970. Kenaf and Roselle in Western Nigeria. World crops Nov/Dec, 1970, PP: 380-386.

Gerald Keller and Brian War Rack, 2003. Statistics for Management and Economics. Zcurt Hinrich Brooks Kole Thomas learnig, pp: 602-695.

Gibert Tukers, 1976. Growth, yields and yields components of safflower as affected by source, rate and time of application of Nitrogen. Agronomic J., 59: 54-56.

Ogunremi, E.A., 1970. Relationships Between Yeilds and some Agronomic Characters of sugar-cane in southern Nigeria. Nig. Agri. J., PP: 1-8.

Rizzi Laura, 2008. Specification error, Multicollinearity and Qualitative covariates. Www site net Nov 19, 2008.

Gardner, B.R. and T.C. Tucker, 1976. Nitrogen effects on cotton: Vegetative and fruiting characteristics. Soil science America Proc. Vol. 31, No.6, Nov-Dec. 1967; pp: 780-791-56.

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Statistical Process Control on Production: A Case Study of Some Basic Chemicals Used in Pure Water Production

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Abstract: Statistics is the art of making decisions about a process or population based on an analysis of the information contained in a sample from that population. In any production process, regardless of how well designed or carefully maintained it is, a certain amount of inherent or natural variability will always exits, Such variability like background noise. When the variability is small, we usually consider this an acceptable level of process performance or the process is within the process control. A process that is operating in the presence of assignable causes is said to be out of control. A data set collected from randomly selected packaged water producers referred to as "Pure water producers" and subjected to laboratory test of the of the level of some basic chemicals used in the production of pure water, such as pH, conductivity (μS/cm; Lead (Pb); Aluminum (Al) and Chloride (Cl). The test shows that most of the chemicals used are out of process control. This is danger to health of the consumers.

Key words: Production, process control, tweaking, tasteless, assignable causes, variability, chemicals

INTRODUCTION

Production processes must perform consistently overtime to be capable of meeting production and design requirements. Statistical process control is a methodology to monitor and benchmark a process to improve its variability, stability and capability. According to Douglas Montgomery (1985) states that Statistical methods play a vital role in quality assurance. They provide the principal means by which product is sampled, tested and evaluated and information in those data used to control and improve the production process. In any production process, regardless of how well designed or carefully maintained it is, a certain amount of natural variability will always exist. The natural variability "background noise "is the cumulative effect of many small, essentially uncontrollable causes. When the background noise of a process is relatively small, we usually consider this an acceptable level of process performance.

There is variability that usually arises from improperly adjusted machines, operator error and/or defective raw materials. Such variability is generally large when compared to the background noise. This variability that is not part of chance cause pattern is referred to "assignable causes".

Veronica and Patrick (1997) stress that control charts are among the most important tools in Statistical process control. They were developed in the 1920's by Dr. Walter Shewhart a scientist at Bell laboratories, the research arm of American Telephone and Telegraph. Statistical control can be used to:

- Monitor and reduce process variability.
- Monitor and maintain the process on target.
- Determine when a process needs "tweaking" (adjusting) and when it does not.
- Establish process stability and detect process changes so that corrective action can be taken.
- Improve quality and productivity by improving the process, which reduces product inspection, scrap and rework at the end of the line.

Background of the study: Pure water is an odourless, tasteless liquid. It has a bluish tint, which may be detected, however, only in layers of considerable pressure. Accessibility and availability of fresh clean water is a key to sustainable development and an essential element in health, food production and poverty reduction (Adekunle, 2004). However an estimated 1.2 billion people around the world lack access to safe water and close to 2.5 billion are not provided with adequate sanitation (Third World Water Forum on Water, 2003). The standard industrialized world model for delivery of safe drinking water and sanitation technology is however, not affordable in much of the developing world. Thus, given the renewed global commitments towards the Millennium Development Goals (MDG) marked for 2015, the importance and contribution of locally sourced low cost alternative drinking water schemes to sustainable access in rural and semi-urban settings of developing nations cannot be over emphasized (UNDESA, 2004).

Statutorily, portable water supply in Nigeria had been by the Government Owned Public Water Utilities (GPWU) in the past. The GPWUs provided their supply from

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conventional water treatments plants that uses water from impounded reservoirs, flowing perennial streams, lakes and deep boreholes. As the country population grows and industries increase, the supply of water by the GPWUs becomes inadequate in quality and quantity. This led to the emergence of some Privately Owned Water Enterprises (POWE) that operated side by side with the GPWUs within the water sector (Onemano and Otun, 2003).

One of the most popular POWE in Nigeria is the sachet water sold in polythene sachet otherwise called 'Pure Water'. The POWEs mainly collect their water as the end product of initially treated water supplied by the GPWUs and do little treatment such as the removal of the suspended solids to make the GPWUs water more potable. They also do some minor treatment on water from natural springs, open wells and deep boreholes. Some also collect water directly from the GPWUs kiosks and later resells them at a higher price (Onemano and Otun, 2003).

The production, marketing and consumption of sachet water have increased tremendously. There are now several brands of these type of packaged water marketed in Nigeria and other developing nations (Ogan, 1992 and Kassenga, 2007). This so called Pure Water in sachets is readily available, easy to serve and the price is affordable and finds patronage from the middle class and members of low socio-economic classes, but there are concerns about its purity. The integrity of the hygienic environment and the conditions where majority of the water in sachets are produced has also been questioned (CAMON, 2007).

We use the Statistical process control chart to monitor the production process of pure water with the assessment of some basic chemicals used in the production and process of pure water.

A control chart includes three horizontal parallel lines: a centre line, an upper control limit above it and a lower control limit below it. The centre (CL) on a control chart points are expected to cluster in the absence of an assignable cause. The centre line is usually set at average, the median, the mode or the target value of the points being plotted. The Upper Control Limit (UCL) line and the Lower Control Limit (LCL) defined a region where most observations are expected to fall. The upper and lower control limits refer to as statistical control limits or Statistical Process Control (SPC) limits reflect the natural variability of the process and are constructed in such a way that when the process is in control most of the points will falls inside the control limits in random fashion. If a point on the control charts falls above the upper limits or below the lower control limits the process is said to be out of control, and assignable causes need to be searched for eliminated.

Many control charts have a common form. Let w be an observed process characteristics that is determined from a sample, such as the sample mean (average)

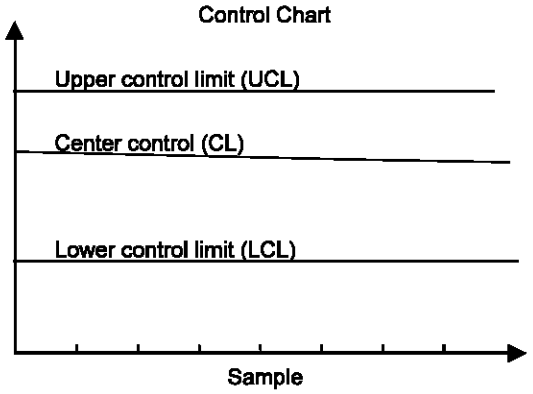

Fig. 1: A typical control chart

x-bar. Let w have a true population mean (μ) and standard deviation (σ).

The centre line, upper control limit and lower control limit have the general form:

$$UCL = \mu + k\sigma$$
$$CL = \mu$$
$$LCL = \mu - k\sigma$$

The upper and lower control limits are chosen to be a distance of K standard deviation (σ) for the mean (μ). It is customary to use k = 3, Which gives 3-sigma control limits that are standard deviation away from the centre. Research as show that if the distribution is normal, approximately 99.73% of the values production will fall between the 3-sigma control limits and 0.27% of the values of the production will fall outside the control limits giving a false alarm approximately only 27 out of 10,000 times.

Objectives of the study: The research work was aimed at ensuring that the production process is monitored to reduce variability and maintain the process target. To determine when a process needs "tweaking" (adjusting) and when it does not. To establish process stability and detect process changes so that corrective action can be taken and finally, to improve quality and productivity by improving the process, which reduces product inspection, scrap and rework at the end of the line.

MATERIALS AND METHODS

A total of hundred sachets (100) from ten different brands of the Pure water were randomly selected in Minna, Niger state which was the study area. Ten samples of each were collected direct from hawkers and wholesalers at different locations of the town which includes; motor parks, markets and other busy areas of the town. These were then transferred to the laboratory for analysis. For physical and laboratory analysis, the physical visual examination of the water samples; odour, and appearance such as colour, turbidity and presence

of floating particles were all noted. While in the laboratory, each sample was subjected to both physical and chemical tests in accordance with standard methods as obtained from the American Public Health Association (APHA), 2005. The chemical tests conducted include chemical analysis of Lead (Pb), chloride (Cl), Iron (Fe) and Aluminum (Al). The physical tests carried out were those of pH and conductivity. The summary of the Raw data is as in appendix1 (Table 2).

Data analysis and interpretation: Data obtained were analyzed using means, standard deviation, standard error of mean and control charts with the use of Instat and Minitab 14 statistical software.

All the tests were conducted between January-June, 2009. The choices of the periods were as a result of the realization of the large production and consumptions of pure water during these periods. The analysis is as follows:

Table 1: Mean, standard deviation, standard error of mean of the chemicals tested in January-June, 2009

Groups	Mean	SD	SEM
pH	7.1600	0.712	0.225
Conductivity (µS/cm)	0.3610	0.1760	0.0556
Iron (Fe^{+2})	0.0870	0.1114	0.0352
Lead (Pb)	0.0340	0.0836	0.0264
Aluminum (Al)	0.0310	0.02558	0.00809
Chloride (Cl)	8.4000	3.3000	1.0400

SD = Standard deviation
SEM = Standard error of mean

From Table 1, the mean values Iron (Fe^{+2}), Aluminum (Al), Conductivity (µS/cm); Chloride (Cl) are less that maximum permitted by Standard organization of Nigerian, while, mean of the Lead (Pb) is greater than the maximum permitted by Standard Organization of Nigerian (SON) standard for portable drinking water as in appendix 1 (Table 3). This shows statistical out of process which invariably has health impact on the consumers.

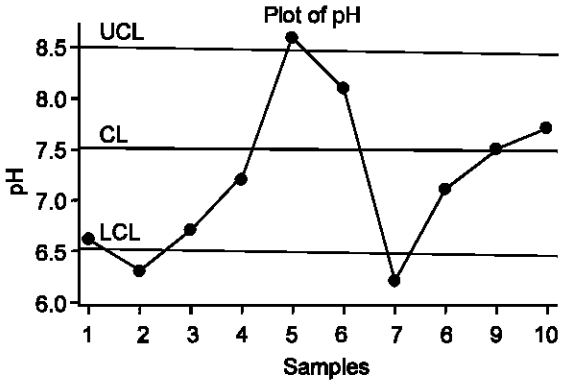

Fig. 2: The control chart for the pH

The upper control limit is 8.5 while the lower control limit is 6.5 from the statistical process chart it seen that three of the points are out of statistical process control, most of the points are at the lower control limit, which according to Veronica and Patrick (1997) when eight points fall at one side the production is out of process control. This might lead to increase of dissolution of certain metals whose concentration above normal could have detrimental health effect.

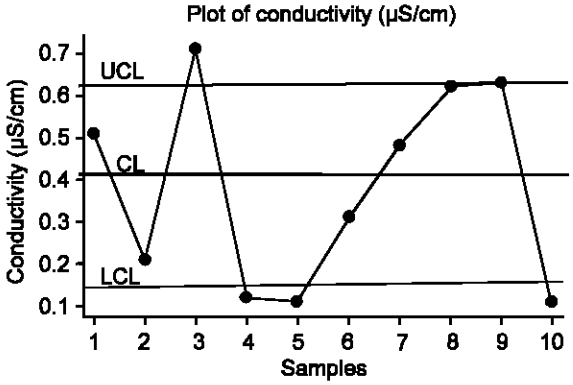

Fig. 3: Plot showing the control chart for conductivity

From the Plot above, it concord with the assertion made by Veronica and Patrick (1997), that an indicator for out of control is that if eight consecutive points are on one side of the centre line or two out of three consecutive points are outside the 2-sigma warning limits on one side of the centre line, then the productions are out of control. Thus, conductivity could be classified has been out-of-control.

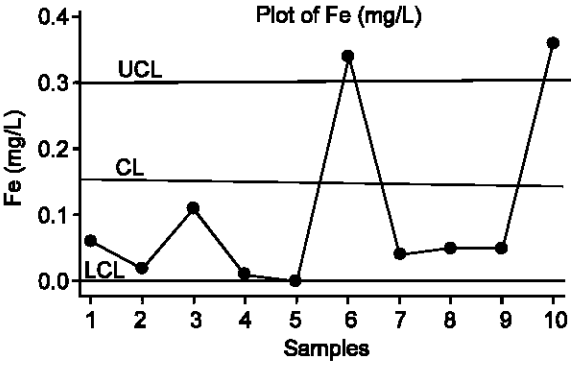

Fig. 4: Plot showing the control chart of Iron

Figure 4 shows that two of the values are out of control and looking at the plot most of the points are on one side of the center, that is , the lower limit. This could lead to water having a metallic taste and could cause stains to laundry.

Table 2: Raw Mean score of parameters analyzed for the duration of work

Sam.	pH	Cond. (µS/cm)	Fe (mg/L)	Pb (mg/L)	Al (mg/L)	Cl (mg/L)
1	6.80	0.38	0.02	0.27	0.02	11.20
2	6.40	0.24	0.01	0.00	0.06	4.33
3	7.00	0.62	0.15	0.02	0.03	4.75
4	7.20	0.13	0.03	0.00	0.03	7.96
5	8.80	0.18	0.00	0.01	0.04	15.74
6	7.20	0.38	0.24	0.00	0.04	7.34
7	6.20	0.51	0.04	0.00	0.01	8.71
8	7.10	0.61	0.03	0.00	0.08	6.80
9	7.30	0.36	0.03	0.03	0.00	9.638
10	7.60	0.20	0.32	0.01	0.00	7.53

Sam. = Samples; Cond. = Conductivity

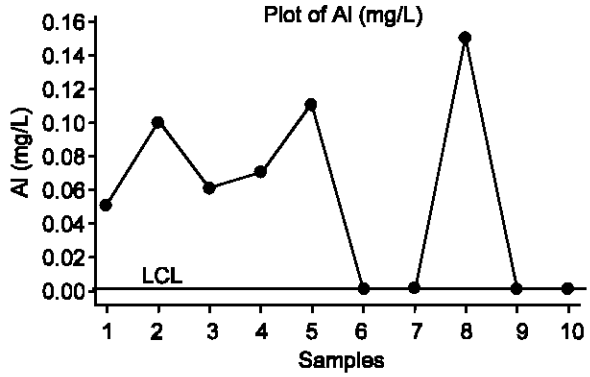

Fig. 5: Plot of the control chart for Aluminum

The range of Aluminum is between 0.00-0.0.155 mg/L which is within the acceptable limit of 0.2 mg/L.

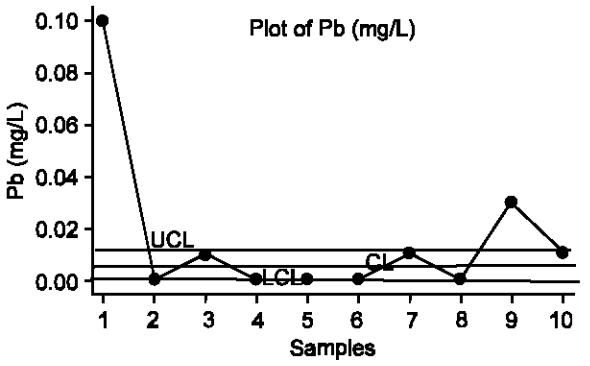

Fig. 6: Plot of control chart showing the responses for Lead (Pb)

From the figure above it was observed that the element Lead (Pb) is out of control. Given the upper control limit to be 0.01 mg/L, showing most of the points to be out of controlled, this could have deleterious health effect as lead is associated with cancer, interference with mental development and vitamin D metabolism and is also implicated in central and peripheral nervous system toxicity.

Table 3: Chemical parameters-inorganic constituents

Parameter	Unit	Maximum permitted	Health impact
Aluminum (Al)	Mg/L	0.2	Potential Neuro-degenerative disorders
Arsenic (As)	Mg/L	0.01	Cancer
Barium	Mg/L	0.7	Hypertension
Cadmium (Cd)	Mg/L	0.003	Toxic to the kidney
Chloride (Cl)	Mg/L	250	None
Chromium (Cr^{6+})	Mg/L	0.05	Cancer
Conductivity	µS/cm	1000	None
Copper (Cu^{+2})	Mg/L	1	Gastrointestinal disorder
Cyanide (CN^{-})	Mg/L	0.01	Very toxic to the thyroid and the nervous system
Fluoride (F)	Mg/L	1.5	Fluorosis, skeletal tissue (bones and teeth) morbidity
Hardness (as $CaCO_3$)	Mg/L	150	None
Hydrogen sulphide (H_2S)	Mg/L	0.05	None
Iron (Fe^{+2})	Mg/L	0.3	None
Lead (Pb)	Mg/L	0.01	Cancer, interference with vitamin D metabolism, affects mental development in infants, toxic to the central and peripheral nervous systems
Magnesium (Mg^{+2})	Mg/L	0.20	Consumer acceptability
Manganese (Mn^{+2})	Mg/L	0.2	Neurological disorder

Source: Nigerian Industrial Standard (NIS) 554: 2007, Nigerian standard for drinking water quality

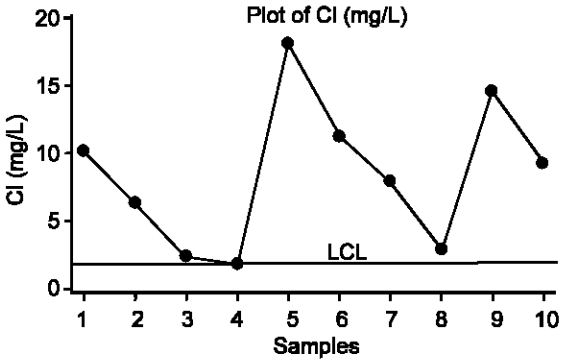

Fig. 7: Plot of control chart showing the responses for chloride

Chloride concentration in the samples has a mean range of 4.33 and 15.74 which is within the accepted limit of 250 mg/L. The range is 15.74-4.33 = 11.31.

RESULTS AND DISCUSSION

The results were compared with Nigerian Standard for Drinking Water Quality as outlined by Standard Organization of Nigeria (SON), 2007. As outlined in the table the mean pH ranges from 6.2-8.8, which indicates that some of samples are falling out of range of the normal pH of drinking water (6.5-8.5). Though, this might not have any direct effect but could lead to increase of dissolution of certain metals whose concentration above normal could have detrimental health effect.

The conductivity has a mean range of 0.13-0.62 µS/cm, while Iron has a mean range of 0.00-0.32 mg/L indication a deviation of the accepted level of 0.3 mg/L, this could lead to the water having a metallic taste and could cause stains to laundry. Lead has a mean range of 0.00-0.03 mg/L with the upper limit well above the accepted limit of 0.01 mg/L this could have deleterious have effect as lead is associated with cancer, interference with mental development and vitamin D metabolism and is also implicated in central and peripheral nervous system toxicity. Aluminum has a mean range of 0.00-0.08 mg/L which is within the accepted limit of 0.2 mg/L. Chloride concentration in the samples has a mean range of 4.33-15.74 which also within the accepted limit of 250 mg/L.

Conclusion and recommendations: Production process control chart can monitor and reduce process variability, determine when a process needs adjusting and when it does not, establish process stability and detect process changes.

As evidence from the results some of the chemicals used in the production of packaged water at times do not conform to standards led out for quality drinking water, the chemical constituents are out of process control; as such this could pose health risk to the consumers. The result also confirms other reports on the health risk of pure water.

We therefore recommend that a statistical process control chart should be plotted periodically productivity by improving the process, which the process production, scrap and rework at the end of the days production.

REFERENCES

Adekunle, L.V., M.K.C. Sridhar, A.A. Ajayi, P.A. Oluwade and J.I. Olawuyi, 2004: An assessment of the health and social economic implications of sachet water in Ibadan Nigeria: A public health challenge. Afr. J. Biomed. Res., 7: 5-8.

American Public Health Association (APHA), 2005. Standard Methods for the Examination of Water and Wastewater. 21st Edn., Washington, DC.

Consumer Affairs Movement of Nigeria (CAMON), 2004. NAFDAC to Ban Pure Water-97% Samples Contaminated. Consumer Link 1:1.

Douglas Montgomery, C., 1985. Introduction to Statistical Quality control. John wiley and sons Inc., USA.

Kassenga, G.R., 2007. The health-related microbiological quality of bottled water sold in Dar es Salam, Tanzania. In Dada, A.C. (2009), Sachet water phenomenon in nigeria: assessment of the potential health impacts. Afr. J. Microbiol. Res., 3: 15-21.

Ogan, M.T., 1992. Microbiological quality of bottled sold in retail outlets in Nigerian, In Dada, A.C. (2009), Sachet water phenomenon in Nigeria: Assessment of the potential health impacts. Afr. J. Microbiol. Res., 3: 15-21.

Onemano, J.I. and J.A. Otun, 2003. Problems on Water Quality Standards and Monitoring in Nigeria: Towards the Millennium Development Goals. 29th WEDC International Conference, Abuja, Nigeria.

Standards Organization of Nigeria (SON), 2007. Nigerian Standards for Drinking Water Quality. Nigerian Industrial Standards NIS 554.

Third World Forum on Water, 2003. Blockade, Myth, Illusions in Development and Cooperation. January, 2003; 30 (1).

UN Department of Economics and Social Affairs (UNDESA), 2004. Urban Agglomerations in Dada, A.C. (2009), Sachet Water Phenomenon in Nigeria: Assessment of the Potential Health Impacts. Afr. J. Microbiol. Res., 3: 15-21.

Veronica Czitron and D. Patrick Spagon, 1997. Statistical case studies for industrial process improvement. America Statistical Association Alexandria, Virginia.

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Physical Growth and Nutritional Status of a Cohort of Semi-Urban Nigerian Adolescents

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Abstract: The nutritional transition in developing countries may lead to imbalances in the growth and nutritional status of adolescents in such countries - events that could result in improper maturation and morbidity in adult life. This study seeks to determine the patterns of physical growth and nutritional status of adolescents living in a low income semi-urban town in Nigeria. Anthropometric data from six hundred and twenty five (625) secondary school students aged 10-19 years (adolescents) were collected and their Body Mass Index (BMI) calculated. Their heights and weights were compared to those of a reference population. Outcome measures for nutritional status were proportion of the population that is stunted (height-for-age < 3rd percentile of the reference data), thin and overweight/obese (BMI-for-age < 5th and > 85th percentiles of the reference data respectively). The girls matched the heights of half of the reference population at all ages, but the boys did not. From age fourteen years, the boys weighed less than half of the reference population while the girls matched or weighed more than the reference population. Under-nutrition was found to affect 19.36% of the population (with stunting accounting for 84.47% of this group), while 13.12% of the population were overweight/obese. The prevalence of thinness and stunting were higher in boys than in girls. Boys were also slightly more obese than the girls. Under- and over-nutrition co-exist in the population and affect more boys than girls. There is an urgent need to address these problems in preventive and curative health care programmes.

Key words: Adolescence, anthropometry, nutritional status, physical growth

INTRODUCTION

Adolescence is a period of rapid growth which begins with pubescence and continues until morphological and physiological changes approximate adult status. Adolescents are persons aged 10-19 years (WHO, 1986). They generally have a low prevalence of infectious diseases when compared to under-five children and chronic diseases when compared to ageing people. As a result of this, they have attracted little health and nutrition attention (Senderowitz, 1995; Wang *et al*., 1998). This is worrisome especially for developing countries where the proportion of adolescents in relation to other age groups is high and is expected to increase in the future (Blum, 1995).

Anthropometry is a useful non-invasive tool in the assessment of physical growth and nutritional status in adolescents (WHO, 1995). Growth may be responsive to nutritional surfeit or deficit. Therefore, anthropometry in adolescents can be used to evaluate nutritional abnormalities like stunting, thinness (under-nutrition) and obesity (over-nutrition). The US National Center for Health Statistics (NCHS) and the first US National Health and Nutrition Examination Survey (NHANES 1) reference data provide veritable tools for comparing anthropometric indices and nutritional status from different populations (Hamill *et al*., 1979; Must *et al*., 1991a and b).

Nigeria, a developing country, like the other countries in that category, is experiencing changes in the dietary patterns of her people, as a result of the prevailing nutritional transition. This transition often comes with changes in habits that predispose to overweight and obesity at adolescence, which tends to persist into adulthood with its sequelae (Serdula *et al*., 1993; Popkin, 1994), while the reverse (under-nutrition), could result in improper maturation (Agarwal *et al*., 1995). In view of these problems and the high poverty level in Nigeria, assessing the physical growth and nutritional status of Nigerian adolescents, dwelling in a low income semi-urban town (where majority of Nigerians live) is important as it would provide data that may help in prevention, intervention and rehabilitation programmes. This study attempts to fill that gap.

MATERIALS AND METHODS

Setting: Nigeria's population (estimated in the year 2000) is distributed as follows: 0-14 years - 44% (male 27,181,020; female 26,872,317); 15-64 years - 53%

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(male 33,495,794; female 32,337,193); 65 years and over - 3% (male 1,729,149; female 1,722,349). The census 2006 preliminary results however put the total population of Nigeria at 140,003,542 (Wikipedia, 2007). A 1999 estimate by the World Health Organization puts life expectancy at birth for males and females at 46.8 and 48.2 years respectively. In the developed world, the figures are much higher (74.7 years and 79.7 years for American males and females respectively) (WHO, 2000). On the other hand, total fertility rate in Nigeria is at 5.66 children per woman while mortality rate is at 74.18 deaths per 1000 live births (Wikipedia, 2007). In Western Europe, the figures contrastingly stand at 1.5 children per woman and 10 deaths per 1000 live births. Nigeria's Gross Domestic Product (GDP) per capita (2004 estimate) stood at $560 (USD) as against $44,670 (USD) in Denmark and $30,941(USD) in the United States of America (USA) (Microsoft, 2007). The 1997 WHO per capita health expenditure estimate for Nigeria was $30 (USD) while Denmark had $1940 (USD) and the USA had $ 3724 (USD) (WHO, 2000). Nigeria, clearly, has the markers of a low income country.

Ajaokuta is a small semi-urban town in Kogi state, North-Central Nigeria. Virtually all the people dwelling in the town are either subsistence farmers or unskilled artisans (only a negligible proportion work in the almost moribund Iron and Steel Complex located there) and therefore belong to the low income socio-economic status group. Tropical tubers like cassava (*Manihot esculenta*) and yam (*Diascoria spp)* and maize (*Zea* mays) are the major agricultural products of these farmers and therefore provide their staple food. Legumes and meat are protein sources for the population. But legumes are seasonal in availability (due to an absence of storage facilities), while meat is expensive and often out of reach of low income people. Ajaokuta typifies the average Nigerian experience and hence its choice as a setting for this study.

Subjects: Students from two public secondary schools in Ajaokuta (with no signs of overt ill-health) whose parents or legal guardians gave informed consent were allowed to participate in the study.

The study was conducted from October to December (the harvest period in Nigeria) of 2007. A total of six hundred and twenty five (625) adolescents (374 males of mean age 14.27±2.15 and 251 females of mean age 14.32±2.27 years) participated in the study. Data on students' ages were obtained from their school records. Age at last birthday was recorded for each student. Height was measured (with the student standing on bare feet) using a non-elastic measuring tape fastened to a vertical rod, to the nearest 0.5 cm. Weight was measured (with the student on bare feet and with light clothing) using an electronic weighing balance, to the nearest 0.1 kg. From the heights and weights got, Body Mass Index (BMI) was calculated using the formula BMI = Weight (kg)/[Height (m)]^2. To assess the growth and nutritional status of the participants, their heights, weights and BMI were compared to those of an age-matched reference population (Hamill *et al*., 1979; Must *et al*., 1991a; Must *et al*., 1991b). Stunting (a measure of chronic under-nutrition) is defined as height-for-age < 3rd percentile of the NCHS percentiles (Hamill *et al*., 1979), while obesity (a measure of over-nutrition) and thinness (a measure of present under-nutrition) are defined as BMI-for-age > 85th percentile and BMI-for-age < 5th percentile of the NHANES 1 reference data (Must *et al*., 1991a; Must *et al*., 1991b) respectively. Stunted and thin adolescents were taken to be undernourished.

The same trained personnel took all measurements in both locations. The weighing balance was appropriately calibrated before use each morning. Measurements were taken between 8 am and 10 am each day throughout the duration of the study. The study protocol was prepared in accordance with the Helsinki Declaration and was approved by the Human Experiments Review Board of the Department of Biochemistry, Kogi State University, Anyigba. Additional approvals were sought and obtained from the principals of participating schools.

Data analysis: Mean values for the different data collected in the appropriate groups were calculated and differences between means separated by one way ANOVA. The Least Significant Difference (LSD) was fixed at 0.05. All data analysis was done using SPSS for windows version 11.0 (SPSS Inc. Chicago, IL). Results are presented as percentages and means with standard deviations from means in table and line graphs respectively.

RESULTS

The means of the anthropometric parameters studied and those of the reference population are given in Fig. 1-3. At ten years, the boys were significantly ($p<0.05$) taller than the girls. By twelve years, the girls had caught up with the boys. Both boys and girls aged 10 and 11 years were taller than 50% (height-for-age >50th percentile) of the reference population. From 12 years onwards, the females were about the same height as 50% of their age-matched counterparts. The boys matched the heights of 50% of the reference data only at ages 12 and 13 years. From age 14 years onwards, the boys were shorter than 50% of the reference population. In fact from age 16-19 years, the boys were shorter than 95% (height-for-age < 5th percentile) of the reference population. The increases in height with age for both sexes were significant ($p<0.05$), except between the ages of 10 and 11 years and 16-19 years.

The males weighed more than 50% of the reference population from 10-13 years. Thereafter, they weighed less than 50% of the reference group, but more than

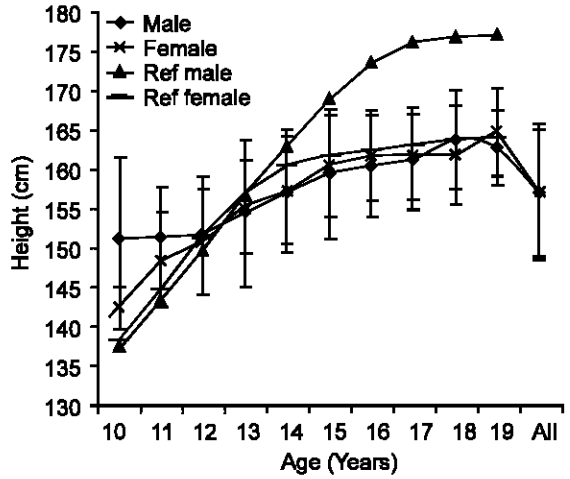

Fig. 1: Comparison of the heights of the participants to the median (50th percentile) heights of an age-matched reference population

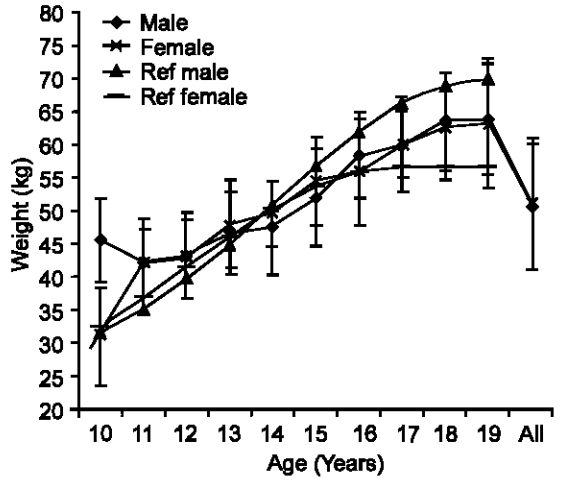

Fig. 2: Comparison of the weights of the participants to the median (50th percentile) weights of an age-matched reference population

25% (weight-for-age >25th percentile) of the same group. The females, at 10 years had a smaller mean weight than that of 50% of their age-matched counterparts. However, at ages 11 and 12 years, the females weighed heavier than 50% of the reference population. Between the ages of 13 and 16 years, the weights of the females matched that of 50% of the reference group. From age 17 years onwards, the females became markedly heavier than 50% of the reference population, but still lighter than 10 % (weight-for-age < 90th percentile) of the same population. In the sampled population, the boys were significantly ($p<0.05$) heavier than the girls (at 10 years), the difference being as much as 14 kg. The girls however equilibrated with the boys at age 11 years and both increased with age while the difference between

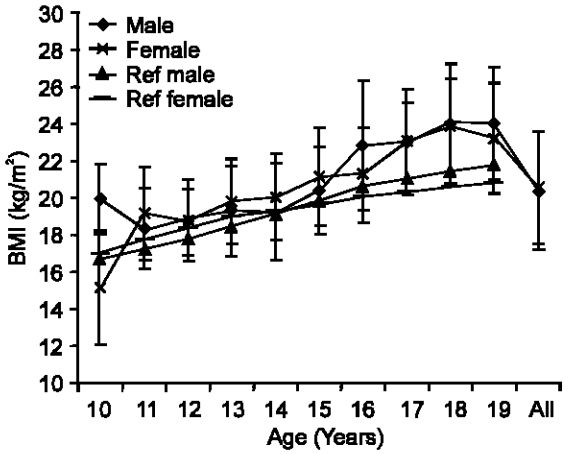

Fig. 3: Comparison of the weight-to-height ratio (BMI) of the participants to the median (50th percentile) BMI of an age-matched reference population

their means was insignificant ($p>0.05$). For both sexes, the differences in the weights of the different ages were significant ($p<0.05$) except between those aged 10 and 11years and those aged 18 and 19 years.

For BMI, the same trend for height and weight was noticed for the boys and the girls. Except at age 14 years, the boys had clearly more BMI than 50% of the reference population, but their BMI was still less than that of the top 15% (BMI-for-age < 85th percentile) of the same population. The females at all ages had higher BMI than 50% of the reference group (except at 10 years). Their BMI were however less than the top 15% of the reference group.

More boys were thin (4.81% compared to 2.39% of the girls), stunted (23.80% compared to 4.78% of the girls) and obese (24.60% for boys and 4.78% for girls) (Table 1). The thin boys were between the ages of 11 and 16 years. Obesity was found in all ages for the boys. Girls aged 10, 12, 18 and 19 years had no case of obesity. Stunting was found in boys from age 12-19 years and in girls from age 13-19 years (though none was found to be stunted at age 17 years). A total of 46.79% of the boys and 86.79% of the girls studied had no detectable nutritional impairment, while 37.28% of the studied population (irrespective of sex) suffer from under- (20.00%) or over- (17.28%) nutrition. Stunting accounted for 80.80% of the undernourished group.

DISCUSSION

Mean BMI for boys and girls of all ages were within the normal ranges of the reference data. Only the heights of the boys aged 14 years and above fell below acceptable ranges. However, as much as 23.80% of the boys and only 4.78% of the girls had height-for-age values lower than the 3rd percentile of the reference data. This

Table 1: Prevalence of thinness, obesity and stunting in the population

Age (Years)	Thinness (%)			Obesity (%)			Stunting (%)		
	Boys (N)	Girls (N)	All (N)	Boys	Girls	All	Boys	Girls	All
10	0.00 (12)	10 (10)	4.55 (22)	50.00	0.00	27.27	0.00	0.00	0.00
11	11.11 (27)	4.55 (22)	8.16 (49)	29.63	18.18	24.49	0.00	0.00	0.00
12	6.25 (32)	0.0 (20)	3.85 (52)	34.38	0.00	21.15	3.13	0.00	1.92
13	3.08 (65)	0.00 (41)	1.89 (106)	18.46	2.44	12.26	6.15	4.88	5.66
14	6.98 (86)	8.70 (46)	7.58 (132)	17.44	2.74	12.12	11.63	10.87	11.36
15	6.90 (58)	0.00 (36)	4.26 (94)	13.79	22.22	17.02	25.86	2.78	17.02
16	3.33 (30)	0.00 (28)	1.72 (58)	43.33	3.57	24.14	46.67	3.57	25.86
17	0.00 (27)	0.00 (23)	0.00 (50)	25.93	4.35	16.00	74.07	0.00	40.00
18	0.00 (24)	0.00 (19)	0.00 (43)	29.67	0.00	16.28	66.67	10.53	41.86
19	0.00 (13)	0.00 (6)	0.00 (19)	38.46	0.00	26.32	69.23	16.67	52.63
Total	4.81 (374)	2.39 (251)	3.84 (625)	24.60	6.37	17.28	23.80	4.78	16.16

disparity in the prevalence of stunting between the sexes agrees with the reports of Jackson *et al.* (2002), Venkaiah *et al.* (2002), Ukegbu *et al.* (2007) and Wamani *et al.* (2007). The same trend was noticed for thinness as more boys than girls had BMI-for-age values below the 5th percentile of the reference data.

The 20.00% prevalence rate for adolescent under-nutrition reported in this study is higher than the 8.7% reported for Jamaica (Jackson *et al.*, 2002), 18% reported for China (Wang *et al.*, 1998) and 18.9% reported (for adolescents enrolled in private school) in Pakistan (Din and Paracha, 2003), but lower than the 23.00%, 36.00% and 53.00% reported for Benin, Nepal and India respectively (Kurtz, 1996) and 42.2% reported for adolescents in a Pakistani public school (Din and Paracha, 2003). The high prevalence of stunting among boys aged 15 years and above agrees with a report from another part of Nigeria (Ukegbu *et al.*, 2007) but is terribly high when compared to the 3% reported in Jamaica (Jackson *et al.*, 2002). This prevalence of under-nutrition in adolescents is high as it suggests that one in five adolescents in the study area is undernourished. It is also of great concern since under-nutrition may affect the maturation (Agarwal *et al.*, 1995; Grantham-McGregor, 1995) and later physical work capacity (Haas *et al.*, 1996) of the individual. Stunting is attributable to low birth weight (Espo *et al.*, 2002), inadequate care and stimulation (Begin *et al.*, 1999), insufficient nutrition and recurrent infections (Cole and Parkin, 1997) and other environmental determinants. For this reason, stunting is said to capture the multiple dimensions of children's health, development and the environment where they live (Wamani *et al.*, 2007).

The prevalence of obese adolescents in the study is 17.28% (24.60% for boys and 6.37% for girls). The figure is a lot higher than the 4% reported for China (Wang *et al.*, 1998) but lower than 19.3% and over 20% found in Jamaica and the United States of America respectively (Jackson *et al.*, 2002; Troina *et al.*, 1995). The current report however shows that obesity was as high in boys as in the USA, but apparently still low in the girls. This clearly disagrees with the reports of Gam *et al.* (1986), Dietz (1994), Jackson *et al.* (2002) and Monyeki *et al.* (2008) that report a higher prevalence of overweight and obesity in girls, but agrees with Ukegbu *et al.* (2007). This prevalence of adolescent obesity is relatively high and is worrisome since obesity in adolescence often persists to adulthood (Popkin, 1994), especially for females (Dietz, 1994).

The fact that a considerably high percentage of the population was stunted and only a very small proportion was thin, suggests that the cause of the noticed under-nutrition in the population is chronic. Thinness disappeared at 15 years in the girls and at 17 years in the boys, yet the percentage of the population that was stunted kept increasing. This suggests an imbalance in the food intake of the population in favour of high energy yielding foods. This is not entirely surprising as the population subsists largely on starchy carbohydrates. Though there was an apparent attempt at catch-up growth in the population, it resulted in increase in weight without a collateral increase in height. The acute consumption of these high energy foods may be responsible for the noticed obesity in the population. Catch-up growth has also been shown to increase the chances of becoming obese (Espo *et al.*, 2002). Martotell *et al.* (1994) however, report that catch-up is incomplete, particularly for those remaining within the same adverse environment. How these affect boys and girls disproportionately is still unclear. The high BMI in some of the boys may be due to a relatively high muscle mass as a result of cultural practices that require males to do the heavy (probably muscle building) tasks.

Conclusion: In conclusion, both under- and over-nutrition co-exist at different prevalence levels in the studied adolescent population. The observed under-nutrition is due largely to chronic deprivation. These findings show the effects of the nutrition crisis in low income areas like Ajaokuta, Nigeria and highlight the dilemma it presents to such societies who still have infectious/ communicable diseases to tackle. Most semi-urban and rural girls marry and conceive as adolescents.

Adolescents also provide a good proportion of the productive work force in such environments. There is, therefore, an urgent need to develop strategies to improve the growth and nutritional status of adolescents. Our study is representative of school-going adolescents in Ajaokuta and may not represent the others. We had few people at the ages of 10 and 19 years, because most people enroll into secondary school at age 11-12 years and graduate at 17-18 years. This therefore calls for a cautious interpretation of the data, especially at those two ages.

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REFERENCES

Agarwal, K.N., D.K. Agarwal and S.K. Upadhydal, 1995. Impact of chronic undernutrition on higher mental function in boys aged 1-12 years. Acta Peadiatr, 84: 1357-61.

Begin, F., E.A. Frongillo Jr. and H. Delisle, 1999. Caregiver behaviours and resources influence child height-for-age in rural Chad. J. Nutr., 129: 680-6.

Blum, R.W., 1995. Global trends in adolescent health. J. Am. Med. Assoc., 265: 2711-9.

Cole, T.J. and J.M. Parkin, 1997. Infection and its effect on growth of young children: a comparison of the Gambia and Uganda. Trans. R Soc. Trop. Med. Hyg., 71: 196-8.

Dietz, W.H., 1994. Critical periods in childhood for the development of obesity. Am. J. Clin. Nutr., 59: 955-9.

Din, Z.U. and P.I. Paracha, 2003. Assessment of nutritional status of adolescent boys from public and private schools of Peshawar. Pak. J. Med. Res., 42: 129-33.

Espo, M., T. Kulmala, K. Maleta, T. Cullinan, M.L. Salin and P. Ashon, 2002. Determinants of linear growth and predictors of severe stunting during infancy in rural Malawi. Acta Paediatric, 91: 1364-70.

Gam, S.M., M. La Velle, K.R. Rosenberg and V.M. Hawthorn, 1986. Maturational timing as a factor in female fatness and obesity. Am. J. Clin. Nutr., 43: 879-83.

Grantham-McGregor, S., 1995. A review of the studies of the effects of severe malnutrition on mental development. J. Nutr., 125: S2233-8.

Haas, J.D., S. Murdoch, J. Rivera and R. Martorell, 1996. Early nutrition and later physical work capacity. Nutr. Rev., 54: S41-8.

Hamill, P.V.V., T.A. Drizd, C.L. Johnson, R.B. Reed, A.F. Roche and W.M. Moore, 1979. Physical growth: National Centre for Health Statistics percentiles. Am. J. Clin. Nutr., 32: 607-629.

Jackson, M., M. Samms-Vaughan and D. Ashley, 2002. Nutritional status of 11-12 years-old Jamaican children: coexistence of under- and over-nutrition in early adolescence. Public Health Nutr., 5: 281-288.

Kurtz, K.M., 1996. Adolescent nutritional status in developing countries. Proc. Nutr. Soc., 55: 321-331.

Martotell, R., L. Kettel-Khan and D.C. Schweder, 1994. Reversibility of stunting: Epidemiological findings in children from developing countries. Eur. J. Clin. Nutr., 4: S45-57.

Microsoft, 2007. Microsoft Encarta encyclopeadia premium suite. (DVD). Microsoft corporation USA .

Monyeki, K.D., M.A. Monyeki, S.J. Brits, H.C.G. Kemper and P.J. Makgae, 2008. Development and tracking of body mass index from preschool age into adolescence in rural South African children: Ellisras Longitudinal Growth and Health Study. J. Health Popul. Nutr., 26: 405-17.

Must, A., G.E. Dallal and W.H. Dietz, 1991a. Reference data for obesity: 85th and 95th percentiles of body mass index (wt/ht^2) and triceps skinfold thickness. Am. J. Clin. Nutr., 53: 839-46.

Must, A., G.E. Dallal and W.H. Dietz, 1991b. Reference data for obesity: 85th and 95th percentiles of body mass index (wt/ht^2) -a correction. Am. J. Clin. Nutr., 1991 54: 773.

Popkin, B.M., 1994. The nutrition transition in low-income countries: an emerging crisis. Nutr. Rev., 52: 285-98.

Senderowitz, J., 1995. Adolescent health: reassessing the passage to adulthood. World Bank Discussion Paper No 272. World Bank, Washington DC.

Serdula, M.K., D. Ivery, R.J. Coates, D.S. Freeman, D.F. Williamson and T. Byers, 1993. Do obese children become obese adults? A review of the literature. Prev. Med., 22: 167-77.

Troina, R.P., K.M. Flegal, R.J. Kuczmarski, S.M. Campbell and C.L. Johnson, 1995. Overweight prevalence and trends for children and adolescents. Arch Ped. Adolesc Med., 149: 1085-91.

Ukegbu, P.O., I.A. Onimawo and A.U. Ukegbu, 2007. Nutritional status and energy intake of adolescents in Umuahia urban, Nigeria. Pak. J. Nutr., 6: 641-6.

Venkaiah, K., K. Damayanti, M.U. Nayak and D. Vijayaraghavan, 2002. Diet and nutritional status of rural adolescents in India. Eur. J. Clin. Nutr., 56: 1119-25.

Wamani, H., A.N. Åstrøm, S. Peterson, J.K. Tumwine, and T. Tylleskär, 2007. Boys are more stunted than girls in Sub-Saharan Africa: a meta-analysis of 16 demographic and health surveys. BMC Pediatr, 7: 17.

Wang, Y., B. Popkin and F. Zhai, 1998. The nutritional status and dietary patterns of Chinese adolescents 1991 and 1993. Eur. J. Clin. Nutr., 52: 908-16.

Wikipedia, 2007. Demography of Nigeria. Retrieved from http://en.wikipedia.org/wiki/demography_of_Nigeria, on Monday 31st December 2007.

World Health Organization, 1986. Young Peoples Health: A challenge for society. Technical report series No 731, WHO Geneva.

World Health Organization, 1995. Physical status: The use and interpretation of anthropometry. Technical report series No 854, WHO Geneva.

World Health Organization, 2000. Health systems: Improving performance. The world health report. WHO Geneva.

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The Significance of Pathogenic Bacteria in the Gut of Swimming Crab, *Callinectes* sp Obtained from Lagos Lagoon and Market Samples Stored at Freezer Temperature (0°C)

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Abstract: An investigation was conducted to isolate and characterize pathogenic bacteria in the gut of swimming crab (*Callinectes*) obtained from Lagos lagoon (28±2°C) and market frozen samples (0°C). The $\log_{10}$ of cfu/ml total value of 7.2 and 4.83 cfu/ml was obtained from fresh lagoon and market samples respectively. Pathogens isolated were *Bacillus cereus*, *Escherichia coli*, *Proteus vulgaris*, *Salmonella* sp, *Streptococcus agalactiae*, *Vibrio* sp, *Staphylococcus aeureus* and *Micrococcus* sp. *Escherichia coli* and *Bacillus subtilis* were more preponderant than other pathogens with $\log_{10}$ cfu/ml values of 5.90 and 3.0 (for Lagoon and market samples) and 5.81 and 4.47 (for lagoon and market samples) respectively. However, the $\log_{10}$ of cfu/ml for market samples for *Bacillus subtilis* was higher than *Escherichia coli*. On blood agar supplemented with sheep red blood cells, most isolates except few were ß- and α-haemolytic, demonstrating that they could be pathogenic strains. The degree of haemolysis were *Bacillus cereus* 34 (76%), *Escherichia coli* 46 (78%), *Salmonella sp* 33 (76.7%), *Vibrio parahaemolyticus* 40 (93%), *Staphylococcus aureus* and *Bacillus subtilis* were more haemolytic than *Micrococcus sp, Streptococcus agalactiae* and *Proteus vulgaris*. The occurrence of these pathogens in the swimming crab is of epidemiological and health significance.

Key words: *Salmonella, Vibrio parahaemolyticus, Escherichia coli, Staphylococcus aureus, Bacillus cereus, Bacillus subtilis,* epidemiological

INTRODUCTION

Crabs are crustaceans found in virtually all parts of the world. In tropical waters, *Callinectes* species are common and most of the landed crabs belong to this genus. Crabs are popular dietary components of many Nigerian dishes. Because of its high calcium content it is recommended for pregnant women. However, they have been implicated in incidences of food intoxication and infection. Crabs and shrimps have been implicated in *Vibrio parahaemolyticus* food poisoning (Silker, 1986) cholera (Baine *et al*., 1974) salmonellosis (Frazier and Westhoff, 1988) shigellosis (Piexotto *et al*., 1979) and Yersinia food infection (Silker, 1986). Other pathogenic microbes has been associated with food poisoning and infection in sea foods; *Bacillus cereus* enterotoxin (Granum *et al*., 1993; Johnson, 1984).

Deaths from staphylococcal food poisoning have been reported (Bergdoll, 1990). This investigator's report also asserted that the offending organism, *Staphylococcus aureus* grow rapidly and produces enterotoxins between 66°F and 99°F (20°C and 37°C) and that the staphylococcal enterotoxins are highly resistant to heat. Bergdoll (1979, 1990) reported that the normal temperature used in cooking will not destroy the toxins and foods containing staphylococcal enterotoxins usually look and taste normal.

The present study was designed to determine the occurrence of pathogenic and health-threatening bacterial species in the gut of the swimming crab (*Callinectes sp)* and to stress the health implication of their presence in this edible seafood.

MATERIALS AND METHODS

Samples of crab of the species *Callinectes* variety were purchased from supermarkets (frozen) and lagoon front of the University of Lagos, in Lagos metropolis. The fresh samples were collected in new sterile polythene bags in the morning and transported to the laboratory immediately for analysis.

Different enrichment and selective cultural methods were used to determine the abundance of bacteria in the gut of the swimming crab. The medium employed were

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among others, Thiosulphate Citrate Bile Salts (TCBS; oxoid), MacConkey agar, Nutrient agar, *Salmonella-Shigella* agar, Blair-Parker agar, Eosin Methylene Blue agar (EMB), Tryptose Soy Agar (TSA), Tryptone, Bile salt, X glucuronide medium (TBX, oxoid) for the cultivation of the pathogens etc.

Isolation of the gut from the crab samples: Using a sterile scalpel and other dissecting instruments under aseptic conditions, the crabs were dissected and the guts obtained and kept in sterile microbiological tubes prior to inoculation into appropriate media.

Isolation of pathogens from the gut samples: About 10.0 g of the dissected guts samples were blended with 90 ml of Phosphate Buffered Saline (PBS). Serial dilution was subsequently made from this stock for the enumeration of bacterial pathogens. For the isolation, identification and enumeration of various bacterial species the methods of Maugeri *et al.* (2004), Olutiola *et al.* (1991) and Oyewole (1990) were employed. Morphology of the isolated organisms was noted and selected colonies from NA, MCA, were transferred to agar slants as pure cultures and stored at 4°C for further studies. Isolates were identified according to the procedure of Oyewole (1990); spore staining, catalase test, IMVC test, sugar fermentation test, nitrate reduction, oxidase text, indole test (Olutiola *et al.*, 1991).

Escherichia coli: For the isolation of *Escherichia coli,* Concentrated samples were inoculated onto plates of Tryptone, Bile salts, X glucuronide medium (TBX; Oxoid) and incubated at 44°C for 18-24 h. Blue colonies were counted and the isolates were identified by using the API 20E system (bioMe´rieux). *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 11228 were used as positive and negative control strains respectively.

***Vibrio*:** The total number of *Vibrio* spp. was obtained by directly inoculating the concentrated samples onto plates of Thiosulphate Citrate Bile Salts (TCBS; Oxoid) and incubating at 37°C for 24 h. Yellow and green colonies were isolated and identified at genus and species level (Alsina and Blanch, 1994a,b; Maugeri *et al.*, 2004). The isolates were confirmed using the API 20E system (bioMe´rieux). The nonpathogenic *Vibrio furnissii* strain NCTC 11218, isolated by Taylor and Barry (1981) and *E. coli* ATCC 25922 were used as positive and negative control strains respectively.

Identification of *B. cereus*: All colonies that were rough in texture, turquiose to peacock blue in color, surrounded by greyish zones of egg yolk precipitate and mannitol negative were picked from plates with the highest dilutions for identification and confirmation. These isolates belonging to the *'Bacillus cereus* group' were identified using staining procedures described by Holbrook and Anderson (1980) for detecting lipid globules and spore location. Differential biochemical tests were carried out as described by Harmon (1982). Motility, hemolytic activity on trypticase soy sheep blood agar, rhizoid growth on nutrient agar and the presence of toxin crystals were determined.

Blood haemolysis of isolates: The method of Samie *et al.* (2007) was used. Briefly, sheep red blood was incorporated into blood agar base (Oxoid, Basingstone, England) after sterilization and cooling to 50°C. Pure cultures of the isolates were streaked on the prepared blood agar plates and incubated at 37°C for 24 h. Plates were thereafter observed for ß, α and no haemolysis. Also the percentages ß- and α-haemolysis were determined for each bacterial type.

RESULTS AND DISCUSSION

Table 1 Number in Log_{10} and types of colony forming units of bacteria in the gut of crab samples from Lagos lagoon (28°C) and those stored at freezer temperature (0°C).

In Fig. 1 the results are average values of all analysis. The abundance of bacteria measured in log_{10} of cfu/ml of fresh crabs from the lagoon was 7.2, which were higher than cfu/ml for the frozen market samples of 4.83. Interestingly, all the isolates from the lagoon samples were also isolated from the frozen market samples. This means freezing does not actually eliminate the pathogens but only helped to reduce their numbers probably below the infective dose. However, it is not inconceivable that potent toxins, which might have been elaborated before and during freezing, may be detrimental to health of consumers when foods are consumed.

Fig. 1 shows the abundance of each pathogen obtained from the crabs. CRB2 (*Escherichia coli*) and CRB4 (*Bacillus subtilis*) occurred more than all other isolates with values of 5.90 and 3.0 (for lagoon and market samples) and 5.81 and 4.47 (for Lagoon and market samples) respectively. However, the cfu/ml for the market samples for CRB4 (*Bacillus subtilis*) was higher than for CRB2 (*Escherichia coli*). The occurrence of large numbers of *Escherichia coli* and *Bacillus subtilis* in these samples is due to the daily and uncontrolled discharge of untreated human wastes into the lagoon. The pathogenic bacteria isolated from the gut of crab include *Bacillus cereus* (CRB1), *Escherichia coli* (CRB2), *Proteus vulgaris* (CRB3), *Bacillus subtilis* (CRB4), *Salmonella* sp (CRB5), *Streptococcus agalactiae* (CRB6), *Vibrio parahaemolyticus* (CRB7), *Staphylococcus aureus* (CRB8) and *Micrococcus* sp (CRB9).

Table 1: Results of Blood haemolysis by isolates (28°C)

Microbial species	No. of Strains tested	No. haemolytic	Type of haemolysis ß	Type of haemolysis α	Type of haemolysis No	% haemolysis ß	% haemolysis α
Bacillus cereus	45	42	34	9	2	76	2.0
Escherichia coli	59	50	46	4	9	78	6.8
Proteus vulgaris	45	18	15	3	27	33	6.7
Bacillus subtilis	58	48	38	10	10	65.5	17.2
Salmonella sp	43	36	33	3	7	76.7	7.0
Streptococcus agalactiae	40	25	14	11	15	35	27.5
Vibrio parahaemolyticus	43	43	40	3	0	93	7.0
Staphylococcus aureus	40	38	35	3	2	87.5	7.5
Micrococcus sp	45	32	20	12	13	44.4	26.7

Table 2: Results of Blood haemolysis by isolates (refrigerator stored, 0°C)

Microbial species	No of Strains tested	No. haemolytic	Type of haemolysis ß	Type of haemolysis α	Type of haemolysis No	% haemolysis ß	% haemolysis α
Bacillus cereus	42	36	33	3	6	78.6	7.1
Escherichia coli	30	26	20	6	4	66.7	20
Proteus vulgaris	39	21	14	7	18	35.9	18
Bacillus subtilis	45	32	23	9	13	51.1	20
Salmonella sp	35	32	30	2	3	85.7	5.7
Streptococcus agalactiae	34	27	13	14	7	38.2	41.2
Vibrio parahaemolyticus	36	33	30	3	3	83.3	8.3
Staphylococcus aureus	30	28	25	3	2	83.3	10
Micrococcus sp	33	22	16	6	11	48.5	18.2

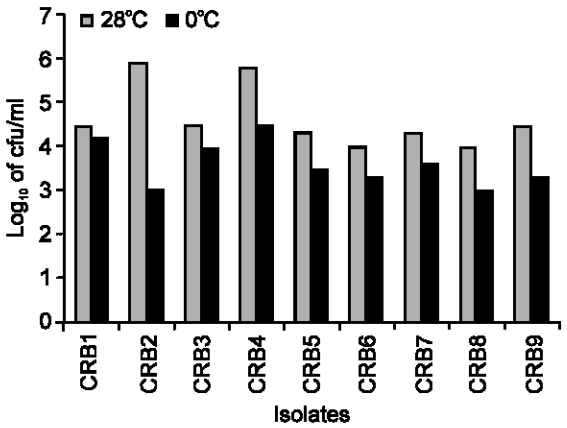

Fig. 1: Composite Bar Chart of Log_{10} of cfu/ml of bacterial pathogens isolated from the gut of the swimming crab (*Callinectes* sp) obtained from Lagos lagoon (28°C) and frozen market samples at 0°C

In blood haemolysis test, *Bacillus cereus* 34 (76%), *Escherichia coli* 46 (78%), *Salmonella sp* 33 (76.7%), *Vibrio parahaemolyticus* 40 (93%), *Staphylococcus aureus* and *Bacillus subtilis* were more haemolytic than *Micrococcus sp, Streptococcus agalactiae* and *Proteus vulgaris* were least haemolytic with values of 20 (44.4%), 14 (35%) and 15 (33%) respectively for samples obtained from the open lagoon at 28°C (Table 1). Similar degree of haemolysis was recorded for isolates obtained from market samples stored at 0°C (Table 2) with percentage haemolytic values ranging from *Vibrio parahaemolyticus* 30 (83.3%), *Staphylococcus aureus* 25 (83.3%), Salmonella sp 30 (85.7%), *Bacillus cereus* 33 (78.6%) and *Escherichia coli* 20 (66.7%). *Bacillus subtilis, Micrococcus sp, Streptococcus agalactiae* and *Proteus vulgaris* had lowest percentage haemolytic values of 23 (51.1%), 16 (48.5%), 14 (38.2%) and 14 (35.9%) respectively. Few numbers of each bacterial isolates were ß-haemolytic except for *Streptococcus agalactiae* (market samples) where the percentage ß-haemolytic strains were more than α-haemolytic strains. It must be stressed that the exponential value of isolates from open lagoon was higher than those from the market samples at 0°C. So the percentage values of crabs stored at 0°C should not be compared to those of lagoon samples in the real sense of it as these values are actually lower than those of stored samples.

The bacterial species isolated from the gut of the swimming crabs are usually found as pathogens associated with the guts of warm-blooded animals. Frazier and Westhoff (1988); Owhe-Ureghe *et al.* (1993) in their studies have implicated *Bacillus cereus* and *Bacilllus subtilis* in infections and food poisoning. The pathogenesis of *Escherichia coli* and its occurrence as faecal contaminant has been reported (Frazier and Westhoff, 1988). Other workers have reported virulence and pathogenic *E. coli* strains in different animals. In humans (Reid *et al.*, 2000; Kaper, 2004); in avian (Audouin gulls) (Carmada *et al.*, 2007); in poultry (JanBen *et al.*, 2001). Hussein (2006) reported the prevalence and pathogenicity of shiga toxin-producing *E. coli* in beef and their products. Schmid-Hempel and

Frank (2007) observed in their study that in *E. coli* as small as 10 cells are infectious enough to establish disease condition, whereas the dosage can be very high in other pathogens like *Vibrio cholerae* (10^3-10^8 cells). The presence of *E. coli* is an indication of the pollution status of Lagos lagoon water where the crabs were harvested. Frazier and Westhoff (1988) and Owhe-Ureghe *et al.* (1993) have repeatedly mentioned *Salmonella* spp as enteric pathogens and a source of food borne infections. *Streptococcus agalactiae* belong to the pyrogenic (pus-producing group that causes mastitis in cow (Frazier and Westhoff, 1988). Owhe-Ureghe *et al.* (1993) asserted that *Staphylococcus aureus* is pathogenic and produce enterotoxins that enhance their pathogenic effect. The isolation of *Vibrio parahaemolyticus* from the gut of crab is in line with the finding of Frazier and Westhoff (1988) that isolated this organism from the alimentary canal of mammals and classified them as potential pathogens. Also Silker (1986) in his study implicated this pathogen as a potential pathogen to consumer of raw shrimps. In addition, Vongxay *et al.* (2006, 2008) reported the prevalence, pathogenesis and occurrence of pandemic *Vibrio parahaemolyticus* in clinical samples and seafood in China, thereby re-emphasizing the public health significance of this pathogen. Pan *et al.* (2007) in their analysis of foods in China isolated haemolysin-related genotypes of *Vibrio parahaemolyticus* from seafood and clinical samples. Beecher *et al.* (1995) in their study observed that *Bacillus cereus* produces distinct exotoxin-mediated and emetic food poisoning syndrome as well as a variety of non-gastrointestinal infections. Also Rusul and Yaacob (1994) working on selected foods isolated *Bacillus cereus* and toxins associated with its growth on foods. Though *Micrococcus* spp occur as commensals, they have also been implicated in opportunistic infections especially in immunocompromised systems such as HIV patients. They have also been implicated in recurrent bacteremia, septic shock, septic arthritis, endocarditis, meningitis and cavitating pneumonia (Microwiki, 2008). Due to their metabolic versatility, they are capable of utilizing wide range of unusual substrates such as pyridine, herbicides, chlorinated biphenyls and oil. They are also involved in the detoxification or biodegradation of many environmental pollutants. Hence their occurrence in the lagoon and hence the gut of the swimming crab is understandable. (Microwiki, 2008). Farley *et al.* (1993) and Farley (2001) revealed that *Streptococcus agalactiae* can cause Group B streptococcus infection. It causes bacterial septicaemia of the newborn which can lead to death or long-term sequelae such as hearing loss. It can cause neonate meningitis, invasive group B streptococcal disease of adult in pregnant, elderly or immunosuppressed. Barbaras *et al.* (2005) and Farley (2001) stressed the danger of group B agalactiae infection in newborns and proffer how it can be prevented. Ip *et al.* (2006) isolated *S. agalactiae* serotype III from nonpregnant adults and describe their invasiveness. *Proteus vulgaris* inhabit the intestinal as well as urinary tract of humans and animals and cause urinary and wound infections. The production of urease by this organism causes UTI obstruction as the urease precipitate organic and inorganic compounds which lead to struvite stone formation.

The colony forming units observed for crabs stored at low temperature (0°C) further strengthen the assertion that low temperature arrests microbial load of foods. It is advised that crabs when harvested from the lagoons or other sources be frozen at temperature of between 0°C and subzero since toxin production by the pathogens especially *Staphylococcus aureus* will be stalled (Bergdoll, 1979, 1990). It should be emphasized that the habit of dumping untreated human excreta into the lagoon as commonly practiced should be discouraged. The Federal Government of Nigeria should pass laws or amend existing ones, prohibiting the discharge of untreated human sewage into the lagoon and, in the event of circumvention, impose stiff penalties to defaulters. The presence of potentially pathogenic bacteria in seawater associated with seafood in this case crabs, can have serious ecological, public health and epidemiological implications. Consequently, consumption of raw and partially cooked *Callinectes* sp. (swimming crab) pose serious danger to consumers of this protein and calcium-rich sea food.

Conclusion: In conclusion, our findings show that the swimming crab, *Callinectes* harbour quite a large variety of life-threatening pathogen bacteria. We are not aware if there had been any report on this subject anywhere regarding the presence of the identified pathogens in the gut of the swimming crab. Reports available so far are those on other seafood especially shrimps and lobsters. With the consortium of pathogens isolated from this crab (which serves as source of protein and mineral ions) it is advised that appropriate campaign is organized by the Nigerian Government to educate the populace on the need to cook crabs properly before consumption. The Ministry of Health should be more functional in this regard.

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REFERENCES

Alsina, M. and A.R. Blanch, 1994a. A set of keys for Biochemical identification of environmental *Vibrio* species. J. Appl. Bacteriol., 76: 79-85.

Alsina, M. and A.R. Blanch, 1994b. Improvement, update of a set of keys for biochemical identification of *Vibrio* species. J. Bacteriol., 77: 719-721.

Baine, W.B., A. Zampien, M. Mazzotti, G. Aniogioni, D. Cureco, M. Digioia, E. Izzo, E.J. Gangorosa and F. Pcchicni, 1974. Epidemiology of cholera in Italy in 1973. Lancet, 2l: 1370.

Barbaras, S., M.D. Apgar, M.D. Grant Greenberg and M.D. Gary Yen, 2005. American Fairly Physician, 71: 905-910.

Beecher, D.J., J.L. Schoeni and A.C. Wong, 1995. Enterotoxic activity of hemolysin BL from *Bacillus cereus*. J. Infect. Immun., 63: 4423-4428.

Bergdoll, M.S., 1990. Staphylococcal food poisoning: In: Cliver, D.O. Ed. Foodborne diseases. San Diego, Calfornia: Academic press, Inc., 85-106.

Bergdoll, M.S., 1979. Staphylococcal Intoxication. In: Rieman, A., Bryan, F.L., Eds. Foodborne infections and intoxications, 2nd Edn. Orlando, Florida, Academic press, Inc., 444-490.

Carmada, A., E. Circella, D. Giovanardi, D. Pennelli, P. Battist, E. Campagnari, G. Bruni and S. Tagliabue, 2007. Avian pathogenic *E. coli* in Audouin gulls (*Larus audouinii*) could they affect the surviving of the bird colonies. Ital. J. Anim. Sci., 6: 317-320.

Farley, M.M., R.C. Harvey and T. Stull, D.J. Smith, A. Schuchat, J.D. Wenger and D.S. Stephen, 1993. A population-based assessment of invasive disease due to group B *Streptococcus* in non-pregnant adult. N. Engl. Med., 328: 1807.

Farley, M.M., 2001. Group B Streptococcal disease in nonpregnant adults. Clin. Infect. Dis., 33: 556.

Frazier, W.C. and D.C. Westhoff, 1988. Food Microbiology. Tata-McGraw-Hill Publishers Co., New Delhi, pp: 410.

Granum, P.E., S. Brynestad and J.M. Kramer, 1993. Analysis of enterotoxin production by *Bacillus cereus* from dairy products, food poisoning incidence and non-gastrointestinal infections. Inter. J. Fd. Microbiol, 17.

Harmon, S.M., 1982. New method for differentiating members of the *Bacillus cereus* group: collaborative study. J. Assoc. Anal. Chem., 65: 1133-1139.

Holbrook, R. and J.M. Anderson, 1980. An improved selective and diagnostic medium for the isolation and enumeration of *Bacillus cereus* in foods. Can. J. Microbial., 26: 753-759.

Hussein, H.S., 2006. Prevalence and pathogenicity of shiga toxin-producing *E. coli* in beef cattle and their products. J. Anim. Sci., http://jas.tass. org october2006.

Ip, M., E.S.C. Cheuk, H.Y. Tsui, F. Kong, T.N. Leung and G.L. Gilbert, 2006. Identification of a *Streptococcus agalactiae* Serotype III Subtype 4 Clone in Association with Adult Invasive Disease in Hong Kong. J. Clin. Microbiol., 44: 4252 - 4254

JanBen, T., C. Schwarz, P. Preikschat, M. Voss, H.C. Phillis and L.H. Wieler, 2001. Virulence associated with genes in avian pathogenic *E. coli* (APEC) isolated from internal organs of poultry having died from colibacillosis. Int. J. Med. Microbiol, 291: 371-378.

Johnson, K.M., 1984. *Bacillus cereus* foodborne illness-an update. J. Fd. Prot., 47: 145-153.

Kaper, J.B., 2004. Pathogenic Escherichia coli. Nat. Rev. Microbiol., 2: 123-140.

Maugeri, T.L., M. Carbone, M.T. Ferra, G.P. Irrera and C. Gugliandolo, 2004. Distribution of potentially pathogenic bacteria as free living and plankton associated in a marine coastal zone. J. Appl. Microbiol., 97: 354-361.

Microwiki, 2008. Issues on *Micrococcus* infections. The Free Encyclopedia, http://en.wikipedia.org/wiki/micrococcus. 10/June/2008.

Olutiola, P.O., O. Famurewa and H.G. Sontang, 1991. An Introduction to General Microbiology-A practical Approach. Ca. Heidelberg verlagsanstaltund Dreuckerei GMbh., Heidelberg, Germany. pp: 520.

Owhe-Ureghe, U.B., O. Afe, D.E. Ekundayo, P. Agbolahor, A. Oboh and P. Orhue, 1993. Bacteriological examination of some "ready to eat" foods marketed in Ekpoma, Edo State Nigeria. Nig. Fd. J., 11: 45-52.

Oyewole, O.B., 1990. A microbiological studies on cassava fermentation for "Lafun" and Fufu" Production. PhD. Thesis, Department of Botany and Microbiology, University of Ibadan, Nigeria.

Piexotto, S.S., G. Pianne, M.O. Hanna and C. Venderzant, 1979. Presence and Growth of *Yersinia enterocolitica* in oyster, shrimps and Crabs. J. Fd. Prot., 42: 947-951.

Pan, E.J., K. Vongxay, B. Shen, G.M. Lou and W.H. Fang, 2007. Analysis of major phenotypes and haemolysin-related genotypes of *V. parahaemolyticus isolates* from clinical and seafood samples. Acta Microbiol. Sinica, 47: 508-51.

Reid, S.D., C.J. Herbelin, A.C. Bumbaugh, R.K. Selander and T.S. Whittam, 2000. Parallel evolution of virulence in Pathogenic *E. coli*. Nature, 406: 64-67.

Rusul, G. and N.H. Yaacob, 1994. Prevalence of *Bacillus cereus* in selected foods and detection of enterotoxin using TECRA-VIA and BCET-RPLA. Inter. J. Fd. Microbiol., 25 (1995): 131-139.

Samie, A., C.L. Obi, L.J. Barrett, S.M. Powell and R.L. Guerrant, 2007. Prevalence, Haemolytic, Haemagglutination activities and Antimicrobial susceptibility profiles of *Campylobacter* spp. Isolated from human diarrhoeal stools in Vhembe District, South Africa. Health Popul. Nutr., 25: 406-413.

Silker, J.H., 1986. New Bacteria in the news. Fd. Technol., 40: 23-25.

Schmid-Hempel, P. and S.A. Frank, 2007. Pathogenesis, virulence and infective dose of pathogens. PloS Pathog., 3: e147.

Taylor, J.A. and G.I. Barry, 1981. Isolation and Characterisation of non-pathogenic *Vibrio furnissii* NCTC 11218. J. Clin. Path., 34: 208-212.

Vongxay, K., S.Y. Cheng, X.Y. Zhou, B. Shen, X.L. He, G.Z. Zhang and W.H. Fang, 2006. Prevalence of *Vibrio parahaemolyticus* in seafood and their processing environments as detected by Duplex PCR. J. Sci. Fd. and Agric., 86: 1871-1877.

Vongxay, K., S.N. Wang, X.F. Zheng, B.B. Wu, H.X. Hu, X.J. Pan, S.Y. Cheng and W.H. Fang, 2008. Pathogenic characterization of *V. parahaemolyticus* isolates from clinical and seafood sources. Int. J. Fd. Microbiol., 2008 accepted.

Vongxay, K., Z.J. Pan, X.F. Zhang, S.Y. Cheng, L.L. Mei, C. Xu, S.N. Wang and W.H. Fang, 2008. Occurrence of pandemic clones of *V. parahaemlyticus* isolates from seafood and clinical samples in a Chinese coastal province. Foodborne pathogens and Dis., 5: 127-134.

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Effect of Slaughtering Age on Chemical Composition of Goat Meat

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Abstract: The research consist of chemical analysis of the goat meat to investigate the relationship between goat meat in different age groups, group A (≤7 m), group B (8-10 m) and group C (≥11 m). Chemical characteristics of goat meat in respect of moisture, protein, fat and ash contents were determined of goat meat processed by butchers under local marketing conditions were investigated during 2008-9. Moisture content of goat meat group A was higher (78.30±0.48%) followed by group B (75.70±0.50%) and group C (73.8±0.061%). Protein, fat, ash content in meat of group A was lowered (15.31±0.68, 1.77±0.24 and 1.20±0.06%, respectively) and increase with advance slaughter age (Group B; 18.43±0.80, 2.71±0.18 and 1.31±0.08% and Group C; 20.30±0.91, 3.07±0.17 and 1.63±0.07. The results conclude the meat of goat slaughtered in advanced age may have an extensive advantage to reduce qualitative and quantitative losses of end products and variation in meat of different age groups animal were found.

Key words: Goat meat, protein, fat, ash content

INTRODUCTION

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

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

Few studies on carcass chemical composition quality of goat meat has appeared in literature (Babiker *et al*., 1990; Mahgoub and Lodge, 1996; Babji *et al*., 2000) and no studies have been reported so far on the same aspects of goat meat particularly in Sindh. Therefore keeping in view the importance of the subject, this study is designed to analyzed the chemical attributes of goat meat available.

MATERIALS AND METHODS

Collection of meat samples: A total of 30 goat meat samples I-e. (ten from each category) were randomly collected from local meat market of Tando Jam. All the samples were grouped according to the age at slaughter as per butcher's information and accredited with A (≤7 m, age), B (8-10 m, age) and C (≥11 m, age) codes. Whereas boneless meat samples. All the samples were brought to Laboratory of department of

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Animal products Technology, Faculty of Animal Husbandry and Veterinary Sciences, Sindh Agriculture University, Tando Jam, for further analysis.

Moisture content: Moisture content was observed according to the method of Association of Official Analytical Chemistry (AOAC, 2000). The fresh minced meat sample (5 g) was transferred in pre-weighed flat bottom aluminum dish, which was transferred to hot air oven at 101±1°C for 3-4 h. Dried sample was then placed in desiccators having silica gel as desiccant. After 1 h, the dish was weighed. Moisture content was calculated by applying the following formula:

$$\text{Moisture}(\%) = \frac{W2 - W3}{W2 - W1} x100$$

W1 = weight of empty dish
W2 = weight of dish + sample
W3 = weight of dish + dried sample

Total protein content: Protein content was determined according to the method as described by AOAC (2000). The sample (2 g) was digested using Micro-Kjeldhal digester in the presence of catalyst (0.35 g copper sulfate and 7 g sodium sulfate/potassium sulfate) where sulfuric acid (20-30 ml) was used as an oxidizing agent and diluted with distilled water (250 ml). The diluted sample (5 ml) was distilled with 40% NaOH using Micro-Kjeldhal distillation unit where steam was distilled over 2% boric acid (5 ml) containing an indicator bromocresol green for 3 min. The ammonia trapped in boric acid was determined by titrating with 0.1N HCl. The nitrogen percentage was calculated using the following formula:

$$N(\%) = \frac{1.4(V_1 - V_2) \text{ x normality of HCl x } 250}{\text{Weight of sample taken x volume of diluted sample}}$$

V_1 = titrated value
V_2 = blank sample value

While protein percentage was determined by conversion of nitrogen percentage to protein by using conversion factor (6.25) assuming that all the nitrogen in meat was presented as protein i.e. protein percentage = N% x CF.

Total fat content: Total Fat Content (TF) was extracted in Soxhlet Extraction Unit as described by AOAC (2000). Soxhlet Extractor was set with reflux condenser and distillation flask which has been previously dried and weighed. Dried meat sample (2 g) was taken in to fat free extraction thimble and placed in extraction apparatus (soxhlet). Then ether (150 ml) was poured in to extraction flask and condenser was joined and placed on electric heater in order to boil the solvent gently. Extraction was carried out for 6 h. The solution was removed. Fat content was calculated by using the following formula:

$$\text{Fat}(\%) = \frac{W1 - W2}{W3} x100$$

W1 = weight of empty distillation flask
W2 = weight of distillation flask + Fat
W3 = weight of sample taken

Ash percentage: Ash percentage was determined by Gravimetric method as described by AOAC (2000) using muffle furnace. The fresh minced meat sample (5 g) was transferred in pre-weighed crucible and transferred to muffle furnace at (550°C) for 4-5 h. Ashed sample was transferred to desiccator having silica gel as desiccant. After 1 h, the dish was weighed. The ash content was calculated by the following formula:

$$\text{Ash}(\%) = \frac{\text{Wt of ashed sample}}{\text{Wt of sample taken}} x100$$

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

RESULTS

Moisture content: Moisture content of goat meat was examined and consequent results are shown in Fig. 1. Average moisture content in meat of group A goats was observed as 78.30±0.48% (range 76.60-80.02%), which is comparatively higher than the mean moisture contents in meat of group B and group C, goats i.e. 75.70±0.50% (range 73.40-77.00%) and 73.80±0.61% (range 72.20-76.60%), respectively. The overall moisture content in goat meat varied in a range 72.20 and 80.02 (mean 75.99±0.50%) There were statistically (one way AOV) highly significant differences ($p<0.001$) observed in meat of different age groups of goat meat (group A, B and C). Further results of LSD (0.05) comparison of means reveals that the moisture content in meat of different age groups are significantly different ($p<0.001$) from each other.

Protein content: Protein content in goat meat was analyzed, and results are depicted in Fig. 2. Protein content varied between 13.12-17.50% in goat meat of group A, 15.31-21.87% in group B meat and 17.50-24.06% in group C goat meat. Result further showed that the protein content in group A meat (average 15.31±0.68%) was lower as compared to group B and group C meat (average 18.43±0.80% and 20.30±0.91%, respectively). The overall average protein content in goat meat ranged between 13.12-24.06% (mean,

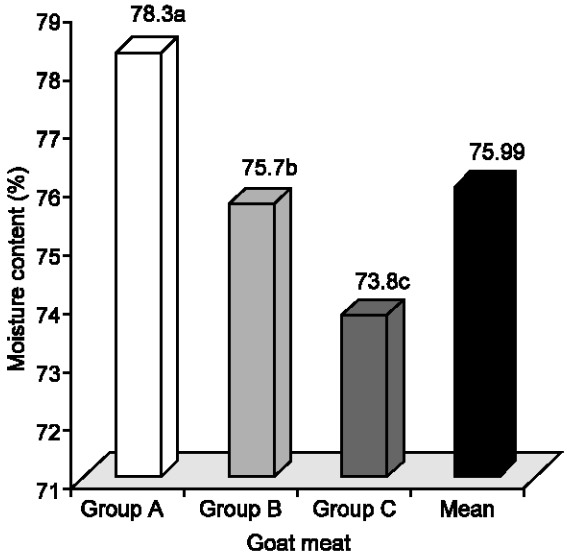

Fig. 1: Moisture content (%age) of goat meat of different age groups

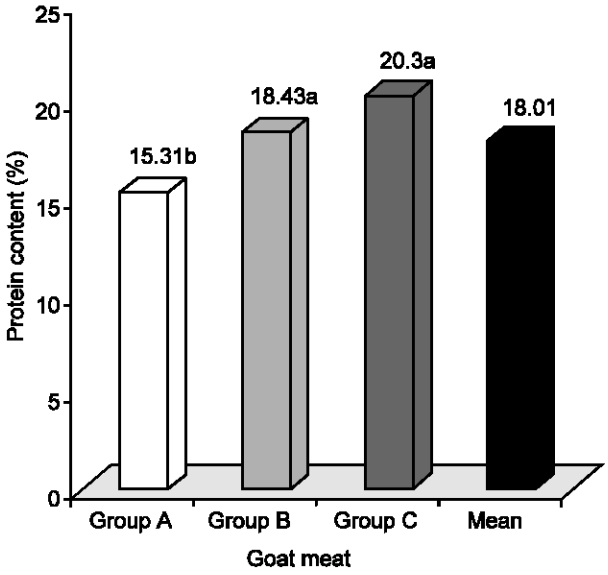

Fig. 2: Protein content (%age) of goat meat of different age groups

18.0±0.64%). It was further observed that protein content was statistically (AOV) different ($p<0.001$) in different age groups of goat meat. However, LSD comparison of means at rejection level of 0.05 revealed that the average protein content in meat of group B and group C goat was not significantly different ($p>0.05$) from each other. While mean of protein content in meat of group A meat was significantly lower ($p<0.05$) from the meat of other groups (B and C) goats.

Fat content: Fat content in goat meat was examined, and results are presented in Fig. 3. A wide variation in fat

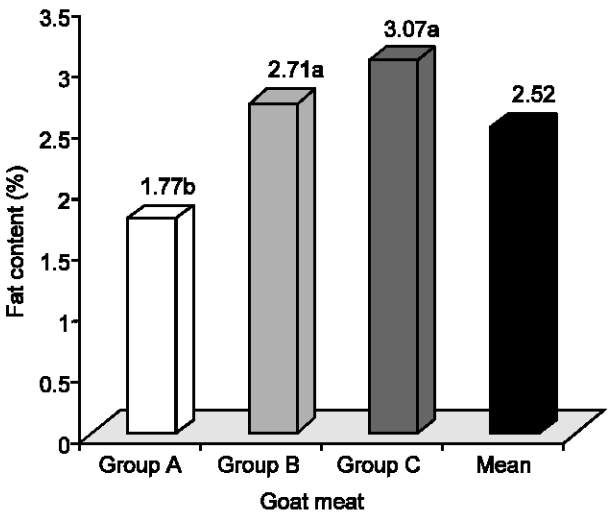

Fig. 3: Fat content (%age) of goat meat of different age groups

content within three groups of goat meat was observed. Fat content in goat meat of group A ranged between 1.0 and 2.5%, in group B between 2.0 and 3.5% and in group C between 2.5 and 3.5%. Furthermore the result showed that the fat content in goat meat of group C was highest (3.07±0.17%) followed by group B (2.71±0.18%) and group A (1.77±0.24%). It was further observed that the fat content was statistically (AOV) different ($p<0.001$) in three groups of goat meat. However, LSD (0.05) comparison of means revealed that the average fat content in goat meat of group A was significantly lower ($p<0.05$) from meat of other groups; while means of group B and group C were not significantly different ($p>0.05$) from each other.

Ash content: The goat meat of different age groups was analyzed for ash content and results are summarized in Fig. 4. Ash content in meat of group A goat averaged 1.20±0.06% (range, 1.0-1.4%), while in group B meat, it was in a range in between 1.0-1.6% (mean 1.31±0.08%) however, ash content in goat meat of group C varied between 1.4 and 1.8% (mean, 1.63±0.07%). One way (ANOVA) revealed significant differences ($p<0.002$) in ash content of different age group of goat meat. However, LSD comparison of means at rejection level of 0.05 revealed that the average ash content in group A and group B meat was not significantly different ($p>0.05$) from each other. While mean of group C meat was significantly ($p<0.05$) higher from other groups (A and B).

DISCUSSION

Moisture content (78.30±0.48) in goat meat with age group of $\leq$7 was remarkably higher than the meat of goat with age group of 8-10 m and with age group of $\geq$ 11 m, i.e. 75.70±0.50 and 73.80±0.61, respectively. It is of

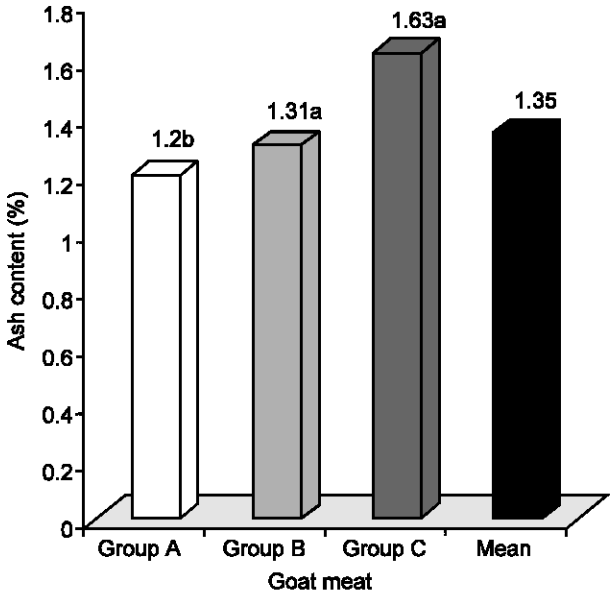

Fig. 4: Ash content (%age) of goat meat of different age groups

interest to point that the decrease in moisture content of goat meat observed in the present study is directly correlates with increase in age of goat. It has been observed that slaughter age had significant effect on decrease in moisture content with advancing slaughter age (Madruga *et al*., 1999 a). While, Stankov *et al*. (2002) reported that the decrease in moisture content in meat has been due to increase in fat content in meat. However in an other study conducted by Beserra *et al*. (2004) the increase in moisture content had been attributed with breeding group and with age.

Meat of goat slaughter at the age of ≥11 revealed remarkably high protein content (20.30±0.91%) compared to meat of goat slaughter at the age of 8-10 m, (18.43±0.80%) and are at the age of ≤7 m, (15.31±0.68%). These results are in agreement with results of Madruga *et al*. (2006) who reported the significant effect of slaughter age on protein content of goat meat. They further reported that meat of goat slaughter at ≥11 m, age was better in chemical composition compared to meat of goat slaughter at the age of ≤7 m and 8-10 m, age. However, there was no significant difference in proximate protein content between Longissimus dorsi and bicep femoris of goat meat. Niedziolka *et al*. (2006) reported the similar trends of protein content of goat meat as observed in present study.

Fat content in young age group ≤7 m of goat meat was considerably low (1.77±0.24%) compared to advance age group 8-10 m (2.71±0.18%) and ≥11 m old age group (3.07±0.17%). There are contradictory results in the literature; fat content is low in younger animal and increased with advancing slaughter age Madruga *et al*. (1999 a). While Akic *et al*. (2001) reported the highest fat content in the meat of old animals as compared to younger animals. This had been confirmed in other study conducted by Stankov *et al*. (2002) they also found the significant differences in slaughtering age (upon reaching sexual maturity). However, Beserra *et al*. (2004) reported the similar results as observe in present study. Ash content (1.20±0.06%) analyzed in group A goat meat was significantly lower followed by in group B goat meat (1.31±0.08%) and in group C goat meat (1.63±0.07%). Considerable research has been conducted in different parts of world on examining the physicochemical changes in goat meat with advancing age. The result of present study is in line with study conducted by Madruga *et al*. (2006) who reported that slaughter age had significant effect on physico-chemical characteristics of meat. Particularly the ash content increased with advancing slaughter age. In another study Pieniak-Lendzion *et al*. (2008) also reported the similar trend of increase in ash content and attributed it with slaughter age.

REFERENCES

AOAC, 2000. Meat and Meat products. In: Official Methods of Analysis. Association of Official Analytical Chemists Inc. Gaithersburg, U.S.A.

Babji, Y., T.R.K. Murthy and A.S.R. Anjaneyulu, 2000. Microbial and sensory quality changes in refrigerated minced goat meat stored under vacuum and in air. Small Rum. Res., 36: 75-84.

Babiker, S.A., I.A. El Khider and S.A. Shafie, 1990. Chemical composition and quality attributes of goat meat and lamb. Meat Sci., 28: 273-277.

Beserra, F.J., M.S. Madruga, A.M. Leite, E.M.C. da Silva and E.L. Maia, 2004. Effect of age at slaughter on chemical composition of meat from Moxoto goats and their crosses. Small Rum. Res., 55: 177-18.

Carlucci, A., A. Girolami, F. Napolitano and E. Monteleone, 1998. Sensory evaluation of young goat meat. Meat Sci., 50: 131-136.

Chowdhury, S.A. and A. Mutalib, 2003. Goat production in Bangladesh present status and future program in goat production in SAARC countries. Azal M. and Usmani R.H. (Eds). Pak. Agric. Res. Council Islamabad, pp: 6-28.

Dahnda, J.S., 2001. Evaluation of crossbred genotype for growth, carcass and meat quality characteristics. Ph.D. thesis university of Queensland, Australia.

Johnson, D.D., J.S. Estridge, D.R. Neubauer and C.H. Mcgowan, 1995. Effect of sex class on nutrient content of meat from young goat. J. Anim. Sci., 73: 296-301.

Lesiak, M.T., D.G. Olson, C.A. Lesiak and D.U. Ahn, 1997. Effects of postmortem time before chilling and chilling temperatures on water-holding capacity and texture of turkey breast muscle. J. Poult. Sci., 76: 552-556.

Madruga, M.S., F.S. Resosemito, N. Narain, W.H. Souza, M.G.G. Cunha and J.L.F. Ramos, 2006. Effect of raising conditions of goats on physico-chemical and chemical quality of its meat. Cienc. Technol. Eleiment, 5: 100-104.

Mahgoub, O. and G.A. Lodge, 1996. Growth and body composition in meat production of Omani Batina goats. Small Rum. Res., 19: 233-246.

Madruga, M.S., S.G.B. Arruda, E.M. Araújo, L.T. Andrade, J.C. Nascimento and R.G. Costa, 1999a. Slaughter age effect on nutritive and sensory value of Mestiço goat meat. Ciencia e Tecnologia de Alimentos, 19: 374-379.

Niedziolka, R., K.P. Lendzion and E. Horoszewicz, 2006. Comparison of the chemical composition and fatty acids of the intramuscular fat of goat kid and ram lambs meat, Elec. J. Polish Agri. Univ., 8: 11-15.

Stankov, I.K., N.A. Todorov, J.E. Mitev and T.M. Miteva, 2002. Study on some qualitative features of meat from young goat of Bulgarian breeds and crossbreeds of goats slaughtered at various ages. Asian-Austr. J. Anim. Sci., 15: 283-289.

Pieniak-Lendzion, K., R. Niedziolka, E. Horoszewicz and T. Brokowska, 2008. Evalution of slaughter value and physicochemical attributes of goat meat. Elect. J. Polish Agric. Univ., 11: 11-116.