
African Journal of Biochemistry Research (AJBR) provides rapid publication (monthly) of articles in all areas of Biochemistry such as Nutritional biochemistry, Analytical biochemistry, Clinical Biochemistry, Human and Plant Genetics, Molecular and Cell Biology, Enzymology, Toxicology, Plant Biochemistry, Biochemistry Education etc. The Journal welcomes the submission of manuscripts that meet the general criteria of significance and scientific excellence. Papers will be published shortly after acceptance. All articles are peer-reviewed.

Submission of Manuscript

Please read the Instructions for Authors before submitting your manuscript. The manuscript files should be given the last name of the first author.

Click here to Submit manuscripts online

If you have any difficulty using the online submission system, kindly submit via this email ajbr@academicjournals.org.

With questions or concerns, please contact the Editorial Office at ajbr@academicjournals.org.
Editorial Board

Dr. Desouky A.M. Abd-El-Haleem
Biological Sciences Department, 
College of Arts and Sciences, 
Qatar University, Doha, 
Qatar

Dr. S.K. Trigun
Biochemistry and Molecular Biology Section, 
Banaras Hindu University 
Varanasi-221005, 
India

Dr. Imed Gallouzi
McGill University, 
Biochemistry Department, 
3655 Promenade Sir William OslerMontreal, 
Quebec, H3G 1Y6, 
Canada

Dr. Ashraf A Khalil
Protein Technology Lab, Mubarak City for Science, 
New Borg Elarab, 
Alexandria, 
Egypt.

Dr. Stanley Mukanganyama
Department of Biochemistry, 
University of Zimbabwe, Box MP 167, 
Mount Pleasant,Harare, 
Zimbabwe

Prof. Salah A. Sheweita
Taibah University, Faculty of Medicine, 
Department of Biochemistry, PO Box 30001, 
Madinah, 
Saudi Arabia

Dr Oluwafemi O Oguntibeju
Department of Clinical Biochemistry, 
School of Medicine, 
Spartan Health Sciences University, 
P.O. Box 324, Vieux Fort, St Lucia, 
West Indies

Dr. Robert L. Brown
USDA ARS, 
Southern Regional Research Center 
1100 Robert E. Lee Blvd., 
New Orleans, LA 70124

Dr. Edward Etoshola
Biomedical Engineering Center 
Davis Heart and Lung Research Institute 
Ohio State University 
473 W. 12th Avenue 
Columbus, OH 43210

G. Suresh Kumar
Senior Scientist and Head 
Biophysical Chemistry Laboratory 
Indian Institute of Chemical Biology 
Council of Scientific and Industrial Research 
Jadavpur, 
Kolkata 700 032, 
India

Xu Lu
Department of Biochemistry and Molecular Biology 
Colorado State University 
Fort Collins, 
CO 80523-1870 
USA

Mohammed A.A Sarhan
Dept. Biological Sciences 
Faculty of Science 
King Khalid University 
Saudi Arabia

Mehrdad Behmanesh
Department Of Genetics 
School Of Science 
P.O.Box 114-175 Tehran Iran 
Iran

Hans Verhagen
Po Box 1 3720 Ba Bilthoven 
The Netherlands 
Netherlands

P.K. Sumodan
Post Graduate Department Of Zoology 
Government College Madappally India 
India

Baleseng Moseki
University Of Botswana 
Botswana
<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. Suraini Abd-Aziz</td>
<td>Universiti Putra Malaysia</td>
</tr>
<tr>
<td>Dr. Mustafa Numan Bucak</td>
<td>Lalahan Livestock Central Research Institute, Lalahan, Ankara, Turkey</td>
</tr>
<tr>
<td>Alparslan Kadir Devrim</td>
<td>Department Of Biochemistry, Faculty of Veterinary Medicine, Kafkas University, Kars, Turkey</td>
</tr>
<tr>
<td>Vasudev R. Thakkar</td>
<td>Sardar Patel University, School of Biosciences, Sardar Patel University, Nagar</td>
</tr>
<tr>
<td>Prof. Emmanuel Anosike</td>
<td>Department Of Biochemistry, University Of Port Harcourt, Nigeria</td>
</tr>
<tr>
<td>Dr. Usama Beshay</td>
<td>New Bourg El-Arab City, Research Area, Alexandria 21934, Egypt</td>
</tr>
<tr>
<td>Dr. Ramar Perumal Samy</td>
<td>Department of Anatomy, Yong Loo Lin School of Medicine, National University of Singapore, Singapore</td>
</tr>
<tr>
<td>Dr. Shin-ichi ONO</td>
<td>Laboratory of Clinical Pharmacy, College of Pharmacy, Nihon University, Japan</td>
</tr>
<tr>
<td>Prof. Lawal Bilbis</td>
<td>Biochemistry Department, Usman Danfodiyo University Sokoto, Nigeria</td>
</tr>
<tr>
<td>Dr. Adriana G. Chicco</td>
<td>Department of Biochemistry, University of Litoral, Santa Fe, Argentina</td>
</tr>
<tr>
<td>Prof. Zia-Ur-Rahman</td>
<td>Department Of Physiology and Pharmacology, University Of Agriculture, Falsalbad, Pakistan</td>
</tr>
<tr>
<td>Dr. Oluwole Ariyo</td>
<td>Allen University, USA</td>
</tr>
<tr>
<td>Prof. Francisco Torrens</td>
<td>Institut Universitari de Ciência Molecular, Universitat de València, Spain</td>
</tr>
<tr>
<td>Prof. Belkhodja Moulay</td>
<td>University of Senia Oran, Algeria</td>
</tr>
<tr>
<td>Dr. Hossam M Ashour</td>
<td>Department of Microbiology and Immunology, Faculty of Pharmacy, Cairo University, Egypt</td>
</tr>
<tr>
<td>Dr. Fidelis Ocloo</td>
<td>Biotechnology and Nuclear Agriculture Research Institute/GAEC, Ghana</td>
</tr>
<tr>
<td>Ass. Prof. Alfonso Baldi</td>
<td>Dept. Biochemistry, Sect. Pathology, Second University of Naples, Italy</td>
</tr>
<tr>
<td>Dr. Anandh Babu Pon Velayutham</td>
<td>Department of Human Nutrition, Foods and Exercise 253 Wallace Hall Virginia Tech, Blacksburg VA 24061, USA</td>
</tr>
<tr>
<td>Dr. Tapan K. Chaudhuri</td>
<td>Department of Biochemical Engineering and Biotechnology, Indian Institute of Technology Delhi, Hauz Khas, New Delhi-110016, India.</td>
</tr>
<tr>
<td>Dr. Rong Zhang</td>
<td>Shenyang Pharmaceutical University, China</td>
</tr>
</tbody>
</table>
Ass. Prof. Tzong-Jih Cheng  
Department of Bio-Industrial Mechatronics  
National Taiwan University  
Taiwan

Dr. Zuyong Xia  
Department of Radiology,  
1201 Welch Rd, Room P089, Stanford, CA 94301  
USA

Dr. Pratap Kumar Das  
Indian Institute of Chemical Biology  
India

Dr. Vasudeo Pandharinath Zambare  
Advanced Enzyme Technologies Ltd  
India

Dr. A M Mujumdar  
Agharkar Research Institute  
India

Prof. Christine Clayton  
ZMBH  
Im Neuenheimer Feld 282  
69120 Heidelberg  
Germany

Prof. Rekik Boulbaba  
ESA Mateur  
Département des sciences et techniques de productions animales  
Tanzania

Dr. Farhad Mirzaei  
National Dairy Research Institute, NDRI  
Karnal  
India

Dr. ROUBHAI Rachid  
Biology Department  
Tebessa University.  
Algeria

Prof. Vaclav Vetvicka  
University of Louisville  
USA

Dr. Ramesh Putheti, Ph.D  
Research scientist  
Actavis Pharmaceuticals  
10065 red run blvd, owings mills Blvd, Maryland, USA 21030  
USA

Prof. Dr. Mustafa NAZIROGLU  
Head of Department of Biophysics  
Medical (TIP) Faculty, Suleyman Demirel University  
Cunur, TR-32260 Isparta  
TURKEY

Dr. José Luis Arias Mediano  
Grupo Investigación Farmacia Práctica (CTS-205)  
Dept. Farmacia y Tecnología Farmacéutica  
Facultad de Farmacia  
Campus Universitario de Cartuja, s/n Universidad de Granada  
18071 Granada.

Ahmed Malki, PhD  
Lecturer of Biochemistry and Molecular Biology  
Biochemistry Department  
Faculty Of Science  
Alexandria University  
Alexandria,  
Egypt

Dr. Alireza Seidavi (PhD)  
Assistant Professor of Animal and Poultry Nutrition,  
Department of Animal Science,  
Islamic Azad University, Rasht Branch,  
Rasht, Iran

Amani S. Awaad  
Professor of pharmacognosy, Chemistry Department  
Faculty of Sciences, King Saud University  
Riyadh, KSA, P.O. Box 22452, Riyadh 11495  
Saudi Arabia

Dr. Abdel-Tawab Mossa  
Environmental Toxicology Research Unit (ETRU),  
Pesticide Chemistry Department,  
National Research Centre,  
Dokki,  
Egypt
Dr. Amal A. Mohamed  
*Plant Biochemistry Department, Agriculture Division - National Research Center, 31-El-Tahrir St., Dokki, Cairo – Egypt*

Dr. Anabella Gaspar  
*Department of Biochemistry, University of Pretoria, South Africa*

Dr. Anna Janecka  
*Department of Biomolecular Chemistry, Medical University of Lodz, Mazowiecka 6/8, 92-215 Lodz, Poland*

Dr. Caser Abdel  
*Horticulture Department, Dohuk University, Iraq*

Dr. David Sheehan  
*Dept Biochemistry, University College Cork, Ireland*

Dr. Dayananda Chandrappa  
*Center for Bioenergy, Department of Life and Physical Sciences, Cooperative Research, Lincoln University, Jefferson City, USA*

Dr. Elsayed Abdelaal  
*Special Graduate Faculty, University of Guelph, Ontario, Canada*

Dr. Etienne Marbaix  
*CELL Unit, de Duve Institute, UCL-75.41, 75 avenue Hippocrate, B-1200 Bruxelles, Belgium*

Dr. Gary L. Firestone  
*Department of Molecular and Cell Biology, University of California, Berkeley, CA, 94720, USA*

Dr. Henryk Zielinski  
*Institute of Animal Reproduction and Food Research, Polish Academy of Sciences, Poland*

Dr. Irshad A. Nawchoo  
*Department of Botany, University of Kashmir, India*

Dr. Luchai Butkhup  
*Department of Biotechnology, Faculty of Technology, Mahasarakham University, Mahasarakham 44000, Thailand*

Dr. Luminita Vladescu  
*Department of Analytical Chemistry, Faculty of Chemistry, University of Bucharest, Romania*

Dr. Mira Debnath  
*School of Biochemical Engineering, Institute of Technology - Banaras Hindu University, Varanasi, India*

Dr. Nilesh S. Panchal  
*Department of Biosciences, Saurashtra University, Rajkot-360005, Gujarat, India*

Dr. Rayappa A. Balikai  
*University of Agricultural Sciences, Dharwad, Karnataka- 580 005, India*
Dr. Saad Tayyab  
*Institute of Biological Sciences, University of Malaya, 50603 Kuala Lumpur, Malaysia*

Dr. Shijun Fu  
*Institute of Health Sciences, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences and Shanghai Jiao Tong University School of Medicine, Shanghai, P. R. China*

Dr. Shiming Zhang  
*Weis Center for Research, Geisinger Clinic, Danville, Pennsylvania, USA*

Dr. Thomas Efferth  
*Department of Pharmaceutical Biology, Institute of Pharmacy and Biochemistry, University of Mainz, Heidelberg, 55128 Mainz, Germany*
Instructions for Author

Electronic submission of manuscripts is strongly encouraged, provided that the text, tables, and figures are included in a single Microsoft Word file (preferably in Arial font).

The cover letter should include the corresponding author’s full address and telephone/fax numbers and should be in an e-mail message sent to the Editor, with the file, whose name should begin with the first author’s surname, as an attachment.

Article Types
Three types of manuscripts may be submitted:

Regular articles: These should describe new and carefully confirmed findings, and experimental procedures should be given in sufficient detail for others to verify the work. The length of a full paper should be the minimum required to describe and interpret the work clearly.

Short Communications: A Short Communication is suitable for recording the results of complete small investigations or giving details of new models or hypotheses, innovative methods, techniques or apparatus. The style of main sections need not conform to that of full-length papers. Short communications are 2 to 4 printed pages (about 6 to 12 manuscript pages) in length.

Reviews: Submissions of reviews and perspectives covering topics of current interest are welcome and encouraged. Reviews should be concise and no longer than 4-6 printed pages (about 12 to 18 manuscript pages). Reviews are also peer-reviewed.

Review Process
All manuscripts are reviewed by an editor and members of the Editorial Board or qualified outside reviewers. Authors cannot nominate reviewers. Only reviewers randomly selected from our database with specialization in the subject area will be contacted to evaluate the manuscripts. The process will be blind review. Decisions will be made as rapidly as possible, and the journal strives to return reviewers’ comments to authors as fast as possible. The editorial board will re-review manuscripts that are accepted pending revision. It is the goal of the AJFS to publish manuscripts within weeks after submission.

Regular articles
All portions of the manuscript must be typed double-spaced and all pages numbered starting from the title page.

The Title should be a brief phrase describing the contents of the paper. The Title Page should include the authors’ full names and affiliations, the name of the corresponding author along with phone, fax and E-mail information. Present addresses of authors should appear as a footnote.

The Abstract should be informative and completely self-explanatory, briefly present the topic, state the scope of the experiments, indicate significant data, and point out major findings and conclusions. The Abstract should be 100 to 200 words in length. Complete sentences, active verbs, and the third person should be used, and the abstract should be written in the past tense. Standard nomenclature should be used and abbreviations should be avoided. No literature should be cited. Following the abstract, about 3 to 10 key words that will provide indexing references should be listed.

A list of non-standard Abbreviations should be added. In general, non-standard abbreviations should be used only when the full term is very long and used often. Each abbreviation should be spelled out and introduced in parentheses the first time it is used in the text. Only recommended SI units should be used. Authors should use the solidus presentation (mg/ml). Standard abbreviations (such as ATP and DNA) need not be defined.

The Introduction should provide a clear statement of the problem, the relevant literature on the subject, and the proposed approach or solution. It should be understandable to colleagues from a broad range of scientific disciplines.

Materials and methods should be complete enough to allow experiments to be reproduced. However, only truly new procedures should be described in detail; previously published procedures should be cited, and important modifications of published procedures should be mentioned briefly. Capitalize trade names and include the manufacturer's name and address. Subheadings should be used. Methods in general use need not be described in detail.
**Results** should be presented with clarity and precision. The results should be written in the past tense when describing findings in the authors’ experiments. Previously published findings should be written in the present tense. Results should be explained, but largely without referring to the literature. Discussion, speculation and detailed interpretation of data should not be included in the Results but should be put into the Discussion section.

The **Discussion** should interpret the findings in view of the results obtained in this and in past studies on this topic. State the conclusions in a few sentences at the end of the paper. The Results and Discussion sections can include subheadings, and when appropriate, both sections can be combined.

The **Acknowledgments** of people, grants, funds, etc should be brief.

**Tables** should be kept to a minimum and be designed to be as simple as possible. Tables are to be typed double-spaced throughout, including headings and footnotes. Each table should be on a separate page, numbered consecutively in Arabic numerals and supplied with a heading and a legend. Tables should be self-explanatory without reference to the text. The details of the methods used in the experiments should preferably be described in the legend instead of in the text. The same data should not be presented in both table and graph form or repeated in the text.

**Figure legends** should be typed in numerical order on a separate sheet. Graphics should be prepared using applications capable of generating high resolution GIF, TIFF, JPEG or Powerpoint before pasting in the Microsoft Word manuscript file. Tables should be prepared in Microsoft Word. Use Arabic numerals to designate figures and upper case letters for their parts (Figure 1). Begin each legend with a title and include sufficient description so that the figure is understandable without reading the text of the manuscript. Information given in legends should not be repeated in the text.

**References:** In the text, a reference identified by means of an author’s name should be followed by the date of the reference in parentheses. When there are more than two authors, only the first author’s name should be mentioned, followed by ‘et al.’. In the event that an author cited has had two or more works published during the same year, the reference, both in the text and in the reference list, should be identified by a lower case letter like ‘a’ and ‘b’ after the date to distinguish the works.

Examples:

Abayomi (2000), Agindotan et al. (2003), (Kelebeni, 1983), (Usman and Smith, 1992), (Chege, 1998; 1987a,b; Tijani, 1993,1995), (Kumasi et al., 2001) 

References should be listed at the end of the paper in alphabetical order. Articles in preparation or articles submitted for publication, unpublished observations, personal communications, etc. should not be included in the reference list but should only be mentioned in the article text (e.g., A. Kingori, University of Nairobi, Kenya, personal communication). Journal names are abbreviated according to Chemical Abstracts. Authors are fully responsible for the accuracy of the references.

Examples:


**Short Communications**

Short Communications are limited to a maximum of two figures and one table. They should present a complete study that is more limited in scope than is found in full-length papers. The items of manuscript preparation listed above apply to Short Communications with the following differences: (1) Abstracts are limited to 100 words; (2) instead of a separate Materials and Methods section, experimental procedures may be incorporated into Figure Legends and Table footnotes; (3) Results and Discussion should be combined into a single section.

Proofs and Reprints: Electronic proofs will be sent (e-mail attachment) to the corresponding author as a PDF file. Page proofs are considered to be the final version of the manuscript. With the exception of typographical or minor clerical errors, no changes will be made in the manuscript at the proof stage.
Fees and Charges: Authors are required to pay a $550 handling fee. Publication of an article in the African Journal of Biochemistry Research is not contingent upon the author's ability to pay the charges. Neither is acceptance to pay the handling fee a guarantee that the paper will be accepted for publication. Authors may still request (in advance) that the editorial office waive some of the handling fee under special circumstances.

All rights Reserved. In accessing this journal, you agree that you will access the contents for your own personal use but not for any commercial use. Any use and or copies of this Journal in whole or in part must include the customary bibliographic citation, including author attribution, date and article title.

Submission of a manuscript implies: that the work described has not been published before (except in the form of an abstract or as part of a published lecture, or thesis) that it is not under consideration for publication elsewhere; that if and when the manuscript is accepted for publication, the authors agree to automatic transfer of the copyright to the publisher.

Disclaimer of Warranties

In no event shall Academic Journals be liable for any special, incidental, indirect, or consequential damages of any kind arising out of or in connection with the use of the articles or other material derived from the AJBR, whether or not advised of the possibility of damage, and on any theory of liability. This publication is provided "as is" without warranty of any kind, either expressed or implied, including, but not limited to, the implied warranties of merchantability, fitness for a particular purpose, or non-infringement. Descriptions of, or references to, products or publications does not imply endorsement of that product or publication. While every effort is made by Academic Journals to see that no inaccurate or misleading data, opinion or statements appear in this publication, they wish to make it clear that the data and opinions appearing in the articles and advertisements herein are the responsibility of the contributor or advertiser concerned. Academic Journals makes no warranty of any kind, either express or implied, regarding the quality, accuracy, availability, or validity of the data or information in this publication or of any other publication to which it may be linked.
ARTICLES

Research Articles:

Production of bacterial amylases and cellulases using sweet potato (*Ipomoea batatas* (L.) Lam.) peels
Olanbiwoninu Afolake Atinuke and Fasiku Samuel

Comparative effects of peel extract from Nigerian grown citrus on body weight, liver weight and serum lipids in rats fed a high-fat diet
Josephine Ozioma Ezekwesili-Ofili and Ngozi Christine Gwacham
Full Length Research Paper

Production of bacterial amylases and cellulases using sweet potato (*Ipomoea batatas.* (L.) Lam.) peels

Olanbiwoninu Afolake Atinuke* and Fasiku Samuel

Department of Biological Sciences, Ajayi Crowther University, Oyo Town, Oyo State, Nigeria.

Received May 7, 2015; Accepted September 15, 2015

Peels of sweet potato (*Ipomoea batatas*) were buried in the soil for 14 days and the isolates associated with the degradation of the peels were obtained using standard microbiological procedures. The bacterial isolates obtained were screened for amylolytic and cellulolytic activities under different pH and temperatures as parameters and optimized for enzyme production. Sixteen (16) bacterial isolates were obtained and characterized and screened for amylase and cellulase production. *Bacillus pumilus* has the highest frequency of occurrence (18.75%) followed by *B. subtilis* (12.50%). After 24 to 48 h of incubation, *B. pumilus* produced highest concentration of amylase at 55°C, pH 6 (5.4 U/mL) while *B. subtilis* had the best cellulase production of 0.75 U/mL at 55°C, pH 7. *B. pumilus* and *Bacillus subtilis* produced the highest amylase and cellulase concentrations and seem to be the potential sources of these enzymes for industrial application.

Key words: Sweet potato peel, amylase, cellulase, bacteria.

INTRODUCTION

Amylases are class of enzymes, which are of important applications in the food, brewing, textile, detergent and pharmaceutical industries. Their most relevant effect is employed during starch liquefaction to reduce their viscosity, production of maltose, oligosaccharide mixtures, high fructose syrup and maltotetraose syrup (Jose and Arnold, 2014). During detergents production, they are applied to improve cleaning effect and are also used for starch de-sizing in textile industry (Aiyer, 2005). α-Amylase is characterized by its random hydrolysis of α-1,4-glucosidic bonds in amylose and amylopectin molecules, while amylopectin α-1,6-bonds are resistant to its cleavage (Parmar and Pandya, 2012). Many micro-organism such as *Bacillus subtilis*, *Bacillus cereus*, *Bacillus polymyxa*, *Bacillus amylo liquefaciens*, *Bacillus coagulans*, *Lactobacillus*, *Escherichia*, *Proteus*, *Bacillus licheniformis*, *Bacillus stheirotherophilu*, *Bacillus megaterium*, *Strepotmyces* sp., *Pseudomonas* sp. etc. were used in α- and β-amylases production. Although, among bacteria, *Bacillus* sp. was widely used for thermostable α-amylase production so as to meet industrial needs (Parmar and Pandya, 2012).

Cellulose is the most abundant biomass on Earth (Tomme et al., 1995). It is the primary product of photosynthesis in terrestrial environments and the most abundant renewable bioresource produced in the
biosphere (Jarvis, 2003; Zhang and Lynd, 2004). Cellulose is commonly degraded by an enzyme called cellulase. This enzyme is produced by several microorganisms, mainly bacteria and fungi (Bahkali, 1996; Magnelli and Forchiassin, 1999; Shin et al., 2000; Immanuel et al., 2006). Cellulases from bacteria are more effective catalysts and less inhibited by the presence of material that has already been hydrolyzed. The greatest potential importance of using bacteria for cellulase production is the ease with which bacteria can be genetically engineered, high growth rate as compared to fungi, often more complex and in multi-enzyme complexes providing increased function and synergy, inhabit a wide variety of environmental and industrial niches (Ariffin et al., 2006; Sadhu and Maiti, 2013). However, the application of bacteria in producing cellulase is not widely used (Sonia et al., 2013). Some bacterial species used in cellulase production are Cellulomonas species, Pseudomonas species, Bacillus species and Micrococcus species (Nakamura and Kappamura, 1982). Cellulases are used: In the textile industry for cotton softening and denim finishing; in laundry detergents for colour care, cleaning; in the food industry for mashing; in the pulp and paper industries for drainage improvement and fibre modification (Cherry and Fidants, 2003).

Amylase and cellulase yields appear to depend upon a complex relationship involving a variety of factors like inoculums size, pH value, temperature, presence of inducers, medium additives, aeration, growth time, and so forth (Immanuel et al., 2006).

This study was therefore designed to isolate high amylase and cellulase producing bacteria from decaying sweet potato peels and to optimise for enzyme production.

MATERIALS AND METHODS

Samples collection

Sweet potatoes (yellow skin) were purchased from Agbowo Market in Ibadan Metropolis, Oyo State, Nigeria.

Sample preparation

The peels of sweet potatoes were carefully scraped off so that the amount of corker removed was kept to a minimum. The scraped peels were buried inside the soil (14 cm deep) in Botanical Garden, University of Ibadan, Oyo State, Nigeria.

Isolation of organism

The buried scrawled peels were exhumed carefully after 14 days and put in a sterile nylon bag and carried to the laboratory. The adhering sand was shaken off and 1 g of the peel was homogenized aseptically using a sterilized mortar and pestle. Serial dilution was carried out and 1 mL of dilution 10⁻⁴ and 10⁻⁶ were mixed with 20 mL of plate count agar, poured on plate and allowed to set. This was incubated for 24 h at 37°C and observed for bacterial growth. Colonies with different morphology (shape, texture and colour) were isolated and purified by sub-culturing several times till pure cultures were obtained. Isolation was carried out in triplicates.

Identification of isolates

Organisms were identified based on their macroscopic, microscopic, physiological and biochemical characteristics of the isolates with reference to Bergey’s Manual of Systematic Bacteriology (Sneath et al., 1986). The biochemical tests carried out are starch hydrolysis, catalase test, Voges Prokauer test, citrate utilization and endospore test.

Growth on carboxymethylcellulose (CMC)

CMC (2%) was prepared with nutrient agar, sterilized and allowed to cool to 45°C. It was poured into Petri dishes. The plates were inoculated with single streak of test organism and incubated at 37°C for 48 h. Presence of clear zones along line of growth indicates that the organism can utilize or break down cellulose and this was used to screen for cellulase production ability of the isolates.

Growth on starch

Starch agar was prepared by adding 1 g of soluble starch to 100 mL of nutrient agar. The mixture was homogenized and sterilized at 121°C for 15 min. This was then dispensed into sterile plates and allowed to set. A single streak of culture was made on the plate and incubated at 37°C for 48 h. After incubation, the plates were flooded with Gram’s iodine. A positive result was indicated by retention of the iodine as a clear zone around the growth region indicating starch hydrolysis while unhydrolyzed starch formed a blue and black colouration with iodine. This was used to screen the bacterial isolates for amylase production.

Extraction of enzymes

The medium used was nutrient broth in which soluble starch and CMC (1%) was added respectively. It was sterilized at 121°C for 15 min, allowed to cool and the test organisms inoculated into it. It was then incubated at 30°C for 48 h after which the culture was centrifuged at 10,000 rpm for 15 min using a refrigerated centrifuge (IEC centra, MP4R model). The cell free culture supernatant was then assayed for amylase and cellulase production and activity. One unit (U) of enzyme activity is expressed as the quantity of enzyme, which is required to release 1 mol of glucose per minute under standard assay conditions (Muhammad et al., 2012).

Amylase assay

Amylase assay was determined using DNSA reagent method of Bernfeld (1955) as modified by Giraud et al. (1991). To 1 mL of culture supernatant was added 1 mL of the substrate containing 1.2% w/v soluble starch in 0.1 N phosphate buffer, pH 6.0. The enzyme substrate reaction was incubated at 45°C for 1 h. The reaction was brought to halt by adding a drop of 5 M NaOH. The amount of reducing sugar produced was determined with 3,5-dinitrosalicylic acid (DNS). 1 mL of DNS reagent was added to filtrate-substrate reaction mixture and was heated in a boiling water bath at 100°C for 10 min. It was cooled with distilled water. The absorbance was measured at 540 nm using spectrophotometer.
Table 1. Frequency of occurrence of bacterial isolate from decaying sweet potato peels.

<table>
<thead>
<tr>
<th>Bacterial isolate</th>
<th>No.</th>
<th>Frequency of occurrence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Bacillus</em> species</td>
<td>7</td>
<td>43.75</td>
</tr>
<tr>
<td><em>Pseudomonas</em> species</td>
<td>3</td>
<td>18.75</td>
</tr>
<tr>
<td><em>Flavobacterium rigense</em></td>
<td>2</td>
<td>12.50</td>
</tr>
<tr>
<td><em>Proteus</em> sp.</td>
<td>1</td>
<td>6.25</td>
</tr>
<tr>
<td><em>Derxia gummosa</em></td>
<td>1</td>
<td>6.25</td>
</tr>
<tr>
<td><em>Azotobacter vinelandii</em></td>
<td>1</td>
<td>6.25</td>
</tr>
<tr>
<td><em>Micrococcus luteus</em></td>
<td>1</td>
<td>6.25</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>16</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Effect of different pH on amylase and cellulase production

Buffer was used to adjust the pH of nutrient broth to 3.0, 4.0, 5.0, 6.0 and 7.0 accordingly. 10 mL of the adjusted nutrient broth was dispensed into screw capped bottles and sterilized at 121°C for 15 min. After cooling, test isolates were inoculated into each bottle and incubated at 37°C for 24 h. Enzymes activities were determined as earlier described.

RESULTS

*Bacillus* species had highest occurrence of bacterial isolate from buried potatoes peels after 14 days (Table 1). *Bacillus* sp. recorded 43.75% of occurrence; followed by *Pseudomonas* with 18.75%. Other bacteria isolated were *Flavobacterium rigense*, *Proteus* sp., *Derxia gummosa*, *Azotobacter vinelandii* and *Micrococcus luteus*. The colonial morphologies of *Bacillus* species isolated were represented on Table 2, they all have raised elevations and cream colour while their texture are either smooth, dull or shiny. Also, they exhibit different colony shapes on the plate, *B. pumilus* is circular, *B. licheniformis* is rhizoid, *B. megaterium* is oval and *B. subtilis* is round.

Table 2. Colonial morphology of *Bacillus* sp. isolated from decaying sweet potato peels.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Shape</th>
<th>Elevation</th>
<th>Surface</th>
<th>Colour</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPA1</td>
<td>Circular</td>
<td>Raised</td>
<td>Smooth</td>
<td>Cream</td>
</tr>
<tr>
<td>SPA2</td>
<td>Rhizoid</td>
<td>Raised</td>
<td>Shiny</td>
<td>Cream</td>
</tr>
<tr>
<td>SPA7</td>
<td>Circular</td>
<td>Raised</td>
<td>Smooth</td>
<td>Cream</td>
</tr>
<tr>
<td>SPB2</td>
<td>Rhizoid</td>
<td>Raised</td>
<td>Dull</td>
<td>Cream</td>
</tr>
<tr>
<td>SPB3</td>
<td>Oval</td>
<td>Raised</td>
<td>Smooth</td>
<td>Cream</td>
</tr>
<tr>
<td>SPB7</td>
<td>Round</td>
<td>Raised</td>
<td>Dull</td>
<td>Cream</td>
</tr>
<tr>
<td>SPB8</td>
<td>Circular</td>
<td>Raised</td>
<td>Smooth</td>
<td>Cream</td>
</tr>
</tbody>
</table>

(Unipec 23 D, Uniscope England). One millilitre of uninoculated blank similarly treated was used to set spectrophotometer at zero. Standard maltose concentrations were prepared within the range of 0.2 - 3.0mg/mL maltose into the requisite medium. The results were then used to construct a standard curve. The spectrophotometer values were then extrapolated as maltose equivalent from the standard curve plotted (Bernfield, 1955).

Effect of different temperatures on amylase and cellulase productions

Nutrient broth was prepared and 10 ml each dispensed into screw capped bottles and sterilized at 121°C for 15 min and allowed to cool. *Bacillus* isolates were inoculated into each bottle and incubated at different temperatures (25, 37, 45, 55 and 65°C) for 24 h. Amylase and cellulase activities were then determined as described earlier.
Table 3. Biochemical characteristics of *Bacillus* sp. isolated from decaying sweet potato peels.

<table>
<thead>
<tr>
<th>Strain code</th>
<th>Grams reaction</th>
<th>Endospore</th>
<th>Catalase</th>
<th>Starch hydrolysis</th>
<th>Voges-Proskauer</th>
<th>Citrate Utilisation</th>
<th>Growth at 55°C</th>
<th>Probable identity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPA1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td><em>B. pumilus</em></td>
</tr>
<tr>
<td>SPA2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td><em>B. licheniformis</em></td>
</tr>
<tr>
<td>SPA7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td><em>B. pumilus</em></td>
</tr>
<tr>
<td>SPB2</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td><em>B. licheniformis</em></td>
</tr>
<tr>
<td>SPB3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td><em>B. megaterium</em></td>
</tr>
<tr>
<td>SPB7</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td><em>B. subtilis</em></td>
</tr>
<tr>
<td>SPB8</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td><em>B. pumilus</em></td>
</tr>
</tbody>
</table>

Table 4. Amylase and cellulase concentration (U/mL) of *Bacillus* sp. at different temperature.

<table>
<thead>
<tr>
<th>Code</th>
<th>Probable identity</th>
<th>Amylase 27°C</th>
<th>Amylase 37°C</th>
<th>Amylase 45°C</th>
<th>Amylase 55°C</th>
<th>Amylase 65°C</th>
<th>Cellulase 27°C</th>
<th>Cellulase 37°C</th>
<th>Cellulase 45°C</th>
<th>Cellulase 55°C</th>
<th>Cellulase 65°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>SPA1</td>
<td><em>B. pumilus</em></td>
<td>2.00</td>
<td>2.40</td>
<td>2.75</td>
<td>2.80</td>
<td>2.70</td>
<td>0.50</td>
<td>0.60</td>
<td>0.60</td>
<td>0.50</td>
<td>0.40</td>
</tr>
<tr>
<td>SPA2</td>
<td><em>B. licheniformis</em></td>
<td>2.00</td>
<td>2.60</td>
<td>2.70</td>
<td>2.70</td>
<td>2.50</td>
<td>0.50</td>
<td>0.60</td>
<td>0.60</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>SPA7</td>
<td><em>B. pumilus</em></td>
<td>2.70</td>
<td>2.80</td>
<td>2.90</td>
<td>2.90</td>
<td>2.80</td>
<td>0.60</td>
<td>0.65</td>
<td>0.65</td>
<td>0.70</td>
<td>0.70</td>
</tr>
<tr>
<td>SPB2</td>
<td><em>B. licheniformis</em></td>
<td>2.00</td>
<td>2.40</td>
<td>2.50</td>
<td>2.60</td>
<td>2.40</td>
<td>0.50</td>
<td>0.60</td>
<td>0.60</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>SPB3</td>
<td><em>B. megaterium</em></td>
<td>2.80</td>
<td>2.90</td>
<td>3.00</td>
<td>3.10</td>
<td>2.90</td>
<td>0.50</td>
<td>0.60</td>
<td>0.60</td>
<td>0.60</td>
<td>0.70</td>
</tr>
<tr>
<td>SPB7</td>
<td><em>B. subtilis</em></td>
<td>2.50</td>
<td>3.70</td>
<td>3.80</td>
<td>3.90</td>
<td>3.80</td>
<td>0.60</td>
<td>0.60</td>
<td>0.70</td>
<td>0.70</td>
<td>0.75</td>
</tr>
<tr>
<td>SPB8</td>
<td><em>B. pumilus</em></td>
<td>2.20</td>
<td>2.30</td>
<td>5.30</td>
<td><strong>5.40</strong></td>
<td>4.90</td>
<td>0.50</td>
<td>0.60</td>
<td>0.65</td>
<td>0.70</td>
<td>0.70</td>
</tr>
</tbody>
</table>

Mean value of triplicate readings. Bold values indicate highest concentration of amylase and cellulase production, respectively at 55°C.

Figure 1. Amylase concentration (U/mL) at different pH.

*pumilus* which is negative to hydrolysis citrate utilization. *B. subtilis* was positive to Voges-Proskauer test, citrate utilization but no growth was recorded at 55°C. *B. licheniformis* is positive to Voges-Proskauer test, citrate utilization and also has the ability to grow at 55°C. *B. megaterium* is negative to both Voges-Proskauer and citrate utilization test.

The effect of different temperatures on amylase and cellulase production of the *Bacillus* species are presented in Table 4, for all the isolates, there was a gradual increase in enzymes activities as the temperature increases with maximum concentration produced at 55°C before a general decline at 65°C. *B. pumilus* SPB8 produced highest concentration of amylase at 55°C (5.4 U/mL) while *B. subtilis* SPB7 produced cellulase best also at 55°C with 0.75 U/mL concentration. Least production of enzymes was noticed at 27°C for all isolates. Figure 1 shows the effect of pH, at pH 6, *B.
**DISCUSSION**

The most predominant bacterial isolates obtained from decaying sweet potatoes peels were identified as *B. pumilus*, *B. licheniformis*, *B. subtilis* and *B. megaterium*. The prevalence of *B. pumilus* and *B. subtilis* isolated in this work conforms to the findings of Lorena et al. (2001) and Madigan et al. (2005) which states that these two organisms are natural inhabitant of soil.

In this study, *B. pumilus* produced the highest concentration of amylase (5.4 U/mL) at 55°C and pH 6 which was also reported by Andrea et al. (2007) which states that *B. pumilus* produced amylase between the pH of 5.8 and 7.5 and at a temperature of 55°C. Effect of temperature on amylase production was observed by varying growth temperature of isolates and optimum temperature was found to be 55°C. This findings agrees with the behaviour of amylases from *Bacillus* spp. isolated from soils as reported by Cordeiro et al. (2003) and Vipal et al. (2011) who reported 50°C as optimum temperature. The effect of temperature on cellulase production was also observed when temperature of the production medium was varied. Cellulase production was highest in the temperature range of 45 - 55°C, with an optimum temperature of 55°C. Similarly, Shaikh et al. (2013) observed that *Bacillus* sp. produced cellulase optimally at 50°C and affirm that the thermostable property of cellulase has been shown to be of interest for industrial applications. Optimum pH for the production of cellulase by all the organisms used in this study ranged from 5 - 7 with pH 5 been the most predominant. This result was in agreement with the findings of others like Goya and Soni (2011), Azzeddine et al. (2013) and Trinh et al. (2013) who reported pH 5, 6 and 7 respectively as the optimum pH for production of cellulase from *Bacillus* spp.

**Conclusion**

This study inferred that decaying sweet potato peels harbour amylolytic and cellulolytic *Bacillus* species and the enzymes produced by these bacteria can be harnessed for industrial application. Optimum temperature for amylase and cellulase production was 55°C, whereas optimum medium pH for amylase and cellulase was 6 and 5, respectively. *B. subtilis* and *B. pumilus* produced the highest concentration of amylase (5.4 U/mL) and cellulase (0.75 U/mL), respectively.

**Conflict of interest**

No conflict of interest among the authors.

**REFERENCES**


Comparative effects of peel extract from Nigerian grown citrus on body weight, liver weight and serum lipids in rats fed a high-fat diet

Josephine Ozioma Ezekwesili-Ofili* and Ngozi Christine Gwacham

Department of Applied Biochemistry, Nnamdi Azikiwe University, PMB 5025 Awka, Nigeria.

Received 24 July, 2015; Accepted September 10, 2015

The effects of ethanolic extracts of five different citrus peels on mean body and liver weight and serum lipid content were investigated in albino rats. Six groups (n=8 each) were fed with a high fat diet for seven days ad libitum before oral daily administration of the peel extracts of orange (OR), lemon (LE), lime (LI), tangerine (TA), grapefruit (GR) and synergistic combination of equal ratios (w/w), that is, (SY), respectively at a dose of 500 mg / kg body weight for 14 days. The positive control group received only the high fat diet (HFD), while the negative control group received only a standard diet (STD). The body weights of the animals were monitored every two days and the animals were sacrificed after the 7th and 14th days of or following the administration of the extracts. All the parameters increased in the positive control group (HFD) compared to the negative control (STD) group. Body and liver weights decreased in all treated groups, as well as serum cholesterol and triglycerides, which decreased significantly in SY and GR groups, p < 0.05. All extracts contained mainly flavonoids and alkaloids while the grapefruit peel extract contained additional saponins that could contribute to the reduction in both body weight and serum lipid content. Conclusively, peel extract from different types of Nigerian citrus which ordinarily serve as waste may synergistically be used to control and manage obesity and associated pathologies.

Key words: Citrus peel, high fat diet, obesity, serum lipids, liver and body weight.

INTRODUCTION

Excessive body weight or obesity has in the last few decades become an emerging serious health concern throughout all cultures, especially when the diets tend towards western type. Obesity is generally associated with an increased risk of excessive fat – related metabolic and chronic diseases such as type two diabetes mellitus, hypertension and dyslipidemia (Bays et al., 2006). Excessive weight gain is also generally linked to the onset of cardiovascular disease, cancers fibroid, renal disease and psychosocial incapacity, amongst others (Abu-Abid et al., 2002; WHO, 2002; Hossain et al., 2007). There is also evidence that obesity is associated with increased morbidity and mortality (Huang et al., 2009). In order to reduce the prevalence of these excessive weight-related diseases, several measures, which include production of low fat diets, dietary restriction, use of...
leptin, induction of thermogenesis and liposuction have been tried out. Most recent studies on the treatment of overweight have focused on the potential role of plant constituents, including polyphenols found in citrus (Sindler, 2001; Murase et al., 2002; Aoki, 2007).

Citrus species, belonging to the family Rutaceae, are one of the main fruit crops grown throughout the world. Citrus fruits have been used by man for centuries for agricultural, medicinal and herbal purposes (Tomar et al., 2013). Several pharmacological properties have been attributed to various members of the citrus species, ranging from anticancer, (Jacob et al., 2000; Silalali, 2002; Enterazi et al., 2009) antimicrobial, (Nannapaneni et al., 2008; Tao et al., 2009; Dhanavade et al., 2011; Kumar et al., 2011; Lawal et al., 2012), antifungal, (Valezquez-Nunez et al., 2013) antityphoid, (Kumar et al., 2011), antioxidant, (Duda-Chodak and Tarko, 2007), anti-inflammatory, (Galati, 1994; Karaca et al., 2008), antiulcer, (Nagaraju et al., 2012), hypolipidemic (Khan et al., 2010), hepatoprotective (Karaca et al., 2008; Kangalkar et al., 2009) and antiadiabetic, (Daniels, 2006; Parmar and Kar, 2007), among others. The peels of the citrus fruits, especially grapefruit and bitter orange, which are rich in flavonoid glycosides, polyphenols and volatile oils have been used in several cultures for weight control, amongst other pharmacological uses (Fujioaka et al., 2006; Stohs and Shara, 2007). Previous studies have demonstrated the effects of these flavonoids on lipid and glucose metabolism in experimental animals and humans (Jung, 2004; Miwa, 2005), specifically on lipid catabolism, glucose transport, the insulin-receptor function, and peroxisome proliferator-activated receptors (PPARs) activation, all of which play essential roles in weight control (Shisheva, 1992; Liang, 2001; Kim, 2003; Lee, 2003).

Nigeria is richly blessed with an all year round availability of a number of citrus fruits, most of which form a huge economic asset to both rural dwellers who cultivate the fruits and the urban vendors. The most commonly sold citrus, sweet oranges (*C. sinensis*), are often sold in the peeled form, thus leaving huge amounts of peels as waste. As part of an ongoing search for local herbal drugs for weight control, this work investigated the comparative and synergistic effects of five locally grown citrus fruits, namely, sweet orange (*C. sinensis* L.), lemon, (*C. limon* L.), lime (*C. aurantifolia* L.), tangerine (*C. reticulate* L.) and grapefruit (*C. paradisi* L.) on mean liver and body weights and serum lipids in albino rats fed a high fat diet.

**MATERIALS AND METHODS**

**Materials**

**Plants**

Citrus species used were orange (*C. sinensis* L. (OR), lemon (*C. limon* L. (LE), grape (*C. paradise* L. (GR), tangerine (*C. reticulate* L. (TA) and lime (*C. aurantifolia* L. (LI) obtained from Eke Awka local market and private compounds in Awka, Anambra State. The samples were authenticated by Professor Clement Okeke, Department of Botany, Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria..

**Chemicals:** All chemicals used in this work are of analytical grade, and products of BDH, (Poole, England), Merck (Germany) and others. Kits by Randox, (UK) were used for the estimation of total serum cholesterol and triacylglycerol.

**Animals**

Male albino rats of about 6 weeks of age (weighing between 200 – 230 g) were purchased from the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. The animals were left to acclimatize for one week under ambient conditions before the experiments. The animals were handled in accordance with the guidelines of the Ethics Committee on Animal Research of the Faculty of Biosciences, Nnamdi Azikiwe University, Awka, Nigeria.

**Methods**

**Extraction and phytochemical analyses**

Around 500 g of each peel were air dried, ground into coarse powder, extracted exhaustively with 70% ethanol using the soxhlet apparatus and concentrated en vacuo at 40°C. The yields of the extracts were calculated and phytochemical analysis was carried out on the citrus peel extracts according to the method of Harborne (2003).

**Experimental procedure**

Diets used consisted of corn flour, rice husk, crayfish, palm oil, multivitamins and mineral salts in the percentage combinations (w/w) as stipulated in Table 1:

<table>
<thead>
<tr>
<th>Table 1. Percentage composition of experimental diets.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Component</strong></td>
</tr>
<tr>
<td>Carbohydrate</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Palm oil</td>
</tr>
<tr>
<td>Crude fiber</td>
</tr>
<tr>
<td>Mineral salt</td>
</tr>
<tr>
<td>Vitamins</td>
</tr>
</tbody>
</table>

*The mineral mix above consisted of calcium (0.8 g), phosphorous (0.6 g), manganese (50 mg), zinc (30 mg), sodium (0.15 g). The vitamins consisted of vitamin A (8000 iu), vitamin D3 (2400 iu), vitamin E (15 mg), vitamin B2 (4 mg), vitamin C (50 mg).*
the negative control, were initially allowed access to the high fat diet (HFD, Table 1) and water *ad libitum* for seven days. At the end of the 7 days the individual weights of the animals were taken and a daily oral dosage regimen of citrus peel extracts (500 mg/kg body weight of animal) was administered for 14 day as follows:

Group one was fed HFD and orange peel extract (OR)
Group two was fed with HFD and lemon peel extract (LE)
Group three was fed with HFD and lime peel extract (LI)
Group four was fed with HFD and tangerine peel extract (TA)
Group five was fed with HFD and grapefruit peel extract (GR)
Group six was fed with HFD and synergistic (equal) combinations of the extracts (w/w) (SY)
Group seven was fed with HFD only (HFD; positive control)
Group eight was fed with standard diet only (STD, Table 1; negative control).

The weights of the animals were taken at 2 days interval after overnight fasting, the rats were sacrificed after the 7th and the 14th day of administration of citrus peel extract by cardiac puncture and the serum was collected. The total serum cholesterol and triacylglycerol were determined spectrophotometrically using Randox kits supplied by Randox, (UK). The liver weights at necropsy were also determined.

**Statistical analyses**

All statistical analyses were performed using Graph Pad Prism (version 4.0). A level of *p* < 0.05 was considered significant. Data were presented as mean ± SEM. The data were tested by ANOVA, followed by Bonferroni’s pair-wise comparison test.

**RESULTS AND DISCUSSIONS**

There was high significant increase (*p* < 0.05) in the mean body weight, as expected, in the rats fed on a high fat diet (Figure 1). The body weight was, however, significantly reduced (*p* < 0.05) by concurrent administration of a daily oral dose of citrus peel (500 mg/kg body weight) for all test groups in the 1st week. The groups LE, LI and TA regained weight in the 2nd week, however, OR, GR and SY groups, significantly and consistently lost weight (*p* < 0.05). The relative liver weight increased significantly (*p* < 0.05) for the high fat fed group by the 14th day. The citrus peel extract caused a general non-significant decrease in liver weight by the 7th day when compared with the HFD group (*p* > 0.05), but there was no further significant loss except only for the OR group by the 14th day (Figure 2). The serum cholesterol levels decreased significantly in all the groups when compared with HFD group, by the 7th day, but the decrease was more in the GR, SY, and OR groups by the 14th day at *p* < 0.05 (Figure 3). Similarly, serum triglycerides also reduced significantly in all test groups, especially for SY, GR, OR and LE groups by the 14th day (*p* < 0.05). The least changes were observed for TA and LI groups (Figure 4).

There was also noticeable reduction of appetite and the animals were observed to show signs of tremor and exfoliation of fur for SY, LI, OR and GR groups in the 2nd week but not for LE and TA groups.

On a comparative basis, these results showed that grapefruits (GR) peel extract followed by orange (OR) were the most effective single remedies, while TA was the least effective for weight loss and for the reduction of cholesterol and triglycerides. A synergistic combination of all extracts was most beneficial.

Phytochemical analysis of the citrus peel extracts used in this work showed the presence of mainly flavonoids, alkaloids and additionally saponins in grapefruits (GR) (Table 2). The relative quantities and identities of the
individual components were however not determined. Polymethoxylated flavones (PMFs - tangeretin, nobiletin, hesperidin, sinensten and naringin) found in the peels and in smaller in amounts in the juices of a variety of citrus fruits have been isolated from tangerine, orange, grapefruit (Rouseff and Ting, 1979). PMFs showed effects in reducing cholesterol (especially LDL cholesterol, by 30 to 40%, although treatment did not appear to have any effect on levels of HDL cholesterol) and to suppress appetite in previous animal studies, suggesting health benefits in cardiovascular health (Hakim and Harris, 2004; Kurowska and Manthey, 2004).

There was noticeable reduction of appetite for SY, LI, OR and GR groups in the second week, observed by the increasing amount of leftover food per day (actual weight not determined). This may have been due to the presence of polyphenols, as well as pectin in the peel extracts. Pectin, though not soluble in pure alcohol, is extractible in the hydroacholic solution (70%, v/v) used in this work. Pectin reduces appetite by swelling into a gel in the stomach to give a feeling of fullness for at least 4 h (Rayburn et al., 1998). Another mechanism for weight loss may include stimulation of β-3 cell receptors, thus eliciting thermogenesis, leading to increased lipolysis and metabolic rate (Preuss et al., 2002). It has also been determined that PMFs also help reduce cortisol levels.
Cortisol is a stress hormone, higher levels of which have been linked to weight gain. The use of PMFs to reduce systemic and local cortisol concentrations (liver and adipose tissue), has been beneficial in promoting blood sugar control and weight loss (Talbot, 2009). Grapefruit peels have been reported to promote weight loss by reducing insulin spike after a meal, thus the body processes more food for use as energy and less is stored as fat. Grapefruit extract was the most effective single extract in this work.

The mechanisms by which cholesterol and triglycerides were reduced could be due to interaction of extract with bile acids thus preventing reabsorption of the bile acid, and therefore, cholesterol or by inhibition of β HMG CoA reductase and acyl CoA cholesterol acyl transferase (ACAT), thereby preventing de novo synthesis, or by increased lipase activity (Bok, 1999). Hesperidin and naringin, and their aglycones hesperetin and naringenin, have been reported to decrease plasma and hepatic cholesterol and triacylglycerol by inhibiting these hepatic enzymes in experimental animals (Lee, 1999; Lee, 2003; Kim, 2003). A study also demonstrated that hesperidin and naringin were beneficial for improving hyperlipidemia and hyperglycemia in type-2 diabetic animals by partly regulating the fatty acid and cholesterol metabolism and affecting the gene expression of glucose-regulating enzymes; they also markedly enhanced hepatic and adipocyte PPARγ protein expression (Jung et al., 2006).

Furthermore, naringenin increased hepatic fatty acid oxidation through up-regulation of the gene expression of enzymes involved in peroxisomal β-oxidation and white adipose tissues in mice (Huong, 2006; Hamendra and Anand, 2007; Fukuchi et al., 2008).

Other positive effects of citrus extracts reported include antiviral, antiulcer, anticancer, antioxidant, diuretic, anti-allergy, antihypertensive, antimutagenic, relief of stomach upset, distension and asthma (Kim et al., 2000; Murakami et al., 2000; Kanaze et al., 2008).

Other constituents of citrus peels include essential oils which have lipolytic, antimicrobial, antioxidant and anti-inflammatory effects; (Hyang-Sook, 2006; Kanaze et al., 2008; Oliveira et al., 2014) and also vitamin C which contributes to effective digestion and weight loss by increasing acidity thereby increasing calcium assimilation.
and replacement of fat in cells.

No negative side effects were reported in previous work in the animals that were fed with PMFs. However, in this work, the animals showed signs of tremor and exfoliation of fur for SY, OR and GR groups, both in the second week. Synephrine, the major alkaloid of C. aurantium, similar in structure to epinephrine was reported to exhibit milder ephedrine-like effects, which range from CNS stimulation, energy boost, appetite suppression to increased fat metabolism without the cardiovascular side effects of nervousness, dry mouth and high blood pressure (Pellati et al., 2002). A number of adrenergic alkaloidal amines (synephrine, n-methyl tyramine, hordemine, octopamine and tyramine) have been reported in the Mediterranean citrus, C. aurantium, as major ingredients of dietary supplements for weight loss (Pellati et al., 2002). The citrus peels used in this work contained alkaloids, although not classified.

Results obtained in this work and other previous reports that assessed the effects of these citrus flavonoids on lipid and glucose metabolism have led to the conclusion that the extracts from peels of Nigerian grown citrus could prevent the development of obesity through the modulation of lipid and glucose metabolism, with grapefruit peels being the single most effective peels while synergistic effect gave best results. Grapefruit peels have been reported to contain the highest total phenolics and the highest total antioxidant activity, followed by sweet orange peels, while tangerine peels had the least (Li et al., 2006; Londono-Londono et al., 2010).

Conclusively, peels from different types of Nigerian citrus which ordinarily serve as waste may synergistically be used to control and manage weight problems and associated pathologies. However, despite the positive effects of citrus extracts in weight reduction in this work, there may be risk of cardiovascular toxicity, due to the possible presence of adrenergic amines such as synephrine, n-methyl tyramine etc. which may have epinephrine-like action. Dosage control may be required to reduce the adverse effects. It has, however been reported that different extraction processes may result in different products with varying concentrations and ratios of PMFs. The extraction process can therefore be selected and modified as desired to shift the ratios of the component PMFs (Kawai et al., 1999) to enhance the beneficial and reduce the untoward effects, although the soil composition may have an effect on the concentrations of constituents. However, additional studies are needed to validate these conclusions.

Conflict of interests

The authors did not declare any conflict of interest.

REFERENCES


