African Journal of Food Science

Volume 8 Number 4, April, 2014
ISSN 1996-0794

Academic Journals

ABOUT AJFS

The **African Journal of Food Science (AJFS)** (ISSN 1996-0794) is published bi- monthly (one volume per year) by Academic Journals.

African Journal of Food Science (AJFS) provides rapid publication of articles in all areas of Food Science such as Sensory analysis, Molecular gastronomy, Food safety, Food technology 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 published in AJFS are peer-reviewed.

Submission of Manuscript

Click here to Submit manuscripts online. A manuscript number will be mailed to the corresponding author shortly after submission.

Please read the **Instructions for Authors** before submitting your manuscript.

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

Editors

Editorial Board

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 Power point 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:

Chikere CB, Omoni VT and Chikere BO (2008). Distribution of potential nosocomial pathogens in a hospital environment. Afr. J. Biotechnol. 7: 3535-3539.

Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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 Food Science 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

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.

African Journal of Food Science

Table of Contents: Volume 8 Number 4 April, 2014

ARTICLES

academicJournals

Vol. 8(4), pp. 176-183, April 2014
DOI: 10.5897/AJFS2013.1092
Article Number: 557347144263
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Review

Importance of lecithin for encapsulation processes

Adriana Rodrigues Machado[1]*, Leticia Marques de Assis[1], Maria Inês Rodrigues Machado[2] and Leonor Almeida de Souza-Soares[1]

[1]Federal University of Rio Grande (FURG), School of Chemistry and Food, P.O. Box: 474, CEP: 96201-900, Rio Grande/RS - Brazil.
[2]Department of Food Science and Technology, Federal University of Pelotas (UFPEL), Brazil.

Received 03 October, 2013; Accepted 25 March, 2014

The crude soy and rice lecithin are used in studies of liposome formulation. They have been used as flavor encapsulators, flavor masking, antioxidants, and nutrient protective against degradations in the gastro intestinal tract. This study aimed to show the importance of rice and soy lecithin in its varied uses as liposomes in different areas. The paper indicates the importance of soy and rice lecithin, both crude and purified (phosphatidylcholine) ones, as feasible for the encapsulation of various materials, from medical and pharmaceutical to food ones.

Key words: Lecithin, phospholipids, vesicles.

INTRODUCTION

Lecithin is the name given to a mixture of glycolipids, triglycerides, and phospholipids (for example phosphatedylcholine, phosphatidylethanolamine, and phosphatidylinositol). However, in biochemistry, the term lecithin is usually used as a synonym for pure phosphatidylcholine - a phospholipid which is the main component of the phosphate fraction obtained from egg yolk (in Greek *lekithos* - λεκιθος, from which the name of the compound was derives) or from soy and rice beans, from which it is extracted by mechanical or chemical means using hexane. Lecithin is marketed in high purity as a food supplement for medical use (Mertins, 2004).

Lecithin is commercially used as an emulsifier and lubricant in various economic activities such as food or pharmaceutical industry. For example, it is used as an emulsifier in chocolate and in the production of coating for foods. Lecithin is considered as a non-toxic surfactant which is well tolerated by the organism, since it is an integral part of cell membranes and can be completely metabolized. It was classified in the United States by the Food and Drug Administration as generally recognized as safe product for human consumption. Also, it is recognized as a food additive by the European Union under the number EE322 (Zambelli and Moreira, 2009).

Another advantage of soy lecithin mainly arising is that it can be used as a vitamin stabilizer food ingredient to protect the vitamins A and E against oxidation, and as a source of choline, inositol and other growth promoter components (Meyers, 1990).

Thus, soy lecithin and rice, both of which are important by-products of soy oil (SO), and rice bran oil (OFA) respectively, the main difference of rice and soya lecithin is related to the composition and characteristics, for rice lecithin can replace soya lecithin in food and industrial

*Corresponding author. E-mail: adriana.rodriguesmachado@yahoo.com.br

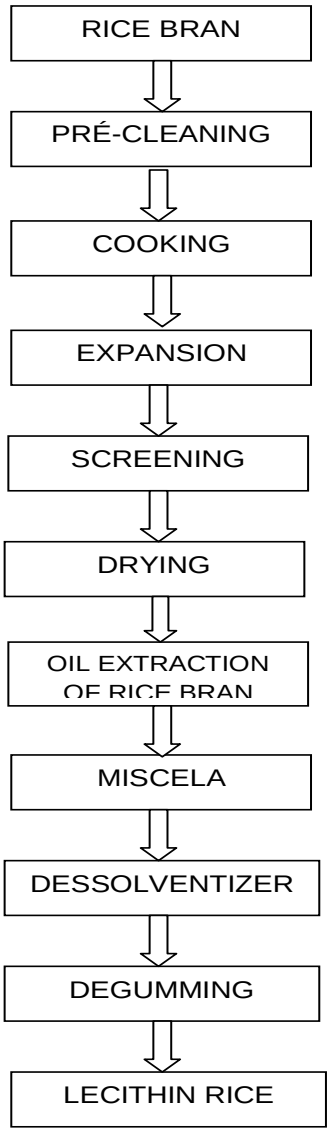

Figure 1. Flowchart for obtaining lecithin rice.

products; compared to rice lecithin is more rich in phospholipids, which soybean lecithin and also marketed at a lower cost (Orlandelli, 2008).

This review study aimed to show the importance of rice and soy lecithin in its diverse applications as liposomes in different areas.

DEVELOPMENT

Obtaining the lecithin

Lecithin is obtained by hydration and separation from the crude oil according to Figures 1 and 2, which show the flowcharts for obtaining the rice and soy lecithin.

Physico-chemical characterization of soy lecithin and rice

Peroxide index, insoluble in acetone (AOCS, 1993) and refractive index using the AOCS Cc 7-25 (AOCS, 1993) method and a: for physico-chemical characterization of lecithin, soy and rice as some specific analyzes are performed Abbé refractometer. Reading takes place on a scale which directly yields the absolute refractive index at 40°C. Assim atom pH which can be determined by potentiometer Digimed - DM-20 at a temperature of 20°C by the method of Institute Adolfo Lutz (2008) acidity index, according to the volumetric method by titration with 0.1 N NaOH, and the results expressed as % citric acid, the second method of Institute Adolfo Lutz (2008).

Purification of rice and soy lecithin

There are several methods of purifying the crude lecithin, but the most common are: Purification of crude lecithin by extraction and column chromatography to obtain the phosphatidylcholine (Mertins et al., 2008; Machado et al., 2013).

Phosphatidylcholine

Phosphatidylcholine (soy lecithin/rice) is a natural phospholipid of molar mass equal to 780 g/mol and = 0.05 g/cm^3 (Reis, 2010; apud Willard et al., 1998). It is a part of the molecular structure of biological membranes and is also present in blood plasma as a constituent of lipoproteins. It is biocompatible, biodegradable, and has cleansing action. The structure of phosphatidylcholine (Figure 3) is formed by two long hydrocarbon chains, one saturated and one unsaturated constituting the non-polar or hydrophobic portion of the molecule. The polar or hydrophilic portion is formed by glycerol, phosphate group, and choline (Mertins, 2004).

Phosphatidylcholine is a yellowish, hygroscopic and little stable solid (Figure 3). It is easily decomposed at high temperatures and degraded by the action of oxygen when exposed to air and moisture for long periods. Its main degradation product is the lysophosphatidylcholine resulting from hydrolysis of the ester function on the carbon at positions 1 or 2 of glycerol, giving a molecule with only one non-polar chain (Reis, 2010). Its presence dramatically increases the permeability of liposome membranes and reduces the capacity of retaining encapsulated material (Lutz et al., 1995).

Phosphatidylcholine is obtained from a by-product in the manufacturing process of soybean oil and rice bran oil. This raw material comprises a mixture of a large number of fatty acids, lipids, proteins, phospholipids, and pigments of different molecular structures with phosphatidylcholine between 10 and 20%. Its laboratory

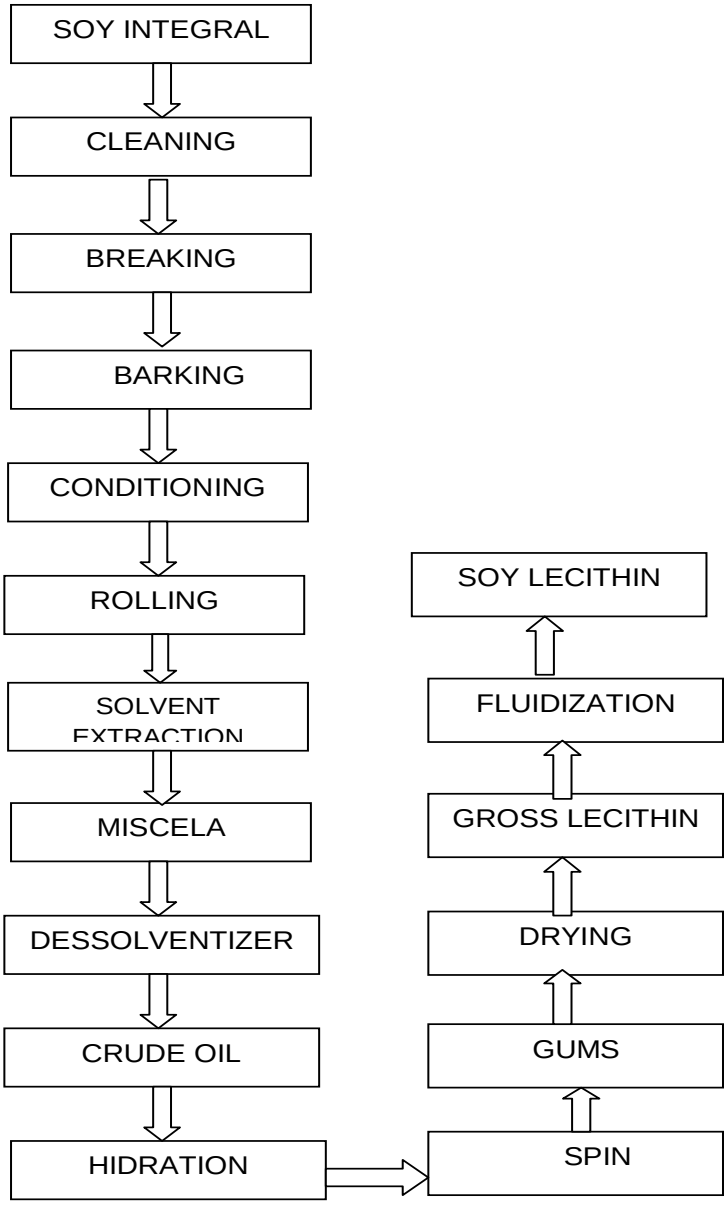

Figure 2. Flowchart for obtaining soy lecithin.

and industrial purification is performed by column chromatography. The most accessible processes use columns of silica or alumina and mixtures of chloroform: methanol as eluent (Mertins, 2004). Phosphatidylcholine today is widely used in the pharmaceutical and cosmetic industries and as an emulsifier in the production of liposomes as well as in food and paint industries as a stabilizer and emulsifier (Maron et al., 2007).

Liposomes

The first description of the colloidal behavior of phospholipids such as lecithin, among others, as well as the formation of lipid vesicles occurred in 1961 by Bangham and colleagues. It was in this study Bangham and Horne (1964) described the formation of these phospholipid vesicles in dilute aqueous solutions and it was found that "Liposomes are artificial vesicles of smaller spherical shape that can be produced from natural phospholipids and non-toxic cholesterol (Cruz et al., 2009).

Thus, some of the nanostructures used for encapsulation are called liposomes (Figure 4), which are microscopic vesicles composed of one or more concentric lipid bilayers separated by aqueous medium. They are widespread in the medical and pharmaceutical industry. In the food industry they are used as flavor encapsulators, taste masking, antioxidants, and nutrient

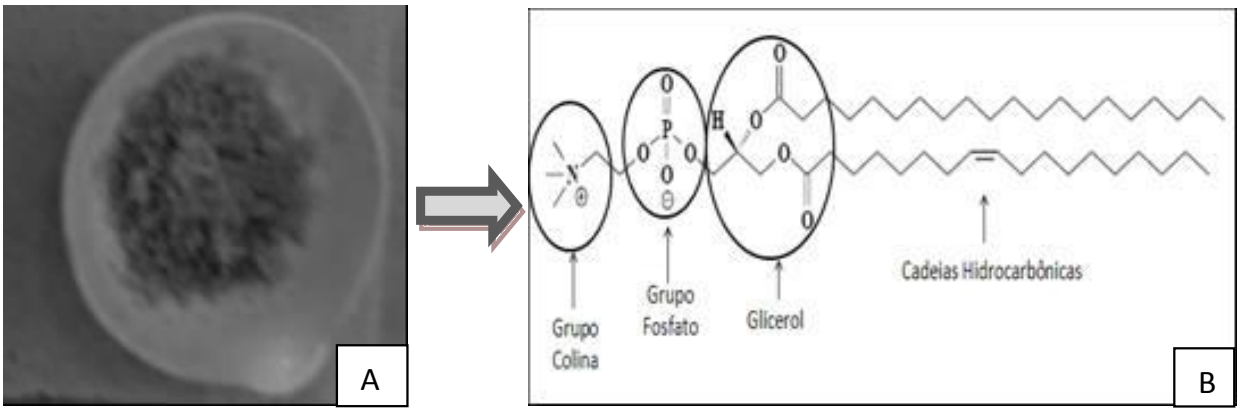

Figure 3. (A) Purified soy lecithin and (B) molecular structure of phosphatidylcholine. Sources: Figure A (Machado et al., 2012) and B (Mertins, 2004).

protective against degradation in the gastro intestinal tract.

The liposomes can incorporate various types of substances regardless of their molecular weight, solubility or electric charge, they can encapsulate hydrophilic and/or lipophilic substances, where the hydrophilic ones are in the aqueous compartment and the lipophilic ones are inserted or adsorbed into the membrane. Since liposomes are biodegradable, biocompatible and non-immunogenic, they are highly versatile for research, therapeutics, and analytical applications (Edwards and Baeumner, 2006; New, 1990; Puisieux et al., 1995). These vesicles consist primarily of phospholipids (either synthetic or natural ones), sterols, and an antioxidant (Vemuri and Rhodes, 1995).

The lipids that most be used in the formulations of liposomes are those with a cylindrical shape as phosphatidylcholines, phosphatidyl serine, phosphatidyl glycerol, and sphingomyelin, which are likely to form a stable bilayer in aqueous solution. Phosphatidylcholines are the most used in studies of liposome formulation, since they have great stability against variations in pH or salt concentration in the medium.

Phospholipids are characterized by a phase transition temperature (Tc), in which the membrane goes from a gel phase, where the lipid hydrocarbon chain is in the ordered state, to a liquid-crystal phase where the molecules are in more free motion and the grouped hydrophilic radicals become completely hydrated. The length and saturation of lipid chain influence the Tc value. Thus, membranes composed of different lipid may show different levels of fluidity at the same temperature (Frézard et al., 2005; Lasic, 1998).

The permeability of liposomes is relatively low when the temperature is lower than the liposome Tc, and this is measured by the flow rate at which the solution exits the aqueous compartment through the bilayer. However, this property depends on the nature of the solute and on membrane fluidity (Frézard, 1999).

Liposomes were developed to improve the biodistribution of compounds at specific locations in the body. Thus, they became recognized as carriers of biologically active compounds, with the ability to enhance and/or modify the activity of the compounds with which they are associated. This effect depends on the chemical composition and the phospholipid structure (Gómez-Henz and Fernandez-Romero, 2005).

Liposomes can be produced in a range of sizes, from a few nanometers to several micrometers (Figure 3). They are distinguished by the following categories: small unilamellar vesicles (SUVs), the smallest in the size range, with diameters ranging from 20 to 80 nm and the membrane formed by a single phospholipid bilayer; large unilamellar vesicles (LUVs), intermediate in the size range, with a diameter ranging from 80 nm to 1 µm and the membrane formed by a single phospholipid bilayer; small multilamelar vesicles (MLVs), with an average diameter between 400 nm and a few micrometers and the membrane formed by a phospholipid bilayer concentrically arranged (Mertins, 2008), and giant unilamellar vesicles (GUVs), larger than 1 µm, sometimes reaching tens of microns (Figure 3), comparable to the size of a eukaryotic cell (Lasic, 1995; Santos, 2002).

Liposomes may carry lipophilic substances within their lipid bilayer, thus allowing an increase in the transport of compounds to specific cells or tissues and enhancing the potency and/or reducing the toxicity of the encapsulated agent.

Liposome rating

Liposomes can be classified in terms of composition and intracellular delivery mechanism into five types: conventional liposomes, pH sensitive liposomes, cationic liposomes; Immunoliposomes and long-circulating liposomes.

Otherwise, the vesicle size is a critical parameter for

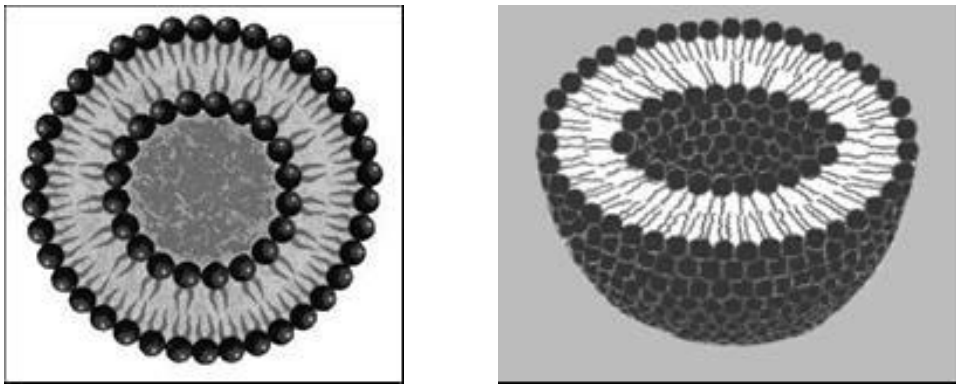

Figure 4. Liposome structure and cross section of a unilamellar liposome and its features. The polar heads are in gray while the hydrophobic heads are in black. Source: Machado, 2012.

determining circulating half-life of the liposomes, and size and number of bilayers influence the degree of drug encapsulation within liposomes. Thus, the liposomes were typically classified according to their size and number of bilayers (Sharma Sharma, 1997 cited by Laouini et al., 2012.): The small unilamellar vesicles (SUV): 20-100 nm; large unilamellar vesicles (LUV): > 100 nm; The giant unilamellar vesicles (Head): > 1000 nm; oligolamellar vesicle (OLV). 100-500 nm and multilamellar vesicles (MLV) > 500 nm (Bordi et al., 2006 cited by Laouini et al., 2012).

Techniques for the preparation of liposomes

The type of liposome is essentially constrained by their method of preparation which, several techniques can be used:

a) Hydration of the lipid film: As classical preparation, phospholipids are dissolved in an organic solvent, a flask glass.The solvent is evaporated under constant rotation of the balloon so as to produce a thin film phospholipids in inner wall of the balloon; subsequently, water or buffer hidration is added to the film. Agitation, ultrasonic agitation, sonication and heating can be applied at this stage to assist in the formation of double layers that will be self-assemble into liposomes encapsulating water or buffer inside process (Mertins, 2008).

b) Method of preparation of liposomes based on the reverse phase evaporation: The phospholipids are dissolved in organic solvent, then adding an aqueous portion causes formation of two phases; phospholipids tend to deposit on the interface water/organic solvent polarity by interactions of the polar extremities with water and interactions between apolaridade hydrocarbon chains and the organic solvent. The mixture is subjected to sonication; formation of reverse micelles where water droplets are surrounded by phospholipids and the mixture becomes calm. The organic solvent is evaporated and the reverse micelles concentrated. Finally, the stirring llipossomas are formed, and the system becomes a milky liquid and an aqueous portion may be added to accelerate the formation of structures (Mertins, 2008). The advantage of this method is the high encapsulation efficiency.

c) Method of preparation of liposomes DRVs type, based on the dehydration and rehydration process: This method consists of mixing a suspension of small empty liposomes (prepared in water), freeze-dried after mixing. The preparation of this rehydration under specific conditions of temperature (> Tt) and lipid concentration leads to obtaining liposomes with a high encapsulation rate, referred to as DRVs ("dehydration rehydration vesicles") and allow a high rate of encapsulation (Frézard et al., 2005).

The classic method of lipid film hydration for production of nanosized liposomes is still used in research because of its simplicity and low cost (Mertins, 2004). Atomization, lyophilization, agitation, sonication and freeze-thaw extrusion were applied to standardize the structures, as complementary techniques (Figures 4 and 5).

Chemical stability of liposomes

The stability of liposomes can be affected by chemical, physical and biological processes (Batista et al., 2007). The chemical instability depends on the composition of the liposomes, which involves the prevention of the ester hydrolysis and the oxidation of the unsaturation located in the lipid chain (Batista et al., 2007).

Physical instability: Some of the major processes that cause instability of the liposomes are the aggregation and fusion of vesicles and the leakage of encapsulated material.

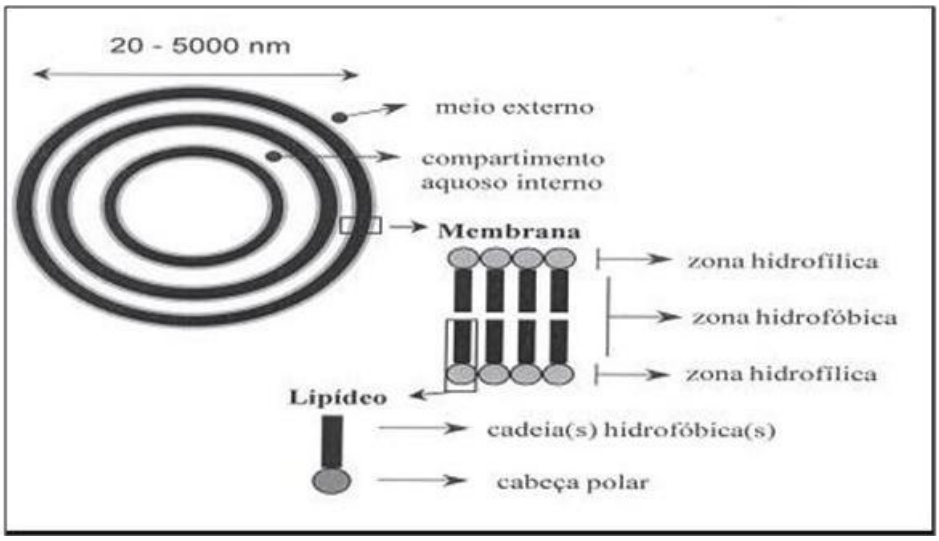

Figure 5. Structural features of liposomes. Source: Frezard et al. (2005). (Quimica Nova).

Biological instability: Liposomes must move and retain the material long enough for effective access and interaction with the target, typically in the blood, in the capillary walls and sometimes in the cells of extravascular areas (Batista et al., 2007).

Use of liposomes in different fields

Liposomes are widely used in the fields of medicine, pharmaceutics and food science. In the pharmaceutical field, they are used in the treatment of cancer, the development of vaccines, gene therapy, and the therapy of infectious and parasitic diseases. Besides, a new composition refers to the so-called transfersomes or ultradeformable or elastic liposomes, which are formed by phospholipids and surfactants (Santana et al., 2012).

The latter provides the membrane elasticity, which facilitates the conduction of these structures through the skin, including some cases reaching the deep layers. Some liposomal products approved by the Food and Drug Administration (FDA) and already marketed include: Ambisome (liposomal amphotericin B), indicated for the treatment of systemic fungal infections and for the treatment of leishmaniasis; Daunoxome (liposomal daunorubicin) for the treatment of Kaposi's sarcoma; Doxil (stealthy liposomal daunorubicin) for the treatment of Kaposi's sarcoma, ovarian cancer and breast cancer; Depocyt (liposomal cytarabine) for the treatment of meningitis, and Visudyne (liposomal verteporfin) for the macular degeneration with laser treatment (Santana et al., 2012).

In the area of food science, vitamins and minerals typically are added to foods in order to fortify them. However, they may give a strange taste, react with other ingredients or change the color of the product. These drawbacks can be avoided if such additions are made in encapsulated products. Encapsulation protects and enhances the stability of vitamins in some extremes of humidity and temperature as well as enables controlled release in the intestinal tract (Janovsky, 1993). There are several works using this process, such as: liposomal encapsulation of ciprosina (proteases used as rennet) to avoid loss during cheese processing (Picón et al., 1994); beta-carotene incorporation into liposomes through hydration of dried phospholipid particles produced by atomization; assessment of pediocin antimicrobial activity encapsulated against strains of *Listeria* (Mello et al., 2009); assessment of the encapsulation efficiency of liposomes containing nanometer size spirulina (Machado et al., 2011a); liposome preparation for protein source encapsulation (Machado et al., 2011b); importance of soy lecithin in the preparation of liposomes for commercial casein encapsulation (Machado et al., 2011b), and liposomal nanoencapsulation of phenolic compounds (Assis et. al., 2012b).

Liposomal nanoencapsulation of food

Nanoencapsulation is the incorporation, absorption or scattering of combinations of solid, liquid or gaseous bioactive compounds in small vesicles with a diameter at nanometer scale. The combinations of incorporated bioactive compounds can be protected against degradation and improve stability and solubility (such as solubilization of hydrophilic components in hydrophobic matrices and vice versa) (Assis et al., 2012a; Klaypradit and Huang, 2008; Jafari et al., 2008). Nanoliposomes are vesicles composed of phospholipid bilayers trapping materials in their aqueous compartments. Its unique properties have triggered numerous applications in various

scientific and technological fields. Nanoliposomes can provide controlled release of various bioactive agents, including food and nutraceutical ingredients, in the right place at the right time, so they increase the efficiency and cellular uptake of the encapsulated material. Reactive, sensitive or volatile additives (vitamins, enzymes, antioxidants, weight loss etc.) can be transformed into stable ingredients using nanoliposomes (Mozafari, 2007).

Encapsulation of Spirulina platensis strain LEB 18 as a source of proteins in liposomes using the method of hydration of the lipid film, sonication or homogenization associated as complementary treatments, was efficient for the preparation of nano lipospheres. The homogenization process yielded particles with average size larger and morphologically more uniform when compared to the sonication process. The purified phosphatidylcholine proved viable as wall material for the formation of Spirulina platensis strain liposomes LEB 18 as the material obtained was presented at the nanoscale and visually encapsulated (Machado, 2012).

Morais et al. (2003) used the method of reverse phase evaporation for the preparation of liposomes hydrolyzed casein. Although there are several studies of the use of soybean lecithin as wall material, in a comparative manner, the process of encapsulation by liposomes using soybean lecithin as rice behave similar way, both the classic method of preparing liposomes, as the method by reverse phase evaporation. An advantage of rice lecithin and its superiority as the phospholipid content compared to soybean lecithin.

Characterization of liposomes prepared with lecithin

Liposomes in their physicochemical characterization has the average size specified, observing the average diameter of the particles and their polydispersity indices (PDI) by light scattering technique in the Zetasizer equipment (Castro and Lima, 2006). The Zeta potential is defined as the surface potential of the particles. It is determined by electrophoretic mobility measurements that correspond to the speed of the particles in suspension, the larger the surface, the greater is the load speed of particles moving toward the oppositely charged electrode (Assis, 2007).

Toxicity of nanoencapsulated food

Miller and Senjen (2010) warned about the increasing use of nanotechnology in food production, by means of nanoparticles, nanocapsules and nanoemulsions in processing and packaging food, without proper regulation. Although nanotechnology may provide improvements in industrial performance, nutritional quality and food packaging efficiency, it can also bring risks to human health and the environment. Examples include nanoparticles of silver, titanium dioxide and zinc oxide which are used in nutritional supplements and in packages showing high toxicity to cells.

Given the potential risks associated with nanotechnology uses in agriculture and food, Miller and Senjen (2010) advocated a moratorium on the development of food products, packaging and agrochemicals until the specific security of nanotechnology is discussed and regulated under the following aspects: (a) the regulation of nanomaterials as new substances; (b) the expansion of the definition of nanomaterials; (c) transparency in the evaluation on the security of nanomaterials; (d) product labeling, and (e) greater involvement of society in the discussions of security and the sustainability of agricultural and food production.

However, the impact on the environment and human health is still controversial, mainly due to the lack of toxicological studies. As a consequence, assessment processes and risk management for such nanomaterials are hampered.

Conclusions

This study highlights the importance of soy and rice lecithin, both crude and purified (phosphatidylcholine) ones, as feasible for the encapsulation of various materials, from medical and pharmaceutical to food ones. Liposomes currently are seen as a promising source for the development of several technologies. However, its state of the art is at a level that still requires caution. Several studies on the subject are in progress and each day brings new publications, which makes their study very dynamic.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors wish to acknowledge FAPERGS, Brazil, for the financial support. The English version of this manuscript was carried out by Rodrigo da Rosa Pereira through the English Language Support to Student Scientific Production at Federal University of - FURG.

REFERENCES

AOCS (American Oil Chemists Society) (1993). Official methods and recommended practices. 4. ed. Champaign, v. 3.

Assis DN (2007). Biodistribuição do fluconazol marcado com 99m tecnécio, livre e encapsulado em nanocápsulas, em um modelo experimental de infecção com Cândida albicans.Programa de Pós-Graduação em Ciências Farmacêuticas da Universidade Federal de Minas Gerais.

Assis LM, Machado AR, Mello MB, Micheletto YMS, Badiale-Furlong E, Souza-Soares LA (2012b). Encapsulation efficiency of phenolic extracts in the microalgae Chlorella Vulgaris in liposomes. 16th World Congress of Food Science and Technology-IUFoST, Foz do Iguaçu , Paraná, Brasil.

Assis LM, Machado AR, Mello MB, Micheletto YMS, Motta AS, Badiale-Furlong E, Souza-Soares LA (2012a). Nanoencapsulation of phenolic extract in liposomes. 16th World Congress of Food Science and Technology-IUFoST, Foz do Iguaçu, Paraná, Brasil.

Bangham AD, Horne RW (1964). Negative Staining of Phospholipids and Their Structural Modification by Surface-Active Agents As Observed in the Electron Microscope. J. Mol. Biol. 8:660-668.

Batista CM, Carvalho CMB, Magalhães NSS (2007). Lipossomas e suas aplicações terapêuticas:estado da arte. Rev. Bras. Cienc. Farm. [online]. 43(2):167-179. ISSN 1516-9332.

Bordi F, Cametti C, Sennato S (2006). Advances in PlanarLipid Bilayers and Liposomes edited by Academic Press. pp. 281-320.

Castro NC, Lima EM (2006). Desenvolvimento e caracterização de lipossomas unicelulares e microcápsulas de colágeno para a encapsulação da isotretioína. Congresso de pesquisa, ensino e extensão da UFG- CONPEX, Goiânia.

Cruz MEM, Simões SI, Crow ML, Martins MB, Gaspar MM (2009). Formulation of nanoparticulate drug delivery systems (NPDDS) for macromolecules. In: drug delivery nanoparticles: formulation and characterization "Yashwant Pathak, Deepak Thassu (eds). Informa Healthcare USA, Inc. New York, Vol 191, Series: Drugs and the Pharmaceutical Science, Chapter 3, pp. 35-49. ISBN 978-142007804-6.

Edwards KA, Baeumner AJ (2006). Liposomes in analyses. Talanta 68(5):1432-1441.

Frézard F(1999). Liposomes: from biophysics to the design of peptide vaccines. Braz. J. Med. Biol. Res. 32(2): 181-189.

Frézard F, Schettini DA , Rocha OGF, Demicheli C(2005). Lipossomas: propriedades físicoquímicas e farmacológicas, aplicações na quimioterapia à base de antimônio. *Quimica Nova*, São Paulo. 28:511- 518.

Gómez-Hens A, Fernández-Romero JM (2005). The role of liposomes in analytical processes. Trends Anal. Chem. 24:9.

Institute Adolfo Lutz (2008).Normas Analíticas do Instituto Adolfo Lutz: Métodos Químicos e Físicos para a Análise de Alimentos. 4ª. ed. São Paulo.

Jafari SM, Assadpoor E, Bhandari B, HE Y(2008). Nano-particle encapsulation of fish oil by spray drying. Food Res. Int. 41(2):172-183.

Janovsky C (1993). Encapsulated ingredients for the baking industry. Cereal Food World 38(2):85-87.

Klaypradit W, Huang YW (2008). Fish oil encapsulation with chitosan using Ultrasonic Atomizer. LWT: Food Sci. Technol. 41:1133.

Laouini A, Jaafar-Maale J, Climayem-Blouza I, Sfar S, Charcosset C, Fessi H (2012). Preparation, Characterization and Applications of Liposomes: State of the Art. J. Colloid Sci. Biotechnol. 1:147-168.

Lasic DD (1995). Liposomes - From Physics to Applications. Amsterdam, Elsevier.

Lasic DD (1998). Novel application of liposomes. Trends Biotechnol. 16:307-321,

Lutz J, Augustin AJ, Jager, LJ, Bachmann D, Brandi M (1995). Acute toxicity and depression of phagocytosis *in vivo* by liposomes: Influence of lysophosphatidylcholine. Life Sci. 56:99.

Machado AR (2012).Avaliação biológica da spirulina em tamanho micro e nanométrico e estudo da sua encapsulação em lipossomas . p.149. Universidade Federal de Rio Grande-FURG, Dissertação de mestrado, em engenharia e Ciências de Alimentos Rio Grande RS.

Machado AR, Assis LM de, Badiale-Furlong E, Souza-Soares LA (2011b). IMPORTÂNCIA DA LECITINA DE SOJA NA ELABORAÇÃO DE LIPOSSOMAS PARA ENCAPSULAÇÃO DE CASEINA COMERCIAL.VII Simpósio gaucho de nutrição-SIGAN.

Machado AR, Assis LM de, Krolow MZ, Badiale-Furlong E, Souza-Soares LA (2011a). ELABORAÇÃO DE LIPOSSOMAS PARA ENCAPSULAÇÃO DE UMA FONTE PROTEICA. XIII Encontro de Pós-graduação. Pelotas.

Machado AR (2012).Avaliação biológica da spirulina em tamanho micro e nanométrico e estudo da sua encapsulação em lipossomas. p.149. Universidade Federal de Rio Grande-FURG, Dissertação de mestrado, em engenharia e Ciências de Alimentos Rio Grande RS.

Machado AR, Cavalheiro PL, Hackbart HCS, Souza-Soares LA (2013). Tecnica de cromatografia em coluna para purificação da lecitina obtida da degomagem do óleo de arroz. In: SOCIEDADE BRASILEIRA DE ÓLEOS E GORDURAS-20ANOS.

Maron LB, Covas CP, Silveira NP, Pohlmann A, Mertins O (2007). LUVS .RECOVERED WITH CHITOSAN: A NEW PREPARATION FOR VACCINE DELIVERY. J. Liposome Res. 17:155.

Mello MB, Jantzen MM, Malheiros Pda S, Brandelli A, Motta A de S (2009). AVALIAÇÃO DA ATIVIDADE ANTIMICROBIANA DA PEDIOCINA ENCAPSULADA FRENTE A LINHAGENS DE *Listeria.* XVIII,Congreso de Iniciação Cientifica.QXI Encontro de Pós-Graduação e I Mostra Cientifica.UFPEL.

Mertins O(2004). *Dissertação de Mestrado*; Estudo fisico-quimicos e estruturais de lipossomas compósitos de fosfatidilcolina e quitosana. 206p. Universidade Federal do Rio Grande do Sul, Brasil.

Mertins O, Sebben M, Schneider PH, Pohlmann AR, Silveira NP(2008). da.Caracterização da pureza de fosfatidilcolina da soja através de RMN de ^{1}H e de^{31}P.*Química. Nova* [online]. 31(7):1856-1859. ISSN 0100-4042.

Meyers SP (1990). Aquaculture diets with lecithin. Feed International, Lousiana, june, p.17-19.

Miller G, Senjen R (2010). Out of the laboratory and on to our plates: nanotechnology in food & agriculture. Disponível em: <http://nano.foe.org.au>. Acesso em: novembro de 2010.

Morais HA, Barbosa CMS, Delviv FM, Silva VDM, Mansur HS, Oliveira MC (2003). Estabilidade e avaliação sensorial de lipossomas contendo hidrolisados de caseína. Braz. J. Food Technol. 6:213.

Mozafari MR (2007). Nanomaterials and Nanosystems for Biomedical Applications. Dordrecht, The Netherlands: Springer.

New RRC (1990). (Ed.). *Liposomes:* A practical approach, IRL/ Oxford University Press, Oxford, p.432.

Orlandelli MCFS(2008). Desacidificação do OFA através do processo de extração líquido-líquido descontínuo utilizando álcoois.Dissertação (mestrado). Universidade Estadual de Campinas, Faculdade de Engenharia Química. Campinas, SP.

Picón A, Gaya P, Medina M, Nunez M (1994). Microencapsulation of cyprosins from flowers of cynara cardunculus l. In: Dehydrationrehydration liposomes. Biotechnol. Lett. 16(10): 1031-34.

Puisieux F, Couvreur P, Delattre J, Devisaguet JP (1995). (Eds.). Liposomes, new systems and new trends in their applications. Paris*:* Éditions de Santé. p. 266.

Reis MFT (2010). Micelas reversas formadas por surfactante/solvente orgânico/água: estudo termodinâmico e aplicação para extração de proteínas da semente de jaca. Universidade estadual do sudoeste da Bahia - UESB Dissertação-Mestrado em Engenharia de Alimentos, Itapetinga,BA.

Santana MHA, Martins F, Alves GP (2012).Nanotecnologia Aplicada ao Desenvolvimento de Produtos Farmacêuticos. Janeiro de 2010.Disponível em <http://www.racine.com.br >Acesso: Fevereiro de 2012.

Santos NC, Castanho MARB (2002). Lipossomas: a bala mágica acertou. *Química nova*, São Paulo, 25:6.

Sharma A and Sharma US (1997). Liposomes in drug delivery: progress and limitations. Int. J. Pharm. 154:123-140.

Vemuri S, Rhodes CT (1995). Preparation and characterization of liposomes as therapeutic delivery systems: a review. Pharm. Acta Helvetica, Berne. 70:95-111.

Willard DM, Riter RE, Levinger NE(1998). Dynamics of Polar Solvation In Lecithin/Water/Cyclohexane Reverse Micelles. J. Am. Chem. Soc. 120:4151.

Zambelli R, Moreira S (2009). Definição de lecitina. Engenharia de Alimentos. Universidade Federal do Ceará, Brasil. Disponível em:<http://engenhariadealimentosufc.blogspot.com.br/2009_11_01_ archive.html> Acesso: Janeiro de 2011.

academicJournals

Vol. 8(4), pp. 184-189, April 2014
DOI: 10.5897/AJFS2013.1121
Article Number: 15FA30344278
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Physico chemical and organoleptic characteristics of Muscovy drake meat as influenced by cooking methods

Omojola A. B.[1], Hammed S.[1], Attoh-Kotoku V.[2], Wogar G. S. I.[3], Iyanda O. D.[1] and Aremo J. O.[1]

[1]Meat Science Laboratory, Animal Science Department, University of Ibadan, Ibadan, Nigeria.
[2]Animal Science Department, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana.
[3]Animal Science Department, University of Calabar, Calabar, Nigeria.

Received 05 December, 2013; Accepted 25 March, 2014

The study was conducted to determine the effect of various cooking methods - grilling, deep frying, pan frying and roasting on quality attributes of Muscovy drake meat. A total of one hundred and eighty (180) Muscovy drakes fillets weighing between 118-130 g were used in a completely randomized design. Cooked samples were analyzed for proximate composition and physical characteristics. Organoleptic characteristics were evaluated using a nine-point hedonic scale. Deep fried meat samples had the highest (P<0.05) cooking loss (52.37%), while pan and deep fried meat samples had the highest shear force values of 4.10 and 3.91 kg/cm^3, respectively in comparison to values of 2.68 and 2.64 kg/cm^3 for roasted and grilled samples. The water holding capacity (WHC) of the meat was not affected significantly (P>0.05). The moisture content varied from 71.64% in the raw meat to 35.57% in deep fried fillets. Protein and ash content increased across the treatments after cooking. Pan fried (13.95%) and roasted duck fillets (13.92%) had higher fat than the raw sample (12.92%). Except for colour, other organoleptic parameters were not affected significantly. The use of deep frying method should be minimized since it resulted into meat with the least nutrient composition.

Key words: Muscovy drake, cooking method, organoleptic characteristics, physical attribute.

INTRODUCTION

Over the years, duck meat has been reported to be uniquely tasty and nutritious (Omojola, 2007). It has been appreciated for these qualities especially when food was in short supply. Today, duck meat is still very popular and in strong demand in many areas of the world especially Asia. Preference with regard to the breeds of duck and methods of preparation vary widely. In North America, parts of Europe, Australia and in many areas of the world roasted duck meat are a popular item in the menus of restaurants. Roasted, braised or barbecued duckling is also popular among homes and gourmets (Hird et al., 2005). More recently duck parts, such as breast and legs have become more available, which offers more options for diet conscious consumers. Pre-cooked parts which can be quickly heated in a microwave are also becoming more available in developed countries. Increased

E-mail: omojolababs@yahoo.com.

availability of duck meat and an increase in the available processed products prepared with duck meat is evidence of a movement to the large scale production of duck products (Hird et al., 2005).

However, duck meat consumption is reducing in Nigeria most probably because of several factors such as taboo on duck meat consumption and lack of technical know-how on duck husbandry (Oluyemi, 1979). Among people who have never tried duck or those who rarely eat it, there appears to be two concerns. The first concern seems to be lack of knowledge on how to properly prepare duck meat while the other is the somewhat higher fat content of duck, which is true of whole duck but not of leg meat and skinless breast meat (USDA, 2008).

The purpose of cooking is to make meat palatable, digestible and microbiologically safe (Tornberg, 2005). During this process, meat undergoes many changes, both physical and chemical, including weight loss, modifications of water-holding capacity, texture, muscle fibre shrinkage, colour and aroma development (Walsh et al., 2010) that are strongly dependent on protein denaturation and water loss. Quality characteristics of cooked meat products are also dependent on the composition and characteristics of the muscles, the heating method, as well as the time/temperature evolution during cooking (Christensen et al., 2000). The heating profile affects the sequence and extent of meat protein denaturation in the cooking process and, consequently, the physical and sensory properties of the final product (Riva and Schiraldi, 1994).

However, this can lead to undesirable modifications, such as a decrease in the nutritional value, mainly due to vitamin and mineral losses, and changes in the fatty acid composition associated with lipid oxidation (Rodriguez-Estrada et al., 1997). The use of different cooking methods on duck meat and its effect on the properties of the cooked product are interesting and worthy of investigation. The interaction between the raw ingredients and the cooking procedure has, however, not been studied in depth, even though heat treatment has a significant impact on the composition and physicochemical characteristics of the final food (Ratnajothi, 2010). The objective of the present work, therefore, was to determine the influence of commonly used cooking methods of - grilling, deep frying, pan frying and roasting on nutritional value and eating qualities of Muscovy drake meat.

MATERIALS AND METHODS

Sample preparation

A total of one hundred and eighty fillets weighing between 118-130 g were excised from the breast portion of eighteen (18) matured Muscovy drakes within one hour post-mortem. The meat samples were trimmed of any visible fat, ligaments and bones and kept frozen -2°C for 24 h after which the meats were properly thawed before cooking.

Cooking

After thawing, the fillets were distributed randomly to the four cooking conditions of: grilling, deep-frying, pan-frying, and roasting in a completely randomized design. Raw fillets were analyzed and served as the standard.

Cooking methods

Pan-frying

The Muscovy drake breasts were fried for 5 min per side without fat or oil in a Teflon-coated pan, which was preheated and the surface temperature was measured as 180°C.

Deep-frying

Fresh soybean oil$^{(R)}$ was used. The Muscovy drake breasts were fried for 10 min in a commercial stainless steel deep-fat fryer, when the temperature of oil reached 180°C.

Gas grilling

The drake breasts were grilled for 10 min per side, total cooking time was 20 min, the distance between samples and burner was about 15 cm. The surface temperature of the samples was about 200°C.

Roasting

The breasts were placed in a gas oven for roasting for 20 min at 200°C. The temperature of cooking was monitored by the thin chromium-aluminum thermocouples and cooking terminated when the core temperature of the meat reached the degree of well done (80°C). No condiment, spice or salt were applied to the fillets in each of the cooking procedure before and after cooking.

Parameters measured

Tenderness

Once fillets reached the desired internal temperature endpoint, they were cooled to room temperature for a minimum of two hours at which time an average of six 1.0 cm diameter cores were removed for shear force (tenderness) measurement using a Warner- Bratzler attachment to an Instron Universal Testing Machine.

Cooking loss

Fillets were weighed prior to and two hours post-cooking to determine cooking loss which is expressed as a percentage of raw fillet weight.

Proximate composition

After mincing the cooked samples, they were homogenized with a mixer (Moulinex 320) prior to determination of dry matter (ISO, 1997), total fat (ISO, 1973), protein (ISO, 1978) with a conversion

Table 1. Physical properties of Muscovy drake meat as affected by different cooking methods.

Parameter	Cooking method						
	Grilling	Deep frying	Pan frying	Roasting	SEM	F-Val	P-Val
Cooking loss (%)	44.40[b]	52.37[a]	43.36[b]	43.02[b]	0.94	4.61	0.003
Shear force (Kg/cm^3)	2.64[b]	3.91[a]	4.10[a]	2.68[b]	0.13	16.83	0.001
Water holding capacity (%)	21.14	21.11	17.94	18.24	0.71	2.70	0.092

[abc] Means with the same superscript along the same row are not significantly different (P>0.05).

factor of 6.25 and ash (ISO, 1998). All analyses were performed in duplicate per cooking procedure.

Water holding capacity (WHC)

This was determined using the press method as modified by Tsai and Oeckerman (1981). Approximately 0.5 g sample was taken from the differently cooked drake breast and weighed into a 9cm diameter Whatman No 1 filter paper (Model C, Carver, Inc Wabash IN, USA) and pressed between two 10.2×10.2 cm plexi glasses at approximately 35.2 kg/cm^3 for 1 min. The area of the free water was measured using a compensatory planimeter (Planix 5000, Tamaya Technics Inc, Tokyo, Japan) and percent free water calculated based on sample weight and moisture content (Tsai and Oeckerman, 1981). Percent bound water (WHC) was calculated as 100% minus free water percent. Six determinations were performed for each cooking trial.

Taste panel evaluation

Samples for sensory evaluation were taken from each of the treatment groups after cooking to the desired internal temperature. A total of 20 trained individuals aged between 22 and 35 years (40% male and 60% female) were employed to assess the cooked meat samples. Equal bite size from each treatment was coded, replicated thrice and served in odourless plastic plates. Each sample was evaluated independent of the other. The samples were evaluated on a 9-point hedonic scale for colour, flavour, tenderness, juiciness and overall acceptability.

Statistical analysis

All data obtained were subjected to analysis to variance and where statistical significances were observed, the means were compared using the Duncan's Multiple Range Test (Duncan, 1955). The SAS (1999) computer software package was used for all statistical analysis.

RESULTS AND DISCUSSION

Cooking procedures (methods) can have a dramatic effect on the final appearance and eating quality of meat, as well as its nutritional value. During cooking, meat loses approximately 20-40% of its weight (Davey and Gilbert, 1974; Martens et al., 1982; Bertola et al., 1994; Palka and Daun, 1999; Aaslyng et al., 2003). This is ascribed to a temperature-induced, structural shrinkage, which causes fluid to be expelled from the meat (Davey and Gilbert, 1974). The aspects of cooking methodology that are particularly crucial are final endpoint temperature, the time the meat spends at higher temperatures and the presence of moisture or fat.

Physical properties

Cooking loss measurement is a rapid and valid method of assessing the impact of heat treatment on meat, because it reflects the degree of its juiciness, as well as certain economic aspects (Bertram et al., 2004). Cooking loss of all samples is shown in Table 1. Deep fried meat samples had the highest (P<0.05) cooking loss (52.37%) while there was no noticeable statistical differences between values obtained for the other cooking methods. The variation observed in the percentage cooking loss especially for the high value in deep fried meat may be due to high temperature involved in deep frying which might have led to loss of fat and shrinkage in the fried meat. According to Bertram et al. (2004) the strong correlation between cooking loss and shrinkage of meat can be explained by the fact that the shrinkage appearing during cooking causes loss of meat liquid, which resulted in loss in mass.

Cooking loss is the reduction in weight of meat during cooking process. This loss of weight has been shown to consist of mainly water but a substantial loss of lipid can also occur. The degree of cooking loss will depend greatly on the cooking procedure employed. Davey and Gilbert (1974) found that the temperature at which cooking loss increased in meat corresponded to the temperature at which isolated collagen shrunk. It may therefore be reasonable to suggest that the differences in cooking loss values observed in the present study could be due to a difference in the force generated by collagen shrinkage on the myofibrils. Collagen shrinkage before its solubilization may not have been severe enough in the slow cooking methods of grilling, pan frying and roasting to generate a force able to expel water (King et al., 2003).

Shear force

Shear force measures the degree of toughness, the higher the value the tougher the meat. Tenderness is considered as the most important trait of meat quality attributes (Cross et al., 1986) that determines the perception of consumers to a particular type of meat.

Table 2. Proximate composition of Muscovy drake meat as it is affected by different processing methods.

Parameter (g/100 g)	Cooking method							
	Raw	Grilling	Deep frying	Pan frying	Roasting	SEM	F- Val	P- Val
Moisture	71.64[a]	45.89[c]	35.57[e]	38.38[d]	53.18[b]	1.68	3021.51	0.000
Protein	21.91[d]	33.43[a]	28.74[c]	30.91[b]	31.28[b]	0.55	107.97	0.000
Fat	12.92[b]	11.52[c]	8.66[d]	13.85[a]	13.92[a]	0.26	2146.94	0.000
Ash	2.12[b]	7.20[a]	7.02[a]	7.08[a]	7.02[a]	0.26	3726.86	0.000

[abc] Means with the same superscript along the same row are not significantly different (P>0.05).

Shear force results are reported in Table 1. Pan fried and deep fried meat samples had the highest shear force values of 4.10 and 3.91 kg/cm^3, respectively in comparison to values of 2.68 kg/cm^3 for roasted and 2.64 kg/cm^3 for grilled samples. The high shear force in fried samples is probably indicative of the sample surface consistency and to surface crust formation and the higher dehydration of the centre that the samples underwent during cooking. The high shear force value obtained in this study for frying methods was similar to that of Apata et al., (2012) who observed high shear force values for fried rabbit meat. Cooking temperatures and methods thus dramatically affect the tenderness of meat cuts (Combes et al., 2003).

Water holding capacity (WHC)

The WHC, which is the ability of meat to retain its water during application of external forces is important in meat processing, and the overall eating quality of meat revolves around it (Omojola, 2008). WHC is related to the denaturation of proteins that leads to different longitudinal shrinkage (Offer et al., 1984; Tornberg, 2005) and is dependent on the heating rate (Bertram, et al., 2006). The four cooking methods used in this study did not affect the WHC of the meat (P>0.05) (Table 1) probably because cooking meat for a long period of time at lower temperatures can have the same effect on water retention as does cooking at higher temperatures for short periods of time (Bertram et al., 2006).

Proximate analysis

Results in Table 2 showed the proximate composition of the raw meat, and after undergoing four cooking procedures. The moisture content significantly varied from 71.64% in the raw meat to 53.18% in roasted, 45.89% in grilled, 38.38% in pan fried and 35.57 in deep fried samples (P<0.05). Differences between dry-heat and moist heat cooking methods have previously been reported (Sainsbury et al., 2011) with higher losses of moisture in dry-heat cooking procedures. Each of the cooking procedures used in this study led to reduction in moisture compared to the raw meat (Table 2), when considering the same weight of raw and cooked meats. The percent reduction in moisture ranged from 25.77% for roasted duck meat to 35.94% for grilled, 46.43% for pan fried and 50.35% for deep fried fillets. The high moisture loss recorded for deep fried samples could be due to replacement of the water matrix by fat because deep frying is primarily a dehydration process, which means that water and water-soluble substances are extracted from the product being deep fried and transferred to the cooking fat, and at the same time the product absorbed the surrounding fat (Choe and Minutes, 1997).

The consumption of pan fried and roasted duck meat implied the intake of 7.20 and 7.74%% more fat while the consumption of grilled and deep fried meat indicated a reduction in fat consumption of 10.99 and 32.97% less fat than the composition of the raw product. Pan fried and roasted duck fillets under our cooking conditions showed a relative increase of fat in relation to the raw product, while samples that were either grilled or deep fried had lower fat contents than the raw samples.

The ash values obtained for the different processing methods did not differ from each other. The processing methods employed in this work equally increased ash content of the duck meat. This agreed with Vaclavik and Christian (2007), who stated that minerals tend to have higher heat stability and are less affected by cooking methods. No loss of ash was observed during cooking. The increase in percentage ranged from 231.13% for deep fried and roasted samples to 233.96 and 239.62% for pan fried and grilled samples respectively.

Taste panel evaluation

The results of the taste panel evaluation (Table 3) indicated that none of the cooking procedures influenced the sensory score of flavour, juiciness, tenderness and overall acceptability significantly (P>0.05). Tenderness which is regarded as the most important sensory attribute affecting meat acceptability (Cross et al., 1986; Quali, 1990; Warkup et al., 1995) was rated between a score of slightly tough (4.50) to intermediate (5.13) on a nine point hedonic scale. The panelists were however not able to

Table 3. Effect of different cooking methods on organoleptic properties of Muscovy drake meat.

Parameter	Cooking method						
	Grilling	Deep frying	Pan frying	Roasting	SEM	F- Val	P-Val
Colour	5.00[a]	3.20[c]	3.47[bc]	4.17[b]	0.24	10.28	0.004
Flavour	5.60	5.43	4.73	4.57	0.22	1.51	0.286
Tenderness	4.50	5.03	5.10	5.13	0.15	0.91	0.478
Juiciness	4.78	5.01	5.76	5.23	0.12	6.54	0.150
Overall acceptability	5.07	5.20	4.80	5.07	0.12	0.40	0.761

[abc] Means with the same superscript along the same row are not significantly different (P>0.05).

detect any difference in the tenderness of Muscovy drake meat irrespective of the cooking method employed. A similar trend was observed for flavor, juiciness and overall acceptability. Colour score for grilled duck meat was rated highest with a value of 5.00 (intermediate score on a nine point hedonic scale). Deep and pan fried samples were rated lowest with values of 3.20 and 3.47%, respectively which were significantly lower (P<0.05) compared to the value 5.00 for grilling while the colour score for roasted fillets was 4.17. There is possibility that the high temperature and the oil used in frying might have affected the colour of the product resulting to a lower score compared to the other meat products. Apata et al., (2012) stated that different cooking methods greatly affect the colour of processed rabbit eliciting the lowest scores. The low score recorded by the panelist for each of the eating quality traits is probably an indication of the level of acceptance of duck meat irrespective of the cooking method employed.

Conclusion

Cooking influenced the nutrient content of meat in different ways depending on the cooking process. In this study, it was observed that processing methods affected the physical properties of drake breast meat differently, except for water holding capacity. Grilled duck breast meat was the tenderest. Nutrient composition of processed drake breast meat was affected by different cooking methods with deep fried fillets having the lowest nutrient composition.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Aaslyng MD, Bejerholm C, Ertbjerg P, Bertram HC, Andersen HJ (2003). Cooking loss and juiciness of pork in relation to raw meat quality and cooking procedure. J. Food Qual. Prefer. 14: 277- 288.

Apata ES, Koleosho TS, Apata CO, Okubanjo AO (2012). Influence of sex and processing methods on physicochemical and organoleptic quality of rabbit meat. Afr. J. Food Sci. 6 (15):407-411.

Bertola NC, Bevilacqua AE, Zaritzky NE (1994). Heat treatment effect on texture changes and thermal denaturation of proteins in beef muscle. J. Food Process. Preserv. 18:31-46.

Bertram HC, Engelsen SB, Buska H, Karlssona AH, Andersen HJ (2004). Water properties during cooking of pork studied by low-field NMR relaxation: Effects of curing and the RN-gene. Meat Sci. 66:437-446.

Bertram HC, Wu Z, Straadt IK, Aagard M, Aaslying MD (2006). Effects of pressurization on structure, water distribution, and sensory attributes of cured ham: Can pressurization reduce the crucial sodium content? J. Agric. Food Chem. 54(26):9912-9917.

Choe E, Minutes DB (1997). Chemistry of deep- frying oils. J. Food Sci. 72(5):R77-R86

Christensen M, Purslow PP, Larsen LM (2000). The effect of cooking temperature on mechanical properties of whole meat, single muscle fibres and perimysial connective tissue. Meat Sci. 55:301-307.

Combes S, Lepetit J, Darche B, Labas F (2003). Effect of cooking temperature and time on Warner Bratzler tenderness measurement and collagen content in rabbit meat. Meat Sci. 66:91-96.

Cross HR, Savell JW, Francis JJ (1986). National consumer retail beef study. In: Proceedings 38thAnnual Reciprocal Meat Conference 39: 112-114.

Davey CL, Gilbert KV (1974). Temperature-dependent cooking toughness in beef. J. Sci. Food Agric. 25:931-938.

Duncan DB (1955). Multiple range and multiple F tests. Biometrics 11:1-42

Hird H, Chisholm J, Brown J (2005). The detection of commercial duck species in food using a single probe-multiple species-specific primer real-time PCR assay. Eur. Food Res. Technol. 221:559-563.

ISO (1973). Meat and meat products - Determinutesation of total fat content. ISO 1443:1973. International Organization for Standardization, Geneva, Switzerland.

ISO (1978). Meat and meat products - Determinutesation of nitrogen content. ISO 937:1978. International Organization for Standardization, Geneva, Switzerland.

ISO (1997). Meat and meat products - Determinutesation of moisture content. ISO 1442:1997. International Organization for Standardization, Geneva, Switzerland

ISO (1998). Meat and meat products - Determinutesation of total ash. ISO 936:1998. International Organization for Standardization, Geneva, Switzerland.

King DA, Dikeman M.E, Wheeler TL, Kastner CL, Koohmaraie M (2003). Chilling and cooking rate effects on some myofibrillar determinutesants of tenderness of beef. J. Anim. Sci. 81:1473-1481.

Martens H, Stabursvik E, Martens M (1982). Texture and color changes in meat during cooking related to thermal denaturation of muscle proteins. J. Texture Stud. 13:291-309.

Offer G, Restall D, Trinck J (1984). Water-holding in meat. In: Recent Advances in Chemistry of Meat. A. J. Bailey, ed. The Royal Society of Chemistry, London. pp. 71-83.

Oluyemi JA (1979). Potentialities of the indeginous species of poultry for meat and egg production in Nigeria. NAPRI pp. 163-186.

Omojola AB (2007). Carcass and organoleptic characteristics of duck

meat as influenced by breed and sex. Int. J. Poult. Sci. 6(5):229-334.
Omojola AB (2008). Yield and organoleptic characteristics of suya (an intermediate moisture meat) prepared from three different muscles of a matured bull. Afr. J. Biotechnol. 7(13):2254 - 2257.
Palka K, Daun H (1999). Changes in texture, cooking losses in myofibrillar structure of bovine M. semitendinosus during heating. Meat Sci. 51:237-243.
Quali A (1990). Post-mortem changes in muscle tissue. J. Muscle Foods 1:129-134.
Ratnajothi H (2010). The impact of heat-moisture treatment on molecular structures and properties of starches isolated from different botanical sources. Crit. Rev. Food Sci. Nutr. 50:835-847.
Riva M, Schiraldi A (1994). A DSC investigation of the effects of heating rate on cooking indexes of ground meat. Ital. J. Food Sci. 6:43-58.
Rodriguez-Estrada MT, Penazi G, Caboni MF, Bertacco G, Lercker G (1997). Effect of different cooking methods on some lipid and protein components of beef hamburgers. Meat Sci. 45:365-375.
Sainsbury J, Schonfeldt HC, Van Heerden SM (2011). The nutrient composition of South African mutton. J. Food Compos. Anal. 24:720-726.
SAS (1999). Statistical Analysis System Institutes. User's guide. SAS Institute Inc. Cary, N. C.
Tornberg E (2005). Effects of heat on meat proteins- Implications on structure and quality of meat products. Meat Sci. 70:493-508.
Tsai TC, Oeckerman HW (1981). Water holding measurement of meat. Meat Sci. 46:697-701.
USDA (2008). National Nutrient Database for Standard Reference, Release 21 (PDF).United State Department of Agriculture. http://www.nal.usda.gov/fnic/foodcomp/ Data/SR21 /nutrlist/ sr21 w601.pdf.
Vaclavik VA, Christian EW (2007). Essentials of Food Science. 3rd Edition, Springer. p. 590.
Walsh H, Martins S, O' Neill EE, Kerry JP, Kenny T, Ward P (2010). The effects of different cooking regimes on the cook yield and tenderness of non-injected and injection enhanced forequarter beef muscles. Meat Sci. 84:444-448.
Warkup CC, Marie S, Harrington G (1995). Expression of tissue proteinases and regulation of protein degradation as related to meat quality Ed. A. Quali, D. Demeyer and F.J.M. Smulders. Ecceamst. Ultrechit. The Netherlands. pp. 225-23.

academicJournals

Vol. 8(4), pp. 190-195, April 2014
DOI: 10.5897/AJFS2014.1141
Article Number: 640609344279
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Household food processing methods to enhance iron and zinc bioavailability in formulated haricot bean and maize complementary food

Getenesh Berhanu[1], Addisalem Mesfin[1], Afework Kebebu[1], Susan J Whiting[2] and Carol J Henry[2]*

[1]School of Nutrition, Food Science and Technology, Hawassa University, Hawassa, Ethiopia.
[2]College of Pharmacy and Nutrition, University of Saskatchewan, Canada.

Received 13 February, 2014; Accepted 25 March, 2014

This study aimed to test the nutritional quality of white haricot bean-maize porridge, a potential complementary food made using household food processing. Focus group discussions were conducted with mothers and revealed that traditional processing practices were soaking, germination and roasting. Although few used pulses in complementary foods (only maize), they expressed preference for white haricot bean to incorporate as a pulse in food for infants and young children. Germination (for 48 or 72 h) and roasting methods of household processing and preparation methods were used during preparation of white haricot bean flour, and soaking and roasting were selected in preparation of maize flour. Proximate nutrient analysis was done on processed and unprocessed flours using standard methods. There were no significant differences in iron (p=0.114), and zinc (p= 0.326) between 48 and 72 h germinated white haricot bean. However, processed products showed significant reduction of phytate (p= 0.001). Community acceptability test was undertaken with 36 mother-child pairs. There were no significant mean differences among porridge samples for sensory attributes. This study shows that processing such as germination of pulse is necessary for improved bioavailability of iron and zinc, and that pulse-cereal porridge is suitable as a complementary food.

Key words: Phytate, iron, zinc, porridge, maize flour, haricot bean flour, germination, roasting, soaking.

INTRODUCTION

Appropriate infant and child feeding, including complementary feeding, is critical for child growth, development and survival (WHO and UNICEF, 2008). Poor households subsist on monotonous staple-based diets and intakes of animal-source foods are low (Gibson et al., 2008). Lack of diversity in the diet is strongly associated with inadequate intake and risks of deficiencies of essential micronutrients such as vitamin A, iron and zinc in young children (Hawkes and Ruel, 2011). In Ethiopia, the most recent national survey indicates that 21.4% of under 5 children are mildly anemic, 20.4% are moderately anemic and 2.5% are severely anemic. Cite Reference (Central Statistics Agency (CSA) CSA, 2012) Previous studies conducted in southern Ethiopia indicated the presence

*Corresponding author: E-mail: cj.henry@usask.ca. Tel: 1-306-966-5836 Fax: 1306-966-6377.

of zinc and iron deficiency (Hambidge et al., 2006; Stoecker et al., 2009; Gibson et al., 2008). The main source of zinc is animal source foods such as meat which is lacking in many rural poor households. Young children are in the rapid growth phase where requirements for nutrients are increased, yet in Ethiopia, it is common to find their diet is mainly cereal based which is low in iron and zinc, and high in phytate content (Kebebu et al., 2013).

Locally available foods such as pulses are rich source of protein, vitamins and minerals. In resource-limited households, malnutrition is attributable not solely to insufficient amounts of food but also to the poor nutritional quality of the available food supply, especially among plant-based diets (WHO and UNICEF, 2008; Gibson et al., 2008). Low bioavailability of nutrients, arising from the presence of antinutrients such as phytate is another factor that limits the quality of predominantly plant-based diets (Hawkes and Ruel, 2011; Central Statistics Agency (CSA), 2012). Given the heavy reliance of low-income populations on cereals as a food source, the negative effects of low mineral bioavailability on mineral status and subsequent health are potentially quite substantial. Combining pulses with other plant-based protein sources such as cereal grains can generate a more complete protein than either alone and can also provide vitamins and minerals not found in either by itself, especially in complementary foods (Egounlety, 2002).

Household food-processing and preparation methods such as soaking, fermentation, roasting and germination can enhance the bioavailability of micronutrients in plant-based diets by decreasing phytate content and improving overall digestibility and absorption of nutrients (Hotz and Gibson, 2007). Using home based recipes with easily available pulse products and traditional ways of food processing methods should encourage greater acceptance of products with improved nutrient availability in areas where the usual diet is of poor nutritional quality (De Onis et al., 2012). Therefore, this study aimed to test the nutritional quality of a pulse-cereal complementary food after simulating household food processing procedures to decrease phytate content. A white haricot bean and maize complementary food was formulated, and various household processing methods were tested, after which acceptance was determined by community women and their children.

METHODOLOGY

This study was conducted at Hawassa Zuria woreda, in Sidama zone, Southern Nations and Nationalities People Region, Ethiopia. Hawassa Zuria woreda is situated 22 km south east of the regional capital of Hawassa. Main crops are maize, red paper, enset (also called false banana, *Enset ventricosum*), potato, and red and white haricot (kidney) beans, according to the Hawassa Zuria woreda health report of 2010. This study area was selected because previous studies conducted in the region indicated the presence of zinc and iron deficiency in children and adults (Hambidge et al., 2006; Stoecker et al., 2009; Gibson et al., 2008; Aubuchon-Endsley et al., 2011). Ethical approval was obtained from Hawassa University's Ethical approval committee.

Study design

Laboratory-based experimental study design was used for this study. For the community consultation, three Focus Group Discussions (FGD) took place with mothers who were living in the study area, to find out (1) the traditional ways of pulse based foods processing practices; (2) whether pulses were used in preparation of complementary foods in the community; (3) overall feeding practices of infants and young children; (4) the most common pulses available in the area; and (5) their knowledge, attitudes and beliefs regarding incorporating pulses in preparation of complementary foods. Discussion in a private area was facilitated by principal investigators, and tape recorded. The answers to the five questions were grouped, and emerging themes regarding potential for pulse-cereal complementary foods of high nutritional quality were summarized to inform the research on complementary food preparation and acceptability.

The community consultation indicated that the complementary foods normally made were maize-based gruels, and that the preferred pulse was white haricot bean. The food processing methods in use in the community were germination and roasting. A series of laboratory-based studies then tested these household processing methods applied to preparation of a pulse-cereal porridge as complementary food. The ingredients were purchased in the local market, and the flours and complementary food that were formulated in the food laboratory at Hawassa University, were analyzed. Once formulation was completed, the resulting porridges were tested for acceptance in the community.

Preparation of white haricot bean flour

The white haricot beans were first cleaned of defective grains, stones and other debris, then washed and soaked in clean tap water for 12 h. After draining, the beans were left to germinate at room temperature for 48 or 72 h, respectively. These two batches of germinated seeds were rinsed, and then dried in the sun to facilitate removal of the hulls and aid in removing moisture. The sun dried beans were roasted using a hot plate which is a commonly available household appliance. After 5 min of roasting, the beans were milled using the community's milling machine, to obtain smooth and consistent particle sizes. The resulting flours were stored in airtight polyethylene plastic containers until further use.

Preparation of maize flour

Defective grains, stones, and other debris were removed from the locally obtained maize. After washing in clean tap water, the maize was soaked overnight at room temperature. The soaked maize grains were thoroughly washed and sun dried. The dried maize was roasted for five minutes. After milling to a smooth and consistent particle size, maize flour was stored in an airtight polyethylene plastic bag.

Preparation of porridge

Three types of porridge were prepared from maize and haricot bean mix. Porridge was chosen because it has smooth consistency and is palatable for young children. FAO/WHO/UNICEF (1985) guidelines

are to add up to 40% pulses into cereal-based products. The unprocessed (control) porridge was prepared from 70 g unprocessed maize flour and 30 g unprocessed haricot bean flour and same amount of oil and salt as that of treatment porridges. Two treatment foods were prepared, one was by using white haricot bean flour that had undergone 48 h of germination, and the other from beans germinated for 72 h. Both treatment porridges were prepared from 70 g of processed maize flour blended with 30 g white haricot bean flour, and cooked in boiling water with oil, salt and a measured amount of water.

Laboratory analysis of prepared flours

All chemicals and reagents used in the laboratory analysis were analytical grade. Each laboratory determination was carried out on separate fresh samples using standard methods. Iron and zinc levels were analyzed at the Saskatchewan Food Industry Development Center (Saskatoon SK, Canada) and phytate analyzed at University of Saskatchewan (Saskatoon SK, Canada). Other proximate analyses were done at Ethiopian Health and Nutrition Research Institute (Addis Ababa, Ethiopia) (AOAC) 2000). Determinations included moisture content (AACC international method 44-15.02), protein (Kjeldahl method), calcium, iron and zinc (inductive coupled plasma mass spectrometry, ICP-MS), crude fat and ash (AOAC 4.5.01, 2000; AOAC 923.03, 2000), crude fiber (AOAC 962.09) and phytate [(modified Wade Reagent Method of Gao et al., (2007).

Acceptability testing

Thirty six (36) mothers and their young children aged 7-20 months performed field sensory evaluation using a modified five-point grade scale whereby five and one represented the highest and the lowest orders of preference, respectively. Terminologies for sensory evaluation were appearance, flavor, taste, texture (mouth feel) and overall acceptability. Each mother-child pair was given a sample of each of the three porridges in random order, and in duplicate. Mothers were told to taste the food and feed their children with it. Prior to conducting the sensory tests mothers were asked not to feed their children for an hour prior to conducting the sensory tests. The objectives of the study were explained to mothers and they were instructed to give their honest opinions. Mothers assigned a score to the preference of their children based on the facial expression and their general reaction after tasting the food mixtures. Samples were served with similar utensils.

Statistical analysis

Analysis of recorded, transcribed and translated consultation discussions were performed using thematic analysis technique. Themes developed by comparing findings from each theme across study sites (by kebele/district) to look for similarities and differences in response within and between communities. Initial conclusions were triangulated by comparing responses between target populations to examine the relationships between their demand, experiences and perceptions. The findings presented are consistent within subgroups and across study sites.

Analysis of experimental data was conducted using SPSS version 16.0. Means and standard deviations were calculated for proximate laboratory results and acceptability (sensory attributes) of the complementary foods. Factorial analysis of variance (ANOVA) and Duncan's multiple significant tests were conducted to determine significantly different means. Independent sample t-test was conducted to see the significance difference in processed and unprocessed maize samples. Differences were considered significant at $p < 0.05$.

RESULTS

In the community group consultation, the common pulses in the area were red and white haricot beans. The former was grown only during time of plentiful rain. While most participants agreed they ate red haricot bean during the coffee ceremony, at which time it was boiled and roasted after soaking; they also mentioned that red haricot bean is considered a "poor person's diet". The white haricot bean was preferred, especially when mixed in the national dish "Kocho", so that the resulting food remained white.

Respondents knew the health benefits of pulses including their benefits to infants. However, they did not incorporate pulses in an infant's diet, raising two major problems: pulse was not available in the dry season, and they did not know how to prepare complementary foods using pulses. Their usual complementary food was prepared from maize as a form of a thick porridge or a thin gruel, to which they might add oil/fat, powdered milk, potato and/or egg. Thus they had no experience with any traditional food processing method specifically for adding pulses in infant food. However, they roasted or boiled pulses after a short period of soaking.

Composition of haricot bean flour after soaking

The analysis of the three haricot bean flours is shown in Table 1. There were no significant differences in iron and zinc content among groups, thus germination had no detrimental effect on these minerals. Phytate concentrations, however, were lower in both geminated flours, which would lead to improvement in mineral bioavailability.

While there were significant differences between the ungerminated and germinated flours in protein, fat and carbohydrate, other differences showed no pattern to germination. Significant differences between 48 and 72 h germination included fat, energy and ash content.

Maize flour that was processed by soaking and roasting showed no differences to unprocessed flour except in iron content, where levels appeared to improve with processing (Table 2).

Acceptability

There were no significant mean differences among porridge samples except for overall acceptability. Here, the bean porridge made with 72 h germinated bean flour was liked less well than that made with the 48 h germinated bean flour porridge and the bean porridge made with ungerminated beans. As the mean acceptability score was 4.0 for all samples, it appeared that all of the porridges were moderately well liked and not very different (Table 3).

Table 1. Proximate analysis of mineral, phytate and macronutrients of ungerminated and germinated haricot bean flour, Mean (SD) of triplicate analysis.

Component per 100 g	Content of samples		
	No germination	48 h germination	72 h germination
Zinc (mg)	3.21 (0.28) [a]	3.45 (0.05)[a]	3.34 (0.01)[a]
Iron (mg)	6.03 (0.08)[a]	6.09 (0.01)[a]	5.90 (0.09)[a]
Phytate (µg)	206.7 (58.7)[a]	166.1 (10.0)[b]	163.4 (75.0)[b]
Protein (%)	25.00 (0.16)[a]	27.5 (0.25)[b]	28.17 (0.59)[a]
Fat (%)	1.35 (0.50)[a]	2.54 (0.90)[b]	4.22 (0.98)[c]
CHO (g%)	60.24 (0.15)[a]	55.36 (0.77)[ab]	53.79 (1.21)[b]
Energy (kcal)	353.1 (0.3)[b]	354.3 (1.5)[b]	365.9 (5.0)[a]
Fiber (g)	6.72 (0.13)[a]	6.20 (1.13)[a]	5.29 (0.98)[a]
Ash (g%)	4.03 (0.04)[a]	4.25 (0.08)[b]	4.02 (0.12)[a]
Moisture (g%)	9.37 (0.03)[a]	10.34 (0.45)[b]	9.79 (0.15)[a]

Values in the same row with different superscript letters are significantly different from each other at $p<0.05$.

Table 2. Proximate analysis of mineral, phytate and macronutrients of unprocessed and processed maize, Mean (SD) of triplicate analysis.

Component per 100 g	Content of sample	
	Unprocessed	Processed#
Zinc (mg)	1.86 (0.10)[a]	2.02 (0.07)[b]
Iron (mg)	1.65 (0.05)[a]	1.80 (0.01)[a]
Phytate (µg)	143.5 (6.9)[a]	134.0 (7.0)[a]
Protein (%)	7.88 (0.05)[a]	8.68 (0.42)[b]
Fat (%)	6.65 (1.24)[a]	8.21 (0.53)[a]
CHO (g%)	73.84 (1.22)[b]	71.45 (0.53)[a]
Energy (kcal)	386.7 (6.5)[a]	394.5 (1.6)[a]
Fiber (g)	1.74 (0.34)[a]	2.71 (0.09)[b]
Ash (g%)	1.92 (0.24)[a]	2.14 (0.26)[a]
Moisture (g%)	9.70 (0.31)[a]	9.50 (0.47)[a]

Values in the same row with different superscript letters are significantly different from the other value at $p<0.05$. # Maize was processed by soaking and boiling

Table 3. Children's acceptability based on mothers' perception at Hawassa Zuria woreda (n=36) June 2013.

Sample	Appearance	Flavor	Taste	Texture	Overall
Unprocessed 70% maize and 30% haricot bean	4.46 (.05)[a]	4.26 (.15)[a]	4.21 (.01)[a]	4.33 (.04)[a]	4.33 (.01)[b]
Processed 70% maize and 30% 48 h germinated haricot bean	4.25 (.04)[a]	4.14 (.11)[a]	4.02 (.12)[a]	4.09 (.02)[a]	4.14 (.01)[b]
Processed 70% maize and 30% 72 h germinated haricot bean	4.36 (.39)[a]	4.04 (.21)[a]	3.48 (.68)[a]	3.97 (.19) [a]	4.01(.09)[a]
Significance (p value)	0.696	0.478	0.313	0.110	0.026*

*indicates significance at $p < 0.05$.

DISCUSSION

Traditional food processing methods for pulses include soaking, germination, frying, fermentation, boiling, roasting, and blanching (Walingo, 2009). These processing procedures of plant-based food components are known to improve digestibility and to remove or reduce anti-nutritional factors such as phytate (Gebrelibanos et al., 2013). Further, these methods may enhance organoleptic properties of food, thus increasing acceptability. While considered "traditional", these methods are being quickly forgotten (Walingo, 2009). Our results show that by germinating white haricot beans, the resulting flour had significantly lower level of phytate, which is known to reduce iron and zinc absorption (Sandburg et al., 1999; Hawkes and Ruel, 2011). This is in agreement with studies on germination of pulses wherein germination increased protein content and dietary fiber, and also reduced phytate content and increased mineral bioavailability (Ghavidel and Prakash, 2006). Some researchers have shown improved zinc content (El-Adawy, 2002), which we did not observe. Ibnouf (2007) found that roasting reduced phytate content of beans, and this might have influenced the phytate contact of the bean flours. However, roasted maize did not show a decline in phytate.

Red and white haricot beans are common in the Hawassa Zuria woreda, of Ethiopia, yet most people there consider red haricot bean as a "poor" person's food. When consumed, it was common to boil or roast beans, and there was a positive attitude towards the benefit of red or white haricot beans in the study community. Beans were known to "replace meat" (as a protein source) by the community members we interviewed. However it was not common to incorporate pulses into complementary foods, thus mothers/caregivers did not know how to prepare complementary food using haricot bean. This attitude was observed previously in a different area of southern Ethiopia (Kebebu et al., 2013). Also of note was the finding that few community members used any processing methods, except soaking and roasting.

Finding that processing of the beans reduced levels of phytate in the bean flours has importance to the promotion of complementary foods made from pulses. Phytates have a high binding capacity to minerals such as iron and zinc and reduce their bioavailability (Sandberg et al., 1999). Germination induces hydrolysis of phytate (which is more accurately called inositol-hexaphosphate), and this prevents binding of divalent cations. While the fall in phytate cannot be attributed to germination alone, we did not see a drop in phytate level in processed maize, which was processed by roasting. Overall, there was no difference in phytate levels between germinating for 48 h and for 72 h. Therefore, the shorter time would be preferred.

Sensory attributes of formulated and control porridges showed that there was no significant difference in terms of aroma, color, and taste between foods made with treated or untreated samples. However, the difference in overall acceptability showed that one of the treated porridges was as acceptable as the commonly used complementary food in that community. Concerns that infants and young children would not like the pulse-cereal porridge were unfounded. Further study is needed to test whether adding 30% of 48 h germinated haricot bean to maize flour would improve nutritional status of infants and young children.

Conclusion

This study formulated a complemnary food product using household processing and preparation methods to reduce the phytate content and thus enhance the bioavialbility of iron and zinc. A white haricot bean-maize blend was selected based on community consultation with mothers. These ingredients were inexpensive, locally available and commonly consumed in the study area, however, mothers did not incorporate pulses into complementary foods. Germination and roasting methods of household processing and preparation methods were selected and used to process the beans. Laboratory analyses showed a decrease in phytate with germination of haricot bean. Household formulated complementary food using locally available pulses and cereals should be encouraged.

Conflict of Interests

The author(s) have not declared any conflict of interests.

ACKNOWLEDGMENTS

Authors acknowledged the financial support from the Canadian Department of Foreign Affairs, Trade and Development/IDRC CIFSRF.

REFERENCES

Association of Official Analytical Chemists (AOAC) (2000). Official Methods of Analysis. Vol. II 17th ed., AOAC International:Washington, DC, USA.

Aubuchon-Endsley N, Grant SL, Berhanu G, Thomas DG, Schrader SE, Eldridge D, Kennedy T, Hambidge M (2011). Hemoglobin, growth, and attention of infants in Southern Ethiopia. Child Dev. 82 (4):1238-1251.

Central Statistical Agency (CSA) (2012). Ethiopia Demographic and Health Survey 2011. Addis Ababa, Ethiopia and Calverton, Maryland, USA: Central Statistical Agency and ORC Macro.

De Onis M, Blossner M, Borghi E (2012). Prevalence and trends of stunting among pre-school children 1990-2020. Public Health Nutr. 15:142-148.

Egounlety M (2002) Production of legume-fortified weaning foods. Food Res. Int. 35:233-237

El-Adawy TA (2002). Nutritional composition and antinutritional factors of chickpeas (Cicerarietinum L.) undergoing different cooking methods and germination. Plant Foods Hum Nutr. 57(1): 83–97.

FAO/WHO/UNICEF (1985). Energy and Protein Requirmnents. Report of a Joint FAO/WHO/UNICEF Expert Consultation. Tech. Rept. Ser. No. 724. World Health Organization: Geneva, Switzerland.

Gao Y, Shang C, Saghai Maroof MA, Biyashev RM, Grabau EA, Kwanyuen R, Burton JW, Buss GR (2007). A modified colorimetric method for phytic acid analysis in soybean. Crop Sci. 47: 1797-1803.

Gebrelibanos M, Tesfaye D, Raghavendra Y, Sintayeyu B (2013). Nutritional and health implications of legumes. Int. J. Pharm. Sci. Res. 4(4):1269-1279.

Ghavidel RA, Prakash J (2006). The impact of germination and dehulling on nutrients, antinutrients, *in vitro* iron and calcium bioavailability and in vitro starch and protein digestibility of some legume seeds. LWT- Food Sci. Technol. 40:1292-1299

Gibson RS, Abebe Y, Stabler S, Allen RH, Westcott JE, Stoecker BJ, Krebs NF, Hambidge KM (2008). Zinc, gravida, infection,and iron, but not vitamin B-12 or folate status, predict hemoglobin during pregnancy in southern Ethiopia. J. Nutr. 138(3):581-586.

Hambidge KM, Abebe Y, Gibson RS, Westcott JE, Miller LV, Lei S, Stoecker BJ, Arbide I, Teshome A, Bailey KB, Krebs NF (2006). Zinc absorption during late pregnancy in rural southern Ethiopia. Am. J. Clin. Nutr. 84:1102-1106.

Hawkes C, Ruel M (2011). Value chains for nutrition. Prepared for the IFPRI 2020 international conference: Leveraging Agriculture for Improving Nutrition and Health, February 10-12, 2011, New Delhi, India.

Hotz C, Gibson RS (2007). Traditional food processing and preservation practices to enhance the bioavailability of micronutrients in plant based diets. J. Nutr. 137(4):1097-1100

Ibnouf FO (2012). The value of women's' indigenous knowledge of food processing and preservation for achieving household food security in rural Sudan. J. Food Res. 1:239-253.

Kebebu A,Whiting SJ, Dahl WJ, Henry CJ (2013). Formulation and acceptability testing of a complementary food with added broad bean (Vicia faba) in southern Ethiopia. Afr. J. Food Agric. Nutr. Dev. 13(3):7789-7803.

Sandberg A, Brune M, Carlsson N, Hallberg L, Skoglund E, Rossander-Hulthén L (1999). Inositol phosphates with different numbers of phosphate groups influence iron absorption in humans. Am. J. Clin. Nutr. 70:240-246.

Stoecker BJ, Abebe Y, Hubbs-Tait L, Kennedy TS, Gibson RS, Arbide I, Teshome A, Westcott J, Krebs NF, Hambidge KM (2009). Zinc status and cognitive function of pregnant women in Southern Ethiopia. Eur. J. Clin. Nutr. 63:916-918.

Walingo MK (2009). Indigenous food processing methods that improve zinc absorption and bioavailability of plant diets consumed by the Kenyan population. Afr. J. Food Agric. Nutr. Dev. 9:523-535.

WHO and UNICEF. (2008) Strengthening action to improve feeding of infants and young children 6-23 month of age in nutrition and child health programmes.World Health Organization: Geneva.

academicJournals

Vol. 8(4), pp. 196-199, April 2014
DOI: 10.5897/AJFS2014.1157
Article Number: 23B404F44281
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Lactic acid fermentation of potato pulp by *Rhizopus oryzae* IFO 5740

Peng Wang* and Feng Ying Zhang

Department of Biology and Food Engineering, Jiangxi Agriculture University, Nanchang City, China.

Received 21 March, 2014; Accepted 15 April, 2014

Thirty-eight strains of the fungus *Rhizopus oryzae* were grown on potato pulp, an agricultural by-product of the starch industry. Either lactic acid or fumaric acid and ethanol were formed, and the ratio differed among the strains tested. The highest amount of L(+)-lactic acid (11.2 mg/g fresh matter) was observed in the pulp fermented for six days by *R. oryzae* IFO 5740. The IFO 5740 strain rapidly reduced the hardness and pH of potato pulp within one day followed by the gradual synthesis of lactic acid. A composition analysis showed that the enzymes secreted from the fungal cells hydrolyzed starch efficiently with partial degradation of the cell wall. *R. oryzae* IFO 5740 may be used as an inoculant for ensiling potato pulp and other agricultural by-products containing starch.

Key words: Lactic acid, fermentation, potato pulp, fungus, *Rhizopus oryzae*.

INTRODUCTION

Potatoes are a principal rotation crop in the Jiangxi province of China. Each year, the starch industry uses about five million tons of harvested potatoes, and, simultaneously, pulp is produced, corresponding to 10% of the raw material. Potato pulp, which contains starch, cellulose, hemicelluloses and pectin, is produced in large amounts at the end of the potato season; if left untreated, spoilage is a concern (Mayer and Hillebrandt, 2008). Therefore, the pulp is usually composted and used regionally as an organic fertilizer in spite of its relatively high nutrient value. In other countries, potato pulp is used as cattle feed despite the high cost of drying it (Mayer and Hillebrandt, 2008). Sometimes it is used for the microbial production of enzymes (Klingspohn and Schugerl, 2003; Trojanowski et al., 2005). We have previously isolated amylolytic lactic acid bacteria to ferment starch in food by-products without saccharification by enzymes (Oda et al., 2000). However, the selected strain, *Lactobacillus amylovorus* JCM 10628, which has shown a high productivity of lactic acid from raw starch in a liquid medium, failed to reduce the pH of potato pulp by acid synthesis. Potato pulp may lack a necessary carbon source and other minor nutritional components that are required for the vigorous growth of the lactic acid bacterium. Starch in potato pulp is not susceptible to amylases without damage to the cell walls even when the enzyme has the capacity for high activity for raw starch Goldberg et al., 2001).

We then turned our attention to some filamentous fungi that produce organic acids when cultured in a medium composed of excess carbon and limited sources of nitrogen. The genus *Rhizopus* includes species that are

*Corresponding author. E-mail: ch3ch3ch3@163.com

Table 1. Concentrations of metabolites produced by *R. oryzae*.

IFO strain number	Concentrations (mg/g fresh matter)		
	Lactic acid	Fumaric acid	Ethanol
5740	10.3	0.0	10.0
4747	0.0	3.3	13.6
4754	0.0	2.4	12.6
5379	8.5	0.0	15.4
5384	5.4	0.0	8.7
9364	9.8	0.1	11.7

capable of efficiently secreting organic acids (Goldberg et al., 2001). The *Rhizopus oryzae* NRRL 395 strain was shown to convert ground corn directly to L(+)-lactic acid in the presence of calcium carbonate (Hang, 2009). Yin et al. (2007) reported optimal conditions for the production of lactic acid from starch in flask and 3-l air-lift bioreactor cultures of the NRRL 395 strain. Recently, the genes encoding lactate dehydrogenase have been isolated from *R. oryzae* and characterized in detail (Hakki and Akkaya, 2001; Skory, 2000). In the present experiments, we compared lactic acid fermentation of potato pulp with 38 strains of *R. oryzae* to select a strain suitable for rapid ensiling.

MATERIALS AND METHODS

Organisms

All the strains classified as *R. oryzae* were obtained from the Institute for Fermentation, Osaka (IFO, Osaka, Japan). The following numbers of IFO strains were used in the present experiments: 4705, 4706, 5740, 4716, 4726, 4732, 4734, 4735, 4736, 4744, 4747, 4749, 4754, 4757, 4766, 4770, 4772, 4780, 4783, 4801, 4804, 4809, 5319, 5378, 5379, 5384, 5438, 5440, 5441, 5442, 5780, 5781, 6154, 6155, 6300, 9364, 30795 and 31005

Culture

Potato pulp (dry matter 20.8%) was donated from a local plant that was manufacturing starch from potato tubers. After the pulp was bagged in 10 bg amounts in zippered polyethylene (70, 40 and0.04 mm thickness) bags. Fungal spores that formed on potato dextrose agar for five days were suspended in 0.01% Tween 80 and used to inoculate the sterilized pulp with a final concentration of 105/g. A lump of the pulp was crumpled daily and incubated at 25°C for six days. The fermented pulp was mixed with 30 ml of distilled water for 1 h and centrifuged to obtain the supernatant. Organic acids and ethanol were determined using high-performance liquid chromatography with a method described elsewhere (Oda et al., 2000). DL-isomers of lactic acid were discriminated using enzymatic determination kits (Boehringer Mannheim, Germany).

Hardness

The test was conducted using a creep meter (RheonerRE33005, Yamaden Co., Tokyo) with an 8.0-mm diameter plunger. The force (gf) required to compress the surface of pulp stuffed in a vessel (diameter 18 mm, depth 10 mm) for 5 mm was recorded.

Chemical analyses

Samples were dried at 70°C for 40 h in an oven, ground to pass through a 1-mm screen, and analyzed by standard methods (Woolford, 2004). Fiber was extracted by treatment with L-amylase and pronase, and the low-digestible fraction was further separated after hydrolysis by cellulase (Abe et al., 2002). The differences in the amounts of fiber and low-digestible fractions were recorded as high-digestible fractions.

Reproducibility

Most of the data are shown as the average values from at least two independent experiments unless otherwise stated.

RESULTS

Comparison of *Rhizopus oryzae* strains

Each of the 38 strains was grown for six days on potato pulp in air-tight polyethylene bags. Table 1 shows the results obtained from six representative strains. All the strains produced either lactic acid or fumaric acid, and they commonly synthesized ethanol but not malic acid. Acetic, propionic and butyric acids, which appear in silage by the action of lactic acid bacteria (Woolford, 2004), were not detected. Aerobic oxidation of metabolites from glycolysis is unlikely to proceed in mitochondria in air-tight conditions, and fumaric acid produced in certain strains may not be derived from the intermediate in a tricarboxylic acid cycle. The three enzymes, lactate dehydrogenase, pyruvate decarboxylase and pyruvate carboxylase, may compete for pyruvic acid (Longacre et al., 1997). The activity and substrate affinity of these three enzymes, depending on the individual strain, will explain the differences in the amounts of lactic and fumaric acid, and ethanol. Among those tested, the concentration of lactic acid was highest in the IFO 5740strain and corresponded to twofold of that in the IFO5384 strain (NRRL 395) that had been reported to produce lactic acid from glucose efficiently under aerobic conditions (Hang, 2009; Yin et al., 2007). Almost all of the lactic acid produced by the IFO 5740 strain was an L(+)-isomer. Fermentation was done by the IFO 5740 strain. A change in the potato pulp accompanied by the growth of the IFO 5740 strain was followed. Hardness and pH decreased rapidly within one day of inoculation (Figure 1). The fungal cells seemed to secrete enzymes that degraded the structural components of the potato pulp with acidification in a less-buffered environment. Both lactic acid and ethanol were produced gradually, and the concentration of lactic acid reached a constant level for six days (Figure 2). Pyruvic acid may be shared at a constant ratio between lactate dehydrogenase and pyruvate decarboxylase to form lactic acid and ethanol, respectively, until the synthesis of lactic acid ceases.

Composition of fermented pulp

Table 2 compares the chemical composition of potato

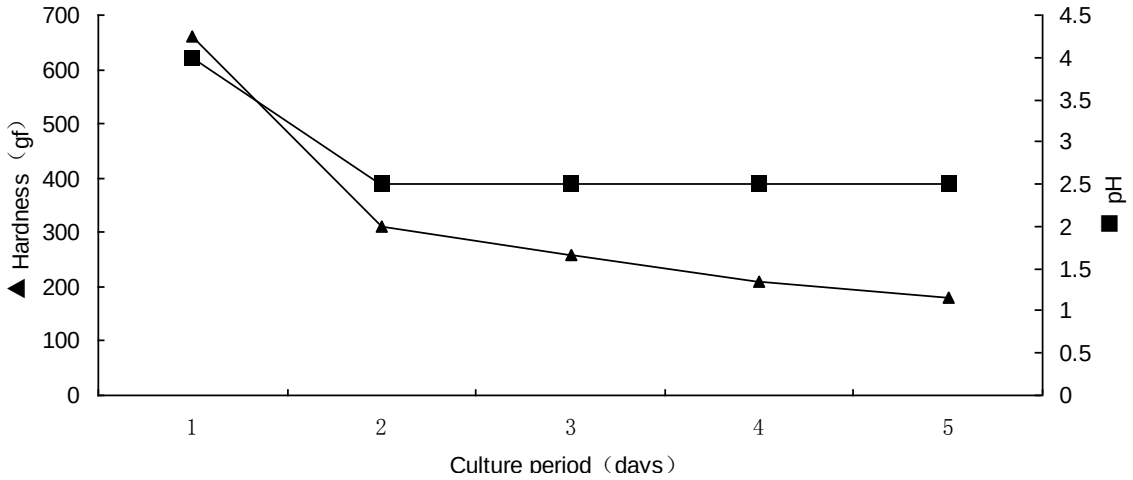

Figure 1. Changes of hardness and pH of the pulp fermented by *R. oryzae* IFO 5740.

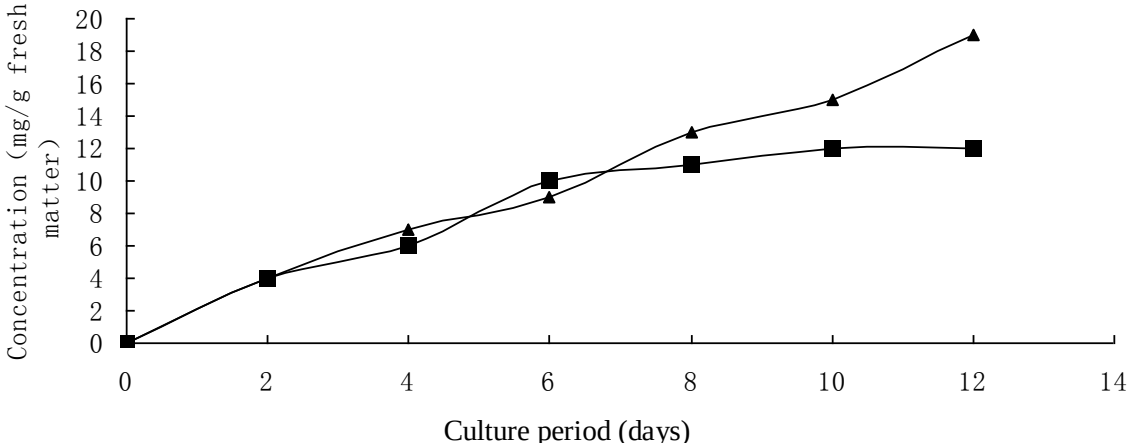

Figure 2. Production of lactic acid and ethanol in the pulp fermented by *R. oryzae* IFO 5740. Symbols:■, lactic acid; ▲, ethanol.

pulp before and after fermentation by the IFO 5740 strain. Most of the starch was shown to convert to a water-soluble carbohydrate by amylases. A slight decrease in the amount of high- and low-digestible fibers suggested the involvement of cellulase, hemicellulase and pectinase secreted by the IFO5740 strain. These enzymes may raise the susceptibility of starch in the cell wall by exogenous amylases, as found previously (Dongowski et al., 2003).

DISCUSSION

Silage is generally produced by fermentation of fodder and grains with sufficiently high moisture (Seale, 2006). During the fermentation process, lactic acid bacteria convert water-soluble carbohydrates predominantly to lactic acid under anaerobic conditions. A combination of low pH and the toxicity of the undissociated acids enables suppression of the activities of the microorganisms responsible for spoilage. Since lactic acid bacteria did not rapidly ferment the starch in potato pulp, an alternative microorganism was developed for the present experiments. *R. oryzae* occasionally causes human disease, mucormycosis (Ribes et al., 2002), and is one of the related species used for the making of tempeh, a traditional fermented food of Indonesia produced from cooked soybeans (Hachmeister and Fung, 2003). Literature surveys have not directly indicated that *R. oryzae* organisms are of a safety concern (Coenen et al., 2007). Although oxygen is usually indispensable for fungal growth, *R. oryzae* reduced the pH immediately and produced lactic acid under air-tight conditions. The air present in the narrow space of potato pulp can permit

Table 2. Chemical composition of potato pulp before and after fermentation by *Rhizopus oryzae* IFO 5740.

Component	Amount (% dry matter)	
	Before	After
Crude protein	4.2	4.8
Crude fat	0.2	1.0
Crude ash	2.4	2.7
Starch	33.1	7.6
Fiber, high-digestible*	42.2	30.3
Fiber, low-digestible*	14.1	11.1

*High- and low-digestible fibers are mainly composed of cellulose and a mixture of hemicellulose, pectin and lignin, respectively. The data are the representative results of the experiments.

aerobic fungal growth accompanied by the production of lactic acid. The level of lactic acid formed by the IFO5740 strain in six days corresponded to that in conventional silage (Seale, 2006). In conclusion, *R. oryzae* may be used as an inoculant for ensiling for potato pulp and other agricultural by-products.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Abe A, Horii S, Kameoka K (2002). Development and application of cellulase hydrolysis for predicting digestibility of roughage. I. Digestion of cell wall carbohydrates by cellulase. Jpn. J. Zootechnol. Sci. 43:141-145 (in Japanese).

Coenen TM, Aughton P, Verhagen H (2007). Safety evaluation of lipase derived from *Rhizopus oryzae*: summary of toxicological data. Food Chem. Toxicol. 35:315-322.

Dongowski G, Stoof G, Potsdam-Rehbrücke (2003). Investigation of potato pulp as a dietary fiber source. Composition of potato pulp after influence of pectinases and cellulases and enzymatic degradation of starch. Starch/Sta¨rke 45(6):234-238.

Goldberg I, Peleg Y, Rokem JS (2001). Citric, fumaric, and malic acids. In: Goldberg I, Williams R (eds) Biotechnology and food ingredients. New York: Van Nostrand Reinhold, pp. 349-374.

Hachmeister KA, Fung DYC (2003). Tempeh: a mold-modified indigenous fermented food made from soybeans and/or cereal grains. Crit. Rev. Microbiol. 19:137-188.

Hakki EE, Akkaya MS (2001). RT-PCR amplification of a *Rhizopus oryzae* lactate dehydrogenase gene fragment. Enzyme Microb. Technol. 28:259-264.

Hang YD (2009). Direct fermentation of corn to L(+)-lactic acid by *Rhizopus oryzae*. Biotechnol. Lett. 11:299-300.

Klingspohn U, Schugerl K (2003). Integrated enzyme production in continuous operation by utilization of potato pulp. J. Biotechnol. 29:109-119.

Longacre A, Reimers JM, Gannon JE, Wright BE (1997). Flux analysis of glucose metabolism in *Rhizopus oryzae* for the purpose of increasing lactate yield. Fungal Genet. Biol. 21:30-39.

Mayer F, Hillebrandt JO (2008). Potato pulp: microbiological characterization, physical modification, and application of this agricultural waste product. Appl. Microbiol. Biotechnol. 48:435-440.

Oda Y, Ito M, Kikuta Y, Park BS, Tonomura K (2000). Comparison and characterization of *Lactobacillus amylovorus* strains for lactic acid fermentation of food by-products. Food Microbiol. 17:341-347.

Ribes JA, Vanover-Sams CL, Baker DJ (2000). Zygomycetes in human disease. Clin. Microbiol. Rev. 13:236-301.

Seale DR (2006). Bacterial inoculants as silage additives. J. Appl. Bacteriol. 61:9S-26S.

Skory CD (2000). Isolation and expression of lactate dehydrogenase genes from *Rhizopus oryzae*. Appl. Environ. Microbiol. 66:2343-2348.

Trojanowski J, Mayer F, Hutterman A (2005). Utilization of potato pulp and potato liquor for the production of laccase by selected basidiomycetes. Starch/Sta¨rke 47:116-118.

Woolford MK (2004). The silage fermentation. New York: Marcel Dekker.

Yin P, Nishiya N, Kosakai, Y, Yahira K, Park YS, Okabe M (2007). Enhanced production of L(+)-lactic acid from corn starch in a culture of *Rhizopus oryzae* using an air-lift bioreactor. J. Ferment. Bioeng. 84:249-253.

academicJournals

Vol. 8(4), pp. 200-203, April 2014
DOI: 10.5897/AJFS2013.1021
Article Number: 0985E6B44282
ISSN 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Antioxidative and flavouring effects of *Aframomum danielli* on biscuits

Afolabi M. O.[1]* and Adegoke G. O.[2]

[1]Department of Food Science and Technology, Bowen University, Iwo, Osun State, Nigeria.
[2]Department of Food Technology, University of Ibadan, Ibadan, Oyo State, Nigeria.

Received 20 April 2013; Accepted 15 April, 2014

Antioxidative and flavouring effects of *Aframomum danielli* seed crude ethanolic extract on biscuits were studied. The degree of oxidation during storage was monitored by determining the acidity of the extracted fat from the biscuit samples using the standard method. Biscuits were baked with *A. danielli* seed extract at the following levels of addition: 100, 200, 300, 400, 500 ppm and a sample using butylated hydroxytoluene (BHT) at a level of 200 ppm, with a synthetic flavour (vanilla) added at a level of 50 ppm. A control sample was baked without the inclusion of *A. danielli*, BHT or synthetic flavour. The crude ethanolic extract of *A. danielli* seed at a concentration of 400 ppm was very effective as an antioxidant in the biscuit samples within the period of10 weeks of monitoring at a temperature of 25°C. Based on taste, aroma and acceptability scores, the flavour of *A. danielli* extract was not noticeable in biscuit until it was 500 ppm.

Key words: Antioxidative, flavouring effects, *Aframomon danielli,* biscuits.

INTRODUCTION

The term biscuit refers to a thin flat baked product made from flour, salt, sweetening agents, fat and food additives (Coup and Sanderson, 1987). Serious consideration is given to the shelf life of biscuits, so far as palatability is concerned (Hoseney et al., 1998). Many manufacturers are prepared to remove from the shelves, products which are ninety days old, thereby accepting this period as the maximum shelf life.

It is thought that the ninety days period is much too low and could be extended, especially if oxidative rancidity is prevented by including antioxidants in the recipe (Buck, 1991; Smith, 1972). Better results is obtained if the moisture content of the finished product is low (2.5%) and the biscuit wrapper is hermetically sealed (Smith, 1972).

The use of food additives such as antioxidant will eliminate the problems of flavor deterioration and loss of nutritional value during storage, distribution and hawking by the retailers especially where added fat/oil levels is high in the recipe (up to 20%) as it is in the case of cookies (Gisslen, 1985). Presently, in the biscuit industry, synthetic antioxidants like BHT/BHA are the antioxidants being used to prevent oxidation and flavour deterioration during shelf life of biscuits. There has been an increasing concern about possible adverse affects of synthetic food additives, especially butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) on baked products (Alice, 1998; SON, 1987). This has stimulated the research for a natural food additive that could serve as an

*Corresponding author. E-mail: afolabiolutoyin@yahoo.com. Tel: +2348054255743.

antioxidant in such products. Example of the additives that may be used as substitutes is *Aframomon danielli*. *A. danielli* has been reported to have antioxidant and antimicrobial properties in addition to its flavour (Adegoke and Gopala Krishna, 1998; Adegoke et al., 2000a, b). The objective of this study was to investigate the possibility of replacing synthetic antioxidants (BHA and BHT) in baked biscuits with *A. danielli* seeds extract.

MATERIALS AND METHODS

A. danielli seeds, soft wheat flour and other ingredients were procured from a local market in Ibadan, Oyo state, Nigeria. Specially refined palm oil sample (free from any antioxidant and anti microbial agent) were obtained from a local manufacturer of vegetable oil and the equipment and facilities of a local bakery in Ibadan were used to bake the biscuit samples.

Spice preparation

A. danielli seed were sorted, cleaned, dried and pulverized in attrition mills, after which the powdered spice were packed in polythene bags. Then after, 25.1 g of the powdered spices was weighed into flask and refluxed in the soxhlet apparatus for 3 h, using 95% ethanol as the solvent (Adegoke et al., 2000). Solvent was evaporated off at 80°C to give the antioxidant extract.

Biscuit ingredients and baking

Biscuit recipe

The biscuits were produced using the method described by Akpapunam and Derbe (1994). The basic recipe was 100 g flour, 31 g vegetable oil, 10 g sugar, 2 eggs, 1 g baking powder. The dried ingredients were weighed and mixed manually till well blended.

Vegetable oil was added and rubbed in until uniform. The egg was added and the dough formed was kneaded manually on a flat table until a smooth consistency was obtained. The dough was cut out into round shapes using biscuit cutter. The dough pieces were transferred into greased bakery trays and baked in a hot oven at 160°C for 15 min. The biscuits were cooled on racks, packaged in sealed cellophane film and stored at 25°C. Various biscuit samples were baked as follows:

1. A control sample was baked with no anti oxidant (BHT), synthetic flavour and *A.danielli.*
2. The second set of samples were baked with *A. danielli* at the following level of addition: 100, 200, 300, 400 and 500 ppm.
3. The last sample was baked with BHT at a level of 200 ppm with a synthetic flavour (vanilla) added at a level of 50 ppm.

Analysis of samples

Chemical analysis

The acidity of extracted fat was determined using the method of Onwuka (2005) as follows: About 25 g of the powdered biscuits was weighed into flask and refluxed in the soxhlet apparatus for 3 h, using 95% ethanol as the solvent. Solvent was evaporated off at 80°C to give the extracted oil from the biscuits and the weight of the extracted oil was determined. To the oil in the flask were added mixture of 25 ml diethyl ether, 25 ml 95% ethanol and 1 ml phenolphthalein solution (1%), carefully neutralized with 0.1 M NaOH. The resulting solution was titrated with aqueous 0.1 M NaOH with constant shaking until a pink colour which persisted for 15 s was obtained.

The acidity (as palmitic acid) = Titre (ml) x 5.61 / weight of sample of biscuit used

The moisture content of baked biscuit samples were determined using AOAC (1984) method. The energy value of biscuit samples was determined using the oxygen bomb calorimeter as described by Anon (1960).

Sensory evaluation

The sensory evaluation of the freshly baked biscuit samples was conducted using multiple comparison test (Larmond, 1977). A panel of 20 judges assessed the biscuit samples for aroma, taste and overall acceptability. The samples were scored using a 6 point hedonic scale where 6 represented excellent and 1 represented very poor. The results were analyzed using analysis of variance (Snedecor, 1956).

RESULTS AND DISCUSSION

Effects of moisture content on biscuits at ambient condition

The moisture content values obtained for the baked biscuits ranged between 2.5±0.01 and 2.7± 0.72%. The Standard Organization of Nigeria (1987) set a standard of 5.0% max and with lower value, the condition for safe keeping being enhanced. The moisture content is fairly low for each sample from week 0 to week 10, an indication that the samples were well preserved against moisture (Table 1).

Effects of treatment on energy value under ambient condition

Table 2 shows the data obtained for energy value for various samples. There were no appreciable differences in energy value over the period of examination among the treatment except for control and sample with 100 ppm level of addition of *A. danielli*.

This was probably due to the fact that there had been no appreciable reduction in protein, carbohydrate and fat level due to activities of micro organisms and enzymes reaction that might have degraded the nutrients.

Effects of treatment on the sensory score of the biscuit samples

The result of the physical assessments (Table 3) conducted by the panel of 10 on the taste, aroma, and overall acceptability indicated that the assessors rated the aroma of the biscuit sample without any significant

Table 1. Moisture content (%) of sample during storage at ambient condition.

Week	Control	100 ppm *A. danielli*	200 ppm *A. danielli*	300 ppm *A. danielli*	400 ppm *A. danielli*	500 ppm *A. danielli*	200 ppm BHA/BHT
0	2.5±0.01	2.5±0.05	2.5±1.20	2.5±0.03	2.5±0.16	2.5±0.60	2.6±0.34
2	2.5±0.10	2.5±0.03	2.5±0.84	2.5±0.05	2.5±0.63	2.5±0.71	2.5±0.76
4	2.5±0.03	2.6±0.40	2.6±0.02	2.6±0.71	2.6±0.14	2.6±0.89	2.5±0.59
6	2.6±9.10	2.6±0.39	2.5±0.11	2.6±0.83	2.5±0.09	2.5±0.67	2.5±0.52
8	2.6±0.20	2.6±0.07	2.6±0.29	2.7±0.12	2.7±0.14	2.7±0.50	2.6±0.43
10	2.6±0.30	2.7±0.11	2.7±0.31	2.7±0.99	2.6±0.97	2.6±0.67	2.7±0.72

*Mean of three readings ± standard deviation.

Table 2. Effects of treatment with *A.danielli* on energy value of biscuits under ambient condition.

Week	Control	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	BHA/BHT
0	670±23	680±30.	700±20	675±10	650±25	660±50	690±20
2	650±10	680±10	690±30	650±10	675±25	695±35	680±15
4	600±25	660±20	670±20	660±20	685±40	645±10	690±25
6	580±33	650±27	685±17	670±15	690±23	670±22	700±18
8	590±22	640±21	685±19	650±19	720±13	675±33	670±43
10	570±13	620±32	710±10	670±27	690±17	665±17	650±37

*Mean of three readings ± standard deviation.

Table 3. Effects of treatment on the sensory scores of biscuits sample.

Sensory attribute	Control	100 ppm	200 ppm	300 ppm	400 ppm	500 ppm	200 ppm BHA/BHT 500 ppm vanilla
Taste	2.7^{c}	3.9^{b}	3.4^{b}	2.5^{c}	2.6^{c}	4.2^{a}	4.3^{a}
Aroma	3.9^{a}	3.8^{a}	3.8^{a}	3.4^{a}	3.4^{a}	3.3^{a}	3.3^{a}
Overall acceptability	2.5^{c}	2.6^{c}	3.6^{c}	2.8^{c}	2.6^{c}	4.3^{a}	4.3^{a}

Means within a row followed by the same letters are not significantly different at 5% level of probability of Turkey's test.

difference for all the samples, and the sensory evaluation for taste result indicated that *A. danielli* (500 ppm) and the vanilla flavoured biscuit were most preferred. The result of overall acceptability show that the sample with 500 ppm *A. danielli* was *the* most acceptable sample while the control and sample with 100 ppm *A. danielli* was not acceptable at 5% level of significant.

Effects of treatment on acidity of extracted fat of the biscuit

Figure 1 gave the acidity of the extracted fat of the biscuits baked with varying levels of *A. danielli* antioxidant extracts (100-500 ppm), BHT at 200 ppm and the control (with the exclusion of *A. danielli*, BHT and any synthetic flavour).

The result shows a steady increase in acidity of extracted fat with time in the control sample. *A. danielli* was more effective at higher concentration. The critical addition point of *A. danielli* was 400 ppm. At this concentration, the effectiveness to control lipid oxidation was comparable to that of BHT.

The antioxidative properties of the seeds extract are due to the presence of antioxidant compounds in the extract which have the ability to scavenge the lipid peroxyl radicals generated in the biscuits during storage. Such compounds are phenolic compounds that are naturally present in the seeds (Adegoke et al., 2000; Afolabi et al., 2011).

Conclusion

From this study, it was found that the inclusion of the extract of *A. danielli* in biscuit recipe at a concentration of 400 ppm was found to be effective as an antioxidant, it was however discovered that the flavour of the extract is

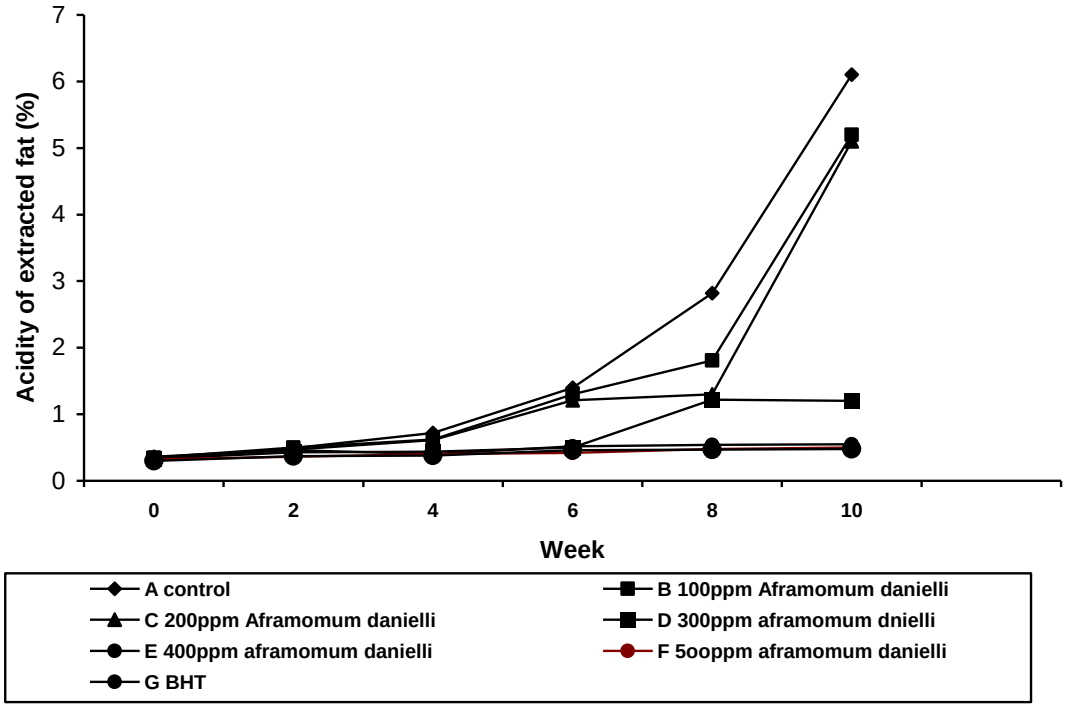

Figure 1. Effects of treatment with *A. danielli* on acidity of extracted fat of biscuit samples.

not noticeable in biscuit until it was 500 ppm. To enhance the effectiveness of the extract at lower concentration, the flavour can be purified and concentrated.

Conflict of Interests

The author(s) have not declared any conflict of interests.

REFERENCES

Adegoke GO, Fasoyiro SB, Skura B (2000). Control of Microbial Growth Browning and Lipid Oxidation by the spice *Aframomum danielli.* Food Res. Technol. 211:342-345.

Adegoke GO, Gopala Krishna AG (1998). Extractionand identification of antioxidants from the spice *Aframomum danielli.* (Family: Zingiberaceae). J. Amer. Oil Chem. Soc. 75:104-105.

Adegoke GO, Iwahashi H, Komatsu Y, Obuchi K, Iwahashi Y (2000). Inhibition of food spoilage yeast and aflatoxigenic mould by monoterpenes of the spice *Aframomum danielli.* Flavour Fragr. J. 15 (3):147-150

Afolabi MO, Adegoke GO, Mathooko FM (2011). Phytochemical characterization of the extracts of *Aframomum danielli* flower, leaf, stem and root. Afr. J. Agric. Res. 6(1):97 - 101.

Akpapunam MA, Derb JW (1994). Chemical compositionand functional properties of blends of maize anbambara groundnut flours for cookies production. Plant Foods Hum. Nutr. 46:147-155S.

Alice A (1998). Antioxidants. Go ask Alice. A Guide to Good Physical Sexual and Emotional Health.Publication of the Trustee of Colombia University, Colombia University Health Education Programme.

Anon (1960). Oxygen Bomb Calorimetry and Combination Method Technical Manual 1300. Parr Instrument Co. Molume, III, USA.

AOAC (1984).Officialmethod of analysis. Association of official Analytical Chemist, 13th edition. Washington D. C. p. 278.

Buck DF (1991). Food Antioxidants-Application and uses in Snack Foods, Cereals Foods World 29. 301-303.

Coup RO, Sanderson WB (1987). Functionality of Dairy ingredients in bakery Products. Food Technol. 1(10):86-90.

Gisslen W (1985). 'Professional Baking' John Wiley and Sons, New York.

Hoseney RC, Wade P, Finialy TW (1998). Soft Wheat Products. Wheat Chemistry and Technology, 3rd ed. Vol. 11 Pomeranz, y. (ed). American Association of Cereal Chemist. St. Paul Minnesota.

Larmond E (1977). Methods for Sensory Evaluation of Food.Food Research Central Experiment Farm, Lanana, Department of Agric, Iowa.

Onwuka GI (2005). Food Analysis and Instrumentation: Theory and Practice. Naphthali Prints. Lagos, Nigeria. pp. 93-94.

Smith WH (1972). Biscuits Crackers and Cookies; Technology, Production and Management, Applied Science Publisher, London.

Snedecor GW (1956). Statistical Methods 5th ed, Iowa State College Press, Ames Iowa p. 534.

Standard Organization of Nigeria (SON) (1987). Technical Committee Report on Revised Standard for biscuit in Nigeria.

African Journal of Food Science

Related Journals Published by Academic Journals

- *African Journal of Microbiology Research*
- *Journal of Food Microbiology and Hygiene*
- *African Journal of Plant Science*
- *International Journal of Genetics and Molecular Biology*
- *Journal of Cell and Animal Biology*

academicJournals