OPEN ACCESS

African Journal of Food Science

September 2018
ISSN 1996-0794
DOI: 10.5897/AJFS
www.academicjournals.org

ACADEMIC JOURNALS
expand your knowledge

ABOUT AJFS

The African Journal of Food Science (AJFS) (ISSN 1996-0794) is published 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.

Contact Us

Editorial Office: *ajfs@academicjournals.org*

Help Desk: *helpdesk@academicjournals.org*

Website: *http://www.academicjournals.org/journal/AJFS*

Submit manuscript online *http://ms.academicjournals.me/*

Editors

Editorial Board Members

African Journal of Food Science

Table of Content: Volume 12 Number 9 September 2018

ARTICLES

Vol. 12(9) pp. 210-215, September 2018
DOI: 10.5897/AJFS2018.1710
Article Number: 0782A3558055
ISSN: 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Temperature effects on cooked food in the tropics: A case study of local soups in Abraka, Delta State, Nigeria

Nwagbara M. O.[1*], Obisesan A.[2] and Ozabor F.[2]

[1]Department of Soil Science and Meteorology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria.
[2]Department of Geography and Environmental Management, University of Port-Harcourt, Port Harcourt, River State, Nigeria.

Received 6 March, 2018; Accepted 23 May, 2018

This study examined the effects of temperature on cooked food in Abraka, Delta State, Nigeria. It utilized the survey research design. Primary data about cooked food conditions between hours of reheating were generated from women in the area using copies of checklist. Temperature data were collected for a period of one year from Department of Geography and Regional Planning, Delta State University, Abraka. The data were analyzed using analysis of variance (ANOVA) and Pearson's product moment correlation (PPMC). Results indicate that, incidents of soup souring were related to temperature at $p < 0.05$, although the relationship was direct ($r = 0.72$), revealing that as temperature increases soup sours or as temperature reduces soup taste was sustained longer. Again, melon, banga, and owo soups went sour within 6 h of previous heating as temperature reaches 31°C, while, vegetable, okra, and ogbono soups stayed beyond 6 h at 31°C. The ANOVA revealed that, there was a significant difference in soup sour incidence at $p < 0.05$ ($F = 274$) at different seasons (December, January, and February [DJF], May, June, and July [MAM], June July and August [JJA], September, October, and November [SON]) of the year thereby emphasizing the effects of weather on soup behavior after cooking. Based on these findings and dangers that soured soup can cause to human health and the loss of money, there should be proper kitchen ventilation in the absence of refrigerator and/or power supply. Choice of soup to cook should also be made with utmost consideration of prevailing air temperature in the tropics where air temperature is generally high.

Key words: Tropics, local-soup, sour, weather, cooked-food.

INTRODUCTION

Man's survival on earth depends largely, on the quality of food and water available to him for consumption (Dolan et al., 2010). Food and water are basic materials required by man for energy and growth. Food is also required for building up of immune system, which helps the body fight against diseases that may try to attack the human body from time to time (Baxter, 2008). However, food can become a source of poison, when proper cautions were

*Corresponding author. E-mail: monwagbara@yahoo.com.

not taken, before, during and after cooking, due to bacteria activities (Schmutz and Hoyle, 2011).

Generally, there are bacteria everywhere (water, air, and soil) (Dolan et al., 2010; Omaye, 2004; Kotsonis and Burdock, 2008). Therefore, allowing cooked food to stay out of cold environments (>4.5°C) or in warm-hot environment (4.5 to 60.5°C) for a long time may lead to bacterial contamination (USDA, 2011a, b; USFDA, 2009). Such bacteria include *Escherichia coli, Staphylococcus aureus*, and *Salmonella enteritidis* (Schmutz and Hoyle, 2011). Nevertheless, all raw food and vegetable have various levels of bacteria infestation (USFDA, 2010) which are killed or reduced during cooking (USDA, 2011a). Conversely, if the cooked food is not properly preserved and in time, some bacteria that did not die during cooking or those reintroduced during scooping or air infiltration, will be incubated by temperature, and consequently, begins to grow in numbers that are potentially harmful to human health (Schmutz and Hoyle, 2011). A good environment for bacteria to grow is one typical of 4.5 to 60.5°C temperature (Hutt et al., 2007; Jones, 1995; Kotsonis and Burdock, 2008) and it takes about 20 min for bacteria to double in number in such environment. Based on this, USDA (2011b) advised that food must not be left unconsumed after 2 h of cooking or removal from freezers, and if temperature of that environment is up to 32°C, cooked food must be consumed within an hour from cooking or re-heating period.

In the developed world, these above precautions are practicable, not only because of difference in climate characteristics, but also because of stable power supply, which allows for refrigerating cooked food consistently. However, a sharp contrast exists in Nigeria (a developing country), where power supply is very poor or in some cases non-existent. Furthermore, alternative sources of power are very expensive to acquire, since technological advancement is low. Furthermore, the cost of refrigerating sets makes them beyond the reach of many Nigerians. As a result of these, cooked food is mostly left for long hours at the mercy of temperature and other environmental factors of the tropical climate where Nigeria is positioned, thereby leading to contamination of food and consequently food wastage. Because of poverty, some locals still consume such soured foods and end up having some medical complications (Ozabor and Obaro, 2016; Ostertag et al., 2002).

In Abraka, the Benin Electric Distribution Company (BEDC) supply power on an average of an hour per day, thereby making refrigerating of food after cooking impossible. Local temperature is also high (ranging between 23 and 32°C), thus making reheating of food after cooking to be the only means of its preservation. As a result of economic activities engaged in by locals, food is left for up to 6 to 7 h without reheating, as such bacteria are allowed to incubate and multiply. The implication of this is that soups go soured and in most cases are poured away, while in some other cases are still consumed, since some locals are very poor (Efe, 2006). The consequences of these include, triggering some health conditions and exacerbating poverty situation in the area, since the already poor has to spend more money to replace food that had already been cooked. Ironically, to the best of the knowledge of the researchers, no known work exist regarding the subject matter especially in the tropics. This work thus examined effects of temperature on different local soups in Abraka, Delta State, Nigeria.

MATERIALS AND METHODS

Description of the study area

Abraka is an area in Delta State, Nigeria. To the east, the area is bordered by Ukwani Local Government Area, at the west by Edo State, at the north and south by Ethiope River and Ughelli North, respectively. It is situated on latitudes 5° 46' and 5° 48' north of the equator and longitudes 6° 05' and 6° 08' east of the Greenwich meridian. The area extends over 168.4 km^2 (Olomo and Ajibola, 2006). Abraka is located within the tropical environment, based on the classification of Koppen (1918) and Strahler (1965). Monthly temperature ranges between 23 and 32°C, while monthly rainfall ranges between 25.4 and 457 mm (Efe, 2006). The major economic activities of the locals include farming, transportation, trading, service, etc. These activities signify that people will have to leave their homes in the morning to come back in the evening or at night. This coupled with the poor power supply situation in the area implies that, cooked food is left at the mercy of prevailing temperatures, which are hot enough to allow for bacteria incubation, which could affect soup and food tastes in the area.

Study design and sampling technique

The study adopted the survey research design which involved the use of questionnaire in the area (Nwagbara et al., 2017). Copies of check lists were administered to women in the area to generate information about soup conditions after cooking. To be able to achieve proper sample for the study, the total population of registered residential buildings were generated from the archive of Ministry of Lands, Survey, and Urban Development, Oria, Abraka. After which, the Taro Yamane formula was applied to the total registered residential buildings (7643) and a sample size of 380 were derived using alpha 0.05 levels. However, for the purpose of efficient distribution of the check list to respondents, the systematic sampling technique was used to select 1 in every 3 streets in the area. After which 2 copies of the check lists were randomly distributed to households in the area. Furthermore, daily temperature data were obtained from the Department of Geography and Regional Planning, Delta State University, Abraka and for a period of one year (June 1, 2016 to May 31, 2017). The need for this was to help the researchers find out the relationship between outdoor and indoor temperatures. Also, a thermometer was placed in the kitchens of the houses to which checklists were administered. This was done to find out the relationship between outdoor and indoor temperatures and from there find out the relationship between the incidences of soup sour in the various houses.

Analysis of variance (ANOVA) was used to find out the statistical difference between soup tastes both at seasonal and daily intervals. Again, the Pearson's Product Moment correlation (PPMC) was used to determine the statistical relationship between temperature

Table 1. Frequently cooked soups and their ingredients in Abraka.

Soup type	Ingredients
Owo	Cassava flakes, red oil, beef or fish or both, potash, seasoning cubes, water, pepper, salt, crayfish, prawn.
Ogbono	Red oil, beef or fish or both, seasoning cubes, water, pepper, salt, crayfish, prawn, onion, *Irvingia*.
Egusi	Red oil, beef or fish or both, seasoning cubes, water, pepper, salt, crayfish, prawn, onion, melon seeds.
Vegetable	Red oil, beef or fish or both, seasoning cubes, water, pepper, salt, crayfish, prawn, onion, pumpkin leaf, water leaf and *Celosia argentea*
Okra	Red oil (optional), beef or fish or both, seasoning cubes, water, pepper, salt, crayfish, prawn, onion, *Abelmoschus esculentus*
Tomatoes	Red oil or ground nut oil, beef, chicken or fish or both, seasoning cubes, water (optional), curry, pepper, salt, crayfish, prawn, onion, and tomatoes
Banga	Palm fruits, beef, fish (dry of fresh), seasoning cubes, water, curry, pepper, salt, crayfish, prawn, onion

and soup sour incidences.

The basic soups that are cooked at least twice a week by a household in Abraka were determined by a focused group discussion and informal experience of the researchers and they include the following soups: owo, ogbono, egusi, vegetable, okra, tomato and banga. The ingredients used for the soups are listed in Table 1.

RESULTS AND DISCUSSION

In Table 2, egusi soup has the highest cases of soup sour, with 5212 cases. The next to that is owo soup (2231) and then banga soup (1432). However, tomato soup recorded the lowest cases of soup sour in the area. The variation in soured soup cases, center on the variation in preparation techniques, which reduces the activities of bacteria in the soup after preparation. Within the temperature limit for cooked food to get spoilt (4.5 to 60.5°C) as identified by USDA (2011a), only vegetable, okra and tomato soups were able to last up to 6 h or beyond without going bad or changing taste (Table 3). This also reveals that these soups are able to resist high temperature more than other local soups such as egusi, owo, and banga in tropical environments such as Abraka.

Table 4 shows that there is a strong correlation between outdoor and indoor temperatures at $p < 0.05$. Nevertheless, in Table 5, there was a strong relationship ($r = 0.72$) between temperature and cases of soup sour, thus revealing that as temperature increases, soup sours, and conversely as temperature reduces, soup taste is sustained. This finding is also consistent with that of Schmutz and Hoyle (2011) and USDA (2011b), who identified that the rates at which foods go bad after preparation is related to temperature fluctuations.

However, temperature was only able to explain 51.84% of soup sour cases, while leaving the remaining 48.16% possibly to poor cooking techniques, contaminated ingredients, etc. The correlation model was also significant at $P<0.05$, implying that, soup sour cases are significantly related to temperature in Abraka.

Furthermore, the seasonal characterisation of soup sour cases is shown in Table 6. Here, May, June, and July (MAM), is the season with the highest soup sour cases (2649), the next to that, is September, October, and November (SON) season with 2633 cases. June July and August (JJA) recorded the lowest cases of soup sour (1621). It is expedient to state that, these seasonal distributions are not accidental. For example, temperature in Abraka is usually low during JJA which also coincides with the peak of rainy season in the area. Therefore, the heavy rains have a moderating effect on temperature and consequently on the soup sour cases in the area.

Similarly, although December, January, and February (DJF) belong to the dry period, the harmattan winds that blow into the area during the period have a lowering effect on temperature, as such temperature has reduced effect on soup at this time as compared to other periods of the year such as MAM and SON where convective heating exacerbate temperature conditions, which in turn results in high soup sour cases.

Nonetheless, the effects of temperature is established in Table 7, where the ANOVA analysis indicated that there is significant seasonal variation in soup sour cases at $P < 0.05$ in the area.

As shown in Table 8, within the day soup sour cases were significantly different at $p < 0.05$. However, between 7 am and 12 noon, the highest amount (4475) of soup sour cases are recorded, while the lowest is recorded between 8pm and 5am. Reasons advanced for this variation is that in the tropical environments, there exists a sharp or rather steep change in temperature between 6 am and 12 noon and this quickly creates room for bacteria incubation, resulting in the high amounts of soup sour in that period of the day.

Table 2. Reported cases of soup sour incidence.

Soup type	Sour taste incidence
Owo	2231
Ogbono	1067
Egusi	5212
Vegetable	823
Okra	842
Tomato	231
Banga	1432

Table 3. Duration and temperature effect on the different local soups.

Soup type	Temperature range (±)	Average duration (h)
Owo	4.5-32	4
Ogbono	4.5-32	±6
Egusi	4.5-32	≤4
Vegetable	4.5-32	>6
Okra	4.5-32	>6
Tomato	4.5-32	>6
Banga	4.5-32	5

Table 4. Relationship between outdoor and indoor temperatures.

Sample size	Indoor and outdoor temperature (Pearson's)	P value (0.05)
365	0.842	0.00

Table 5. Relationship between temperature and local soups.

Sample size	Soup sour and temperature (Pearson's)	P value (0.05)
365	0.721	0.00

Again, the effects of time of day temperature is shown in Table 9, where the ANOVA analysis indicated that there is significant daily time variation in soup sour cases at $P < 0.05$ in the area.

CONCLUSION AND RECOMMENDATIONS

This study identified the effects of temperature on incidences of soup sour in Abraka, Delta State, Nigeria. Investigations revealed that, egusi soup is the most susceptible to temperature as compared to the other local soups investigated. On a time scale of 1 to 6 h under 4.5 to 32°C, egusi soup mostly went sour in less than 4 h, while okra and tomato soups were able to retain original taste after 6 h of previous heating. Furthermore, soup sour cases were strongly correlated with temperature. This, by extension, established that there is variation in the seasonal occurrence of soup sour, as most cases of soup sour were recorded in the dry season.

However, what is not established in the current study is a laboratory analysis which should tell what types of bacteria and chemical components are involved in the soured soups.

This is a limitation which this study has not handled of which is being suggested for further investigation. Nevertheless, whenever a soup sours, money is lost. This is not a small measure by exacerbating existing poverty conditions in the study area in particular and developing countries of tropics in general if nothing is done to avert this problem.

From the current study, the following recommendations

Table 6. Seasonal variation in soup sour events.

Season	Subset for alpha = 0.05			
	N	1	2	3
JJA	92	1621.4520	-	-
DJF	91	-	2115.9434	-
SON	92	-	-	2633.1635
MAM	90	-	-	2649.8890
Sig.	-	1.000	1.000	0.884

Table 7. Significance of seasonal variation in soup sour events.

Sum of variance	Sum of squares	Df	Mean square	F	Sig.
Between groups	655679.205	3	218559.735	274.001	0.000
Within groups	1272558.696	361	3525.093	-	-
Total	1928237.901	364	-	-	-

Table 8. Daily time variation in soup sour events.

Time of the day	Subset for alpha = 0.05			
	N	1	2	3
8 pm - 5 am	365	1801.5252	-	-
4 pm - 6 pm	365	-	2741.2954	-
7 am - 12 noon	365	-	-	4475.1794
Sig.	-	1.000	1.000	0.884

Table 9. Significance daily time variation in soup sour events.

Sum of variance	Sum of squares	df	Mean square	F	Sig.
Between groups	1276667.420	2	638333.710	126.537	0.000
Within groups	5508740.936	1092	5044.635	-	-
Total	6785408.356	1094	-	-	-

could be made:

(i) There should be proper kitchen ventilation in the absence of refrigerator and/or power supply.
(ii) Choice of soup to cook should be made with utmost consideration of prevailing air temperature in the area and other areas in the tropics since air temperatures are generally high.
(iii) For private consumption, there is need to reduce the quantity of soup cooked per time.
(iv) There is need to wash food ingredients properly before cooking, so as to reduce the bacterial load that may be inherent of such ingredients themselves which may quicken souring with increasing temperature.
(v) Proper public orientation should be initiated by Delta State government using electronic media to sensitize the locals on the effects of temperature on soups.
(vi) The use of food thermometers by all who cook is recommended as it will help them identify the internal food temperature of food especially during reheating.
(vii) Finally, improvement on power supply in Abraka is highly recommended. With an improved power supply, the residents would be able to utilize their refrigerating sets which have already become cupboards and homes for pests.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Baxter SD (2008). Nutrition for Healthy Children and Adolescents Aged 2 to 18 Years. In Handbook of Nutrition and Food, Berdanier CD, Dwyer J, Feldman EB (Eds), CRC Press: Boca Raton, FL, USA P 295.

Dolan LC, Matulka AR, Burdock GA (2010). Naturally occurring food toxins. Toxins 2(9):2289-2332.
Efe SI (2006). Quality of rainwater harvesting for rural communities of Delta State, Nigeria. Environmentalist 26(3):175-181.
Hutt PB, Merrill RA, Grossman LW (2007). Food and Drug Law, 3rd ed. Foundation Press: New York, NY, USA P 369. Available at: https://www.amazon.com/Food-Drug-Law-University-Casebook/dp/1609301757
Jones JMJ (1995). Food Safety. Eagan Press: St. Paul, MN, USA pp. 71-87.
Koppen W (1918). Klassification der Klimate nach Temperatur, Niederschlag and Jahreslauf. Petermanns Geographische Mittenlungen. Available at: https://ci.nii.ac.jp/naid/10012396395/
Kotsonis FN, Burdock GA (2008). Food Toxicology. In Casarett and Doull's Toxicology: The Basic Science of Poisons, 7th ed. Klaassen CD, Ed. McGraw-Hill: New York, NY, USA pp. 1191-1236.
Nwagbara MO, Ozabor F, Obisesan A (2017). Perceived Effects of Climate Variability on Food Crop Agriculture in Uhunmwode Local Government Area of Edo State, Nigeria. Journal of Scientific Research and Reports 16(3):1-8.
Olomo OV, Ajibola OO (2006). Process Factors Affecting the Yield and Physicochemical Properties of Starches from Cassava Chips and Flour. Agricultural Mechanization in Asia, Africa and Latin America 37(1):65-69.
Omaye ST (2004). Toxicity of Nutrients. CRC Press: Boca Raton, FL, USA. pp. 205-213.
Ostertag E, Becker T, Ammon J, Bauer-Aymanns H, Schrenk D (2002). Effects of storage conditions on furocoumarin levels in intact, chopped, or homogenized parsnips. Journal of Agricultural and Food Chemistry 50(9):2565-2570.
Ozabor F, Obaro HN (2016). Health Effects of Poor Waste Management in Nigeria: A Case study of Abraka in Delta State. International Journal of Environment and Waste Management, 18(3):195-204.
Schmutz E, Hoyle E (2011). Effects of temperature on food. Clemson Cooperative Extension, Home and Garden Information Center (HGIC). Available at: https://hgic.clemson.edu/factsheet/effects-of-temperature-on-food/
Strahler AN (1965). Introduction to Physical Geography. New York: John Wiley & sons. Available at: https://onlinelibrary.wiley.com/doi/abs/10.1002/sce.3730510498
United States Food and Drug Administration (USDA) (2009). Food Code Available at: https://www.fda.gov/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/ucm2019396.htm
United States Food and Drug Administration (USFDA) (2010). Bad Bug Book: Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. BBB-Gemphylotoxin. Available online: https://www.hsdl.org/?abstract&did=446972
United State Department of Agriculture, Food Safety and Inspection Service (2011a). USDA Revises Recommended Cooking Temperature for All Whole Cuts of Meat, Including Pork, to 145 °F. Available at: https://fonddulac.uwex.edu/usda-revises-recommended-cooking-temperature-for-whole-cuts-of-meat/
United State Department of Agriculture, Food Safety and Inspection Service (2011b). How Temperatures Affect Food. Available at: https://www.fsis.usda.gov/wps/portal/fsis/topics/food-safety-education/get-answers/food-safety-fact-sheets/safe-food-handling/how-temperatures-affect-food/ct_index
United States Food and Drug Administration (USFDA). (2009). Food Code. Available at: https://www.fda.gov/Food/GuidanceRegulation/RetailFoodProtection/FoodCode/ucm2019396.htm
United States Food and Drug Administration (USFDA). (2010). Bad Bug Book: Foodborne Pathogenic Microorganisms and Natural Toxins Handbook. BBB-Gemphylotoxin. Available at: https://www.henrythehand.com/wp-content/uploads/2011/02/Bad-Bug-Book.pdf

Vol. 12(9) pp. 216-229, September 2018
DOI: 10.5897/AJFS2018.1695
Article Number: A65613458057
ISSN: 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

A comparative study on stability of different types of coconut (*Cocos nucifera*) oil against autoxidation and photo-oxidation

Subajiny Sivakanthan[1], Dilini Bopitiya[2] and Terrence Madhujith[3*]

[1]Department of Agricultural Chemistry, Faculty of Agriculture, University of Jaffna, Sri Lanka.
[2]University College of Ratmalana, University of Vocational Technology, Ratmalana, Sri Lanka.
[3]Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Sri Lanka.

Received 19 February, 2018; Accepted 25 June, 2018

This study aimed to compare the stability of five types of coconut (*Cocos nucifera*) oil available in Sri Lanka. The types of coconut oil studied were coconut pairing oil (CPO), white coconut oil (WCO), refined bleached and deodorized coconut oil (RBDCO), virgin coconut oil (VCO) and coconut oil (CO). Oils were exposed to elevated temperature (60±5°C) in the presence of excess air or oxygen to induce autoxidation and florescent light (2650 lux) to induce photo-oxidation in 28 days of storage. Samples were taken on 0, 1, 3, 5, 7, 14, 21 and 28 days to assess the level of oxidation by peroxide value (PV), conjugated dienes (CD), conjugated trienes (CT) and thiobarbituric acid reactive substances (TBARS) with time. WCO and VCO possessed the highest oxidative stability against autoxidation followed by CO and CPO, while, RBDCO showed the lowest stability. WCO and VCO possessed highest stability against photo-oxidation. CO was more susceptible to photo-oxidation among the oils examined followed by CPO and RBDCO. WCO and VCO possessed similar stability against both autoxidation and photo-oxidation. VCO has highest monounsaturated fatty acid: polyunsaturated fatty acid ratio (4.28) and suitable n6/n3 ratio (5:1); hence applicable as whole purpose oil which is resistant to processing and storage conditions with good nutritive value.

Key words: Autoxidation, coconut oil, fluorescent, oxidative stability, photo-oxidation.

INTRODUCTION

Coconut (*Cocos nucifera*) is one of the major plantation crops cultivated in Sri Lanka over many decades and coconut oil is the widely used edible oil in the country. Coconut fat accounts for 80% of fat intake among Sri Lankans (Amarasiri and Dissanayake, 2006). In 2015, the total area under coconut cultivation was approximately 455,000 ha which produced almost 3,056 million nuts, while coconut oil production was approximately 52,790 MT (Central Bank of Sri Lanka, 2017). Coconut oil is the major dietary source of medium chain triacylglycerols (MCTs). The MCTs are quickly absorbed and utilized when as consumed compared to long chain triacylglycerols

*Corresponding author. E-mail: madujith@yahoo.com. Tel: +9-481-239-5306. Fax: +9-481-239-5212.

(Krishna et al., 2010). MCTs may reduce the incorporation and storage of dietary fats and oil in adipose tissue, thus, reducing body fat (Adhikari et al., 2010). In addition, coconut oil is considered to be the most stable for deep frying because of high level of saturation as compared to other edible oils. Therefore, recently, there is a trend to encourage the use of coconut oil for deep frying to reduce the health risks arising from *trans* fats (Manchanda and Passi, 2016).

Oxidative stability is an important indicator to determine quality and shelf life of edible oils (Choe and Min, 2006). Most plant oils in their natural forms do not possess sufficient oxidative stability, which limits their application for food processing and long term storage (Comandini et al., 2009). Lipid oxidation causes deterioration of the oils leading to loss of essential fatty acids and alteration of sensory qualities such as flavour, aroma and colour; thus reducing palatability. Moreover, oxidation of lipids produces primary and secondary oxidative products such as peroxides, hydroperoxides, aldehydes, ketones, acids and alcohols which adversely affect the sensory quality, nutritive value and safety of the oil (Choe and Min, 2006; Vidrih et al., 2010).

Chemical mechanisms such as autoxidation and photo-oxidation are responsible for the oxidation of lipids during processing and storage (Choe and Min, 2006). Autoxidation and photo-oxidation of lipids are influenced by heat, light, composition of fatty acids, type of oxygen and minor compounds such as metals, pigments, phospholipids, free fatty acids, mono- and di-acylglycerols, thermally oxidized compounds and antioxidants. In contrast, phenolic compounds, especially carotenoids, decrease autoxidation in oil, while tocopherols, chlorophylls and phospholipids exhibit both antioxidant and pro-oxidant activity depending on the oil system and storage conditions (Shahidi and Zhong, 2005; Choe and Min, 2006).

Triplet ($^3O_2$) and singlet ($^1O_2$) oxygen act as the initiators of oxidation of the lipids (Ahmed et al., 2016). Unsaturated fatty acids undergo autoxidation, which is initiated by triplet oxygen via the free radical chain reaction through attack on alpha methylene of the carbon double bonds leading to the formation of hydroperoxides (primary oxidation product), which are further decomposed to form secondary oxidation products (Rukmini and Raharjo, 2010). Lipid autoxidation involves three steps: initiation, propagation and termination stages. In the initiation step, when lipid is exposed to an initiator (heat, light or metal ions), hydrogen atom of double bond is abstracted and alkyl radical is formed. This free radical abstracts hydrogen from other lipid molecules and reacts with the hydrogen to form hydroperoxide, which is a tasteless and odourless compound and another alkyl radical. Alkyl radical also reacts with triplet oxygen to form peroxy radical, which abstracts hydrogen from other lipid molecules and reacts with hydrogen to form hydroperoxide and another alkyl radical. Radicals react with each other to form non-radical species and the reaction is terminated (Choe and Min, 2006; Lee et al., 2004). Hydroperoxide further break down via several steps into secondary oxidation products such as carbonyl compounds responsible for objectionable odour and taste such as aldehyde, ketones, acids, alcohols, acids, esters and short-chain hydrocarbons via monomolecular and bimolecular reactions (Choe and Min, 2006).

Singlet oxygen initiates photo-oxidation via the direct attack of the extremely electrophilic singlet oxygen on the unsaturated fatty acids. In the presence of light, photosensitizers such as chlorophyll and porphyrin convert triplet oxygen into singlet oxygen, which is a highly reactive non-radical molecule. If wavelength of solar light is less than 220 nm, unsaturated fatty acids cannot absorb light; however, photosensitizers can absorb light energy and convert triplet state sensitizer to singlet state sensitizer (Ahmed et al., 2016). Two types of photo-oxidation mechanisms have been proposed; an electron or a hydrogen atom transfers between an excited triplet sensitizer and a substrate, producing free radicals or radical ions; and triplet oxygen can be excited by light to singlet oxygen, which reacts with the double bond of unsaturated fatty acids, producing an allylic hydroperoxide (Gordon, 2001; W¹sowicz et al., 2004; Song et al., 2007; Galano et al., 2015; Ahmed et al., 2016). The autoxidation is comparatively a slower process than photo-oxidation (Rukmini and Raharjo, 2010).

Although, the oxidative stability of some oils is higher compared to other edible oils due to lower amounts of polyunsaturated fatty acids and presence of natural antioxidants, natural photosensitizers in oils such as chlorophyll may initiate photo-oxidation (Kim et al., 2000). Coconut oil is an example for saturated oils. Thus, greater concern should be given to photo-oxidation than auotoxidation of coconut oil. Generally, the oils are packaged in transparent plastic or glass bottles during storage display of retail markets. The opacity of the packaging material to light is of fundamental importance to minimize the deterioration of edible oils by photo-oxidation (Méndez and Falqué, 2007). Therefore, prevention of light exposure during storage of vegetable oils is necessary to preserve the quality and extend its shelf-life (Gargouri et al., 2015).

Different types of coconut oil are available in Sri Lanka. Coconut pairing oil (CPO) is extracted from fresh pairings resulting from the desiccated coconut, coconut cream and coconut milk industry and contains free fatty acid content of maximum 0.8%. Virgin coconut oil (VCO) is manufactured from fresh kernel either through dry or wet process without high heat treatment. Coconut oil (CO) is prepared from traditional copra (dried coconut kernel). Refined and bleached and deodorized coconut oil (RBDCO) is obtained from crude coconut oil by refining process. White coconut oil (WCO) is obtained from best

quality copra and contains free fatty acid percentage of less than 0.8%. These oils may differ in their susceptibility to oxidation because of the processing methods which may lead to differences in the presence of minor components. For example, chemical refining may remove major proportion of natural antioxidants (Ayyildiz et al., 2015). Thus, the knowledge on the stability of different types of coconut oil for autoxidation and photo-oxidation will be useful to suit the oil type for different cooking or processing techniques and to select suitable packaging materials to extend their shelf life. In this backdrop, the aim of this study was to examine and compare the stability of five types of oils from coconut available in Sri Lankan market against autoxidation and photo-oxidation in order to understand the impact of storage conditions such as temperature and florescent light on the oxidation of selected oils and to select the oil which is most resistant to oxidation during processing and storage.

MATERIALS AND METHODS

The study was carried out at the Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Sri Lanka.

All oil samples were collected from a local oil mill within three days after manufacture. It was ensured that the oils do not contain any additive. Oil samples in glass bottles wrapped with aluminum foil were stored at -20°C after flushed with nitrogen. After an oil sample was drawn for a particular analysis, the bottle was flushed with nitrogen again, covered with the original cap and sealed with parafilm. All the chemicals used in this study were of analytical grade with highest purity available (>99.5%) and obtained from Sigma Chemicals Company (MO, USA).

Determination of fatty acid composition of oils

Fatty acids profile of selected oils was determined by gas-liquid chromatography (GLC). Fatty acid methyl esters (FAMEs) were prepared according to the method explained by Christie (1992). To prepare FAMEs, 3 mL of 0.5 M sodium methoxide and 0.3 mL of dichloromethane were added to the 0.2 g of oil sample. The mixture was kept at 50°C for 30 min. The mixture was allowed to cool to room temperature and 5 mL of deionized water was added. Then, 0.1 mL of acetic acid and 0.5 mL of hexane was added and centrifuged at 1500 rpm for 10 min at 5°C. The distinct upper layer of hexane containing FAMEs was separated for analysis. Analysis of FAMEs was carried out on GLC (GC-14B, Shimadzu, Japan) equipped with Flame Ionization Detector (FID) and capillary column (SP^{TM} 2560, 100 m × 0.25 mm ID, 0.20 µm film). The initial column oven temperature was maintained at 140°C for 5 min and increased to 220°C at the rate of 4°C/min, then maintained at that temperature for 10 min. Both injector and detector temperatures were maintained at 260°C. Helium was used as the carrier gas at flow rate of 30 mL/min. The injection volume was 1 µL at split ratio of 100:1. Fatty acids were identified by comparison of their retention time with authentic standards (Supelco^{TM} 37 component FAME mix).

Extraction of phenolic fraction

Phenolic fraction present in 50 g of oil was extracted into methanol by passing oil through a gravity column packed with silica (60-Å pore diameter) according to the method described by Steel et al. (2005). Hexane and methanol (1:1) mixture was used for conditioning the column and hexane and ethyl acetate (9:1) mixture was used for washing the column. The oil sample thoroughly mixed with hexane was introduced to the column and the phenoilc fraction bound to silica was subsequently extracted into methanol. The extract was recovered after desolventizing *in vacuo* at 40°C. The prepared extracts were stored under frozen condition (-20°C) after flushing with nitrogen. The frozen extracts were thawed and appropriately diluted before chemical analysis.

Determination of total phenolic content

The total phenolic content (TPC) of the extracts was determined colorimetrically using Folin-Ciocalteu's reagent as described by Thaipong et al. (2006) with minor modifications. Twenty microliters of oil extract and 1.58 mL of deinozed water were mixed with 100 µL of Folin-Ciocalteu's reagent, left for 3 min and subsequently 300 µL of sodium carbonate (0.7 M) was added and vortexed. The absorbance of the resulting mixture was read at 725 nm using a UV visible spectrophotometer (UV 1601, Shimadzu, Japan) after leaving for 30 min at room temperature (27°C). The results were expressed as mg of gallic acid equivalents (GAE) per liter of extract using a gallic acid (50 to 500 mg/L) standard curve.

Determination of oxidative stability of oils

Oxidative stability of selected oils was examined by accelerated oxidative stability tests. The rate of oxidation was monitored by the measurement of peroxide value (PV), conjugated dienes (CD), conjugated trienes (CT) and thiobarbituric acid reactive substances (TBARS) with the time.

Preparation of samples for *Schaal* oven test method

The oxidative stability of the investigated oil samples was studied using the *Schaal* oven accelerated oxidation test (Shahidi et al., 1997). The test was performed in an oven at constant temperature. Sixteen samples (5 mL) of each oil type were placed in glass vials (2 cm ID × 4.5 cm) and kept in the oven at the temperature of 60±5°C for up to 28 days without lids to facilitate the oxidation. Two samples from each oil type were drawn on days 0, 1, 3, 5, 7, 14, 21 and 28, and the level of oxidation of oil samples was analyzed.

Preparation of samples for accelerated photo-oxidation method

The stability against light induced oxidation of selected oils was evaluated under fluorescent light (2650 lux) (Khan and Shahidi, 1999) up to 28 days of storage period. Sixteen samples (5 mL) of each oil type were placed in glass vials (2 cm ID × 4.5 cm) and glass vials were placed in a polypropylene box (70 cm length x 35 cm width x 25 cm height) equipped with 80 Watt cool white fluorescent lights fixed on the lid above the surface of the oil containers. The remaining open space of the box was covered with aluminum foil. The fluorescent radiation was at a level of 2650 Lux and the temperature inside the container was maintained at 27±1°C. Two samples from each oil type were drawn from the box on days 0, 1, 3, 5, 7, 14, 21 and 28 to assess the level of oxidation.

Determination of PV

PV of the oil samples was measured according to the modified IDF

method (Hornero-Méndez et al., 2001). The sample (0.01 to 0.05 g) was dissolved in 1 mL of chloroform/acetic acid (2:3), with addition of 100 µL Fe (II) solution, mixed for 15 s in a vortex mixer and left in the dark for 10 min. Deionized water (2 mL) was added and 4 mL of diethyl ether was added. Organic phase was discarded and remaining ether in the aqueous phase was removed under N_2 current for a few seconds. Aqueous phase (1 mL) was transferred into another tube and 100 µL of saturated ammonium thiocyanate solution was added. After 10 min, absorbance at 470 nm was read against water blank using a UV visible spectrophotometer (UV 1601, Shimadzu, Japan). A reaction blank also prepared. PV was calculated and the results were expressed as meq/kg of oil using a Fe (III) solution (0 to 40 µg/mL) calibration curve.

Determination of TBARS

The sample of oil (0.05 to 0.10 g) was thoroughly mixed with 5 mL 1-butanol solution and 5 mL of 0.2% (w/v) thiobarbituric acid (TBA) in 1-butanol solution was added. Prepared solution was incubated for 2 h in a water bath maintained at 95°C and cooled immediately. Absorbance of the solution was taken at 532 nm using a UV visible spectrophotometer (UV 1601, Shimadzu, Japan). The results were expressed as mg of malonaldehyde equivalent/g of oil using TMP (1×10^{-6} - 1×10^{-5} M) as standard (Yi et al., 2011).

Determination of CD and CT

CD and CT values were determined by IUPAC II.D.23 analytical method (Paquot, 1979). Oil sample (0.01 to 0.03 g) was thoroughly mixed with 25 mL of iso-octane using vortex mixer for 15 s. Then, the absorbances were measured separately at 233 nm for CD and 268 nm for CT using a UV visible spectrophotometer (UV 1601, Shimadzu, Japan). The results were calculated as extinction values ($E_{1\%}$) using following equation.

$$E_{1\%} = \frac{A_\lambda}{(C\times l)}$$

Where, A_λ is the absorbance measured at either 233 or 268 nm; C represent the concentration of oil solution (g/100 mL); l is the path length of the cuvette (cm).

Statistical analysis

The data were analyzed using Minitab 16 (Minitab Inc., UK) and Microsoft (Excel) procedures. All measurements were performed in triplicate and results are expressed as mean ± SD. The ANOVA tables were constructed using GLM procedure. Duncan's new multiple range test was used to determine significant differences at 0.05 significant levels.

RESULTS AND DISCUSSION

PV of all oils ranged between 0.21 and 0.42 meq/Kg. As per the Codex Standard (Codex Alimentarius, 1999), the maximum limit of peroxide values for any refined oil and virgin oils are 10 and 20 meq/kg, respectively. Thus, initial quality of the oil sample used in this study complied with this standard.

Fatty acid composition of oils

The fatty acid composition of oils is presented in Table 1. All oil samples were characterized by five to seven different types of saturated fatty acids of chain length C6 to C22 and three to six different types of unsaturated fatty acids of chain length C18 to C22. Palmitic acid (C16:0) and stearic acid (C18:0) were common in all saturates.

The amount of saturated fatty acid of the selected oils ranged from 87 to 93%. Lauric acid (43-52%) was most predominant followed by myristic acid (16-18%). Moreover, oils contained considerable amounts of short chain fatty acids such as caproic, caprylic and capric. The average content of oleic acid and lauric acid were significantly higher in WCO and VCO as compared to that of other coconut oils tested. Results of this study appeared identical with those of earlier published findings in the context of major fatty acids of the respective oils (Vidrih et al., 2010). It is recommended that the *n*-6/*n*-3 fatty acid ratio in the diet should be between 4:1-5:1 and 10:1 (Candela et al., 2011). The most suitable ratios were found in all types except CO (Table 1).

In addition to fatty acid composition, the presence of minor components in edible oils contributes to their oxidative stability (Bendini et al., 2009; Shinagawa et al., 2017). Due to this reason, even though all types of coconut oils selected for this study contained more than 87% of saturated fatty acid, oxidative stability of these oils may differ. Table 2 shows the total phenolic content of the oils. WCO contained highest amount of total phenolic content followed by VCO, whereas, CPO and RBDCO had the lowest. Lowest level of total phenolic content of RBDCO indicates that refining process reduced the phenolic content. VCO, WCO and CO did not undergo refining chemical refining process. However, the lower total phenolic content of the CO than VCO and WCO could be due to the heat applied during the processing. Szydłowska-Czerniak and Łaszewska (2015) reported that the refining process of rapeseed oils reduced the antioxidant capacity by about 60% and total phenolic content by above 80%.

Oxidative stability of oils against autoxidation

Accelerated storage tests have been used extensively in researches to evaluate the stability of edible oils against oxidation. In this test, the oxidation of oils is accelerated by keeping the oil at 60 to 65°C. By increasing the temperature of storage, the oxidation rate is increased (accelerated). One day of storage at this condition is equal to one month of storage at ambient temperature (Evans et al., 1973). Therefore, the present study has evaluated the stability of oils for 28 months of storage at the ambient temperature.

Table 3 summarized the rate of increment of PV, CD, CT and TBARS per day of the oils tested to study their

Table 1. Percentage of saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids of each type of oil.

Fatty acid	CPO	WCO	RBDCO	VCO	CO
SFA	92.29[a]	90.72[a]	92.86[a]	87.88[b]	91.36[a]
MUFA	4.19[d]	6.81[b]	5.18[c]	9.76[a]	5.82[c]
PUFA	2.81[a]	2.45[b]	1.37[c]	2.28[b]	2.83[a]
MUFA/PUFA	1.75[d]	2.78[c]	3.78[b]	4.28[a]	2.06[c]
n-6/*n*-3	9:1	4:1	4:1	5:1	24:1

Values (means) with different superscript letters in the same row indicate significant differences ($p < 0.05$). CPO, coconut pairing oil; WCO, white coconut oil; RBDCO, refined bleached and deodorized coconut oil; VCO, virgin coconut oil; CO, coconut oil; SFA, saturated fatty acids, MUFA, monounsaturated fatty acids; PUFA, polyunsaturated fatty acids.

Table 2. Total phenolic content of oils.

Oil	TPC (mg GAE/ g extract)
WCO	28.37 ± 0.46[a]
VCO	24.52 ± 0.34[b]
CO	20.40 ± 0.41[c]
CPO	12.78 ± 0.03[d]
RBDCO	12.64 ± 0.28[d]

Values (means (n=3) ± SD) with different letters in the same column imply significant differences ($p < 0.05$).

Table 3. Rate of value increment (per day) of PV, CD, CT and TBARS of different oils stored at 60±5°C during storage period.

Parameter	CPO	WCO	RBDCO	VCO	CO
PV	0.471	0.044	0.044	0.029	0.030
CD	0.025	0.034	0.044	0.022	0.021
CT	0.013	0.017	0.025	0.022	0.035
TBARS	0.163	0.064	0.199	0.112	0.127

oxidative stability against autoxidation. PV is an indicator of the extent of primary oxidative products formed in oils (Yi et al., 2011). The effect of storage condition at 60±5°C on the formation of primary oxidative products is expressed as PV. The PV of the oils with storage time is shown in the Figure 1 and the fold increment of PV with duration of storage is presented in Table 4. Development of PV was observed in all samples except VCO during the experimental period. Level of oxidation gradually increased with the storage time in all oil samples. However, rate of formation of hydroperoxides decreased with extent of duration. Li et al. (2014) reported that the higher rate of increment during early storage, decrease the rate during the latter period during the storage of oil blends under accelerated condition for 24 days. Instability of hydroperoxides under higher degree of oxidation could be the reason for lower detection values. In the later stages of lipid oxidation, the hydroperoxides are broken down into secondary oxidative products (Ullah et al., 2003). Autoxidation is affected by temperature. Since these oils were stored at 60±5°C, which might have accelerated autoxidation and rapid production of secondary oxidative products. The PV varied among oils due to different oxidative stabilities of the oils. During the first three days of storage, no significant decrease ($p>0.05$) in level of the primary oxidative products was observed in WCO and VCO. The induction period of the oils that contained considerable value of MUFA: PUFA ratio and high antioxidant activity is high. The rapid oxidation that occurred in CPO and RBDCO indicated that the oxidative stability deteriorated quickly during autoxidation. Based on the PV, highest oxidative stability

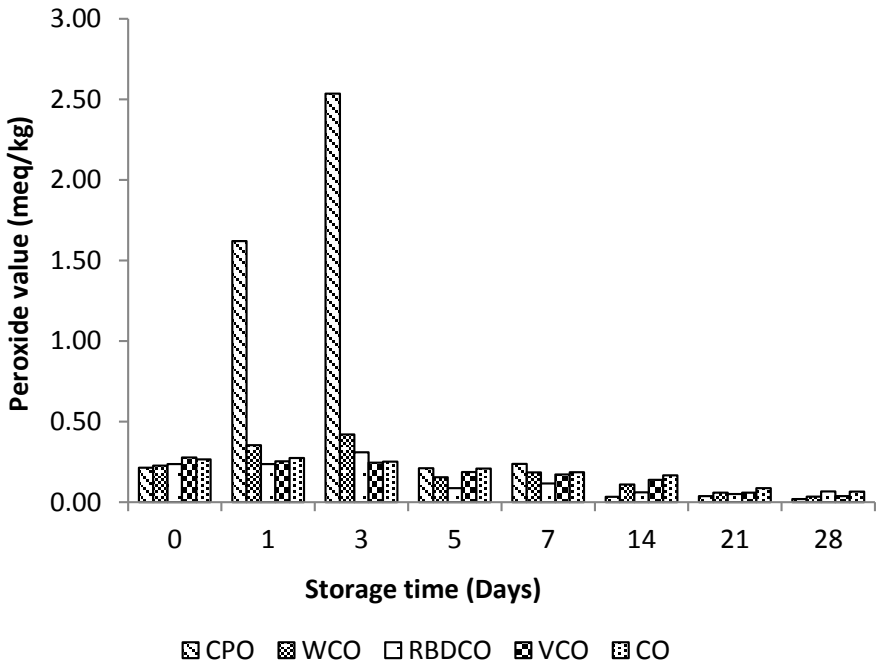

Figure 1. Peroxide value of oils stored under *Schaal* oven conditions at 60±5°C.

Table 4. Fold increment of PV of different oils stored at 60±5°C up to 28 days.

Storage time (days)	CPO	WCO	RBDCO	VCO	CO
1	7.58±0.20^{b}	1.56±0.01^{a}	1.00±0.02^{b}	0.91±0.01^{a}	1.03±0.03^{a}
3	11.85±0.37^{a}	1.19±0.44^{ab}	1.31±0.01^{a}	0.88±0.01^{a}	0.94±0.01^{b}
5	0.98±0.05^{c}	0.37±0.02^{c}	0.37±0.01^{d}	0.67±0.01^{b}	0.78±0.01^{bc}
7	1.11±0.07^{c}	1.20±0.02^{bc}	0.49±0.01^{c}	0.62±0.01^{c}	0.70±0.01^{b}
14	0.16±0.01^{d}	0.59±0.02^{c}	0.26±0.02^{e}	0.50±0.01^{d}	0.62±0.02^{b}
21	0.17±0.01^{d}	0.54±0.01^{c}	0.21±0.02^{e}	0.21±0.01^{e}	0.33±0.03^{d}
28	0.09±0.01^{d}	0.58±0.03^{c}	0.28±0.01^{e}	0.13±0.01^{f}	0.24±0.02^{c}

Values (Means (n=3) ± SD) with different letters in the same column imply significant differences ($p < 0.05$).

was observed in VCO. Highest oxidation rate was executed in CPO, RBDCO and WCO.

CD value is also useful for monitoring the early stages of lipid oxidation that formed almost immediately after peroxides are produced (Xia and Budge, 2017). The oxidation of the tested oils expressed as CD value is presented in Figure 2 and fold increment of the values are shown Table 5.

All oil samples revealed the increase of CD value during the experiment. Therefore, oxidative stability of oils decreased with storage conditions. The CD values of all oils were slowly decreased after third or fifth day of storage since they would have completed conjugation of unsaturated fatty acids available. Based on the CD values, the highest oxidative stability was observed in VCO followed by CPO and CO. The rate of autoxidation of RBDCO was the highest under accelerated storage conditions.

The oxidation of the tested oils expressed as CT value is presented in Figure 3 and the fold increment of CT value of oils obtained during experimental period is presented in the Table 6. The CT values of all oils tested increased with the storage time. According to the data shown in Table 3, the rate of formation of CT value per day was lowest in WCO followed by CPO, VCO and RBDCO.

TBARS value is an indicator for the extent of secondary lipid oxidative products, aldehydes (Ross and Smith, 2006). Data illustrated in Table 7 and Figure 4 indicated that the TBARS value of the oils fluctuated with experimental time. During the first few days of the storage period, WCO, CPO and CO showed the highest level of

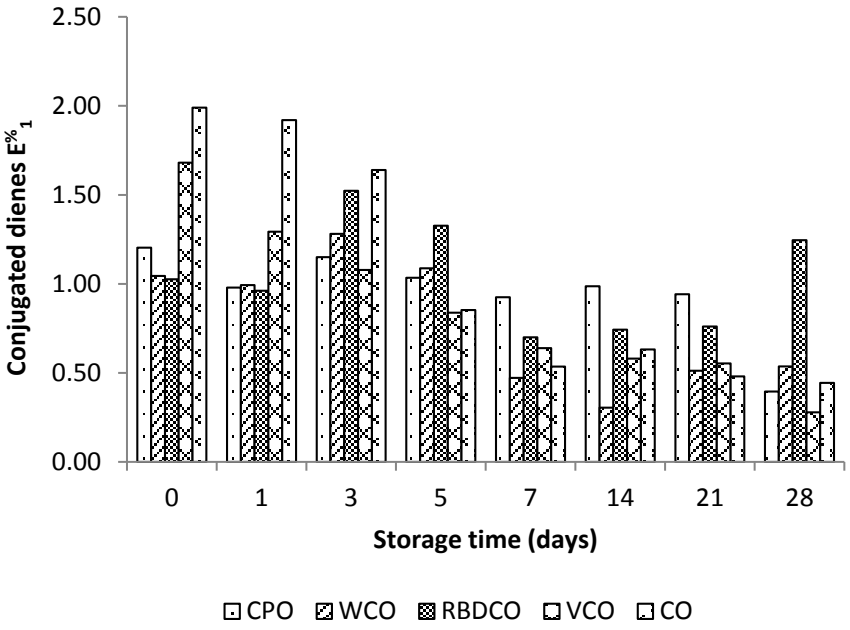

Figure 2. CD of different oils stored under *Schaal* oven conditions at 60±5°C.

Table 5. Fold increment of CD of different oils stored at 60±5°C up to 28 days.

Storage time (days)	CPO	WCO	RBDCO	VCO	CO
1	0.81±0.03^{ab}	0.95±0.10^{a}	0.94±0.14^{bc}	0.77±0.01^{a}	0.96±0.04^{a}
3	0.96±0.01^{a}	1.23±0.24^{a}	1.48±0.14^{a}	0.64±0.06^{ab}	0.82±0.04^{a}
5	0.86±0.11^{a}	1.04±0.19^{a}	1.29±0.04^{a}	0.50±0.10^{bc}	0.43±0.07^{b}
7	0.77±0.09^{ab}	0.45±0.13^{b}	0.68±0.01^{c}	0.38±0.01^{c}	0.27±0.06^{bc}
14	0.82±0.01^{ab}	0.29±0.02^{b}	0.72±0.14^{c}	0.35±0.02^{cd}	0.32±0.01^{bc}
21	0.78±0.11^{ab}	0.49±0.06^{b}	0.74±0.01^{c}	0.33±0.02^{cd}	0.24±0.02^{bc}
28	0.33±0.05^{b}	0.51±0.01^{b}	1.21±0.02^{ab}	0.17±0.01^{d}	0.22±0.04^{c}

Values (Means (n=3) ± SD) with different letters in the same column imply significant differences ($p < 0.05$).

TBARS value and then reduction was observed. This could be attributed to the rapid production of tertiary oxidative product by these oils. Therefore, they did not respond positively for tests which evaluate secondary oxidative product. Thus, the increasing temperature may quicken the breakdown of fatty acids in the above mentioned oils. VCO and RBDCO positively responded to the test with the accelerated heating. TBARS values of these oils increased with time. As data shown in Table 3, based on the rate of development of TBARS value per day, WCO and VCO possessed higher oxidative stability as compared to other oils examined. RBDCO showed higher rate of oxidation.

Based on results obtained during the oxidation in the oven, WCO and VCO possessed the highest oxidative stability. Despite higher oxidation rate as measured by PV and CD value, WCO showed the lowest rate of formation of TBARS and CT (Table 3). Therefore, WCO can be categorized as oil which has the strongest oxidative stability against autoxidation. Oxidation rate of both primary and secondary oxidative parameters was lower for VCO. Therefore, oxidative stability of VCO was more or less similar to that of WCO. Oxidative stability of the oil tested can be arranged in descending order as WCO, VCO, CO, CPO and RBDCO.

High oxidative stability of VCO and WCO is mainly due to its higher phenolic content (Table 2) than other types of oils examined as well as fatty acid composition, in particular, to the high MUFA to PUFA ratio (Table 1). Even though the RBDCO contain higher MUFA: PUFA ratio than WCO, RBDCO showed poor oxidative stability than WCO, which could be attributed to the loss of minor components in RBDCO during refining. Several studies reported that the refined oils have less stability against

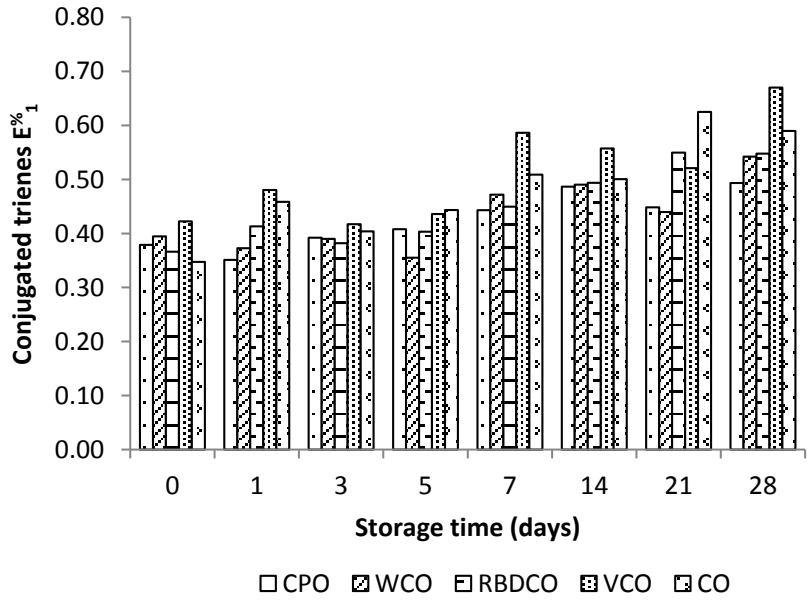

Figure 3. CT of different oils stored under *Schaal* oven conditions at 60±5°C.

Table 6. Fold increment of CT of different oils stored at 60±5°C up to 28 days.

Storage time (days)	CPO	WCO	RBDCO	VCO	CO
1	0.93±0.02^{c}	0.94±0.04^{bc}	1.13±0.37^{c}	1.14±0.10^{ab}	1.32±0.03^{cd}
3	1.03±0.09^{bc}	0.99±0.01^{bc}	1.04±0.50^{c}	0.99±0.02^{b}	1.16±0.01^{d}
5	1.08±0.05^{abc}	0.90±0.06^{c}	1.10±0.37^{c}	1.03±0.13^{b}	1.28±0.08^{cd}
7	1.17±0.04^{ab}	1.20±0.06^{abc}	1.23±0.70^{b}	1.39±0.07^{ab}	1.46±0.03^{bc}
14	1.28 ± 0.05^{a}	1.24±0.11^{ab}	1.35±0.38^{ab}	1.32±0.33^{ab}	1.44±0.16^{bcd}
21	1.18±0.09^{a}	1.11±0.08^{abc}	1.50±0.61^{a}	1.23 ±0.06^{ab}	1.80±0.15^{a}
28	1.30±0.04^{a}	1.37±0.12^{a}	1.50±0.38^{a}	1.59±0.02^{a}	1.70±0.03^{ab}

Values (means (n=3) ± SD) with different letters in the same column imply significant differences (p <0.05).

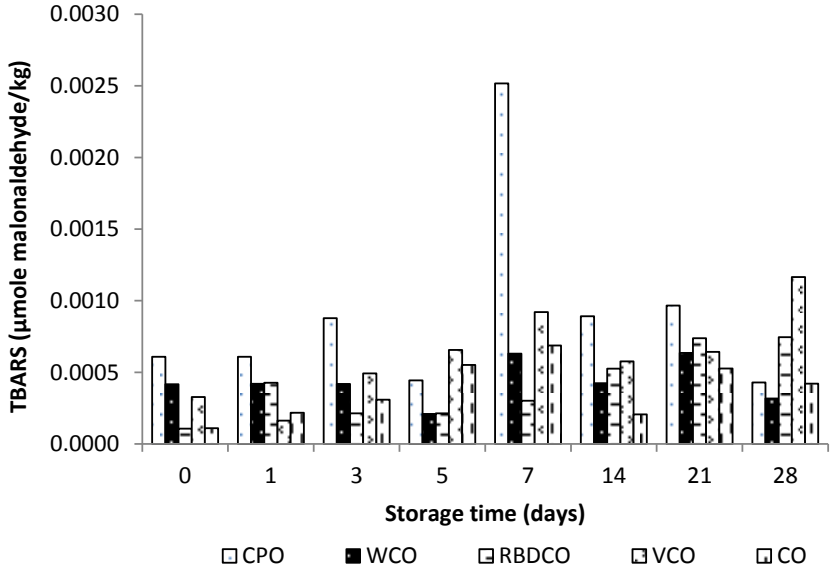

Figure 4. TBARS values of oils stored under *Schaal* oven conditions at 60±5°C.

Table 7. Fold increment of TBARS of different oils stored at 60±5°C up to 28 days.

Storage time (days)	CPO	WCO	RBDCO	VCO	CO
1	1.00±0.01^{cd}	1.01±0.01^{b}	4.00±0.02^{c}	0.50±0.23^{c}	1.99±0.01^{e}
3	1.44±0.18^{bc}	1.01±0.03^{b}	2.00±0.02^{e}	1.50±0.21^{bc}	2.82±0.32^{d}
5	0.73±0.09^{d}	0.50±0.01^{d}	2.00±0.05^{e}	2.00±0.46^{b}	5.03±0.02^{b}
7	4.14±0.17^{a}	1.51±0.04^{a}	2.83±0.23^{d}	2.81±0.61^{a}	6.27±0.33^{a}
14	1.47±0.34^{b}	1.02 ± 0.03^{b}	4.93 ± 0.18^{b}	1.76 ± 0.69^{bc}	1.88 ± 0.02^{e}
21	1.59±0.25^{b}	1.53±0.02^{a}	6.92±0.50^{a}	1.96±0.03^{b}	4.80±0.06^{b}
28	0.71±0.10^{d}	0.76±0.01^{c}	6.99±0.31^{a}	3.55±0.52^{a}	3.84±0.03^{c}

Values (means (n=3) ± SD) with different letters in the same column imply significant differences ($p < 0.05$).

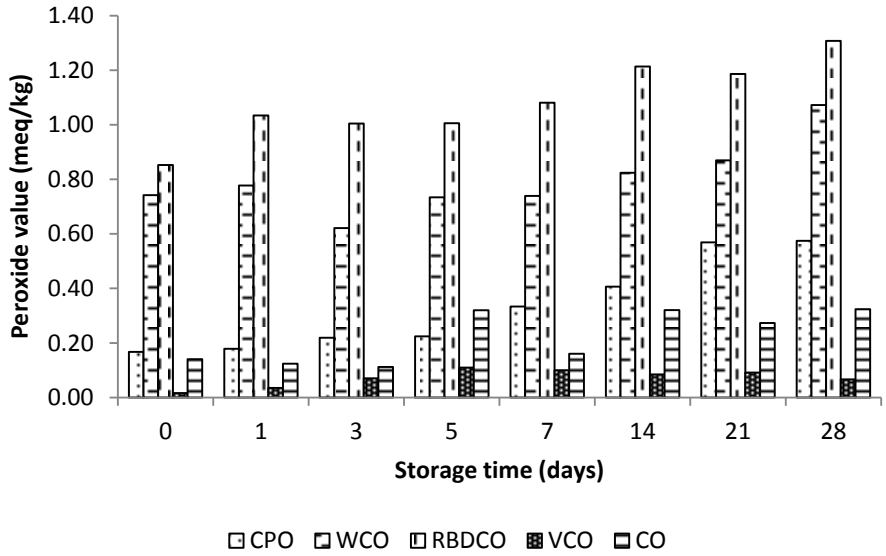

Figure 5. Peroxide value of different oils stored under fluorescent light at 27±1°C.

Table 8. Rate of increment (per day) of PV, CD, TBARS and CT of different oils stored under fluorescent light at 27±1°C during storage.

Parameter	CPO	WCO	RBDCO	VCO	CO
PV	0.087	0.025	0.014	0.115	0.061
CD	0.011	0.014	0.014	0.006	0.018
TBARS	0.340	0.066	0.205	0.120	0.525
CT	0.040	0.013	0.023	0.019	0.058

oxidation than their unrefined counterparts attributed to the loss of natural antioxidants during chemical refining process (Velasco and Dobarganes, 2002; Medina-Juárez and Gámez-Meza, 2011; Szydłowska-Czerniak and Łaszewska, 2015). Same reason is applicable in the present study.

Oxidative stability of oils under fluorescent light

The rate of increment of PV, CD, CT and TBARS per day of tested oils is summarized in Table 8. The oxidation of edible oils exposed to fluorescent light as measured by PV is shown in Figure 5 and Table 9. According to the results, the peroxide value of oils increased up to 28 days of storage under fluorescent light. The oxidative stability of oils decreased with the storage period. At the end of the storage period, PV of WCO and RBDCO exceeded the acceptable level which is less than 1 meq/kg (Khan and Shahidi, 1999). The concentration of peroxides and hydroperoxides was low in all oils tested during 28 days of storage under the fluorescent light. Despite the fact

Table 9. Fold increment of PV of different oils stored under fluorescent light at 27±1 °C up to 28 days.

Storage time (days)	CPO	WCO	RBDCO	VCO	CO
1	1.07±0.09^{e}	1.05±0.02^{cd}	1.21±0.03 ^{cd}	2.14±0.32^{b}	0.88±0.01^{d}
3	1.31±0.09^{d}	0.84 ± 0.01^{e}	1.18±0.02 ^{d}	4.32±0.88^{ab}	0.80±0.05^{d}
5	1.34±0.11^{d}	0.99±0.02^{d}	1.18±0 .02^{d}	6.73±0.80^{a}	2.28±0.04^{a}
7	1.99±0.10^{c}	1.00±0.03^{d}	1.27±0.04^{c}	6.14±0.60^{a}	1.15±0.01^{c}
14	2.43±0.08^{b}	1.11±0.01^{bc}	1.42±0.01^{b}	5.20±0.35^{a}	2.28±0.02^{a}
21	3.40±0.19^{a}	1.17±0.01^{b}	1.39±0.03^{b}	5.60±0.41^{a}	1.95±0.02^{b}
28	3.43±0.19^{a}	1.45±0.01^{a}	1.53±0.04^{a}	4.08±0.43^{ab}	2.31±0.02^{a}

Values (means (n=3) ± SD) with different letters in the same column imply significant differences (p <0.05).

Table 10. Fold increment of CD of different oils stored under fluorescent light at 27±1°C up to 28 days.

Storage time (days)	CPO	WCO	RBDCO	VCO	CO
1	0.98±0.01^{b}	0.97±0.10^{b}	1.25±0.10^{a}	0.83±0.05^{a}	1.26±0.16^{a}
3	1.00±0.12^{b}	1.10±0.10^{ab}	1.29±0.11^{a}	0.91±0.20^{a}	1.24±0.33^{a}
5	1.04±0.07^{b}	1.01±0.01^{b}	1.22±0.02^{a}	0.66±0.26^{a}	1.15±0.09^{a}
7	1.15±0.10^{ab}	1.12±0.02^{ab}	1.18±0.09^{a}	0.83±0.06^{a}	1.35±0.44^{a}
14	1.17±0.06^{ab}	1.19±0.06^{ab}	1.32±0.16^{a}	0.75±0.06^{a}	1.29±0.18^{a}
21	1.28±0.09^{a}	1.23±0.04^{ab}	1.36±0.17^{a}	0.88±0.07^{a}	1.40±0.09^{a}
28	1.29±0.01^{a}	1.36±0.04^{a}	1.48±0.02^{a}	0.96±0.10^{a}	1.55±0.31^{a}

Values (means (n=3) ± SD) with different letters in the same column imply significant differences (p <0.05).

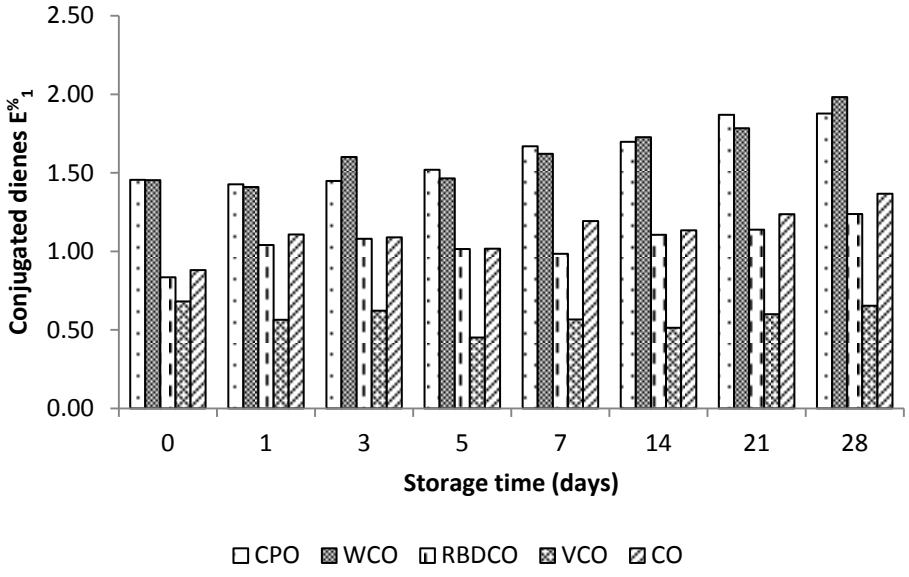

Figure 6. Conjugated dienes of different oils stored under fluorescent light at 27±1°C.

that initial stages of photo-oxidation progressed rapidly, the level of oxidation is less as compared to that of autoxidation. In this experiment, RBDCO and WCO showed higher oxidative stability against the fluorescent light oxidation as measured by increment of PV per day VCO was more susceptible to photo-oxidation (Table 8).

The CD values and fold increments of CD values of tested oils under fluorescent light are presented in Figure 6 and Table 10, respectively. The variation of CD values during experiment period followed a similar trend as PV,

Table 11. Fold increment of CT of different oils stored under fluorescent light at 27±1°C up to 28 days.

Storage time (days)	CPO	WCO	RBDCO	VCO	CO
1	0.88±0.36[a]	0.95±0.18[a]	0.99±0.24[a]	0.65±0.63[a]	1.07±0.73[a]
3	1.02±0.50[a]	0.95±0.06[a]	1.10±0.34[a]	0.46±0.60[a]	1.40±0.41[a]
5	0.80±0.04[a]	0.90±0.04[a]	0.93±0.05[a]	0.52±0.50[a]	1.52±0.02[a]
7	0.88±0.19[a]	1.05±0.15[a]	1.17±0.09[a]	0.78±0.55[a]	1.16±0.08[a]
14	1.16±0.21[a]	0.94±0.22[a]	1.16±0.44[a]	0.61±0.04[a]	1.78±0.30[a]
21	1.03±0.38[a]	0.86±0.18[a]	0.84±0.05[a]	0.59±0.18[a]	2.24±0.34[a]
28	0.98±0.35[a]	0.91±0.11[a]	1.03±0.05[a]	0.95±0.46[a]	2.17±0.81[a]

Values (means (n=3) ± SD) with different letters in the same column imply significant differences ($p < 0.05$).

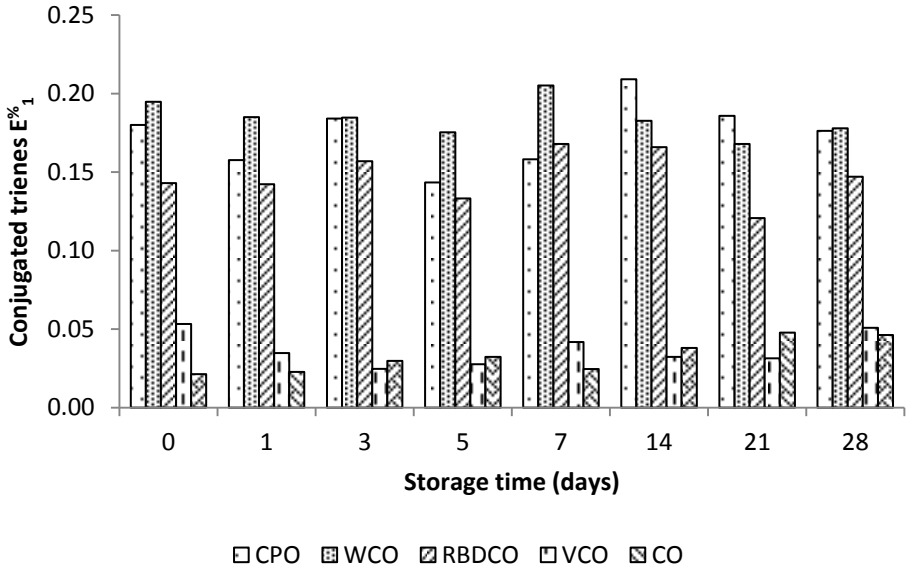

Figure 7. Conjugated trienes of different oils stored under fluorescent light at 27±1°C.

VCO, WCO, CPO, RBDCO and CO showed more or less constant fold increment throughout the time of illumination.

Fold increment of CT value and development of CT value of the oils obtained during experimental period is presented in the Table 11 and Figure 7. The oxidation of all oils tested increased with the storage time. Therefore, the effect of fluorescent light on oxidation was obvious. CPO, WCO, RBDCO, VCO and CO showed an ability to maintain a statistically more or less constant fold of increment throughout the experiment. This may be due to the presence of higher amount of antiphoto-oxidative compounds of theses oils. According to the results obtained as rate of formation of CT value per day (Table 8), CO showed lowest stability followed by CPO, RBDCO, VCO and WCO.

According to the data illustrated in the Figure 8 and Table 12, level of oxidation has increased with the extended storage life in all the oil samples. The results suggested that the oxidation of these oils accelerated by the fluorescent light effect. First few days of the storage period of CO and VCO showed the highest level of TBARS value and then reduction was observed. In the later stages of lipid oxidation, the secondary oxidative products are broken down into tertiary oxidative products (Dąbrowska et al., 2015). The TBARS values of other oils excluding the aforementioned oils were well correlated with the storage time. Based on the data shown in Table 8, WCO showed higher photo-oxidative stability. CO showed the highest oxidative rate followed by CPO and RBDCO.

The role of light in the oxidation process is clearly shown by the rate of oxidation. Based on the results presented as PV, CD, TBARS and CT values, CO is more susceptible to photo-oxidation among the oils examined. The second lowest photo-oxidative stability was exhibited by CPO followed by RBDCO. Based on the results, the susceptibility of these oils for photo-oxidation

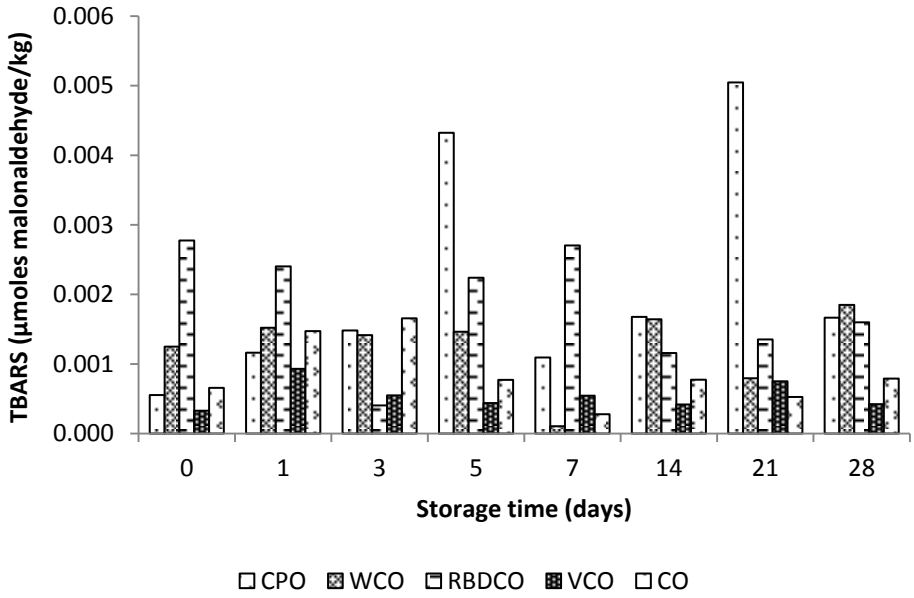

Figure 8. TBARS value of different oils stored under fluorescent light at 27±1°C.

Table 12. Fold increment of TBARS of different oils stored under fluorescent light at 27±1°C up to28 days.

Storage time (days)	CPO	WCO	RBDCO	VCO	CO
1	2.10±0.41^{c}	1.22 ± 0.21^{a}	0.87 ± 0.03^{ab}	2.84±0.26^{a}	2.24±0.11^{a}
3	2.68±0.12^{c}	1.13±0.23^{a}	0.15±0.01^{d}	1.67±0.03^{c}	2.52±0.03^{a}
5	7.82±0.22^{b}	1.17±0.15^{a}	0.81±0.02^{b}	1.33±0.01^{c}	1.17±0.01^{b}
7	1.98±0.05^{c}	0.08±0.01^{c}	0.97±0.02^{a}	1.66±0.05^{c}	0.42±0.12^{c}
14	3.03±0.21^{c}	1.31±0.25^{a}	0.42±0.02^{c}	1.27±0.01^{c}	1.18±0.12^{b}
21	9.13±0.53^{a}	0.64±0.14^{b}	0.49±0.08^{c}	2.29±0.04^{b}	0.80±0.01^{bc}
28	3.01±0.11^{c}	1.48±0.25^{a}	0.58±0.03^{c}	1.29±0.02^{c}	1.20±0.12^{b}

Values (means (n=3) ± SD) with different letters in the same column imply significant differences ($p < 0.05$).

can be arranged as WCO<VCO<RBDCO<CPO<CO. High content of total phenolics could be the reason for higher stability of WCO and VCO against photo-oxidation than other types of oils. Furthermore, pigments such as riboflavin and porphyrins (chlorophyll) can act as initiators of lipid photo-oxidation (W¹sowicz et al., 2004). Therefore, the presence of pigments in addition to the less total phenolics could have led to less stability of CPO and CO.

Conclusion

In conclusion, WCO and VCO showed higher stability against both autoxidation and photo-oxidation than RBDCO, CPO and CO. This may be attributed to the high phenolic and other minor natural compounds as well as high MUFA to PUFA ratio. While the unrefined oils contain high amount of phenolic and other natural antioxidants including vitamins, which act to increase the oxidative stability, presence of prooxidants such as free fatty acids, trace metals and others may act to reduce the stability. Thus, it is necessary to quantify all minor compounds present in these oils to make a clear conclusion regarding the stability of these oils against oxidation. Thus, further studies are needed to identify and quantify the minor compounds (pro-and antioxidants) in order to identify the exact reason for the variation in the oxidative stabilities among these various types of coconut oils. In addition, further research is necessary to examine the effect of packaging materials on the susceptibility of these oils to oxidation, which will help to minimize the oxidative deterioration and thus improve the organoleptic, nutritional and economic value of the coconut oils.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

Financial assistance provided by National Research Council (NRC) of Sri Lanka is highly appreciated.

REFERENCES

Ahmed M, Pickova J, Ahmad T, Liaquat M, Farid A, Jahangir M (2016). Oxidation of Lipids in Foods. Sarhad Journal of Agriculture 32(3):230-238.

Amarasiri WADL, Dissanayake AS (2006). Coconut fats. Ceylon Medical Journal 51(2):47-51. Available at: https://www.ncbi.nlm.nih.gov/pubmed/17180807

Adhikari P, Shin J, Lee JH, Hu JN, Zhu XM, Akoh CC, Lee KT (2010). Production of trans-free margarine stock by enzymatic interesterification of rice bran oil, palm stearin and coconut oil. Journal of the Science of Food and Agriculture 90(4):703-711.

Ayyildiz HF, Topkafa M, Kara H, Sherazi STH (2015). Evaluation of fatty acid composition, tocols profile, and oxidative stability of some fully refined edible oils. International Journal of Food Properties 18(9):2064-2076.

Bendini A, Cerretani L, Salvador M, Fregapane G, Lercker G (2009). Stability of the sensory quality of virgin olive oil during storage: an overview. Italian Journal of Food Science 21(4):389-406.

Candela CG, López LMB, Kohen VL (2011). Importance of a balanced omega 6/omega 3 ratio for the maintenance of health. Nutritional recommendations. Nutricion Hospitalaria 26(2):323-329.

Central Bank of Sri Lanka (2017). Annual Report 2016. Published by the Central Bank of Sri Lanka, Colombo). Available at: https://www.cbsl.gov.lk/en/publications/economic-and-financial-reports/annual-reports/annual-report-2016

Choe E, Min DB (2006). Mechanisms and factors for edible oil oxidation. Comprehensive Reviews in Food Science and Food Safety 5(4):169-186.

Christie WW (1992). Detectors for high-performance liquid chromatography of lipids with special reference to evaporative light-scattering detection. Advances in Lipid Methodology 1:239-271.

Codex Alimentarius (1999). Codex Standards for Named Vegetable Oils CODEX-STAN 210:1-3.

Comandini P, Verardo V, Maiocchi P, Caboni MF (2009). Accelerated oxidation: comparative study of a new reactor with oxidation stability instrument. European Journal of Lipid Science and Technology 111(9):933-940.

Dąbrowska M, Zielińska A, Nowak I (2015). Lipid oxidation products as a potential health and analytical problem. Chemik 69(2):89-94. Available at: http://www.chemikinternational.com/year-2015/lipid-oxidation-products-as-a-potential-health-and-analytical-problem/

Evans CD, List GR, Moser HA, Conan IC (1973). Long term storage of soybean and cottonseed salad oils. Journal of the American Oil Chemists' Society 50(6):218-222.

Galano JM, Lee YY, Durand T, Lee JCY (2015). Special Issue on "Analytical Methods for Oxidized Biomolecules and Antioxidants" The use of isoprostanoids as biomarkers of oxidative damage, and their role in human dietary intervention studies. Free Radical Research 49(5):583-598.

Gargouri B, Zribi A, Bouaziz M (2015). Effect of containers on the quality of Chemlali olive oil during storage. Journal of Food Science and Technology 52(4):1948-1959.

Gordon MH (2001). The development of oxidative rancidity. In: Pokorny J, Yanishlieva N, Gordon M (eds) Antioxidants in Food – Practical Applications. CRC Press, Washington, pp. 7-22. Available at: http://www.scirp.org/(S(i43dyn45teexjx455qlt3d2q))/reference/ReferencesPapers.aspx?ReferenceID=1395596

Hornero-Méndez D, Pérez-Gálvez A, Mínguez-Mosquera MI (2001). A rapid spectrophotometric method for the determination of peroxide value in food lipids with high carotenoid content. Journal of the American Oil Chemists' Society 78(11):1151-1155.

Paquot C (1979). Standard methods for the analysis of oil, fat and derivatives. 6th edition. Pergamon Press: Toronto.

Khan MA, Shahidi F (1999). Rapid oxidation of commercial extra virgin olive oil stored under fluorescent light. Journal of Food Lipids 6(4):331-339.

Kim JP, Jung MY, Kim J, Kim SY (2000). Antiphotooxidative activity of protoberberines derived from Coptis japonica makino in the chlorophyll-sensitized photooxidation of oil. Journal of Agricultural and Food Chemistry 48(4):1058-1063.

Krishna GAG, Raj G, Bhatnagar AS, Kumar PPK, Chandrashekar P (2010). Coconut oil: chemistry, production and its applications-a review. Indian Coconut Journal 53(3):15-27.

Lee J, Koo N, Min DB (2004). Reactive oxygen species, aging, and antioxidative nutraceuticals. Comprehensive Reviews in Food Science and Food Safety 3(1):21-33.

Li Y, Ma W, Qi B, Rokayya S, Li D, Wang J, Feng H, Sui X, Jiang L (2014). Blending of soybean oil with selected vegetable oils: impact on oxidative stability and radical scavenging activity. Asian Pacific Journal of Cancer Prevention 15(6):2583-2589.

Manchanda SC, Passi SJ (2016). Selecting healthy edible oil in the Indian context. Indian Heart Journal 68(4):447-449.

Medina-Juárez LA, Gámez-Meza N (2011). Effect of refining process and use of natural antioxidants on soybean oil. In Soybean-Biochemistry, Chemistry and Physiology. InTech pp. 435-460.

Méndez AI, Falqué E (2007). Effect of storage time and container type on the quality of extra-virgin olive oil. Food Control 18(5):521-529.

Ross CF, Smith DM (2006). Use of volatiles as indicators of lipid oxidation in muscle foods. Comprehensive Reviews in Food Science and Food Safety 5(1):18-25.

Rukmini A, Raharjo S (2010). Pattern of peroxide value changes in virgin coconut oil (VCO) due to photo-oxidation sensitized by chlorophyll. Journal of the American Oil Chemists' Society 87(12):1407-1412.

Shahidi F, Amarowicz R, A. Abou-Gharbia H, Adel Y. Shehata A (1997). Endogenous antioxidants and stability of sesame oil as affected by processing and storage. J. Am. Oil Chem. Soc. 74(2): 143-148. https://doi.org/10.1007/s11746-997-0159-0

Shahidi F, Zhong Y (2005). Lipid oxidation: Measurement methods. In: Shahidi F (Eds,). Bailey's industrial oil and fat products. John Wiley and Sons, Inc.

Shinagawa F, Carvalho de Santana F, Araujo E, Purgatto E, Jorge MANCINI-Filho (2017). Chemical composition of cold pressed Brazilian grape seed oil. Food Science and Technology 38(1):164-171.

Song W, Bardowell S, O'Shea K (2007). Mechanistic study and the influence of oxygen on the photosensitized transformations of microcystins (cyanotoxins). Environmental Science and Technology 41(15):5336-5341.

Steel CJ, Dobarganes MC, Barrera-Arellano D (2005). The Influence of natural tocopherols during thermal oxidation of refined and partially hydrogenated soybean oils. Grasas Aceites 56:46-52.

Szydłowska-Czerniak A, Łaszewska A (2015). Effect of refining process on antioxidant capacity, total phenolics and prooxidants contents in rapeseed oils. LWT-Food Science and Technology 64(2):853-859.

Thaipong K, Boonprakob U, Crosby K, Cisneros-Zevallos L, Hawkins Byrne D (2006). Comparison of ABTS, DPPH, FRAP, and ORAC assays for estimating antioxidant activity from guava fruit extracts. Journal of Food Composition and Analysis 19(6-7):669-675.

Ullah J, Hamayoun M, Ahmad T, Ayub M, Zarafullah M (2003). Effect of light, natural and synthetic antioxidants on stability of edible oil and fats. Asian Journal Plant Science 2(17-24):1192-1194.

Velasco J, Dobarganes C (2002). Oxidative stability of virgin olive oil. European Journal of Lipid Science and Technology 104(9-10):661-676.

Vidrih R, Vidakovic S, Abramovi H (2010). Biochemical parameters and oxidative resistance to thermal treatment of refined and unrefined vegetable edible oils. Czech Journal of Food Sciences 28(5):376-384.

W¹sowicz E, Gramza A, Hêœ M, Jeleñ HH, Korczak J, Ma³ecka M, Mildner-Szkudlarz S, Rudziñska M, Samotyja U, Zawirska-Wojtasiak R (2004). Oxidation of lipids in foods. Poland Journal of Food Nutrition Science 13/54:87-100.

Xia W, Budge SM (2017). Techniques for the analysis of minor lipid oxidation products derived from triacylglycerols: Epoxides, alcohols,

and ketones. Comprehensive Reviews in Food Science and Food Safety 16(4):735-758.
Yi J, Andersen MH, Skibsted L (2011). Interactions between tocopherols, tocotrienols and carotenoids during autoxidation of mixed palm olein and fish oil. Food Chemistry 127(4):1792-1797.

Vol. 12(9) pp. 230-237, September 2018
DOI: 10.5897/AJFS2018.1725
Article Number: 6D93A8C58059
ISSN: 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Isolation and identification of probiotic Lactobacillus species from traditional drink kunun-zaki fortified with paddy rice and sweet potatoes

Victor-Aduloju A. T.[1*], Anyamene C.[2], Ogbu K. N.[3] and Ishiwu C. N.[1]

[1]Department of Food Science and Technology, Faculty of Agriculture, Nnamdi Azikiwe University Awka, Anambra State, Nigeria.
[2]Department of Applied Microbiology and Brewing, Faculty of Biosciences, Nnamdi Azikiwe University Awka, Anambra State, Nigeria.
[3]Department of Agricultural and Bioresources Engineering, Nnamdi Azikiwe University Awka, Anambra State, Nigeria.

Received 25 May, 2018; Accepted 25 June, 2018

The present study was conducted to determine the probiotic properties of the lactic acid bacteria (LAB) isolated from Kunun-zaki processed with different thickeners. Kunun-zaki was processed using 50:50 and 80:20 millet and sorghum. A total of seven isolates that is, *Lactobacillus* (*Lb.*) *lactis, Lactobacillus casei, Lactobacillus brevis, Lb. acidophilus, Lactobacillus fermentum, Lactobacillus delbrueckii* and *Lactobacillus plantarum* were isolated. The isolated LAB showed good growth in the presence of 1% NaCl but none at 10% NaCl except *L. plantarum*. The susceptibility of isolated *Lactobacillus* toward selected pathogenic organisms was determined using standard agar well diffusion method. *Lb. plantarum* was predominant and showed the most significant antimicrobial inhibition against tested pathogenic strains (*Pseudomonas aeruginosa, Staphylococcus haemolyticus* and *Klebsiella pneumoniae*) tested followed by *Lb. casei*. The results of this study showed that LAB species isolated from Kunun-zaki processed fulfill the most common criteria of probiotic bacteria. Therefore, numerous probiotic products can be developed from Kunun-zaki.

Key words: Probiotics, kunun-zaki, *Lactobacillus* spp., antimicrobial inhibition.

INTRODUCTION

Lactobacillus spp. represents a highly diverse group of Gram-positive micro-aerophilic bacteria that micro-scopically appear as long to short rods (MacFaddin, 2000). Species within this genus are generally catalase-negative, either homo or heterofermentative with regard to hexose metabolism (Hasan and Frank, 2001). Certain species of lactobacilli are important and are gaining increasing attention in food fermentation industry because of their biotechnologically interesting properties (Roy et al., 2000). Based on their generally regarded as

*Corresponding author. E-mail: yemtop2013@gmail.com.

safe (GRAS) status, lactobacilli have been extensively studied for their molecular biology in order to improve their specific beneficial characteristics (Daly and Davis, 1998). The largest group of probiotic bacteria in the intestine is lactic acid bacteria (LAB) (Diplock et al., 2001).

Probiotics are live microorganisms that are similar to beneficial microorganisms found in the human gut and have emerged as a major balancing factor influencing gastrointestinal physiology and function. In the food industry, LAB is widely used as starter cultures and has been recognized to be part of human microbiota (Holzapfel and Wood, 1995). The criteria for the *in-vitro* selection of lactobacilli to be used as health- promoting probiotic ingredients in food and pharmaceutical preparations include antibiotic tolerance as well as the production of lactic acid that inhibits the growth of other microorganisms which allow them to be established in the intestinal tract (Oskar et al., 2004).

LAB is very significant to human health due to the production of some antimicrobial substances and ability to inhibit pathogenic bacteria (Flórez et al., 2005). Furthermore, the bacteria are also used as a starter culture in the production of various foods. Certain *Lactobacillus* strains are considered important owing to their role in various foods and feed fermentations, production of many important metabolites and owing to their role in the prevention of food spoilage. Furthermore, they play a role in combating intoxication and infection by acting as antagonists against other pathogens through the production of antimicrobials and bacteriocin (Hirano et al., 2003; Oyetayo, 2004).

Kunun-zaki is one of the most highly consumed cereal-based non-alcoholic, non-carbonated beverages in Nigeria (Ayo et al., 2004). It is a fermented non-alcoholic cereal beverage whose popularity is due to its characteristic sweet-sour taste typical of LAB fermented foods of African origin (Efiuvwevwere and Akoma, 1995). Cereals used for Kunun-zaki include: sorghum (*Sorghum bicolor*), millet (*Pennisetum typhoideum*), maize (*Zea mays*), rice (*Oryza sativa*), acha (*Digitalis exilis*) or wheat (*Triticuma estivum*) and other cereals could be used in non-composite proportions (Ayo-Omogie and Okorie, 2016).

Kunun-zaki is normally flavoured with a combination of spices which includes ginger (*Zingiber officinale*), cloves (*Eugenia aromatica*), black pepper (*Pipper guinese*), cinnamon (*Xylopia acthiopica*) and together with saccharifying agents such as paste of sweet potato tubers, malted rice, malted sorghum, crude extract from dried *Cadaba farinose* stems are also added (Adebayo et al., 2010).

Research has been done on the production of Kunun-zaki and many strains of probiotic bacteria have been isolated from different sources. However, not much studies has been carried out on isolation of probiotic *Lactobacillus* strains from Kunun-zaki processed with sweet potatoes and paddy rice thickener, so there is little or no information on it. The aim of this study was to isolate and identify probiotic *Lactobacillus* species from traditional drink Kunun-zaki fortified with different thickeners.

MATERIALS AND METHODS

Collection of raw materials for Kunun-zaki production

Millet (*Pennisetum glaucum*), sorghum (*Sorghum bicolor*), ginger (*Zingiber officinale*), red pepper (*Capsicum annum*), clove (*Eugina caryophyllata*) and paddy rice (*Oryza sativa*) were all purchased from Eke Awka market, Awka South Local Government, Anambra State, Nigeria. These grains were sorted, cleaned and stored in plastic containers at ambient temperature before being used.

Collection of pathogenic microorganisms

Three pathogens (*Staphylococcus haemolyticus, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) were collected from Department of Microbiology, Chwukwuemeka Odumegwu Ojukwu Teaching Hospital, Awka, Anambra State, Nigeria. The isolates were confirmed by morphological and biochemical tests according to Cooper and Lawrence (1996). The isolates were sub-cultured on nutrient agar plates and incubated for 24 h at 35-37ºC. The colonies were picked and stored on slants at 4° C until when needed.

Preparation of sweet potatoes

Sweet potatoes (*Ipomea batatas*) was purchased from Government House Market Awka, Nigeria. The potatoes were washed, sliced into 2 cm and sundried for 5 days then ground into powder.

Preparation of ground malted rice paste

The 250 g of paddy rice to be used was washed with tap water and soaked in 500 ml of tap water (1:2 w/v) for 12 h and then drained. The drained grains were couched by covering them with a moist cloth for 4 to 5 days at ambient temperature (30°C) to germinate and then dried in the sun for 3 days. The dried malted rice was washed and ground into a paste.

Preparation of Kunun-zaki

Kunun-zaki was prepared from 50:50 and 80:20% millet and sorghum with modification on ingredients according to Adelekan et al. (2013). 500 g of cereal grains were washed with potable water, drained and steeped in 1000 ml of tap water (1:2 w/v) in a bucket for 8 h after which the grain was washed and mixed with 60 g of spices (ginger 40 g, clove 10 g, red pepper 10 g), these were washed and then ground to paste (Figure 1). The slurry was divided into two unequal portions (1:3 w/w). The larger portion was gelatinized by the addition of boiling water (1:1v/v) in a plastic container and stirred vigorously (2 to 3 min) following which it was cooled to about 50°C. The slurry was allowed to sediment and ferment for 12 h. The fermented samples were sieved using a clean muslin cloth (mesh 350 µm). The supernatant liquid was decanted and the filtrate (Kunun-zaki) was packed in the sterile container for subsequent analysis (Figure 1). The paddy rice and sweet potatoes based Kunun-zaki were produced differently by applying the aforementioned procedures. Kunun-zaki produced without any

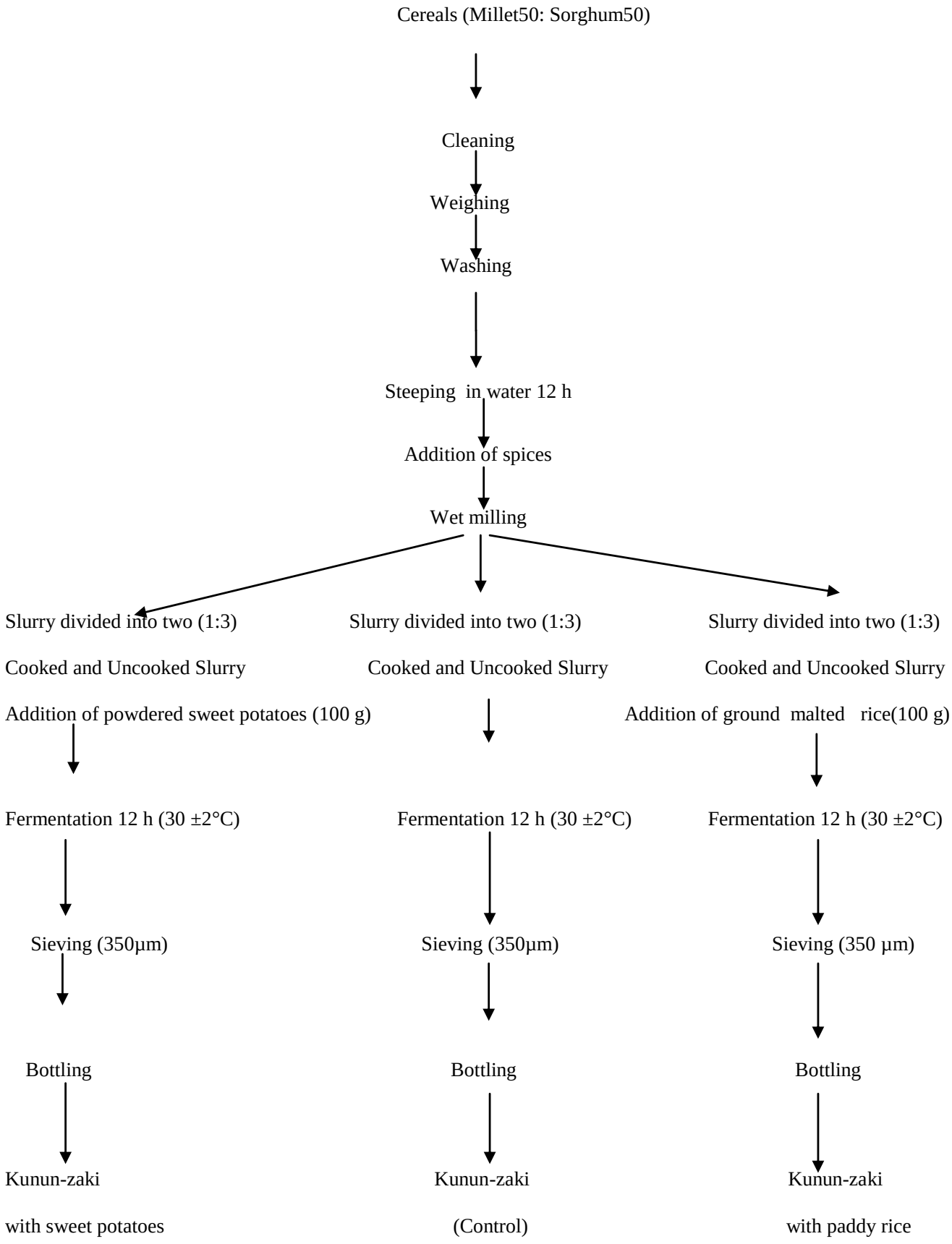

Figure 1. Flow chart for the production of different Kunun-zaki samples.

thickener served as control. Figure 1 showed the preparation of Kunun-zaki.

Isolation of lactic acid bacteria

The bacteria *Lactobacillus* spp. were isolated from Kunun-zaki samples by using modified MRS broth and MRS agar (Tharmaraj and Shah, 2003). Additionally, 0.05% cysteine was added to MRS to improve the specificity of this medium for isolation of *Lactobacillus* spp. (Hartemink et al., 1997). The pH of the media was adjusted to 6.5. All media and glassware's were autoclaved for 15 min at 121°C before use. 1 ml of each sample was separately suspended in 100 ml of MRS broth of pH 6.5 and homogenized. Five-fold dilutions were made from each homogenized sample, and all dilutions were incubated for 24 h at 37°C under an anaerobic condition in the presence of 5 % CO_2. A loopful of each culture was streaked on to the MRS agar plate and plates were incubated under anaerobic condition at 37°C for 24 h. The single colony of *Lactobacillus* was isolated by observing their colony morphology and some biochemical tests (Gram staining, catalase and motilitytest), and the culture were maintained in MRS broth at pH 5.5.

Identification

The isolated bacteria were identified as *Lactobacillus* species by observing their morphological characteristics: Gram staining, motility test, catalase test, indole, oxidase and milk coagulation activities and 1-10% NaCl tolerance test (Issazadeh et al., 2013).

Sugar fermentation test

MRS broth at pH 6.5 was put into a screw-capped test tube and phenol red (0.01 g per L) was added into the tube as pH indicator (Devriese et al., 1993). After autoclaving, 1 ml of different sterilized sugar solutions (10%) was inoculated into the different tube. Then, 200 µl of an overnight bacterial culture was inoculated into the broth medium and incubated anaerobically at 37°C for 24 h. As a pH indicator, phenol red was included in the medium; acid production changed the medium from its original color to yellow (Manero and Blanch, 1999). After adding the proper amount of broth, Durham tubes were inserted into each culture tube in order to observe gas production (Herrero et al., 1996).

Screening of isolated *Lactobacillus* species for probiotic properties

Tolerance of isolated LAB to acidic pH

The tolerance of the probiotic bacteria to acidic pH was tested *in vitro* as described by Pelinescu et al. (2009). 1 ml of each LAB culture at 1×10^{8} CFU/ml was inoculated into sterile MRS broth and incubated anaerobically at 37°C overnight, then sub-cultured into fresh MRS broth tubes of pH 2 to 4 (broth was adjusted by a pH meter using HCl and NaOH) and incubated anaerobically at 37°C for 24 h. After incubation, 1 ml inoculums from each tube was inoculated into MRS agar medium using pour plate technique and incubated anaerobically at 37°C for 48 h. The growth (indicated by presence or absence of growth) of the LAB on MRS agar was used to designate isolates as pH tolerant.

Sensitivity to temperature

The selected LAB cultures were inoculated into 10 ml sterile MRS broth and incubated anaerobically at varying temperatures (from 15 to 45°C) for 48 to 72 h. Thereafter, 1 ml inoculums were transferred to MRS agar plates by pour plate method and incubated at 37°C for 48 h. The growth of LAB on MRS agar plates was used to designate isolates as temperature tolerant (Tambekar and Bhutada, 2010).

NaCl tolerance

Tested LAB cultures were inoculated into 10 ml sterile MRS broth with NaCl concentration between 1 to 10% and incubated at 37ºC for 48 h. Growth was monitored by visual inspection of the test tubes and NaCl tolerance was evaluated after 1 ml was platted using sterile MRS agar, allowed to set and incubated at 37ºC for a period of 48 h (Tambekar and Bhutada, 2010). Positive control experiments were made of tubes containing LAB cultures without additional NaCl, while negative control experiments were tubes with added NaCl but without LAB cultures.

Assay for antimicrobial activity

Antimicrobial activities of probiotics were determined by the agar well diffusion method as described by Tajehmiri et al. (2014). A 0.2 ml of a 24 h broth culture was aseptically introduced into the sterile petri dishes. The sterilized medium at 45 to 50°C was poured into petri dishes. The agar depth was 4 mm. A 26 ml medium was used for the plate with 90 mm diameter. Wells were made on the agar plates using a sterile cork borer of 5 mm diameter. A 100 µl of the supernatants of isolated probiotics were placed into each well. A negative control was 100 µl of the broth without organisms. The culture plate was incubated at 37°C for 48 h, and the resulting zones of inhibition were measured using a ruler calibrated in millimeter. Each experiment was replicated three times and the results were expressed as average values. Isolates which gave an inhibition zone bigger than 10 mm was determined to have antimicrobial activity.

Statistical analysis

Data were subjected to analysis of variance (ANOVA) using Minitab 16.0 statistical software (Minitab Inc., State College, PA, USA), and they were characterized descriptively as means ± standard deviation. Statistically, significant means were separated using Duncan Multiple Range test (DMRT). The significance level adopted was $P\leq0.05$.

RESULTS AND DISCUSSION

Isolation and identification

Seven *Lactobacillus* bacterial isolates were obtained from five samples of Kunun-zaki. The organisms include *Lactobacillus lactis, Lactobacillus casei, Lactobacillus brevis, Lactobacillus acidophilus, Lactobacillus fermentum, Lactobacillus delbrueckii* and *Lactobacillus plantarum.* Microscopically, the isolates were: Gram positive, rod-shaped, oxidase negative, catalase negative, non-motile, indole- negative, starch hydrolysis negative and absence of endospores. The carbohydrates fermentation patterns of the isolates and their characteristics are listed in Table 1. This is in agreement with the study of Adelekan et al. (2013) and Okoronkwo

Table 1. Physiological and biochemical properties of *Lactobacillus* species isolated from Kunun-zaki.

Variable	Isolate 1	Isolate 2	Isolate 3	Isolate 4	Isolate 5	Isolate 6	Isolate 7
Gram reaction	+	+	+	+	+	+	+
Catalase	-	-	-	-	-	-	-
Oxidase	-	-	-	-	-	-	-
Motility	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-
pH3	+	+	+	-	+	+	+
pH5	+	+	+	+	+	+	+
15°C	+	+	-	+	+	-	+
45°C	+	+	+	+	+	+	+
CO_2 from glucose	+	-	-	-	+	-	+
Production of acid	-	-	-	-	+	-	+
Glucose	+	+	+	+	+	+	+
Lactose	+	+	-	+	+	+	+
Sucrose	+	-	+	+	+	-	+
Mannitol	+	+	-	-	+	-	+
Sorbitol	-	+	-	-	+	-	+
Gala	+	+	-	+	+	-	+
Arabinose	+	+	+	+	+	-	+
Probable identity	*L. lactis*	*L. casei*	*L. brevis*	*L. acidophillus*	*L. fermantum*	*L. delbrueckii*	*L. plantarum*

+ = Positive reaction; -= Negative reaction.

Table 2. LAB isolated from Kunun-zaki processed with natural thickeners.

Isolate	Source of isolation	Significant LAB isolated
1	Millet- Sorghum at 50:50%	*Lactobacillus lactis*
2	Millet- Sorghum+ Paddy Rice at 50:50%	*Lactobacillus casei*
3	Millet- Sorghum + Sweet Potatoes at 50:50%	*Lactobacillus brevis*
4	Millet- Sorghum + Sweet Potatoes at 50:50%	*Lactobacillus acidophilus*
5	Millet and Sorghum at 80:20%	*Lactobacillus fermentum*
6	Millet and Sorghum at 80:20%	*Lactobacillus delbrueckii*
7	Millet- Sorghum + Paddy Rice at 80:20%	*Lactobacillus plantarum*

(2014). The identified LAB are organisms which are naturally present in the human gut also they are the organisms that cause fermentation in Kunun-zaki (Oluwajoba et al., 2013). Table 2 showed that *L. fermentum* and *L. delbrueckii* were isolated from Kunun-zaki made from millet and sorghum at 80:20%. The colonies of *Lactobacillus* isolates appeared rough, dull white, 0.1 to 0.5 mm in diameter, and demonstrated medium to short rods.

Sugar fermentation

Isolate 7 tentatively identified as *Lactobacillus plantarum*fermented all the sugars used. *L. lactis, L. brevis, L. acidophilus* and *L. delbrueckii* did not produce gas from glucose and other sugars, and also showed variation in sugar fermentation patterns. Sorbitol was only fermented by three isolates.

Sensitivity to temperature and pH

The growth of the *Lactobacillus* species as presented in Table 1 showed that all the isolates were able to grow at high temperature but *L. brevis* and *L. delbrueckii* showed no growth at 15°C. According to Ibourahema et al. (2008), the bacterial capability to grow at high temperature is a good characteristic as it could be interpreted as indicating an increased rate of growth and lactic acid population. LAB are acidophilic which means they are tolerant to low pH (Klaenhammer and Kullen, 1999). However, this needs to be differentiated from a condition of high concentration of free acids because the free acids may cause growth inhibitors (Mohd and Tan, 2007).

Table 3. Sodium chloride tolerance of isolated *Lactobacillus* spp..

LAB	1%	2%	3%	4%	5%	6%	7%	8%	9%	10%
A	++	+	+	+	+	+	+	+	+	+
B	-	-	-	-	-	-	-	-	-	-
Lacobacillus lactis	++	+	+	+	+	+	+	+	+	-
Lactobacillus casei	++	+	+	+	+	+	+	+	+	-
Lactobacillus brevis	++	+	+	+	+	+	+	+	+	-
Lactobacillus acidophillus	++	+	+	+	+	+	+	+	+	-
Lactobacillus fermentum	++	+	+	+	+	+	+	+	+	-
Lactobacillus delbruekii	++	+	+	+	+	+	+	+	+	-
Lactobacillus plantarum	++	+	+	+	+	+	+	+	+	+

++ = Good growth; + = visible growth; - = no growth. A=Positive control (Tubes with lactic acid bacteria cultures without NaCl); B=Negative control (Tubes with NaCl but without cultures); LAB= Lactic acid bacteria.

Table 4. Inhibition zones (mm) induced by lactic acid bacteria against the pathogenic bacteria.

	Inhibition zone (mm)		
LAB isolates	***Pseudomonas aeruginosa***	***Staphylococcus haemolyticus***	***Klebsiella pneumoniae***
Lactobacillus lactis	8.43±0.05^{f}	7.57±0.02^{g}	6.82±0.03^{g}
Lactobacillus casei	13.57±0.02^{b}	14.03±0.03^{b}	11.83±0.02^{b}
Lactobacillus brevis	8.69±0.04^{e}	8.46±0.06^{e}	8.80±0.04^{c}
Lactobacillus acidophilus	9.71±0.55^{c}	9.42±0.06^{c}	8.46±0.02^{d}
Lactobacillus fermentum	9.46±0.04^{d}	8.14 ±0.01^{f}	7.25±0.04^{e}
Lactobacillus delbrueckii	5.15±0.02	9.23±0.01^{d}	7.04±0.06^{f}
Lactobacillus plantarum	13.86±0.03^{a}	14.25±0.03^{a}	13.19±0.03^{a}

Each data is the mean of triplicate determination with standard error.; Different superscripts within the same column with each test are significantly different (P≤5).

Tolerance to NaCl

In this study, all the isolated probiotic candidates were able to grow at 1 to 9% NaCl, whereas fairly grow at 7% but completely failed at 8 to 10% NaCl (Table 3). The tolerance of all the isolates to high NaCl concentration (4 to 9%) further indicated their potential to survive the harsh conditions and bile salt of the intestine (Cullimore, 2000; Topisirovic et al., 2006).

Antimicrobial activity of lactic acid bacteria

The results of probiotics antimicrobial effect on selected pathogenic organisms are shown in Table 4. The comparison of the inhibitory zones caused by the seven strains of probiotics (*L. lactis, L. casei, L. brevis, L. acidophilus, L. fermentum, L. delbrueckii* and *L. plantarum*) showed that the antimicrobial effect of *L. plantarum* and *L. casei* was significantly different from other strains. *L. plantarum* had the highest zone of inhibitory on all the pathogenic organisms (Table 4). *L. delbrueckii* exhibited lowest zone of inhibition on *Pseudomonas aeruginosa* (5.15±0.02 mm). Antibacterial activity of *L. lactis* against *Staphylococcus haemolyticus* and *Klebsiella pneumoniae* showed lowest zones of inhibition (Table 4). The observed variation in the inhibition of the test pathogens by the LAB is an indication that the organisms possess varying abilities to exert antimicrobial effects on pathogens and this corroborates the study of Azcarate-Peril et al. (2004) that antimicrobial effect exerted by lactic acid bacteria are strain specific. In general, tolerance to sodium chloride salts has been considered a condition for colonization and metabolic activity of bacteria in the host intestine (Anadon et al., 2006). The low resistance of *Lactobacillus delbrueckii* toward *Pseudomonas aeruginosa* may be an indication of their potential to survive the temperature of the human gut since temperature is an important requirement for bacterial growth, and the selected temperature range was chosen to simulate the normal human body temperature. This factor is very important in determining the effectiveness of probiotics since growth and viability during storage and use is one of the important determining factors for the functionality of probiotics (Tambekar and Bhutada, 2010) (Tables 1 to 4).

Conclusion

L. plantarum has been shown to be the most effective

probiotic which exerted the highest antimicrobial effect against the test pathogens. Lactic acid bacteria from Kunun-zaki may act as a reservoir of antimicrobial-resistance genes. These bacteria could act as bio-therapeutic microorganisms and might be good probiotic drink.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENT

The authors are grateful to the Tertiary Education Trust Fund (tet fund) for providing financial support under "Grants for Institutional Based Research Fund" in conducting this research work.

REFERENCES

Adebayo GB, Otunda GA, Ajao TA (2010). Physcio-chemical, microbiological and sensory characteristics of kunun prepared from millet, maize and guinea-corn and stored at selected temperatures. Advance Journal of Food Science and Technology 2(1):41-46.

Adelekan AO, Alamu AE, Arisa NU, Adebayo YO, Dosa AS (2013). Nutritional, microbiological and sensory characteristics of malted soy-kunu zaki: an improved traditional beverage. Advances in Microbiology 3(04):389-397.

Anadon A, Larranaga RM, Martinez AM (2006). Probiotic for animal nutrition in the European Union, regulation and safety assessment. Regulatory Toxicology and Pharmacology 45(1):91-95.

Ayo HN, Okorie EI (2016). *In vitro* probiotic potential of autochthonous lactic acid bacteria and microbiology of Kunu made from mixed grains. British Microbiology Research Journal 14(4):1-10.

Ayo JA, Umaianze G, Gaffa T (2004). Microbiological evaluation of kunu zaki and Zoborobo drink (beverage) locally produced and sold in a polytechnic community in Nigeria. Nigerian Food Journal 22(1):119-126.

Azcarate-Peril AM, Altermann E, Hoover-Fitzula RL, Cano RJ, Klaenhammer, TR. (2004). Identification and inactivation of genetic loci involved with *Lactobacillus acidophilus* acid tolerance. Applied and environmental microbiology 70(9):5315-5322.

Cooper R, Lawrence JC (1996). The isolation and identification of bacterial from wounds. Journal of Wound Care 5(7):335-340.

Cullimore DR (2000). Practical Atlas for Bacterial Identification. CRC/Lewis Publishers, London P 209.

Daly C, Davis R (1998). The biotechnology of lactic acid bacteria with emphasis on applications in food safety and human health. Agricultural and Food Science 7(2):251-265.

Devriese LA, Pot B, Collins MD (1993). Phenotypic identification of genus *Enterococcus* and differentiation of phylogenetically distinct enterococcal species and species groups. Journal of Applied Bacteriology 75(5):399-408.

Diplock PAT, Aggett M, Ashwell F, Bornet EF, Roberfroid WH, Holzapfel P, Haberer RJ, Geisen BB, Schillinger M (2001). Taxonomy and important features of probiotic microorganisms in food nutrition. The American Journal of Clinical Nutrition 73(2):365-373.

Efiuvwevwere BO, Akoma AO (1995). The Microbiology of 'kunun-zaki', a cereal beverage from Northern Nigeria, during the fermentation (production) process. World Journal of Microbiology and Biotechnology 11(5):491-493.

Flórez BA, Delgado S, Mayo B (2005). Antimicrobial susceptibility of lactic acid bacteria isolated from a cheese environment. Canadian Journal of Microbiology 51(1):51-58.

Hartemink R, Domenech VR, Rombout FM (1997). LAMVAB - A new selective medium for the isolation of lactobacilli from faeces. Journal of Microbiological Methods 29(2):77-84.

Hasan NA, Frank JF (2001). Applied Dairy Microbiology. 2nd Edition. New York: Marcel Dekker, Inc., pp. 152-155.

Herrero MM, Gonzalez B, Suarez JE (1996). Evaluation of technologically important trains in lactic acid bacteria isolated from spontaneous fermentations. Journal of Applied Bacteriology 81(5):565-570.

Hirano T, Yoshida T, Sugiyama N, Koide IM, Yokochi T (2003). The effect of *Lactobacillus rhamnosus* on enterohemorrhagic *Escherichia coli* infection of human intestinal cells *in vitro*. Microbiology and Immunology 47(6):405-409.

Holzapfel WH, Wood BJB (1995). The Genera of Lactic Acid Bacteria in the Lactic acid. 1st Edition. Blackie Academic and Professional Publishing, London P 398.

Hoque F, Akter KM, Hossain MSM, Rahman MM, Billah KMD (2010). Isolation, identification and analysis of probiotic properties of *Lactobacillus* spp. from selective regional yoghurts. World Journal of Dairy and Food Sciences 5(1):39-46.

Ibourahema C, Dauphin RD, Jacqueline D, Thonart P (2008). Characterization of lactic acid bacteria isolated from poultry farm in Senegal. African Journal of Biotechnology 7(12):2006-2012.

Issazadeh K, Azizollahi A, Abadi M, KazemiDarsanaki R, Alikhani F, Dadras H, Tajehmiri A (2013). Isolation, identification and analysis of probiotic properties of Lactobacillus spp. from traditional yoghurts in North of Iran. Journal of Pure and Applied Microbiology 7(4):2965-2971 Available at: http://www.microbiologyjournal.org/archive_mg/jmabsread.php?snoid=1584&month=&year=

Klaenhammer TR, Kullen MJ (1999). Selection and design probiotic. International Journal of Food Microbiology 50(1-2):45-57.

MacFaddin JF (2000). Biochemical Tests for Identification of Medical Bacteria. 3rd edition. Lippincott Williams and Wilkins, USA.

Manero A, Blanch C (1999). Identification of *Enterococcus* ssp. With a biochemical key. Applied and Environmental Microbiology 65(10):4425-4430.

Mohd AF, Tan IK (2007). Isolation of lactic acid bacteria from Malaysian food and assessment of the isolates for industrial potential. Bioresource Technology 98(7):1380-1385.

Okoronkwo CU (2014). Isolation and characterization of lactic acid bacteria involved in the fermentation of millet and sorghum sold In Nkwo-Achara Market, Abia State. Journal of Environmental Science Toxicology and Food Technology 8(9):42-45.

Oskar A, Meydani SN, Russell RM (2004). Yogurt and gut function. The American Journal of Clinical Nutrition 80(2):245-256.

Oluwajoba SO, Akinyosoye F, Oyetayo VO (2013). Comparative sensory and proximate evaluation of spontaneously fermenting Kunu-zaki made from germinated and un-germinated composite cereal grains. Food Science and Nutrition 1(4):336-349.

Oyetayo VO (2004). Phenotypic characterization and assessment of the inhibitory potential of *Lactobacillus* isolates from different sources. African Journal of Biotechnology 3(7):355-357.

Pelinescu DR, Sasarman E, Chifiruc MC, Stoica I, Nohita AM, Avram I (2009). Isolation and identification of some *Lactobacillus* and *Enterococcus* strains by a polyphasic taxonomical approach. Romanian Biotechnological Letters 14(2):4225-4233.

Roy P, Ward D, Mondou F (2000). Molecular identification of potentially probiotic lactobacilli. Current Microbiology 40(1):40-46.

Tajehmiri A, Darsanaki RK, Moslem MN, Lozoumi Z, Kolavani MH, Aliabadi MA (2014). Antimicrobial activity of Lactobacillus spp. isolated from commercial yoghurt against pathogenic bacteria. Journal of Pure and Applied Microbiology 8(3):2211-2215.

Tambekar DH, Bhutada SA (2010). Studies on antimicrobial activity and characteristics of bacteriocins produced by *Lactobacillus* strains isolated from milk of domestic animals. The Internet Journal of Microbiology 8(2):1-6.

Tharmaraj N, Shah NP (2003). Selective enumeration of *Lactobacillus delbrueckii* ssp *bulgaricus*, *Streptococcus thermophilus*, *Lactobacillus acidophilus*, Bifidobacteria *Lactobacillus casei*, *Lactobacillus rhamnosus* and Propionibacteria. Journal of Dairy Science 86(7):2288-2296.

Topisirovic M, Kojic D, Fira N, Golic I, Strahinic Lozo J (2006). Potential of lactic acid bacteria isolated from specific natural riches food production and preservation. International Journal of Food Microbiology 112(3):230-235.

Vol. 12(9) pp. 238-245, September 2018
DOI: 10.5897/AJFS2017.1662
Article Number: 8A1BA3058096
ISSN: 1996-0794

http://www.academicjournals.org/AJFS

African Journal of Food Science

Full Length Research Paper

Proximate composition, physical characteristics and mineral content of fruit, pulp and seeds of *Parinari curatellifolia* (Maula) from Central Malawi

Lesten Eliez Chisomo Chatepa[1*], Kingsley Masamba[2] and Macdonald Jose[1]

[1]Department of Basic Science, Faculty of Agriculture, Lilongwe University of Agriculture and Natural Resources, P.O. Box 219, Lilongwe, Malawi.
[2]Department of Food Science and Technology, Faculty of Food and Human Sciences, Lilongwe University of Agriculture and Natural Resources, P.O. Box 219, Lilongwe, Malawi.

Received 6 October, 2017; Accepted 5 July, 2018

In this study, proximate composition, physical characteristics and mineral content of fruit, pulp and seeds of *Parinari curatellifolia* (Maula) fruits from Bunda forests in Central Malawi were determined. Proximate composition included crude protein, crude fat and crude fiber, while physical characteristics included mass, length, thickness of whole fruit and kernels and minerals included potassium, magnesium, manganese, copper, zinc, iron and phosphorus. Total carbohydrate contents were also determined. All the results have been presented on dry matter basis. Results on crude protein ranged from 3.9±0.03 to 15.61±0.05% with pulp registering the lowest values (P<0.05) while the highest values were registered in kernels. Results on crude fat revealed that kernels had the highest values (46.05±0.19%) compared to values obtained in pulp or a mixture of pulp and peels while for crude fiber, the highest values (21.39±0.28%) were obtained in whole fruit. Furthermore, results on ash content showed that the highest values (5.71±0.25 %) were obtained in fruit peels while the lowest values (1.58±0.15 %) were registered in whole fruit. Lastly, results on carbohydrate content revealed that the highest values (84.95±0.14%) were obtained in the pulp while the lowest values (34.34±0.21%) were registered in kernels. The findings from this study have shown that there are significant differences in nutrient and mineral composition in the whole fruit, seeds and pulp of *P. curatellifolia* from Central Malawi and therefore these findings can be useful in nutritional planning regarding consumption of the *P. curatellifolia* fruit.

Key words: Maula *(Parinari curatellifolia),* indigenous fruits, kernels, minerals, crude protein, nutrition, Bunda forest.

INTRODUCTION

Global human population is estimated to reach 9.6 billion by 2050 and 10.9 billion by 2100 respectively. On the other hand, it is expected that the population would rise to 8.2 billion by 2050 reaching 9.6 billion by the 21st

*Corresponding author. E-mail: lecchatepa@yahoo.com; lchatepa@bunda.luanar.mw.

century in developing worlds with a 5 times increase in the least developed countries like Malawi (UNPD, 2012).

Sub-Saharan Africa (SSA) population growth, Malawi inclusive, is expected to increase by 1.2% annually by 2050 triggering urbanization and improved individual income (UNPFA, 2008; Thornton, 2010; Théwis and Galiş, 2012). It has previously been reported that SSA has the highest number of food insecure people where 214.1 million people have been reported to be chronically undernourished in 2012 to 2014 with a prevalence of 23.8% (FAO et al., 2014).

Furthermore, it is widely acknowledged that global climate is changing. Global surface temperature has been estimated to increase between 1.8 and 4.08°C by 21st century (IPCC, 2007a, b). In lower latitudes, like in SSA, temperature changes have been projected to be 1 to 2.8°C with crop productivity in tropics and subtropics dropping by 10 to 20% by 2050 (Jones and Thornton, 2003) creating food insecurity because of dependence on rain-fed agriculture (IPCC, 2007a).

Cereal production for a number of crops in SSA would decline by 3.2% spiking prices by more than 4% in 2050 (Gachene et al., 2015; Ringler et al., 2010; Ringler et al., 2011) culminating in declining of cereal demand by 3.6 MMT representing 1.5% decrease by 2050 (Ringler et al., 2010). In SSA, 23.2% of the population, live on calorie-deficient food (FAO et al., 2015) with about 600,000 children expected to suffer from malnutrition by 2050 as a result of climate change (Ringler et al., 2010). Many people in rural and peri-urban populations of southern Africa, face food shortage (FAO, 2000), resulting in food acute malnutrition (Akinnifesi et al., 2004).

In SSA, many authors have previously reported that 4 in 5 rural people are poverty stricken depending on wild fruit harvesting to secure food supply during times of hunger/famine (Loghrust, 1986; Lockett and Grivetti, 2000; Makonda and Gillah, 2007; Msuya et al., 2010). Indigenous fruit trees (IFTs) are food and nutrient sources for SSA people in the form of minerals and vitamins from fruit consumption (Chirwa and Akinnifesi, 2008) while the surplus fruits have been used for sale (Akinnifesi et al., 2004). With respect to Malawi, many people experience severe food shortage in both dry and rainy season (October to February) forcing them to depend on wild products like indigenous fruits (Akinnifesi et al., 2004; Mtupanyama et al., 2008).

These fruits are consumed in different forms and some are used as medicines by the rural marginalized communities (Benhura et al., 2012). Indigenous fruits, like *Parinari curatellifolia* fruit extracts are used as cardiac tonic and for the treatment of heart diseases like hypertension whereas the leaf extracts treat body inflammation and anemia (Peni et al., 2010). Despite being significant in people lives, wild edible fruits have been underutilized with little attention in investigating their various potentials (Salih and Yahia, 2015). In view of increasing food insecurity from the effects of growing human population and climate change, new and nonconventional sources of food from indigenous fruit trees (IFTs) should be explored. Therefore research intensification in exploration of the significance of underutilized crops like fruits from IFTs is of paramount importance.

P. curatellifolia, locally known as Maula, is an IFT that grows in Sub-Saharan Africa, Malawi inclusive. *P. curatellifolia* grows naturally in Bunda forest and belongs to the *Chrysobalanceae* family (Oladimeji and Bello, 2011). It bears greenish with grey spots (Benhura et al., 2013) ovoid in shape fruits of 3-5 cm long and 2.4-4 cm in diameter (FAO, 1982). The fruits contain woody seed stones containing kernels which have high oil content and the kernels are eaten raw in the form of nuts (Saka and Msonthi, 1994; Katende et al., 1995; Benhura et al., 2012). The kernels have been reported to contain high levels of nutrients like crude protein, 33.1±0.12%, carbohydrates, 52.38±0.01% (Oladimeji and Bello, 2011), and crude fat, 37.75 % (Orwa et al., 2009).

Efforts have been made to explore the uses, growth, domestication and commercialization of wild fruit trees (IFTs) in SSA countries, Malawi inclusive, including that of *P. curatellifolia* (Mtupanyama et al., 2008; Kwesiga et al., 2000; Akinnifesi et al., 2004, 2006, and 2007). However, limited information exists on the nutritional value of these indigenous fruits in Malawi (Saka and Msonthi, 1994). The significance of foods for human consumption is based on their nutrients, energy and mineral contents. Against this background of having limited information on nutritional value of indigenous fruits such as *P. curatellifolia,* this current study was carried out with the aim of characterizing the physical properties and nutritional value, in terms of proximate, mineral and chemical composition of Maula fruits, with respect to the fruit, pulp and seeds, from Bunda Forest in Central Malawi.

MATERIALS AND METHODS

Sample collection and preparation

Ripe Maula *(Parinari curatellifolia)* fruits were collected from Bunda Forest which is under Lilongwe University of Agriculture and Natural Resources, Bunda campus, in Lilongwe district in Central Malawi in the months of September and October. The fruits were washed using distilled water and the peels and mixture of pulp and peels and pulp were manually removed (Figure 1) from the woody seed stone and were partially sundried for 24 h. The fruits were sundried at 37°C on a stainless metal sheet which was placed on a concrete floor to prevent dust accumulation (Brennand, 1994; Ahmed, 2013). The fruits were finally dried in the forced air laboratory oven at 60°C to avoid cooking them for 2 days (Wijewardana et al., 2016) (Figure 1). The woody seed stones were sundried for 5 weeks for seed kernel collection. The seed kernels were removed from the woody seed stone by crushing the stones with a hard stone as shown in Figure 2. Some fruits were sundried for 14 days and crushed together with kernels before finally being dried in the oven for 48 h at 60°C (Ahmed, 2013) (Figure 2). The dried whole fruits, pulp,

Figure 1. A: *Parinari curatellifolia* fruits, B: Peels, C: peeled fruits, D: fruit stones.

Figure 2. A: Removing kernels from the stones, B: kernels in the container, C: Kernels.

mixture of pulp and peels and peels were ground through a 1 mm sieve using a Thomas-WILEY model 4 Laboratory Mill before analyzing the chemical properties. The ground samples were used in the analysis for proximate composition; dry matter (DM), ash, crude protein (CP), crude fat and crude fiber (CF) using Association of Official Analytical Chemists (AOAC, 1996) methods.

Physical characteristics determination

The fruits and kernels were evaluated for their physical characteristics as described by Benhura et al. (2013) where 20 fruits and kernels were randomly selected, thoroughly cleaned and weighed on JP-2000 electronic balance to the nearest 0.01 g. The 20 fruits and kernels were weighed each at a time with its stone and peels weighed too. The physical characteristics of the seeds and kernels in terms of length (long axis) and thickness (short axis) were evaluated by using Mitutoyo vernier caliper.

Proximate composition

The analysis of dry matter, mineral ash, crude protein, crude fat, crude fiber and calculation of carbohydrate content in the samples were carried out using methods described in Association of Official Methods of Chemical Analysts (AOAC 1990), AOAC, (1996) and AOAC, (2005) respectively.

Dry matter using oven method

Dry matter (DM) was determined by drying the samples in a laboratory drying oven at 105°C for 5 h. The crucibles were thoroughly washed, dried in the oven, cooled in a desiccator and weighed. 2.5 g of the sample was weighed into the crucible and dried to constant weight. The sample dry matter in percentage was calculated as the fraction of the dried weight to that of the original one multiplied by 100 (AOAC, 1996; AOAC, 1990).

Ash using muffle furnace

Ash content was determined by igniting 2.5 g of the samples weighed in a porcelain crucible in the muffle furnace at 550°C for 2 h. The amount of ash content in percentage was calculated as shown below:

$$\%Ash = [(W_a-W_t)/ (W_0-W_t)] \times 100 \text{ (AOAC, 1990)}.$$

Where:

W_0 is weight of crucible and sample before igniting the sample, W_a is weight of crucible and ash and W_t is weight of crucible only.

Crude fat

Crude fat was analyzed by extracting 2.5 g of the sample weighed in the porous extraction thimble by using petroleum ether in a soxhlet apparatus for 16 h. The soxhlet apparatus was equipped with a water cooled condenser fitted on to the weighed 250 ml flat bottomed quick fit flask containing petroleum ether as a fat solvent. The solvent was boiled at 40°C continuously to extract the fat from the sample. The mixture of fat and solvent was collected in the flask and the solvent was evaporated at 40°C in a vacuum rotary evaporator. Thereafter, the flask was dried and re-weighed and crude fat content was calculated as a percentage of the dry weight of the sample as shown below:

$$\% \text{ Crude fat} = [(A-B) / C] \times 100 \text{ (AOAC, 1996)}.$$

Where A = weight of flask + oil, B = weight of flask only, C = weight of dry sample.

Crude fiber

Crude fiber was determined by boiling 1.5 g of the samples in 200

ml of weak Sulphuric acid (1.25%) and Sodium Hydroxide (1.25%), with few drops of anti-foaming agents being added, for 30 min respectively. The residues were filtered and washed three times with hot water and then washed with 95% ethanol and dried at 105°C for 5 h to constant weight. The dried residues were ignited in a muffle furnace at 550°C for 2 h. The crude fiber, in grams, was calculated as the difference between the weight of the residues and ash and converted as a fraction of the sample weight in percentages as shown in the equation below:

% Crude fiber = [(Loss of weight on ignition / sample weight)] x 100 (AOAC, 1990)

Crude protein using Kjeldahl method

Crude protein (CP) content of the samples was analyzed by using micro- Kjeldahl method, and the N content was converted to CP by multiplying by 6.25. The method involves digestion of the samples in concentrated (98 %) sulphuric acid with selenium tablet as a catalyst, distillation of the digests into weak acids (4 % boric acid) and titration of the distillates with 0.1 M Hydrochloric (HCl) acid (AOAC, 1990).

Carbohydrates

Carbohydrates content was calculated by difference using the following formula; 100 % - (CP % + CF % + crude fat % + Ash %) as described in AOAC (2005).

Determination of mineral composition

1.0 g of each sample was weighed in porcelain crucibles which were ignited in a muffle furnace at 550°C to constant weight. The ash was dissolved in 3 ml of 3 M Hydrochloric (HCl) acid, transferred to 100 ml volumetric flask and diluted to the 100 ml mark (AOAC, 2005). 0.75 ml of the diluted digested samples were placed in 20 to 25 ml glass vials and diluted with 9 ml of distilled water. Standards were prepared by adding 0.0 ml, 0.1 ml, 0.2 ml, 0.3 ml, 0.4 ml and 0.5 ml into 20 to 25 ml vials and diluted with 9 ml of distilled water. 2.0 ml of phosphovanadomolybdate /molybdate reagent (solution) was added in each vial and absorbance was measured after 1 h of color development (AOAC, 2005). Phosphorus was determined by a DR 5000 WAGTECH projects ultra-violet visible spectrophotometer at 860 nm wavelength. Potassium (K) was analyzed using Flame Photometer while magnesium, iron, manganese, copper and zinc were analyzed using PG990 atomic absorption spectrophotometer (AAS) (AOAC, 2005).

Statistical analysis

Data from the laboratory chemical analyses were done in triplicates and the mean value of each chemical parameter was calculated using Microsoft excel. The data was statistically analyzed by using analysis of variance (ANOVA) in Microsoft Excel ToolPak. T-test two-sample with unequal variances was used to compare mean values and significance was accepted at P≤ 0.05 level.

RESULTS AND DISCUSSION

Physical characteristics of *P.curatellifolia* seeds and kernels

Results on the physical characteristics of *P. curatellifolia* fruit are presented in Tables 1 and 2 respectively. The ripe fresh fruit weighed on average 22.69±0.69 g and this observation was in agreement with findings reported by Benhura et al. (2013) in Zimbabwe who found an average weight of 23.0±3.0 g for Waterfalls samples. The mean proportions of pulp and pulp and stone as a fraction of fruit were 46.75±0.9 and 90.09±0.50% respectively. However, the pulp proportion obtained in this study was lower than 51.1, 48.9 and 50.1% for *P. curatellifolia* fruit obtained from Amby, Waterfalls and Acadia in Zimbabwe respectively as reported by Benhura et al. (2013). *P. curatellifolia* kernel from this study weighed 352.8±11.4 mg and was 6.38±0.16 mm long with a thickness of 3.66±0.13 mm. The kernels from this study weighed less than 14000±3000, 21000±4000 and 23000±3000 mg for *P. curatellifolia* kernels when compared with kernels from Amby, Waterfalls and Acadia in Zimbabwe respectively (Benhura et al., 2013). Similarly, the kernels from this study were shorter than 22±3, 30±6 and 30±6 mm for *P. curatellifolia* kernels from Amby, Waterfalls and Acadia in Zimbabwe (Benhura et al., 2013). These differences could probably be attributed to the differences in ecological zones from where the fruit was grown (Tables 1 and 2).

Proximate composition

Results on proximate composition of *P. curatellifolia* fruits and its different parts in percentages are shown in Table 3.

Dry matter composition

Results on dry matter composition revealed that there were significant differences for the different fruit parts. Dry matter composition ranged from 88.66±0.15 to 99.31±0.04% for pulp and peels, and fruit respectively. Maula fruits registered higher dry matter values than ($P>0.05$) kernels, peels, pulp and ($P<0.05$) pulp and peels respectively.

Crude protein composition

Crude protein content ranged from 3.90±0.03 to 15.61±0.05% for pulp and kernel respectively. Crude protein content in pulp was the lowest ($P<0.05$) compared to the mixture of pulp and peels, kernels and ($P>0.05$) peels. The high crude protein value in the mixture of pulp and peels compared to that registered in the pulp could be attributed to the peels which are known to contribute

Table 1. Physical Characteristics of *Parinari curatellifolia* fruit**.**

Parameter	Value (Mean ± SE)
Mass of fruit (g)	22.69±0.69
Pulp + stone fraction (as % of fruit)	90.09 ±0.50
Pulp fraction (as % of fruit)	46.75±0.90
Pulp + peel fraction (as % of fruit)	56.67±0.77
Mean peel fraction (as % of fruit), n=20	9.91±0.50
Mean stone fraction (as % of fruit), n=20	43.33±0.77
Mean fruit length (mm), n=20	38.30±0.48
Mean fruit thickness (mm), n=20	30.84±0.56

Mean ± SE= mean ± standard error, n=20.

Table 2. Physical characteristics of *Parinari curatellifolia* kernel.

Parameter	Value (Mean ± SE)
Mass of kernel (mg)	352.8±11.4
Mean kernel fraction (as % of fruit), n=20	1.59±0.08
Mean kernel fraction (as % of stone), n=20	3.69±0.19
Mean kernel length (mm), n=20	16.78±0.40
Mean kernel width (mm) , n=20	6.38±0.16
Mean ernel thickness (mm), n=20	3.66±0.13

Mean ± SE= mean ± standard error, n=20.

the extra crude protein to the pulp (Benhura et al., 2012). The crude protein content in pulp was higher than 3.4 and 3.0 respectively as compared to the values reported by other researchers in Tanzania and Malawi (Ndabikunze et al., 2006; Saka and Msothi, 1994). The findings in this study have revealed that pulp, peels and mixture of pulp and peels are poor sources of proteins for humans. Crude protein content in the kernel was 15.61±0.05% which is lower than the value of 33.10±0.12% (Oladimeji and Bello, 2011) and 27.10 (Ndabikunze et al., 2006) for research conducted in Nigeria and Tanzania. Crude protein value of 15.61±0.05% for the kernels was lower than 24.70% for raw *Arachis hypogeae* seeds as reported by other researchers for studies conducted in Nigeria (Ayoola et al., 2012). These findings have shown that *P. curatellifolia* kernels are good sources of proteins for human nutrition.

Crude fat composition

Results on crude fat content showed that the range was from 2.02±0.47 to 46.05±0.19% for pulp and kernels respectively. Crude fat content in fruit was the lowest (P<0.05) and kernels had the highest (P<0.05) crude fat contents. Crude fat content in pulp was 3.99±0.08% which was higher (P<0.05) than 2.07±0.23 and 3.70±0.05% for the mixture of pulp and peels and peels only respectively. The values for crude fat were 0.9 and 1.5 higher than the values previously reported by other researchers (Ndabikunze et al., 2006; Saka and Msothi, 1994). Interestingly, crude fat values in kernels were high by different values as compared to values reported by other researchers: 1.77 (Ogungbenle and Atere, 2014) and 5.11±0.10% (Oladimeji and Bello, 2011) but lower than 47.00% (Ndabikunze et al., 2006) as reported for studies conducted in Nigeria and Tanzania. Crude fat value of 46.05±0.47 for *P. curatellifolia* kernel was higher than 39.10% (Kumar et al., 2013) but similar to 47.00±0.03 (Atasie et al., 2009) for groundnuts (*Arachis hypogaea*) as reported in other studies done in India and Nigeria respectively. The high crude fat and crude protein contents in kernels have shown that the kernels are potential sources of energy and essential amino acids and could therefore be used both as food for humans and feed for animals. The *P. curatellifolia* kernels flour could be used as an alternative of groundnut (*Arachis hypogaea*) flour in preparation of traditional vegetable soup by the less privileged rural communities.

Ash composition

Ash content was found to be the highest (P<0.05) in peels compared to that of kernels, fruit, pulp, mixture of pulp and peels respectively. However, ash content in pulp was equal (P>0.05) to that of kernels and mixture of pulp and peels. The ash content in the pulp was 2.46±0.09% which was low compared to the value of 3.9% reported

Table 3. Proximate composition of *Parinari curatellifolia.*

Sample	DM % (Mean±SE)	Ash % (Mean±SE)	CP % (Mean±SE)	Crude fat % (Mean±SE)	CF % (Mean±SE)	NFE % (Mean±SE)
P. Fruit	99.31±0.04^{a}	1.58±0.15^{a}	ND	2.02±0.47^{a}	21.39±0.28^{a}	ND
Pulp	90.6± 0.4^{a}	2.46±0.09^{b}	3.90±0.03^{a}	3.99±0.08^{b}	4.71±0.06^{b}	84.95±0.14^{a}
Pulp + Peels	88.66±0.15^{b}	2.85±0.06^{b}	5.14±0.10^{b}	2.07±0.23^{a}	6.25±0.11^{c}	83.70±0.28^{b}
Peels	95.59±0.03^{a}	5.71±0. 25^{c}	4.17±0.03^{c}	3.70±0.05^{b}	19.44±0.13^{d}	66.97±0.35^{c}
Kernels	99.27±0.04^{a}	2.43±0.02^{b}	15.61±0.05^{d}	46.05±0.19^{c}	1.58±0.04^{e}	34.34±0.21^{d}

Mean ± SE= mean ± standard error. For each parameter, means with same superscript were not significantly different (P>0.05). ND= not determined.

by Ndabikunze et al. (2006) in Tanzania but was higher than 1.8% (Saka and Msothi, 1994) as reported in Malawi. However, ash content in kernels was found to be 2.43±0.02% which was lower than the value of 2.65±0.21% reported in Nigeria by Oladimeji and Bello (2011). The ash content in kernels was lower than 4.55% (Kumar et al., 2013) but higher than 1.48% (Ayoola et al., 2012) for *A. hypogaea* as reported by other authors for studies previously conducted in India and Nigeria respectively.

Crude fiber

The crude fiber content ranged from 1.58±0.04 to 21.39±0.28% for kernels / nuts and whole fruit respectively. The kernel had the lowest (P<0.05) crude fiber content followed by pulp (4.71±0.06%), mixture of pulp and peels (6.25±0.11%) and peels (19.44±0.13%) respectively. The crude fiber content in the pulp was lower than 5.4 and 5.5% as reported in Tanzania and Malawi respectively (Ndabikunze et al., 2006; Saka and Msonthi, 1994). However, crude fiber content in kernels was lower than 5.45 and 1.6±0.1% as compared to the values reported in Nigeria by other researchers (Ogungbenle and Atere, 2014; Oladimeji and Bello, 2011). On the other hand, the crude fiber values for the kernels were lower than 3.7±0.3% (Atasie et al., 2009) and 2.91% (Kumar et al., 2013) for raw groundnuts seeds as reported in Nigeria and India respectively.

Carbohydrate composition

Results on carbohydrate content showed that the range was from 34.34±0.21 to 84.95±0.14% for kernels and pulp respectively. Pulp registered the highest (P<0.05) carbohydrate content compared to the mixture of pulp and peels, peels and kernels. Carbohydrate content in pulp was higher than 21.40±0.30, 27.50±0.50 and 28.90±0.40% respectively as compared to the values obtained for samples collected from Cranborne, Greendale and Prospect in a related study in Zimbabwe (Benhura et al., 2012). However, carbohydrate content for pulp from this study was comparable to the value of 88.20% reported in Malawi (Saka and Msonthi, 1994). The kernel carbohydrate content of 34.34±0.21% was lower than 52.38±0.01% (Oladimeji and Bello, 2011) but higher than 26.00% (Ndabikunze et al., 2006) in comparisons to values obtained in related studies in Nigeria and Tanzania respectively. Furthermore, the carbohydrates content for the kernels obtained in this study was higher than 17.41 (Ayoola et al., 2012) and 25.30% (Kumar et al., 2013) for *A. hypogaea* observed in other studies in Nigeria and India respectively. The high carbohydrate contents in pulp have demonstrated that the pulp could be a potential source of energy for consumers in Malawi.

Mineral composition

Results on different minerals obtained in *P. curatellifolia* fruit, pulp, mixture of pulp and peels, peels and kernels, in mg 100 g^{-1}, are presented in Table 4. Potassium content ranged from 680.20±4.88 to 736.24±6.29 for kernels and peels respectively. Potassium content in pulp was 712.65±12.02 which was higher (P<0.05) than 710.86±10.51 but lower (P>0.05) than 736.24±6.29 for mixture of pulp and peels and peels respectively. However, potassium content in pulp, mixture of pulp and peels and peels were higher (P<0.05) than that of kernels. The potassium content in pulp was higher than 22.3 (Ndabikunze et al., 2006), 103.68 (Saka and Msonthi, 1994) and 15±1.0% (Benhura et al., 2013) as reported in Tanzania and Zimbabwe. Potassium content in kernels was higher than 459 from a related study in Nigeria (Ogungbenle and Atere, 2014).

Iron content ranged from 295.49±0.68 to 403.81±4.34 for pulp and peels respectively. Peels had the highest (P>0.05) iron content compared to the mixture of pulp and peels which was high (P<0.05) compared to that of pulp and kernels respectively. Pulp iron content was higher than 103.0 (Saka and Msonthi, 1994) but lower than 700±200, 500±100, 800±200 (Benhura et al., 2013) reported in studies conducted in Malawi and Zimbabwe respectively. Iron content in the kernels was high compared to 13.2 obtained in a related study conducted

Table 4. Mineral composition of *P. curatellifolia* (mg100 g^{-1} DM).

Sample	K (Mean±SE)	Mg (Mean±SE)	Mn (Mean±SE)	Cu (Mean±SE)	Zn (Mean±SE)	Fe (Mean±SE)	P (Mean±SE)
P. Fruit	ND	ND	ND	ND	ND	ND	ND
Pulp	712.65±12.02^{a}	213.06±8.25^{a}	24.27±0.58^{a}	6.26±0.00^{a}	17.57±0.67^{a}	295.49±0.68^{a}	405.23 ± 2.17^{a}
Pulp + Peels	710.86±10.51^{a}	216.08±13.61^{a}	31.53±0.00^{a}	112.74±0.54^{b}	17.85±0.58^{a}	398.78±0.56^{b}	370.57±10.47^{b}
Peels	736.24±6.29^{b}	200.01±7.64^{b}	29.33±0.27^{b}	70.23±0.61^{c}	37.57±1.07^{b}	403.81±4.34^{b}	382.56±4.54^{b}
Kernel	680.20±4.88^{c}	222.45±2.72^{c}	45.79±1.77^{c}	12.59±0.76^{d}	42.1±0.58^{c}	357.02±3.65^{c}	540.67±4.87^{c}

Mean ± SE= mean ± standard error. For each parameter, means with same superscript were not significantly different (P>0.05). ND= not determined.

in Nigeria (Ogungbenle and Atere, 2014).

Phosphorus content ranged from 370.57±10.47 to 405.23±2.17 for mixture of pulp and peels and kernels respectively. Phosphorus in kernels was the highest (P<0.05) compared to pulp, mixture of pulp and peels and peels respectively. Phosphorus content in pulp was higher than 200±0.0 (Benhura et al., 2013) and 339 (Saka and Msonthi, 1994) from other studies conducted in Zimbabwe and Malawi respectively. *P. curatellifolia* kernels had high phosphorus content compared to 180 (Ndabikunze et al., 2006) and 196 (Ogungbenle and Atere, 2014) obtained from related studies conducted in Tanzania and Nigeria. Furthermore, phosphorus content for the kernels was higher than 340.2 for *A. hypogaea* as observed in India (Kumar et al., 2013) (Tables 3 and 4).

Conclusion

Results from the study have shown that *P. curatellifolia* fruits from Malawi contain essential nutrients and minerals which are essential for the nutritional well-being of less privileged communities in Malawi. The results have also revealed that there are differences in nutrient and mineral composition in different parts of the *P. curatellifolia* fruit. The high carbohydrate content in the pulp present in *P. curatellifolia* have demonstrated that *P. curatellifolia* fruit is a potential source of energy which can benefit consumers from low resource communities in Malawi. It is therefore highly recommended that people should be encouraged to consume *P. curatellifolia* fruits to get good nutrition. It is further recommended that future studies should use *P. curatellifolia* from different regions in Malawi to find out to what extent the nutrient and mineral composition can be influenced by the differences in ecological zones.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Ahmed N (2013). Different drying methods: their applications and recent advances. International Journal of Food Nutrition Safety 4(1):34-42.

Akinnifesi FK, Kwesiga FR, Mhango J, Mkonda A, Chilanga T, Swai R (2004). Domesticating priority miombo indigenous fruit trees as a promising livelihood option for smallholder farmers in Southern Africa. Acta Horticulture 632:15-30.

Akinnifesi FK, Kwesiga F, Mhango J, Chilanga T, Mkonda A, Kadu CAC, Kadzere I, Mithofer D, Saka JDK, Sileshi G, Ramadhani T, Dhliwayo P (2006). Towards the development of miombo fruit trees as commercial tree crops in southern African. Forests, Trees and Livelihoods 16:103-121.

Akinnifesi FK, Ajayi OC, Sileshi G, Kadzere I, Akinnifesi AI (2007). Domestication and commercialization indigenous fruit and nut tree crops for food security and income generation in Sub-Saharan Africa. Paper presented at the New Crops International Symposium, 3-4 September, Southampton, United Kingdom. Available at: www.worldagroforestry.org/downloads/Pubications/PDFS/pp1537.pdf

Association of Official Analytical Chemists (AOAC) (1990). Official methods of analysis. 15th Edn., Washington DC.

Association of Official Analytical Chemists (AOAC) (1996). Official methods of analysis. 18th edn. AOAC International, Arlington, VA.

Association of Official Analytical Chemists (AOAC) (2005). Official methods of analysis. Food composition, additives and natural contaminants. Aldric, RC 15th edn. Association of Official Analytical Chemists Inc. USA.

Atasie VN, Akinhanmi TF, Ajiodu CC (2009). Proximate analysis and physico-chemical properties of groundnut (*Arachis hypogaea L.*). Pakistan Journal of Nutrition 8(2):194-197.

Ayoola PB, Adeyeye A, Onawumi OO (2012). Chemical evaluation of food value of groundnut (*Arachis hypogaea L.*) seeds. American Journal of Food Nutrition 2(3):55-57.

Benhura C, Benhura MAN, Muchuweti M, Nyangura SF, Gombiro PE (2012). Proximate analysis of *Parinari curatellifolia* fruit pulp of fruit from parts of Harare and a rural area in Zimbabwe. Pakistan Journal of Nutrition 11(7):541-544.

Benhura MAN, Muchuweti M, Gombiro, PE, Benhura C (2013). Properties of (*Parinari curatellifolia*) (Hacha or Chakata) fruits from different parts of Harare, Zimbabwe. African Journal of Food Agricultural Nutrition Development 13:4.

Brennand CP (1994). Home drying of food. All archived publications. Paper 606. Available at: http://digitalcommons.usu.edu/extensionhistall/606

Chirwa PW, Akinnifesi FK (2008). Ecology and Biology of *Uapaca kirkiana, Strychnos cocculoides* and *Scelerocarya birrea* in Southern Africa. In: Akinnifesi FK, Leakey RRB, Ajayi OC, Sileshi G, Tchoundjeu Z, Matakala P, Kwesiga FR (eds.). Indigenous fruit in the tropics: Domestication, Utilisation and Commercialisation. CABI, UK. P 322.

Food and Agriculture Organization (FAO) (1982). Fruit-bearing forest trees: technical notes. pp. 34-177.

Food and Agriculture Organization (FAO) (2000). World forest resources assessment, FAO, Rome, Italy.

Food and Agriculture Organization (FAO), International Fund for Agricultural Development (IFAD), World Food Programme (WFP) (2014). The state of food insecurity in the world 2014. Strengthening the enabling environment for food security and nutrition. Rome, FAO.

Available at: http://www.fao.org/3/ai4030e.pdf
FAO, IFAD, WFP (2015). The state of food insecurity in the World. Meeting the 2015 international hunger targets. Taking stock of uneven progress. Rome, FAO. Available at: http://www.fao.org/3/a-i4646e.pdf
Gachene CKK, Karuma AN, Baaru MW (2015). Climate Change and crop yield in Sub-Saharan Africa. Springer International Publishing Switzerland. In: R. Lal et al., (eds), Sustainable Intensification to Advance Food Security and Enhance Climate Resilience in Africa. Available at: https://link.springer.com/chapter/10.10007/978-3-319-09360
International Panel on Climate Change (IPCC) (2007a). Climate change 2007: Impacts, Adaptation and Vulnerability. Summary for policy makers. Online at http://www.ipcc.cg/SPMapr07.pdf. 2017.08.028
International Panel on Climate Change (IPCC) (2007b). Climate change 2007: The physical science basis. Contribution of working groups 1 to the Fourth Assessment Report of the International Panel on Climate Change, Solomon S, Qin D, Manning M, Chen Z, Marquis M, Avery KB, Tignor M, Miller HL (eds.), Cambridge and New York: Cambridge University Press. Available at https://www.ipcc.ch/publicationsanddata/ar4wg1/en/spmsspmprojections-of.html
Jones PG, Thornton PK (2003). The potential impacts of climate change on maize production in Africa and Latin America in 2055. Global Environment Change 13(1):51-59.
Katende AB, Brinie A, Tengnas BO (1995). Useful trees and shrubs for Uganda, identification, propagation and management for agricultural and pastoral communities. Regional soil conservational unit (RSCU). Swedish International Development Authority (SIDA).
Kumar BS, Shankar SR, Vasanthi RP, Vishnuvardhan KM, Purushothan M (2013). Comparative physico-chemical, proximate and mineral analysis on raw and roasted seeds of groundnut. Communications in Plant Sciences 3(3-4):25-29.
Kwesiga F, Akinnifesi FK, Ramadhani T, Kadzere I, Saka J (2000). Domestication of indigenous fruit trees of the miombo in Southern Africa. Pp8-24 in Shumba EM, Lusepani E, Hangula R (eds,). The Domestication and Commercialization of Indigenous Fruit Trees in the SADC Region. SADC Tree Seed Centre Network.
Lockett C, Grivetti L (2000). Food related behaviours during drought: a study of rural Fulani, North-eastern Nigeria. International Journal of Food Science and Nutrition 51:91-107.
Loghrust R (1986). Household food strategies in response to seasonality and famine. Institute of Development Studies Bulletin 17:27-35.
Makonda FBS, Gillah PR (2007). Balancing wood and non-wood products in Miombo woodlands. Working Papers of Finnish Forest Research Institute 50:64-70 Available at: http://www.melta.fi/julkaisut/workingpapers/2007/mwp050.html
Msuya T, Kideghesho JR, Mosha TCE (2010). Availability, preference and consumption of indigenous forest food in the Eastern Arc Mountains, Tanzania. Ecology of Food Nutrition 49:208-227.
Mtupanyama YM, Mwase WF, Stedje B, Kwapata MB, Bokosi JM, Hvoslef-Eide AK (2008). Indigenous knowledge of rural communities in Malawi on socio-economic use, propagation, biology, biodiversity and ecology *of Uapaca kirkiana* Muell. African Journal of Biotechnology 7(14):2386-2396.
Murphy J, Riley JP (1962). A modified single solution method for the determination of phosphorus in natural waters. Analytica chimica acta 27:31-36.
Ndabikunze BK, Mugasha AG, Chcimshama SAD, Tiisekwa BPM (2006). Nutritive value of selected forest/woodland edible fruits and nuts in Tanzania. Tanzania Journal of Agricultural Science 7(1):27-33.
Oladimeji AO, Bello MO (2011). Proximate analysis and fatty-acid profiles of mobola plum seed. Elixir Applied Chemistry 41:5942-5943.
Ogungbenle HN, Atere AA (2014). The chemical, fatty acid and sensory evaluation of *Parinari curatellifolia* seeds. British Biotechnology Journal 4(4):379-386.
Orwa C, Mutua A, Kindt R, Jamnadass R, Anthony S (2009). Agroforestree database: a tree reference and selection guide version 4.0. Available at: http://www.worldagroforestry.org/sites/treedbs/treedatabases.asp
Peni IJ, Elinge CM, Yusuf H, Itondo AU, Agaie BM, Mbongo AN, Chongo E (2010). Phytochemical screening and antibacterial activity of Parinari curatellifolia stem extract. Journal of Medicinal Plant Research 4(20):2099-2102.
Ringler C, Zhu X, Cai X, Koo J, Wang D (2010). Climate change impacts on food security in Sub-Saharan Africa: Insights from comprehensive climate change scenarios. International food policy research institute discussion paper no. 1042. International Food Policy Research Institute, Washington, DC.
Ringler C, Zhu T, Cai X (2011). Sub-Saharan Africa: insights from comprehensive climate change modeling. The impact of climate change on food outcomes. IFPRI Research 20:1-2.
Saka JDK, Msonthi JD (1994). Nutritional value of edible fruits of indigenous wild trees in Malawi. Forest Ecology Management 64:245-248.
Salih NK-EM, Yahia EM (2015). Nutritional value and antioxidant properties of four wild fruits commonly consumed in Sudan. International Food Research Journal 22(6):2389-2395.
Théwis A, Galiş A M (2012). Livestock production. Prospects over the next decades and alternatives protein bio-resources. AgroLife Science Journal 1:29-38.
Thornton PK (2010). Livestock production: recent trends, future prospects. Philosophical Transactions of the Royal Society of London B: Biological Sciences 365:2853-2867.
United Nations Population Division (UNPD) (2012). The 2012 Revision. Population Division of the Department of Economic and Social Affairs of the United Nations, World population prospects. Available at: https://esa.un.org/unpd/wpp/publications/Files/WPP2012_HIGHGHTS.pdf
United Nations Population Funds (UNPFA) (2008). The state of the World population 2007: unleashing the potential of urban growth. United Nations Population Fund. http://www.unfpa.org/swp/swpmain.htm.2017.06.016
Wijewardana RMNA, Nawaranthne SB, Wickramasinghe I (2016). Effect of various dehydration methods on proximate composition and retention of antioxidants in different fruit powders. International Food Research Journal 23(5):2016-2020.

Related Journals: