



African Journal of Food Science

Volume 11 Number 3, March 2017
ISSN 1996-0794



*Academic
Journals*

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://academicjournals.org/AJFS>

Submit manuscript online <http://ms.academicjournals.me/>

Editors

Thaddeus Chukwuemeka Ezeji

*Ohio State University and Ohio State
Agricultural and Development Center (OARDC)
Department of Animal Sciences
1680 Madison Avenue
Wooster, Ohio 44691 USA.*

Prof. Kofi E. Aidoo

*Department of Biological and Biomedical Sciences
Glasgow Caledonian University
Cowcadden Road
Glasgow G4 0BA.*

Dr. Barakat S.M. Mahmoud

*Food Safety/Microbiology
Experimental Seafood Processing Laboratory
Costal Research and Extension Centre
Mississippi State University
3411 Frederic Street
Pascagoula, MS 39567
USA.*

Prof. Dr. A.N. Mirsa

*Department of Biosciences and Biotechnology,
School of Biotechnology
Fakia Mohan University,
Vyasa Vihar, Balsore-756019, India.*

Dr. Neela Badrie

*Department of Food Production,
Faculty of Science and Agriculture,
University of the West Indies,
St. Augustine, Republic of Trinidad and Tobago,
West Indies.*

Prof. Yulong Yin

*Institute of Subtropical Agriculture (ISA),
The Chinese Academy of Science (CAS),
Hunan, Changsha 410125,
Post Box 10,
China.*

Dr. Hu Xiao-Qing

*State Key Lab of Food Science and Technology,
Jiangnan University,
1800 Lihu Ave., Wuxi 214122,
China.*

Dr. R. A. Siddique

*Department of Veterinary Biochemistry
College of Veterinary Science and Animal Husbandry
Navsari Agricultural University,
Navsari, 396450
Gujarat, India.*

Dr. Brnčić Mladen

*Faculty of Food Technology and Biotechnology;
Pierottijeva 6; 10000 Zagreb.*

Dr. Jianbo Xiao

*Institute of Chinese Medical Sciences
State Key Laboratory of Quality Research in Chinese
Medicine University of Macau, Macau*

Dr. Petr Konvalina, Ing

*University of South Bohemia in Ceske Budejovice,
Faculty of Agriculture, Studentska 13, České Budějovice,
Czech Republic*

Dr. Ashish Kumar Singh

*Senior Scientist, Dairy Technology Division
National Dairy Research Institute, Karnal-132001
Haryana,
India.*

Dr. K. Pandima Devi

*Department of Biotechnology
Alagappa University
Karaikudi- 630 003
Tamil Nadu
India.*

Editorial Board

Dr. Chakradhar Reddy

*Division of Gastroenterology
University of Miami/Jackson Memorial Hospital
Miami, Florida, U. S. A.*

Dr. Sara Chelland Campbell

*Department of Nutrition, Food and Exercise Sciences
Florida State University
Tallahassee, Florida
U. S. A.*

Dr. Naveen Dixit

*University of Florida
Institute of Food and Agricultural Sciences
Southwest Florida Research and Education Center
U. S. A.*

Dr. M. Ayub Hossain

*Bangladesh Agricultural Research Institute
Gazipur-1701 Bangladesh.*

Dr . Aline Lamien-Meda

*Department of Biochemistry and Phytochemistry
Institut für Angewandte Botanik und Pharmakognosie
Veterinärmedizinische Universität Wien, Veterinärplatz 1,
A-1210 Wien,
Austria.*

Dr. Olalekan Badmus

*Research and development Team,
Thames water,
Leeds University,
United kingdom.*

Dr. Rui Cruz

*ADEA-Escola Superior de Tecnologia
Universidade do Algarve
Campus da Penha, Estrada da Penha
8005-139 Faro
Portugal.*

Prof. Zheng

*Key Laboratory for Biotechnology on Medicinal Plants of
Jiangsu Province, Xuzhou Normal University,
Xuzhou 221116,
China.*

Dr. Khaled A. Osman

*Department of Plant Production and Protection
College of Agriculture & Veterinary Medicine,
Qassim University,
Buriadah, Al-Qassim
P.O. Box 6622
Saudi Arabia.*

Dr. Olusegun Olaoye

*Division of Food Sciences
University of Nottingham
United Kingdom.*

Dr. Anastasios Koulaouzidis

*Staff Gastroenterologist
Centre of Liver & Digestive Disorders
Royal Infirmary of Edinburgh
51 Little France Crescent
United Kingdom.*

Dr. Ding

*Department of Respiratory Diseases,
General Hospital of Chinese People's Armed Police Forces
Beijing,
China.*

Dr. Ashok Kumar Malik

*Department of Chemistry,
CDLU, Sirsa,
Haryana*

Dr. Chongbi Li

*Biotechnology Field.
Institute of Biopharmaceutical Engineering ,
Zhaoqing University,
China.*

Dr. Odara Boscolo

*National Museum / Federal University of Rio de Janeiro)-
Phanerogamic systematic and ethnobotany Laboratory-
Botany Department,
do Rio de Janeiro, Brazil*

Dr. José Lavres Junior

*University of São Paulo,
Center for Nuclear Energy in Agriculture,
São Paulo - Brazil*

Dr. Gokben Ozbey

*Firat University,
Vocational School of Health Services,
Engineering Campus,
Elaziğ
Turkey.*

ARTICLES

Factors associated with poor food safety compliance among street food vendors in the Techiman Municipality of Ghana	50
Akuu Joshua Amaami, Danyi Dominic and Dapaah Collins	
Effect of extraction techniques on the quality of coconut oil	58
Nurah Tijani Oseni, WMADB Fernando, Ranil Coorey, Isona Gold and Vijay Jayasena	
Antioxidant potential use of bioactive peptides derived from mung bean hydrolysates (<i>Vigna Radiata</i>)	67
Carlos M. Guerra A., Walter Murillo and Jonh J. Méndez A.	
Effect of processing on proximate and mineral composition of black climbing (<i>P. coccineus</i> L.) bean flour	74
Mengistu Tadesse Mosisa	

Full Length Research Paper

Factors associated with poor food safety compliance among street food vendors in the Techiman Municipality of Ghana

Akuu Joshua Amaami*, Danyi Dominic and Dapaah Collins

Catholic University College of Ghana, P. O. Box 363, Sunyani Brong Ahafo, Ghana, West Africa.

Received 5 September, 2016; Accepted 2 November, 2016

This study assessed various factors associated with poor compliance of street food vendors to safety measures in the Techiman Municipality with emphasis on the World Health Organization's five keys to safer food policy. Two sets of questionnaires were designed to collect data from 150 respondents (140 food vendors and 10 officials of the Environmental Health and Sanitation Agency) in the Techiman Municipality. Data was collected based on the level of awareness, food hygiene/handling practices and effectiveness of regulatory bodies in the Techiman municipality. Overall, awareness of food hygiene was high (91.4%) and depended on vendor's educational level (Chi-square = 7.810, $P < 0.05$). The ability to maintain a clean food preparation area was generally poor. Most food vending sites (68%) were dirty and most respondents disposed of their waste in polythene bags (56.4%). Only a few food vendors washed their hands after scratching themselves (32%) or handling money (22%). Separation between raw materials from cooked food was also poor. Overall, 25% of the vendors always stored raw and cooked food separately, 29% stored them separately sometimes, while 47% did not. Food was however kept at safe at temperature 82%. The hygiene and safety of raw materials used in cooking was in doubt. Most food vendors (69%) considered price important and purchased cheap raw materials. Finally, the effectiveness of regulatory bodies was generally fair (50%). This might be a result of some challenges faced by regulatory bodies in terms of logistics and resources (90%) and also lack of cooperation from food vendors (10%). The study concluded that there is high awareness of food safety among food vendors in the Techiman municipality. Yet food hygiene and handling practices are poor. This might be due to challenges faced by food vendors in terms of finances (65.7%) and pressure from consumers (34.3%). There is also poor regulation by regulatory bodies which might also be due to a lack of logistics and resources (90%). Current regulations in the municipality regarding general food hygiene practices should be reviewed and strengthened to focus on a risk based approach.

Key words: Compliance, food safety, food vendors, food hygiene.

INTRODUCTION

The street food industry plays an important role in cities and towns of many developing countries. It also

contributes substantially to meeting food demands of city dwellers and provides an income to many female-headed

households (Tracy, 2011). It is estimated that street foods contribute up to 40% of the daily diet of urban consumers in developing countries (Afoi et al., 2015). The term street food as quoted by Tracy (2011) refers to a “wide variety of ready-to eat foods and beverages sold and sometimes prepared, in public places”. Street food may be consumed where it was purchased or can be taken away and eaten elsewhere (Tracy, 2011). The people who sell these foods are referred to as street food vendors (WHO, 2008). Food passes through a lot of steps in the food supply chain before it gets to the consumer. Food should be handled, prepared and stored in ways that prevent the occurrence of foodborne illnesses like cholera and gastroenteritis (WHO, 2008).

Millions of people fall ill and many suffer from serious disorders, long-term complications or die as a result of eating unsafe food (FAO, 2007). Foodborne and waterborne diarrheal diseases kill an estimated 2.1 million people annually, most of whom are children in developing countries (Fleury et al., 2008). The high prevalence of diarrheal diseases in these countries suggests major underlying food and water safety problems. One out of every three Africans suffers foodborne illness every year (WHO, 2011). In Ghana, it is estimated that the total number of out-patients that report with a foodborne disease is about 420,000 per year, with 65,000 dying annually. Twenty-five percent are children under five years (Mensah et al., 2009).

Street food safety is influenced by many environmental factors. These include knowledge and awareness of food safety measures, poor food hygiene and low socio-economic status of food vendors, poor attitude of food vendors towards food safety, socio-cultural beliefs and trust (Thilde, 2008), and limited effectiveness of food safety regulatory bodies. All these play a role in determining the safety and quality of street food consumed by people.

In 2009 The World Health Organization adopted *Five Keys to Safer Food* for the street food sector to be used as the basis for global training of street food vendors. The policy requires that street food is prepared and served under good environmental conditions. In so doing, food vendors must maintain good personal hygiene, Separate raw and cooked foods, cook foods thoroughly, keep foods at safe temperatures and use both safe water and safe raw materials. This policy has achieved results in many WHO nations (WHO, 2010). However, Ghana and for that matter Techiman Municipality is retrogressing in its practice of food hygiene and safety (Thilde, 2008). A survey carried out by the Techiman Municipal assembly in 2010 revealed that about 70% of street food vendors in

the Municipality did not comply with the WHO safer food policy (Techiman Municipal Assembly, 2010). This led to the establishment of food bye-laws in 2012 by the Environmental Health and Sanitation Agency (EHSA) for food vendors which were poorly implemented (EHSA, 2012).

The above situation has led to contamination of street food with microorganisms and toxic substances like pesticide residues, heavy metals, myotoxins, dust and smoke (WHO, 2007) and proliferation of foodborne illness in the Techiman Municipality. In 2012, 12,072 cases of diarrheal disease were recorded with 1.2% mortality. In 2013, 15,228 diarrheal cases were recorded with 1.9% mortality (Holy Family Hospital, 2013). Cases of foodborne illness are on the increase with a corresponding increase in mortality. Foodborne illness can cause social and economic burdens beside death (Agyen, 2012). Children suffer most in such situations since they comprise 75% of street food consumers in Ghana (Micah et al., 2012). This research therefore seeks to identify the most important factors leading to the poor compliance by street food vendors with the WHO safer food policy guidelines in the Techiman Municipality.

MATERIALS AND METHODS

Informed consent

Ethical clearance was first obtained from the Faculty of Public Health and Allied Sciences of the Catholic University College of Ghana, Fiapre and the Environmental Health Unit of Techiman Municipal Assembly before the study. The nature, purpose and procedure of the study were explained to each respondent and they were given the option to either accept or decline to respond to the questionnaires. Respondents were assured of confidentiality and anonymity.

Study area

The study was carried out in the Techiman Municipality. The Municipality lies between longitudes 10 49' East and 20 30' West and latitudes 80 00' North and 70 35' South. Techiman Municipal Assembly is one of the 27 Municipal/District Assemblies in the Brong Ahafo Region of Ghana and Techiman is its capital. The total land size of the Municipal Assembly is 669.7 km². This area forms about 1.69% of the regional land area of 39,557 km². The population of Techiman is 206,856 with females forming 51%. Techiman Municipal Assembly is situated in the central part of the Brong Ahafo Region and shares common boundaries with four other districts; three in Brong Ahafo Region and one in Ashanti Region. The Wenchi Municipal, Kintampo South is to the Northeast. Nkoranza South District is to the South-East and Offinso North District (in the Ashanti Region) is to the south.

*Corresponding author. E-mail: akuu.joshua@yahoo.com.

Table 1. Socio-demographic characteristics of respondents.

Variable	Category	Frequency	%
Gender	Male	42	30.0
	Female	98	70.0
	Total	140	100.0
Age	15-25	36	25.7
	26-35	67	47.9
	36-45	21	15.0
	Above 45	16	11.4
	Total	140	100.0
Marital status	Married	76	54.3
	Single	46	32.9
	Divorced	15	10.7
	Widowed	3	2.1
	Total	140	100.0
Educational level	Senior High School leavers (SHS)	22	15.7
	Junior High School leavers(JHS)	64	45.7
	None	54	38.6
	Total	140	100.0

Study population**Inclusion criteria**

The study population was stationary registered street food vendors above the age of fourteen in the Techiman Township. 10% of hot and cooked registered food vendors above 14 years in Techiman Municipality were chosen due to their maturity and level of knowledge. Stationary street food vendors were used because they were easy to locate during the sample process using the register of food vendors in the Municipality.

Exclusion criteria

Mobile street food vendors were excluded due to difficulties in locating and sampling.

Data collection tools

Two sets of questionnaires were designed for the study, one for street food vendors and the other for environmental health officials. While the views of street food vendors were assessed based on knowledge and food hygiene practices, regulatory protocols used by environmental health officials were also examined. As the questionnaires were administered to the food vendors, an observation on their surrounding was made to find out the hygienic nature of the vending site.

Data analysis

Analysis was done using Statistical Package for Social Science (SPSS) version 20 and Microsoft Excel. In this study, the statistical

significance, dependence and association levels were set at 5% ($P \leq 0.05$).

RESULTS**Demographic/background information**

The socio-demographic characteristics of the 140 food vendors included in the study are shown in Table 1. Overall, 30% of the food vendors were male and 70% were female with 25.7% between the ages of 15-25 years, 47.9% between 26-35 years, 15% between 36-45 years and remaining 11.4% 45 years or older. With respect to marital status, 54.3% were married, 32.9% were single, 10.7% were divorced and 2.1% were widowed. In terms of educational level, 15.7% were Senior High School leavers, 45.7% were Junior High School leavers and 38.6% had no formal education.

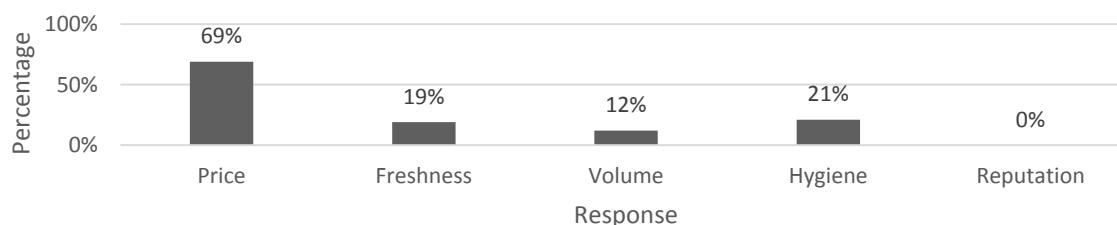
Knowledge of food hygiene and safety by street food vendors**Awareness of food safety and contamination**

The results indicated high awareness of food safety and contamination concerns by street food vendors. Overall, 128 (91.4%) of vendors were aware of food safety concerns and 12 (8.6%) were not. There was strong evidence of relationship between awareness and

Table 2. Chi-square computation between awareness of food safety and contamination.

Category	Awareness of food safety and contamination			Chi-square	P-value
	Yes	No	Total		
SHS	22	0	22	7.810	0.10
Basic	61	3	64		
None	45	9	54		
Total	128	12	140		

Source: Field survey, 2014

**Figure 1.** Factors considered by food vendors when buying raw food material.

educational level ($P < 0.05$) (Table 2).

Food hygiene practices

Factors considered when buying raw foodstuffs and storage of cooked and raw foods

Figure 1 shows that 69% of respondents consider only the price before buying raw material for cooking. They target the least expensive products irrespective of quality. Among vendors, 19% considered the freshness of raw materials but 21% of the respondents indicated that, they buy the raw food materials from the source (farms) and considered the hygiene condition of the source before buying. On the practice of storing raw and cooked food separately, 25% of the vendors always stored raw and cooked food separately, 29% sometimes stored them separately, while 47% did not.

Hand washing among street food vendors

Figure 2 indicates that majority of the respondents (90, 94 and 98%) washed their hands adequately after blowing their noses, visiting the toilet and eating, respectively. However, few (22%) food vendors washed their hands after touching money. Overall, 36% of vendors washed after handling raw materials, 34% after handling garbage and 32% after scratching themselves before serving food to consumers. Money is a very good source of

microorganisms since it passes through the hands of many people. Hand washing practices among street food vendors in the Techiman Municipality were generally poor, with only 48% practicing continuous hand washing after handling food.

Temperature of food and waste disposal

None of the respondents could tell the actual temperature of the food they sold to customers. However, majority of food vendors (82%) indicated that they served food while it was still hot with the remaining 18% serving cold foods to consumers (Figure 3).

Overall 28.6% of the vendors dispose of their waste in bins, 56.4% in polythene bags, 10.7% in baskets and 4.3% on the ground (Table 3).

Environmental hygiene

Hygiene of vending sites was generally very poor. This conclusion was drawn from the responses of the environmental health officers and personal observations during the administration of questionnaires. According to their responses, 68% indicated the vending sites were dirty, 20% very dirty but only 12% of the environmental health officers indicated that vending sites were clean. During the administration of the questionnaire, the researchers observed that there is a problem with the waste disposal system. After sweeping and collection of

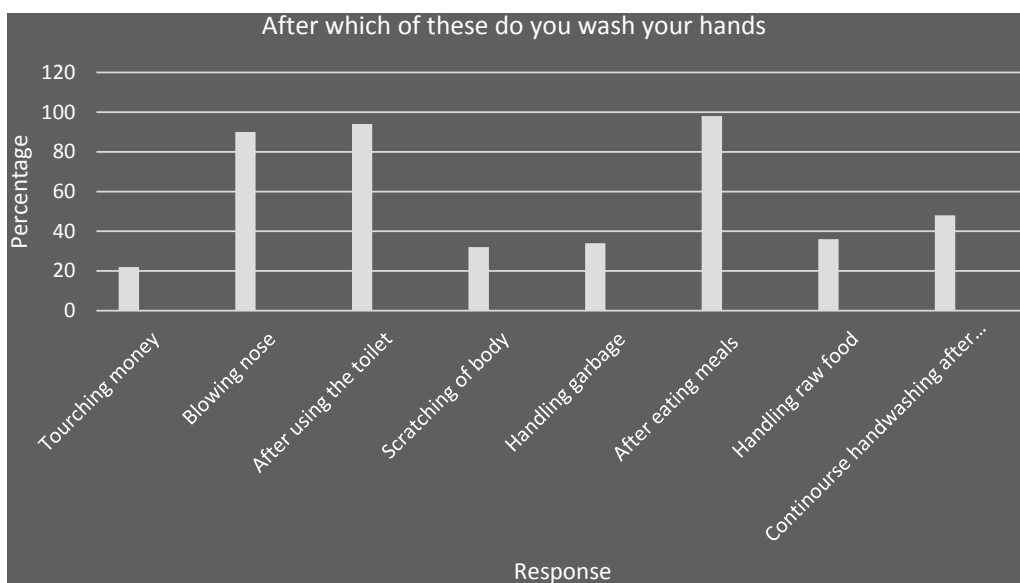


Figure 2. Percentage distribution showing Hand washing practices among street food vendors in the Techiman municipality

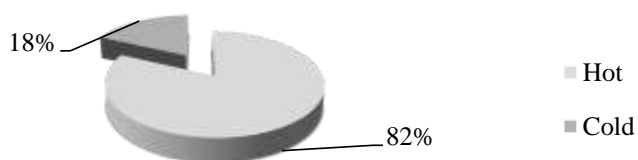


Figure 3. Temperature of food served.

garbage, it is still left there for some time which generate flies and are sometimes been blown away by wind which could further affect the safety of the food they prepared and sold. This observation was in line with the responses of the environmental health officers.

Challenges faced by food vendors in practice good food hygiene

From Table 4, most of the food vendors (65.7%) identified financial constraints as an obstacle in adhering to good food hygiene practices. Pressure from consumers to purchase the food whilst it is not yet ready for consumption was also stated by 34.3% of food vendors as a hindrance to food safety.

Effectiveness of regulatory bodies in ensuring hygienic food practices

This section queried 10 Environmental Health Officers in

Table 3. Disposal of waste by vendors.

Waste disposal	Frequency	%
In bin	40	28.6
Polythene	79	56.4
Basket	15	10.7
On the ground	6	4.3
Total	140	100.0

Table 4. Challenges faced by food vendors as they practice food safety.

Challenge	Frequency	%
Financial constraints	92	65.7
Pressure from consumers	48	34.3
Total	140	100

the Techiman Municipality on the effectiveness of their regulatory agencies in enforcing the regulations. The Environmental health offices were established in every District and Municipal Assemblies in Ghana to enforce by-laws to promote the health and safety of the people. The Techiman Municipal Assembly office was represented by 10 officials (Table 5). The work of the regulatory bodies is described as good if they are able to enforce the by-laws effectively. Fair if they are able to perform half of their duties and poor if they are not able to perform their

Table 5. Effectiveness of regulatory bodies.

Variable	Category	Frequency	%
Rating of effectiveness in ensuring enforcement	Good	3	30.0
	Fair	5	50.0
	Poor	2	20.0
	<i>Total</i>	10	100.0
Challenges associated with regulating activities of street food vendors	Lack of logistics and resources	9	90.0
	Lack of cooperation from vendors	1	10.0
	<i>Total</i>	10	100.0

duties.

Thirty percent (30%) rated the effectiveness of ensuring bye-laws as good, 50% as fair and 20% as poor. With regards to the challenges associated with regulating the activities of street food vendors, 90% stated lack of logistics and resources as a challenge and 10% stated lack of cooperation from vendors as a challenge.

DISCUSSION

Knowledge on food hygiene or safety

Increasing the education and knowledge of food vendors and consumers in hygiene matters has been recommended as a means of improving food handling practices, and thus, the safety of food (WHO/FAO, 2009). In this study, awareness of food safety and contamination was high; 128 (91.4%) among respondents. Only 12 (8.6%) were not aware. This is in contrast with the analysis by Tambekar et al. (2009) who showed that street food vendors are mostly uninformed about good hygiene practices and causes of diarrheal diseases. There was however a strong relationship between awareness and educational level ($P < 0.05$). The likelihood of being knowledgeable on food hygiene and safety increases with the educational level attained by food vendors. This raises many questions and concerns since 45.7% of the respondents were junior high school leavers and if education increases awareness of food hygiene, then many consumers are at risk of foodborne illness.

Food contaminants can enter the food supply chain at any point from the farm to the table. Farm animals and their manure can carry human pathogens without any clinical manifestations. Likewise fresh vegetables and grains can harbor pathogens or mycotoxins without any discernible loss of quality (FAO, 2007). Therefore, any raw food could potentially be contaminated before reaching the caterer. However, most food vendors (67%) did not consider this scenario because price was indicated as the most important determinant when purchasing raw

food materials. This means that they are more likely to purchase less expensive products for cooking which may affect the quality of food. Overall, 19% of vendors considered the freshness of raw materials, 21% of caterers who indicated that they buy the raw food materials from the farms use the hygiene practices and conditions at the source to determine the kind of raw materials to buy. However, no one took into consideration the reputation of the manufacturer when purchasing the raw materials, especially ingredients and other food items that have been packaged. This might be due to lack of experience and awareness, bad behavior of vendors and financial constraints.

On the practice of storing raw and cooked foods separately, 25% of the vendors always stored raw and cooked food separately, 29% sometimes stored them separately, while 47% did not. This agrees with the findings of Donkor et al. (2009) who reported that 27% of vendors always stored raw and cooked food separately, 23% stored them separately most times, while 49% did not do this often. Proper cooking will eliminate most microbial hazards. Studies have shown that cooking or serving foods at a temperature of 60 to 70°C or above can help ensure that it is safe for consumption. Microorganisms can multiply very quickly if food is stored at room temperature. By holding at temperatures below 5°C or above 60°C, the growth of microorganisms is greatly slowed or stopped (WHO, 2010). Most food vendors (82%) served food while it was still hot while 18% served cold foods to consumers. This may be as a result of increased awareness and the culture of the people.

Hands should be washed before handling food and often during food preparation. Hands must be always washed after visiting the toilet in order to minimize the chance for transmitting disease (Addo et al., 2014). The World Health Organisation (2010) also indicated that most the number of food-related illnesses and deaths could be substantially reduced by using proper food handling techniques and hand washing practices. These assertions were shared in the study as the majority of the respondents (90, 94 and 98%) washed their hands

adequately after blowing their nose, visiting the toilet and after eating, respectively. However, the response to the following activities with respect to hand washing contrasted the assertion of Addo et al. (2014). Few vendors (22%) washed their hands after touching money, 36% after handling raw materials, 34% after handling garbage, 32% or after scratching themselves, however 48% practiced continuous hand washing with regard to serving food. This might be as a result of pressure from consumers or lack of knowledge.

According to Donkor et al. (2009), waste bins with lids should be used and emptied on a regular basis. As stated by Addo et al. (2014), fomites in poor environmental hygiene can help transmit diarrheal diseases. In this study, waste disposal was poor with 28.6% of vendors disposing their waste in a bin, 56.4% in polythene bags, 10.7% in a basket and 4.3% on the ground which might be due to financial constraints to buy bins and poor education as stated by WHO/INFOSAN (2010).

Food vendors in the Techiman Municipality followed poor environmental hygiene practices. The study found that 68% of the vendor sites dirty and 20% were very dirty and with only 12% characterized as clean. These results were slightly different from that of Donkor et al. (2009) who scored 60% of the food vendor sites as hygienically. This calls for increased attention since most food contamination could be prevented by keeping the vending units and locations clean (WHO, 2012). Financial constraints (65.7%) and pressures from consumers (34.3%) were the main factors affecting personal and environmental hygiene with respect to food safety. Factors like a low level of education and lack of discipline or poor care are also likely influencing these findings. Food laws and regulations attempt to protect the health of consumers. These laws have been kept in place to guide food providers to ensure proper food handling and to ensure the serving of wholesome food to the general public (Rosaline, 2008). In Ghana, the Food and Drug Board is charged with educating and training food manufacturers and handlers on safe food handling procedures. They are supported by environmental health officers, the Environmental Protection Agency, and the district assemblies. They also help in inspecting facilities where food is being cooked for compliance with current safety standards (Rosaline, 2008).

In this study, 50% of environmental health officers rated the effectiveness of the food safety bye-laws as good and 20% as very good. The regulatory authorities failed to carry out their roles effectively mainly because of poor institutional capacity, lack of coordination, shortage of personnel and funds (FAO, 2007). This observation was also evident in the current study with 90% of regulatory officials stating a lack of logistics and resources as a challenge associated with regulating the activities of street food vendors and the remaining 10% lamenting a lack of cooperation from vendors as a challenge inhibiting

them from performing effectively.

Conclusions

The study concludes that there is high awareness of food safety and contamination (91.4%) among food vendors. Food hygiene practices are poor in the Techiman Municipality, likely due to financial constraints (65.7%) and consumers pressure (34.3%). Ineffective enforcement of the food safety bye-laws by regulatory authorities was likely due to challenges in logistics and resources (90%) and a lack of cooperation among food vendors (10%). We therefore recommend that current regulations be reviewed and strengthened to focus on a risk-based approach.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Addo HO, Addo KK, Langbong B (2014). Water Handling and Hygiene Practices on the Transmission of Diarrheal Diseases and Soil Transmitted Helminthic Infections Communities in Rural Ghana. *Civil Environ. Res.* 6(1):2224-5790.
- Agyen FG (2012). Safe Food Handling: Knowledge, Perceptions and Self-Reported Practices of Turkish Consumers. *Int. J. Business Manag.* 7(24):1-11.
- Emmanuel A, Mangai JM, Kayong EA, Afoi BB, Goshit JD, Naman K, Innocent O. (2015). Assessment of Practice of Food Safety and Hygiene among Food Vendors within Jos North Local Government Area of Plateau State, Nigeria. *Int. J. Med. Health Res.* 1(2):83-86.
- Donkor ES, Kayang BB, Quaye J, Akyeh ML (2009). Application of the WHO Keys of Safer Food to Improve Food Handling Practices of Food Vendors in Rural Communities in Ghana. *International. J. Environ. Recourses and Public Health*, 6:2833-2842.
- Environmental Health and Sanitation Agency (EHSA) (2012). Techiman Municipality Food Safety Bye- Laws. Municipal Assembly. Page 5 Brong Ahafo, Ghana.
- Fleury MD, Stratton J, Tinga C, Charron DF, Aramini J, (2008). A descriptive analysis of hospitalization due to acute gastrointestinal illness in Canada, 1995-2004. *Canadian J. Public Health* 99(6):489-493.
- FAO (Food and Agriculture Organization) (2007). Spotlight: School Children, Street Food and Micronutrient Deficiencies in Tanzania. FAO: Rome, Italy.
- Holy Family Hospital. (2013). Hospital Annual Report for the Year 2013. Biostatistics Unit. Techiman Municipality, Brong Ahafo Region, Ghana.
- International Food Safety Authorities Network. (2010). Basic steps to improve safety of street vended food. Safety of street-vended food. INFOSAN Information Note, 3.
- Mensah P, Manu DY, Darko, KO Abblordey A (2009). Streets foods in Accra, Ghana: how safe are they? *Bull. World Health Organ.* 80(7):546-554.
- Micah EB, Colecraft EK, Lartey A, Aryeetey R, Marquis GS (2012). Street Foods Contribute to Nutrient Intakes Among Children from Rural Communities in Winneba and Techiman Municipalities, Ghana. *Afr. J. Food, Agric. Nutr. Dev.* 12(1):5789-5801.
- Rosaline SG (2008). Inadequate feeding practices and impaired growth among children from subsistence farming households in

- Sidama, Southern Ethiopia. *Matern. Child Nutr.* 5(3):260-267.
- Tambekar DH, Kulkarni RV, Shirsat SD, Bhadange DG (2011). Bacteriological Quality of Street Vended Food Manipuri: A Case Study of Amravati City in India. *Biosci. Discov.* 12(3):350-354.
- Techiman Municipal Assembly (2010). Food Vendors Survey. Environmental Health and Sanitation Agency Annual Report. Techiman Municipality, Brong Ahafo Region.
- Thilde R (2008). Street Food Quality; A Matter of Neatness and Trust. A Qualitative Study of Local Practices and Perceptions of Food Quality, Food Hygiene and Food Safety in Urban Kumasi, Ghana. Masters Thesis., submitted to the Public Health Institute, University of Copenhagen, Denmark.
- Tracy L (2011). Vietnamese Street Food. Kindle Edition. UK: Hardie Grant Group.
- WHO (World Health Organization) (2010). Prevention of Foodborne disease: Five Keys to Safer Food. Retrieved on June 23, 2015 from <http://www.who.int/mediacentre/factsheets/fs237/en>.
- WHO (World Health Organization) (2012). Manual for Integrated Foodborne Disease Surveillance in the WHO African Regions. Geneva: WHO.
- WHO (World Health Organization) (2008). Knowledge and Prevention. The Five Keys to Safer Food. Food Safety and Zoonoses. Retrieved on June 23, 2015 from <http://www.who.int/foodsafety/en/>.

Full Length Research Paper

Effect of extraction techniques on the quality of coconut oil

Nurah Tijani Oseni^{1,2*}, WMADB Fernando^{3,4}, Ranil Coorey¹, Isona Gold² and Vijay Jayasena⁵

¹School of Public Health, Faculty of Health Science, Curtin University, Australia.

²Biochemistry Division, Nigerian Institute for Oil palm Research, P.M.B. 1030, Benin City, Nigeria.

³Centre of Excellence in Alzheimer's Disease Research and Care, School of Medical Sciences, Edith Cowan University, 270 Joondalup Drive, Joondalup, 6027, Australia.

⁴McCusker Alzheimer's Research Foundation, Hollywood Medical Centre, 85 Monash Avenue, Suite 22, Nedlands, 6009, Australia.

⁵School of Science and Health, University of Western Sydney, Locked Bag 1797, Penrich, NSW 2751, Australia.

Received 26 July, 2016; Accepted 7 December, 2016

Coconut oil (*Cocos nucifera* L.) has a unique role in the diet as an important physiologically functional food. The health and nutritional benefits that can be derived from consuming coconut oil have been recognized in many parts of the world for centuries. The aim of this study was to compare the quality parameters of coconut oil under different common extraction techniques. Six different techniques of coconut oil extraction were employed to produce virgin coconut oil (VCOs) and refined coconut oil (RCO). VCOs were produced using enzymatic, chilling and thawing, centrifugation, natural-fermentation and induced-fermentation processes. The highest oil yield (83%) was from RCO and also RCO had a significantly higher peroxide value (1.06 meq/kg oil) than VCO samples. Antioxidant activity of RCO was significantly ($p < 0.5$) lower than those of VCO samples, with induced-fermentation having the highest antioxidant activity of 28.3%. Interestingly, enzymatic extraction resulted in higher quantity of short-chain triglycerides. Although, there was no method which could result significantly in high quantity of all the tested parameters, induced-fermentation showed relatively high oil yield and antioxidant activity.

Key words: Antioxidants, coconut oil, extraction, fatty acids, quality.

INTRODUCTION

Coconut oil is a vegetable oil extracted from coconut palm (*Cocos nucifera* L.). Coconut is the most extensively grown and used palm in the world with approximately 12 million hectare in cultivation (FAO, 2014) serving as a major source of income and food for about 10 million

families from over 80 countries (Perera et al., 2010). In coconut oil producing countries, extraction process is still crude and usually involves the use of locally sourced equipment that gives oil with poor quality (Bawalan, 2011).

*Corresponding author. E-mail: nurahoseni@gmail.com. Tel: +234 8137611473.

Virgin coconut oil (VCO) is defined as the oil resulting from the fresh and mature kernel of the coconut through mechanical and natural means, either with the use of heat or not, provided that it does not lead to alteration or transformation of the oil (APCC, 2009). There are no specific processing prerequisites that are established for coconut oil production (Marina et al., 2009a). However, several methods to produce VCO are found to measure up with the definition of the VCO (Marina et al., 2009a; Bawalan and Chapman, 2006; Nevin and Rajamohan, 2010; Raghavendra and Raghavarao, 2010). These methods can be largely divided into wet and dry methods. In wet method, the coconut meat/kernel does not go through drying process, while in dry method, the kernel is heated under specific conditions to remove the moisture in it, while preventing scorching and microbial invasion. Wet method can be further divided into chilling and thawing, fermentation, enzymatic and pH method or any of these in combination as the main aim is to destabilize the coconut milk emulsion (Raghavendra and Raghavarao, 2010). In dry method, the kernel is dried using controlled heating and subsequently pressed mechanically to obtain the oil (APCC, 2009). The method of extraction influences the quality and grade of the oil (Amri, 2011). Moisture content, free fatty acid, peroxide value and antioxidant content are common oil quality parameters. While saponification value and fatty acid profile are identification parameters. These parameters can be used to compare oil to determine how extraction conditions impacts on quality.

VCO retains its naturally occurring phytochemicals which produce a distinctive coconut taste and smell. The oil is pure white when the oil is solidified, or crystal clear like water when liquefied. The oil contains high lauric acid (C-12) as medium chain fatty acid (MCFAs). MCFAs are burned up immediately after consumption and therefore the body uses it immediately to make energy rather than store it as body fat (Enig, 1996). Coconut oil contains about 90% saturated fats, with 60% being medium chain triglycerides (MCFAs) (Nagao and Yanagita, 2010). A few clinical trials and animal studies using a formulation of MCFAs reported significant health benefits such as the reduction of body weight, inflammatory disease, metabolic syndrome and serum cholesterol concentration (Han et al., 2007). MCFAs are broken down once consumed almost immediately by enzymes in the saliva and gastric juices, without the need for pancreatic fat-digesting enzymes due to its low molecular weight (Marten et al., 2006). Apart from the MCFAs, the antioxidant profile of coconut oil also assists in the earlier mentioned health benefits of coconut oil (Marina et al., 2009c).

Despite this link between coconut oil and health benefits, the impact of the different common coconut oil extraction method on quality parameters is yet to be fully examined collectively. There is therefore, need to evaluate the common methods used in the extraction of

coconut oil to determine which of the method optimizes the MCFAs and antioxidant activity of coconut oil. The current study aims to address this knowledge gap by investigating the effect of extraction techniques of coconut oil with respect to its quality parameters.

MATERIALS AND METHODS

Sample collection and preparation

Dehusked coconut fruits with the shell from one batch were purchased from the local Coles Supermarket in Bentley, Western Australia. Coconuts were de-shelled, cleaned and shredded using a Robot Coupe Blixer 4 V.V at a speed set at 3 for 5 min. All chemicals were of analytical and HPLC grade and were purchased from Sigma Aldrich, Australia.

Coconut oil extraction

Six different techniques were used to extract coconut oil. Induced fermentation (IF), natural fermentation (NF), enzyme (EV), centrifugation (CE), chilling and thawing (CH) produced VCO and other RCO. All extractions were conducted in duplicate.

Induced fermented VCO (IF VCO)

Shredded coconut meat (500 g) was mixed with water at 70°C at a ratio of 1:1. The mixture was kneaded by hand for 5 min and strained through a cheese cloth to obtain coconut milk. The coconut milk was allowed to settle for 6 h. The resulted upper layer of coconut cream was collected by decanting and inoculated with (*Lactobacillus (L.) plantarum* ATCC 14917 (5% w/w)) previously activated in MRS medium. The inoculated cream was allowed to ferment at 40°C for 10 h. After the fermentation, mixture was centrifuged at 4000 rpm (3220 xg) using Eppendorf centrifuge 5810-R (Hamburg, Germany) for 30 min at room temperature (20°C±2) to obtain the coconut oil. Coconut oil was heated to 50°C using Contherm thermotec 2200 (Lower Hutt, New Zealand) to remove aromatic compounds, weighed, flushed with nitrogen and stored in dark brown bottles at 5°C prior to analysis.

Natural fermented VCO (NF VCO)

Shredded coconut meat (500 g) and water at 70°C at a ratio of 1:2 was kneaded by hand for 5 min. Mixture was strained through a cheese cloth to obtain coconut milk. The coconut milk was left to ferment naturally for 16 h at 40°C. Oil was separated from fermented curd by centrifuging at 4000 rpm (3220 xg) using an Eppendorf centrifuge 5810-R (Hamburg, Germany) for 30 min at room temperature. The separated coconut oil was heated at 50°C using Contherm thermotec 2200 (Lower Hutt, New Zealand) to remove aromatic compounds. It was then weighed, flushed with nitrogen and stored in dark brown bottles at 5°C.

Enzymatically extracted VCO (EV VCO)

Shredded coconut meat (500 g) was mixed with water (1:4) and the temperature of mixture was brought to 40°C using a water bath (Grant OLS200, Cambridge, UK) (Che Man et al., 1996; Mansor et al., 2012; McGlone et al., 1986). Amylases (1%) from *Aspergillus oryzae*, pectinase (1%) from *Aspergillus niger* and proteases (1%)

from *Streptomyces griseus* purchased from Sigma-Aldrich (Australia) were added to the coconut mixture and temperature was maintained at 40°C and agitated for 3 h using a shaking water bath. After 3 h, the solution was centrifuged at 4000 rpm (3220 ×g) using Eppendorf centrifuge 5810-R (Hamburg, Germany) for 30 min at room temperature to obtain upper coconut oil layer. Coconut oil was weighed, flushed with nitrogen and stored in dark brown bottles at 5°C.

Centrifugation (CE VCO)

Coconut meat (500 g) which was grated was mixed with water (1:1) to extract the coconut milk. Centrifugation (Eppendorf centrifuge model 5810-R, Hamburg, Germany) was done twice (4000 rpm) to destabilise the oil-water emulsion for 30 min at room temperature. Initial centrifugation was to obtain the cream and the second centrifugation separated the cream into three layers (oil, cream and aqueous). The top oil layer was decanted, weighed, flushed with nitrogen and stored in dark brown bottles at 5°C prior to analysis.

Chilling and thawing (CH VCO)

Grated coconut meat (500 g) was mixed with water (1:1), hand kneaded for 5 min and filtered to extract coconut milk. Coconut milk was centrifuged at 3220 ×g for 10 min and the upper layer of cream was removed for chilling. Chilling was done at 0°C for 6 h and then the chilled cream was thawed slowly at room temperature to extract the oil (Raghavendra and Raghavarao, 2011). Centrifugation (Eppendorf centrifuge model 5810-R Hamburg, Germany) was applied (4000 rpm) for 30 min at room temperature to obtain coconut cream. Coconut cream was further centrifuged at 4000 rpm for another 30 min to produce CT VCO. Oil was weighed, flushed with nitrogen and stored in dark at 5°C prior to analysis.

Refined coconut oil (RCO)

Coconut meat (shredded, 500 g) was oven dried using Contherm thermotec 2200 (Lower Hutt, New Zealand) at 75°C (to a moisture content of 7%) Amri (2011). Oil was extracted from the dried coconut by solvent extraction using n-hexane in a Soxhlet apparatus (Buchi E-816, Flawil, Switzerland). Thermal cycle was done at 80°C for 8 h. Solvent was recovered using a rotary evaporator at 40°C under vacuum (Ixtaina et al., 2011). Solvent extracted oil was refined according to Canapi et al. (2005). The coconut oil was preheated to 80°C and 85% phosphoric acid (0.1% w/w) was added and temperature maintained at 85°C for 20 min. One percent of bleaching earth/activated carbon (10:1) was added to the oil and temperature was further adjusted to 95°C under vacuum for another 20 min. The bleaching earth was removed by filtration with aid of vacuum using Whatman No. 1. Oil was deodorized by heating under pressure and high temperature (240°C) for 1.5 h (Lindberg/Blue M™ Vacuum Oven). Oil was weighed, flushed with nitrogen and stored in dark at 5°C prior to analysis.

Determination of physiochemical parameters

Analysis for each physiochemical parameter was carried out in triplicates except otherwise stated.

Oil yield

Oil yield (%) was calculated compared to total oil content. Total oil

content was determined according to the procedure Am 5-04 (AOCS, 2009). All analysis was carried out in duplicates.

$$\text{Oil Yield (\%)} = \text{Weight of oil extracted (g)} \div \text{Total oil content (g)} \times 100$$

Moisture and volatile content

Determination of moisture and volatile content was performed using AOCS Method Ca 2b-38 (AOCS, 2009). Samples (20 g) were heated at a temperature of 110±5°C in a pre-dried beaker until cessation of rising bubbles of steam and incipient smoking. Heated samples were cooled to room temperature in a desiccator and re-weighed. The moisture and volatile content was calculated by difference.

$$\text{Moisture and volatile (\%)} = \text{Initial weight} - \text{Final weight} \div \text{Initial weight} \times 100$$

Free fatty acid (FFA)

Free fatty acid value was determined using the AOCS Official Methods Ca 5a-40 (AOCS, 2009). All measurements were expressed as the percentage of free fatty acid (as lauric).

$$\text{FFA (Lauric \%)} = \text{mL of alkali} \times \text{Molarity} \times 20 \div \text{Mass of test portion}$$

Fatty acid composition (FAC)

Preparation of fatty acid methyl esters (FAMES) were carried out using 12% BCl₃-Methanol according to the Sigma Aldrich method (Sigma-Aldrich, 1997) with slight modification to allow for sufficient volatility of FAMES by the GC-FID. Toluene (5 ml) was added to 0.1 g oil to dissolve. BCl₃-methanol (10 ml) was added to the mixture and flushed with nitrogen gas. Mixture was left at 60°C for 10 min in a water bath. FAMES were extracted twice using 20 ml of hexane. The FAMES mixture was washed five times with water to remove any trace of BCl₃ and dried with anhydrous sodium sulphate. FAMES were filtered into 100 ml flask and made to mark with hexane.

The fatty acids from the FAMES were analyzed according to Coorey et al. (2012). One microliter of FAMES was injected via an auto sampler into a gas chromatography (Perkin Elmer model, Autosystem XL, USA) coupled with flame ionization detector running at 250°C and SGE forte BPX 70 capillary column (30 m × 0.32 mm × 0.25 μm) using helium as the carrier gas set at 20 ml/min with injector running at 200°C. The oven temperature was set at 80°C for 2 min and increased to 130°C (45°C/1 min) and left for 10 min. It was further increased to 172°C (2°C/1 min) for the final 6 min. Peak identification were compared with the standard FAMES obtained from Sigma Chemicals, Australia.

$$\text{Fatty acids (\%)} = (\text{FA concentration} \times \text{dilution factor} \times \text{extraction volume}) \times 100 \div \text{Sample weight}$$

Saponification value

Saponification value (SV) was ascertained using AOCS Official Methods Cd 3-25 (AOCS, 2009). Two grams of filtered oil was mixed with 0.5 N ethanolic potassium hydroxide and boiled under reflux for 60 min. The mixture was left to cool slightly at room temperature and subsequently titrated with 0.5 N hydrochloric acid until the colour changed from pink to colourless. A blank sample was also carried using same method without oil.

$$\text{SV} = (\text{Volume of blank titrant} - \text{Volume of sample titrant}) \times M(\text{HCl}) \times 56.1 \div \text{Weight of oil}$$

Table 1. Comparison of major differences of each extraction method.

Techniques	(Coconut Meat:Water)	Inoculum/Enzyme	Temperature/Duration
IF	1:1	<i>L. plantarum</i>	40°C/10 h
NF	1:2	Nil	40°C/16 h
Enzymatic	1:4	Amylase, pectinase, protease	40°C/3 h
Centrifugation	1:1	Nil	Nil
CH	1:1	Nil	5°C/6 h
RCO	Nil	Hexane	80°C/8 h

Peroxide value (PV)

PV was quantified according to the standard method of IUPAC (1992). The oil sample (5 g) was thoroughly mixed with a mixture of acetic acid:chloroform (3:2 v/v, 25 ml) and saturated KI solution (1 ml), before incubating in the dark for 1 h. After adding water (75 ml), the mixture was titrated with a standard solution of sodium thiosulphate (0.01 N) using a starch solution as an indicator.

$$PV = \frac{\text{Volume of sample titrant} - \text{Volume of blank titrant} \times N(\text{Na}_2\text{S}_2\text{O}_3) \times 1000}{\text{Weight of oil}}$$

Triglycerides (TAG)

The triglycerides were identified following AOCS official method Ce 5b-89 AOCS (2009) as modified by Cunha and Oliveira (2006). Oil (0.2 g) previously dehydrated with anhydrous sodium sulphate and filtered was dissolved in 4.0 ml of acetone and homogenized by stirring. The mixture passed through a 0.22 µm disposable LC filter disk and analyzed using a reversed-phase high performance liquid chromatography (HPLC) (Hewlett-Packard model 1100 Waldbronn, Germany) equipped with Evaporative Light Scattering (ELS) detector (Alltech 2000ES, USA). Acetone dissolved oils (10 µl) were eluted with acetone: acetonitrile using Apollo C18 (5 µm; 250 × 4.6 mm) column (Alltech Grace, USA) operating at room temperature. TAG peaks were identified by comparing retention time with TAG standards (Sigma Aldrich, Australia) and compared on the basis of retention time of TAG standards using chemstation software.

Antioxidant activity

The antioxidant activity was determined according to (Ramadan and Wahdan, 2012). Oil (10 mg) was mixed with 100 µl of toluene and 390 µl of freshly prepared toluenic-DPPH solution (10⁻⁴ M). Mixture was vortexed for 20 s and left at room temperature for 60 min. Decrease in absorbance of toluenic-DPPH solution with oil and without oil (control) using toluene without DPPH as blank was measured at 515 nm using a Novaspec II visible recording spectrophotometer (Pharmacia Biotech, Cambridge, England).

$$\text{Antioxidant activity(\%)} = \frac{\text{Absorbance of control} - \text{Absorbance of sample} \times 100}{\text{Absorbance of sample}}$$

Statistical analysis

Extraction of coconut oil was carried out in duplicates. All chemical analyses were conducted in triplicate. Analysis of variance (ANOVA) was carried out on the results using IBM SPSS version 22 (IBM Corp., NY, USA). Significant differences among means were determined at p<0.05 using Tukeys' test.

RESULTS AND DISCUSSION

Oil yield

The oil yield of the different techniques of extractions revealed the differences in the quantity of oil extracted. Significantly (p < 0.05), higher oil yield was observed from RCO compared to the different VCO methods (Table 1). Among the VCO extracted oil, centrifugation had a significantly low oil yield. The low oil yield from centrifugation may be attributed to the speed of centrifugal force used (4000 rpm) as no other means was combined to destabilize the oil-water emulsion as seen in the other methods which employed enzymes, bacteria or physical means such as chilling. Later studies may need to optimise the centrifugal force that gives maximum oil yield if employed singularly. Nour et al. (2009) suggested that the yield of oil was directly proportional to the centrifugal force used in extraction. The higher yield of oil from RCO may be due to the use of hexane which is a non-polar solvent capable of dissolving fats coupled with prolonged exposure to heat, that is, 80°C for 8 h.

The oil yield of the enzyme assisted extraction (65.74%) was low compared to earlier studies. Che Man et al. (1996) reported that higher oil yield (73%) using cellulase, protease, α amylase and polygalacturonase (1% w/w each) at 60°C and pH 7. McGlone et al. (1986) also reported that 80% of oil recovery using similar enzymes used in this study. The results from enzyme extraction using α-amylase (from *A. oryzae*), pectinase (from *A. niger*) and proteases (from *Streptomyces griseus*) indicated that there is need to determine the optimal temperature and pH at which the enzymes are most active in order to achieve higher oil yield. Chih et al. (2012) showed that oil yield of olive oil can be increased by enzyme treatment.

Inducing fermentation (*L. plantarum*) led to a significant increase in yield of oil (p < 0.05) as compared to natural fermentation (Table 2). The effect of *L. plantarum* in destabilizing coconut emulsion was also reported by Che Man et al. (1997) with oil yield (95%) compared to control (84%). This result suggests that the use of microorganisms such as *L. plantarum* in the production of VCO may lead to the optimization of the oil yield of coconut.

Table 2. Physiochemical properties of coconut oil extracted from different techniques.

Extraction method	Oil yield (%)	FFA (%)	Moisture and Volatiles (%)	Peroxide value (meq/kg oil)	Saponification value (Mg KOH/G)
EVCO	65.74±2.19 ^a	3.28±0.2 ^c	0.39± 0.01 ^b	0.43±0.04 ^b	259±0.82 ^{ab}
NFVCO	68.13±2.4 ^a	0.36±0.05 ^a	0.11±0.02 ^a	0.68±0.02 ^a	257±0.96 ^a
IFVCO	77.67±2.26 ^b	0.3±0 ^a	0.12±0.02 ^a	0.66±0 ^a	254±8.7 ^{ab}
CHVCO	69.31± 0.47 ^a	0.08±0 ^b	0.15±0.04 ^a	0.43±0.03 ^b	261±3.9 ^b
CEVCO	54.4±1.1 ^d	0.17±0.01 ^{ab}	0.34±0.03 ^b	0.34±0.07 ^b	250±9.4 ^{ab}
RCO	83.23±3.38 ^c	0.06±0 ^d	0.02±0.1 ^c	1.06±0.22 ^c	256±7.1 ^{ab}
Standards	NA	≤ 0.5 ²	0.2 ^{1,2}	<15 ¹ <3 ²	248-265

Means (n=6) within the same column with different superscripts are significantly different at $p < 0.05$. NA= not available. Standards for CODEX and APCC are coded 1 and 2, respectively.

FFA

FFA of coconut oil from different extraction is as shown in Table 2. All samples except for enzymatic extraction had FFA within the Asian and Pacific Coconut Community (APCC) Standard (APCC, 2009) for virgin coconut oil (0.5%). The relatively high FFA from enzymatic extraction could be due to the enzymatic hydrolysis of triglycerides in coconut oil resulting in the increase in FFA content. FFA are formed from the hydrolysis of an ester by lipase or moisture (Choe and Min, 2006). According to Raghavendra and Raghavarao (2011), hydrolytic rancidity could be due to hydrolysis of triglycerides of fats and oils by enzymes resulting in an increase in FFA of oil and fats.

Moisture and volatile

Moisture and volatile matter are an important determinant of oil quality (Choe and Min, 2006). It is desirable to keep the moisture content low as it will increase the shelf life by preventing oxidation and rancidity processes. High moisture content promotes hydrolytic rancidity of fats and oils (Raghavendra and Raghavarao, 2011). RCO had significantly lower ($p < 0.05$) moisture content than all VCO samples. Marina et al. (2009a) also reported that lower moisture content of RCO compared with VCO. VCO from IF, CH and NF were in accordance to APCC standards (APCC, 2009). Enzymatic and centrifugation techniques had moisture content of 0.39 and 0.34%, respectively which were both above APCC set standard of <0.2%. As separation of water and oil phase is based on the centrifugal force used (Nour et al., 2009), it may be necessary for future studies to determine the optimum centrifugation technique. The high moisture (0.39%) in coconut oil from enzymatic extraction may have led to the high FFA observed.

Peroxide value

All oil samples had peroxide values below the CODEX

and APCC limit (Table 2). This indicates that samples were highly stable against oxidative rancidity. Overall, RCO had significantly higher peroxide value (1.06 ± 0.22) $p < 0.05$ compared to the VCO samples. Other studies carried out by Raghavendra and Raghavarao (2011), Dayrit et al. (2011), and Gopala Krishna et al. (2010) comparing RCO and VCO also found a higher peroxide value in RCO samples. According to Cunha and Oliveira (2006), higher degree of unsaturation in fats and oils increased the chances of oxidative rancidity. Coconut oil generally has low percentage of unsaturated fats making it relatively stable to oxidation (Gopala Krishna et al., 2010). The higher peroxide value in RCO could be due to the high temperature used in its refining. Heat has been suggested as a factor that enhances oxidative rancidity (Marina et al., 2009a). VCO methods of producing coconut oil is a better option to RCO method in controlling oxidative rancidity, as it requires the use of low heat as opposed to RCO that uses high heat.

Saponification value

Saponification value is an indication of the degree of saturation, where high values correspond to shorter chain fatty acids in the glycerol bond (Marina et al., 2009a). Coconut oil has a relatively high saponification value due to its high concentration of short and medium chain triglycerides (Gopala Krishna et al., 2010). All the oil samples had saponification value within CODEX standard of 248 to 265 mg KOH/g of oil (FAO, 2009).

Fatty acid composition

The fatty acid compositions of coconut oil from different extraction are presented in Table 3 along with CODEX standard (FAO, 2009). Coconut oil is predominantly comprised of MCFAs. MCFAs are saturated fatty acids with a carbon chain of 6 to 12 atoms. Of these MCFAs, lauric acid (C12) is predominant with antiviral and antimicrobial properties similar to monolaurin in human

Table 3. Fatty acid composition of coconut oil produced from different techniques and Codex standard.

Extraction method	C6	C8	C10	C12	C14	C16	C18	C18:1	C18:2
EVCO	0.65±0.09 ^a	8.44±0.0 ^a	7.05±0.0 ^a	47.15±0.0 ^d	18.85±0.02 ^a	8.3±0.2 ^d	2.02±0.03 ^a	6.32±0.35 ^{ab}	1.68±0.002 ^b
NFVCO	0.83±0.0 ^b	9.02±0.0 ^d	7.29±0.0 ^b	49.81±0.0 ^c	18.25±0.01 ^b	6.62±0.02 ^{ab}	2.01±0.01 ^a	5.36±0.03 ^c	1.21±0.01 ^c
IFVCO	1.02±0.0 ^d	8.22±0.0 ^b	8.38±0.0 ^c	48.94±0.00 ^{cd}	19.01±0.05 ^c	6.26±0.0 ^c	2.00±0.01 ^a	6.68±0.01 ^a	0.27±0.0 ^d
CHVCO	0.66±0.0 ^a	8.36±0.0 ^a	7.07±0.0 ^a	49.37±0.00 ^{ac}	19.36±0.01 ^d	6.87±0.0 ^a	2.56±0.054 ^b	5.27±0.02 ^c	0.49±0.01 ^e
CEVCO	0.68±0.01 ^a	8.25±0.0 ^b	6.24±0.0 ^d	50.12±0.01 ^c	19.69±0.01 ^e	6.36±0.0 ^{bc}	2.18±0.02 ^c	6.18±0.05 ^b	0.44±0.0 ^a
RCO	1.12±0.0 ^d	8.90±0.0 ^d	6.16±0.0 ^e	49.33±0.05 ^c	18.46±0.01 ^e	8.91±0.0 ^e	2.01±0.02 ^a	5.08±0.005 ^c	0.43±0.0 ^a
CODEX	ND-0.7	4.6-10	5.0-8.0	45.1-53.2	16.8-21.0	7.5-10.2	2.0-4.0	5.0-10	1-2.5

Means (n=6) within the same column with different superscripts are significantly different at p<0.05. ND: Not detected.

milk (Mansor et al., 2012). The lauric content of all oils was not significantly different from each other (Table 3). This indicates that extraction method does not enhance the lauric content for coconut oil. Myristic acid (C14), the next highest MCFA after lauric acid, showed similar trend of non-significant difference among methods (18.25 to 19.69%). These values obtained for Myristic acid were similar to myristic content of VCO samples reported by Mansor et al. (2012) and Marina et al. (2009c), but slightly lower than the values obtained by Raghavendra and Raghavarao (2011) which was 22.3%. Overall, all the percentage of fatty acids had their values within CODEX standard for coconut oil except for caproic acid (C6) which had values slightly higher than CODEX (<0.7) (FAO, 2009), NF, IF and RCO had values 0.83, 1.02 and 1.12%, respectively,

Antioxidant activity

There have been increasing studies suggesting that consumption of food containing phenolic antioxidant may help fight against several disease (Marina et al., 2009c). These studies showed that

increase in phenolic content leads to an increase in antioxidant activity (Marina et al., 2009c; Marina et al., 2009b)

The antioxidant activity of the VCO samples and RCO is as shown in Figure 1. Percentage antioxidative activity of free radical scavenging system (RSA) was high in oil from IF (28.29%). NF, enzymatic, and centrifugation quenched DPPH radical by 19.7, 24.23 and 23.51%, respectively. The lowest antioxidant activity from VCO extracted oil sample was from CH (17.32%).

RSA of RCO was significantly lower than VCOs with ratio of 1:4 compared with IF (Figure 1). Earlier study comparing the ability of coconut oil from different extraction methods (fermentation, CH and RCO) to quench DPPH radicals also showed that fermentation gave significantly higher RSA compared to RCO (1:3) (Marina et al., 2009b). The low antioxidant activity of RCO may be due to the exposure to high heat during the extraction process (Seneviratne et al., 2008).

Marina et al. (2009b) reported that the reason for low RSA in CH compared to other VCOs may be due to more processing steps involved. Seneviratne et al. (2008) also concluded that the possibility of slight heat employed in other

processing methods may enhance RSA, but excess heat will lead to reduction in RSA as observed by oil sample from RCO method. The extent of heat required to enhance the antioxidant properties of coconut oil is not established Marina et al. (2009c). However, the reduced RSA of RCO which uses high heat above 200°C is in agreement with other reported studies by Nevin and Rajamohan (2004), Seneviratne et al. (2008), and Marina et al. (2009b).

Triacylglycerol (TAG) composition

The major triglycerides in coconut oil are the MCTs, with equivalent carbon number ranging from 32 to 42. Triglyceride composition is used to distinguish coconut oil from other lauric acid containing oils, that is, palm kernel oil due to its high composition of short chain triglycerides (C30 to C34) and lower composition of long chain triglycerides (C44 to C54) (Amri, 2011). VCO sample from enzymatic extraction was the only oil with C26 and C28 with values of 3.38 ± 0.17 and 2.11 ± 0.05% (Table 4). The major triglycerides present in VCO samples consist of 23.31 to

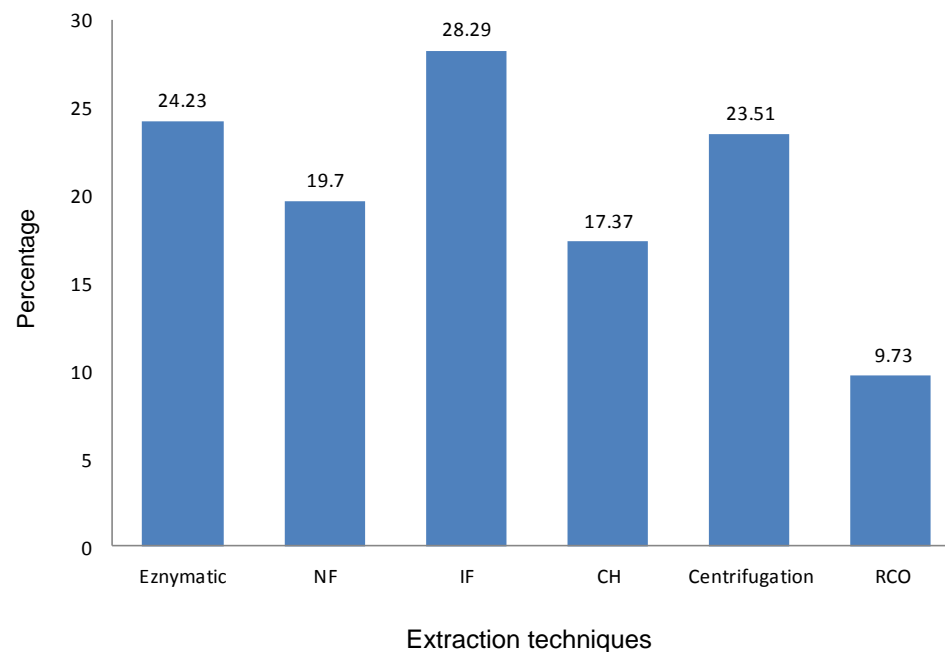


Figure 1. Antioxidant activity (%) of coconut oils extracted with different techniques.

Table 4. Triacylglycerol composition of coconut oil produced from different techniques and Codex standard.

Extraction method	C24	C26	C28	C30	C32	C34	C36	C38	C40	C42	C44	C46	C48	C50
EVCO	0.49±0.08 ^{bc}	3.38±0.17	2.11±0.05	3.66±0.08 ^b	15.75±0.31 ^{bc}	21.69±0.25 ^a	23.31±0.19 ^c	15.74±0.29 ^a	8.37±0.026 ^b	3.53±0.82 ^{ab}	2.36±1.33 ^b	0.63±0.07 ^a	ND	ND
NFVCO	0.78±0.1 ^b	ND	ND	3.93±0.8 ^c	19.38±1.17 ^b	21.72±0.17 ^a	26±0.51 ^a	15.87±0.11 ^a	7.8±0.14 ^a	3.4±0.57 ^{ab}	0.74±0.08 ^a	0.3±0.02 ^a	ND	ND
IFVCO	0.45±0.01 ^c	ND	ND	4.04±0.04 ^a	15.98±0.31 ^a	21.84±0.18 ^a	26.12±0.2 ^{ab}	17.18±0.16 ^b	9.31±0.11 ^c	3.89±0.5 ^a	0.92±0.03 ^a	ND	ND	ND
CHVCO	0.7±0.1 ^{ab}	ND	ND	3.75±0.03 ^{bc}	19.57±0.24 ^b	24.57±0.01 ^b	25.74±0.14 ^a	14.74±1.00 ^c	7.42±0.03 ^d	2.82±0.42 ^{bc}	0.24±0 ^a	ND	ND	ND
CEVCO	1.03±0.05 ^d	ND	ND	ND	21.59±0.28 ^d	23.29±0.31 ^c	27.51±0.12 ^d	16.32±0.4 ^{ab}	7.94±0.04 ^a	2.11±0.38 ^c	0.27±0.06 ^a	ND	ND	ND
RCO	0.65±0.1 ^c	ND	ND	ND	14.79±0.12 ^c	17.73±0.03 ^d	26.63±0.11 ^b	20.13±0.04 ^d	11.88±0.02 ^e	6.03±0.02 ^d	ND	1.57±0.05 ^b	0.52±0.02	0.14±0.02

Means (n=6) within the same column with different superscripts are significantly different at p<0.05. ND: Not detected. %RSA.

27.51% of C36, 17.73 to 24.57% of C34, 14.79 to 21.59% of C32, 14.47 to 20.13% of C38 and 7.42

to 11.88% of C40. These values were in agreement with the values of VCO samples

reported by Marina et al. (2009a). Overall, RCO had higher composition of longer chain triglycerides

(C38 to C50) than shorter chain triglycerides (C24 to C34) and was the only oil sample which contained C46, C48 and C50. It indicates higher degree of unsaturation in coconut oil produced by RCO. Similar findings of higher long chain triglycerides in RCO compared to VCO was reported by (Gopala Krishna et al., 2010).

Conclusion

RCO shows more economic prospect for coconut oil extraction due to higher oil yield. The method of extraction does not significantly alter the fatty acid composition of coconut oil. Also the triglyceride of coconut oil extracted from enzymatic, centrifugation, natural fermentation, induced fermentation, chilling and thawing and refined coconut oil is relatively similar. However, with regards to oil quality, VCO is a better option for coconut oil extraction compared to RCO, with higher phenolic antioxidant activity. Of the VCO methods of extraction, induced fermentation using *L. plantarum* holds promising prospect due to its relatively high oil yield compared to other VCO methods and its higher level of antioxidant activity compared to other methods. On average, the physicochemical parameters of the coconut oil produced from different methods did not vary from set standards

CONFLICTS OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

- Amri IN (2011). The Lauric (Coconut and Palm Kernel) Oil. Vegetable Oils in Food Technology. Wiley-Blackwell. Pp.169-197.
- AOCS (2009). Official methods and recommended practices of the AOCS / editors of analytical methods, David Firestone. Urbana, Ill. AOCS.
- APCC (2009). Quality standard virgin coconut oil. Available at: www.apccsec.org/apccsec/admin/files/11VCOStandardFlyer.pdf. Pp.5-6.
- Bawalan DD (2011). Processing manual for virgin coconut oil, its products and by-products for Pacific Island Countries and Territories. Available at: <http://www.spc.int>.
- Bawalan DD, Chapman KR (2006). Virgin coconut oil production manual for micro- and village-scale processing. Available at: <ftp://ftp.fao.org/docrep/fao/009/ag094e/ag094e00.pdf>. P 80.
- Canapi EC, Agustin YTV, Moro EA, Pedrosa EJ, Bendaño MLJ (2005). Coconut oil. Bailey's Industrial Oil and Fat Products. Hoboken: John Wiley & Sons, Inc.
- Che Man YB, Asbi AB, Azudin MN, Wei LS (1996). Aqueous enzymatic extraction of coconut oil. J. Am. Oil Chem. Soc. 73(6):683-686.
- Che Man YB, Karim MIB, Teng CT (1997). Extraction of coconut oil with *Lactobacillus plantarum* 1041 IAM. J. Am. Oil Chem. Soc. 74(9):1115-1119.
- Chih H, James A, Jayasena V (2012). Addition of enzymes complex during olive oil extraction improves oil recovery and bioactivity of Western Australian Frantoio olive oil. Int. J. Food Sci. Technol. 47(6):1222-1228.
- Choe E, Min DB (2006). Comprehensive Reviews in Food Science and Food Safety Mechanisms and Factors for Edible Oil Oxidation. Comp. Rev. Food Sci. Food Saf. 5:169-186.
- Coorey R, Grant A, Jayasena V (2012). Effects of chia flour incorporation on the nutritive quality and consumer acceptance of chips. J. Food Res. 1(4):85-95.
- Cunha SC, Oliveira MBPP (2006). Discrimination of vegetable oils by triacylglycerols evaluation of profile using HPLC/ELSD. Food Chem. 95(3):518-524.
- Dayrit FM, Dimzon IKD, Valde MF, Santos JER, Garrovillas MJM, Villarino BJ (2011). Quality characteristics of virgin coconut oil: Comparisons with refined coconut oil. Pure Appl. Chem. 83(9):1789-1799.
- Enig MG (1996). Coconut: In Support of Good Health in the 21st Century. 301:1-27.
- FAO (2009). Codex Alimentarius Stan 201. Standard for Named Vegetable Oils. Available at: <http://www.fao.org/fao-who-codexalimentarius/en/>.
- FAO (2014). FAOSTAT, FAO, ROME. Retrieved October 17, 2014, from <http://www.fao.org/faostat/en/#data/QC>.
- Gopala KAG, Raj G, Bhatnagar AS, Prasanth KP, Chandrashekar P (2010). Coconut Oil: Chemistry, Production and Its Applications - A Review. Indian Coconut J. 53(3):15-27.
- Han JR, Deng B, Sun J, Chen CG, Corkey BE, Kirkland JM, Guo W (2007). Effects of dietary medium-chain triglyceride on weight loss and insulin sensitivity in a group of moderately overweight free-living type 2 diabetic Chinese subjects. Metabolism 56(7):985-991.
- IUPAC (1992). Standard methods for the analysis of oils, fats, and derivatives. 7th ed., Oxford: Pergamon.
- Ixtaina VY, Martínez ML, Spotorno V, Mateo CM, Maestri DM, Diehl BWK, Nolasco SM, Tomás MC (2011). Characterization of chia seed oils obtained by pressing and solvent extraction. J. Food Compos. Anal. 24(2):166-174.
- Mansor TST, Che Man YB, Shuhaimi M, Abdul Afiq MJ, Ku Nurul FKM (2012). Physicochemical properties of virgin coconut oil extracted from different processing methods. Int. Food Res. J. 19(3):837-845.
- Marina AM, Che Man YB, Amin I (2009c). Virgin coconut oil: emerging functional food oil. Trends Food Sci. Technol. 20(10):481-487.
- Marina AM, Che Man YB, Nazimah SAH, Amin I (2009a). Chemical Properties of Virgin Coconut Oil. J. Am. Oil Chem. Soc. 86(4):301-307.
- Marina AM, Che man YB, Nazimah SAH, Amin I (2009b). Antioxidant capacity and phenolic acids of virgin coconut oil. Int. J. Food Sci. Nutr. 60(Sup. 2):114-123.
- Marten B, Pfeuffer M, Schrezenmeir J (2006). Medium-chain triglycerides. Int. Dairy J. 16(11):1374-1382.
- McGlone OC, Canales AL, Carter JV (1986). Coconut oil extraction by a new enzymatic process. J. Food Sci. 51(3):695-697.
- Nagao K, Yanagita T (2010). Medium-chain fatty acids: functional lipids for the prevention and treatment of the metabolic syndrome. Pharm. Res. 61(3):208-212.
- Nevin KG, Rajamohan T (2004). Beneficial effects of virgin coconut oil on lipid parameters and *in vitro* LDL oxidation. Clin. Biochem. 37(9):830-835.
- Nevin KG, Rajamohan T (2010). Effect of Topical Application of Virgin Coconut Oil on Skin Components and Antioxidant Status during Dermal Wound Healing in Young Rats. Skin Pharmacol. Physiol. 23:290-297.
- Nour AH, Mohammed FS, Yunus RM, Arman A (2009). Demulsification of virgin coconut oil by centrifugation method: a feasibility study. Int. J. Chem. Technol. 1(2):59-64.
- Perera L, Perera SACN, Bandaranayake CK, Harries HC (2010). Coconut. In: J. Vollmann & I. Rajcan (Eds.). Oil Crops. Springer, Dordrecht, Heidelberg, London, New York. pp. 369-396.
- Raghavendra SN, Raghavarao KSMS (2010). Effect of different treatments for the destabilization of coconut milk emulsion. J. Food Eng. 97(3):341-347.
- Raghavendra SN, Raghavarao KSMS (2011). Aqueous extraction and enzymatic destabilization of coconut milk emulsions. J. Am. Oil Chem. Soc. 88(4):481-487.
- Ramadan MF, Wahdan KMM (2012). Blending of corn oil with black cumin (*Nigella sativa*) and coriander (*Coriandrum sativum*) seed oils: Impact on functionality, stability and radical scavenging activity. Food Chem. 132(2):873-879.

Seneviratne KN, Sudarshana D, Dissanayake M (2008). Variation of phenolic content in coconut oil extracted by two conventional methods. *Int. J. Food Sci. Technol.* 43(4):597-602.

Sigma-Aldrich (1997). Technical documents: BCl3-Methanol 12%. <http://sigmaaldrich.com>.

Full Length Research Paper

Antioxidant potential use of bioactive peptides derived from mung bean hydrolysates (*Vigna Radiata*)

Carlos M. Guerra A.*, Walter Murillo and Jonh J. Méndez A.

Departamento de Química, Grupo de Investigación de Productos Naturales, Facultad de Ciencias. Universidad del Tolima. Ibagué, Colombia.

Received 5 September, 2016; Accepted 14 November, 2016

The antioxidant activity of bioactive peptides obtained from hydrolyzed mung bean (*Vigna radiata*) grown in Espinal Tolima was evaluated. Alkaline hydrolysis was performed with 1 N NaOH. Mung bean protein concentrate obtained was 82%. The enzymatic hydrolysis was performed using a randomized block design, with commercial enzymes: Alcalase®, trypsin® and Flavourzyme®. The kinetics of the hydrolysis degree (DH) was measured based on time. The factor evaluated was the reaction time in minutes and the response variable was the degree of hydrolysis (DH), which was 43.21% for Alcalase, 41.20% for Trypsin and 38.41% for Flavourzyme. Two experiments used for measuring the antiradical activity *in vitro* were positive for the three types of enzymes. The optimal antiradical capacity was obtained at 30 min for Alcalase and Trypsin, and 45 min, respectively for Flavourzyme. Antioxidant activity *in vitro* such as, ABTS, DPPH, ORAC and FRAC correlated with the *in vivo* assays. Mung bean hydrolysates could have antioxidant effects, a good alternative when incorporated into diet, as a dietary supplement or added to a food matrix.

Key words: Antioxidant, bioactive peptides, hydrolyses, mung bean.

INTRODUCTION

Enzymatic protein hydrolysates have been used to improve the functional properties of food products, formulate pharmaceuticals in specific clinical application, and obtain bioactive peptides (Torruco-Uco et al., 2008). Today, there is a great interest in protein hydrolysates and their various applications and uses. It is known that after hydrolysis, the biofunctional properties of proteins can be improved (Torruco-Uco et al., 2009). Generally, the resulting peptides have different biological activities in addition to being an energy source of essential amino

acids (Betancur-Ancona et al., 2004; Tecson-Mendoza et al., 2009). Most bio-functional properties are based on amino acids sequences in the polypeptides, the type of enzyme used, and methods of recovery of the peptide. Bioactive peptides are often small peptide chains consisting of 2 to 15 amino acid residues (Segura-Campos et al., 2010; Megias et al., 2004). However, some studies mention that the bioactive peptides derived from food are only between 2 and 9 amino acid residues (El-adawy, 2000). Although, there may be exceptions

*Corresponding author. E-mail: cmguerra@ut.edu.com.

because there are peptides with 20 amino acid residues, as lunasin peptide extracted from soybeans with anticancer activity tested in rats had 43 amino acid residues and a molecular weight of 5400 Da (Jeong et al., 2002). Bioactive peptides have been isolated from various sources, both animal and vegetable. Among the former are casein, cheese whey-based milk, fermented milk, muscle of chicken and fish, and the latter are those of plant origin which have been isolated from wheat gluten, soy, sunflower, spinach, etc (Das Neves et al., 2006; Megias et al., 2004). These peptides have various biological activities such as antihypertensives (Jenssen et al., 2004), opioids (Muñoz, 2011), antioxidants (Gómez et al., 2013) anticholesterolemic, (Torrugo.Uco et al., 2008), antimicrobial (Benitez et al., 2008), anticariogenic (Montano-Perez And Vargas-Albores, 2002), antithrombotic (Rojano et al., 2012) anti-cancer and immunomodulators (Martinez and Martinez, 2015), among others.

Mung bean is an important source of protein for human consumption in Asia. The bean is used for animal food in some countries. The main protein in mung bean seeds is vicilin (8S), which represents 89% globulin, followed by 7.6% of 11S, and 3.4% of the basic 7S globulin (Tecson-Mendoza et al., 2009). Only basic 7S and 11S have disulfide bonds. By isolating the main protein of mung bean, it is possible to use it to obtain many desirable features when added to processed foods, such as changes in foaming, emulsification and water absorption (El-adawy, 2000). The natural antioxidants based on peptides isolated from plant samples are effectively used for extending the storage period of food (Gómez et al., 2013; Benitez et al., 2008). The antioxidant activities are affected by the composition and sequences of amino acid present in those particular peptides and the molecular weight of the peptides (Montano-Perez And Vargas-Albores, 2002). In Colombia, mung bean is currently used for its high nutritional value as a flour in feeding pigs, fish and poultry. Similarly, it is used as green manure and sown and incorporated as organic matter at the beginning of growth to improve the physical, chemical and biological soil properties, and reduce weed populations and disease incidence. Mung bean hydrolysis products act as antioxidants in a food matrices. The values obtained are similar to reported values for some foods in the database of United States Department of Agriculture

MATERIALS AND METHODS

Chemicals

2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), (±) -6-Hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid (Trolox) and potassium persulphate were purchased from Sigma-Aldrich.

Plant material

Mung bean was collected in the Municipality of Espinal Tolima. The

seeds were cleaned by hand and grinded in an electric grinder brand Thomas Wiley. The flour obtained was passed through a mesh screen 200 µm. Afterwards it was defatted with hexane in a Soxhlet apparatus by 8 h. Once evaporated, the hexane passed through a mesh screen of 100 µm to achieve a more homogeneous particle size. It was stored in airtight containers for later use.

Proximal composition of flour

The proximate composition of the mung bean flour was analyzed to determine crude protein, crude fat, ash, moisture, phosphorus, potassium, calcium and magnesium. The composition was determined according to the procedures described by AOAC (AOAC, 1990).

Mung bean protein concentrate (MBPC)

The protein concentrate was fractionated using an established method Betancur-Anaconda et al., 2004), with some modifications. To raise the pH 1 N NaOH was added to the flour suspension to reach pH 9 and stirred for one hour at 450 rpm. The suspension was centrifuged at 9000 g × 15 min (centrifuge Hermle-Z32HK). The supernatant was adjusted to pH 4.5 with 1 N HCl, to isoelectric point of globulin, filtered and centrifuged at 15000 g for 20 min. The precipitate was washed with deionized water several times, and subsequently was lyophilized at -50°C.

Enzymatic hydrolysis

The method described by Pedroche et al 2002, was used for the hydrolysis of the protein concentrate. A randomized block design was used for it, where the blocks used were commercial enzymes (Alcalase® 2.4 L FG, Flavourzyme® 500 mg, and trypsin Novo Nordisk, Bagsvaerd, Denmark), reaction times (0, 5, 15, 30, 45 and 60 min) and the response variable was the degree of hydrolysis (DH). Hydrolysis was performed by individual treatments with the above enzymes, carrying out digestion with Alcalase®, Flavourzyme®, Trypsin® up to 60 min. A hydrolysis curve was obtained from the pH-stat technique using the following parameters of hydrolysis: enzyme/substrate ratio (E/S) 3% for all enzymes; pH 8 at 50° C for Alcalase, pH 7 at 50°C for Flavourzyme and pH 8 at 37°C for trypsin. The hydrolysis was stopped by heating at 85°C for 15 min. The hydrolyzate was clarified through a 0.45 µm filter. The filtrate was centrifuged at 12,000 g × 10 min, lyophilized and stored in a freezer at -20°C.

The DH% for each enzyme was calculated by the pH-stat method, using the following equation:

$$\%DH = \frac{h}{h_{total}} \times 100 = \frac{V_{NaOH} * N_b}{MP * \alpha * h_{total}} \times 100$$

$$\alpha = \frac{1}{1 + 10^{pK - pH}}$$

Where: %DH is the degree of hydrolysis; h is the number of broken peptide; h_{total} is total number of bonds available for hydrolysis proteolytic; V_{NaOH} is the total volume of NaOH consumed expressed in mL to keep the pH constant during the reaction. N_b is normality of the NaOH; MP is the mass of the protein; α is the degree of dissociation of the protein.

DPPH radical scavenging activity assay

The methodology described by Braca et al., 2002, was followed,

Table 1. Composition of the proximal mung bean flour cultivated in the Tolima Espinal.

% Humidity	% Ash	pH	E.E	% Protein	P	Ca	K	Mg
8.33	3.8	6.77	0.65	18.43	0.54	0.36	1.77	0.24

with slight modification. One milliliter of either hydrolysate or peptide solution at different concentrations (0-1 mg/ml) was mixed with 4 ml of 0.15 mM DPPH (in 95% ethanol). The mixture was then shaken vigorously using a mixer. The reaction mixture was incubated for 30 min in the dark at room temperature. The absorbance of the resulting solution was measured at 517 nm with a spectrophotometer. Ethanol and Trolox was used as a control and standard sample, respectively. The radical scavenging capacity of the samples was measured as a decrease in the absorbance of DPPH radical and it was calculated using the following equation:

$$\% \text{ DPPH} = \left(\frac{A_c - A_m}{A_c} \right) \times 100$$

Where: % DPPH, DPPH radical scavenging activity, expressed as a percentage. A_c : Absorbance control; A_m : Absorbance of the reaction mixture

From these values, the percent inhibition of 50% of radical (IC_{50}) of both samples, as pattern was determined using a linear regression model.

ABTS radical stabilizing activity

The methodology described by Kuskoski et al., 2004, was followed. The radical $ABTS^+$ was obtained by a mixture of ABTS (7 mM) and potassium persulfate (2.45 mM final concentration). This mixture was allowed to stand for 16 h at room temperature, time after which it was diluted with ethanol to achieve an absorbance of 0.7 ± 0.02 at 734 nm. The sample was prepared by mixing 3.43 mL $ABTS^+$ solution adjusted with 70 μ L of the hydrolysates at different concentrations (0.2 to 200 μ g/mL final concentration) and recording the absorbance values after 6 min of reaction. TROLOX was used as pattern (0.1-1 μ g/mL).

$$\% \text{ ABTS} = \left(\frac{A_{\text{ABTS}} - A_{6\text{min}}}{A_{\text{ABTS}}} \right) \times 100$$

Where: %ABTS is ABTS radical stabilizing activity, expressed as a percentage; A_{ABTS} is absorbance of $ABTS^+$ before adding the sample; $A_{6\text{min}}$ is absorbance of the reaction mixture at 6 min.

Oxygen radical absorbance capacity (ORAC)

The methodology described by Zapata et al. (2014) was used. Trolox® was used as standard, under controlled conditions (temperature to 37 ° C and pH 7.4.) Absorbance was performed at 493 nm λ excitation and excitation slit 10 nm λ emission 515 and emission slit 15 with 1% attenuator. For the calibration of the technique 1×10^{-2} M of fluorescein solutions AAPH and phosphate buffer 0.6 M (75 mM, pH 7.4) were used. 30 μ L aliquot of sample was mixed with 21 μ L of fluorescein, 2.899 mL of phosphate buffer and 50 μ L of AAPH. The protective effect of the antioxidant was calculated using the differences in areas under the decay curve of fluorescein between the target and the sample, and compared to the standard curve.

The results were expressed in μ mol /micromoles Trolox equivalents per liter of sample (micromol Trolox / 100 g dry product), according to the following equation:

$$\text{ORAC} = \left(\frac{AUC_{\text{SAMPLE}} - AUC_{\text{CONTROL}}}{AUC_{\text{TROLOX}} - AUC_{\text{CONTROL}}} \right) f[\text{TROLOX}]$$

Where AUC_{SAMPLE} is the area under the curve of the sample, AUC_{CONTROL} area under the curve for the control, AUC_{Trolox} area under the curve for the Trolox, f is the factor of dilution of the sample. The fluorescence was measured in a fluorescence spectrophotometer PerkinElmer® LS55.

Antioxidant activity reducing Fe^{+3} : FRAP Assay

This method evaluates the reducing power of a sample based on its ability to reduce ferric iron (Fe^{+3}) complexed with 2, 4, 6-tri (2-pyridyl)-s-triazine (TPTZ) to ferrous form (Fe^{+2}) which has an absorbance maximum at a wavelength between 590 and 595 nm. 50 μ L of sample was added to 900 μ L of a solution of FRAP (acetic acid –sodium acetate, pH 3.4 Buffer acid, TPTZ, $FeCl_3$, in 10:1: 1), after 30 min of reaction the absorbance was determined at a wavelength of 593 nm. This value was compared with the reference curve constructed with ascorbic acid as a primary standard, and the results were expressed as ascorbic acid equivalents (AEAC).

Anti-hemolytic activity

The anti-hemolytic activity was carried out following the methodology proposed by Nabavi et al., 2011 and Aguillon et al., 2013. Blood was centrifuged at 2500 rpm for 10 min; plasma and leucocytes were removed. Red cells were washed three times with isotonic saline phosphate buffer (PBS: 22.2 mM Na_2HPO_4 , 5.6 mM KH_2PO_4 , 123.3 mM NaCl and 10 mM glucose in distilled water, pH 7.4). The activity was determined using the following equation:

$$\% \text{ AAH} = \left(\frac{A_{\text{b control}} - A_{\text{b hydrolyzed}}}{A_{\text{b control}}} \right) \times 100$$

Where: %AAH is anti-hemolytic activity, expressed as a percentage. $A_{\text{b control}}$ is absorbance of control in this case ascorbic acid is used; hydrolyzed A_{b} is absorbance of the sample, in this case the hydrolysates.

RESULTS AND DISCUSSION

The results of the composition of mung bean seeds flour are shown in Table 1. Protein percentage agrees with that reported in the literature. Potassium concentration is comparatively high for plants (López Bellido, 1996). This suggests that mung bean can be a good source of Pottasium.

Enzymatic hydrolysis

The degree of hydrolysis for each enzyme is shown in Table 2. The highest degree of hydrolysis was obtained

Table 2. Percentage of hydrolysis for enzymes at different reaction times.

Hydrolysis time	Alcalase %DH	Trypsin %DH	Flavourzyme%DH
5'	28.81 ± 1.8	21.61 ± 1.1	19.21 ± 1.2
15'	33.61 ± 1.96	32.17 ± 1.4	24.01 ± 2.1
30'	43.21 ± 1.5	41.29 ± 1.9	33.61 ± 1.9
45'	38.41 ± 1.7	40.81 ± 1.2	38.41 ± 1.8
60'	40.81 ± 1.3	38.45 ± 1.8	37.45 ± 1.3

Data reported are the average of three different determinations in time ± standard deviation.

Table 3. Antioxidant activity of mung bean hydrolysates obtained with alcalase.

Hydrolysis time	Alcalase			
	IC ₅₀ ABTS (µg/mL)	IC ₅₀ DPPH (µg/mL)	TEAC (mM Trolox / 100 g sample)	mg. Ascorbic Acid/ 100 g sample)
5'	67.62 ± 1.69	163.22 ± 1.49	24.06 ± 0.51	226.34 ± 1.65
15'	54.25 ± 1.70	153.25 ± 1.50	32.09 ± 0.48	298.56 ± 1.97
30'	51.13 ± 1.91	141.13 ± 1.61	44.01 ± 0.65	327.31 ± 2.05
45'	52.52 ± 1.72	152.52 ± 1.42	52.16 ± 1.47	398.89 ± 2.13
60'	54.65 ± 1.83	169.65 ± 1.93	55.02 ± 3.20	427.12 ± 2.22

Values expressed as the average of three determinations ± standard deviation.

Table 4. Antioxidant activity of mung bean hydrolysates obtained with trypsin.

Hydrolysis time	Trypsin			
	IC ₅₀ ABTS (µg/mL)	IC ₅₀ DPPH (µg/mL)	TEAC (mM Trolox / 100 g sample)	mg. Ascorbic acid/ 100 g sample)
5'	110.31 ± 1.53	190.31 ± 1.53	12.23 ± 0.71	427.33 ± 2.63
15'	81.89 ± 0.71	171.89 ± 1.71	16.34 ± 0.52	478.65 ± 2.89
30'	82.39 ± 1.82	152.39 ± 1.82	19.32 ± 0.74	552.27 ± 2.96
45'	95.50 ± 1.93	195.50 ± 1.73	22.29 ± 1.25	580.36 ± 3.42
60'	116.29 ± 2.04	216.29 ± 1.89	29.59 ± 1.93	569.61 ± 3.02

Values expressed as the average of three determinations ± standard deviation.

after 30 min for both Alcalase **A**, trypsin **T**, and Flavourzyme **F**. The optimal hydrolysis time was 45 min. Similar result was observed in the degree of hydrolysis of Alcalase and trypsin enzymes, compared to Flavourzyme enzyme.

The degree of hydrolysis obtained is slightly higher than those reported in previous studies of legumes such as beans and soybeans, where the values of hydrolysis are between 20 and 31% for alcalase and 18 and 51% for flavourzyme (Martínez Ayala et al., 2015 and Viogue et al., 2008). The highest degree of enzymatic hydrolysis was obtained after 30 and 45 min in the three enzymes respectively, compared to 60 and 90 min reported in previous works for alcalase and flavourzyme (Betancur-

Anaconda et al., 2004; Megías et al., 2004).

Evaluation of antioxidant activity *in vitro*

The results of DPPH and ABTS antiradical activity, antioxidant activity as well as the ORAC and the FRAP, are presented for the three enzymes Alcalase, Trypsin and Flavourzyme in Tables 3 to 5 respectively.

The degree of hydrolysis for 30 min results in higher antiradical activity ABTS for alcalase and trypsin with IC₅₀ values of 51.13 and 82.39 µg/mL, respectively. In the case of Flavourzyme enzyme the highest IC₅₀ value was 93.44 µg/mL.

Table 5. Antioxidant activity of mung bean hydrolysates obtained with Flavourzyme.

Hydrolysis time	Flavourzyme			
	IC ₅₀ ABTS (µg/mL)	IC ₅₀ DPPH (µg/mL)	TEAC (mM Trolox / 100g sample)	mg. Ascorbic acid/ 100 g sample)
5'	223.32 ± 1.61	236.82 ± 1.71	10.21 ± 0.42	498.75 ± 2.98
15'	175.65 ± 1.52	195.95 ± 1.72	14.12 ± 0.37	507.62 ± 2.77
30'	143.13 ± 1.84	106.03 ± 1.74	17.04 ± 0.74	522.29 ± 3.12
45'	123.44 ± 1.65	93.44 ± 1.93	26.17 ± 1.01	518.93 ± 3.22
60'	174.92 ± 1.65	94.92 ± 1.85	43.41 ± 1.97	535.67 ± 3.31

Values expressed as the average of three determinations ± standard deviation.

Table 6. Oxygen radical absorbance capacity (ORAC) of selected foods, Release 2.0.

NDB No.*	Description	ORAC mmol TE/100 g
99459	Sumac, bran, raw	312.40
99465	Sorghum, bran, black	100.80
99464	Sorghum, bran, red	71.00
2030	Spices, pepper, black	34.05
16032	Beans, kidney, red,, mature seeds, raw	8.61
16014	Beans, black, mature, seeds, raw	8.49
16040	Beans, pink, mature, seeds, raw	8.32
16069	Lentils, raw	7.28
14097	Alcoholic beverage, wine, table, red, Cabernet Sauvignon	4.52
99586	Mangosteen, raw	2.51
99070	Tea, green, brewed	1.25
11052	Beans, snap, green, raw	0.80

*5-Digit Nutrient Databank number. Source: USDA data base of antioxidant activity, May, 2010.

According to the values obtained *in vitro* DPPH antiradical activity, an increased capacity of hydrolysis was observed at 30 min time with trypsin and Alcalase enzymes, with values of 141.13 g/mL and 152.39 µg/mL respectively; for flavourzyme enzyme hydrolysis timewhere most capacity antiradical activity *in vitro* is presented at 45 min with 123.44 µg/mL

ORAC assay

Antioxidant activities as measured by the ORAC method for mung bean hydrolysates with enzymes Alcalase, Flavourzyme and trypsin, at different times of hydrolysis are presented in Table 6. The ORAC values vary between 55.02 and 22.29 mmol Trolox / 100 g sample. ORAC values for some foods reported in the database of the United States Department of Agriculture (USDA) are shown in Table 6 (Haytowitz and Bhagwat, 2010).

Interestingly, only the protein hydrolysates bean exceeds the antioxidant potential of the seed, in which the secondary metabolites of phenolic origin are included. As can be seen, bean seeds have values between 0.80

and 8.61 mmol Trolox/100 g sample, ORAC values much lower than those obtained with mung bean hydrolysates. This means that mung bean hydrolysates obtained with Alcalase, Flavourzyme and trypsin Enzymes have a very high antioxidant potential. Some researchers suggested an intake between 3.0 to 5.0 ORAC/day, in order to promote oxidative balance in blood plasma and body tissues (Prior et al., 2003; Rojano et al., 2012). Therefore, mung bean hydrolysates could be a good alternative when incorporated into diet, either in the form of encapsulation or added to food matrix.

FRAP assay

In this test, the values of the redox potential of Fe⁺³ - TPTZ are comparable with that of ABTS; FRAP values for the hydrolysates are slightly higher than those reported in studies on foods like fruits and juices (Prior et al., 2003; Rojano et al., 2012).

Hydrolysates obtained in Tables 4 and 5 with trypsin and Flavourzyme had values of 535.67 and 580.36 (mg Ascorbic Acid/100 g sample) at 60 and 45 min respectively,

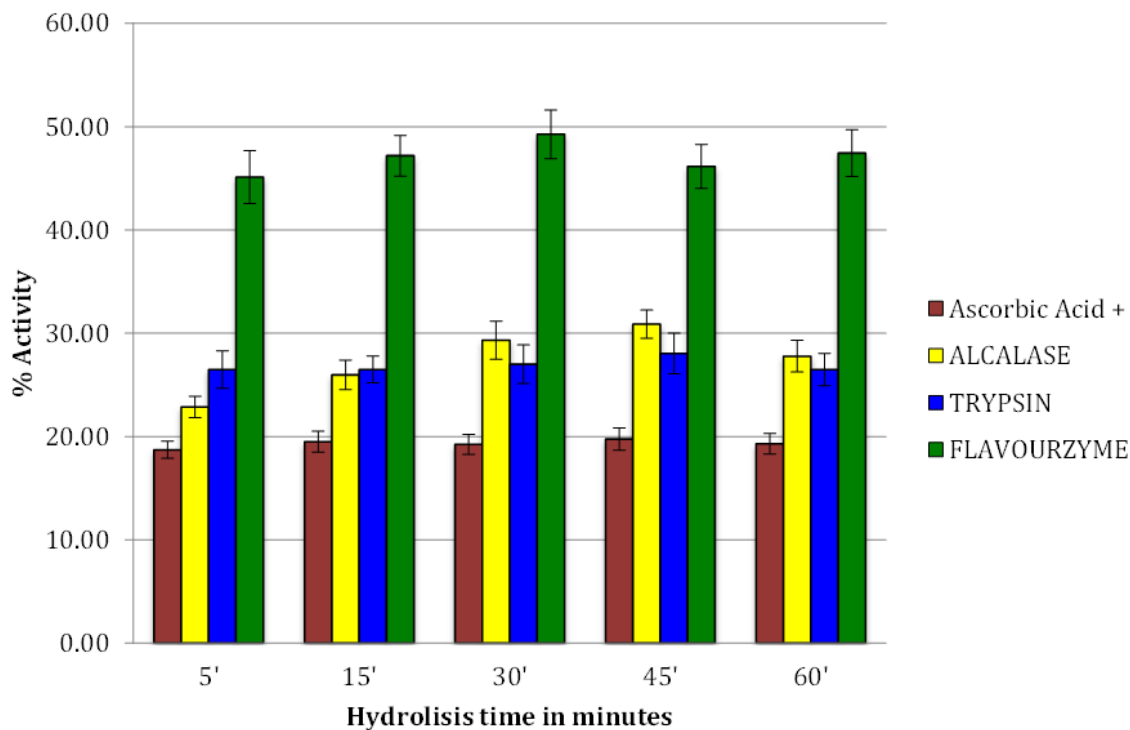


Figure 1. Anti-hemolytic activity of MBPC with alcalase, flavourzyme and trypsin. Values expressed as the mean of three determinations \pm standard deviation.

whereas for Alcalase the highest value of 427.12 (mg Ascorbic Acid/100 g sample) was obtained at 60 min.

Anti-hemolytic activity

The results of the anti-hemolytic activity of mung bean hydrolysates at different times of hydrolysis are presented in Figure 1.

The results obtained in anti hemolytic activity of different hydrolysates mung bean against ascorbic acid used as a positive control are shown in Figure 1, being hydrolysates obtained with Flavourzyme the highest percentage of activity against those obtained with Alcalase and trypsin.

The hydrolysates show more ability to inhibit the hemolysis H_2O_2 -induced than positive control. The antihemolytic activity of trypsin was the lowest compared to the other hydrolysates with Flavourzyme and Alcalase. All enzymatic hydrolysates were significantly different compared to ascorbic acid used as positive control.

Conclusion

The degree of enzymatic hydrolysis (%DH) of protein concentrates mung beans grown in the municipality of Espinal Tolima was more efficient with Alcalase and trypsin at 30 min with values of 43.21 and 41.29%, compared to 45 min used for Flavourzyme enzyme where a value of 38.45% was obtained. According to the values

obtained for the two trials of antiradical activity *in vitro*, a higher capacity was observed in the hydrolysis time of 30 min with the enzyme Alcalase ABTS and DPPH respectively. Similar case with trypsin Enzyme, where at the same hydrolysis time (30'), and the highest values of antiradical activity *in vitro* was presented. For Flavourzyme enzyme, it is evident that the hydrolysis time where the increased capacity of antiradical activity *in vitro* occurs was at 45 min. A trend almost directly proportional was observed between the degree of hydrolysis (% HD) and antiradical activity, in the samples taken at different times, showing this behavior in each of the enzymes used. The hydrolysates with each one of the enzyme presented a higher value in the antihemolytic activity than those reported by the positive control in ascorbic acid.

Interestingly, only the protein hydrolysates bean exceeds the antioxidant potential of the seed, in which the secondary metabolites of phenolic origin are included. Evaluating antioxidant activity in cells has a very interesting report that correlates with the data obtained in the *in vitro* assays. Therefore, mung bean hydrolysates could be a good alternative when incorporated in diet, either in the form of encapsulation or added in a food matrix.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGMENT

The authors thank the Office of Scientific Research and Development at the University of Tolima for the financial support provided for the implementation of this work, as well as the research groups GIPRONUT and GIRYSOUT part of Tolima University.

REFERENCES

- Aguillon J, Maldonado ME, Loango N, Arango SS, Landázuri P (2013). Antioxidant and antiproliferative activity of ethanolic and aqueous extracts from leaves and fruits juice *Passiflora edulis*. *Perspect. Nutr. Hum.* 15(1):13-25.
- AOAC-Association of Official Agricultural Chemists (1990). *Official Methods of Analysis*, 14th ed. Washington DC.
- Benitez R, Ibarz A, Pagan J (2008). Hidrolizados de proteína: procesos y aplicaciones. *Acta Bioquím. Clín. Latinoam.* 42(2):227-236.
- Betancur-Ancona D, Gallegos-Tintoré S, Chel-Guerrero L (2004). Wet-fractionation of *Phaseolus lunatus* seeds: partial characterization of starch and protein. *J. Sci. Food Agric.* 84(10):1193-1201.
- Braca A, Sortino C, Politi M, Morelli I, Mendez J (2002). Antioxidant activity of flavonoids from *Licania licaniaeflora*. *J. Ethnopharmacol.* 79(3):379-81.
- Das Neves MRA, Campos T, Márquez LUM (2006). Modulação da Pressão Arterial por Hidrolisados Protéicos. *Braz. J. Food Technol.* III JIPCA, 81-86.
- El-Adawy TA (2000). Functional properties and nutritional quality of acetylated and succinylated mung bean protein isolate. *Food Chem.* 70:83-91.
- Gómez LJ, Figueroa OA, Zapata JE (2013). Actividad Antioxidante de Hidrolizados Enzimáticos de Plasma Bovino Obtenidos por Efecto de Alcalasa@ 2.4 L. *Inf. Tecnol.* 24(1):33-42.
- Haytowitz DB, Bhagwat S (2014). *Database for the Oxygen Radical Absorbance Capacity (ORAC) of Selected Foods*, Release 2. U.S. Department of Agriculture Agricultural Research Service. Pp. 10-41.
- Jenssen H, Andersen JH, Uhlén-Hansen L, Gutteberg TJ, Rekdal Ø (2004). Anti-HSV activity of lactoferricin analogues is only partly related to their affinity for heparan sulfate. *Antiviral Res.* 61(2):101-109.
- Jeong JH, Lam Y, De Lumen OB (2002). Barley Lunasin Suppresses Ras-induced Colony Formation and Inhibits Core Histone Acetylation in Mammalian Cells. *J. Agric. Food Chem.* 50:5903-5908.
- Kuskoski EM, Asuero AG, García-Parilla MC, Troncoso AM, Fett R (2004). Actividad antioxidante de pigmentos antocianicos. *Ciênc. Tecnol. Aliment.* 24(4):691-693.
- Lopez Bellido L (1996). Nuevas técnica para determinar la calidad de las legumbres. Resultados de los trabajos realizados en la universidad de córdoba sobre la influencia de suelo y clima en la calidad de garbanzos y lentejas. 158: 77-83. http://www.magrama.gob.es/ministerio/pags/biblioteca/revistas/pdf_DYC/DYC_1995_25_77_83.pdf
- Martínez AO, Martínez DV (2015). Proteínas y péptidos en nutrición enteral. *Nutr. Hosp.* 21(Supl.2):1-14.
- Megías C, Yust MD, Pedroche J, Lquari H, Girón-Calle J, Alaiz M, Millán F, Vioque J (2004). Purification of an ACE Inhibitory Peptide alters Hydrolysis of Sunflower (*Helianthus annuus* L.) Protein Isolates. *J. Agric. Food Chem.* 52(7):1928-1932.
- Megias C, Yust MM, Pedroche J, Lquari H, Girón-Calle J, Alaiz M, Millán F, Vioque J (2004). Purification of an ACE Inhibitory Peptide after Dydrolisis of Sunflower (*Helianthus annuus* L.) Proetin Isolates. *J. Agric. Food Chem.* 52:1928-1932.
- Montano-Perez K, Vargas-Albores F (2002). Péptidos antimicrobianos: un mecanismo de defensa ancestral con mucho futuro. *INCI.* 27(1): 21-27.
- Muñoz EJF (2011). Péptidos Opioides Endógenos, Dolor y Adicción. *Synopsis* 3(1):33-39.
- Nabavi SF, Ebrahimzadeh M, Nabavi S, Eslami B (2011). Antioxidant activity of flower, stem and leaf extracts of *Ferula gummosa* Boiss. *Grasas Aceites* 61(3):244-250.
- Pedroche J, Yust MM, Girón - Calle J, Alaiz M, Millán F, Vioque J (2002). Utilisation of Chickpea Protein Isolates for Production of Peptides with Angiotensin I- Converting Enzyme (ACE)-Inhibitory Activity. *J. Sci. Food Agric.* 82(9):960-965.
- Prior RL, Hoang H, Gu L, Wu X, Bacchiocca M, Howard L, Hampsch-Woodill M, Huang D, Ou B, Jacob R (2003). Assays for hydrophilic and lipophilic antioxidant capacity (oxygen radical absorbance capacity (ORACFL) of plasma and other biological and food samples. *J. Agric. Food Chem.* 51:3273-3279.
- Rojano B, Zapata K, Cortés F (2012). Capacidad atrapadora de radicales libres de *Passiflora mollissima* (Kunth) L.H. Bailey (curuba). *Rev. Cubana Plantas Med.* 17(4):408-419.
- Segura-Campos M, Chel-Guerrero L, Betancur-Ancona D (2010). Efecto de la digestión en la biodisponibilidad de péptidos con actividad biológica. *Rev. Chil. Nutr.* 37(3):386-391.
- Torruco-Uco J, Chel-Guerrero L, Martínez-Ayala A, Dávila-Ortiz G, Betancur-Ancona D (2009). Angiotensin-I converting enzyme inhibitory and antioxidant activities of protein hydrolysates from *Phaseolus lunatus* and *Phaseolus vulgaris* seeds. *Food Sci. Technol.* 42:1597-1604.
- Torruco-Uco JG, Domínguez-Magaña MA, Dávila-Ortiz G, Martínez-Ayala A, Chel-Guerrero LA, Betancur-Ancona DA (2008). Péptidos antihipertensivos, una alternativa de tratamiento de origen natural: una revisión. *Cienc. Tecnol. Aliment.* 6(2):158-168.
- Zapata S, Piedrahita AM, Rojano B (2014). Capacidad atrapadora de radicales oxígeno (ORAC) y fenoles totales de frutas y hortalizas de Colombia. *Perspect. Nutr. Hum.* 16:25-36.

Full Length Research Paper

Effect of processing on proximate and mineral composition of black climbing (*P. coccineus* L.) bean flour

Mengistu Tadesse Mosisa

Department of Chemical Engineering, School of Mechanical Chemical and Material Engineering, Adama Science and Technology University, Adama-Ethiopia.

Received 15 June 2016 Accepted 19 October, 2016

The objective of this study was to determine the effect processing on proximate and mineral compositions of raw and processed seeds of black climbing bean “hepho” (*Phaseolus coccineus* L.). The experiment was conducted on raw, dehulled and undehulled black climbing bean flour. The processing techniques employed were traditional (TC) and pressure cooking (PC), while the raw sample served as control. The processing techniques showed deviations in nutrient content from the raw by the all tested processing techniques particularly, PC which caused a significantly ($p < 0.05$) difference in all the proximate contents except the carbohydrate content, whereas TC showed a significant ($p < 0.05$) difference in only some of the proximate composition. Generally, both the traditional cooking (DTC and UTC) and pressure cooking (DPC and UPC) techniques caused significant difference ($p < 0.05$) in some of the mineral profile of hepho (Ca, P and Fe) except the zinc content which has non-significant ($p > 0.05$) difference. Although all the process technique applied in this study have significant effect in the composition of hepho bean contents; DPC and UPC which have the most suitable techniques to prevent the loss of protein and minerals (Ca, P, Fe and Zn). Hence, the black climbing bean seeds “hepho” is an alternative and cheaper source of protein and contribute to solve the problem of malnutrition which is a prevalent problem in developing world especially, Ethiopia.

Key words: “Hepho” (*Phaseolus coccineus* L.) traditional cooking, pressure cooking, chemical composition.

INTRODUCTION

Protein-calorie deficiency is now viewed as the major nutritional problem in most developing countries including Ethiopia. Due to the high price of animal proteins, much importance is now placed on plant foods as a source of proteins in all developing countries (Yagoub et al., 2008). The dearth in food supply especially of protein is so

enormous that, the developing nations have to depend on cereals, grains, starch roots and tubers for energy and protein need (Akporhonor et al., 2006; Aremu et al., 2009). Legumes which refer to the seeds of Leguminosae include: peas, beans and pulses. Legumes are considered as “poor man’s meat” due to their high protein

E-mail: mengistutadesse706@gmail.com.

Author(s) agree that this article remains permanently open access under the terms of the [Creative Commons Attribution License 4.0 International License](https://creativecommons.org/licenses/by/4.0/)

content and low cost sources (Aremu et al., 2006a). The bean to be used as food, is better to have proximate contents (Derese, 2012) which comprises of 15 to 25 % proteins, 50 to 75 % carbohydrates mostly starch and about 1 to 3 % fat, 2.9 to 4.2 % ash and 3.5 to 6.5 % crude fiber. Further, beans contain considerable amounts of vitamins, minerals and nutritionally useful quantities of many essential amino acids (Vidal-Valverde et al., 1993; Oshodi et al., 1998). Though legumes are important sources of dietary proteins for human and animals, their usefulness have been hindered by the presence of some anti-nutritional factors known as toxins (Onyeike et al., 1995; Aremu et al., 2010).

Nutritional quality is affected by these factors that interact with the intestinal tract such as phytate, tannins and oxalates which reduce protein digestibility and amino acid absorption (Nowacki, 1980; Davis, 1981). However, these substances need to be destroyed either by heat or other treatments otherwise concentration of toxins will exert adverse physiological effects when ingested by man and animals (Liener, 1994). Hephho is the Afan Oromo name for black climbing bean (*Phaseolus coccineus* L.) which is a seed pulse crop, belongs to the *Phaseolus* species and family of Leguminosae (Zelalem, 2002). Therefore, the word hephho, as used by the Oromo, which describes the entire black climbing bean with black seed bean. Hephho bean (*Phaseolus coccineus* L.) is one of the principal food and cash crops legumes grown in both the lowland and medium altitude areas of Ethiopia ranging from 700 to 2000m above sea level and this crop has been known in the country since the 16th century (Shimelis and Rakshit, 2005). Hephho is well known in the North-West parts and Wollega Zones (Western and Eastern Wollega Zones of Oromia region and Bannishangul-Gumuz region in Ethiopia (Derese, 2012). Cranberry bean (*Phaseolus coccineus* L.) are low in fat and loaded with nutrients, and one would probably love to eat more of them if they were not also loaded with flatulence-producing enzymes (Denonck, 1991).

The growth habit of hephho is use in determining type-4 (climbing), seeds size is medium speckled /speckled with black color. Intercropping with maize is planted under Fence around household (supportive) (Aremu et al., 2010). Hephho seed usually reach a harvestable stage within five to six months from planting depending on the environment, the plant dies after the seeds have matured (Tadese and Bekele, 2003). Although relevant literature on chemical compositions of processed *P. coccineus* is not widely available, recent studies have shown that the mature seeds are of good source of essential minerals and amino acids (Aremu et al., 2005, 2008). It has not gained widespread industrial, economic and nutritional importance because its acceptability and utilization have been limited. Black climbing (Hephho) bean is an excellent source of vegetable protein, starch, soluble and insoluble fiber, vitamins (especially B group) and elements (particularly potassium, iron, zinc, magnesium, phosphorus

and manganese).

Results of these contents black climbing is consumed in different forms; such as 'Wett', which is prepared from the bean (both dehulled and undehulled) with addition of salts, pepper and butter and 'Nifero', that is prepared from the hephho bean with addition of maize. Form these consumption forms of "Hephho" or black climbing bean which is obtained directly or indirectly through heating operation of processing methods such as cooking and dehulling, it can effectively reduce the antinutritional factors and improve the nutritional value of the beans that are applied on it. The industrial and nutritional potential of this crop is unknown to the host communities. Therefore, this study was aimed to evaluate the effect of different processing techniques on proximate and elemental compositions of black climbing bean "hephho" (*Phaseolus coccineus* L.); with a view of providing preliminary information towards effective utilization of this legume in various food dishes, to fight protein energy malnutrition.

MATERIALS AND METHODS

Collection of Sample

For the purpose of this study, mature seeds of Hephho beans (*Phaseolus coccineus* L.) were collected randomly from 18 selected households (1 kilogram per house hold) of study site (Bandira, Kubena Hambelta and Horo Hambelta kebeles), Ethiopia. The seeds were thoroughly cleaned and sorted to remove stones and bad ones. The samples were packed in polyethylene bags, kept in an ice-box (to prevent moisture loss), and transported to Food Technology and Process Engineering laboratory of Wollega University and Ethiopian Health and Nutrition Research Institute (EHNRI), Ethiopia, within the same day.

Preparation of processed hephho bean seeds

The experiment was conducted on raw, dehulled and undehulled hephho bean seed. The processing techniques employed were traditional and pressure cooking, while raw sample was served as control.

Cooked seeds

Traditional cooking

The seeds were soaked in water in the ratio (1:10) (wt/vol) and was taken for cooking. The ordinary cooking was done by using local cooking pot for 1 and 2 h, respectively (dehulled and undehulled hephho bean) until they became soft when felt between the fingers following the method justified by the local women. The cooking water was drained off and the seeds were sun dried for two days and ground into fine powder by using an electric mill (NIMA-8300 Burman, Germany) until to pass through 0.425 mm sieve mesh size. Samples were preserved in air-tight bottles in the refrigerator for analysis.

Pressure cooking

The dehulled and undehulled seeds of Hephho were pressure cooked in house hold pressure cooker at 101.31 Kpa (15 psi

Table 1. Proximate composition (g /100 g dry weight) of raw and processed Hephho bean seeds (*Phaseolus coccineus* L.).

Treatment	Moisture	protein	fat	fibre	CHO	energy	Ash
Raw	7.14±0.03 ^a	24.63±0.23 ^b	0.90±0.03 ^{ba}	4.63±0.02 ^a	65.85±0.36 ^{bc}	369.89±0.26 ^{cb}	3.99±0.09 ^b
DTC	7.13±0.03 ^a (-0.1%)	28.16±0.04 ^a (+14%)	0.83±0.04 ^b (-8%)	4.11±0.02 ^{dc} (-11%)	64.29±1.43 ^d (-2%)	378.24±12.78 ^a (+2%)	2.61±0.07 ^d (-35%)
UTC	7.37±0.03 ^a (+3%)	24.67±0.24 ^b (+0.16%)	0.92±0.00 ^a (+2%)	4.06±0.07 ^d (-12%)	67.22±0.23 ^a (+2%)	375.82±0.12 ^a (+2%)	3.14±0.31 ^c (-21%)
DPC	8.67±0.13 ^a (+21%)	24.68±0.10 ^b (+0.20%)	0.97±0.10 ^a (+8%)	4.13±0.22 ^c (-11%)	65.12±0.17 ^{dc} (-1%)	367.87±0.45 ^c (-1%)	4.31±0.04 ^a (+8%)
UPC	8.24±0.03 ^a (+15%)	24.46±0.42 ^b (-0.69%)	0.91±0.02 ^{ba} (+1%)	4.44±0.84 ^b (-4%)	66.57±0.04 ^{ba} (+1%)	372.23±0.24 ^b (+1%)	3.99±0.20 ^b (0%)

Each value represents the mean ±standard deviation of three triplicate determinations. Carbohydrate % calculated as the (100-total of other components). Energy % calculated kJ/100g (protein ×17+fat ×37 + CHO ×17). NB: DTC stands for De-hulled Traditionally Cooked, UTC for Undehulled Traditionally Cooked, DPC for Dehulled Pressure Cooked, and UPC for Undehulled Pressure Cooked." "(+) and (-) indicate increased and decreased from raw mean" Means in the same column with different superscript letters are significantly (p <0.05) different.

pressure), 121°C in tap water (1:10, w/v) for 14 min. The cooking water was drained off and the seeds were sun dried for two days, ground into fine powder by using an electric mill (NIMA-8300 Burman, Germany) until it pass through 0.425 mm sieve mesh size. Samples were preserved in air-tight bottles in the refrigerator for analysis.

Experimental site

Sample preparation and moisture content determination were conducted in the laboratory of Wollega University; Food Technology and Process Engineering Department, but the other Nutritional composition both proximate and mineral analysis was carried out in Ethiopian Health and Nutrition Research institute laboratory. All determinations were carried out in triplicate.

Procedure of analysis

Proximate analysis

The moisture, ash, crude fat, crude fibre, crude protein (N×6.25) and carbohydrate (by difference) were determined in accordance with methods of the Association of, Official Analytical Chemists (2000). All proximate analyses of the legume seeds were carried out in triplicate and reported in percent. All chemicals were of Analytical grade.

Mineral analysis

Phosphorus was determined by Vanadomolybdate colorimetric method (AACC, 2000). Calcium, iron and zinc were determined by Atomic Absorption Spectrophotometer (Buck Scientific's 210VGP, USA). All determinations were done in triplicate and the minerals were reported in mg 100 g⁻¹ sample.

Statistical Analysis

The results obtained from the various analyses were subjected to

Analysis of Variance (ANOVA) using Statistical Package for Social Sciences (SPSS) version 16.0 (SPSS Inc., Chicago IL, USA).

RESULTS AND DISCUSSIONS

Table 1 shows the proximate composition of raw and processed Hephho bean seeds. The moisture, crude protein, crude fat, crude fiber, carbohydrates, and ash values were reported in percentage of dry weight.

Moisture

In all treatment, moisture content was increased except in DTC (Table 1), non-significant differences (P<0.05). The moisture content hephho (black climbing) bean of pressure cooked (PC) and traditional cooking (TC) sample has shown increment from 3.22 to 21.43% (Table 1). These findings were in agreed with the report of Audu and Aremu, (2011), that the moisture content of red kidney bean has increased (33.33%). Generally, the different processing techniques employed in this study increased the moisture content in this order: DTC < UTC < UPC < DPC.

The increased moisture content might be due to water absorption by fibers and other natural chemical components during heat treatment (Ejigui et al., 2005; Mubarak, 2005 and Mittal et al., 2012).

Crude Protein

Crude protein value of 24.63% of raw Hephho bean seed (Table 1) is comparable to some commonly beans (Fasoyiro et al., 2006) (Table 2) though lower as compare

Table 2. Proximate compositions (g/100g) of some under-utilized grain legumes seed* and black climbing (Hepho) bean**.

Legumes	Protein	Fat	Carbohydrate	Ash	Fibre
Hepho bean	24.63	0.90	65.85	3.99	4.63
Common bean	20-27	1-2	60-65	4-5	4-5
Lima bean	19-25	2-3	50-51	4-6	1-2
Winged bean	30-40	15-20	35-45	6-7	3-5
Cowpea	22-26	1-2	50-60	3-4	3-4
Soybean	33-41	17-18	25.30	4-6	4-5
Hyacinth bean	24-28	1-2	65-70	7-9	4-5
Pigeon pea	17-26	1.9-2.8	48-49	3-4	4-6

* Source: Fasoyiro et al. (2006). ** Laboratory analysis of black climbing (hepho) bean.

to the results of selected beans such as winged bean 30 to 40%, soybean 37 to 41% and pigeon pea 28 to 29%. The mean crude protein content for dehulled traditionally cooked (DTC) was significantly ($p < 0.05$) higher, but there was non-significant difference ($p < 0.05$) in the crude protein content for UTC, PC and UPC as compared to the raw seeds. The different processing techniques enhanced crude protein content of Hepho bean in this order: UTC < DPC < DTC (Table 1). UTC increased the crude protein content of the dehulled *P. coccineus* seed by 14%.

Crude Fat

The UTC and UPC seeds increased in crude fat content (Table 1). This result was in agreement with Akajayeju and Ajayi (2011), that cooking enhanced crude fat in African oil bean. DTC decreased the crude fat content by 7.78%. The reduction in crude fat content in the cooked seeds may be due to leaching, while reduction in sprouted seed may be due to metabolic activity taking place in the seeds (Kylan and McCready, 1975).

Crude Fiber

Crude fibre content of 4.63 % of raw Hepho bean seed is within the expected values of most beans (Table 2). The mean crude fiber content of DTC, UTC, DPC and UPC hepho bean seeds were significantly ($p > 0.05$) different when compared with crude fiber content of raw and UPC hepho bean seed.

Similarly, the DPC has significant ($p < 0.05$) different in compared to DTC and UTC. The mean crude fiber content of UPC has non-significant ($p > 0.05$) different in compared to DPC mean fiber content of hepho bean seeds (Abiodun and Adepeju, 2011). In DTC, UTC and UPC processing techniques, the crude fibre content decreased and increased in DPC (Mubarak, 2005). This

suggests that the sample would provide moderate dietary fibre in the diet. Fibre helps to maintain the health of the gastrointestinal tract, but in excess may bind trace elements, leading to deficiencies of iron and zinc (Siddhuraju et al., 1996).

Carbohydrate

The mean total carbohydrate content of DTC, UTC, DPC and UPC Hepho bean seeds were presented in Table 1. Changes in total carbohydrate values of both raw and processed hepho bean seeds reflect change in observed values of other proximate composition. The values of mean total carbohydrate for traditionally cooked has significant ($p < 0.05$) different, when compared with raw hepho bean seeds, while, pressure cooked samples showed non-significant ($p < 0.05$) difference in comparison with raw sample (Table 1). The result has shown that Hepho bean seed contained higher amount of carbohydrate as other dry beans and this indicate that, hepho bean seeds could be a good source of carbohydrate energy.

The UTC and UPC techniques increased the total carbohydrate content in dehulled seeds while decreased the total carbohydrate content in dehulled seeds (Table 1). This was in agreement with Mugendi et al. (2010), which reported the total carbohydrate content of Jack bean was increased by 24.69% by pressure cooking and by 19.38% by convectional cooking. Carbohydrate was calculated by the difference accounted for 65.85% in the raw Hepho bean seed. The carbohydrate content suggests that the seed could be a good supplement to scarce cereal grains as sources of energy and feed formulations. The carbohydrate value was compares with the range value of 60 to 65% of common bean and cowpea as reported by Fasoyiro et al. (2006) (Table 2). The value is however higher than those of soya bean (26.3%) as reported by Temple et al. (1991), cranberry bean (31.5%), red specks coat and

Table 3. Mean mineral composition (mg/100g db) of raw and processed Hephobean seeds (*Phaseolus coccineus* L.).

Treatment	Calcium	Phosphorous	Iron	Zinc
Raw	145.21 ± 0.01 ^a	342.27 ± 0.02 ^a	8.20 ± 0.01 ^a	1.70 ± 0.97 ^a
DTC	110.16 ± 0.02 ^b (-24.14%)	241.91 ± 0.01 ^b (-29.32%)	7.84 ± 0.01 ^b (-4.39%)	1.40 ± 0.01 ^b (-17.65%)
UTC	122.29 ± 0.02 ^c (-15.78%)	250.99 ± 1.41 ^c (-26.66%)	8.01 ± 0.01 ^a (-0.06%)	1.35 ± 0.02 ^c (-20.58%)
DPC	137.82 ± 0.39 ^d (-5.08%)	279.23 ± 0.44 ^d (-18.41%)	8.19 ± 0.30 ^a (-0.12%)	1.54 ± 0.08 ^d (-9.41%)
UPC	139.73 ± 0.09 ^e (-3.77%)	297.05 ± 0.05 ^e (-13.21%)	8.10 ± 0.05 ^a (-1.22%)	1.60 ± 0.32 ^e (-5.88%)

"Mean not followed by the same superscript letters in the same column are significantly different (P<0.05). NB: DTC stands for De-hulled Traditionally Cooked, UTC for Undehulled Traditionally Cooked, DPC for Dehulled Pressure Cooked, and UPC for Undehulled Pressure Cooked." "(+) and (-) indicate increased and decreased from raw mean".

white coat scarlet runner bean, 31.4 and 30.1%, respectively (Aremu et al., 2006b) but lower to those reported for kerstings groundnut (77.3%), moderate brown coat cowpea (82.9%) and small white coat cowpea (77.2%) (Aremu et al., 2006a).

Energy

The energy value (KJ / 100 g) was 367.87 in DPC and 378.24 in DTC sample. Changes in energy values of both raw and processed seeds of Hephobean seed reflect changes in the observed values of other proximate composition (Table 1).

Total ash

The ash content of 3.99 % (Table 1) observed is moderate but higher than those of the wild jack bean (3.0%) (Vidivel and Janardhanan, 2001), melon seeds (3.3%) (Omafuvbe et al., 2004) and *Vigna ublobata* (3.2%) (Khalil and Khan, 1995), castor seeds (*Ricinus communis*) (3.2%) (Onyeike and Acheru, 2002). Since the sample contained fairly high ash content, it may indicate that the legume could provide essential valuable and useful minerals needed for good body development since soya bean which occupies a unique position among leguminous crops has an average ash content of 4.2% (Temple et al., 1991). DTC, UTC, DPC and UPC samples were presented in (Table 1). All the processing techniques employed in this study with exception of DPC decreased the total ash content in the order of DTC

= UTC > UPC (Table 1). However, the DPC increased the mean total ash content. In addition to the effect of process, the black climbing bean (Hephobean) had high proximate composition in compared to other well-known legumes. This indicates the hephobean which had high nutritional value as the other known common beans (Table 2).

The mineral profile of Hephobean seeds is presented in Table 3. The most abundant mineral in the raw seed was phosphorus (342.27 mg/100g), calcium (145.21 mg/100g), while the least concentrated ones is zinc (1.70 mg/100g). Processing techniques significantly affected the contents of tested minerals in Hephobean seed (P<0.05). All the tested processing techniques reduced calcium, phosphorus, iron and zinc.

Calcium

The different processing techniques employed in this study significantly (P < 0.05) reduced the mean calcium content of hephobean seed as compared to the raw seed. This result also showed significance (p < 0.05) difference in the mean calcium content of hephobean among each processing methods under the study (Table 3). The mean calcium content of raw hephobean seeds was found to be 145.21 mg/100g. This was lower than the report of Ejigui et al. (2005), which state that calcium content of red kidney bean is 179.12 mg/100g but, higher than calcium content of mung bean which was found to be 84.00 mg/100g. The mean Calcium content of DTC, UTC, DPC and UPC were presented in Table 3. All the different processing methods in this study decreased the

calcium content of hepho in this order DPC>UTC>DTC>UPC (Table 3). This observation was in agreement with the report of Abiodun and Adepeju, (2011) that cooking in boiling water caused a great loss of Ca (0.75%) content in Cowpea, Eijigui et al. (2005) also reported that there was a great loss mineral from red kidney bean by cooking and dehulling. The loss of divalent metals was due to their binding to protein and also to the formation of a phytate-cation protein and leaching of the mineral during treatment (Mittal et al., 2012).

Phosphorus

The mean phosphorus content of hepho bean was significantly ($p < 0.05$) reduced in all processing methods employed in this study when compared to raw hepho bean seeds. Significances ($p < 0.05$) were also observed in the mean phosphorus values within each processing techniques (Table 3). The mean phosphorus content of raw hepho was 342.27 mg/100g and this value was lower than the finding of Mubarak (2005), in which the mean phosphorus content of raw mung bean was 391 mg/100g, but this was higher than phosphorus content of underutilized legumes as reported by Ekanayake et al. (2012) that was 291.24 mg/100g. However, the value was comparable with that of chickpea 243.00 mg/100g as reported by Mittal et al. (2012). The mean Phosphorus content of DTC, UTC, DPC and UPC of hepho seeds were shown in (Table 3). All the processing methods under this study decreased the phosphorus content. DTC, UTC, DPC and UPC samples decreased as shown in (Table 3). The finding was analogous with the report of Abiodun and Adepeju (2011) which state that cooking in boiling water caused reduction of phosphorus by (0.70%) in Cowpea and also have similar reduction which observed red kidney bean (Eijigui et al., 2005). The loss of element was due to their binding to protein and also the formation of a phytate-cation protein and leaching of the mineral during treatment (Mittal et al., 2012).

The calcium and phosphorus levels are reasonably distributed in the sample. Phosphorus is always found with calcium in the body both contributing to the blood formation and supportive structure of the body (Ogunlade et al., 2005). Low Ca:P ratio (less than 0.5) facilitates decalcination of calcium in the bone leading to low calcium level in the bones while Ca/P ratio above two helps to increase the absorption of calcium in the small intestine (Nieman et al., 1992). The values of Ca/P ratio in the present study are far lower than 0.5.

Iron

DTC, UTC, DPC and UPC hepho seed mean iron content significantly ($p < 0.05$) varied in hepho iron content,

significantly ($p < 0.05$) differences were observed in mean iron content of hepho bean seeds among each tested processing techniques. The UTC and UPC techniques decreased the iron content of hepho (Abiodun and Adepeju, 2011; Mubarak, 2005; Mittal et al., 2012). In Duhan et al. (2000), there was a reduction by cooking which could be as a result of the effect of heat changing in the insoluble chemical species of some trace elements into soluble ones that were extracted in cooking water.

Zinc

All processing techniques significantly ($p < 0.05$) reduced zinc content. However, non-significantly ($p > 0.05$) differences were observed in mean iron content of hepho bean seeds among each tested processing techniques (Table 3). The mean zinc content of raw hepho was 1.70 mg/100g (Table 3) and the result was higher than the report of Ekanayake et al. (2012) which states that, zinc content of underutilized legumes was found to be 1.37 mg/100g; however, this is lower than that of mucuna bean 4.1 mg/100g as reported by Mugendi et al. (2010). The mean Zinc content of raw, DTC, UTC, DPC and UPC, were, 1.40, 1.35, 1.54 and 1.60, respectively (Table 3). UTC and UPC samples decreased the zinc content hepho in the order of UTC>DTC>DPC>UPC.

This might be due to the removal of hull that justifies the presence of elements in hull of hepho. The findings were in agreement with the previous Abiodun and Adepeju (2011) which states that, dehulled cooking reduced the zinc content of Barbara nut by 54.52%.

Conclusion

The traditional cooking techniques caused an increase in the crude protein contents of Hephho bean seeds than pressure cooking methods which reduced crude fiber and fat contents of Hephho dehulled and unde-hulled cooked. The total ash generally decreased in all tested treatments. In contrasts, the all tested treatments have no effect on carbohydrate. However, the study indicated that there was a loss of minerals (calcium, phosphorus, iron and zinc) during all tested treatments. All the processing methods employed in this study had shown a great loss of phosphorus and calcium as they bind to anti-nutrients and cooked after de-hulled which mostly affect iron content of Hephho bean.

In general, in one or other way; the processing treatments had significant effect on chemical composition and mineral profile of Hephho bean seeds. The study showed that black climbing bean seeds "hepho" have high protein (24.63%) and carbohydrate (65.85%) content with nutritionally valuable minerals as comparable with known protein rich plant foods such as soybean and groundnut. Hence, the black climbing bean seeds

“hepho” is an alternative and cheaper source of protein which contributes to solve the problem of malnutrition; a prevalent problem in developing world especially, Ethiopia.

ACKNOWLEDGMENT

The author would like to thank Mr. Dirriba Chewaka, Wollega University and Ethiopian Health and Nutrition Research Institute (EHNRI) for the collaborative and advice that made this study possible.

CONFLICT OF INTERESTS

The author has not declared any conflict of interests.

REFERENCES

- AACC (American Association of Cereal Chemists). (2000). Approve methods of the American association of cereal chemists. American Association of Cereal Chemists, 10thed. St. Paul, MN.
- Abiodun AO, Adeoju AB (2011). Effect of processing on the chemical, pasting and composition of Banbara Nut (*Vigna subterranea* L. verdc) flours. *Adv. J. Food Sci.* 3:224-227.
- Akajayeju O, Ajayi OF (2011). Effects of dehulling on functional and sensory properties of flours from black bean (*Phaseolus vulgaris*). *J. Food Nutr. Sci.* 2:344-349.
- Akporhonor II, Egwaikhide PA, Eguavoen IO (2006). Effect of sprouting on *in vitro* digestibility of some locally consumed leguminous seeds. *J. Appl. Sci. Environ. Manag.* 10(3):55-58.
- AOAC (Association of Official Analytical Chemists) (2000). Official methods of analysis. 17thed. AOAC, Washington, DC.
- Aremu MO, Olaofe O, Basu SK, Abdulazeez G, Acharya SN (2010). Processed cranberry bean (*Phaseolus coccineus* L.) seed flour for the African diet. *Can. J. Plant Sci.* 90:719-728.
- Aremu MO, Olayioye YE, Ikokoh PP (2009). Effects of processing on the nutritional quality of kersting's groundnut (*Kerstingiella geocarpa* L.) seed flours. *J. Chem. Soc. Nig.* 34(2):140-149.
- Aremu MO, Olaofe O, Orjioko CA (2008). Proximate and amino acid composition of bambara groundnut (*Vigna subterranea*), kersting's groundnut (*Kerstingiella geocarpa*) and scarlet runner bean (*Phaseolus coccineus*) protein concentrates. *La Rivista Italiana Delle Sostanze Grasse*, 85:128-134.
- Aremu MO, Olaofe O, Akintayo ET (2006a). A comparative study on the chemical and amino acid composition of some Nigerian underutilized legume flours. *Pak. J. Nutr.* 5(1):34-38.
- Aremu MO, Olaofe O, Akintayo ET (2006b). Compositional evaluation of cowpea (*Vigna unguiculata*) and scarlet runner bean (*Phaseolus coccineus*) varieties grown in Nigeria. *J. Food Agric. Environ.* 4(2):39-43.
- Aremu MO, Olaofe O, Akintayo ET (2005). Nutritional qualities assessment of the presence of hull in some Nigeria under-utilized legume seeds. *Bull. Pure Appl. Sci.* 24:47-52.
- Audu SS, Aremu MO (2011). Nutritional composition of raw and processed pinto bean (*Phaseolus vulgaris* L.) grown in Plateau State, Nigeria. *J. Food Agric. Environ.* 9(3&4):72-80.
- Bultosa G (2007). Common grain nutrient composition and laboratory methods in grain composition analysis. Haramaya University, Ethiopia.
- Davis KR (1981). Effects of processing on composition and Tetrahymena relative nutritive value of green and yellow peas lentils and white peas and beans. *Cereals Chem.* 58:454-560.
- Denonck DG (1991). Systematic and morphology. In: A. Van Schoonhoven and O. Voysest, eds. Common beans research for crop improvement. CAB International, Wallingford, UK. pp. 55-118
- Derese M (2012). Caning quality evaluation of Haricot Bean (*P. vulgaris* L.) varieties grown in Ethiopia. M.Sc. Thesis. Chemical Engineering Department, Addis Ababa University, Addis Ababa, Ethiopia.
- Duhan A, Khetarpaul N, Bishnoi S (2000). Changes in phytates and HCl extractability of calcium, phosphorus and iron of soaked, dehulled, cooked and sprouted pigeon pea cultivar (UPAS-120). *Plant Food Human Nutr.* 57:275-284
- Ejigui J, Lsavoie L, Marin J, Desrosiers T (2005). Influence of traditional processing methods on the nutritional composition and anti-nutritional factors of red peanuts (*Arachis hypogea*) and small red kidney beans (*Phaseolus vulgaris*). *J. BioSci.* 5:597-605.
- Kylen M, McCready RM (1975). Nutrient in seeds and sprouts of alfalfa lentils, mug beans and soybeans. *J. Food Sci.* 40:1008-1013
- Khalil IA, Khan S (1995). Protein quality of Asian beans and their wild progenitor, *Vigna sublobata* (Roxb). *Food Chem.* 52:327-330.
- Liener IE (1994). Implications of anti-nutritional components in soya bean foods. *Crit. Rev. Food Sci. Nutr.* 34:31-67
- Mittal R, Nagi H, Sharma P, Sharma S (2012). Effect of processing on chemical composition and anti-nutritional factors in chickpea flour. *J. Sci.* 2:180-186.
- Mubarak AE (2005). Nutritional composition and anti-nutritional factors of mung bean seeds (*Phaseolus aureus*) as affected by some home traditional processes. *Food Chem.* 89:489-495.
- Mugendi JB, Njagi MNE, Kuria NE, Mwasaru MA, Mureifhi JG, Apostolides Z (2010). Effect of processing technique on Mucuna bean. *Afr. J. Food Sci.* 4:156-166.
- Nieman DC, Butterworth DE, Nieman CN (1992). Nutrition. Wm. C. Brown Publishers, Dubuque, I. A. P 540.
- Nowacki K (1980). Heat stable anti-nutritional factors in leguminous plants. In: Advances in Legume Science, Summeifield RJ, Buntong AH (Eds.). Royal Botanical Gardens, Kew, pp. 171-177.
- Ogunlade I, Olaofe O, Fadare T (2005). Chemical composition, amino acid and functional properties of *Leucaena leucocephala* seeds flour. *Nig. J. Appl. Sci.* 21:7-12.
- Omafuvbe BO, Falade OS, Osuntogun BA, Adewusi SRA (2004). Chemical and biochemical changes in African locust bean (*Parkia biglibosa*) and melon (*Citrullus vulgaris*) seeds during fermentation to condiments. *Pak. J. Nutr.* 3(3):140-145.
- Onyeike EN, Acheru GN (2002). Chemical composition of selected Nigerian oil seeds and physicochemical properties of the oil extracts. *Food Chem.* 77:431-437.
- Onyeike EN, Olungwe T, Uwakwe AA (1995). Effect of heat treatment and defatting on the proximate composition of some Nigerian local soup thickeners. *Food Chem.* 53:173-175.
- Oshodi AA, Esuoso KO, Akintayo ET (1998). Proximate and amino acid composition of some under-utilized Nigerian legumes flour and protein concentrates. *Riv. Ital. Sostanze Grasse.* 75:409-412.
- Siddhuraju P, Vijayakumari K, Janardhanan K (1996). Chemical composition and protein quality of the little-known legume, velvet bean (*Mucuna pruriens* L.). *J. Agric. Food Chem.* 44: 2636-2641.
- Shimelis EA, Rakshit SK (2005). Proximate composition and physico-chemical properties of improved dry bean (*Phaseolus vulgaris* L.) varieties grown in Ethiopia. *Swiss Soc. Food Sci. Technol.* 38:331-338.
- Fasoyiro FB, Ajibade SR, Omole AJ, Adeniyon ON, Farinde EO (2006). Proximate minerals and anti-nutritional factors of some underutilized legumes in south-western Nigeria. *Nutr. Food Sci.* 36:18-23
- Tadese W, Bekele S (2003). Variation and association of morphological and biochemical characters in grass pea (*Lathyrus sativus* L.). *Euphytical Ethiopia* 130: 315-324
- Temple VJ, Odewumi L, Joseph K (1991). Soybeans and soybeans based diets. Proceedings of the 3rd Regional Workshop on Rural Development, July 31-August 2, 1991, Jos, pp.45-50
- Vidivel V, Janardhanan K (2001). Nutritional and anti-nutritional attributes of underutilized legume, *Cassia floribunda* Car. *Food Chem.* 73:209-215.
- Vidal-Valverde C, Frias J, Valverde S (1993). Changes in the carbohydrate composition of legumes after soaking and cooking. *J. Am. Diet Assoc.* 93:547-550.
- Yagoub AA, Mohammed MA, Baker AAA (2008). Effect of soaking, sprouting and cooking on chemical composition bioavailability of minerals and *in vitro* protein digestibility of Roselle (*Hibiscus*

sabdariffa L.) seed. Pak. J. Nutri.7: 50-56.
Zelalem F (2002). Report on informal survey of major beans disease in Metekel Zone, Pawe Agricultural Research Center. pp. 14-19 (unpublished), Ethiopia.



African Journal of Food Science

Related Journals Published by Academic Journals

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

academicJournals