OPEN ACCESS

African Journal of Food Science

August 2020
ISSN 1996-0794
DOI: 10.5897/AJFS
www.academicjournals.org

ACADEMIC JOURNALS
expand your knowledge

About AJFS

The African Journal of Food Science (AJFS) is a peer reviewed open access journal. The journal commenced publication in September 2007. The African Journal of Food Science welcomes for publication papers that investigate and review perspectives on food engineering, handling, processing, storage, preservation, contamination and packaging; sensory analysis. Other aspects covered include food safety, microbiology, nutraceuticals, chemistry, technology and nanotechnology in addition to quality and nutritional evaluation and genetic variation of food.

Indexing

Abstracts on Hygiene and Communicable Diseases, AgBiotechNet, Agricultural Economics Database, Agricultural Engineering Abstracts, Agroforestry Abstracts, Animal Breeding Abstracts, Animal Production Database, Animal Science, Biofuels Abstracts, Botanical Pesticides, CAB Abstracts, CABI's Global Health Database, Chemical Abstracts (CAS Source Index), CNKI Scholar Crop Physiology Abstracts, Crop Science Database, Dairy Science Abstracts, Environmental Impact Environmental Science Database, Field Crop Abstracts, Forest Science, Google Scholar, Grasslands and Forage Abstracts, Horticultural Science, Horticultural Science Abstracts, Irrigation and Drainage Abstracts, Maize Abstracts, Microsoft Academic, Nutrition Abstracts and Reviews Series A: Human and Experimental, Nutrition Abstracts and Reviews Series B: Livestock Feeds and Feeding, Nutrition and Food Sciences, Ornamental Horticulture, Parasitology Database, Plant Breeding Abstracts, Plant Genetic Resources Abstracts, Plant Genetics and Breeding Database Plant Growth Regulator Abstracts, Plant Protection Database, Potato Abstracts, Poultry Abstracts Rice Abstracts, Rural Development Abstracts, Seed Abstracts, Soil Science Database, Soils and Fertilizers Abstracts, Soybean Abstracts, Sugar Industry Abstracts, Tropical Diseases Bulletin Veterinary Bulletin, Veterinary Science Database, VetMed Resource, Weed Abstracts Wheat, Barley and Triticale Abstracts, World Agricultural Economics and Rural Sociology Abstracts

Open Access Policy

Open Access is a publication model that enables the dissemination of research articles to the global community without restriction through the internet. All articles published under open access can be accessed by anyone with internet connection.

The African Journal of Food Science is an Open Access journal. Abstracts and full texts of all articles published in this journal are freely accessible to everyone immediately after publication without any form of restriction.

Article License

All articles published by African Journal of Food Science are licensed under the Creative Commons Attribution 4.0 International License. This permits anyone to copy, redistribute, remix, transmit and adapt the work provided the original work and source is appropriately cited. Citation should include the article DOI. The article license is displayed on the abstract page the following statement:

This article is published under the terms of the Creative Commons Attribution License 4.0
Please refer to https://creativecommons.org/licenses/by/4.0/legalcode for details about Creative Commons Attribution License 4.0

Article Copyright

When an article is published by in the African Journal of Food Science, the author(s) of the article retain the copyright of article. Author(s) may republish the article as part of a book or other materials. When reusing a published article, author(s) should; Cite the original source of the publication when reusing the article. i.e. cite that the article was originally published in the African Journal of Food Science. Include the article DOI Accept that the article remains published by the African Journal of Food Science (except in occasion of a retraction of the article). The article is licensed under the Creative Commons Attribution 4.0 International License.

A copyright statement is stated in the abstract page of each article. The following statement is an example of a copyright statement on an abstract page.
Copyright ©2016 Author(s) retains the copyright of this article.

Self-Archiving Policy

The African Journal of Food Science is a RoMEO green journal. This permits authors to archive any version of their article they find most suitable, including the published version on their institutional repository and any other suitable website.
Please see http://www.sherpa.ac.uk/romeo/search.php?issn=1684-5315

Digital Archiving Policy

The African Journal of Food Science is committed to the long-term preservation of its content. All articles published by the journal are preserved by Portico. In addition, the journal encourages authors to archive the published version of their articles on their institutional repositories and as well as other appropriate websites.

https://www.portico.org/publishers/ajournals/

Metadata Harvesting

The African Journal of Food Science encourages metadata harvesting of all its content. The journal fully supports and implement the OAI version 2.0, which comes in a standard XML format. See Harvesting Parameter

Memberships and Standards

Academic Journals strongly supports the Open Access initiative. Abstracts and full texts of all articles published by Academic Journals are freely accessible to everyone immediately after publication.

All articles published by Academic Journals are licensed under the Creative Commons Attribution 4.0 International License (CC BY 4.0). This permits anyone to copy, redistribute, remix, transmit and adapt the work provided the original work and source is appropriately cited.

Crossref is an association of scholarly publishers that developed Digital Object Identification (DOI) system for the unique identification published materials. Academic Journals is a member of Crossref and uses the DOI system. All articles published by Academic Journals are issued DOI.

Similarity Check powered by iThenticate is an initiative started by CrossRef to help its members actively engage in efforts to prevent scholarly and professional plagiarism. Academic Journals is a member of Similarity Check.

CrossRef Cited-by Linking (formerly Forward Linking) is a service that allows you to discover how your publications are being cited and to incorporate that information into your online publication platform. Academic Journals is a member of CrossRef Cited-by.

Academic Journals is a member of the International Digital Publishing Forum (IDPF).
The IDPF is the global trade and standards organization dedicated to the development and promotion of electronic publishing and content consumption.

Contact

Editorial Office: ajfs@academicjournals.org

Help Desk: helpdesk@academicjournals.org

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

Submit manuscript online http://ms.academicjournals.org

Academic Journals
73023 Victoria Island, Lagos, Nigeria
ICEA Building, 17th Floor,
Kenyatta Avenue, Nairobi, Kenya.

Editors

Dr. Thaddeus Chukwuemeka Ezeji
Ohio State University and
Ohio State Agricultural and Development
Center (OARDC)
Department of Animal Sciences
USA.

Prof. Kofi E. Aidoo
Department of Biological and Biomedical
Sciences
Glasgow Caledonian University
Glasgow
Scotland.

Dr. Barakat S.M. Mahmoud
Food Safety/Microbiology
Experimental Seafood Processing Laboratory
Costal Research and Extension Centre
Mississippi State University
USA.

Dr. Neela Badrie
Department of Food Production,
Faculty of Science and Agriculture,
University of the West Indies,
Trinidad and Tobago.

Dr. Hu Xiao-Qing
State Key Lab of Food Science and Technology,
Jiangnan University,
China.

Dr. Dominic Agyei
Department of Food Science/Te Tari Pütaiao Kai
University of Otago,
Dunedin,
New Zealand.

Dr. Fook Yee Chye
Faculty of Food Science and Nutrition,
Universiti Malaysia Sabah,
Malaysia.

Dr. Adel Shatta
Department of Food Technology,
Faculty of Agriculture,
Egypt.

Dr. Tendekayi Henry Gadaga
Departmentof Environmental Health Science
University of Swaziland
Swaziland.

Editorial Board Members

Dr. K. Pandima Devi
Department of Biotechnology
Alagappa University
Tamil Nadu
India.

Dr. Ashish Kumar Singh
Dairy Technology Division
National Dairy Research Institute,
Haryana,
India.

Prof. Rui Cruz
Department of Food Engineering
Institute of Engineering
University of Algarve, Faro
Portugal.

Dr. Nicole Roberta Giuggioli,
Department of Agricultural, Forest and Food
Sciences (DISAFA)
University of Turin,
Italy.

Table of Content

Modeling the growth dynamics of Lactobacillus lactis CWBI B-1410: Effect of changes in glucose and nitrogenized matter in the Man Rogosa Sharpe (MRS) culture media 186
Modou DIENG, Ndèye Adiara NDIAYE, Mouhamed NDONG, Ndèye Coumba TOURE/KANE

Performance of a fabricated solar-powered vapour compression cooler in maintaining post-harvest quality of French beans in Kenya
Ogumo, E. O., Kunyanga, C. N., Kimenju, J. and Okoth, M. 192

Evaluation of the nutritional and antioxidant potentialities of Capparis corymbosa
Edwige N. ROAMBA, Crépin I. DIBALA, Marcel D. BENGALY, Mamounata DIAO, Kiessoun KONATE and Mamoudou H. DICKO 201

Assessment of microbial quality status of raw beef around Addis Ababa city, Ethiopia 209
Getenesh Teshome, Zerihun Assefa and Abdi Keba

Vol. 14(7) pp. 186-191, August 2020
DOI: 10.5897/AJFS2020.1960
Article Number: 659BB6464539
ISSN: 1996-0794
Copyright ©2020
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJFS

ACADEMIC JOURNALS
expand your knowledge

African Journal of Food Science

Full Length Research Paper

Modeling the growth dynamics of *Lactobacillus lactis* CWBI B-1410: Effect of changes in glucose and nitrogenized matter in the Man Rogosa Sharpe (MRS) culture media

Modou DIENG[1]*, Ndèye Adiara NDIAYE[2], Mouhamed NDONG[3], Ndèye Coumba TOURE/KANE[4]

[1]Laboratoire d'analyses et Essais, École Supérieure Polytechnique / University Cheikh Anta DIOP University in Dakar, Sénégal.
[2]Laboratoire de Microbiologie, École Supérieure Polytechnique / University Cheikh Anta DIOP in Dakar, Sénégal.
[3]Canadian Rivers Institute, Department of Civil Engineering, University New Brunswick, Fredericton, NB E3B 5E1, Canada.
[4]Faculté de Médecine / University Cheikh Anta DIOP in Dakar, Sénégal.

Received 2 May, 2020; Accepted 21 July, 2020

Food preservation is a big challenge for the industries. The mechanisms of preservation involved microorganisms such as lactic acid bacteria. Modeling the micro-organisms growth is a very useful information to well-understand their behavior and to propose the best conditions for food preservation. In this way, this study was to propose a mathematical model of the dynamical growth of *Lactococcus lactis* CWBI-B1410 which play a main role for the bio-conservation of fish. This study is conducted by varying the concentration of glucose and nitrogenized matter. The results showed that the mathematical model predicted the growth of *L. lactis* CWBI-B1410. The curves that are predicted are exactly those that can be observed experimentally. The results showed that the production of biomass by *L. lactis* CWBI-B1410 is obtained on the MRS medium composed of 15 g of glucose per liter and 40 g of nitrogenous material per liter. From the obtained results, it could be clearly advocated that for two tests with the same amount of nitrogenous matter, it is possible to achieve economies of scale in glucose while maintaining the same biomass performance.

Key words: Mathematical models, lactic bacteria, *Lactococcus lactis* CWBI-B1410, glucose, nitrogenous matter.

INTRODUCTION

Quality control and microbiological safety of fish products are a permanent challenge for fishery industry. They always

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

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

consider fish products degradation due to conservation issues (Fabinyi et al., 2017). Fish conservation is very sensitive to environmental conditions that can increase the risk to undesirable micro-organisms growth under certain conditions and then constitute a threat for public health. This system for food production and distribution in fishery industries has to ensure that undesirable microorganisms growth is controlled. Then, understanding the conditions leading to micro- organisms growth is important (Nikolaev, 2010). Different optimization approaches to determining appropriate levels of metabolic enzymes have been used in a number of purely simulation-based studies for various aspects of microbial metabolism, including the production of serine (Nikolaev, 2010), tryptophan (Chen and Zeng, 2017), and bacteriocin produced by a strain of *Streptococcus gallolyticus* (Abdu et al., 2020). The simultaneous production of serine and tryptophan in *E. coli* has also been considered using a multi-objective optimization strategy (Lee et al., 2010). According to Alfredo et al. (2020), silage technology, with the use of lactic acid bacteria, could help extend the shelf-life of orange residue by up to 10 days by keeping it fresh. In order to describe the behavior of microorganisms in foods and particularly their growth based on key ecological food characteristics, many mathematical models have been proposed in the literature during the recent years (Alfredo et al., 2020). Predictive microbiology based on mathematical models is very useful and it helps to understand microbial hazards in a food chain. It has been showed that it is relatively easy and inexpensive to assess and to validate theoretical models that can be used to predict and to quantify the effects of experimental conditions on microorganism growth (Delhalle et al., 2012). These modeling activities result to link the growth parameters to the environmental factors through mathematical equations (Maoura et al., 2006). Comparing the adjustment of primary models on laboratory data has been the subject of several studies (Pal et al., 2008). Currently, it is not possible to select a particular model to represent the most appropriate bacterial growth. The simplest models can often be sufficient to properly represent the basic parameters of growth. The first growth model in predictive microbiology described by Buchanan (1918) described the exponential phase, but it does not take into account the latent phase or the stationary phase. However, Buchanan's model can be used in a first approach to assess the evolution bacteria population and growth rate. An alternative exponential model has been proposed by Brillet et al. (2016). This exponential model takes into account the reaction time and the deceleration phase. Delhalle et al. (2012) described the exponential growth phase which appeared in the form of a linear portion when the evolution of microbial population is represented by the time. For a given bacterium, the value of this maximum growth rate depended on the characteristics of the medium culture (Kyu et al., 2009). When the microbial concentrations are expressed in semi logarithmic mathematical form the maximum specific growth rate can be determined by calculating (Fahimi, 2012). Senegal is known today for its high level of production of sea products (Diop et al., 2008). However, their fishery industry is not efficient due to the weak conservation methods (Diei-Ouadi, 2005). The insufficient safety and toxic substances affected the organoleptic quality and health products reducing the fish production (Diop et al., 2008). Through their work's, Diop et al. (2008) developed a bio-conservation method for fishery industry by using a strain of *L. lactis* CWBI-B1410 isolated from cereals in Senegal. This important discover is very important for fishery industry to increase their productivity by improving the conservation process. However, the biological characteristic of *L. lactis* CWBI-B1410 expressed into mathematical growth model is still not available in the literature. Then, the main objective of this study was to develop a mathematical model to predict the growth of the strain of lactic acid bacterium *L. lactis* CWBI-B1410 in two components of MRS culture medium, glucose and nitrogenous matter.

METHODOLOGY

This study is conducted in the Analysis and Testing Laboratory at École Supérieure Polytechnique de Dakar (University Cheikh Anta Diop), Senegal.

Materials

Two types of substrates were used: glucose and nitrogenous matter. The nitrogenized matter was provided by the culture medium composed of casein peptone (Sharlau, Spain).

The laboratory equipment's were glassware oven at 30°C, Petri dishes, a spectrophotometer Helios TM Gamma UV-VIS, Thermo Fisher Scientific, (UVD 134502, England).

Preparation of the culture medium

The culture medium used in this work was the MRS agar (Man Rogosa Sharpe) with different concentrations of glucose and nitrogenous matter. The pH of the culture medium was adjusted to 6.6 by adding hydrochloric acid (1 N) in order to obtain an optimal pH for growth of *L. lactis* CWBI-B1410. The medium was stored into tubes slants (10 ml/tube). These tubes containing the medium were put in a autoclave for 15 min at 120°C.

Preparation of the inoculum

The strain used in this study was *L. lactis* CWBI-B1410. It is a strain of the laboratory collection in Belgium (the Bio-industries Wallonia Center of Industrial Biology). It was revived by two subcultures Man Rogosa Sharpe liquid medium and stored at a temperature of 4°C in conservation tubes containing MRS agar substrate.

The re-activation of the strain was done by extracting some

Table 1. Various combinations of concentrations of glucose (G) and nitrogenous matter (N.M.).

Glucose (G) g/l MRS	Nitrogenous matter (N.M.) g/l MRS
15	20
20	20
20	40
15	40

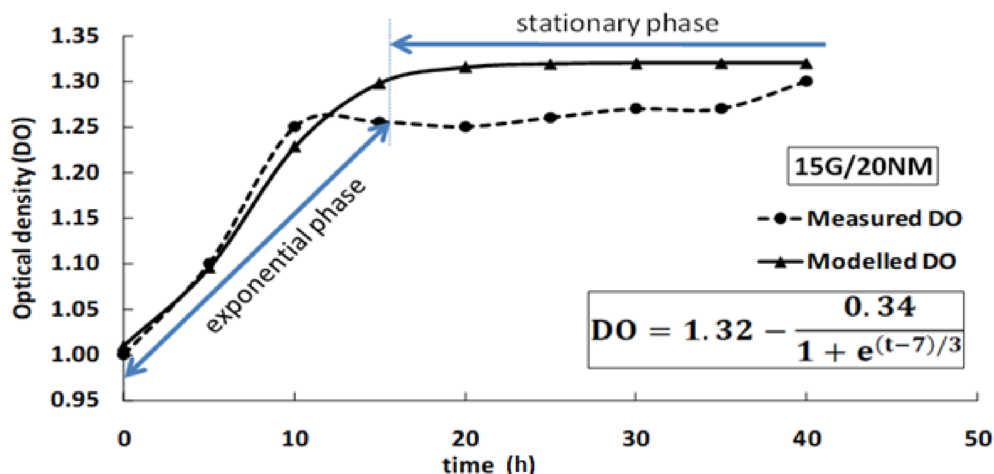


Figure 1. Evolution of biomass as a function of time with 15 g of glucose and 20 g of nitrogenous matter per liter.

colonies from these subcultures and transferred into 10 ml tube containing MRS medium liquid. This tube was then incubated at 30°C during 24 h to get our pre-culture.

After 24 h of incubation, 1 ml of this pre-culture was introduced into an Erlenmeyer flask with a capacity of 250 ml containing 100 ml of MRS liquid medium and incubated at 30°C for 40 hours under constant mixing condition by agitation. The all septical conditions were respected to avoid any external contamination.

Determination methods

The total biomass concentration of *L. lactis* CWBI-B1410 is determined by measuring the optical density (OD) by using a spectrophotometer Helios TM Gamma UV-VIS at 620 nm.

Dieng et al. (2013) showed that the best growth of *L. lactis* CWBI-B1410 are obtained with combinations of different concentrations of glucose and nitrogenized substrate as shown in Table 1.

These associations of glucose (G) and nitrogenous matter (NM) were then tested and mathematical models were proposed by determining the equation for each association. These mathematical models allowed a variation of the biomass according to the quantity of glucose and the nitrogenous matter.

The evolution of the biomass concentration was evaluated by measuring the optical density at 620 nm as a function of time. The data was analyzed with the help of the Excel software. To understand biological phenomena more precisely, the equation parameters have been identified after many simulations in excel to get the best results.

RESULTS

The effect of different combinations of glucose and nitrogenous matter on the growth of *L. lactis* CWBI-B1410 was evaluated and to study if the concentrations of glucose and nitrogenous material vary, number of bacteria varies from one association (Table 1) to another. The biomass measurements performed showed very similar trends for different situations. Figure 1 clearly shows a startup culture at the fifth hour. The four different biomass obtained have almost super imposed curves. It was also noted that the MRS media with a concentration of 40 g/ l crude protein have a higher biomass compared with the media that contain 20 g / l. At the 10th hour, the curves corresponding to associations 15 G / 20 N.M. and 20 G / 20 N.M. had OD_{600nm} close to 1.2 while the curves corresponding to 15 g/40 N.M. and 20 G / 40 N.M., which look better, have an OD_{600nm} of about 1.30 despite the fact that all cultures are seeded with cells in the same physiological state and at the same time. Moreover, from the 10th hour of culture, bacteria *L. lactis* CWBI-B1410 seemed to start the stationary phase for all tests. At the 40th hour, Figure 1 shows that the best growth is presented by the MRS medium 15G/40 N.M. followed by MRS medium 20 G/40 N.M. reaching respectively an

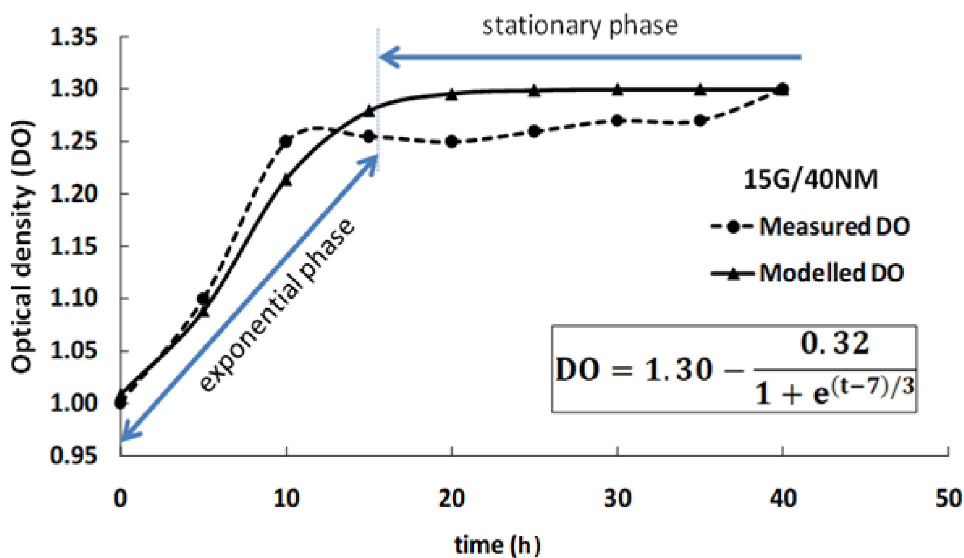


Figure 2. Evolution of biomass as a function of time with 15 g of glucose and 40 g of nitrogenous matter per liter.

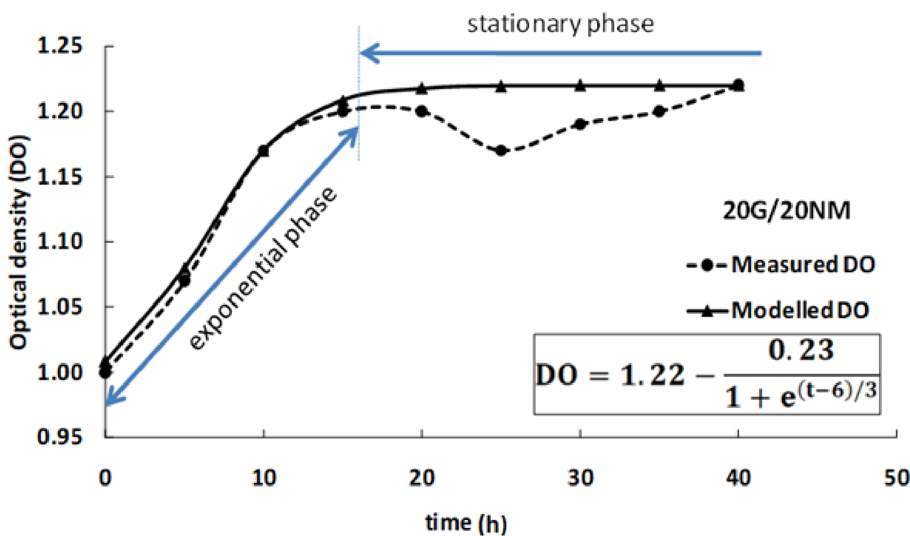


Figure 3. Evolution of biomass as a function of time with 20 g of glucose and 20 g of nitrogenous matter per liter.

OD_{600nm} value of 1.32 and 1.30.

DISCUSSION

These findings are consistent with those of Yaov et al. (2019), which validated a mathematical approach using different experiments with *Escherichia coli*. The approach in this study provided an effective way to predict growth of *L. lactis* CWBI B-1410 by the variation of glucose and nitrogen. The results also shows that for two tests with the same quantity of nitrogenous matter (Figure 1), it is possible to achieve economies of glucose while maintaining the same performance biomass (Figures 2, 3 and 4),. Mataragas et al. (2003) showed that continuous presence of carbon in the MRS medium during culture is necessary but the amount of glucose is not proportional to the amount of biomass in the culture medium. Moreover, increasing the amount of glucose does not necessarily produce a high level of bacteriocin

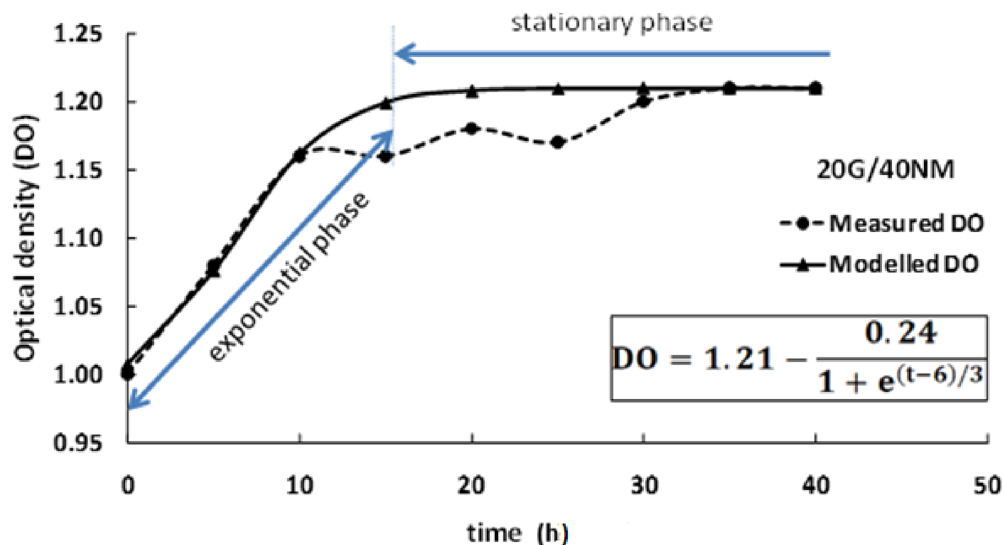


Figure 4. Evolution of biomass as a function of time with 20 g of glucose and 40 g of nitrogenous matter per liter.

(Abbasiliasi et al., 2017). The study showed that the variation in the amount of glucose and nitrogen influenced the growth of *L. lactis*. This is consistent with the results of Thiago et al. (2019), which showed that the biosynthesis of bacteriocins can be influenced by various culture conditions, such as the composition of the medium, pH, temperature and growth kinetics of the microorganisms.

These results are in agreement with the work of Pemmaraju et al. (2016) who showed that *Candida albicans* formed more biofilms in the presence of an important carbon source. Moryl et al. (2013) have been shown that the composition and quantity of the polysaccharide matrix of a biofilm depend on a carbon source such as glucose. According to studies, stressors (salt, oxygen, microbial competition, etc.) can positively influence the production of bacteriocin in lactic acid bacteria grown in MRS broth (Neysens and DE-Vuyst, 2005). The kinetics of growth and the increase in nitrogenous matter present a best fit by a single function called Boltzmann function with the correlation coefficient of $R^2 \geq 0.97$. This value expresses a little error between reality and experimentation. According to the results, it was concluded that there was biomass production by *L. lactis* CWBI-B1410 on the MRS 15 G / 40 N.M.

Conclusion

The main objective of this study was to develop a mathematical model able to predict the evolution of a strain of lactic acid bacterium *Lactococcus lactis* CWBI-B1410 by varying two components of MRS culture medium, glucose and nitrogenous substrate. It was found, according to the type of MRS medium used (standard MRS and modified MRS), from the 1st to 6th hour of the tests showed that the average number of bacteria varies considerably from one medium to another. The application of negative binomial regression showed that the model is globally significant. The model shows a significant statistical difference between the standard MRS medium and the modified MRS medium. This approach has also stimulated the interest of studying a mathematical model for modeling the growth dynamics *L. lactis* CWBI-B1410. To increase the interest of such a model, the effect of pH conditions should be integrated. In future work, the significance of the effect of inoculum size on the length of the lag phase will be also assessed. Finally, for economic reasons, it is important to consider the use of another less expensive source of nitrogenous material such as certain wastes from the food industry as constituents of culture media for the reformulation of the MRS medium in the production of bacteriocins.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abbasiliasi S, Tan JS, Tengku ITA, Bashokouh F, Ramakrishnan NR, Mustafa S, Ariff AB (2017). Fermentation factors influencing the production of bacteriocins by lactic acid bacteria. Royal Society of Chemistry 7:29395-29420.

Abdu A, Arif F, Vikram S, Mathew U (2020). Purification and characterization of nisin produced by a strain of *Streptococcus gallolyticus*. Journal of Medical Microbiology 69:605-616.

Alfredo IGG, Janeth VS, Carolina FG, Mónica CG, Liliana LH, Cristina RDT, German B, Daniel BV, Miguel AAG, Raúl RH, CristÓbal NA (2020). Ensiling as bioprocess for bioconservation of citrus peels. Microbial Services in Restoration Ecology 20:297-314.

Brillet VA, Pilet MF, Courcoux PPH, Leroi F (2016). Optimization of growth and bacteriocin activity of the food bioprotective *Carnobacterium divergens* V41 in an animal origin protein free medium. Frontiers Marine Science 3:128.

Buchanan RE (1918). Life phases in a bacterial culture. Journal of Infectious Diseases 23:109-125.

Chen L, Zeng A (2017). Rational design and metabolic analysis of *Escherichia coli* for effective production of L-tryptophan at high concentration. Applied Microbiology and Biotechnology 101:559-568.

Delhalle L, Daube G, Adolphe Y, Crevecoeur S, Clinquart A (2012). Les modèles de croissance en microbiologie prévisionnelle pour la maitrise de la sécurité des aliments. Biotechnology Agronomy Society Environment 16(3):369-381.

Diei-Ouadi Y (2005). Minced sardinella fillets in fish landing and marketing sites in Senegal. In: FAO fisheries Circular no 999: FIIU/C999 (EN). Roma: FAO.

Dieng M, Mbengue M, Kone PS, Ndiaye K, Tine E, Balde M, Kane NCT (2013). Bioconservation des filets de sole limande frais (*Syacium guineensis*) au Sénégal par utilisation de la souche de *Lactococcus lactis* CWBI-B1410. Revue Africaine de Santé et de Production Animale 11(1):31-36.

Diop MB, Dubois DR, Dortu C, Destain J, Tine E, Thonart P (2008). *In vitro* detection and characterization of bacteriocin-like inhibitory activity of lactic acid bacteria (LAB) isolated from Senegalese traditional food products. African Journal of Microbiology Research 2:206-216.

Fabinyi M, Dressler WH, Pido MD (2017). Fish, trade and food security: Moving beyond ‘availability’ Discourse in Marine Conservation. Human Ecology 45:177-188.

Fahimi N (2012). Study of the interactions between oenological lactic bacteria *Enococcus oeni*. Kinetic analyzes and modeling. Doctoral thesis. University of Toulouse.

Kyu J, Chae MJ, Choi JW, Lee KYK (2009). Effect of different substrates on the performance, bacterial diversity, and bacterial viability in microbial fuel cells. Bioresources Technology 100(14):3518-3525.

Lee FC, Pandu RG, Lee DY (2010). Modeling and optimization of a multiproduct biosynthesis factory for multiple objectives. Metabolic Engineering 12:251-267.

Maoura N, Mbaiguinam M, Gaillardin C, Pourquie J (2006). Suivi technique, analytique et microbiologique de la «bilibili», bière traditionnelle tchadienne». Afrique Science 02(1):69-82.

Mataragas M, Metaxopolous J, Galiotou M, Drosinos EH (2003). Influence of pH and temperature on growth and bacteriocin production by *Leuconostoc mesenteroides* L124 and *Lactobacillus curvatus* L442. Meat Science 64:265-271.

Moryl M, Torzewska A, Jałmużna P, Różalski A (2013). Analysis of *Proteus mirabilis* distribution in multi-species biofilms on urinary catheters and determination of bacteria resistance to antimicrobial agents. Journal Microbiology 62:377-384.

Neysens P, DE Vuyst L (2005). Carbon dioxide stimulates the production of amylovorin L by *Lactobacillus amylovorus* DCE 471, while enhanced aeration causes biphasic kinetics of growth and bacteriocin production. International Journal of Food Microbiology 105:191-202.

Nikolaev EV (2010). The elucidation of metabolic pathways and their improvements using stable optimization of large-scale kinetic models of cellular systems. Metabolic Engineering 12:26-38.

Pal A, Labuza TP, Diez-Gonzalez F (2008). Comparison of primary predictive models to study the growth of *Listeria monocytogenes* at low temperatures in liquid cultures and selection of fastest growing ribotypes in meat and turkey product slurries. Food Microbiology 25(3):460-470.

Pemmaraju SC, Parul A, Pruthi R (2016). Modulation of Candida albicans biofilm by different carbon sources. Mycopathologia 181:341-352.

Thiago S, Adriano B, Sávio LB, Carolina KS, Lisiane FC (2019). Physical and nutritional conditions for optimized production of bacteriocins by lactic acid bacteria – A review. Critical Reviews in Food Science and Nutrition 59(17):2839-2849.

Yaov R, Eynat DG, Maayan B, Kedar K, Uri O, Marcus WF, Tim FC, Judith B, Lilach H (2019). Predicting microbial growth in a mixed culture from growth curve data. Proceedings of the National Academy of Sciences 116(29):14698-14707.

Vol. 14(7) pp. 192-200, August 2020
DOI: 10.5897/AJFS2020.1968
Article Number: 10D5A6164541
ISSN: 1996-0794
Copyright ©2020
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJFS

ACADEMIC JOURNALS
expand your knowledge

African Journal of Food Science

Full Length Research Paper

Performance of a fabricated solar-powered vapour compression cooler in maintaining post-harvest quality of French beans in Kenya

Ogumo, E. O.[1*], Kunyanga, C. N.[1], Kimenju, J.[2] and Okoth, M.[1]

[1]Department of Food Science, Nutrition and Technology, University of Nairobi, Kenya.
[2]Department of Plant Science and Crop Protection, University of Nairobi, Kenya.

Received 15 May, 2020; Accepted 21 July, 2020

The quality and shelf life of French beans can be affected within hours of harvesting if the produce is not cooled after harvest. Solar-powered cooling systems are suitable for use in rural areas that are not connected to the grid. This study aimed at developing a solar powered cooling system to improve the quality of French beans in smallholder farms in Kenya. Freshly harvested French beans were stored under conventional field shed conditions and a solar-powered prototype cooler, after which weight and temperature were measured at intervals of 2 h and later packed in modified, atmosphere packaging bags. The bags were stored for 7 days in a cold room. Accumulated gas levels of oxygen and CO_2 in the packaging bags were measured at the end of the shelf life. Significant differences (P≤0.05) in weight loss between produce stored in the conventional shed and those in the solar cooler prototype were observed. The weight of French beans reduced by 5 and 2.8% after 7 h under a conventional field shed and a fabricated solar cooler respectively. The volume of CO_2 and O_2 released from produce stored in a conventional shed and those in a fabricated solar cooler prototype were significantly different (P≤0.05).

Key words: Conventional cooling method, French beans, temperature, solar cooler, weight loss.

INTRODUCTION

French beans (*Phaseolus vulgaris* L.) require tender care and handling after harvest so as to maintain high quality until the produce gets to the consumer. Speed and efficiency of operations are key during production, transportation and storage (Okello et al., 2007). Deterioration of French beans is very temperature dependent, as an increase in temperature results in increases in metabolic reactions, thereby causing foods to be unfit for consumption due to changes in sensory characteristics and microbial contamination (Rawat, 2015).

Losses after harvest are due to lack of appropriate storage facilities (Kumar and Kalita, 2017). In developing countries, losses as high as 30-40% have been reported due to faults in handling, transportation, storage and marketing have been reported (Atanda et al., 2011).

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

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

Storage losses are mainly due to respiration, evaporation, post-harvest diseases and changes in the chemical composition of the produce (Arah et al., 2016). However, the losses can be reduced by maintaining suitable environmental conditions within the store (Ogumo et al., 2017).

The majority of large exporters has the capacity and is able to meet strict standards. However, small scale and medium sized farmers face a myriad of challenges associated with the implementation of the standards. Optimum temperature and relative humidity control are key to prolonging the storage life and marketable quality of French beans (Basediya et al., 2013). Conventional storage systems such as field-sheds are not only inappropriate but are also becoming unsustainable due to heavy reliance on charcoal and grass which contribute to environmental degradation (Olosunde et al., 2015). Basediya et al. (2013) reported that solar-powered cooling systems maintained a temperature of 21–25°C with 80–90% RH in freshly harvested potatoes. This suggests that it can offer practical solutions to farmers in rural areas. Solar energy is slowly proving to be an alternative source of clean and renewable energy (Zheng et al., 2019).

A cold chain-controlled system is very important in managing the quality of French beans. Freshly harvested French beans have to be cooled immediately to reduce deterioration of quality and loss of weight. French beans require storage temperatures ranging from 5-15ºC (Kitinoja and Thompson, 2010). Any storage below 4-5°C is not ideal, as it may cause chilling injury. Harvested produce continues to respire after harvesting and any delay in cooling the produce will enhance the respiration rate and eventually degradation. Under conventional field conditions, with no refrigerated coolers, storage is normally done in field sheds with no temperature control. At temperatures between 5-10ºC, French beans can be stored for up to 7 days. Generation of electricity contributes to global warming through production of nitrogen dioxide and sulphur dioxide which are both responsible for acid rain, in addition to emissions of carbon dioxide (Gorle et al., 2016). Solar powered cooler systems have a reduced running cost and this is relevant in tropical environments where adequate amounts of solar energy are received throughout the season. Therefore, it is essential to devise a storage system that is solar powered to ensure small scale farmers can afford to store produce with minimum energy input.

MATERIALS AND METHODS

Development of a solar powered cooler prototype

The design was based on the existing coolers so as to overcome the gaps identified in coolers currently used by farmers in Kenya. The prototype was developed based on Hottel-Vhillier (H-V) model that have been developed for solar cooling of meat and milk products.

Design consideration

The cold room rectangular walls were made of 100 mm Expanded Polystyrene (EPS) insulated, sandwiched by 10 mm MDF outer shell and 5 mm confute polyethylene sheeting interior (Figure 1). Extra energy was required to cool the storage chamber was estimated with the Fourier heat conduction equation and appropriate photovoltaics used to generate this energy. Photovoltaics were fitted on the roof to convert solar power to electrical energy for running the cooling system. During the day, excess energy was used to freeze water into ice. A temperature sensor was centrally placed to record temperature fluctuations. A fan, with power of *Ef* (kW) was fitted at the corner to maintain air circulation. Using the Spick platform, data on conditions within the cooler could be configured and transmitted through the internet and notifications would be sent to the user.

Refrigeration operation cycle, temperature and carbon dioxide control

The system was designed to maintain produce temperature between 6 to 10°C and maintain CO_2 levels below 3%. The cold chamber comprises of a compressor, condenser (heat exchanger), radiator with water/ice (phase change material in a radiator, refrigerant (R600), evaporator plate and fan (Figure 2) and the produce chamber. Within the two chambers, two cooling processes occur; the first process begins in the cold chamber. This is where water contained in a heat exchanger/radiator, is used as the phase change material to "store negative thermal energy as ice and is maintained between -1 and 4°C via thermostatic control. The cold chamber control consists of a digital thermostat mounted externally, but with an internal probe. The probe monitors the temperatures between -1 and 4°C by turning off the compressor when the temperature falls below -1°C and again turns it on when the temperature goes above 4°C to start extracting energy from the cold chamber.

In the cold chamber, there is water which is the phase change material held in a radiator. Water provides cooling even without solar power. In addition, two lead acid gel batteries were installed to store energy to run the system when solar energy is unavailable. The water is cooled below 0°C by a typical refrigeration cycle that employs a solar-powered DC compressor. The refrigeration cycle is a closed-loop cycle that consists of a compressor, a condenser, an evaporator plate and a refrigerant (R600) which is organic, biodegradable and Chlorofluorocarbon (CFC) free, thereby making it compliant with current international standards. The compressor compresses the refrigerant, which liquefies at -40°C and at a pressure of 3.5 bar, thereby pushing the refrigerant through the evaporator and forcing it to liquefy and the temperature drops to -40°C.

The refrigerant then absorbs thermal energy from the cold chamber to the condenser/heat exchanger, which is mounted outside the chamber. The thermal energy is released to the exterior of the chamber and exhaled with the help of the fans. It is through this process that the cold chamber gets cooled. The aim of the above process is to keep the phase change material (water) below its freezing point. The main chamber is set to maintain the produce temperature between 6 and 10°C, it achieves this through a closed-loop control system, that includes a DC powered fan blowing cool air from the cold chamber into the produce chamber. The control within this loop is achieved with a second digital thermostat and a digital temperature probe mounted in the produce chamber. The temperature range in the produce chamber is set at 6 to 10°C. When the temperature rises beyond 10°C the thermostat switches on the fan which blows the cold air from the cold chamber into the storage chamber.

This is achieved by a DC fan installed between the walls of the

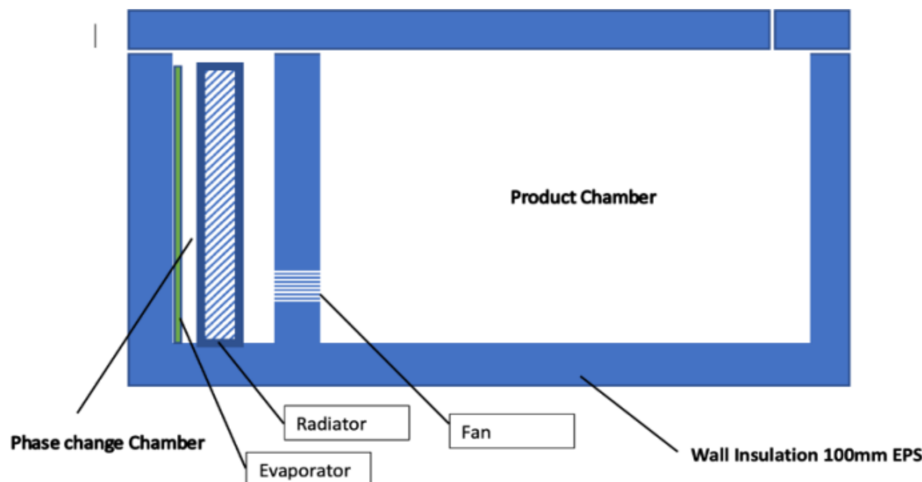


Figure 1. Schematic diagram of the solar cooler.

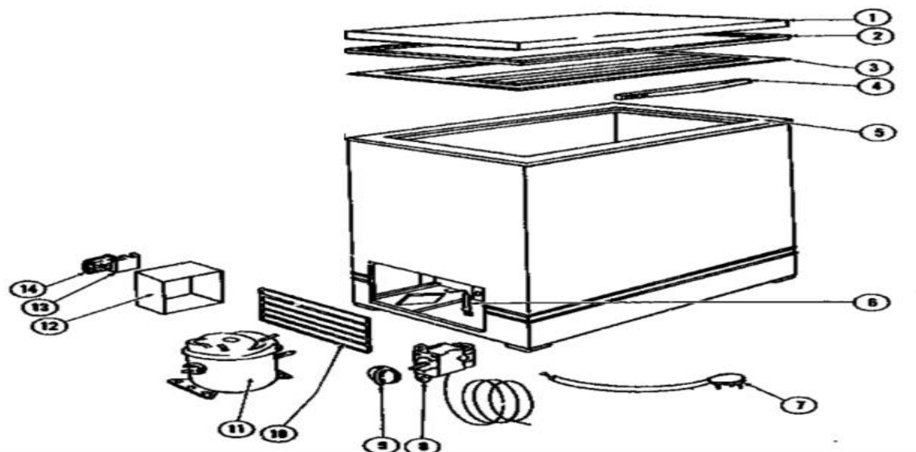


Figure 2. Structural layout of the solar cooler measurements and control system. 1. Lid 100 mm EPS1 insulated; 2. Foam rubber air seal; 3. Evaporator; 4. Sensor Rack; 5. Walls 100 mm Expanded Polystyrene (EPS) insulated sandwiched by 10 mm MDF2 outer shell and 5 mm confute polyethylene sheeting interior; 6. Compressor compartment; 7. 12v DC Power; 8. Plug; 9. External temperature sensor; 10. Dial; 11. Condenser; 12. 24DC Compressor; 13. Danfoss BD50K; 14. 12v DC condenser Fan; 15. Frozen chamber Digital thermostat and compressor controller; 16. Produce chamber digital thermostat and fan controller

cold and produce chamber that blows cold air from the cold chamber into the produce chamber. The system is equipped with sensors, which collect data and send information to the control system. This in turn actuates solenoids and fans that send the data to the spick platform where the data is analyzed in graphical format and can be configured and sent as an email notification to the user. When the produce stored in the cooler respires, it emits CO_2. The storage chamber is equipped with an air quality sensor. When the CO_2 level rises beyond 30000 ppm the control system powers the solenoid, which opens an air valve. A fan located next to the solenoid blows the CO_2 out of the chamber and ensures that the CO_2 levels in the chamber are maintained within the range of 400 - 30000 ppm into (0.01-0.03%) thereby, reducing the rate of breakdown of the produce.

Conventional cooling system

The conventional cooling system was a simple structure with a roof and walls made of iron sheets, with provisions for ventilation provided by use of chicken mesh wires halfway up the sides of the wall. This is the most widely used cooling system by smallholder farmers in Kenya.

Harvesting of French bean

The French bean samples were harvested at different times; 8am, 10am, 12pm and 2pm at a commercial field of beans in, AAA Thika farm and immediately sorted, weighed into 500 g portions and

Table 1. Physiological weight of French beans harvested and stored at 8.00am in conventional and solar powered prototype cooling.

Time of day	Conventional method(g)	Percent change in weight	Solar-powered method(g)	Percent change in weight
8am	500.0^{a}	0.0^{i}	500.0^{a}	0.0^{i}
9am	497.6^{a}	0.4^{h}	497.8^{a}	0.4^{hi}
10am	495.5^{b}	0.9^{gh}	497.2^{a}	0.6^{h}
11am	487.0^{c}	2.6^{de}	496.2^{b}	0.7^{gh}
12pm	484.1^{c}	3.2^{cd}	494.6^{b}	1.1^{gh}
1pm	481.1^{d}	3.7^{bc}	492.0^{b}	1.6^{fg}
2pm	478.4^{e}	4.3^{ab}	489.0^{c}	2.2^{ef}
3pm	475.0^{e}	4.9^{a}	486.8^{c}	2.6^{de}
Mean	487.4	2.5	494.2	1.2
LSD (P≤0.05)	2.1	0.49	2.1	0.49
CV (%)	0.3	21.5	0.3	21.5

Means within column followed by different letters are significantly different based on Fishers Protected LSD test (p ≤0.05).

labeled as per the harvesting time and storage condition; FS for field shade and SC for solar powered cooler. The harvested French bean samples were kept in the two storage conditions until 4pm. At 4pm, the harvested samples were transported to the pack house within the farm and kept in a cold room at temperatures ranging from 4-8°C. The following day, the 500 g portions were re-packaged into two packs of 200 g each, packed into a modified atmosphere packaging (MAP) bag to ensure that the product remained fresh for the longest possible duration. The packed products were stored in cold rooms at temperature between 4-8°C. Quality parameters such as product weight and temperature were monitored during the seven days shelf life window. On the seventh day, the packed products were analyzed for accumulation of oxygen and carbon dioxide.

Determination of physiological weight loss

The weight loss was measured using a digital weighing scale (Constant 14192-1F model, China). The samples were weighed on the first hour of storage and then hourly until 3pm in the afternoon. The mean weight and the mean change in the weight of the samples were determined with time during the storage period and was expressed as a percentage using the following formula; Percentage weight loss = $\frac{W1-W2}{W1} \times 100$ Where W_1 = Initial weight of sample (g); W_2 = Weight of sample after storage (g).

Determination of cooling efficiency and measurement of temperature

Cooling efficiency was determined by dividing the observed temperature decrease by the maximum potential temperature decrease. The cooling efficiency was calculated using the formula by Verploegen et al. (2019):

$$\text{Cooling efficiency} = \frac{\text{Ambient Temperature} - \text{Interior Temperature}}{\text{Ambient Temperature} - \text{wet bulb Temperature}}$$

The interior temperature was determined by reading the temperature sensor at the center of the cooler. A thermometer with the bulb wrapped in wet muslin was used to measure wet bulb temperature.

Data analysis

The data was subjected to a two way analysis of variance using GenStat statistical package version 15 and the least significant difference was used at 5% significance level.

RESULTS

Physiological weight of French beans stored in solar cooler and conventional cooler

The results of the physiological weight loss for the produce stored under both conventional conditions and the solar powered cooler harvested at 8am is presented in Table 1. A significant difference (P ≤ 0.05) in weight loss at different storage durations in the two storage methods was observed. There was higher physiological weight loss in the conventional storage method, compared to the solar powered cooler and it changed from 500 g to 475 g after eight hours of storage. In the solar powered cooler, there was a small reduction in physiological weight from the initial 500 g to 486.8 g after 7 h of storage. Figure 3 shows weight loss in French beans. Weight loss in French beans stored using the conventional method was observed to be 5% of the original weight at harvesting after 7 h of storage, while only 2.6% of the original weight was lost after 7 h of storage for French beans produce stored in the solar powered cooler.

The differences in physiological weight loss were significant (P ≤ 0.05) for the storage duration in the two storage methods (Figure 3). The weight loss of French beans using the conventional storage method was higher compared to those that were stored in the solar powered cooler and it changed from 500 g to 476 g after 6 h of storage. In the solar powered cooler, there was a small reduction in physiological weight from the initial 500 to 490 g after 5 hof storage.

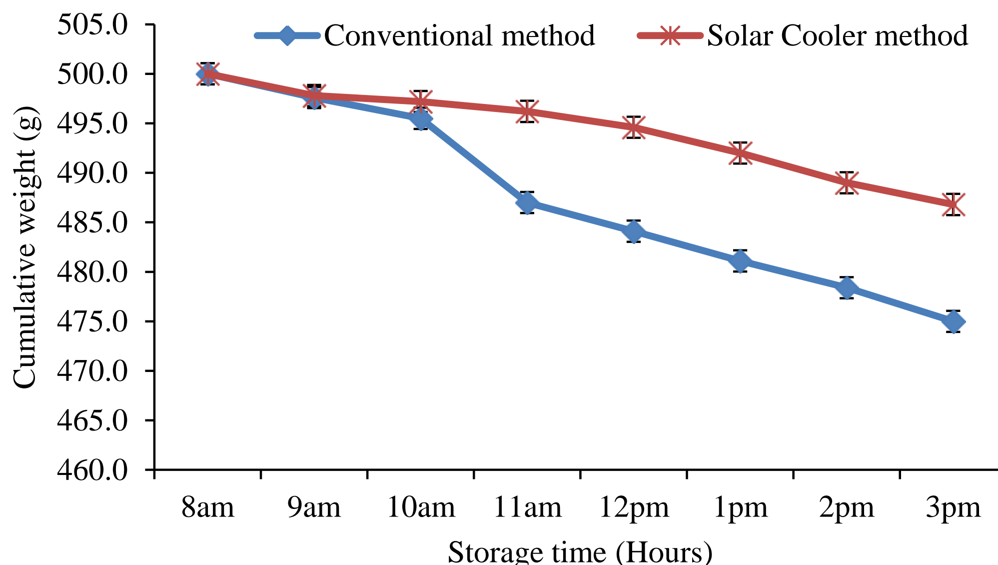


Figure 3. Cumulative weight loss of French beans stored in conventional and solar powered cooler.

Table 2. Physiological weight of French beans stored in conventional and solar powered cooler for produce harvest at 12 noon and 2.00pm.

Time of day	Conventional method (g)	% change in weight	Solar cooler method (g)	% change in weight
12.00pm	500.0^{a}	0.0^{e}	500.0^{a}	0.0^{e}
1.00pm	496.2^{c}	1.5^{c}	498.2^{ab}	0.4^{de}
2.00pm	487.4^{d}	2.5^{b}	497.3^{b}	0.5^{d}
3.00pm	481.4^{e}	3.7^{a}	495.8^{b}	0.8^{d}
Mean	490.1	1.9	497.8	0.4
LSD (P≤0.05)	1.5	0.32	1.5	0.32
CV (%)	0.2	20.8	0.2	20.8
Time of day	Conventional method(g)	% change in weight	Solar Cooler method(g)	% change in weight
2.00pm	500.0^{a}	0.0^{c}	500.0^{a}	0.0^{c}
3.00pm	496.3^{c}	0.7^{a}	498.3^{b}	0.3^{b}
Mean	498.2	0.36	499.2	0.17
LSD (P≤0.05)	0.65	0.12	0.65	0.12
CV (%)	0.1	35.0	0.1	35.0

Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test (P ≤ 0.05).

The results of the physiological weight loss for the produce harvested at noon and at 2pm and stored under both conventional conditions and the solar powered cooler is presented in Table 2. There were significant differences (P≤0.05) between the storage duration in the two storage methods. The weight loss of French beans in the conventional storage method was high compared to those that were stored in the solar powered cooler. For the French beans harvested at 2pm, there was a slight reduction in weight in the two storage methods.

Effects of both cooling systems on the produce temperatures

There was a significant difference in the temperature of produce (p ≤ 0.05) between the storage time in the two cooling methods (Table 3). Under conventional cooling, the temperature of produce was lower in the morning hours and increased in the afternoon, while there were no significant differences in temperature of the produce stored in a fabricated solar-powered cooling system. The

Table 3. Effect of cooling methods on the temperature of French beans harvested and stored at 8am.

Time of day	Conventional cooling (ºC)	Solar-powered cooling(ºC)
8.00am	14.2^{g}	15.6^{cde}
9.00am	14.4^{g}	15.7^{cde}
10.00am	15.1^{f}	15.6^{def}
11.00am	16.2^{c}	15.4^{ef}
12.00pm	17.4^{ab}	15.4^{ef}
1.00pm	17.6^{a}	15.8^{cde}
2.00pm	17.8^{a}	15.9^{cd}
3.00pm	16.8^{b}	15.9^{cd}
Mean	16.2	15.7
LSD (P≤0.05)	0.3	0.3
CV (%)	1.5	1.5

Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test (P ≤ 0.05).

Table 4. Effect of both Conventional and Solar powered cooling systems on the temperature of French beans harvested at 10.00am.

Time of day	Conventional method(ºC)	Solar Cooler method (ºC)
10.00am	17.2^{c}	16.2^{cd}
11.00am	16.4^{c}	16.3^{cd}
12.00pm	17.4^{ab}	16.0^{cd}
1.00pm	18.2^{a}	15.9^{d}
2.00pm	17.7^{a}	15.4^{d}
3.00pm	17.4^{ab}	16.1^{cd}
Mean	17.4	15.9
LSD (P≤0.05)	0.67	0.67
CV (%)	3.1	3.1

Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test (P ≤ 0.05).

produce temperature in the conventional cooler increased by 20% after 7 h of storage. For produce harvested at 10am, there was a significant difference ($p \leq 0.05$) between the storage condition and the storage duration (Table 4). There were significant differences between storage duration and the harvesting time for both the cooling conditions (Table 6). The produce harvested at 12pm and at 2pm had high temperature levels of 18.7 and 18.3°C respectively, however, the temperature levels continued to decline with the number of hours of cooling. In the conventional cooling system, the temperature levels declined by about 7% after 4 h while the temperature levels for produce in the fabricated solar-powered cooling system reduced by about 18%.

Figure 4 shows the relationship between the product temperature and storage duration. The temperature for the produce stored in a conventional shed increased by about 28% from 14 to 18°C after 7 h of storage. The produce stored in a solar powered cooler had an average product temperature of 15.7°C.

Effect of cooling system and harvesting time on cooling efficiency

For the produce harvested at 10 am, the mean degree of cooling for the produce in a conventional cooling system was 0.36 while the solar powered cooling system was 0.68 (Table 5). Consequently, the effectiveness and efficiency of the fabricated solar powered cooling system was 47% more than the conventional cooling method. For the duration of the experiment, for the produce harvested at noon, the mean degree of cooling was 0.4 and 0.95 for both the conventional and solar powered cooler respectively. Consequently, the effectiveness of the solar powered cooling system was 58% more than the conventional cooling method.

Effect of cooling systems on gaseous production

The level of gas released from the produce is presented

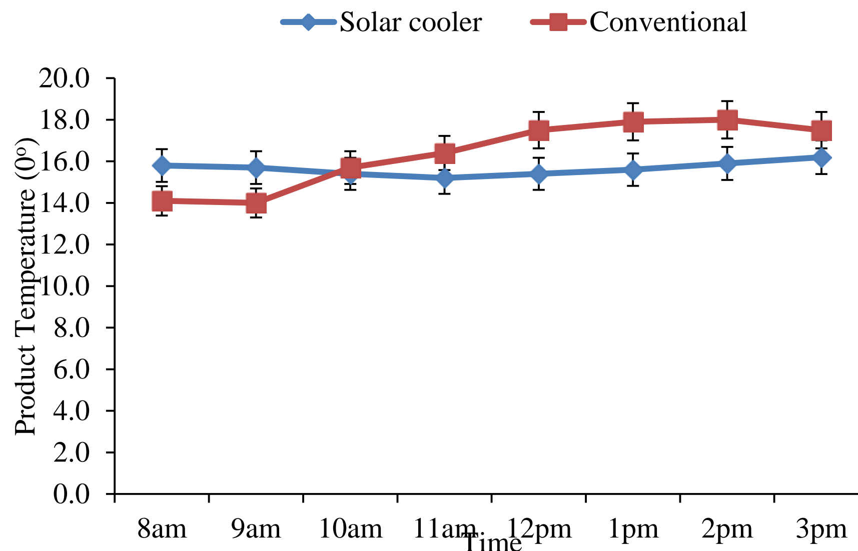


Figure 4. Product temperature loss of French beans stored in conventional and solar powered coolers.

Table 5. The efficiency of the different method of cooling systems on produce harvested at 10am.

Harvesting time- 10am	Conventional method	Solar cooler method
Storage time		
10.00am	0.50[cd]	0.85[a]
11.00am	0.12[e]	0.78[ab]
12.00pm	0.25[de]	0.58[abc]
1.00pm	0.23[de]	0.72[ab]
2.00pm	0.89[a]	0.55[bc]
3.00pm	0.15[e]	0.58[cd]
Mean	0.36	0.68
LSD (P≤0.05)	0.198	0.198
CV (%)	30.0	30.0

Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test (P ≤ 0.05).

in Table 6. There was a significant difference (p ≤ 0.05) in the volume of carbon dioxide released between the two cooling systems. Sample 5A, which was harvested at 8am and stored until 3pm accumulated more CO_2 compared to the rest in the conventional cooling system. There were significant differences (p ≤ 0.05) in the volume of oxygen released by the produce in the conventional cooling system. French samples harvested at 8am, 10am and 12 noon and stored in the conventional shed accumulated more oxygen levels compared with other samples.

Figure 5 shows the reduction in concentration of oxygen and an increase in concentration of carbon dioxide over a period of eight hours causing a gradient. Initially, the amount of CO_2 released is less, but continues to increase with storage time at the same time. The amount of oxygen within the chambers reduces to lower levels after an eight-hour storage period. Thus, inside the cooling chambers, the oxygen amount reduces while the amount of carbon dioxide increases.

DISCUSSION

The study has demonstrated that a fabricated solar-powered cooler was more efficient in maintaining quality and freshness of French beans than the conventional field shed. Produce stored in a fabricated solar-powered

Table 6. Gaseous levels from French beans stored in solar powered and conventional cooling systems.

Sample	Conventional method (Units)		Solar cooler method (Units)	
	Carbon dioxide	Oxygen	Carbon dioxide	Oxygen
1A	13.4[b]	9.9[a]	10.2[a]	10.4[a]
2A	12.6[b]	9.2[a]	9.5[a]	10.7[a]
3A	14.6[ab]	9.3[a]	10.6[a]	9.6[a]
4A	16.7[a]	7.2[b]	10.2[a]	10.6[a]
5A	17.3[a]	7.8[b]	10.5[a]	9.8[a]
Mean	14.9	8.7	10.2	10.2
LSD (P≤0.05)	3.3	1.3	3.3	1.3
CV (%)	18.2	8.8	18.2	8.8

Values followed by the same letter within the same column are not significantly different between the treatments using Fishers Protected LSD test (P ≤ 0.05).

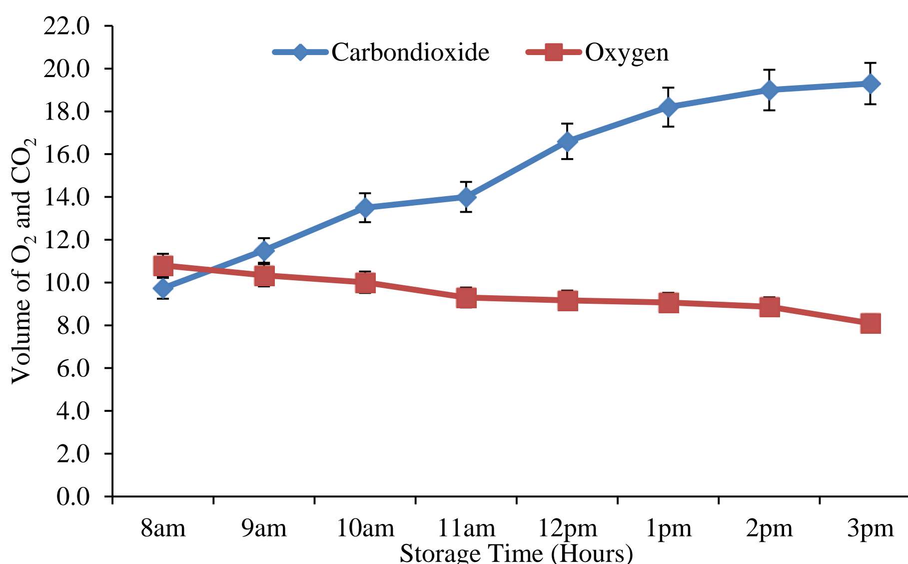


Figure 5. Changes in CO_2 and O_2 concentrations (ppm) with time within the conventional cooling chambers.

cooler had less weight loss, only losing 2.8% weight, compared with a 5% weight loss for those stored in a conventional field shed. Similar results were reported by Dirpan et al. (2018) where the loss in weight of mangoes was lower (3.1%) in cold storage when compared to 14.5% in storage at ambient conditions.

The physiological loss of weight for French beans is a result of both the evaporation of water and respiratory losses (Olosunde et al., 2015). Water evapotranspiration depends on the atmospheric temperature and relative humidity within the store (Dzivama, 2000). Olosunde et al. (2015) stated that low temperature reduces respiratory activity, while high relative humidity reduces the rate of evaporation from the produce. At harvesting, the produce contains field heat and respiratory heat meaning that, in the initial stage there is field heat within the produce in both the conventional and the solar powered cooler. The study has revealed that the fabricated solar-powered cooler was more efficient than the conventional field shed in maintaining produce temperature during storage. French beans stored under conventional cooling had increases in temperature of 18% in 4 h compared to an increase of 7% for the produce stored in the fabricated solar-powered cooler. The increase in temperature could be a result of physiological factors such as respiration that results in heat emission that increases temperature (Sanchez-Mata, 2003).

French bean samples stored in conventional cooling systems accumulated more CO_2 and less oxygen compared with those in a solar-powered cooler. The

difference in CO_2 accumulation can be attributed to the efficiency of the CO_2 sensors installed in the solar-powered cooling system to sense high CO_2 levels and trigger the fans to expel excess CO_2 from the produce chamber. The produce consumes the oxygen inside the system as it respires and CO_2 is released, the volume of oxygen decreases while the volume of CO_2 increases. The high amount of CO_2 within the conventional cooler is an indication of a rapid respiration process that influences the quality of fresh produce like French beans (Kader and Yehoshua, 2000). The accumulation of CO_2 around the produce causes rapid deterioration of the produce by causing a bad flavour, internal breakdown, and other abnormal physiological conditions (Silva, 2008). According to Kader et al. (1989) exposure of a commodity to high levels of CO_2 above the limits can results in increased anaerobic respiration, which in turn causes buildup of ethanol and acetaldehyde that causes off-flavors. Vegetables such as French beans have high respiration rates and an increase in the amount of CO_2 released may have an effect on the shelf life of the produce. Conventional storage systems are characterized by high temperature conditions that lead to faster respiration; an increase in temperature of 10°C doubles the process of respiration (Silva, 2008).

Conclusion

A fabricated solar-powered prototype cooler maintains and reduces the produce temperature, maintains the balance between CO_2 and oxygen and reduces the metabolic activity of the French beans within the optimum level, thereby keeping the produce fresh for longer.

ACKNOWLEDGEMENTS

The authors sincerely appreciate the voluntary support of Mrs. Louise Foxton, Mrs. Sue Homer and Mrs. Bronagh Owako for taking their time to proof-read this manuscript. The authors are grateful to the management of AAA Growers for allowing the study to be conducted in their farm and packhouse in Thika, Kenya. In addition, they also thank Mbili Mbebe, Faith Nkirote, and Nickson Mashati for their support during this study. The authors also acknowledge the support of Engineer Fredrick Jura for his support in designing and fabrication, turning the vision into a reality.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Arah IK, Ahorbo GK, Anku EK, Kumah EK, Amaglo H (2016). Postharvest handling practices and treatment methods for tomato handlers in developing countries: A mini review. Advances in Agriculture. Available at: https://www.hindawi.com/journals/aag/2016/6436945/abs/

Atanda SA, Pessu PO, Agoda S, Isong IU, Ikotun I (2011). The concepts and problems of post-harvest food losses in perishable crops. African Journal of Food Science 5:603-613.

Basediya A, Samuel DVK, Beera V (2013). Evaporative cooling system for storage of fruits and vegetables - A review. Journal of Food Science and Technology 50(3):429-442.

Dirpan A, Sapsal MT, Syarifuddin A, Tahir MM, Ali KNY, Muhammad AK (2018). Quality and storability of mango during Zero Energy Cool Chamber (ZECC). International Journal of Agriculture System 6(2):119-129.

Dzivama AU (2000). Performance evaluation of an active cooling system for the storage of fruits and vegetables. Unpublished PhD Thesis, Department of Agricultural Engineering, University of Ibadan, Ibadan, Nigeria. pp. 34-83.

Gorle RD, Wandhare MM, Khelkar AR, Bhoyar AS, Muley AS (2016). Solar Powered Evaporative Air Cooler with Cooling Cabin for Household Food Items. Available at: http://www.iosrjournals.org/iosr-jmce/papers/vol13-issue2/Version-4/H1302045356.pdf

Kader AA, Ben-Yehoshua S (2000). Effects of super atmospheric oxygen levels on postharvest physiology and quality of fresh fruits and vegetables. Postharvest Biology and Technology 20(1):1-13.

Kader AA, Zagori D, Kerbel, LE (1989). Modified atmosphere packaging of fruits and vegetables. Critical Reviews in Food Science and Nutrition 28:1-30.

Kitinoja L, Thompson JF (2010). Pre-cooling systems for small-scale producers. Stewart Postharvest Review 6(2):1-14.

Kumar D, Kalita P (2017). Reducing postharvest losses during storage of grain crops to strengthen food security in developing countries. Foods 6(1):1-22.

Ogumo EO, Kunyanga CN, Okoth MW, Kimenju J (2017). Current knowledge and performance of existing charcoal coolers in improving the overall quality and shelf-life of French beans. African Journal of Agricultural Research 12(49):3399-3409.

Okello JJ, Narrod C, Roy D (2007). Food safety requirements in African green bean exports and their impact on small farmers. International Food Policy Research Institute.

Olosunde WA, Aremu AK, Onwude DI (2016). Development of a solar powered evaporative cooling storage system for tropical fruits and vegetables. Journal of Food Processing and Preservation 40(2):279-290.

Rawat S (2015). Food Spoilage: Microorganisms and their prevention. Asian Journal of Plant Science and Research 5(4):47-56.

Sanchez-Mata (2003). New Recommendation for building in tropical climates. Building and Environment 28:271-278.

Silva E (2008). Respiration and ethylene and their relationship to postharvest handling in wholesale success: A farmer's guide to selling, postharvest handling, and packing produce (Midwest Edition) Available online at https://eorganic.org/node/2671.

Verploegen E, Ekka R, Gill G (2019). Evaporative Cooling for Improved Vegetable and Fruit Storage in Rwanda and Burkina Faso. D-Lab. Available at: https://dspace.mit.edu/handle/1721.1/121582

Zheng L, Zhang W, Xie L, Wang W, Tian H, Chen M (2019). Experimental study on the thermal performance of solar air conditioning system with MEPCM cooling storage. International Journal of Low-Carbon Technologies 14(1):83-88.

Vol. 14(7) pp. 201-208, August 2020
DOI: 10.5897/AJFS2020.1977
Article Number: 53D7E0164583
ISSN: 1996-0794
Copyright ©2020
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJFS

ACADEMIC JOURNALS
expand your knowledge

African Journal of Food Science

Full Length Research Paper

Evaluation of the nutritional and antioxidant potentialities of *Capparis corymbosa*

Edwige N. ROAMBA, Crépin I. DIBALA*, Marcel D. BENGALY, Mamounata DIAO, Kiessoun KONATE and Mamoudou H. DICKO

Laboratory of Biochemistry, Biotechnology, Food Technology and Nutrition (LABIOTAN), University of Joseph Ki-Zerbo, 09 P. O. Box: 848, Ouagadougou 03, Burkina Faso.

Received 1 June, 2020; Accepted 13 July, 2020

The study was undertaken with the objective of contributing to the search for new sources of dietary antioxidants. It aimed to assess the dietary potential, bioactive compound levels and antioxidant activities of the leaves and fruits of *Capparis corymbosa*, a plant food from Burkina Faso. Standard methods were used to assess the nutritional potential of leaves and fruits as well as the levels of bioactive compounds and antioxidant activities of leaf and fruit fractions. Levels of total sugar were 765.40 ± 134.67 mg/100 g for leaves and 2208.65 ± 194.55 mg/100 g for fruits. Protein levels were 22.722 ± 0.95 mg/100 g for leaves and 13.442 ± 0.91 mg/100 g for fruits. Both organs also had interesting contents of minerals and essential amino acids. Total phenolic levels ranged from 18.36 ± 0.90 g GAE/100 g to 16.46 ± 1.15 g GAE/100 g for leaves and from 4.58 ± 0.15 g GAE/100 g to 1.03 ± 0.31 g GAE/100 g for fruits while those of flavonoids ranged from 2.83 ± 0.23 g EQ/100 g to 2.08 ± 0.25 g EQ/100 g and from 1.78 ± 0.25 g EQ/100 g to 0.93 ± 0.41 mg EQ/100 mg, respectively for leaves and fruits. The butanol extraction fractions had the highest levels of polyphenols of both organs. Monitoring antioxidant activities using DPPH, FRAP and ABTS models showed that leaves and fruits displayed high antioxidant activities but leaves had higher antioxidant activities than the fruits. Antioxidant activity was correlated with total phenolic levels. These results reveal that *C. corymbosa* is an important source of phytonutrients, bioactive compounds and has good antioxidant activity and is therefore an alternative for the fight against diseases linked to oxidative stress and as a food supplement.

Key words: *Capparis corymbosa*, micronutrients, macronutrients, antioxidant activity, oxidative stress.

INTRODUCTION

In Burkina Faso, *Capparis corymbosa* (Capparidaceae) is a plant with great nutritional importance. The species is used as a dietary supplement, gathering or as a food supplement (Ayessou et al., 2018). In addition, many health benefits such as child care (colic and general tiredness) related to the consumption of *C. corymbosa* leaves and fruits have been reported (Busson, 1965; Zerbo et al., 2007). This species is used as a dietary

*Corresponding author. E-mail: dibalacrepin@gmail.com. Tel: 0022670141596.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

supplement in children and type-II diabetic patients (Busson, 1965). Leaf extracts are suggested to be effective against kidney disease, biliary inflammation, rheumatism, etc., and good antioxidants (Zerbo et al., 2007). These different properties of the plant are certainly linked to its chemical composition. Indeed, Nacoulma (1996) and Sereme et al. (2001) reported that the richness of plants in secondary metabolites (tannins, flavonoids, saponosides) and active ingredients would be at the origin of their different properties. Despite all this high potential, little data exist on the phytochemistry, nutritional and antioxidant potential of *C. corymbosa*. Knowledge on antioxidant activities of the plant tissues could allow a better food valorization of the species.

The metabolism of aerobic organisms generates reactive oxygen species (ROS). The main ROS are hydroxyl radical, superoxide radical, and hydrogen peroxide. These ROS can also be generated by exposure to certain exogenous factors such as ionizing radiation, certain drugs, environmental pollutants and some synthetic food additives. ROS impair cellular function, particularly in the inflammatory process, phagocytosis and cellular signal transduction (Garait, 2006). The consequences of chronic exposure to high ROS levels are generally undesirable for the cells. The studies have reported that the accumulation of free radicals is the fundamental root cause of many age-related diseases (tumor proliferation, cataract, pulmonary edema) and affects several organs (muscles, heart, blood vessels, skin, etc). ROS are also factoring which potentiate the development of multifactorial conditions such as degenerative diseases (Parkinson's, Alzheimer's), type-II diabetes, osteoarthritis phenomena, cardiovascular disease, etc (Favier, 2003).

Fortunately, fruits and vegetables are a good source of dietary antioxidants (Valko et al., 2006). Well known natural antioxidants include phenolic compounds, glutathione, nicotinamides, ubiquinone (coenzyme Q_{10}), vitamins (C, E, A and K) and some enzymes (copper, zinc and manganese-dependent superoxide dismutase, catalase, selenium-dependent glutathione peroxidase, etc). Plants (leaves and fruits) are sources of phenolic compounds, vitamins, trace elements and especially beneficial natural antioxidants. Epidemiological studies have shown a positive correlation between the consumption of foods rich in polyphenols and lower risk of developing diseases like age-related neurodegenerative diseases (Mohamed and Darbar, 2010). This is often attributed to the antioxidant potential of phenolic compounds, those containing ortho-dihydro-benzene (catechols) due to their redox properties for eliminating reactive oxygen species or to chelate divalent metals (Atanasova, 2009). In addition, flavonoids and other phenolic compounds may also induce the expression of genes encoding antioxidant enzymes and proteins (superoxide dismutase, glutathione peroxidase, catalase, glutathione, etc). In research perspectives of dietary antioxidants, plant species still underexploited but accessible to rural populations constitute an interesting line of investigation (Garait, 2006).

Therefore, an investigation was conducted on the chemical composition and bioactivities of *C. corymbosa* leaves and fruits.

MATERIALS AND METHODS

Plant

Biological material consisted of leaves and ripe fruits of *C. corymbosa*. Sample collection was conducted during the months of June and July 2018 in the city park Bangréweogo (Ouagadougou). The species has been identified in plant biology laboratory of the University Joseph Ki-Zerbo. The leaves and fruits were dried by ventilation at room temperature (20-25°C) and ground into flour in a microanalytical mill to pass a screen of 0.5 mm. Milling was done at 4°C, and was performed carefully to avoid overheating. The flours were stored at -20°C prior to analysis to prevent endogenous enzymatic or nonenzymatic reactions, after the powders were subjected to different extraction solvents.

Preparation of extracts

Fifty grams (50 g) of powdered plant material as described earlier were extracted with 80% aqueous ethanol (500 ml) in ratio 1/10 (w/v) for 48 h under mechanic agitation (SM 25 shaker, Edmund BÜHLER, Germany) at room temperature (20-22°C). After filtration, ethanol was removed under reduced pressure in a rotary evaporator (BÜCHI, Rotavopor R-200, Switzeland) at approximately 40°C and freeze-dried with Telstar Cryodos 50 freeze-dryer. These ones were filtered and freeze-dried. The extract residues were weighed before packed in waterproof plastic flasks and stored at 4°C until use. The yields of different crude extract were calculated and expressed as grams of extract residues/100 g of dried plant materials.

Fractionation

The aqueous extracts were subjected to sequential liquid-liquid extraction with petroleum ether, dichloromethane, ethyl acetate and n-butanol. Each fraction was then collected and concentrated to dryness under reduced pressure to obtain hexan fraction, dichloromethane fraction, ethyl acetate fraction and n-butanol fraction. The fractions were freeze-dried. The fraction residues were packed in waterproof plastic flasks and stored at 4°C until use.

Determination of carbohydrate content

500 mg of sample were homogenized in 5 ml of 80% hot ethanol. After cooling, the homogenate was centrifuged at 4000 rpm for 10 min. The supernatant was used to estimate the soluble sugar content of fresh leaves. The soluble sugar content was determined using the phenol-sulfuric acid method as described by Dubois et al. (1965) and the absorbance was red at 490 nm. The total sugar content was expressed as µg glucose equivalent/100 gram of sample (µg GE/ 100 g of sample).

Determination of total protein

For proteins extraction, 500 mg of powder of leaves or fruits were

homogenized in 5 ml of 0.1 M NaCl for 5 h at 150 rpm/min at room temperature. The samples were centrifuged at 10000 g during 30 min and the supernatant were collected to determine the protein content. The estimation of water-soluble protein content was performed according to the assay method of Bradford (1976). A volume of 50 µL of sample is added to 250 µL of Bradford reagent (Coomassie Brillant Blue G250). The reading is made using a spectrometer at 595 nm against a blank consisting of 50 µL of sample and 250 µL of buffer solution. BSA served as the standard for the preparation of the calibration curve (0-250 µg/mL (y = 0.0537x + 0.6136; R^2 = 0.9997). Results were expressed in mg per 100 mg of sample.

Determination of minerals and oligo-elements content

The minerals and oligo-elements determination were carried out according to a classical method (Norme international ISO 2171, 2007). A mass of 3 g of the sample was introduced into crucibles before being calcined at 550°C in the muffle furnace for 24 h. The ash obtained was recovered in beakers and dissolved in 5 ml of concentrated hydrochloric acid, add 3 drops of hydrogen peroxide (H_2O_2). The whole was transferred to 100 mL volumetric flasks and made up to the mark with distilled water. Centrifugation was then carried out and the supernatant was recovered in sterile sampling tubes for spectrometric reading. The reading was taken with a Flame Atomic Absorption Spectrophotometer connected to a computer. The analyses permitted the determination of minerals such as calcium, magnesium, potassium and zinc.

Determination of amino acid content

The determination of amino acids was carried out by the PICO-TAG method (Thordser, 2011). For the determination of the complete amino acid profile, 500 mg sample was weighed and placed in a 20 mL volumetric flask. The volumetric flask is filled up to the mark with 0.1 M hydrochloric acid. Approximately 1 mL of the diluted sample is filtered through the 0.45 µm filters and then derivatized with phenylisothiocyanate (PITC) to produce the amino acids phenylthiocarbamyl (PTC). The derivatized sample were added 200 µl of PICO-TAG dilution solution (0.38 µg/µL), the amino acid derivatives were then separated by HPLC (2.3 µg sample/µL and detected by absorption spectrophotometry at values as low as 1 pmol using a wavelength at 254 nm.

Total phenolic content

Total polyphenols were determined by Folin-Ciocalteu method (Lamien-Meda et al., 2008). Aliquots (125 µL) of solution from extract or each fraction in methanol (10 mg/mL) were mixed with 62.5 µl Folin-Ciocalteu reagent (0.2 N). After 5 min, 500 µL of aqueous Na_2CO_3 (75 g/L) were added and the mixture was vortexed. After 2 h of incubation in the dark at room temperature, the absorbencies were measured at 760 nm against a blank (0.5 ml Folin-Ciocalteu reagent + 1 ml Na_2CO_3) on a UV/visible light spectrophotometer (CECIL CE 2041, CECIL Instruments, England). The experiments were carried out in triplicate. A standard calibration curve was plotted using gallic acid (Y= 0.0289x - 0.0036; R^2 = 0.9998). The results were expressed as g of gallic acid equivalents (GAE)/100 g of extract or fractions.

Determination of flavonoid content

The total flavonoids were estimated according to the Dowd method, slightly modified (Hinnebourg et al., 2006). To an aliquot of 0.5 ml of methanol/$AlCl_3$ (2%, w/v) were mixed 0.5 ml of extract or each fraction solution (0.1 mg/mL). After 10 min, the absorbencies were measured at 415 nm against a blank (mixture of 0.5 ml extract solutions and 0.5 mL methanol) on a UV/visible spectrophotometer (CECIL CE 2041, CECIL Instruments, England) and compared to a quercetin calibration curve (Y= 0.0289x-0.0036; R^2 = 0.9998). The data obtained were the means of three determinations. The amounts of flavonoids in plant extracts were expressed as g of quercetin equivalents (QE)/100 g of extract or fractions.

Determination of *in* vitro antioxidant activity

DPPH radical method

Radical scavenging activity of extract or each fraction against stable DPPH (2, 2'-diphenyl-1-picrylhydrazyl, Fluka) was determined with a UV/visible spectrophotometer (CECIL CE 2041, CECIL Instruments, England) at 517 nm as described by Hinnebourg et al., (2006). Extract solutions were prepared by dissolving 10 mg of dry extract in 10 mL of methanol. The samples were homogenized in an ultrasonic bath. Afterwards, 0.5 mL of aliquots which were prepared at different concentrations from each sample of extract were mixed with 1 mL of methanolic DPPH solution (20 mg/mL). After 15 min of incubation in the dark at room temperature, the decrease in absorption was red. All experiments were performed in triplicate and expressed in mmol of ascorbic acid equivalent per mass of extract or fraction.

ABTS radical cation decolorization assay

For the assay (Hinnebourg et al., 2006), ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation ($ABTS^{\cdot+}$) was produced by reacting ABTS with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12 h before use. This mixture was diluted with ethanol to give an absorbance of 0.7 ± 0.02 at 734 nm using a UV/visible spectrophotometer (CECIL CE 2041, CECIL Instruments, England). Afterwards, 10 µL of the diluted sample (1 mg/mL^{-1} in methanol) which was allowed to react with 990 µl of fresh $ABTS^{\cdot+}$ solution and the absorbance was taken 6 min exactly after initial mixing. Ascorbic acid was used as standard. Quercetin was used as positive control.

Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed as previously described (Velazquez et al., 2003). To an aliquot of 0.5 mL of extract or each fraction (1 mg mL^{-1}) was mixed with 1.25 mL of phosphate buffer (0.2 M, pH 6.6) and 1.25 mL of aqueous potassium hexacyanoferrate [$K_3Fe(CN)_6$] solution (1%). After 30 min of incubation at 50°C, 1.25 mL of trichloroacetic acid (10%) was added and the mixture was centrifuged at 2000 × g for 10 min. Then, the upper layer solution (0.625 mL) was mixed with distilled water (0.625 mL) and a freshly prepared $FeCl_3$ solution (0.125 mL, 0.1%). Absorbances were red at 700 nm on a UV/visible spectrophotometer (CECIL CE 2041, CECIL Instruments, England) and ascorbic acid was used to produce the calibration curve (Y= 0.008x-0.0081; R^2 = 0.9999). The iron (III) reducing activity determination was performed in triplicate and expressed in mmol Ascorbic Acid Equivalent per g of extract or fractions. Trolox, a reference compound was used as positive control.

Statistical analysis

The analysis of variance (ANOVA) was performed using XL-STAT

Table 1. Content of carbohydrates and total proteins.

Capparis corymbosa	Carbohydrates (mg/100 g)	Total proteins (mg/100 g)
Leaves	765.41 ± 134.67[b]	22.72 ± 0.95[a]
Fruits	2304.14 ± 194.54[a]	13.44 ± 0.91[b]

The results shown in the previous figure by different letters are statistically distinct (p<0.0001).

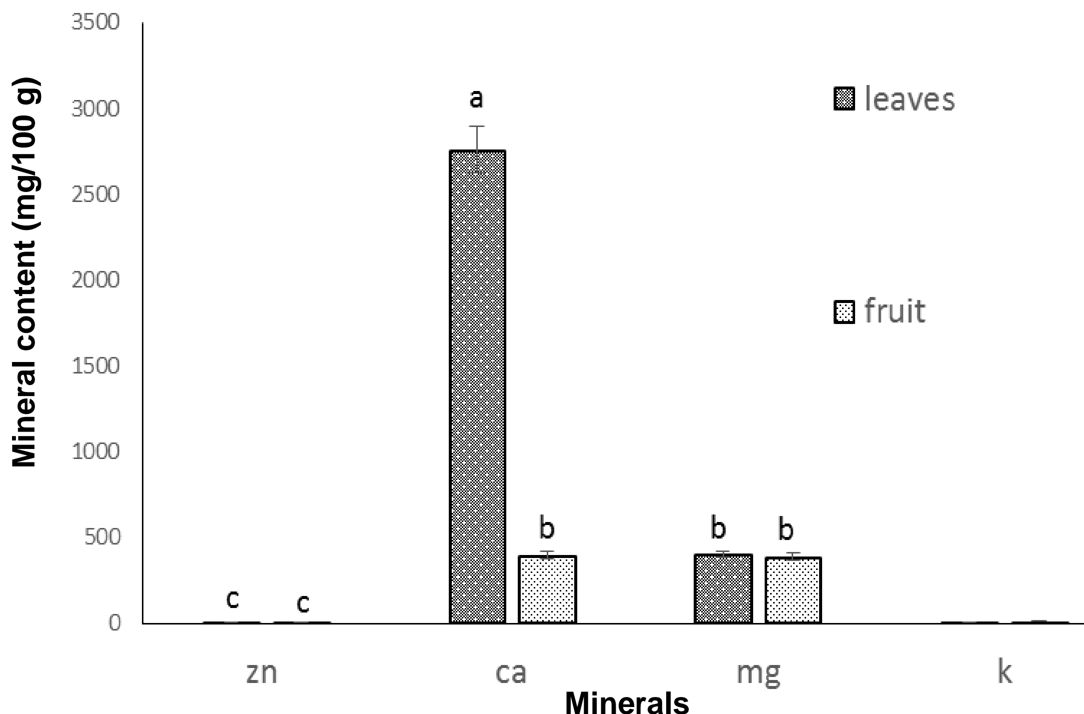


Figure 1. Mineral content of the leaves and fruit. Zn: Zinc, Ca: Calcium; Mg: Magnesium; K: Potassium.

2016 software to determine the variability of the parameters studied. Tukey's test at the 5% threshold was performed for means comparison.

RESULTS

Carbohydrates and protein content

The carbohydrates were determined using glucose as standard. Table 1 presents the carbohydrate and total protein contents expressed, respectively in mg GE/100 g fruit extract and mg GE/100 g leaf or fruit extract. The different contents varied significantly depending on the organs. Thus, in terms of total sugars, the fruits presented the best value (2304.144 mg GE/100 g) while in terms of total protein, the leaves presented the best content (22.722 ± 0.95 mg GE/100 g). The lowest values for total sugars (765.405 ± 19.54 mg GE/100 g) and protein (13.442 ± 0.91 mg GE/100 g) were recorded for leaves and fruit, respectively.

Mineral content of leaves and fruit

The mineral contents (Zn, Ca, Mg and K) expressed in mg/100 g of leaf and fruit powder are as shown in Figure 1. The mineral contents (Zn, Ca, Mg and K) expressed in mg/100 g of leaf and fruit powder are as shown in Figure 1. The results (Figure 1a and b) showed on one hand significant differences between the contents as a function of the minerals and on the other hand as a function of the organs. Calcium had the highest content while Zn and K had the lowest mineral content. In addition, the leaves had the highest Ca content compared to the fruit, which had the highest K content. No significant difference was recorded between the Zn and Mg contents of the leaf and fruit extracts.

Amino acid composition of leaves

The quantification of amino acids was limited to leaf samples only; fruits were not included in this analysis.

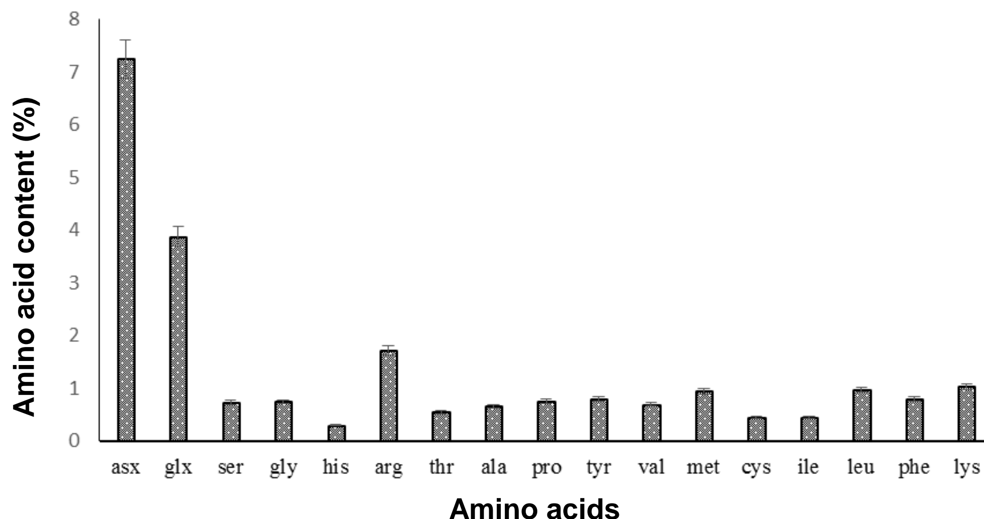


Figure 2. The amino acid composition of leaves *Capparis corymbosa*.

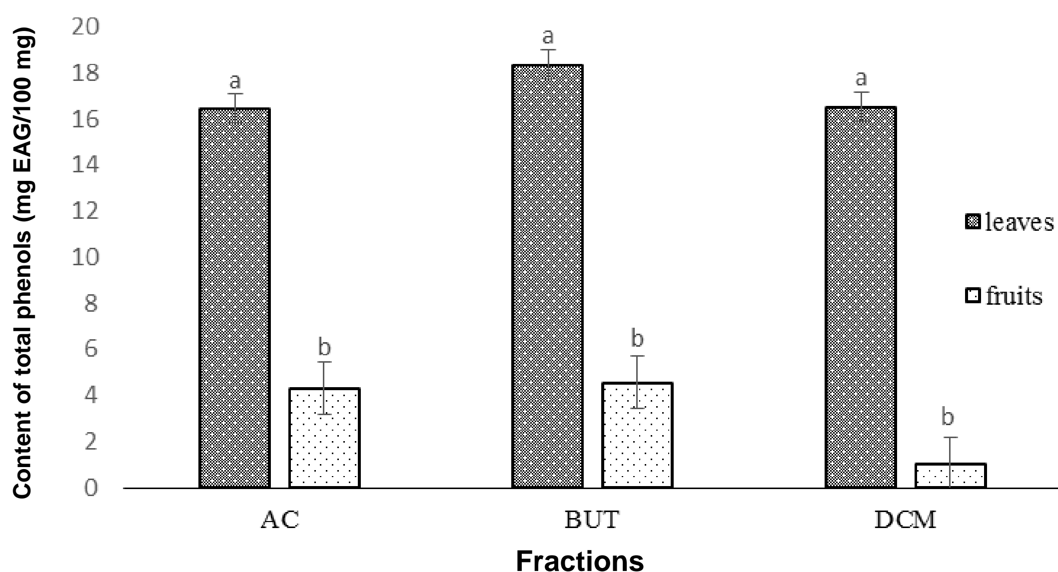


Figure 3. Contents of total phenols. The results shown in the previous figure by different letters are statistically distinct ($p<0.0001$). AC: Ethyl Acetate fraction; BUT: butanolic fraction; DCM: dichloromethane fraction.

Amino acid levels in the leaves are as shown in Figure 2. The analyses detected and quantified 17 amino acids with levels ranging from 0.30 to 7.24%. The highest content was recorded by aspartic acid (7.24%) followed by glutamic acid (3.87%), arginine (1.71%), lysine (1.02%) while the lowest content was observed in histidine (0.29%). The high levels of glutamic acid and aspartic acid could be explained by the conversion of glutamine and aspartate into their respective acids during the derivation process. In addition, apart from Tryptophan, all essential amino acids were detected and quantified in the leaves of *Capparis spinosa*. Their contents ranged from 0.43% for Isoleucine to 1.02% for Lysine.

Total phenolics and flavonoids contents of leaves and fruits

The total phenolic contents of the fractions of the different organs (leaves and fruits) expressed in g AG/ 100 g fraction are as shown in Figure 3. The total phenolic

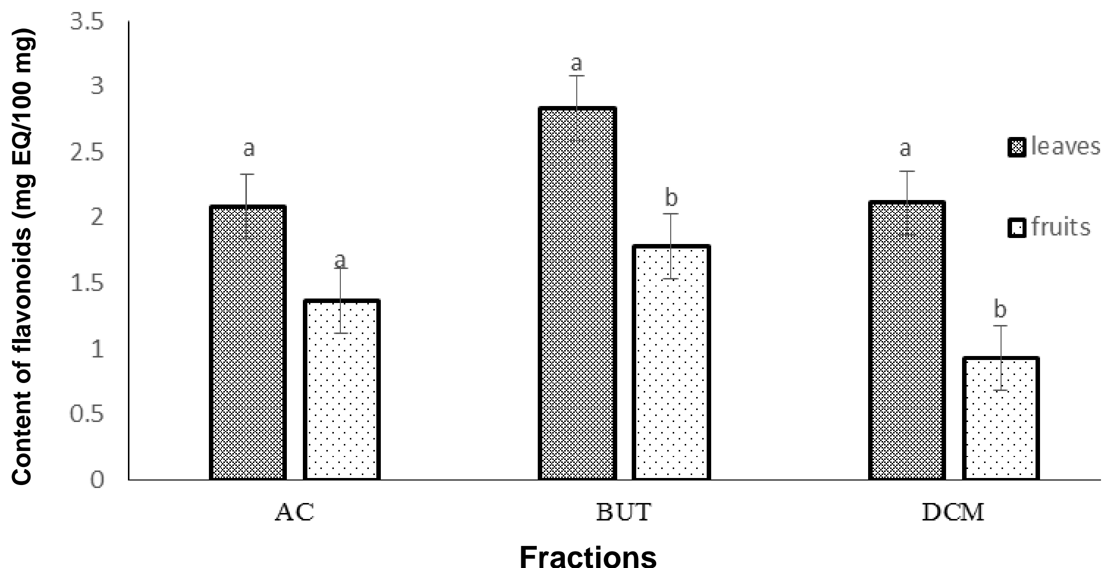


Figure 4. The total flavonoid content. The results shown in the previous figure by different letters are statistically distinct ($p<0.0001$). AC: Ethyl Acetate fraction; BUT: butanolic fraction; DCM: dichloromethane fraction.

contents varied significantly by organ and also by fraction. In general, the different leaf fractions showed higher levels than the fruit fractions. The butanolic fraction had the highest content of phenolic compounds (181.962 ± 3.62 g EAG/100 g for the leaf fraction and 96.947 ± 2.57 g EAG/100 g for the fruit fraction) while the lowest value was recorded in the DCM fraction. (165.316 ± 2.76 g EAG/100 g for the leaf fraction and 37.414 ± 5.28 g EAG/100 g for the fruit fraction).

The total flavonoid content expressed in mg EQ/100 mg of fraction of different fractions of leaves and fruits are as shown in Figure 4. The results show that the total flavonoid content of the leaves and fruit varied significantly in organ function and fractions. The butanol fraction of the leaves (2.83 ± 0.23 g EQ/100 g fraction) presented the best flavonoid content while the lowest concentration was recorded at the fraction DCM fruit (0.93 ± 0.41 g EQ/100 g fraction). In addition, the contents of total flavonoids in leaves were relatively higher than those fruit for all fractions.

Antioxidant activities

In the body, various types of oxidation occur and result in the accumulation of free radicals in the body. Also, the diversity and specificity of antioxidants (radical scavengers, electron donor or hydrogen) necessitate the evaluation of the antioxidant activity by various methods. Thus, three methods (DPPH, FRAP and ABTS) were used to evaluate the antiradical activity of the various fractions. The antioxidant activity of the different fractions is shown in Table 2. Except for the FRAP method, the variation of the various antioxidant activities is not a function of the organ. At the anti-radical activity DPPH (mg EAA/g) while the lowest activity was observed with the DCM fraction (9.54 ± 0.33 mg EAA/g) leaves. At the reducing power of the ABTS radical cation^{+}, the best activity has been recorded with the fraction of the butanol fraction (17.03 ± 0.03 mg EAA/g) fruit while the lowest activity was observed with the DCM fraction (8.01 ± 0.56 mg EAA/g) of the body. For the reduction of Fe^{3+}, the acetate fraction (0.48 ± 0.19 mg EAA/g) of the sheets presented the lowest activity whereas the highest activity with the butanol fraction (1.73 ± 0.18 mg EAA/g) leaves.

The Pearson correlation matrix between the amounts of phenolic compounds, flavonoids and antioxidant activities of the three methods is shown in Table 3. The results showed that the levels of phenolic compounds are negatively correlated respectively to levels of flavonoids ($r = -0.642$) and positively to iron reducer FRAP ($r = 0.643$). In addition, the flavonoid content is negatively and significantly ($r = -0.528$) correlated with iron reducing power while anti-radical activities determined by DPPH and ABTS methods are strongly ($r = 0.811$) correlated positively with each other.

DISCUSSION

This study focused on the evaluation of the nutritional and antioxidant potential of the leaves and fruits of *C. corymbosa*, a food plant of Burkina Faso flora. The study revealed protein and carbohydrate contents ranging from 13.44 to 22.72 mg/100 g and 765 to 20304.144 mg/100 g, respectively. The levels of total phenolics and

Table 2. Comparative table of different methods for antioxidant activity**.**

Capparis corymbosa	**Fractions**	**DPPH**	**ABTS**	**FRAP**
Leaves	Acetate fraction	11.03 ± 0.19[b]	8.92 ± 0.77[b]	0.48 ± 0.19[b]
	Butanolic fraction	12.28 ± 0.19[a]	16.90 ± 0.12[a]	0.64 ± 0.25[b]
	DCM fraction	9.54 ± 0.33[a]	5.48 ± 0.25[b]	1.07 ± 0.02
Fruits	Acetate fraction	11.55 ± 0.22[a]	14.95 ± 0.35[a]	0.90 ± 0.14[a]
	Butanolic fraction	10.94 ± 0.66[b]	17.03 ± 0.03[a]	1.73 ± 0.18[a]
	DCM fraction	10.52 ± 0.71[a]	8.01 ± 0.56[a]	3.07 ± 0.09[a]

DCM: Dichloromethane. The results shown in the previous figure by different letters are statistically distinct ($p<0.0001$).

Table 3. Pearson correlation matrix (fruit and leaves).

Variable	**Polyphenols**	**Flavonoids**	**DPPH**	**ABTS**	**FRAP**
Total phenol	1				
Flavonoids	-0,642	1			
DPPH	0,039	0,267	1		
ABTS	0,419	0,130	0,811	1	
FRAP	0,643	-0,528	-0,362	-0,218	1

The values in bold are different to 0 at a level of significance alpha = 0.05.

flavonoids ranged from 37.414 ± 5.28 to 181.962 g EAG/100 g and 0.93 ± 0.41 to 2.83 g EQ/100 g for total phenolics and flavonoids, respectively. Compared to the values reported on cowpea and *Moringa oleifera*, two plants with a recognized high nutritional potential, *C. corymbosa* appears to be a good source of nutrients, especially proteins and carbohydrates. Indeed, Sombie et al. (2019) reported protein contents ranging from 25.63 to 5.41 mg/100 g and 111 to 551 mg/100 g of fresh cowpea leaves, respectively. In addition, the results generally showed a significant variation in protein, carbohydrate, total phenolic and flavonoid contents between the leaves and fruits. The leaves had higher levels of protein, total phenolics and flavonoids than the fruits, which had higher levels of carbohydrates compared to the leaves.

It appeared that leaves and fruit of *C. corymbosa* are also a good source of micronutrients, essential amino acids and mineral elements such as calcium, zinc, magnesium and potassium that highlight obviously its nutritional potential. This variation in nutrient concentrations in organ function may be due to various factors including genotype and ecotype orders. The found high macronutrient and micronutrient contents in both leaves and fruits of the plant may justify the use of the species as a food. The particularly high concentrations of lysine, histidine, phenylalanine and methionine pave the way for recovery of the species in the human diet given the important physiological roles of these amino acids in the body. These minerals are also essential for the proper functioning of our body. In fact, they contribute to the structure of bones and teeth and are associated with heart rate, muscle contraction, nerve conduction and in the water balance and acid-base of the body (Stadtman, 1992).

The fractions of leaves and *C. corymbosa* fruit presented some interesting levels of total polyphenols and total flavonoids. The presence of bioactive compounds in interesting amounts in fruits and leaves of *C. corymbosa* could be beneficial to health. Indeed, polyphenols and flavonoids are able to counteract the production of reactive oxygen species by neutrophils and therefore reduce the risk of disease (Manallah, 2012). They may inhibit the enzymes involved in the activation of cancer cells (Crozier et al., 2007). In addition, the leaves of fractions exhibited higher levels of polyphenols and flavonoids both in sheets at the level of fruit. The levels of bioactive compounds in organ function have been reported by many authors. Indeed, many secondary metabolites have a protective role for antioxidant plants against environmental aggressors or those pathogens (Garait, 2006). So they were found often in greater concentrations in the skin of the fruit or the outer leaves of leafy vegetables (Al-Snafi, 2015). This variability in levels of compound interest, variable therefore recommends a diet based on the two bodies to benefit both compounds in the leaves than in fruits.

The different fractions of leaves and *C. corymbosa* fruit showed good antioxidant activities. In addition, our results showed that the antioxidant activity evaluated by the FRAP method was strongly correlated with the

polyphenol content. Similar results have been reported. Indeed, numerous studies have shown that the antioxidant properties of a plant product were strongly correlated with their content of phenolic compounds (Cai et al., 2004). The antioxidant potential of the latter is due ALUES redox properties that allow them to act as reducing agents hydrogen donors or electron and metal chelators (Meziti, 2009). The *Capparis* genus species is rich in phenolic compounds which are responsible for many biological activities including antioxidant activity, anticancer and antimicrobial (Turgut et al., 2015). The promising effect of antioxidants from plant health may result from their protective effect based on their ability to eliminate ROS. Some earlier reports *C. corymbosa* of antioxidant activity are very rare in the literature. Therefore, it is very difficult to compare our results with those of previous studies.

Conclusion

C. corymbosa may be good source of essentials amino-acids and also phenolic compounds that display *in vitro* high antioxidant activities. While leaves are rich in proteins, calcium and magnesium, fruits have high level of carbohydrates and minerals (calcium and magnesium). Additionally, butanol fractions of the two bodies have presented the best levels of total flavonoids and total polyphenols. The study also showed a significant variation in nutrient compounds concentrations in organ function which suggests a simultaneous consumption of both bodies.

ACKNOWLEDGEMENT

The West African Biotechnology Network (RABIOTECH, ISP/IPICS project) is appreciated for supporting publication fees and academic mobilities.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Al-Snafi AE (2015). The Chemical Constituents and Pharmacological Effects of *Capparis Spinosa*-an Overview. India Journal of Pharmaceutical Science and Research 5:93-103.

Atanasova M (2009). Revue de Génie Industriel Phénols et flavonoïdes totaux dans les extraits secs des feuilles des bouleaux argentés bulgares (*Betula pendula*). Revue de Génie Industriel 4:21-25.

Ayessou NC, Cissé M, Gueye M, Ndiaye C, Diop CM (2018). Nutritional Potential of Two Leafy Vegetables *Leptadenia hastata* Decne and *Senna obtusifolia* Link Consumed in Senegal. Food and Nutrition Sciences 9:77-85.

Bradford MM (1976). A Rapid and Sensitive Method for the Quantitation of Microgram Quantities of Protein Utilizing the Principle of Protein-Dye Binding. Analytical Biochemistry 72:248-254.

Busson FF (1965). Etude chimique et biologique des végétaux alimentaires de l'Afrique noire de l'Ouest dans leurs rapports avec le milieu géographique. Thèse de Doctorat ès Sciences Naturelles, Université Aix-Marseille, 567p.

Cai Y, Luo Q, Sun M, Corke H (2004). Antioxidant activity phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. Life Science 74:2157-2184.

Crozier S, Woimant F (2007). Infarctus cérébral grave: Quelle prise en charge? Acute management of severe is chemic stroke. Réanimation 16:441-451.

Dubois M, Gilles K, Hamilton JK, Rebers PA, Smith F (1965). Colorimetric method for determination of sugars and related substances. Analytical Chemistry 28:350-356.

Favier A (2003). Le stress oxydant : Intérêt conceptuel et expérimental dans la compréhension des mécanismes des maladies et potentiel thérapeutique. L'actualité Chimique, pp. 108-115.

Garait B (2006). Le stress oxydant induit par voie métabolique (régimes alimentaires) ou par voie gazeuse (hyperoxie) et effet de la Glisodin. Thèse de l'Université Joseph Fourier Grenoble I, 5p.

Hinnebourg I, Damien Dorman HJ, Hiltunen R (2006). Antioxidant activities of extracts from selected culinary herbs and spices. Food Chemistry 97(1):122-129.

Lamien-Meda A, Lamien CE, Compaoré MMY, Meda RTN, Kiendrebeogo M, Zeba B, Millogo-Rasolodimby J, Nacoulma OG (2008). Polyphenol content and antioxidant activity of fourteen wild elible fruits from Burkina Faso. Molecules 13:581-594.

Manallah A (2012). Activités antioxydante et anticoagulante des polyphénols de la pulpe d'olive *Olea europaea* L. Mémoire de Magister de l'Université Ferhat Abbas Setif 1:39-45.

Meziti A (2009). Activité antioxydante des extraits des graines de *Nigella sativa* L étude in vitro et in vivo. Mémoire de magister en Biochimie appliquée, Université de El Hadj Lakhdar, Batna, Algeria, 22p.

Mohamed STS, Darbar BS (2010). Red wine: A drink to your heart. Journal of Cardiovascular Disease Research 1(4):171-176.

Nacoulma OG (1996). Plantes médicinales et Pratiques médicales Traditionnelles au Burkina Faso : cas du plateau central/ Tomes I & II. Thèse d'Etat en Biochimie, Université de Ouagadougou (Burkina Faso), 320p et 260 p.

Norme internationale ISO 2171 (2007). Céréales légumineuses et produits dérivés. Dosage du taux de cendres par incinération à 550°C, 6p.

Sereme A, Millogo-Rasolodimby J, Kouda-Bonafos M, Guinko S, Nacro M (2001). Vertus thérapeutiques des Anacardiaceae en liaison avec leurs métabolites et leur richesse en tanins. Annales de Botanique de l'Afrique de l'Ouest, 00(0):63-71.

Sombie PAED, Sama H, Sidibe H, Kiendrébeogo M (2019). Effect of Organic (Jatropha Cake) and NPK Fertilizers on Improving Biochemical Components and Antioxidant Properties of Five Cowpea (Vigna unguiculata L. Walp.) Genotypes. Journal of Agricultural Science 11(10):48-62.

Stadtman ER (1992). Protein oxidation and aging. Science 257:1220-1224.

Thordser KL (2011). Amino acid analysis of savannah tree seeds. Danish Technological Institute 31p.

Turgut NH, Kara H, Arslanbaş E, Mert DG, Tepe B, Güngör H (2015). Effect of *Capparis spinosa* L . on cognitive impairment induced by D-galactose in mice via inhibition of oxidative stress. Journal of Medical Sciences 45:1127-1136.

Valko M, Rhodes Cl, Moncol I, Izakovic M, Mazur M (2006). Free radicals, metals and antioxidants in oxidative stress-induced cancer. Chemico-Biological Interactions 160:1-40.

Velazquez E, Tournier HA, Mordujovch de Buschhiazzo P, Saavedra G, Schinella GR (2003). Antioxidant activity of Paraguayan plant extracts. Fitoterapia 74:91-97.

Zerbo P, Millogo-Rasolodimby J, Nacoulma-ouedraogo OG, Van Damme P (2007). Contribution à la connaissance des plantes médicinales utilisées dans les soins infantiles en pays San, au Burkina Faso. International Journal of Biological and Chemical Sciences 1(3):262–274.

Vol. 14(7) pp. 209-214, August 2020
DOI: 10.5897/AJFS2019.1844
Article Number: 0D5A86D64641
ISSN: 1996-0794
Copyright ©2020
Author(s) retain the copyright of this article
http://www.academicjournals.org/AJFS

ACADEMIC JOURNALS
expand your knowledge

African Journal of Food Science

Full Length Research Paper

Assessment of microbial quality status of raw beef around Addis Ababa city, Ethiopia

Getenesh Teshome*, Zerihun Assefa and Abdi Keba

Ethiopian Institute of Agricultural Research P. O. Box 2003, Addis Ababa, Ethiopia.

Received 24 July, 2019; Accepted 26 September, 2019

The aim of this study was to assess the microbial quality of raw beef of butcher shops around Addis Ababa city. A total of 60 samples (N=60) of raw beef were randomly collected from Dukem (N=20), Kara (N-20) and Burayu (N=20) and biologically tested for total aerobic mesophilic bacteria, total coliform, yeast and mold, *Staphylococcus* species, *Bacillus* species and psychrophilic bacteria count using standard procedures. A mean of total aerobic mesophilic bacteria, yeast and mold, *Staphylococcus* spp., *Bacillus* spp. and psychrophilic bacteria count showed highly significant difference across the locations (P<0.05) except for total coliform. Overall, mean for total aerobic mesophilic bacteria, total coliform, yeast and mold, *Staphylococcus* spp., *Bacillus* spp. and psychrophilic bacteria count was 8.34, 4.69, 6.01, 5.36, 5.45, 4.26 log 10 cfu/g, respectively. Microbial quality of raw beef collected in Dukem site was the lowest compared to Kara and Burayu while Burayu site had the highest microbial count. The presence of high microbial count in this study might indicate improper meat handling and poor sanitary condition of slaughter houses, personnel, transportation and storage. Thus, to reduce the risks of food borne bacterial infections, there is a need to educate and be aware to practice good sanitation and safe meat handling techniques for butcher shops and personnel.

Key words: *Bacillus*, raw beef, total coliform, total aerobic mesophilic, psychrophilic, staphylococcus, yeast, mold.

INTRODUCTION

Food-borne diseases remain the most significant food safety hazards worldwide associated with beef (Maripandi and Al-Salamah, 2010) and resulting from ingestion of bacteria, toxins, and cells produced by microorganisms present in food (Clarence et al., 2009). Food and Agricultural Organization (FAO) of the United Nations and the World Health Organization (WHO) state that illness due to contaminated food is perhaps the most widespread health problem and important cause of reduced economic productivity (Käferstein, 2003). In the United States, 76 million illnesses, 325,000 hospitalizations and 5,000 deaths are caused by food-borne diseases in each year (31 known pathogens cause 9.4 million illnesses, 56,000 hospitalizations, and 1300 deaths) (Mead et al., 1999; Scallan et al., 2011). World Health Organization (WHO) has reported that 50 million children under five years of age get diarrheal diseases each year, due to the contaminated water and food stuffs

*Corresponding author. E-mail: gete.tesh@gmail.com.

Author(s) agree that this article remain permanently open access under the terms of the Creative Commons Attribution License 4.0 International License

(Tavakoli and Razipour, 2008). It occur commonly in developing countries particularly in Africa because of prevailing poor food handling and sanitation practices, inadequate food safety regulation, weak regulatory systems, lack of financial resources to invest in safer equipment and lack of education for food-handlers (WHO, 2004). Microorganisms, chemicals, and parasites are the leading contaminants associated with food borne acute gastroenteritis, among these; microbes are the most common contributor factor (Mohammed, 2011). Monthly, Prevalence of food borne acute gastroenteritis reported in China (10.9%) (Chen et al., 2018), Italy (8.9%), France (2.6%) and Canada (9.2%).

Contaminated raw meat is one of the major sources of food borne illnesses (Bhandare et al., 2007). Meat provides suitable media for growth of spoilage and pathogenic microorganisms. Health status of butcher shop workers, cloths and knives, wooden boards, and weighing scales can act as a source of microbial contamination (Abebe et al., 2019; Ali et al., 2010). A great diversity of microbes inhabits fresh meat generally, but different types may become dominant depending on pH, composition textures, storage temperature, and transportation means of raw meat (Adu-Gyamfi et al., 2012)

The microbial quality and safety of raw meat products can be estimated by the use of indicator microorganisms, including total aerobic plate count, coliform count and *Escherichia coli* count (Kim and Yim, 2016). The microbiological contamination of meat can occur during processing and manipulation, such as skinning, evisceration, storage and distribution at slaughter houses. Fecal matter is a major source of contamination and could reach carcasses through direct deposition, as well as by indirect contact through contaminated equipment, workers, installations and air (Pal, 2007). In Ethiopia, the consumption of raw meat has associated with cultural practices and widespread raw beef consumption habit that can be a potential source for food borne illnesses. Raw meat is available in open-air local retail shops without appropriate temperature control and purchased by households and served at restaurants as raw, slightly cooked or well cooked (Siddiqui et al., 2006)

However, there is limited information on raw beef microbial quality status in the country. Hence, this study addresses microbial quality status of raw beef from three potential butcher shops; Burayu, Dukem and Kara sites which are located around Addis Ababa city.

METHODS

Study design and sample collection

A cross sectional study was conducted in retail meat shop to determine bacteriological quality of raw beef between January 2017 and 2018 G.C. Three potential butcher shops were selected purposively around Addis Ababa city and a total of sixty samples (n=60) from Dukem (n=20), Kara (n=20) and Burayu (n=20) collected early morning to minimize level of contamination due to environmental temperatures. 1 kg of cut meat collected randomly from each butcher shops and collected samples were immediately transported in insulated ice containers to Holetta Dairy Research Laboratory for microbial analysis.

Meat sample preparation

A representative samples were prepared aseptically using a sterile knife to cut smaller size. Then, 25 g of beef samples were weighed and homogenized into 225 ml of 0.1% sterile buffered peptone water using a sterile blender. Samples were centrifuged for 2 min and supernatant was transferred to test tubes for microbial analysis (FDA, 2001). Collected raw beef samples were biologically tested for total aerobic mesophilic bacteria, total coliform, yeast and mold, *Staphylococcus* species, *Bacillus* species and psychrophilic bacteria count.

Microbial analysis

Total aerobic mesophilic bacteria count

A total aerobic mesophilic bacterial count was done according to FDA (2001) using plate count agar (Oxoid, CM 0325). Serial dilution was done and 1 ml of the sample from appropriate dilution was plated in duplicate using pour plate method. The plates were incubated at 35°C for 48±2 h and the result was expressed as colony forming units per g (cfu/g).

Total coliform count

A total coliform count was done according to FDA (2001) using violet red bile agar (Oxoid, CM 0107). Then, serial dilution was done and 1 ml of the sample from appropriate dilution was plated in duplicate using pour plate method. The plates were incubated at 35°C for 24 h and the result was expressed as colony forming units per g (cfu/g).

Yeast and mold count

Yeast and mold count were carried out according to (FDA, 2001) using Potato Dextrose Agar (Himedia, M096) media. A 1ppm antibiotic (Streptomycin and Chloramphenicol) was added to sterilized PDA media. Serial dilution was done and one ml of the sample from appropriate dilution was plated in duplicate using pour plate method. The plates were incubated at 25°C for 5days in dark place and the result was expressed as colony forming units per g (cfu/g).

Staphylococcus spp.

Enumeration of *Staphylococcus* spp. was done using Baird-Parker Agar medium according to FDA (2001). Serial dilution was done and 0.1 ml of the sample from appropriate dilution was plated in duplicate using spread method. The plates were incubated at 35°C±2 for 48 h and colonies of *Staphylococcus* spp. were expressed as colony forming units per gram (cfu/g).

Table 1. Microbial load of raw beef across the study locations (log_{10} cfu/g).

Microbial group	Dukem		Burayu		Kara	
	Mean ±SD	Range	Mean ±SD	Range	Mean±SD	Range
TAMB	8.09±0.18^{b}	7.75-8.47	8.82± 0.32^{a}	7.53 -9.46	8.10±0.36^{b}	8.18- 8.55
TC	4.66±0.48^{a}	4.0-5.42	4.69±0.37^{a}	3.73- 5.46	4.73 ±0.27^{a}	4.00-5.19
YM	6.13±0.23^{a}	5.63-6.36	6.15±0.34^{a}	5.63 -6.24	5.76±0.17^{b}	5.79-6.49
SSp	4.74±0.24^{b}	4.1-4.97	5.70±0.39^{a}	5.07-6.17	5.66±0.41^{a}	4.80-6.31
BSp	5.05±0.44^{c}	4.46-5.75	5.80±0.55^{a}	4.26- 6.2	5.51±0.65^{b}	4.64-6.46
PYB	3.96 ±0.34^{c}	3.15-4.33	4.17 ±0.2^{b}	4.39-4.87	4.66±0.24^{a}	3.85- 4.85

TAMB=Total aerobic mesophilic bacteria, TC=total coliform, YM=yeast and mold, SSpp=*Staphylococcus* species, BSp= *Bacillus* species, PYB=psychrophilic bacteria. All values are mean ±SD and means with different superscripts within a column are significantly different (P<0.05).

Psychrophilic

Psychrophilic bacterial count was done according to APHA (1984) using nutrient agar medium. Serial dilution was done and 1 ml of the sample from appropriate dilution was plated in duplicate using pour plate method. The plates were incubated at 6.5°C for 5 to 7 days and the result was expressed as colony forming units per g (cfu/g).

Bacillus spp.

The enumeration of *Bacillus* spp. was done using MYP agar base by spreading method (FDA, 2001). Serial dilution was done and 0.1 ml of sample from appropriate dilution was taken and spread in duplicate. Then, the plates were allowed to incubate for 24 h at 30°C and colonies of *Bacillus* spp. were expressed as colony forming units per gram (cfu/g).

Biochemical conformation

Staphylococcus and *Bacillus* spp. isolates were confirmed using morphologically, physiologically and biochemical test.

Statistical analysis

Data was analyzed using Statistical Analysis Software (SAS Inc., Cary, USA, version 9) and ANOVA was applied to compare the means of study sites. Microbial count of cfu results was log transformed before the statistical analysis and the mean separation was done by using Duncan's Multiple Range Test.

RESULTS AND DISCUSSION

Total aerobic mesophilic bacteria

The mean of total aerobic mesophilic bacteria in Dukem and Kara was 8.09 and 8.10 log_{10} cfu/g which was almost the same. This may indicate similarity of sanitary and hygienic practices between two locations. The study showed, there were highly significant differences (P<0.05) across the locations (Table 1). The mean count of total aerobic mesophilic bacteria at Burayu was the highest compared to Dukem and Kara samples. High level of total aerobic bacteria might indicate, the possibility of oxygen demanding microorganism on raw beef. The overall mean of total aerobic mesophilic bacteria in this study revealed 8.34 log_{10} cfu/g. However, HPA (2009) indicated aerobic mesophilic count must be less than 7 log cfu/g for raw meat. The present result is relatively higher than mean of fresh meat of 4.53 and 5.210 log_{10} cfu/g in Bahirdar and Adama town (Melkamnesh and Mulugeta, 2017; Gebeyehu, et al. (2013) and Elsharawy et al. (2018) revealed that aerobic plate count of 5.6 Log CFU/g from beef samples collected from Ismailia city abattoir, Egypt.

High total aerobic mesophilic bacteria count reveals potential indicators for possible presence of pathogenic microorganisms, poor sanitation and cross contamination. According to Firew et al. (2014) observational study in Jigjiga town indicated that poor sanitation and dusty environment and full remains of slaughtered animals such as bones, horn, head and other body parts observation were correlated with total aerobic mesophilic bacterial count. Hence, consumption of raw beef meat has healthy risk unless heat treating (frying) or other optimal food processing methods. Abebe et al. (2019) reported that Bisheftu butcher shops had poor hygienic condition and butcher shops workers had a low education status. Unclean slaughter houses and butcher shops, handling of meat and hot environmental condition are main source of contamination (Bhandare et al., 2007). Firew et al. (2014) revealed that, only 67% of the vendors in Jigjiga town had relatively good personal hygiene with respect to cleanness of their cloths and visible body parts.

Total coliform

The mean of total coliform count from Dukem, Burayu and Kara was 4.66, 4.69 and 4.73 log_{10} cfu/g, respectively. Total coliform count from three study

locations ranged between 4.66 and 4.73 log_{10} cfu/g. This indicates microbial quality of each samples is in close proximity due to comparable hygienic condition and post handling practices. The overall mean of total coliform count in this study was 4.69 log_{10} cfu/g. The higher coliform counts correlate with the higher levels of food-borne pathogens of fecal origin (Jay, 2000). According to HPA (2009), raw meat is categorized as unacceptable if the count of Enterobacteriaceae and coliform is greater than 4 log_{10} cfu/g. But, less total coliform count was observed contrast to Francis et al. (2015) who reported the mean of total coliform count of raw beef collected from local super market in Ghana was 7.23 log_{10} cfu/g. However, the present result was higher than that of Firew et al. (2014) finding who reported the mean of total coliform of raw beef was 4.45 log_{10} cfu/g in South East Ethiopia, Jigjiga town.

Yeast and mold

Yeast and mold counts in raw beef collected from the study site had highly significant difference across the location (P<0.05). Yeast and mold counts of beef samples collected from Kara had significant difference from beef samples collected from Dukem and Burayu sites (P<0.05). The mean value of yeast and mold counts obtained from this study was 6.01 log_{10} cfu/g. The minimum and maximum mean value of yeast and mold in this study was 5.76 and 6.49 cfu/g, respectively. Sanjay (2019) indicated that, high load of yeast and mold has direct relation with sanitation parameters. The mean of yeast and mold was less compared to yeast and mold in raw beef. This might happen due to the difference of pH in raw beef collected from different study locations.

Staphylococcus spp.

Staphylococcus spp. count in raw beef collected from the study sites had highly significant differences (P<0.05) across the study areas (Table 1). The mean value of *Staphylococcus* spp. count from all study areas was 5.4 log_{10} cfu/g. The result of this study was relatively lower than 5.5×10^5 cfu/g reported by Gebeyehu et al. (2013) in Adama town. The present result of *Staphylococcus* spp. figure may indicate poor sanitary condition in slaughter houses, transportation and blucher shops or due to contamination from the skin, mouth, or nose of the handlers can be major cause of high prevalence. Risk factors study by Adugna et al. (2018) who reported high prevalence of *Staphylococcus* from swap samples collected from cutting table, knife and hooks support the study. The current study correlated with risk factors study, conducted at Addis Ababa City, in which a total of 24 butcher shops were interviewed and 75% of the workers did not wear aprons and 58.3% of them did not cover their hair; 41.1% of the butcher shop workers used only water for cleaning (Adugna et al., 2018).

Bacillus spp.

There was a significant difference in mean value of *Bacillus* spp. count for raw beef among the study locations (P<0.05). The present study showed the highest mean value *Bacillus* spp. count was found in Burayu samples (5.80 log_{10} cfu/g), while the lowest mean value was found in Kara (5.05 log_{10} cfu/g). The overall mean value of *Bacillus* spp. count of raw beef was 5.45 log_{10} cfu/g. The result obtained is higher than Bradeeba and Sivakumaar (2012) who reported the mean value of raw beef of *Bacillus* spp. count was 2.68 log_{10} cfu/g in retail shops. Detection of *Bacillus* spp. to this level may indicate the favorable condition for *Bacillus cereus* presence which is food borne pathogen which can survive in harsh environments including normal cooking temperature. Thus, the possibility of raw beef consumption can be health risk and cause either emetic or diarrheal syndrome survives. Although, it is recommended to process raw beef, there will be possibility outbreak if processed food subjected tenderization processes. This happens under slow cooling and storage large amounts of cooked foods at temperatures between 10 and 50°C favor *Bacillus* spp.

Psychrophilic bacteria

The mean value of psychrophilic bacteria count of raw beef samples in Dukem, Burayu and Kara was 3.96, 4.17, and 4.66 log_{10} cfu/g, respectively. The overall mean value of psychrophilic bacteria count in raw beef was 4.26 log_{10} cfu/g. The result obtained was greater than the finding by Bradeeba and Sivakumaar (2012) which found 3.56 log_{10} cfu/g retail shops in Tamil Nadu. High load of psychrophilic bacteria at fresh meat might indicates possibility the flora survive even under refrigerated condition. This study can be supported by the work of Bouzid et al. (2015) who revealed psychrophilic bacteria presence in frozen meat.

The results indicated that, the highest total aerobic mesophilic bacteria, yeast and mould, *Staphylococcus* spp. and *Bacillus* spp. count were recorded from Burayu samples. Whereas, Dukem had the lowest total aerobic mesophilic bacteria, total coliform, yeast and mold, *Staphylococcus* spp., *Bacillus* spp. and psychrophilic bacteria count (Figure 1).

CONCLUSION AND RECOMMENDATION

Based on the present results, microbial load of raw beef

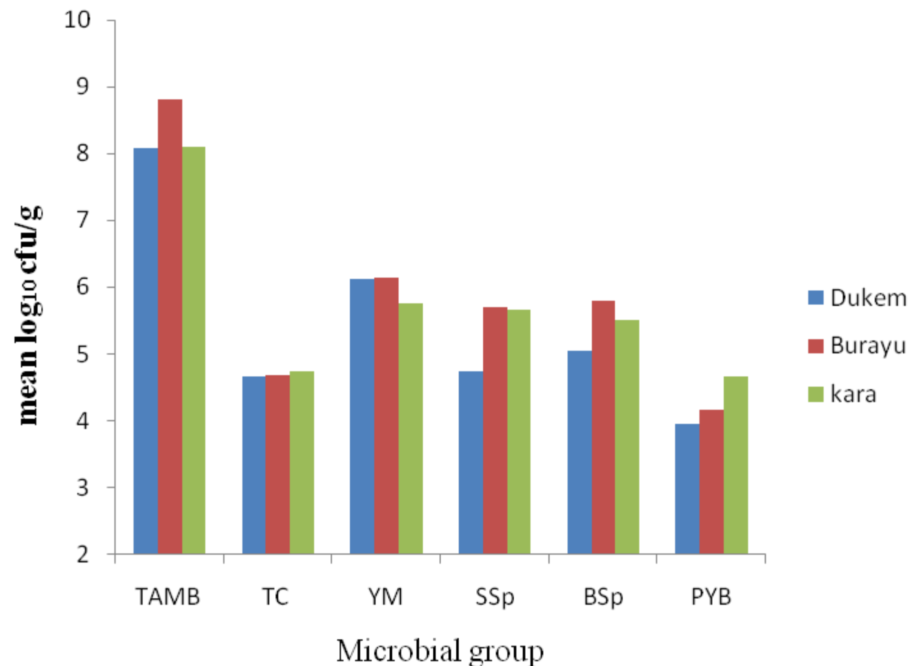


Figure 1. Mean microbial load of raw beef across the study locations. TAMB=Total aerobic mesophilic bacteria, TC=total coliform, YM=yeast and mold, SSpp=*Staphylococcus* species*, BSp=Bacillus* species*, PYB*=psychrophilic bacteria.

in the study locations is high which can be indicated to poor hygienic conditions in slaughter house, transportation and butcher shops. Hence, consuming raw beef has a healthy risk for consumers. Selling raw beef in open place and unhygienic environment, lack of cold storage transportation, lack of awareness on food safety and poor sanitation level of slaughtering houses can be main risk factors for high contamination level of raw beef for bacteria. Thus to reduce the risks of food borne bacteria, there is need to educate on practicing good sanitation and handling techniques guidelines, monitoring safety status and enforcing food safety rule and regulations for public health security and enhance safe meat production. Developing an appropriate Hazard Analysis Critical Control Point system will be important program to identify and control the hazards to improve meat microbial quality supplied to consumer and ensure food safety and quality.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Abebe B, Dereje T, Chaluma N (2019). Investigation of Bacteriological Quality of Meat from Abattoir and Butcher Shops in Bishoftu, Central Ethiopia. International Journal of Microbiology Article ID 6416803:8.

Ali N, Farooqui A, Khan A, Kazmi S (2010). Microbial contamination of raw meat and its environment in retail shops in Karachi, Pakistan. Journal of Infection in Developing Countries 4(6):382-388.

Adu-Gyamfi A, Torgby-Tetteh W, Appiah V (2012). Microbiological Quality of Chicken Sold in Accra and Determination of D10-Value of *E. coli*. Food and Nutrition Science 3(5):693-698

Adugna F, Pal M, Girmay G (2018). Prevalence and Antibiogram Assessment of Staphylococcus aureus in Beef at Municipal Abattoir and Butcher Shops in Addis Ababa, Ethiopia. BioMed Research International Article ID 5017685:7.

American Public Health Association (APHA) (1984). Compendium of Methods for Microbiological Examination of Food. Speck, M.L. Ed. American public Health Association, Washington, DC.

Bhandare SG, Sherikarv AT, Paturkar AM, Waskar VS, Zende RJ (2007). A comparison of microbial contamination on sheep/goat carcasses in a modern Indian abattoir and traditional meat shops. Food Control 18(7):854-858.

Bradeeba K, Sivakumaar PK (2012). Assessment of microbiological quality of beef, mutton and pork and its environment in retail shops in chidambaram, tamil nadu. International Journal of Plant, Animal and Environmental Science 3:1.

Bouzid R, Guemour D, Zidane K, Aggad H, Bendella A, Saegerman C (2015). Hygienic quality of minced meat retailed in western Algeria. Journal of Virology and Microbiology, pp. 5-14.

Chen Y, Yufeng W, Jiangen S, Baifeng C, Shushu D, Lei D, Jiajia D (2018). The correlation between family food handling behaviors and food borne acute gastroenteritis: a community-oriented, population-based survey in Anhui, China. BMC Public Health 18:1290.

Clarence Y, Obinna CN, Shalom NC (2009). Assessment of bacteriological quality of ready to eat food (Meat pie) in Benin City metropolis, Nigeria. African Journal of Microbiology Research 3(6):390-399.

Elsharawy NT, Ahmad AM, Abdelrahman HA (2018). Quality Assessment of Nutritional Value and Safety of Different Meat. Journal of Food Microbiology Safety Hygiene 3:132.

Food and Drug Administration (FDA) (2001). Bacteriological Analytical Manual U.S.

Firew T, Gulelat D, Ketema B, Haile A (2014). Microbiological quality and safety of street vended raw meat in Jigjiga town of Somali Regional State, Southeast Ethiopia. African Journal of Microbiology Research 8(48):3867-3874.

Francis AH, Abraham AG, Victoria A (2015). Microbiological and parasitological quality of local beef retailed in Accra and radiation sensitivity of *Salmonella* sp. International Journal of Current Microbiological and Applied Sciences 4(4):86-96.

Gebeyehu A, Yousuf M, Sebsibe A (2013). Evaluation of Microbial Load of Beef of Arsi Cattle in Adama Town, Oromia, Ethiopia. Journal of Food Process Technology 4:234.

Health Protection Agency (HPA) (2009). Guidelines for assessing the microbial safety of ready to eat foods. London: Health Protection Agency.

Jay JM (2000). Food-borne gastroenteritis caused by *Escherichia coli*. In: Jay J (Ed.), Modern Food Microbiology (5th ed., 531-543). New York, NY: Chapman and Hall.

Käferstein F (2003). Food safety as a public health issue for Developing Countries. Focus 10, brief 2 of 17. 2020 Vision for Food, Agriculture and the Environment. Washington, DC., USA.

Kim JH, Yim DG (2016). Assessment of the microbial level for livestock products in retail meat shops implementing HACCP system. Korean Journal of Food Science 36:594-600.

Maripandi A, Al-Salamah AA (2010). Multiple-antibiotic resistance and plasmid profiles of *Salmonella enteritidis* isolated from retail chicken meats. American Journal of Food Technology 5:260-268.

Mead PS, Slutsker L, Dietz V (1999). Food-related illness and death in the United States. Emerging Infectious Diseases 5(5):607-625.

Melkamnesh A, Mulugeta K (2017). The Bacteriological Quality, Safety, and Antibiogram of *Salmonella* Isolates from Fresh Meat in Retail Shops of Bahir Dar City, Ethiopia. International Journal of Food Science Article ID 4317202:5.

Mohammed FA (2011). The incidence of enterobacteriaceae causing food poisoning in some meat products. Journal of Food Science Technology 3(2):116-21.

Pal M (2007). Zoonoses, 2nd edn. Satyam Publishers, Jaipur, India pp. 104-105.

Sanjay M (2019). Relationship of Sanitation Parameters with Microbial Diversity and Load in Raw Meat from the Outlets of the Metropolitan City Biratnagar, Nepal. International Journal of Microbiology. Article ID 3547072:17.

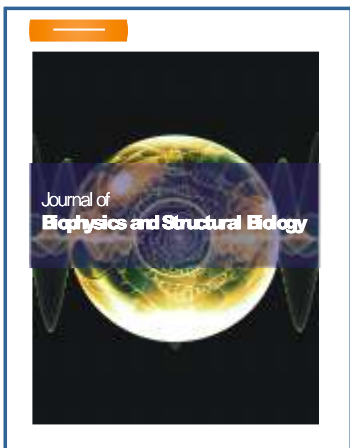
Scallan E, Hoekstra RM, Angulo FJ (2011). Food borne illness acquired in the United States-major pathogens. Emerging infectious Diseases 17(1):7-15.

Siddiqui FJ, Rabbani F, Hasan R, Nizami SQ, Bhutta ZA (2006). Typhoid fever in children: some epidemiological considerations from Karachi, Pakistan. International Journal of infectious Disease10:215-222.

Tavakoli HR, Razipour M (2008). Microbial quality of cooked meat foods in Tehran Universities Restaurants, Pakistan Journal of Medical Science 24:595-599.

World Health Organization (WHO) (2004). Regional Office for Africa “Developing and Maintaining Food Safety Control Systems for Africa Current Status and Prospects for Change”, Second FAO/WHO Global Forum of food Safety Regulators, Bangkok, Thailand pp. 12-14.

Related Journals:



www.academicjournals.org