EXTENDED ABSTRACTS
of Research Presentations

Food Techno 2018
Innovation to Application

04th Annual Research Session

INSTITUTE OF FOOD SCIENCE & TECHNOLOGY SRI LANKA (IFSTSL)

04th August 2018, *Mihilaka Medura*, BMICH, Colombo

Extended Abstracts of the Research Presentations

FoodTechno 2018

Fourth Annual Research Session of the IFSTSL

04th August 2018

Mihilaka Medura, BMICH Colombo, Sri Lanka

Organized by the Institute of Food Science & Technology Sri Lanka (IFSTSL)

The research work reported in this publication were supported by the respective institution(s) and/or interested individuals. The authors of the research papers and/or the supporting institution(s) have equity ownership in developing products related to the reported research.

Citation Guide for this Publication:
Author(s) name, (2018), *Title of the research*. Extended Abstracts of the FoodTechno 2018, 4th IFSTSL Annual Research Session (04th August), Institute of Food Science & Technology Sri Lanka, *pp.*

***Publisher*:** Institute of Food Science & Technology Sri Lanka,
No.1, 1/1, Anderson Road, Colombo-05, Sri Lanka

***Publishing Year*:** 2018

***Printer*:** Nethwin Printers (Pvt.) Ltd., Daladasewane Api Padanama, Getambe, Peradeniya, Sri Lanka

ISSN: 2579-2024

Fourth Annual Research Session of the IFSTSL - 2018

(04th August 2018 at Mihilaka Medura, BMICH Colombo, from 8.30 a.m. to 5.10 p.m.)

PROGRAM

8.30 – 9.00 a.m.	Registration of participants
9.00 – 9.05 a.m.	Traditional lighting of the oil lamp
9.05 – 9.10 a.m.	Welcome address by Dr. Eresha Mendis *Coordinator/ FoodTechno 2018*
9.10 – 9.15 a.m.	Address by Dr. Sujeewa Gunaratne *President/ IFSTSL*
9.15 – 9.40 a.m.	Keynote speech by Mr. Ravi Jayawardena *Group CEO/ Maliban Biscuit Manufactories (Pvt) Ltd*
9.40 – 10.10 a.m.	***TEA***

TECHNICAL SESSION I (*Session Chair: Dr. R. A. U. J. Marapana*)
(Head/ Dept. of Food Science & Technology, Faculty of Applied Science, University of Sri Jayewardenepura)

10.10 – 10.35 a.m.	ANTIMICROBIAL ACTIVITIES OF PROBIOTICS FROM FINGER MILLET AND BANANA ***Divisekera D.M.W.D.**, Samarasekera J.K.R.R., Hettiarachchi C., Iqbal C.M., Gooneratne J. and Gopalakrishnan S.*
10.35 – 11.00 a.m.	EXTENSION OF SHELF-LIFE OF FRESH-CUT TOMATO AND LETTUCE USING CHEMICAL TREATMENTS ***Madusanka G.D.**, Rajapakse R.P.N.P. and A.C. Pathirage*
11.00 – 11.25 a.m.	ANTIBACTERIAL AND METAL CHELATING PROPERTIES OF PEPTIDES DERIVED FROM OVALBUMIN USING PROTEASE ENZYME UNDER DIFFERENT CONDITIONS ***Madushani M.A.A.**, Aruppala A.L.Y.H. and Abeyrathne E.D.N.S*

11.25 – 11.50 a.m.	RICE BRAN AS AN INGREDIENT IN FUNCTIONAL BEVERAGE, AND COMPARISON OF PHYSICOCHEMICAL AND SENSORIAL CHARACTERISTICS OF SELECTED EXTRACTION METHODS ***Gamakumara G.K.M.E.A.*** *and Perera O.D.A.N.*
11.50 – 12.15 p.m.	DEVELOPMENT OF "KIRIKAWADI" CASSAVA FLOUR SUBSTITUTED PASTA PRODUCT ***Jeevana T.*** *and Perera O.D.A.N.*
12.15 – 1.15 p.m.	***LUNCH***

TECHNICAL SESSION II (*Session Chair: Emeritus Professor T.S.G. Fonseka*)
(Faculty of Livestock & Fisheries, Wayamba University of Sri Lanka)

1.15 – 1.40 p.m.	EVALUATION OF GAHALA AND INNALA FLOURS AS WHEAT FLOUR SUBSTITUTES FOR BREAD MAKING *Attanayaka A.M.V.C. and* ***Gunathilake K.D.P.P.***
1.40 – 2.05 p.m.	FORMULATION AND QUALITY EVALUATION OF JACKFRUIT SNACK THROUGH VALUE ADDITION OF LOCAL JACKFRUIT (*Artocarpus heterophyllus* L.) ***Rathnayake R.M.M.L.****, Dalukdeniya D.A.C.K. and Jayasooriya M.C.N.*
2.05 – 2.30 p.m.	PUTRESCINE TREATMENTS ENHANCE QUALITY AND EXTEND THE POSTHARVEST LIFE OF LIME (*Citrus aurantifolia* Swingle) IN COLD STORAGE CONDITION *Gamage K.G.N.M.,* ***Champa W.A.H.*** *and Mendis B.E.P.*
2.30 – 2.55 p.m.	BIOACTIVE PROPERTIES OF THREE SRI LANKAN MEDICINAL PLANTS AND THEIR CHEMICAL PROFILING ***Wickramarachchi D.,*** *Visvanathan R., Nizar A., Bawazeer M., Tissera B., Qader M., Rateb M.E. and Liyanage R.*
2.55 – 3.15 p.m.	***TEA***

TECHNICAL SESSION III (*Session Chair: Dr. Niranjan Rajapakse)*

(Head/ Dept. of Food Science & Technology, Faculty of Agriculture, University of Peradeniya)

3.15 – 3.40 p.m.	THE AMOUNT OF ACTIVE HYDROGEN IN PERISHABLES, JUICES AND CHANGES WITH THERMAL AND NON-THERMAL (ULTRASONIC AND MICROFILTRATION) PROCESSING TECHNIQUES ***Amunugoda P.N.R.J.*** *and Jayasinghe G.D.D.R.*
3.40 – 4.05 p.m.	THE BEHAVIOR OF SRI LANKAN FOOD PROCESSING COMPANIES TOWARDS VOLUNTARY STANDARD CERTIFICATIONS ***Karunasiri J.P.R.N.****, Mendis B.E.P. and De Alwis M.*
4.05 – 4.30 p.m.	EVALUATION & COMPARISON OF REFRESHING READY TO SERVE (RTS) BEVERAGE OF COCONUT HAUSTORIUM WITHOUT CHEMICAL PRESERVATIVES ***Senarath S.A.C.T****. and Perera O.D.A.N*
4.30 – 4.55 p.m.	VALIDATION OF AN IN- HOUSE METHOD DEVELOPED FOR THE DETECTION OF COLIFORMS IN MILK POWDER BASED ON ISO 4831:2006 STANDARD METHOD ***Weragala W.N.K.*** *and Arampath P.C.*
4.55 – 5.05 p.m.	Award of certificates
5.05 – 5.10 p.m.	Vote of thanks by Ms. Choshani Dalukdeniya/ Joint Secretary IFSTSL

Message from the President of the IFSTSL

The Fourth Annual Research Session, FoodTechno 2018, organized by the Institute of Food Science and Technology, Sri Lanka (IFSTSL) is scheduled to be held on 4th August 2018, parallel to the Profood/Propack-AgBiz Exhibition at the BMICH.

The theme of this research session is Innovation to Application, where IFSTSL expects to disseminate research knowledge acquired in universities, research institutes and other line agencies to the food industry seeking possible applications targeting innovation. The institute of food science and technology, in its mandate clearly identifies its service to the food sector specially aiming at the developments of the food industry in Sri Lanka. To accomplish this objective, it requires connecting different stakeholder groups of the Sri Lankan food sector, specially the universities, research entities and the food industry in a common platform. The Annual Research Session commenced in 2015, and from there onwards, each year the research being presented has increased in quality as well as in quantity. With the valuable learnings received as organizers, the IFSTSL is now organizing the Annual Research Session in concurrence with the Profood/Propack-AgBiz Exhibition, which is the largest food exhibition in Sri Lanka and where the food industrialists get together to showcase their innovative and production capacities and capabilities to the public in Sri Lanka. We also continue to request the service of judges from food industries, mainly because the applicability of research in food science could be best assessed by food industry itself. It is commendable that the research presenters have been positively accepting the comments, and made progress in their research, as well as positive changes towards commercial applicability of their work.

As the president of the Institute of Food Science and Technology, I wish to extend my sincere thanks to our editor Dr. Eresha Mendis, the coordinator of the Annul Research Session, and Dr. Niranjan Rajapakse, our past president, for their untiring efforts to make this research session a great success. Further, I offer my sincere thanks to the members of Executive Committee of the IFSTSL who supported this year's IFSTSL activities, especially encouraging this very important event.

I wish all the participants a successful Annual Research Session 2018.

Dr. Sujeewa Gunaratne
President/ IFSTSL

Message from the Coordinator of FoodTechno 2018

I am glad that the Institute of Food Science and Technology Sri Lanka (IFSTSL) continue its effort to hold its research session "Food Techno: Innovations to Application" annually and this year for the fourth consecutive time. Food Techno, I personally see as one of the important events organized by the IFSTSL in accomplishing its mandate which foresee effective link between research entities with the food industry. The food industry in Sri Lanka is one of the leading industries and growing at a smart pace. Sri Lankan food industry continues its efforts to expand its territories in the world food market though with lots of practical challenges. In this challenging effort, diversification in terms of products and processes becomes vital and innovations strengthen the path. Not many SL food manufacturing companies successfully utilizes the concept of branding through innovations which is one of the main ways to become competitive. Higher numbers of product innovations and innovative processes that can be applied for the betterment of the SL food industry are resulted from the research carried out by research entities in Sri Lanka including Universities. However, that knowledge is not satisfactorily brought to the notice of the industry and lag in scientific compilations. Thus the objective of this research session to present research findings to the industry especially during Profood/Propack-AgBiz exhibition where the industry gather largely seeking promotions is timely and meaningful. However, success of any effort would depend on the collaboration and efforts of all the parties involved. Therefore, as the coordinator of this event I expect the fullest cooperation from the food industry, who is the main target group of the event to support FoodTechno in accomplishing its target. I thank all the research presenters of this year for disseminating their research findings at this important forum.

I wish all the very best for "Food Techno" to continue its efforts in years to come!

Dr. Eresha Mendis
Coordinator/ FoodTechno 2018

Extended Abstracts of the Research Presentations

FoodTechno 2018- *Fourth Annual Research Session of the IFST$_{SL}$*

(04th August 2018 at Mihilaka Medura, BMICH Colombo, from 8.30 a.m. to 5.10 p.m.)

TABLE OF CONTENTS

TECHNICAL SESSION I

TECHNICAL SESSION II

TECHNICAL SESSION III

ANTIMICROBIAL ACTIVITIES OF PROBIOTICS FROM FINGER MILLET AND BANANA

Divisekera D.M.W.D*, Samarasekera J.K.R.R., Hettiarachchi C.[1], Iqbal Choudhary M.[2], Gooneratne J. and Gopalakrishnan S.[3]
Industrial Technology Institute, Colombo 07, Sri Lanka

Summary

Out of twenty five potential lactic acid bacteria (LAB) isolated from nondairy substrates finger millet and banana varieties of Sri Lanka, eight isolates exhibiting superior probiotic attributes and demonstrated safety to consume orally by rats were further investigated for antimicrobial activity in-vitro. A significant difference ($P < 0.05$) in antibacterial activity was observed among the all tested probiotic strains against both drug sensitive and multi drug resistant pathogens causing infections in humans and animals. Newly isolated promising probiotic strains were deposited in the culture collection of the International Centre for Chemical and Biological Sciences, Pakistan. These probiotic candidates can be used as industrial starter cultures to produce nondairy probiotic food, claiming their bio-functionality after confirming their safety in clinical trials.

Keywords: Antimicrobial activity, Banana, Finger millet, Non-dairy probiotics

Introduction

Antimicrobial resistance is one of the health threats on global populations including Sri Lankans. Probiotics are gaining recognition in commercial pharmaceutical applications as they are known to confer functional health benefits to the host. Expanding beyond satisfaction of hunger, reduction of health care cost, increase of life expectancy and improvement of quality of life has increased the demand for probiotic choices as self-medications that are alternative to antibiotics. The drift towards vegetarianism, high prevalence of lactose intolerance in many populations around the world, high cholesterol content in dairy products and allergenic milk proteins are the key contributory factors for the development of novel non-dairy probiotic foods and supplements (Vijayendra and Gupta, 2012). Considering the available resources of non-dairy substrates such as cereals, fruits, vegetables, pulses and fish, and its potential safety, probiotic pharmaceutical preparations could be developed, free from issues associated with dairy probiotics. Particularly, raw flours of finger millet and unripe banana are reported to possess high contents of dietary fibre and resistant starch make them suitable as prebiotic substrate for the growth of probiotic bacteria with potential

[1] *Department of Chemistry, Faculty of Science, University of Colombo, Sri Lanka*
[2] *International Centre for Chemical and Biological Sciences, University of Karachi, Pakistan*
[3] *International Crops Research Institute for the Semi-Arid Tropics, Hyderabad, India*
** Corresponding author: mb.wasu@gmail.com*

health benefits (Jayawardana *et al*., 2017). Furthermore, recent technological advances have made it possible to alter the structural characteristics of cereal and fruit matrices, thus making them more resourceful as food substrates. The present study isolate, identify and assess the antibacterial activity of probiotic lactic acid bacteria (LAB) from substrates of non-dairy origin; fermented flour of finger millet and banana varieties grown in Sri Lanka.

Methodology

Finger millet (*Elucinecoracana*) varieties; ravi, ravanaand oshadha and banana (*Musa* species) varieties; kolikuttu, ambulnadee, kandula, rathkesel, anamalu, seeni, seeniparakum and nethrappalamin unripecondition were selected for the study. Samples were obtained from the germplasms of Department of Agriculture. The millet grains were washed with sterile water and fleshy parts of banana samples were soaked in 1% (w/v) solution of sodium metabisulphite for 10 min to avoid enzymatic browning, both the samples were at 35 ± 2 ^{O}C till the moisture content reduced to < 10%. The samples were ground in a variable Speed Rotar Mill to prepare flour.

Batters were prepared by mixing flour with sterilized tap water in 1:3 (w/v) ratios, and fermented at room temperature (24 ± 3 ^{O}C) for 18 h (Ogunremi *et al.*, 2015). Fermented batters were serially diluted and LAB was isolated using de Man Rogosa and Sharpe Agar (Bassyouni *et al*., 2012).All isolates were phenotypically and biochemically characterized. Isolates with typical phenotypic and biochemical characteristics of LAB were identified using 16S rRNA sequencing, their partial sequence was deposited in gene bank and phylogenetic relationships were predicted. They were further investigated for survival in simulated gastric conditions (acid, bile, salt, temperature, phenol and gastric juice) (Aswathy *et al.*, 2008).

The safety attributes of the isolates including antibiotic resistance, presence of virulence causing enzymes were studied and their cell aggregation and adhesion activity was investigated in-vitro. Selected probiotic strains were evaluated for repeated dose acute and sub-chronic oral toxicity in wistar rats. Eight potential probiotics of three different doses of 10^8, 10^{10} and 10^{12} CFU/ml were fed daily for 14 days during acute toxicity and for 8 weeks during sub-chronic toxicity. Total 350 wistar rats including 150 rats for acute toxicity testing (3 male+3 female in one treatment group) and 200 rats for sub-chronic toxicity testing (4 male+4 female in one treatment group) were used. Feed and water intake and animal behavior were investigated daily throughout the study. Animal weight, blood biochemistry, hematology, tissue histopathology, bacterial translocation in blood and tissues were observed at the end of experiment.

Anti-bacterial activity of probiotic candidates was studied against drug sensitive organisms; *Escherichia coli* ATCC 2592, *Klebsiella pneumoniae* ATCC 35594, *Staphylococcus aureus* ATCC 6571, *Streptococcus sanguinis* ATCC 10556, *Streptococcus salivarius* ATCC 13419, *Enterococcus faecalis* ATCC49532, *S. flexneri* ATCC 12022, *Acinetobacter baumannii* ATCC 17978, *Streptococcus mutans* ATCC 25175, *Streptococcus pyogenes* ATCC 700294 and multi drug resistant organisms; *Escherichia coli* ATCC 35218, *Staphylococcus aureus* 16 EMRSA NCTC 13143, *Klebsiella pneumoniae* ATCC 700603, *Enterococcus faecalis* ATCC 700802, *Salmonella enterica* ATCC 700408 (Tharmaraj and Shah, 2009).

Results and Discussion

Two hundred and forty bacterial colonies were isolated from fermented flour of above varieties of finger millet and banana. Among them, 25 isolates possess typical LAB phenotypic and biochemical characteristics including fifteen from finger millet; *Paenibacillus* species (MF480545.1), *Bacillus cereus* (MF480550.1), *Streptococcus lutetiensis* (MF574476.1), *Brevibacillus borstelensis* (MF480552.1), *Lactobacillus plantarum* (MF405176.1), *Lactobacillus fermentum* (MF033346.1), *Bacillus cereus* (MF480468.1), *Lactobacillus fermentum* (MF405134.1), *Lactococcus lactis* subspecies lactis (MF480428.1), *Enterococcus faecium* (MF420431.1), *Bacillus cereus* (MF754478.1), *Bacillus cereus* (MF574479.1), *Bacillus cereus* (MF574477.1), *Pediococcus acidilactici* (MF480434.1) and *Enterococcus lactis* (MF574475.1) and ten from banana; *Enterococcus durans* (MF405179.1), *Enterococcus gallinarum* (MF480436.1), *Lactobacillus plantarum* (MF405177.1), *Weissellacibaria* (MF480445.1), *Enterococcus hirae* (MF480429.1), *Enterococcus faecium* (MF574466.1), *Lactobacillus curieae* (MF405178.1), *Enterococcus durans* (MF480435.1), *Pediococcusacidilactici* (MF480433.1) and *Enterococcus faecium* (MF480430.1).

Among the 25 LAB strains, five from finger millet, *L. plantarum, L. fermentum, L. lactis* sub species lactis, *E. faecium* and *P. acidilactici* and three from banana *E. durans, E. faecium* and *L. curieae* exhibited superior survival in simulated gastric conditions indicating growth at tested concentrations of acid, bile, salt, phenol and gastric juice and temperature between 30 - 42^{O}C. They were free from virulence (hemolysis, DNAse and gelatin hydrolyzing) causing enzymes and did not demonstrate major antibiotic resistant pattern *in-vitro*. *Enterococcus durans* demonstrated highest auto-aggregation of 76.53 ± 0.59%, *L. fermentum* and *E. durans* exhibited the highest co-aggregating activity of 67 ± 0.21% and *E. durans* exhibited highest adhesion of 72.5 ± 5.90% and 74.16 ± 4.89% to HCT-116 and HT-29 cell lines, respectively.

In 14 days repeated dose acute oral toxicity study, and in eight week sub-chronic toxicity study no treatment-related sign of toxicity, mortality or abnormal hematology and biochemistry of rat blood were observed in both male and female rats who received 10^8, 10^{10} and 10^{12} CFU/ml of LAB strains.

A significant difference ($P < 0.05$) in antibacterial activity was observed among the all tested LAB isolates against drug sensitive pathogens (Table 1a) as well asmulti drug resistant pathogens (Table 1b). The LAB isolated from fermented flour of finger millet, *L. plantarum, L. fermentum, L. lactis* subspecies lactis, *E. faeciumand, and P. acidilactici*, showed inhibition against multi drug resistant pathogens except *K. pneumonia* and *E. faecalis*. Similar anti-bacterial activity was observed from LAB isolated from fermented flour of banana. The anti-bacterial activity is one of the key parameters to evaluate the efficacy of potential probiotic candidates that makes them ideal to use in alternative therapy to treat bacterial infections. The anti-bacterial activity of the isolated LAB in this study revealed that these strains can inhibit both drug sensitive as well as multi drug resistant human pathogens which cause infections in gut, skin and respiratory track.

Conclusion

Flour of banana and finger millet varieties grown in Sri Lanka are good substrates for probiotics. These findings can be used to develop commercial non-dairy probiotic food with bio-functional claims such as anti-bacterial activity. This is the first report of investigating non-dairy substrates to produce probiotics in Sri Lanka.

Table 1(a): Anti-bacterial activity of lactic acid bacteria against drug sensitive pathogens.

	Anti-bacterial activity (zones of inhibition in mm)							
Pathogenic strain	***L.plantarum* (R17)**	***L.fermentum* (RV02)**	***L.lactis* subspecies *lactis* (RV19)**	***E.faecium* (RV28)**	***P. acidilactici* (O24)**	***E. durans*(K08)**	***E.faecium* (SP26)**	***L.curieae*(AN18)**
Escherichia coli	15.33 ±0.33^{b}	16.67 ± 1.45^{b}	15.33 ± 0.33^{b}	17.33 ± 0.33^{b}	15.33± 0.33^{b}	16.66 ± 0.33^{b}	15.00 ± 0.57^{b}	16.66 ± 0.33^{b}
Klebsiella pneumoniae	15.33 ±0.33^{c}	14.66 ± 0.33^{c}	15.00 ± 0.00^{c}	18.33 ± 0.33^{b}	18.33± 0.33^{b}	14.33 ± 0.33^{c}	15.33 ± 0.33^{c}	18.66 ± 0.33^{b}
Staphylococcus aureus	10.33 ±0.33^{d}	13.33 ± 0.33^{c}	12.66 ± 0.33^{c}	13.33 ± 0.33^{c}	13.00 ± 0.57^{c}	15.66 ± 0.33^{b}	10.66 ± 0.66^{d}	11.66 ± 0.33^{cd}
Streptococcus sanguinis	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}
Streptococcus salvarius	14.66 ± 0.33^{d}	16.00 ± 0.00^{c}	16.00 ± 0.00^{c}	10.66 ± 0.33^{e}	14.00 ± 0.00^{d}	20.33 ± 0.33^{b}	0.00 ± 0.00f	0.00 ± 0.00^{f}
Shigellaflexenari	14.33 ± 0.33^{b}	15.66 ± 0.33^{b}	16.00 ± 0.00^{b}	12.33 ± 0.66^{c}	15.66 ± 0.33^{b}	13.00 ± 0.00^{c}	17.00 ± 0.00^{b}	13.66 ± 0.66^{c}
Enterococcus faecalis	8.33 ± 0.33^{d}	0.00 ± 0.00^{e}	0.00 ± 0.00^{e}	0.00 ± 0.00^{c}	12.66 ± 0.33^{e}	0.00 ± 0.00^{e}	16.66 ± 0.66^{b}	12.66 ± 0.33^{c}
Acinetobacterbaumannii	0.00 ± 0.00^{g}	15.33 ± 0.33^{c}	11.33 ± 0.33^{f}	13.00 ± 0.00^{e}	14.33 ± 0.33^{d}	17.33 ± 0.33^{b}	14.33 ± 0.33^{d}	13.00 ± 0.00^{e}
Streptococcus mutans	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	11.00 ± 0.57^{b}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}
Streptococcus pyogenes	0.00 ± 0.00^{e}	12.00 ± 0.00^{c}	12.33 ± 0.33^{c}	15.00 ± 0.57^{b}	13.66 ± 0.33^{bc}	13.33 ± 0.33^{c}	10.33 ± 0.33^{d}	10.33 ± 0.33^{d}

Data is expressed as mean ± SEM, n=9. With in a row mean values superscripted with different letters are significantly different ($P < 0.05$). The letters R, RV and O refers to lactic acid bacteria isolated from Ravi, Ravana and Oshadha varieties of finger millet, respectively. The letters K, SP and AN refers to lactic acid bacteria isolated from Kolikuttu, Seeniparakum and Ambulnadee varieties of banana, respectively. Inhibition zones < 4 mm in diameter: weak activity, inhibition zones of 5–9 mm in diameter: average activity, inhibition zones > 10 mm in diameter: strong activity.

Table 1(b): Anti-bacterial activity of lactic acid bacteria against multi drug resistant pathogens.

Pathogenic strain	Anti-bacterial activity (zones of inhibition in mm)							
	L.plantarum (R17)	*L.fermentum* (RV02)	*L.lactis* subspecies *lactis* (RV19)	*E.faecium* (RV28)	*P. acidilactici* (O24)	*E. durans*(K08)	*E.faecium* (SP26)	*L.curieae*(AN18)
Escherichia coli	15.33 ± 0.33^{c}	16.00 ± 0.00^{c}	18.33 ± 0.33^{b}	16.66 ± 0.33^{bc}	15.33 ± 0.33^{c}	16.00 ± 0.00^{c}	14.33 ± 0.66^{cd}	17.66 ± 0.66^{cd}
Staphylococcus aureus 16*	17.33 ± 0.33^{c}	15.00 ± 0.00^{b}	17.66 ± 0.66^{c}	18.66 ± 0.66^{b}	15.66 ± 0.33^{b}	20.33 ± 0.33^{bc}	16.00 ± 0.00^{bc}	19.66 ± 0.66^{b}
Klebsiella pneumonia	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	12.33 ± 0.88^{b}	0.00 ± 0.00^{c}
Eenterococcusfaecalis	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	0.00 ± 0.00^{c}	13.33 ± 0.61^{b}	0.00 ± 0.00^{c}
Salmonella enterica	12.00 ± 0.00^{d}	15.33 ± 0.33^{b}	9.66 ± 0.88^{d}	16.00 ± 0.00^{b}	15.66 ± 0.66^{b}	13.33 ± 1.20^{bd}	15.00 ± 0.00^{bc}	13.33 ± 0.33^{bd}

Data is expressed as mean ± SEM, n=9. With in a row mean values superscripted with different letters are significantly different ($P < 0.05$).LAB intracellular cell free extracts were testedat500 µg/ml concentration. The letters R, RV and O refers to lactic acid bacteria isolated from Ravi, Ravana and Oshadha varieties of finger millet, respectively. The letters K, SP and AN refers to lactic acid bacteria isolated from Kolikuttu, Seeniparakum and Ambulnadee varieties of banana, respectively.* Epidemic Methicillin Resistant. Inhibition zones < 4 mm in diameter: weak activity, inhibition zones of 5–9 mm in diameter: average activity, inhibition zones > 10 mm in diameter: strong activity

References

Aswathy, R.G., Ismail, B., John, R.P. & Nampoothiri, K.M. (2008). Evaluation of the probiotic characteristics of newly isolated lactic acid bacteria. Appl Biochem & Biotechnol, 151- 244-255.

Bassyouni, R.H., Abdel-all, W.S., Fadl, M.G., Abdel-all, S. and Kamel, Z. (2012), Characterization of Lactic Acid Bacteria Isolated from Dairy Products in Egypt as a Probiotic. J.Life Sci, 9, 2924-2930.

Jayawardana, S. A. S., Divisekera, D.M.W.D., De Silva, A.B.G.C.J., Yathursan, S., Samarasekera, J. K. R. R.and Gooneratne, J. (2017), Ensuring human health, food and nutritional security through novel cereal and fruit based prebiotics and probiotics. Abstracts of papers, 1st Indo Sri Lanka joint research symposium, Sri Lanka, 12-13.

Ogunremi, O. R., Agrawal, R. andSanni, A. I. (2015), Development of cereal-based functional food using cereal-mix substrate fermented with probiotic strain –PichiakudriavzeviiOG32. J. Food Sci&Nutr, 3(6), 486–494.

Tharmaraj, N. and Shah, N.P. (2009), Antimicrobial effects of probiotics against selected pathogenic and spoilage bacteria in cheese-based dips. Int J. Food Res, 16, 261-276.

Vijayendra, S.V.N. and Gupta, R.C. (2012), Assessment of probiotic and sensory properties of dahi and yoghurt prepared using bulk freeze-dried cultures in buffalo milk. Ann Microbiol, 62, 939-947.

EXTENSION OF SHELF-LIFE OF FRESH-CUT TOMATO AND LETTUCE USING CHEMICAL TREATMENTS

Madusanka G.D., Rajapakse R.P.N.P. and A.C. Pathirage[1,*]
Department of Food Science & Technology, Faculty of Agriculture,
University of Peradeniya, Sri Lanka

Summary

During storage, fresh-cut lettuce (*Lactuca sativa* L.) and tomato (*Lycopersicon esculentum* Mill.) undergo rapid quality deterioration due to high water content and elevated metabolic reactions. The objective of this research was to extend the shelf-life of fresh-cut lettuce and tomato using chemical treatments. Dipping these commodities in food grade chlorine was used as a pretreatment measure to reduce their surface microbial load. Dipping in calcium chloride was performed to improve tissue firmness while dipping in ascorbic acid aimed to reduce browning. Surface microbial load was reduced significantly ($p<0.05$) by the treatment of food grade chlorine releasing sanitizing agent. Firmness was maintained significantly ($p<0.05$) by treating 0.2% calcium chloride in lettuce and 0.1% calcium chloride in tomato. Browning was significantly reduced ($p<0.05$) by treating 1.0% ascorbic acid in both lettuce and tomato. Shelf-life of fresh-cut lettuce could be increased up to 5 days of storage period at 4 ^{O}C by dipping in 200 ppm food grade chlorine for 2 minutes as a pretreatment with a combination of 0.2% calcium chloride and 1.0% ascorbic acid for 3 minutes. Shelf-life of fresh-cut tomato could be increased up to 5 days of storage period at 4 ^{O}C by dipping in 100 ppm food grade chlorine solution for 2 minutes as a pretreatment with a combination of 0.1% calcium chloride and 1.0% ascorbic acid for 3 minutes. Thus the chemical treatment levels used in this study that selected based on safe concentrations were become effective in increasing shelf life of fresh-cut lettuce and tomato.

Keywords: Shelf life; Fresh-cut; lettuce (*Lactuca sativa* L.); Tomato (*Lycopersicon esculentum* Mill.); Ascorbic acid; Calcium chloride

Introduction

Fruits and vegetables are perishable entities that continue to change after harvesting. During minimal processing, plant tissues incur physical damage and it triggers certain metabolic reactions (Brecht, 1995). The soft, soggy appearance or fermented odor of freshly cut product may give rise to consumer rejection prior to consumption. Textural changes are occurred due to enzymatic and non-enzymatic reactions due to cell damage (Barrett and Ni, 2005). Because freshly cut vegetables are living tissues even after treatment also.

[1] *HJS Condiments (Pvt) Ltd, Biyagama, Sri Lanka*
**Corresponding author: ananda.pathirage@hjs.hayleya.com*

In fresh-cut fruit and vegetable industry chlorine is used as the most common sanitizer due to its easiness in use and the ratio of efficacy and cost effectiveness. There are strict regulations in chlorination treatment due to the possibility of forming carcinogenic chlorinated compounds in water (Rico *et al.*, 2007). However, maintaining levels based on recommended values provides opportunity for the food processors to use it in as an effective sanitizer in fresh commodities.

According to the Code of Federal Regulations (2000), maximum allowable level of calcium ion content that could present in final product of fresh diced tomato is 0.08% by weight (Queralt *et al*., 2010). Calcium ions contribute in increasing the tissue firmness by making cross links between the carboxyl groups of pectin chains. Chilled storage is also essential to extend the shelf-life of the product (Garcia and Barret, 2002).

Methodology

The two types of vegetables used in the experiments were ice berg lettuce (*Letuca sativa*) and tomato (*Lycopersicon esculentum*). They were purchased from Dikkanda garden, Bandarawela (up country wet zone) were grown under greenhouse conditions. Sound, matured, fresh, firm and white to light green colored uniform size lettuce heads without brown spots and reddish colored uniform size tomato fruits were selected. Selected fresh samples were stored at 4°C and were processed within one day.

Fresh lettuce leaves and fresh whole tomato were washed twice with cold water (4 °C). Then treated with chlorine (calcium hypochlorite, thasco chemical industry, Japan) solutions with concentrations of 50, 100, 200 ppm respectively for 2 and 4 minutes separately.

Then microbiological analysis (total plate count) was done 24 hours after treatment. The colony forming units (CFU) were determined using the equation described by Sri Lanka Standard 516: Part 1 (1991) for TPC.

Sample of rinsed lettuce and tomato pieces were separately dipped in cold water (4 °C) as a control for 3 minutes. Separate samples were dipped in Calcium chloride solutions (0.1% and 0.2%) for 3 minutes. Similarly, another set of samples were dipped in ascorbic acid concentrations (0.5, 1.0 and 1.5%) for 3 minutes. Another set of samples were dipped in prepared combinations of both Calcium chloride and ascorbic acid.

Degree of browning was measured using calorimeter (CS-10, china) and reported L*, a* and b* values; color coordinates were determined by CIELAB coordinate color space system. Cutting strength was measured by fruit firmness tester (Forli'- Italy) and expressed as cutting strength in Newton (N) values. An untrained panel of 40 individuals was used for evaluation of color, flavor, aroma, texture and overall acceptability of the final products after 5 days from the production date. A 9-scale hedonic test was used to analyze the preference.

Data obtained from different calcium chloride levels and ascorbic acid levels were fitted into a complete randomized design (CRD) and subjected to analysis of variance (ANOVA). SAS 9.1 version software and Procedures of ANOVA were used for data analysis. A 2-way analysis of variance was conducted for treatments with calcium chloride and ascorbic acid. Mean separation was done by using Duncan's multiple range test (DMRT) at $p<0.05$. ANOVA was followed to find out the significance difference between untreated product and treated product in relation to microbiological characteristics. The sensory evaluation was tested using the Minitab 14 version.

Results and Discussion

Chlorine 200 ppm treatment showed the lowest mean in terms of total plate count. Both washing time durations of 200 ppm treatment resulted the same mean (Fig. 1)

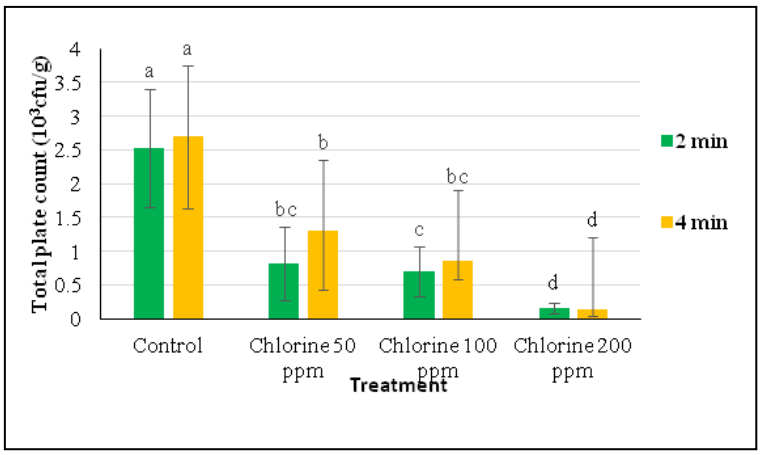


Figure 1: Total plate count of fresh lettuce treated by different chlorine concentrations and dipping times

Firmness was significantly improved by adding calcium chloride (Table 1).

Table 1 Firmness and degree of browning of fresh cut lettuce with respect to different ascorbic acid and calcium chloride levels after 5 days storage

Treatment	Firmness (N)	Degree of browning (ΔE)
C.C 0% + A.A. 0%	0.3 ± 0.18^{d}	11.02 ± 0.14^{ab}
C.C. 0% + A.A. 0.5%	0.4 ± 0.13^{d}	10.60 ± 0.33^{bc}
C.C. 0% + A.A. 1.0%	0.45 ± 0.21^{d}	10 ± 0.16^{cd}
C.C. 0% + A.A. 1.5%	0.55 ± 0.13^{d}	10.02 ± 0.06^{cd}
C.C. 0.1% + A.A. 0%	0.95 ± 0.30^{abcd}	11.58 ± 0.34^{a}
C.C. 0.1% + A.A. 0.5%	0.7 ± 0.48^{bcd}	10.47 ± 0.18^{bc}
C.C. 0.1% + A.A. 1.0%	1.25 ± 0.22^{abc}	**9.28 ± 0.10^{d}**
C.C. 0.1% + A.A. 1.5%	0.95 ± 0.36^{abcd}	9.99 ± 0.18^{cd}
C.C. 0.2% + A.A. 0%	1.21 ± 0.15^{abc}	10.51 ± 0.02^{bc}
C.C. 0.2% + A.A. 0.5%	1.4 ± 0.25^{ab}	10.48 ± 0.34^{bc}
C.C. 0.2% + A.A. 1.0%	**1.48 ± 0.20^{a}**	**9.2 ± 0.16^{d}**
C.C. 0.2% + A.A. 1.5%	**1.45 ± 0.10^{a}**	10.44 ± 0.77^{bc}

a,b.c.d Means are with the same letters within a row are not significantly different ($p<0.05$).
C.C. = Calcium chloride, A.A. = Ascorbic acid
(Degree of browning was measured as ΔE values; high degree of browning results in high ΔE values).

However, firmness was not significantly maintained by ascorbic acid treatment ($p>0.05$). Degree of browning was significantly retarded by adding of ascorbic acid. However, there was a synergistic effect of ascorbic acid with calcium chloride to retard the degree of browning.

There was a significant difference ($p<0.05$) between treated and untreated final product of fresh cut lettuce in color, aroma, texture, flavor and overall acceptability at the end of the storage period (Fig. 2). The treated final product of fresh cut lettuce had slightly higher mean value in color, texture and overall acceptability. However, existing final product of fresh cut lettuce had slightly higher mean value in aroma and flavor.

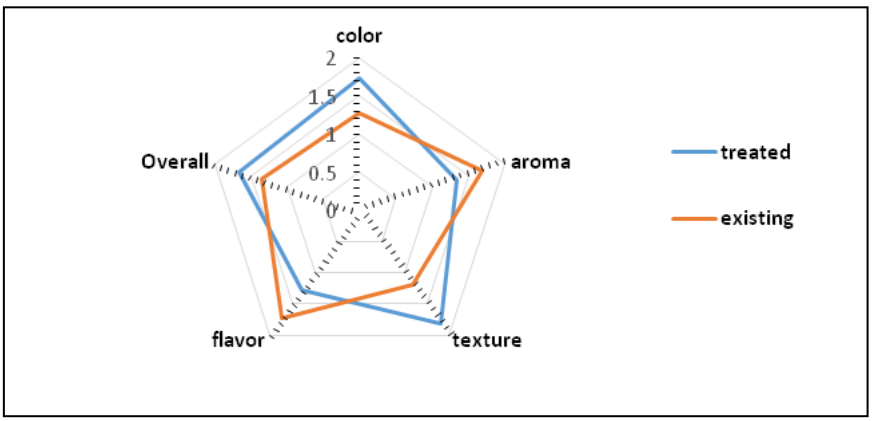


Figure 2: Effect of sensory properties on treated and existing final product of fresh-cut lettuce.

In fresh-cut tomato, both the chlorine treatments (100 and 200 ppm) showed the lowest mean in terms of total plate count (Fig 3). Both washing time durations of 200 ppm treatment resulted the same mean of total plate count.

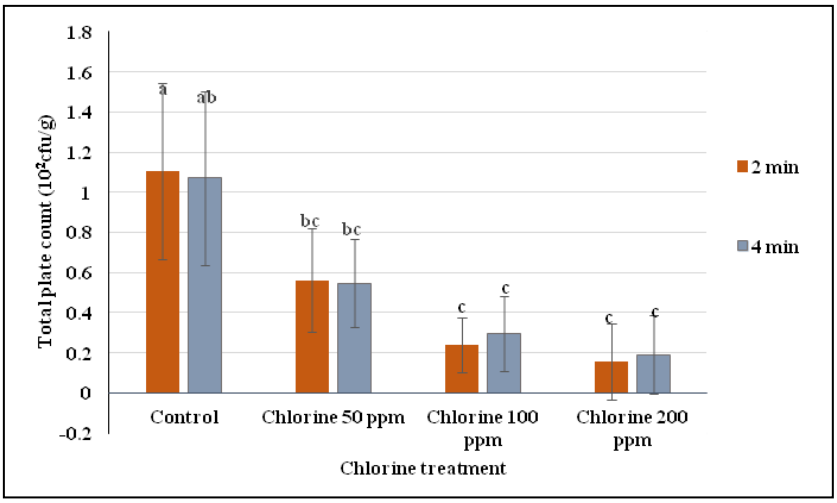


Figure 3: Total plate count of fresh tomato treated by different chlorine concentrations and dipping times.

According to the sensory evaluation, the treated final product of fresh cut tomato had a slightly higher mean value in texture and overall acceptability (Fig 4). However, existing final product of fresh cut lettuce had slightly higher mean value in flavor.

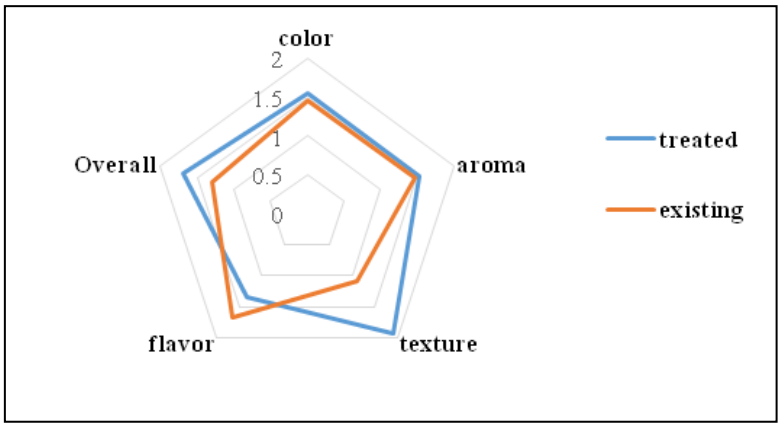


Figure 4. Effect of sensory properties on treated and existing final product of fresh-cut tomato.

Conclusions

Shelf-life of fresh-cut lettuce and fresh-cut tomato were increased up to 5 days of storage period using chemical treatments used in this study. Shelf-life of fresh-cut lettuce can be extended up to 5 days of storage period at 4 °C by dipping in 200 ppm food grade chlorine for 2 minutes as a pretreatment with the combination of 0.2% calcium chloride and 1.0%ascorbic acid for 3 minutes. Shelf-life of fresh-cut tomato was increased up to 5 days of storage period at 4 °C by dipping in 100 ppm food grade chlorine concentration for 2 minutes as a pretreatment with the combination of 0.1% calcium chloride and 1.0% ascorbic acid for 3 minutes.

References

Brecht, J. K. (1995). Physiology of lightly processed fruits and vegetables. Journal of hortscience, 301, 8–22.

Garcia, E. and Barrett, D. M. 2002. Preservative treatments for fresh-cut fruits and vegetables. In: Fresh-cut fruits and vegetables. Science, Technology and Market. O. Lamikanra (ed.). CRC press, Boca Raton, Florida.

Queralt, A.V., Remon, A. M., Lacueva, C. A., Raventos, R. M. L. 2010. Changes in phenolic profile and antioxidant activity during production of diced tomatoes. Food chemistry 126(2011): 700-1707. DOI:10.1016/j.foodchem.2010.12.061.

Rico, D., Martin-Diana, A. B., Barat, J. M., Barry-Ryan, C., 2007. Extending and measuring the quality of fresh-cut fruit and vegetables: A review. Trends in food Science and Technology 18(7): 373-386. https://doi.org/10.1016/j.tifs.2007.03.011

ANTIBACTERIAL AND METAL CHELATING PROPERTIES OF PEPTIDES DERIVED FROM OVALBUMIN USING PROTEASE ENZYME UNDER DIFFERENT CONDITIONS

Madushani M.A.A., Aruppala A.L.Y.H. and Abeyrathne E.D.N.S.*
Department of Animal Science, Uwa Wellassa university, Badulla, Sri Lanka

Summary

Ovalbumin is the major and most abundant protein in chicken egg white with high biological and nutritive value. Therefore, Ovalbumin hydrolysates (OVH) are widely used in manufacturing of dietary supplements and functional food. Research was carried out to compare the peptides produce from ovalbumin using protease enzyme with different temperature, time and pH condition and check its hydrolysates for its some functional properties as antimicrobial and metal chelating properties. Ovalbumin was dissolved with 20mg/ml concentration and hydrolyzed using protease enzyme under different temperature conditions ranging from 37 to 50^{O}C, pH from 6.5 – 8.0 for 0-24 hours. Level of hydrolyzing was observed with 15% SDS-PAGE gel electrophoresis system and visual observation. Best hydrolysates were subjected to antimicrobial assay and Fe^{2+} chelating activity. All the OVH did not show good Fe^{2+} chelating activity. However, there was a significant difference in Fe^{2+} chelating activity among the treatments ($p < 0.05$) and trt12 (50^{O}C/ pH 6.0 for 3 hours) showed highest chelating activity (11.19%±0.57) than the rest. According to the antimicrobial assay most of the OVH showed good antimicrobial property against Salmonella spp. and *E. coli* spp. There was a significant difference of antimicrobial property among the hydrolysates ($p < 0.05$).

Keywords: Hydrolyzed ovalbumin, Antimicrobial, Antibacterial, Metal chelating, Fe^{2+} chelating,

Introduction

Proteins and their peptides have ability to inhibit bacterial activity by their biological mechanisms such as iron binding proteins and have possibilities for combating infectious diseases (Mine, 2007). They inhibit the growth of pathogenic microorganisms, without affecting their hosts. Peptides found in some animals may also affect microorganisms; for example, protegrin, a porcine defensing, act against *Escherichia coli*, *Listeria monocytogenes* and *Candida albicans* (Miyakawa *et al.*, 1996). The separation and purification of bioactive peptides which will involve development of automated and continuous system is an important field for food chemists. Egg is the largest biological cell known which originates from one cell division and is composed of various important chemical substances that forms the basis

**Corresponding author: sandunabeyrathne@yahoo.com, sandun@uwu.ac.lk*

of life. The avian egg is an important source of nutrition, containing all of the proteins, lipids, vitamins, minerals and growth factors required by the developing embryo, as well as a number of defenses factors to protect against bacterial and viral infection (Mine, 2007).Therefore, the study was aimed with hydrolyzing of ovalbumin using protease enzyme under different conditions which differ from the optimum values and conducted to determine the differences in crude peptides and functional properties of ovalbumin hydrolysates such as antibacterial and metal chelating property.

Methodology

Freeze dried pre-prepared Ovalbumin powder (separated by ammonium sulfate and citric acid combination Abeyrathne *et al.,* 2013) was hydrolyzed using protease enzyme mixed at ration of 1:100 (Enzyme: Ovalbumin) for 0, 3, 6, 9, 12 and 24 hours under following conditions (Table 1). After incubation enzyme was inactivated under 100°C for 15 minutes using heat block. Hydrolysates were prepared according to the method described in Abeyrathne *et al.,* (2014). Level of hydrolysis was checked using 15% SDS polyacrylamide gel electrophoresis.

Table 1. Hydrolyzing time temperature conditions of ovalbumin using protease enzyme

Temperature	37˚C	40˚C	45˚C	50˚C
pH	6.0	6.0	6.0	6.0
	6.5	6.5	6.5	6.5
	7.0	7.0	7.0	7.0
	8.0	8.0	8.0	8.0

For the determination of antibacterial activity, *Salmonella* spp. and *Escherichia coli* were used as reference bacteria culture (Locally isolated bacteria cultures). The bacteria cultures were inoculated separately on nutrient agar plate and incubated at 37°C for 48 hours (Abbaz *et al.,* 2016). Agar well diffusion method was performed according to Abbaz *et al.,* (2016) with some modifications.

Fe^{2+} chelating activity was measured using the method described in Abeyrathne *et al.,* (2014) with some modifications. 100 µL of hydrolysates, 900 µL of distilled water and 1mL of 100 ppm $FeSO_4$ were vortex mixed in a 50 mL falcon tube. It was incubated for 5 min at room temperature. Then 900 µL of 11.3% of TCA was added and centrifuged at 2500 × g for 10 min. Then 1 mL of supernatant was transferred to a culture tube. 1mL of distilled water, 800 µL of 10% ammonium acetate, 200 µL of ferroin color indicator (Diluted ferroin color indicator in ratio of 1:1) and vortex mixed. It was incubated at room temperature for 5 min. Absorbance were measured at 562nm and Fe^{2+} chelating activity was calculated by following equation,

Fe^{2+} chelating activity (%) = [1- (sample absorbance/ blank absorbance)] × 100

Data was analyzed by using Minitab 17.0 version statistical software. One way ANOVA was used to analyze the data in functional properties analysis (statistical

significance was considered at $P < 0.05$). Three replicates were used for each trial and mean value was taken as for the final analysis.

Results and Discussion

Findings of the antibacterial activity (*Salmonella* spp. and *E. coli*) and Fe^{2+} chelating activity of ovalbumin hydrolysates were based on the following treatments, (**trt1**-37 ^{O}C / pH 6.0 for 9 hrs, **trt2**-37 ^{O}C / pH 6.5 for 12 hrs, **trt3**-37 ^{O}C / pH 7.0 for 9 hrs, **trt4**-37 ^{O}C / pH 8.0 for 3 hrs, **trt5**-40 ^{O}C / pH 6.5 for 24 hrs, **trt6**-40 ^{O}C / pH 7.0 for 12 hrs, **trt7**-40 ^{O}C / pH 8.0 for 6 hrs, **trt8**-45 ^{O}C / pH 6.0 for 6 hrs, **trt9**-45 ^{O}C / pH 6.5 for 6 hrs, **trt10**-45 ^{O}C / pH 7.0 for 9 hrs, **trt11**-45 ^{O}C / pH 8.0 for 12 hrs, **trt12**-50 ^{O}C / pH 6.0 for 3 hrs, **trt13**-50 ^{O}C / pH 6.5 for 9 hrs, **trt14**-50 ^{O}C / pH 7.0 for 6 hrs, **trt15**-50 ^{O}C / pH 8.0 for 24 hrs). Results of present study revealed that the antibacterial property of ovalbumin hydrolysates depends on concentration of the peptide samples. Ovalbumin hydrolysates which have higher concentration showed good antibacterial activity than other concentration series. However, ovalbumin hydrolysates which were produced under **trt1** (37 ^{O}C / pH 6.0 for 9 hrs), **trt2** (37 ^{O}C / pH 6.5 for 12 hrs), **trt3** (37 ^{O}C / pH 7.0 for 9 hrs), **trt4** (37 ^{O}C / pH 8.0 for 3 hrs), **trt5** (40 ^{O}C / pH 6.5 for 24 hrs), **trt6** (40 ^{O}C / pH 7.0 for 12 hrs), **trt7** (40 ^{O}C / pH 8.0 for 6 hrs), **trt8** (45 ^{O}C / pH 6.0 for 6 hrs), **trt9** (45 ^{O}C / pH 6.5 for 6 hrs) showed inhibition activity than positive control. 20mg/mL of ovalbumin hydrolysates (trt1-trt9) showed higher antibacterial property against salmonella spp. compared with the Augmentin as the positive control ($p < 0.05$). Conferring the results, Ovalbumin hydrolysates produced under **trt1** (37 ○C/ pH 6.0 for 9 hrs), **trt2** (37 ^{O}C / pH 6.5 for 12 hrs), **trt3** (37 ^{O}C / pH 7.0 for 9 hrs), **trt4** (37 ^{O}C / pH 8.0 for 3 hrs), **trt5** (40 ^{O}C / pH 6.5 for 24 hrs), **trt6** (40 ^{O}C / pH 7.5 for 12 hrs), **trt9** (45 ^{O}C / pH 6.5 for 6 hrs), **trt10** (45 ^{O}C / pH 7.0 for 9 hrs), **trt11** (45 ^{O}C / pH 8.0 for 12 hrs), **trt15** (50 ^{O}C/ pH 8.0 for 24 hrs) showed high antibacterial activity than positive control (Augmentin) and there were difference of antibacterial activity against *E. coli* spp. among different hydrolysates ($p < 0.05$) and It clearly described with the finding of Pellegrini *et al.* (2001) and Pellegrini (2003) as most of the antimicrobial peptides are derived through proteolytic digestion of proteins. Proteolytically digested ovalbumin (by trypsin and chymotrypsin) and hydrolysates have good antimicrobial property (Pellegrini *et al.,* 2004). Similar to that, ovalbumin hydrolysates produced by protease enzyme under some conditions showed good antibacterial property and there was significant difference of antibacterial property among hydrolysates ($p < 0.05$).

Ovalbumin hydrolysates produced using protease followed by trypsin enzyme (two enzyme treatment) have 90% iron chelating activity (Abeyrathne *et al.,* 2014). According to this study, ovalbumin hydrolyzed with protease enzyme also showed lower iron chelating activity (Figure 1). However, there were significant differences of iron chelating activity among those hydrolysates which were produced under different conditions and trt12 (50 ^{O}C/ pH 6.0 for 3 hrs) showed highest iron chelating activity (11.19±0.57%) compare with Ovalbumin hydrolysates have good Fe^{2+} chelating activity (Abeyrathne *et al.,* 2014). However, Fe^{2+} chelating activity of these hydrolysates was comparatively low compare with the finding of Abeyrathne *et al.,* (2014). It is in conflict with the previous studies and it may be due to different hydrolysis conditions

and enzyme which used to hydrolyze ovalbumin or differences of peptides (different cleavage sites). Therefore, further studies are necessary to analysis of peptides to find amino acid sequence.

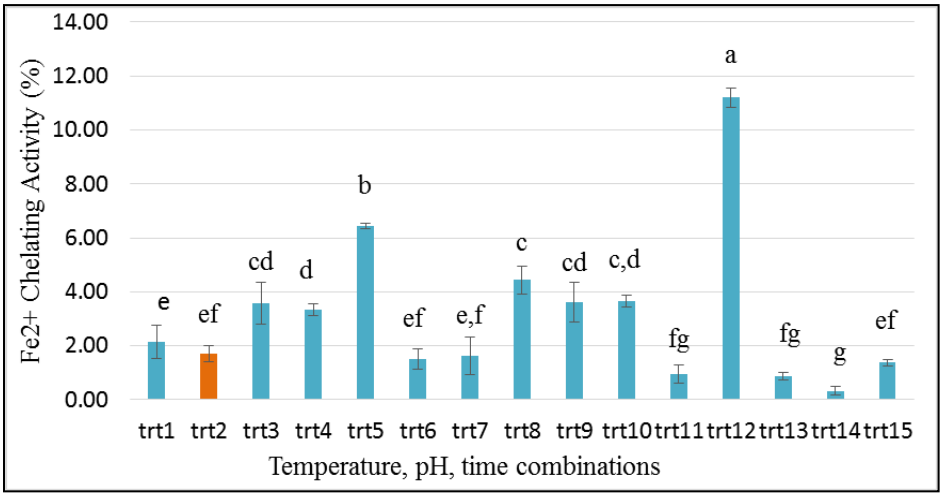


Figure 1 – Fe^{2+} chelating activity of ovalbumin hydrolysates which were produced by protease enzyme under different conditions

Conclusions

Research revealed that all ovalbumin hydrolysates showed antibacterial activity against Salmonella spp. and *E. coli* spp. However, Peptides derived from the conditions trt 1 and trt 9 are good for applicable in food industry as antibacterial agent even though they lack with Fe^{2+} chelating activity. Further studies are required to confirm the large scale application of these peptides in food industry.

References

Abbas, S.Z., Hussain, K., Hussain, Z., Ali, R., and Abbas, T. (2016), Anti-Bacterial Activity of Different Soaps Available in Local Market of Rawalpindi (Pakistan) against Daily Encountered Bacteria. Pharm Anal Acta 7:522. doi: 10.4172/2153-2435.1000522.

Abeyrathne, E.D.N.S., Lee, H.Y. and Ahn, D.U., (2013), Egg white proteins and their potential use in food processing or as nutraceutical and pharmaceutical agents—A review. Poultry Science, 92(12), pp.3292-3299.

Abeyrathne, E.D.N.S., Lee, H.Y., Jo, C., Nam, K.C. and Ahn, D.U., (2014), Enzymatic hydrolysis of ovalbumin and the functional properties of the hydrolysates. Poultry science, 93(10), pp.2678-2686. Dávalos, A., Miguel, M., Bartolomé, B., & López-Fandiño, R., 2004. Antioxidant activity of peptides derived from egg white proteins by enzymatic hydrolysis. Journal of Food Protection, 67, pp.1939-1944.

Bianchi AA, Baneyx F., (1999), Stress responses as a tool to detect and characterize the mode of action of antibacterial agents. Appl Environ Microbiol, 65, pp.5023–5027.

Dávalos, A., Miguel, M., Bartolomé, B., & López-Fandiño, R., (2004), Antioxidant activity of peptides derived from egg white proteins by enzymatic hydrolysis. Journal of Food Protection, 67, pp.1939-1944

Mine, Y. (2002), Recent advances in egg protein functionality in the food system. World's Poult. Sci. J., 58, pp31-39.

Mine, Y. (2007), Egg proteins and peptides in human health, chemistry, bioactivity and production. Curr Pharm Des, 13(9), pp.875–884. doi: 10.2174/138161207780414278.

Miyakawa Y., Ratnakar P., Rao A.G., Costello M.L., Mathieu-Costello O., Lehrer R.I., (1996), In vitro activity of the antimicrobial peptides human and rabbit defensins and porcine leukocyte protegrin against M ycobacterium Tuberculosis. Infect Immun, 64, pp.926–932.

Pellegrini A., 2003. Antimicrobial peptides from food proteins, Curr. Pharm. Des, 9, pp.1225–1238.

Pellegrini A., Dettling C., Thomas U., Hunziker P., (2001), Isolation and characterization of four bactericidal domains in the bovine h lactoglobulin, Biochim. Biophys. Acta, 1526, pp.131–140.

Pellegrini A, Hulsmeier AJ, Hunziker P, Thomas U. (2004), Proteolytic fragments of ovalbumin display antimicrobial activity. Biochim Biophys Acta, 1672(2), pp.76–85.a

RICE BRAN AS AN INGREDIENT IN FUNCTIONAL BEVERAGE, AND COMPARISON OF PHYSICOCHEMICAL AND SENSORIAL CHARACTERISTICS OF SELECTED EXTRACTION METHODS

Gamakumara G.K.M.E.A. and Perera O.D.A.N.*
Department of Food Science and Technology, Faculty of Livestock, Fisheries, and Nutrition, Wayamba University of Sri Lanka

Summary

Rice bran is a nutritionally and functionally rich ingredient still with limited food applications. Most of the rice bran produced globally as well as in Sri Lanka, are used in animal feed industry. However, the rice bran oil industry has now reached to a leading industry due to its potential applications. The research conducted revealed that the selected extraction methods; blending and autoclaving are suitable for further product development, and if the selected raw rice bran is rich in high nutrients, the final extract will be more nourished. Blending with the ratio of 1:5 (rice bran: water) showed best physicochemical and sensorial properties compared to other extractions. The new rice bran beverage also gave excellent sensorial properties. In future, both the rice producers and rice millers will obtain the benefit of earning a reasonable price for rice bran. And consumers will get the benefit of the new functional beverage for a healthy life.

Keywords: Rice bran, New beverage, Extraction methods, Functional property

Introduction

In the process of conversion of paddy into rice, nowadays people practice rice milling in commercial scale. Usually, milling process is carried out in two steps called de-husking and rice polishing. Rice bran is produced during the rice polishing stage, which is considered as the most biologically significant by-product of rice milling. More than half of the world population is consuming rice (Huang & Lai, 2016), and including Sri Lanka, for most of them rice is a dietary staple. This massive need have to be fulfilled by rice milling, at the same time resulting a huge collection of the most important by product of the process, rice bran. Antioxidants such as; γ-oryzanol, tocopherol (α, β, γ, and δ), and tocotrienol (α, β, γ, and δ), along with other phenolic compounds like flavonoids (Tricin) are in the rice bran as functional components. Apart from them protein, lipid, carbohydrate, dietary fiber, and vitamins (B_1, B_2, B_6, E) are the important nutritive components of the rice bran.

However, the utilization of rice bran is limited to only few areas like production of rice bran oil, and animal feed industry. However, the food application of rice bran for human consumption is still at an unsatisfactory level.

**Corresponding author: aannpp2003@gmail.com*

This research is focusing on comparing four selected rice bran extraction methods for the development of a new functional beverage from rice bran using a locally available rice variety BG 406 of Sri Lanka.

Methodology

BG 406 rice variety was obtained from Bathalegoda rice research centre, Sri Lanka and milled to obtain the rice bran. After sieving and setting the moisture content to 21% of raw rice bran, microwave stabilization was done. Stabilized rice bran were packed and stored at room temperature. Two extraction methods were practiced with two ratio combinations per each. Extraction methods were; blending (B) and autoclaving (A), and ratios were; 1:5 and 1:10 (rice bran: water). Blending was carried out for 15 minutes, and autoclaving was done at 120 ^{O}C for 15 minutes. After extraction, samples were filtered thrives. Then each sample (A 1:5, A 1:10, B 1:5, and B 1:10) and the stabilized rice bran were analyzed for tested parameters with analytical grade chemicals.

To select the sample with best sensory properties among four rice bran extracts, and to evaluate them for the development of new rice bran beverage, two sensory evaluations were conducted using 30 semi-trained panelists at each time. Five point hedonic scale was used for ranking.

The best rice bran sample selected from the sensory analysis was further developed with flavor addition. Sugar and fresh milk were used as ingredients. Beverage was homogenized after mixing the appropriate combination of sugar and milk, and then it was filled into sterilized bottles. Pasteurization was done at 90 ^{O}C for 20 minutes, and stored under refrigerated conditions.

Crude protein, crude fat, ash, and moisture contents of the stabilized rice bran and four extracted samples were determined (AOAC, 2000). Crude fiber content was determined using Weende method. Carbohydrate content was taken by difference.

Newly formulated rice bran beverage was observed for the stability for seven days by measuring the height of the precipitate in 25 mL volumetric flasks at 4 ^{O}C storage. Treatments given for the beverage were; homogenization for 5 minutes (control), and homogenization plus addition of a series of CMC (Carboxy Methyl Cellulose) concentrations (0.1%, 0.2%, 0.3%, and 0.4%).
Data collected from triplicates of each analysis was analyzed using SPSS 2016 software and results are shown in mean values.

Results and Discussion

Moisture content of stabilized rice bran (5.28 % ± 0.02) is comparatively lower than that of moisture content of raw rice bran (11.08%), probably is due to the heat generation during the microwave heating (Table 1). It is said that the rate of hydrolysis

of fats into free fatty acids is decreasing with the decrement of moisture content of rice bran, proving the effectiveness of microwaving for stabilization. (Fernando and Hewavitharana, 1993, cited in, Patil *et al.*, 2016)

Table 1. Proximate composition of BG 406 rice bran and four extracts.

Sample	Composition* %					
	Moisture	Lipid	Protein	Ash	Carbohydrate	Fiber
RB	5.28 ± 0.02	26.87 ± 0.80	18.77 ± 0.80	9.62 ± 0.15	34.55	10.19 ± 1.46
A 1:5	94.68 ± 1.22	23.90 ± 0.21	12.08 ± 0.54	0.74 ± 0.01	61.05	2.23 ± 0.90
A 1:10	96.95 ± 1.30	29.23 ± 0.06	16.64 ± 1.90	0.35 ± 0.02	51.09	2.69 ± 0.79
B 1:5	93.47 ± 1.01	10.59 ± 0.35	19.57 ± 0.73	0.78 ± 0.00	68.82	0.24 ± 0.08
B 1:10	96.85 ± 1.28	36.99 ± 0.78	18.98 ± 0.12	0.40 ± 0.07	43.36	0.27 ± 0.01

* Mean ± SD

Rice bran is considered as a good source of lipids. Here also, considerable lipid content could be seen in the stabilized rice bran. A significant difference ($p < 0.05$) of lipid content could be seen among each sample. The effects of high water content, and friction and abrasion forces might have caused B 1:10 sample to release more lipids from the bran particles, whereas low water content of B 1:5, even with same forces have shown a lesser lipid content. Compared to other varieties, BG 406 rice bran has higher lipid content. (Issara and Rawdkuen, 2014) B 1:10 seems a better method for extraction in terms of lipid content of the final extract.

Same as for lipid, BG 406 rice bran contain much higher protein content than other researched rice varieties. Blending resulted much similar protein content as in rice bran for the extracts. However, it is very essential to determine the amino acid profile of each extracts comparing with the rice bran before selecting a best method for the product development.

Total ash content of rice bran is almost similar to other rice bran varieties tested in previous studies. Still, the extractions were very poor in ash content compared to the rice bran. This might be due to the dilutions of the extracts. The ash content of less water added samples; A 1:5 and B 1:5 give evidences for this reasoning as these two contain higher ash content compared to other two extracts (0.74 and 0.78 respectively).

Same as for ash, crude fiber content in the rice bran has been diluted when preparing the extracts. The fiber content, in some cases had reduced even by ten folds. Importantly, autoclaving is much more acceptable in terms of fiber content compared to blending. Forces during the blending might have caused the fiber disruption in blending.

As mentioned in Faccin *et al*. (2009), the carbohydrate content in rice bran extracts is the comparatively most significant difference between other nutritive beverages. The same scenario can be seen in these four extracts as well. Heat and frictional forces in both autoclaving and bending treatments have caused releasing out carbohydrates from the bran particles. So the carbohydrate contents of the extracts are much higher than that of the carbohydrate content of the rice bran.

The sample which has only gone through homogenization has shown the best stability over the tested seven days (Fig. 1). All the other CMC added samples have gradually reduced the precipitate height. Here the precipitate height was measured comparative to a volume scale of a 25.0 mL measuring cylinder.

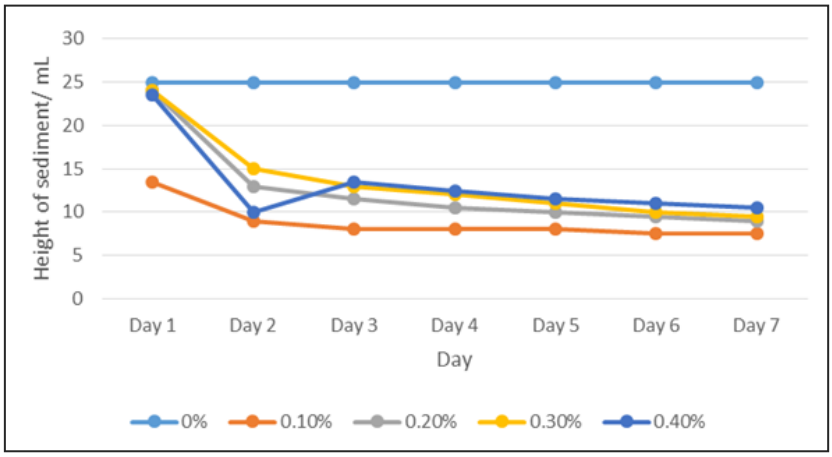
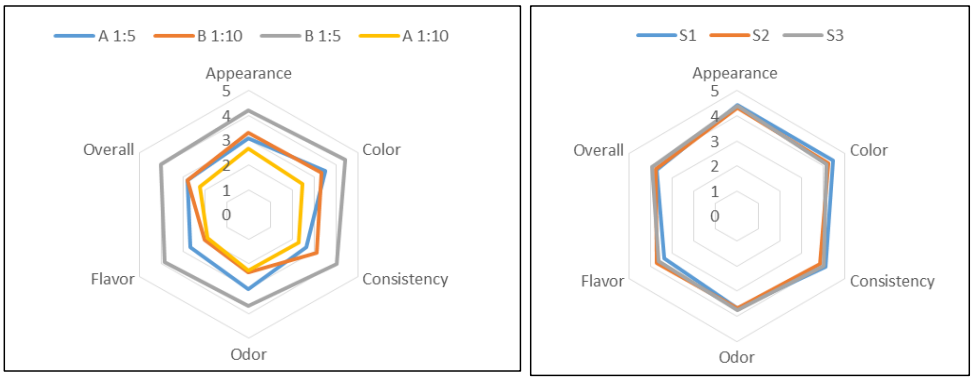


Figure 1. Stability of homogenized, and different CMC% s added samples over a period of 7 days.

According to results, B 1:5 sample has shown the best sensory characteristics among all other extracts, and considering this, B 1:5 method was selected for further product development (Fig 2). According to the sensory analysis of the developed beverage beverage, there was no significant difference among tested attributes (Colour, Consistency, Odour, Flavour, and Overall acceptability) of different flavor combined samples ($p>0.05$ for all attributes).



A **B**

Figure 2. (A) Sensory profile of four extracts of rice bran. (B) Sensory profile of three rice bran beverage samples with different ratios of milk:sugar (S1 – 5% : 5%, S2 – 7.5% : 5%, S3 – 7.5% : 7.5%).

Conclusions

According to the physicochemical properties of four extracts, blending method is more acceptable for further product development referring to the protein, ash, and carbohydrate content. According to the sensorial properties, B 1:5 extract can be used for further analysis and product development. Results showed that the new rice bran beverage has better sensory properties further confirming the potential of rice bran in developing beverages with improved characteristics.

References

Faccin, G. L. F., Ieira, N. V, Adélia, L., Iott, M., Luiz, P., Arreto, M. B., & Mante, E. R. A. (2009). Chemical , Sensorial and Rheological Properties of a New Organic Rice Bran Beverage, 16(3), 226–234. https://doi.org/10.1016/S1672-6308(08)60083-9

Huang, Y., & Lai, H. (2016). ScienceDirect Original article Bioactive compounds and antioxidative activity of colored rice bran. Journal of Food and Drug Analysis, 24(3), 564–574. https://doi.org/10.1016/j.jfda.2016.01.004

Patil, S. S., Kar, A., & Mohapatra, D. (2016). Stabilization of rice bran using microwave: Process optimization and storage studies. Food and Bioproducts Processing, 99, 204–211. https://doi.org/10.1016/j.fbp.2016.05ice .002

Utthapon, I. and Saroat, R. (2014). Organic rice bran milk production and its natural quality, 82–88.

DEVELOPMENT OF "KIRIKAWADI" CASSAVA FLOUR SUBSTITUTED PASTA PRODUCT

JeevanaT. and Perera O.D.A.N.*
Department of Food Science and Technology, Faculty of Livestock, Fisheries, and Nutrition, Wayamba University of Sri Lanka

Summary

Cassava (Manihot esculenta) is a cheap and reliable source of food for more than 700 million people in the developing countries. Cassava is an important tuber crop and is one of the major sources of carbohydrate that fulfils the dietary requirements of Sri Lankan families with low income. This study was carried out to develop a pasta with a portion of wheat flour replaced with cassava flour. The developed product was analyzed for proximate composition along with physio-chemical analysis and also cooking characteristics of pasta. The cooked weight of cassava flour added pasta was comparatively higher than the pasta made out from wheat flour and optimal cooking time of cassava flour added pasta was low compare to the cooking time needed by the pasta made out from wheat flour. Thus, this study is mainly focused on the development of pasta (Tagliatelle shape pasta) using Sri Lankan "Kirikawadi" Cassava cultivar with the intention of replacing a portion of wheat flour with cassava flour to reduce the cost of product and increase the utilization of cassava via the conversion raw cassava into flour.

Keywords: "Kirikawadi" cassava, Pasta, Tagliatelle

Introduction

Pasta is a cereal based food product consumed all over the world in current lifestyle, as they are healthy, low cost, tasty with appreciated sensorial properties with extended shelf life and convenient for transportation with easy preparation (Bergman *et al*, 1994). Conventional pasta products are produced with Triticum durum wheat semolina and also it can be produced with normal wheat flour. Durum wheat contributes only for 5% in the world wheat production and it is very much higher in price than normal bread wheat. Therefore to meet the emerging demand for the pasta products, it is necessary to find novel non-conventional ingredients for the pasta production. Cassava is rich in carbohydrate and it is the main source of carbohydrate for many peoples in developing countries. Howevert, it has a short storage life and begins to deteriorate within 2 to 3 days after uprooting. However the shelf life can be increased by flour preparation and the cassava flour can be used to prepare different foods and apart from that it can also be used in other relevant industries such as garment, bakery, food, pharmaceutical *etc*. Department of agriculture has recommended "six" varieties of cassava and from those

**Corresponding author: aannpp2003@gmail.com*

recommended six varieties, "kirikawadi" cassava cultivar was selected for processing for the study.

Methodology

Flour preparation: "Kirikawadi" cassava cultivar was peeled, washed, sliced and blanched (5 min at room temperature). Then, the sliced cassava was dried at 65°C for 24h until it reached a constant weight. Then, dried sample was ground, sieved by 0.425mm siever, weighed, packaged and stored in freezer until further analysis (Alves *et al.*, 2002). The moisture, ash, crude protein, crude fat and crude fiber of flour were determined using AOAC (2000) standard methods. Carbohydrate was calculated by the difference. Cyanide content was determined by the method described by Ubwaet al (2015) using ninhydrin based spectrometry method. Flour color was determined by chromameter, total phenolic content was determined by folin ciocalteu method described in Maurya and Singh (2010), total flavonoid content was determined according to tangilazuchchi *et al.* (2010), DPPH radical scavenging activity was determined as per Hatana *et al.* (1988) and total plate count (TPC), yeast and mould count (YMC), pH, titrable acidity were also determined. Wheat flour also was sieved by 0.425 mm sieve. Different proportions of wheat and cassava flour (T1-90:10, T2-80:20, T3-70:30) were mixed with 30ml water and 2g salt to prepare the dough. Then, the dough samples were kneaded, rested, sheeted, cut and oven dried at 121oC for 30 min. The best ratio of wheat cassava pasta was selected through sensory analysis. Proximate composition, color, cyanide content, total phenolic content, total flavanoid content and DPPH radical scavenging activity, total plate count and yeast mold count of selected wheat cassava pasta (W/C/P) and 100% wheat pasta (W/P) were determined by previously mentioned method. Texture was determined by texture analyzer. Cooking loss, cooking time, cooked weight, swelling index and water absorption index were analyzed for (W/P) and (W/C/P). The data obtained were analyzed using analysis of variance (ANOVA) completely randomized design (CRD).

Results and Discussion

The proximate compositions of cassava flour, 100% wheat flour pasta (W/P) and 70% wheat and 30% cassava flour pasta (W/C/P) are shown in Table 1. Cyanide content of cassava flour, 100% wheat flour pasta and 30% cassava included pasta were 2.0599±0.0213, 1.0132±0.0555 and 1.1098±0.0131 (mg/kg) respectively. Total phenolic content, total flavanoid content and DPPH radical scavenging activity of cassava flour, 100% wheat flour pasta and 30% cassava flour included pasta are shown in Table 2. The phenolic, flavanoid content and DPPH activity were reduced with substitution of cassava flour. Cooking characteristics of pasta are shown in Table 3. Cooking loss (%), cooked weight (g), swelling index (%) and water absorbance index (%) were increased in cassava added pasta product and optimum cooking time (min) was reduced with addition of cassava flour. Color of cassava flour, (W/P) and (W/C/P) are shown in Table 4. The greenness (a*) and yellowness (b*) were increased with the

addition of cassava flour as well as lightness (L*) was reduced. Total plate count of cassava flour, (W/P) and (W/C/P) were 0.2×10^2, 0.44×10^2 and 0.14×10^2 respectively and yeast mold count was found nil. The firmness value for (W/P) and (W/C/P) were 3.5±0.8888N and 4.2666±0.2039N respectively and the stickiness value for (W/P) and (W/C/P) were 0.1667±0.0577N and 0.4N respectively.

Table 1. Proximate composition of cassava flour, 100% wheat flour pasta and 30% cassava flour substituted pasta.

Nutrition composition	Cassava flour	100% wheat flour pasta	30% cassava flour substituted pasta
Moisture (%)	6.0383±0.1059	3.3905±0.1780	6.7718±0.1573
Ash (%)	2.3395±0.0431	2.2848±0.0244	2.2272±0.0089
Crude protein (%)	3.1301±0.0539	10.25±0.0817	8.6333±0.1067
Crude fat (%)	2.7646±0.0101	7.8991±0.3705	6.8970±0.0984
Crude fiber (%)	12.5636±0.3004	7.1688±0.1403	9.2334±0.0782
Carbohydrate (%)	73.1639±0.0862	69.1398±0.2361	66.2373±0.1263

Table 2. Total phenolic content, total flavanoid content and DPPH radical scavenging activity of cassava flour, 100% wheat flour pasta and 30% cassava flour substituted pasta.

Antioxidant activity	cassava flour	100% wheat flour pasta	30% cassava flour substituted pasta
total phenolic content	51.7574±0.6247	88.4023±0.6411	85.1803±0.2082
total flavanoic content	18.9649±0.2127	10.0526±0.2631	5.7192±0.4088
DPPH radical scavenging activity	65.0084±2.7544	2.9463±1.3286	2.7613±1.0652

Table 3. Cooking characteristics of pasta.

Characters	100% wheat flour pasta	30% cassava flour substituted pasta
Cooking loss (%)	5.1774±0.0272	7.5606±0.02968
Cooked weight (g)	6.1174±0.7338	13.9598±0.1971
Optimum cooking time (min)	8.2±0.02	7.54±0.02
Swelling index (%)	2.4061±0.0041	2.5126±0.0121
Water absorbance index (%)	143.5622±1.3635	205.2659±0.0844

Table 4. Color of "Kirikwadi" cassava flour and pasta.

Color	Amount in Cassava flour (g)	Amount in 100% wheat flour pasta (g)		Amount in 70%wheat and 30% cassava flour pasta(g)	
		Dry pasta	Cooked pasta	Dry pasta	Cooked pasta
L*	89.1666±0.8082	76.6±3.0789	53.36±0.2	70.8±2.4269	57.6±0.2
a*	2.6666±0.05777	1.9±1.1135	1.633±0.1	5.1333±1.2583	3.4±0.1
b*	14.6666±0.3052	16.7333±0.7371	12.3±0.1	18.8667±0.4509	17.96±0.1

Conclusions

The cooked weight of cassava added pasta (13.9598±0.1971 g) was comparatively higher than the pasta made out from wheat flour (6.1174±0.7338 g) and optimal cooking time of cassava added pasta (7.54±0.02 min) was low compare to the cooking time needed by the pasta made out from wheat flour (8.2±0.02 min) which indicate that, the incorporation of cassava into pasta facilitates to obtain a better quality product.

References

AlirezaSadeghi, M. and Bhagya, S. (2008), Quality characterization of pasta enriched with mustard protein isolate. Journal of Food Science, 73(5), 229–237.

Bashir, K., Aeri, V. and Masoodi, V. (2012), Physio-Chemical and Sensory Characteristics of Pasta Fortified With Chickpea Flour and Defatted Soy Flour. Journal of Environmental Science, Toxicology and Food Technology, 1(5). PP. 34-39.

Eleazu, C.O. and Eleazu, K.C. (2012), Determination of the Proximate Composition, Total Carotenoid, Reducing Sugars and Residual Cyanide Levels of Flours of 6 New Yellow and White Cassava (Manihot esculenta Crantz) Varieties. American Journal of Food Technology, 7: 642-649.

Maurya, S. and Singh, D. (2010), Quantitative Analysis of Total Phenolic Content in Adhatoda vasica. Extracts International Journal of Pharmaceutical Technological Research, 2(4): 2403-2406.

Somendrika, M.A.D., Wickramasinghe. I., Wanapala. M.A.J. and Peiris, S. (2016), Analyzing proximate composition of macro nutrients of Sri Lankan cassava variety "Kirikawadi". Pakistan Journal of Nutrition, 15(3): 283-287.

Ubwa, S.T., Otache, M.A., Igbum, G. O. and Shambe, T. (2015), Determination of Cyanide content in three sweet Cassava Cultivars in Three Local government Areas of Benue State, Nigeria. Food and Nutrition Sciences, 6, 1078-1085.

EVALUATION OF GAHALA AND INNALA FLOURS AS WHEAT FLOUR SUBSTITUTES FOR BREAD MAKING

Attanayaka A.M.V.C. and Gunathilake K.D.P.P.*
Department of Food Science and Technology, Faculty of Livestock, Fisheries, and Nutrition, Wayamba University of Sri Lanka

Summary

Gahala (*Colocasia esculenta*) and Innala (*Plectranthus rotundifolius*) are two of the underutilized yams growing in Sri Lanka. The present study was carried out to evaluate the potential of the flours obtained from these yams as wheat flour substitutes for bread making. The study consisted of flour preparation, proximate analysis, and evaluation of rheological properties, preparation of bread, & sensory evaluation at different substitution levels (10-30%). Flour preparation was done by drying and grinding of yams according to published methods. AOAC. approved methods were used for determining the proximate composition. Rheological properties were evaluated by Farinograph. Loaves of bread were prepared and were evaluated for sensory properties. According to proximate composition, the carbohydrate content of the flours of Gahala and Innala were 82.36±2.82%, 81.94±2.59%, and 81.29±2.68% respectively. Protein, fat, moisture, ash and fibre of the flours were 1.27-1.80%, 1.92-2.32%, 10.42-11.43%, 1.75-2.03%, 1.56-1.79% respectively. Farinograms evaluation revealed that 10% incorporation of Gahala flour results in 67% of water absorption, 6 minutes of dough development time, 2 minutes of arrival time and 23.5 minutes of stability. As for Innala the respected values were 60%, 7.5 minutes, 1 minute, and 20 minutes. It was found that, with the substitution levels of yams flour into wheat flour increases, declining in bread rise during the baking process. The results were in agreement with the results of the rheological study. The sensory study revealed that both yams' flour up to 10% substitution level can be used to develop bread which is insignificant in overall acceptability with normal wheat bread. Thus, it was concluded that 10% was the most suitable level of substitution among selected levels when considering the rheological and sensory properties. Further studies are suggested to develop bread using yam flours proposed in this study with improved sensory properties.

Keywords: Gahala (*Colocasia esculenta*), Innala (*Plectranthus rotundifolius*), Sensory property, Rheological property

Introduction

Due to the expensiveness, high demand, and geographical scarcity of wheat flour, there have been many attempts to explore wheat flour substitutes in food product development. Especially with regard to bread making numerous efforts have been made

**Corresponding author: kdppgunathilake@yahoo.com*

notably in tropical areas. Wheat favours the temperate zone, hence the tropical countries have to import wheat. Majority of the tropical countries are developing countries including Sri Lanka. However, sole replacement of wheat flour has not shown positive results throughout the years. The concept of composite flour bread has given more remunerative results. Substitute flours have been prepared mostly out of cereals, yams, and roots. Abundant crops that have not been potentially used in many countries have been considered as sources for flour preparation. By using underutilized potential crop sources it is possible to cut off the high cost for wheat flour importation as well as fulfilling the demand for bread. Sri Lanka provides favorable growing conditions for Gahala (Taro - *Colocasia esculenta*), Innala (Coleus – *Plectranthus rotundifolius*), and Kiriala (Arrow leaf elephant ear - *Xanthosoma sagittifolium*) which have been identified as potential flour and bread making sources. Thus, the focus of this study was to check out the suitability of theses yam flours to substitute wheat flour at different levels, in bread making.

Methodology

All purpose wheat flour was obtained from the local market. Gahala and Innala were obtained from Makandura area. All the chemicals used for experiments were analytical grade and the ingredients used for bread making were food grade.

Gahala and Innala were washed well, and hand peeled. The yams were dipped in 5% citric acid, and then grated into chips to have the thickness around 5 mm. They were then dried in a dehydrator at 70^{O}C for 18 h. By the use of a laboratory scale grinder, the dried chips were powdered. The powder was sifted through a 250 µm sieve. The gained flour samples were sealed and packed in air tight polythene containers. Composite flour blend levels contained 10%, 20%, and 30% yam flour incorporation and the rest with wheat flour.

Proximate analysis was conducted in order to analyse the nutritional composition of each flour type. Kjeldahl method, Soxhlet method were used to determine the crude protein content and crude fat content of flour respectively (AOAC, 1995). Carbohydrate: Subtraction method was used for total carbohydrate analysis. Hence, moisture and ash contents were also determined (AOAC, 1995). Moisture content was determined by the heating sample (2-3 g) at 130°C, in an air oven for 1 hr. (AACC, 2000). Ash content was determined by the heating sample (5 g) at 550°C for overnight in a furnace. (AOCC, 2012).

Rheological properties of flours added with two yam flours at different substitution levels were assessed according to the method described by AACC, (2000). The water absorption, arrival time, dough stability, and dough development time of the dough were measured by the resulted farinogram.

Bread making and sensory evaluation

Bread was prepared according to the typical bread making procedure (Giannou *et al*., 2003). Sensory analysis was conducted using 30 semi-trained panellists. Appearance, crust color, crumb grain, taste, and overall acceptability were evaluated.

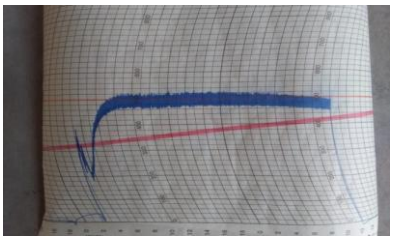
Results and Discussion

Proximate composition of the Gahala and innala flours is shown in Table 1. All the yams majorly consist of carbohydrates.

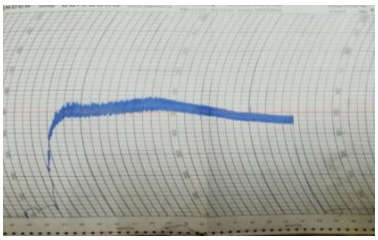
Table 1. Amounts of nutritional compounds with regard to Gahala and Innala flour.

Compound	Gahala	Innala
Carbohydrate	82.36±0.7	81.93±3.7
Moisture	10.41±0.4	11.01±0.6
Ash	1.74±0.1	2.00±0.1
Fat	1.91±0.1	2.04±0.2
Protein	1.80±0.2	1.44±0.2
Fiber	1.75±0.3	1.56±0.1

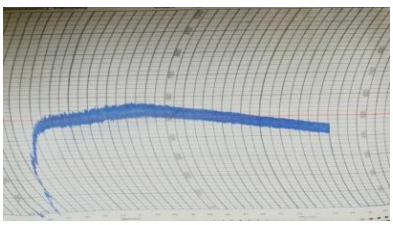
Figure 1 and 2 shows the farinograms for different blend levels of Gahala and Innala flour with wheat flour. Table 2 shows the mixing behaviour of two flours at different substitution levels.



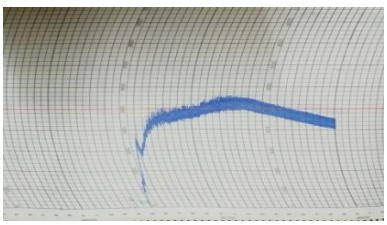
100% Wheat flour



10% of Gahala incorporation



20% of Gahala incorporation



30% of Gahala incorporation

Figure 1: Farinograms of different blend levels of Gahala flour with wheat flour.

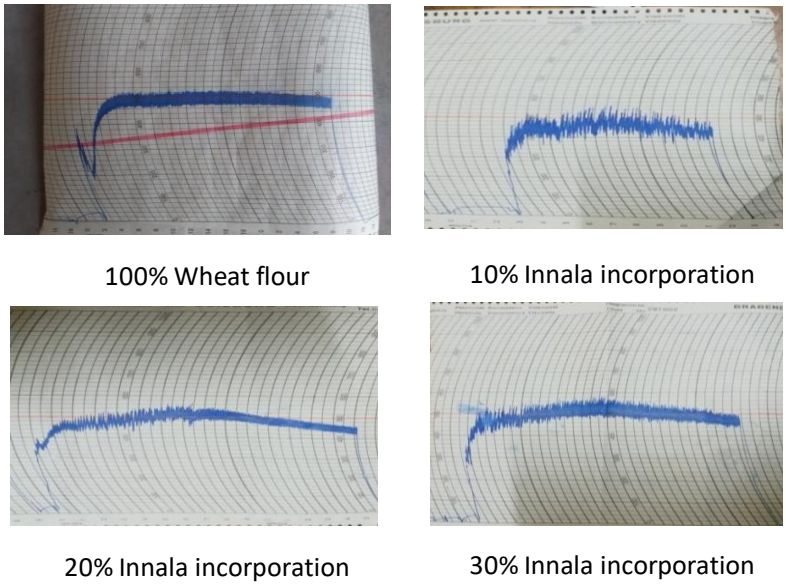


Figure 2: Farinograms of different blend levels of Innala flour with wheat flour.

Table 2: Mixing behaviors of flour blends.

	Level of yam flour substitution (%)	Water absorption (%)	Dough development time (min)	Arrival time (min)	Stability (min)
Gahala	10	67.0	6	2	11.5
	20	71.0	7.5	3	9
	30	74.5	8.5	3.5	8
Innala	10	60.0	7.5	1	12
	20	60.5	8.5	2	12.5
	30	59.5	5.5	2	6

Farinogram evaluation revealed that 10% incorporation of Gahala flour results in 67% of water absorption, 6 minutes of dough development time, 2 minutes of arrival time and 23.5 minutes of stability. As for Innala the respected values were 60%, 7.5 minutes, 1 minute, and 20 minutes. It was found that, with the substitution levels of yam flours into wheat flour increases, declining in bread rise during the baking process. The results were in agreement with the results of the rheological study. This could be probably due to variation in dough development characteristics upon addition of more than 10% yam's flour, which may have contributed to over dilution of gluten forming protein resulting in weakening of the dough. Similar studies have been done for the determination of supplementation of wheat flour with cowpea flour by Sharma, *et al* (1999) and they described that the changes in hydrating properties of two proteins may be another reason for differences in dough characteristics.

Figure 3 and 4 shows the front and cross section views of bread prepared from different composite flours of Gahala and innala.

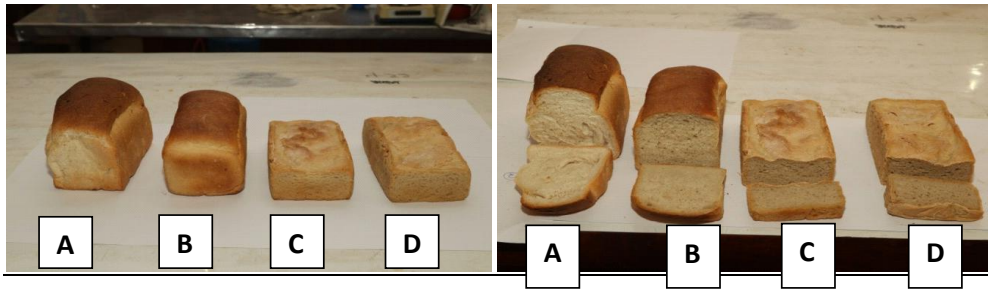


Figure 3: Front views and cross section views of Gahala incorporated wheat flour bread. (A: 100% Wheat flour, B: 10% Gahala flour+ 90% Wheat flour, C: 20% Gahala flour+ 80% Wheat flour, D: 30% Gahala flour+ 70% Wheat flour)

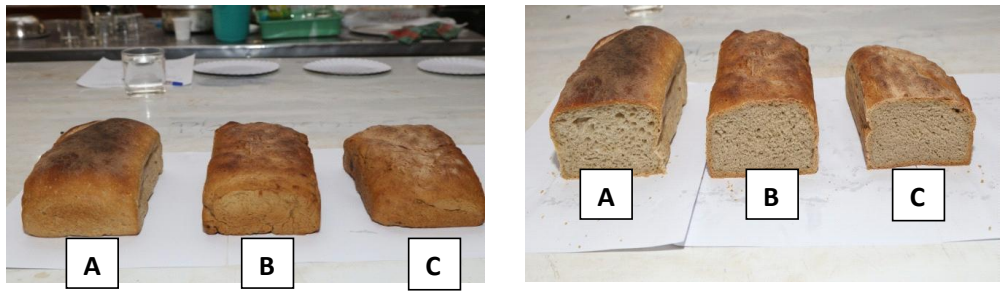


Figure 4: Front views and cross-section views of Innala incorporated wheat flour bread.(A: 10% Innala flour+ 90% Wheat flour, B: 20% Innala flour+ 80% Wheat flour, C: 30% Innala flour+ 70% Wheat flour)

The sensory study revealed that both yams' flour up to 10% substitution level can develop bread which is insignificant in overall acceptability with normal wheat bread. Table 3 and 4 show the sensory evaluation scores of bread samples at different levels of gahala and innala flour substitutions respectively. It is evident from the results that the scores for each sensory parameter decreased significantly with the increase in yam's flour. Thus, these results show that up to 10% substitution is possible to produce bread with acceptable sensory qualities.

Table 3. Sensory properties of Gahala flour incorporated bread.

Percentage of Gahala flour incorporation	Sensory attribute					
	General appearance	Crust color	Crumb grain	Texture	Taste	Overall acceptability
0%	21.2±2.9	8.8±1.1	17.1±3.0	16.1±2.4	20.8±3.0	86.0
10%	21.0±2.6	7.2±1.2	15.1±3.0	15.6±1.9	20.8±2.7	80.2
20%	20.6±3.2	7.4±1.3	15.3±2.4	15.2±2.0	20.5±3.2	62.3
30%	13.2±3.9	5.3±1.6	7.9±3.2	9.9±1.9	18.7±3.2	55.9

Table 4. Sensory properties of Innala flour incorporated bread.

Percentage of Innala flour incorporation	Sensory attribute					
	General appearance	Crust color	Crumb grain	Texture	Taste	Overall acceptability
0%	20.2±2.7	8.8±1.1	18.1±3.0	17.1±2.4	20.8±3.0	87.2
10%	14.0±1.9	8.2±1.2	14.1±3.0	16.6±1.9	20.8±2.7	78.2
20%	10.6±3.2	4.4±1.3	10.3±2.4	11.2±2.0	17.0±3.2	47.3
30%	9.2±3.3	3.3±1.6	9.4±3.2	11.6±1.9	18.4±3.2	44.9

Conclusion

It can be concluded that 10% was the most suitable level of substitution among selected levels when considering the rheological and sensory properties of final product. Further studies are suggested to produce bread incorporated with yam flours proposed in this study with improved sensory properties.

References

AOAC, 2003. Official methods of analysis. 15 ed. Washington, DC, USA: Association of Official Analytical Chemists.

Giannou .V, Kessoglou .V, and Tzia .C., 2003, Quality and safety characteristics of bread made from frozen dough", Trends in Food Science & Technology 14 (2003) 99–108

Sharma, S., Bajwa, U. and Nagi, H.P.S. (1999). Rheological and baking properties of cowpea and wheat flour blends. Journal of the Science of Food and Agriculture, 79:657- 662.

FORMULATION AND QUALITY EVALUATION OF JACKFRUIT SNACK THROUGH VALUE ADDITION OF LOCAL JACKFRUIT (*Artocarpus heterophyllus* L.)

Rathnayake R.M.M.L.*, Dalukdeniya D.A.C.K. and Jayasooriya M.C.N.
Department of Food Science and Technology, Faculty of Applied Sciences, Sabaragamuwa University of Sri Lanka, Belihuloya.

Summary

Alternatives for the classical wheat based snacks which comes under "healthy snacks" category is an emerging trend in current food industry. This study was carried out to formulate a ready- to- eat, gluten free snack from locally available jackfruit (*Artocarpus heterophyllus* Lam) as a value added product. Blanched jackfruit bulbs were ground into a pulp by maintaining the ^{O}Brix value at 140. The snack formula was prepared by utilizing most of the edible part of jackfruit bulb, jackfruit seeds used as flour and black gram flour to enhance the textural properties along with nutritional value. Garlic powder, pepper and salt were used as additives. Snacks were prepared by molding to obtain a 2 mm uniform thickness. Deep fat frying was carried out to make the snack palatable. Three formulations were developed using ingredients into different ratios and the best product was selected by 7 point hedonic scale sensory test. Product was vacuum packaged in aluminum laminated polypropylene bags. Nutrition information of the snack was found as 7.44±0.07% protein, 28.45±1.08 % Crude fat, 1.55±0.07 % Ash, 1.79±0.04 % fiber, 61.24 % carbohydrates, 1.31±0.17 % and 0% gluten. The formulated snack is gluten free, organic, no chemical preservatives added and suitable for vegans.

Keywords: Healthy snacks, Value- addition, Jackfruit, Gluten

Introduction

Snacks specially produced from wheat flour based products have expanded during last few decades. However, due to reported unhealthy conditions associated with gluten protein, consumers look for gluten free food products thus the avoidance of wheat has become a trend in recent food product development efforts of the industry (Jones and Sheats, 2016). Apart from grains and cereals, Jackfruit and Jackfruit seed flour has been tested in substituting wheat flour and researchers have developed various products successfully (Prakash *et al.,* 2009).

Jackfruit is the largest edible fruit in the world (Naik, 1949) and belongs to the Moraceae family. It is commonly grown in South East Asian countries including Sri Lanka and Brazil (Jagadeesh, 2007). Although the nutrient composition varies a little with the cultivar and region (Goswami *et al.,* 2011), Jackfruit is rich with various

**Corresponding author: minolirathnayaka@gmail.com*

nutrients such as carbohydrates, proteins, carotene, vitamin C, vitamin B6, calcium, zinc etc. (Naik, 1949). Even though Jackfruit has myriad of nutritional, functional benefits, the value addition is not prominent. In Sri Lanka, during the peak production period (June- July) considerable amount of this perishable commodity goes waste due to lack of proper postharvest management and lack of knowledge on proper possessing methods to make use of them.

Therefore, this was focused to produce a value added snack from jack fruit by preserving its health benefits and providing easily consumable snack as a solution for a busy lifestyle.

Methodology

Unripen matured jackfruit was purchased from local market and then cleaned, peeled out, bulbs separated and washed. Seeds were removed and flesh part was blanched. Jackfruit pulp was obtained by grinding the flesh. The ^{O}Brix value of the pulp was maintained at 14. Jackfruit seeds were cleaned and boiled for 1 hour to eliminate the anti-nutritional compounds. Water was allowed to drain off, seeds were cut into small pieces and dried in a hot air oven dryer at 65^{O}C for 5 hours. Then they were ground into flour.

Black gram seeds were cleaned to remove debris and stones and shade dried at room temperature (25^{O} C) for 1 hour and ground in to flour.

Garlic was peeled off, cleaned, chopped and dried in a hot air oven dryer at 65^{O}C for 4-5 hours and powdered. All the powders were sieved by a 200 μm mesh sieve to obtain a fine powder.

Three formulations were prepared by mixing ingredients into different ratios as shown in Table 01. Snacks with equal size and shape (approximate thickness of 2 mm) were obtained by feeding the mixtures into a mold and then deep fried using coconut oil. Finally, product was vacuum packaged in aluminum laminated polypropylene bags.

Table 01- Ingredients of prepared formulae.

Ingredient (%)	Formula		
	982	**615**	**374**
Jack fruit pulp	73	71	75
Jack fruit seed flour	14	15	13
black gram flour	8.5	9.5	7.5
Garlic powder	2.4	2.4	2.4
Pepper	0.6	0.6	0.6
Salt	1.5	1.5	1.5

The composition of the jackfruit snack was selected though a 7- point scale hedonic sensory test, evaluated by 30 untrained panelists and the statistical data were analyzed by (Kruskal- Wallis non- parametric test) using Minitab 17 software. Proximate analysis (Crude protein, crude fat, moisture, fiber, ash and carbohydrate contents) for the final product was done following AOAC methods and the gluten content was determined by the AACC method.

Results and Discussion

The basic raw material of this study; jackfruit (*Artocarpus heterophyllus* L.) flesh, which is firm crisp in texture. The textural and organoleptic properties of jackfruit bulb, essentially aid for the development of the organoleptic properties, color, flavor and texture in the final product. The pretreatment of flesh, blanching cause to develop a desirable yellow color and soften the flesh which is highly effective in turning flesh to a fine pulp. The time temperature combination (75 ^{O}C for 4 minutes) of blanching process was determined by considering the temperatures which did not exceed the destruction of jack fruit proteins as described by Ahmed and Chatterjee (1989).

Through the preliminary tests, it was identified that, when the pulp was thicker (starchier in nature), it resulted a coarse texture for the final snack and vice versa. As described by Goswami *et al.* (2011) this starchy nature could mainly depend on the variety, and the region of origin. Therefore maintaining a constant thickness for the jack fruit pulp was obviously essential in large scale production of this product. Thus, ^{O}Brix value 14 selected as the parameter which imparted desirable crispness and hardness to the product.

According to the sensory evaluation, sample 982 obtained mean value of 5.50 for appearance, 5.27 for color, 5.14 for aroma, 5.49 for taste, 5.26 for crispness, 5.51 for texture and 5.15 for overall acceptability. Therefore, sample 982 was selected as the most acceptable product out of three developed products.

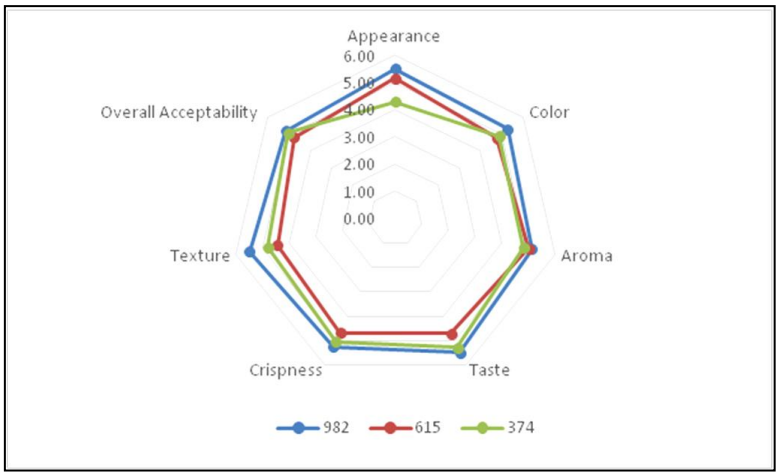


Figure 01- Web diagram of the sensory evaluation.

The results indicate a higher crude fat content which may be due to high oil uptake during deep frying of the snack. Vacuum frying is an effective and alternative method for conventional deep oil frying due to low frying temperatures (Maity *et al.*, 2014), moreover it can also prevent the destruction of heat unstable nutrients and functional compounds. Therefore further processing of the formulated jackfruit snack by using vacuum frying may result in improved organoleptic properties and nutritional qualities with a lower fat content. Gluten content in the snack is 0%, so the snack is totally gluten free, thus it is a good alternative for people who have gluten intolerance.

Table 02- Proximate composition of the developed jackfruit snack.

Compound	In 100 g of product
Carbohydrates	61.24
Crude Proteins	7.44±0.07
Crude Fat	28.45±1.08
Ash	1.55±0.07
Moisture	1.31±0.17
Fiber	1.79±0.04

The cost estimate for ingredients for 60 g of jackfruit snack is around Rs. 36.18. Therefore, mass scale production may be profitable. Shelf-life of the product needs to be determined and advance packaging should be designed to prevent flavor losses from the product and to maintain textural properties.

Table 03- Cost of production.

Ingredient	Unit Price (Rs.)	Required amount	Price (Rs.)
Jack fruit	75.00 (1 kg)	73g	5.48
Jack fruit seed flour	-	14g	-
Black gram seeds	400.00 (1kg)	8.5g	3.40
Garlic	350.00 (1kg)	2.4g	0.84
Pepper powder	212.00 (100g)	0.6g	1.27
Salt	50.00 (400g)	1.5g	0.19
Coconut oil	175.00 (350 ml)	Approx. 50 ml	25.00
Total			36.18

Plate 01- Finished Jackfruit snack.

Conclusions

Jackfruit and jackfruit seeds are effective substitutions or alternatives those comprised of high nutritional value as a raw material, for the wheat and other unhealthy snack products in the current market specially to produce gluten free products. This will also be a good source to popularize jackfruit among the busy urban population as well as for making a profit out of wasting underutilized food crop commodities

Acknowledgment

Authors are willing to express their sincere gratitude to ELA Lanka (PVT) LTD for their financial support and Mr. T. Prishanna who mediated the financial support from the company.

References

AACC, 2000. Approved Methods of the American Association of Cereal Chemists. 10th Edn., American Association of Cereal Chemists Press, St. Paul, MN.

Association of Official Analytical Chemists, 1995. AOAC Official methods of analysis. 15th Edn., Association of Official Analytical Chemists Inc, New York.

Ahmed, H. and Chatterjee, B.P., 1989. Further characterization and Immunochemical studies on the carbohydrate specifically of jackfruit (Artocarpus integrifolia) lectin. Journal of Biological Chemistry, 264(16), pp 9365- 9372.

Goswami, C., Hossain, M.A., Mortuza, M.G. and Islam, R. 2010. Physicochemical parameters of Jackfruit (Artocarpus heterophyllus Lam) seeds in different growing Areas. Int. J. BioRes, 2(10), pp 01- 05.

Jagadeesh, S.L., Reddy, B.S., Swamy, G.S.K., Gorbal, K., Hegde, L. and Raghavan, G.S.V. 2007. Chemical composition of jackfruit (Artocarpus heterophyllus Lam.) selections of Western Ghats of India. Food Chemistry, 102(1) 361-365.

Jones, J.M. and Sheats, D.B., 2016. Consumer Trends in Grain Consumption. St. Catherine University, St. Paul, MN, USA ã Elsevier Ltd.

Maity T., Bawa, A.S. and Raju, P.S., 2014. Effect of vacuum frying on changes in quality attributes of jackfruit (Artocarpus heterophyllus) bulb slices. International journal of food science, 2014

Naik, K.C., 1949. South Indian Fruits and Their Culture. P. Varadachery & Co. Madras. pp. 300-302.

Prakash, O., Kumar, R., Mishra, A. and Gupta, R. 2009. Artocarpus heterophyllus (Jackfruit): An overview. Pharmaccognosy Review, 3(6), p. 353-358.

PUTRESCINE TREATMENTS ENHANCE QUALITY AND EXTEND THE POST HARVEST LIFE OF LIME (*Citrus aurantifolia* Swingle) IN COLD STORAGE CONDITION

Gamage K.G.N.M.[1], Champa W.A.H.* and Mendis B.E.P.[1]
Institute of Postharvest Technology, Anuradhapura, Sri Lanka

Summary

Lime, *Citrus aurantifolia* Swingle, is a popular ingredient in the dishes and drinks of many countries because of its aroma and acidity. In Sri Lanka there is a constant demand for lime throughout the year though it shows seasonal pattern of fruiting. Putrescine is a naturally occurring polyamine. Polyamines are natural compounds involved in many growth and developmental processes in all cells. This study objected to extend the post harvest life of lime using polyamine. During the storage time peel lightless (L*) and the yellowness (b*) and total soluble solids increased. But peel chlorophyll content, titratable acidity and greenness a* value were decreased. Juice pH value showed a slight increase with the storage time. Considering all the variations and physical observations limes treated with low and medium putrescine concentration (0.5 and 1.0 mM) showed better physical and chemical composition and extend the shelf life up to 56 days while control showed 42 days in same condition.

Keywords: Lime, Putrescine, Postharvest life, Cold storage

Introduction

Lime is a popular fruit which use all over the world various dietary purposes like drinks and fruits. When considering the Sri Lankan food pattern lime is almost an essential ingredient of most of the dishes. But the fruiting pattern of lime is seasonal while the demand of the market stays constant. Due to that reason price of lime during off season rapidly rises and the availability in market becomes limited. If there is a possibility of extending the shelf life of lime simultaneously preserving its quality, it would be a solution to this problem.

Postharvest treatment of putrescine has used in extending and improving the quality of different fruit and vegetable varieties. For instance, postharvest dip treatment of polyamines (spermine, spermidine or putrescine) has been reported as an effective means for prolonging storage life and maintaining quality of 'Flame Seedless' grapes (Champa , 2014, 2015). Also in cold storage condition tomato fruits that treated with putrescine have been observed as the best treatment which recorded lowest cumulative physiological loss in weight (Ramesh Babu *et al.*, 2015). Therefore, this study was conducted to extend the shelf life of lime using the dip treatment of putrescine in different concentrations under cold storage conditions.

[1] *Postgraduate Institute of Agriculture, University of Peradeniya, Sri Lanka*
** Corresponding author: harindra74@gmail.com*

Methodology

Limes at optimum maturity stage (based on peel colour, fruit size and weight) were obtained from medium scale commercial orchards nearby the institute. Fruits free from visual defects were selected and divided into 4 groups containing 100 fruits for each group. Putrscine dihydrochloride (AR, Sigma Aldrich) was used to prepare the solutions of different concentrations i.e. 0.0 mmol/L – control, 0.5 , 1.0 and 1.5 mmol/L. Surfactant tween 20® at 0.1% was also added prior to dip the fruits in each concentration including the control to enhance the absorption. The treatment time was 15 minutes. Then, fruits were air dried and stored in cold room (10-13 0C, 90-95% RH) in medium size plastic crates (600×300×320 mm). Observations were taken in two weeks intervals.

Peel colour was measured according to the CIE Lab method using a potable Chroma meter (CR 400, Japan) as L*, a* and b* values. To determine chlorophyll content peel of the fruit was scraped and ground using mortar and pestle. Then sap was extracted in 85% acetone and filtered under vacuum conditions. Absorbance was measured using spectrometer (DR 600, Germany) and concentration was calculated based on absorbance.

The fruits which removed peel for chlorophyll extraction was cut into two and juice was extracted by a squeezer and was filtered through a muslin cloth. pH value of the juice was measured using an electronic pH meter (Model 420, USA). Total soluble sugar (TSS) content was measured as 0Brix using a potable digital refractometer (ATAGO PAL 3800, Japan). To measure titratable acidity (TA) 10 ml of juice was diluted into 100 ml in 100 ml volumetric flasks. From that diluted juice 10 ml was taken and titrated using 0.1 M sodium hydroxide (AR) with the presence of phenolphthalein as the indicator.

Statistical analysis was performed using ANOVA (Analysis of Variance) with Minitab 16 statistical software package.

Results and Discussion

With the storage time peel lightless (L*) & yellowness (b*) increased while peel greenness (a*) decreased (Table 01). According to the statistical analysis peel lightless (L*) in 42 days interval from storage was not significant ($p<0.05$) where it showed significant different among treatments in 14, 28 and 56 days intervals from storage. Peel yellowness or a* value was showed a significant difference ($p<0.05$) among treatments in 14, 28, and 42 days from the storage while it was not significantly differ in 56 days interval. But peel yellowness (b*) showed significant difference among treatments in all time intervals. In similar studies done by Ma-Jun et al., in 1966 during the entire storage period, untreated mango fruits retained the higher values of ‘a’ and ‘b’, whereas minimum values for ‘a’ and ‘b’ were recorded in putrescine at 2.0 mmol/L treated fruits. These results may have caused the inhibition of chlorophyll degradation in skin tissues by polyamines with the inhibition of peroxidase activity (Jawandha *et al.*, 2012)

Table 1. Effect of various concentrations of putrescine on external colour (L*, a* and b*) of lime during cold storage (10-13 ^{o}C, 90-95 % RH).

Colour scale and Putrescine concentration in mmol/l	At harvest	Time (days of storage)			
		14	28	42	56
L*					
0.0	47.88±0.7	55.10±1.40^{b}	79.64±1.89^{a}	79.90±0.70^{a}	80.55±0.40^{a}
0.5		53.70±0.28^{b}	73.46±0.80^{b}	79.49±0.71^{a}	80.53±0.40^{ab}
1.0		52.93±0.53^{b}	73.66±0.86^{b}	79.95±0.60^{a}	80.53±0.68^{a}
1.5		60.39±0.57^{a}	74.19±1.01^{b}	80.07±0.24^{a}	80.07±0.82^{b}
P-value (α=0.05)		0.000	0.001	0.207	0.010
a*					
0.0	20.07±0.37	20.70±0.65^{ab}	15.23±0.73^{a}	10.36±0.44^{b}	9.69±0.06^{a}
0.5		19.52±0.34^{bc}	12.41±0.52^{b}	11.59±0.34^{a}	9.59±0.21^{a}
1.0		21.46±0.07^{a}	12.10±0.60^{b}	11.26±0.81^{a}	9.80±0.13^{a}
1.5		18.46±1.17^{c}	11.98±0.85^{b}	10.92±0.23^{a}	9.90±0.12^{a}
P-value (α=0.05)		0.004	0.001	0.003	0.181
b*					
0.0	35.23±1.06	40.86±0.33^{b}	58.27±0.62^{a}	56.72±0.73^{a}	42.81±0.66^{c}
0.5		36.77±0.20^{c}	47.87±1.49^{c}	46.40±0.55^{d}	42.51±0.16^{c}
1.0		40.73±0.27^{b}	51.86±1.11^{b}	49.77±0.21^{c}	43.92±0.73^{b}
1.5		55.92±0.12^{a}	52.79±0.54^{b}	51.47±0.27^{b}	49.25±0.64^{a}
P-value (α=0.05)		0.000	0.000	0.000	0.000

(Data in columns that do not share the same letter superscripts are significantly different at $p < 0.05$)

Peel chlorophyll content is significantly differ ($p<0.05$) among the treatments in all time intervals (Table 2). At the end of 56 days in cold storage, limes treated with 1.5 mmol/L of putrescine showed a highest amount of chlorophyll while minimum concentration was shown by the limes treated with 1.0 mmol/L of putrescine (Table 2). In comparative to the control, limes treated with 0.5 mmol/L and 1.5 mmol/L of putrescine showed a higher amount of chlorophyll. . According to previous studies, the retardation of chlorophyll loss in musk melon with exogenous application of polyamines has been attributed to reduced hydrolytic activities acting on chloroplast

thylakoid membranes (Lester, 2000). Therefore, putrescine could have effect on this activity.

Table 2. Effect of various concentrations of putrescine on peel chlorophyll content of lime during cold storage (10-13 ^{O}C, 90-95 % RH).

Putrescine concentration (mM)	Chlorophyll Content (mg/l) in different time intervals from storage (days)				
	At harvest	14	28	42	56
0.0	10.24+0.03	1.714±0.01^{d}	1.076±0.03^{a}	0.670±0.02^{b}	0.350±0.01^{c}
0.5		2.883±0.00^{a}	1.381±0.03^{a}	0.871±0.02^{a}	0.490±0.03^{b}
1.0		2.403±0.00^{b}	1.399±0.02^{a}	0.905±0.02^{a}	0.301±0.01^{c}
1.5		2.092±0.02^{c}	1.345±0.02^{a}	0.885±0.00^{a}	0.588±0.01^{a}
P-value		0.000	0.082	0.000	0.000

(Data in columns that do not share the same letter superscripts are significantly different at $p < 0.05$)

pH value of the both treated limes and control was significantly ($p<0.05$) increased with the time of storage (Table 3). Both control and the highest concentration of putrescine treatment showed the highest pH value while medium concentration of putrescine treatment showed the lowest in 56 days from storage. According to Malik *et al*. (2006) higher titratable acidity in putrescine treated fruits may be due to the decreased hydrolysis of organic acids and subsequent accumulation of organic acids (Ramesh Babu *et al*, 2015).

Table 3. Effect of various concentrations of putrescine on juice pH of during cold storage (10-13 ^{O}C, 90-95 % RH).

Putrescine concentration (mM)	pH value in different time intervals from storage (days)				
	At harvest	14	28	42	56
0.0	2.47±0.01	2.43±0.00^{b}	2.31±0.00^{a}	2.24±0.01^{ab}	2.26±0.00^{a}
0.5		2.42±0.00^{c}	2.22±0.00^{b}	2.24±0.01^{b}	2.25±0.01^{a}
1.0		2.43±0.00^{b}	2.21±0.00^{b}	2.21±0.00^{c}	2.23±0.01^{b}
1.5		2.52±0.00^{a}	2.22±0.00^{b}	2.25±0.01^{a}	2.26±0.01^{a}
P-value		0.000	0.000	0.000	0.001

(Data in columns that do not share the same letter superscripts are significantly different at $p < 0.05$)

Total soluble sugar content in lime juice gradually increased, but the rate of increase is significantly different among different treatments ($p< 0.05$) (Table 4). In 56 days of storage period a highest TSS was showed by control while lowest was exhibited by 0.5 mmol/L of putrescine treated limes. As literature shows Mango cv. Langra treated with putrescine, total soluble solids of fruits increased with the advancement of storage period in all the treatments. During the entire storage period, highest TSS content was recorded in the control fruits (Jawandha *et al.*, 1996) and our results also on par with these findings.

Table 4. Effect of various concentrations of putrescine on TSS of lime during cold storage (10-13 ^{O}C, 90-95 % RH).

Putrescine concentration (mM)	**TSS (^{0}Brix) in different time intervals from storage (days)**				
	At harvest	**14**	**28**	**42**	**56**
0.0	8.1±0.2	8.4±0.05^{a}	8.5±0.05^{a}	8.9±0.05^{a}	9.0±0.1^{a}
0.5		8.2±0.05^{b}	8.4±0.00^{ab}	8.4±0.05^{c}	8.4±0.1^{c}
1.0		8.1±0.05^{b}	8.3±0.05^{b}	8.5±0.05^{c}	8.5±0.1^{c}
1.5		8.3±0.05^{a}	8.5±0.05^{a}	8.6±0.05^{b}	8.7±0.1^{b}
P-value		0.002	0.019	0.000	0.000

(Data in columns that do not share the same letter superscripts are significantly different at $p < 0.05$)

Titrtable acidity of lime juice was significantly ($p<0.05$) differ among the treatments and slightly decreased with the time (Table 5). At the end of the storage time, highest amount of titratiable acidity was recorded in limes treated with 0.5 mmol/L of putrescine while the control fruits recorded the minimum.

Table 5. Effect of various concentrations of putrescine on titratable acidity of lime during cold storage (10-13 ^{O}C, 90-95 % RH).

Putrescine concentration (mM)	**Titrtable acidity value (g of citric acid equiv./100 ml juice) in different time intervals from storage (days)**				
	At harvest	**14**	**28**	**42**	**56**
0.0	0.738±0.00	0.653±0.0^{b}	0.627±0.0^{d}	0.621±0.0^{c}	0.618±0.0^{d}
0.5		0.659±0.0^{a}	0.653±0.0^{b}	0.640±0.0^{a}	0.634±0.0^{a}
1.0		0.678±0.0^{a}	0.640±0.0^{c}	0.634±0.0^{b}	0.627±0.0^{b}
1.5		0.672±0.0^{a}	0.627±0.0^{a}	0.622±0.0^{c}	0.617±0.0^{c}
P-value		0.000	0.000	0.000	0.000

Conclusions

Considering the results of the study it can conclude that postharvest dip treatments of putrescine dihydrucloride (0.5 mM and 1.0 mM) extend the postharvest life of lime *Citrus aurantifolia* Swingle up to 56 days with better physical and chemical composition compared to the control in the same storage condition which is commercially acceptable for 42 days.

References

AOAC, (2005). Official Method of Analysis of AOAC International. 18th ed. Suite 500 481, North Frederick Avenue, Gaithersburg, Maryland, 20877-2417. USA.

Harindra Champa W. A., Gill M. I. S., Mahajanb B. V. C. and Arora N. K. (2014). Postharvest treatment of polyamines maintains quality and extends shelf life of table grapes (Vitis vinifera L.) cv. Flame Seedless . Postharvest Biology and Technology, (91) 57–63.

Ramesh Babu, Kulbir Singh, Jawandha S.K., Alam M. S. , Jindalar S. K. Khurana D. S. Narsaiaha K. (2015). Post-harvest application of putrescine to extend shelflife and quality of. Journal of Vegetable Science , 42 (1) : 58-62.

Jawandha S. K., Gill M. S., Nav Prem Singh, Gill P. P. S. and Singh. N. (2012). Effect of post-harvest treatments of putrescine on storage of Mango cv. Langra. African Journal of Agricultural Research , 7(48) 6432-6436.

Xiao-Hua Sun, Jing-Jing Xiong, An-Dan Zhu, Li Zhang, Qiao-Li Ma, Juan Xu, Yun-Jiang Cheng. (2012). Sugars and organic acids changes in pericarp and endocarp tissues of pumelo. Scientia Horticulturae , 142,112–117.

BIOACTIVE PROPERTIES OF THREE SRI LANKAN MEDICINAL PLANTS AND THEIR CHEMICAL PROFILING

Wickramarachchi D[1]., Visvanathan R., Nizar A.[2], Bawazeer M.[3], Tissera B.[3], Qader M.[3], Rateb M.E.[3] and Liyanage R.*

National Institute of Fundamental Studies, Hantana Road, Kandy, Sri Lanka

Summary

In this study methanol extracts of *P. emblica* (Pe), *C. auriculata* (Ca) and *H. indicus* (Hi) were screened for inhibitory activities of enzymes α-amylase and α-glucosidase. Hexane and ethyl acetate fractions of Pe displayed α-amylase and α-glucosidase enzyme inhibitory activity whereas, only the ethyl acetate fractions of Ca and Hi showed good inhibitory activity for both enzymes. In addition, water extracts of these three plants showed a significant inhibition for α-amylase and α-glucosidase enzymes. Results of qualitative phytochemical analysis for methanolic crude extracts revealed the presence of alkaloids, flavonoids, tannins and terpenoids in all three plants whereas saponins and steroids were found only in Ca and Hi. Moreover, all organic extracts of the three plants exhibited good antioxidant activity and hexane extract of Pe showed good inhibitory activity against pathogenic Gram +ve and –ve bacteria. The GCMS profile of the hexane fractions of Pe identified the presence of α-amyrin, β-amyrin, sitosterol and stigmasterol metabolites which may be responsible for the observed high bioactivity.

Keywords: Bioactivity, Medicinal plants, Enzyme activity

Introduction

Diabetes mellitus is one of the major non-communicable diseases currently affecting mankind. Currently, around 2.8% of the world's population suffer from this and it is estimated to reach 5.4% by 2025 (Patel *et al.* 2012). Diabetes mellitus type II has become the most prevalent form of diabetes due to the complications linked with the disease. Effective control of blood glucose level is vital to improve the quality of life of the patients. Currently available anti-diabetic drugs are reported to cause several side effects specially dysfunction of kidneys. Hence, recently there is increased interest on natural anti-diabetic agents of low toxicity (Lee & Jeon 2013).

Throughout decades, medicinal plants have played an important role in the field of health care (Srivastava *et al.* 2011). The, herbal medicine play a vital role in improving the health and quality of life (Ye *et al*. 2010; Grover *et al.* 2002). Moreover, herbal

[1] *Postgraduate Institute of Science, University of Peradeniya, Sri Lanka*

[2] *Faculty of Agriculture, University of Jaffna, Sri Lanka*

[3] *University of the West of Scotland, High Street, Paisley PA1 2BE, Scotlanda*

** Corresponding author: ruvini@ifs.ac.lk*

supplements can be used as an adjuvant or as favourable alternative therapy for diabetic patients.

Sri Lanka being a tropical country possesses a high diversity especially in plants. The plants have been found to be a good source of phytochemicals carrying diverse biological activities. Currently, only few studies have been accompanied on the active compounds from commonly consumed Sri Lankan medicinal plants. Importantly, there should be more studies to find the natural compounds from plants exhibiting recognizable biological activities for developments of drug leads.

The present study assessed in vitro biological activity with special reference to anti-diabetic activity. The selected medicinal plants are *Aegle marmelos* (beli- flowers), *Aegle marmelos* (beli-fruits), *Aerva lanata* (polpala), *Cassia auriculata* (ranawara), *Coscinium fenestratum* (venival), *Hamides musindicus* (iramusu), *Phyllanthus emblica* (nelli), *Scoparia dulcis* (walkottamalli), *Sida rhombifolia* (babila), and *Tinospora cordifolia* (rasakinda) which are extensively used in Sri Lankan Ayurvedic medicine. First-of-all ten plant extracts were screened for its anti-diabetic activity and among them the plants with high anti-diabetic activity were subjected to further analysis.

Methodology

The selected medicinal plants were purchased from an ayurvedic store in 2017 and were identified and authenticated by the Herbarium unit of the Peradeniya Botanical Gardens, Peradeniya, Kandy, Sri Lanka. The plant samples were washed with running tap water and air dried under shade for one week. The dried sample was visually screened for any fungal contamination. Next the dried plant samples were ground into powder (100 g) and sonicated with methanol (400 ml) for 30 minutes. Solutions were filtered using cotton wool and filtrates were collected. The powders were re-extracted three times. The filtrates were evaporated to dryness using a rotary evaporator at 40^{O}C. All crude extracts were screened for their α-amylase and α-glucosidase inhibitory activity. Crude extracts with good enzyme inhibitory activity were further partitioned using hexane, dichloromethane and ethyl acetate. Each fraction was collected separately and evaporated. Each fraction was further assessed for α-amlylase, and α- glcosidase enzyme inhibition activity, antioxidant activity (against ABTS radicals) and antimicrobial activity.

The α-amylase inhibitory activity of the plant crude extracts was assessed by the glucose oxidase method (GOD) described by Visvanathan *et al.* 2016 and IC50 were calculated. The α-glucosidase inhibitory effect of plant crude extracts was assessed by the method described by Ye *et al.* (2010).

The antimicrobial activity of the plant extracts were assessed using the method described by Napagoda *et al.* 2018 with slight modifications. The minimum inhibitory concentrations (MIC) of the plant extracts were calculated. Staphylococcus aureus (G +ve), Escherichia coli (G –ve) and Pseudomonas aeruginosa (G –ve) were used for the assay. The antimicrobial activity, as well as the minimum inhibitory concentrations (MIC) of plant extracts was determined by the nutrient broth method in 96-well microtitre plates as described by Bussman *et al,* (2010) and Napagoda *et al.,* (2018) with slight modifications.

The ABTS radical scavenging activity of the plant extracts were determined by the method described by Shalaby & Shanab (2013) with some modifications. Trolox was used as the standard reference solution.

Methanolic crude extracts of antidiabetic active plants were tested for alkaloids, flavonoids, tannins, saponnins, terpenoids and steroids (Gul *et al.* 2017; Baba & Malik 2015; Kumar *et al.* 2013; Ramamurthy and Sathiyadevi 2017; Vaghasiya, Dave and Chanda 2011).

Powdered crude extracts of Nelli, Ranawara and Iramusu (2 g) were stirred with diluted HCL (8ml) and then filtered. Filtrates were treated with few drops of Dragondroff's reagent. Formation of orange colour precipitate indicated the presence of alkaloids.

Methanolic plant extracts of Nelli, Ranawara and Iramusu (1ml) were mixed with diluted NaOH (1 ml). Formation of intense blue colour indicated the presence of flavonoids.

Methanolic extracts of Nelli, ranawara and Iramusu (2ml) were mixed with few drops of 1% lead acetate. Formation of yellow colour precipitate indicated the presence of tannins.

The presence of saponins was determined by Frothing test. The crude dry powders of Nelli, Iramusu and Ranawara were vigorously shaken with distilled water and was allowed to stand for 10 min and classified for saponin content as follows: No froth indicate absence of saponins and stable froth more than 1.5 cm indicated the presence of saponins.

Chloroform (2 ml) and concentrated H_2SO_4 were added with the 5ml of methanolic plant crude extracts. Formation of red colour indicated the presence of steroids.

Chloroform (2 ml) was added to 5 ml of methanolic plant extracts of Nelli, Ranawara and Iramusu and evaporated on the water bath and then boiled with 3 ml of concentrated H_2SO_4. A grey colour formed which showed the entity of terpenoids.

To analyze the volatile organic compounds is the hexane extracts, 1 mg of hexane extract residue was dissolved in 2 mL of HPLC grade hexane and filtered through a membrane filter (0.2 um PTFE filter) before analysing using GCMS (Agilent 7820A gas chromatography system coupled to Agilent 5975 series quadrupole mass spectrometer) working on EI mode, Thermo HP-5MS column (30 cm x 250 µm x 0.25 µm). 1 uL of the samples was injected. Analysis was performed at 50°C for 5 min, then (50-250°C) over 35 min using Helium as a carrier gas with a flow of 1.2 mL/min. Compounds were identified using NIST 11.0 library of mass spectra on an Agilent ChemStation software.

Results and Discussion

Enzyme inhibitory activities of tested plants are shown in Table 01. Among ten studied medicinal plants, methanolic extracts of *Phyllanthus emblica* (nelli), *Cassia auriculata* (ranawara), and *Hamidesmus indicus* (iramusu) showed highest inhibition for both α-amylase and α-glucosidase enzyme assays. Nelli showed significant inhibitory activity for α-amylase and α-glucosidase assay with an IC50 value of 21.57±0.14 µg/mL and 16.42±0.23 µg/mL respectively. Ethyl acetate fractions of all three plants exhibited highest inhibition for both enzymes. Moreover, hexane fraction of nelli demonstrated significant inhibition for α-amylase and α-glucosidase assays. Therefore, in nelli both

polar and non polar compounds could be responsible for its bioactivity while in ranawara and iramusu only polar compounds could be responsible for their bioactivity. In addition, all extracts of three plants exhibited good radical scavenging activity.

Hexane extract of nelli showed significant inhibitory activity against three pathogenic Gram +ve and Gram –ve bacteria, while dichloromethane and ethyl acetate fractions of ranawara showed moderate inhibitory activity against pathogenic bacteria while iramusu extracts didn't showed any antimicrobial activity for less than 500 ug/mL. Phytochemical analysis was carried for plants with highest inhibition for α-amylase and α-glucosidase enzyme assays. According to the phytochemical analysis, alkaloids, flavonoids, tannins and terpenoids were present in all three plant extracts whereas saponins and steroids were found only in ranawara and iramusu (Table 2). The GCMS profile of nelli hexane fraction identified the presence of secondary metabolites such as α-amyrin, β-amyrin, sitosterol and stigmasterol metabolites which may be responsible for the observed high bioactivity. Secondary metabolites are naturally occurring chemical compounds, serve as survival functions of the plant and has beneficial uses for humans as drug leads in pharmaceutical industry.

Table 01- Percentage Inhibition Values for Alpha Amylase and α-glucosidase **.**

Plant	Percentage Inhibition α-amylase for 2000 ppm solution (%)	Percentage Inhibition α-glucosidase for 2000 ppm solution (%)
C. fenestratum	69.52	-
T. cordifolia	71.80	-
S. rhombifolia	76.45	-
S. dulcis	**93.73**	-
A. marmelos (Flower)	**93.26**	-
P. emblica	**96.30**	**97.89**
C. auriculat	**98.10**	**98.46**
H. indicus	**91.19**	**98.99**
A. lanata	71.56	
A. marmelos (Fruit)	71.33	

Table no 02- Phytochemicals Present in *Phyllanthus emblica* (nelli), *Cassia auriculata* (ranawara), and *Hamides musindicus* (iramusu).

Plant	Alkaloids	Flavonoids	Tannins	saponins	steroids	Terpenoids
P. emblica	+	+	+	-	-	+
C.auriculat	+	+	+	+	+	+
H. indicus	+	+	+	+	+	+

Conclusions

Among the studied medicinal plants (ten), Pe, Ca and Hm demonstrated good biological activity by inhibiting both α-amylase and α- glucosidase enzyme. According to the results, both non-polar and polar compounds were responsible for the enzyme inhibitory activity of nelli while moderately and/or high polar compounds were responsible for the enzyme inhibitory activity of ranawara and iramusu. The presence of secondary metabolites α-amyrin, β-amyrin, sitosterol and stigmasterol and tocopherol could be the reason for the observed high bioactive properties of hexane fraction of nelli. Studied three plants, Nelli, Ranawara and Iramusu shows potential to be used as alternative herbal supplements to control diabetes among population after detailed research on their activities.

References

Baba, S.A. & Malik, S.A., 2015. Determination of total phenolic and flavonoid content, antimicrobial and antioxidant activity of a root extract of Arisaema jacquemontii Blume. Integrative Medicine Research, 9(4), pp.449–454.

Bussmann, R.W., Malca-García, G., Glenn, A., Sharon, D., Chait, G., Díaz, D., Pourmand, K., Jonat, B., Somogy, S., Guardado, G. and Aguirre, C., 2010. Minimum inhibitory concentrations of medicinal plants used in Northern Peru as antibacterial remedies. Journal of Ethnopharmacology, 132(1), pp.101-108.

Grover, J.K., Yadav, S. & Vats, V., 2002. Medicinal plants of India with anti- diabetic potential. Journal of Ethnopharmacology, 81(1), pp.81–100.

Rahman Gul, Syed Umer Jan, Syed Faridullah, Samiullah Sherani, and Nusrat Jahan, Preliminary Phytochemical Screening, Quantitative Analysis of Alkaloids, and Antioxidant Activity of Crude Plant Extracts from Ephedra intermedia Indigenous to Balochistan, The Scientific World Journal, vol. 2017, pp.1-7.

Lee, S.H. & Jeon, Y.J., 2013. Anti-diabetic effects of brown algae derived phlorotannins, marine polyphenols through diverse mechanisms. Fitoterapia, 86(1), pp.129–136. Food Chemistry, 211, pp.853–859.

Napagoda, M., Gerstmeier, J., Butschek, H., Lorenz, S., Kanatiwela, D., De Soyza, S., Qader, M.,Nagahawatte, A., Wijayaratne, G.B., Svatoš, A. and Jayasinghe, L., 2018. Lipophilic extracts of Leucas zeylanica, a multi-purpose medicinal plant in the tropics, inhibit key enzymes involved in inflammation and gout. Journal of Ethnopharmacology, 224, pp. 474-481.

Patel, D.K. et al., 2012. An overview on antidiabetic medicinal plants having insulin mimetic property. Asian Pacific Journal of Tropical Biomedicine, 2(4), pp.320–330.

Ramamurthy V and Sathiyadevi M, 2017. Preliminary Phytochemical Screening of Methanol Extract of Indigofera. Journal of Molecular Histology & Medical Physiology, 2(1), pp.1–5.

Shalaby, E.A. & Shanab, S.M.M., 2013. Comparison of DPPH and ABTS assays for determining antioxidant potential of water and methanol extracts of Spirulina platensis. INDIAN Journal of Geo-Marine Sciences, 42, pp.556–564.

Srivastava, S.., 2011. Costus speciosus (Keukand): A review. Der Pharmacia Sinica, 2 (1), pp.118–128.

Visvanathan, R., Jayathilake, C. & Liyanage, R., 2016. A simple microplate based method for the determination of α-amylase activity using the glucose assay kit (GOD method). Food Chemistry, 211, pp. 853–859.

Y. Vaghasiya, R. Dave and S. Chanda, 2011. Phytochemical Analysis of Some Medicinal Plants from Western Region of India. Research Journal of Medicinal Plants, 5(5), pp.567–576.

Ye, X.P., 2010. α-Glucosidase and α-Amylase Inhibitory Activity of Common Constituents from Traditional Chinese Medicine Used for Diabetes Mellitus. Chinese Journal of Natural Medicines, 8(5), pp.349– 352.

THE AMOUNT OF ACTIVE HYDROGEN IN PERISHABLES, JUICES AND CHANGES WITH THERMAL AND NON-THERMAL (ULTRASONIC AND MICROFILTRATION) PROCESSING TECHNIQUES

Amunugoda P.N.R.J.* and Jayasinghe G.D.D.R.
Food Technology Section, Modern Research and Development Complex, Industrial Technology Institute, Halbarawa Garden, Thalahena, Malabe. Sri Lanka.

Summary

The present study evaluated total antioxidant status of specific food by determining relative amount of active Hydrogen (rH). Antioxidants in foods prevent oxidation or quenches free radicals by donating one electron or H-, thus the amount of active H provides an indicator of the total antioxidant potential of a food system. The total antioxidant potential is a better indicator compared to the determination of the activity of one specific antioxidant since it provides a cumulative effect. The results indicate significant difference ($p<0.05$) in active hydrogen among different raw juices, thermally and non- thermally processed (micro filtrated, ultrasonically and ultraviolet treated) juices. The higher values of active hydrogen were found in Avocado (*Persea americcana*), Katuanoda (*Annona muricata*), Nelli (*Phyllanthus emblica* L.), Lime (*Citrus auaurantifolia*), Papaya (*Carica papaya* L.), Mango (*Mangifera indical* L.) and Tomato (*Solanum lycopersicum*). There were much higher values of active Hydrogen found also in Orange (*Citrus reticulate*), Pomegranate (*Punic agranatum* L.), Mangusteen (*Garcinia mangostana* L.), Wood apple (*Limonia acidissima*), Beet root (*Beta vulgaris*) and Water melon (*Citrullus lanatus*). The amount of active Hydrogen was increased with the processing compared to unprocessed juices.

Keywords: Free radicals, Total antioxidants, Active hydrogen, Non-thermal processing

Introduction

Free radicals in the human body are unstable, highly reactive and energized molecules having unpaired electrons. The majority of free radicals that damage biological systems are oxygen free radicals, and these are more generally known as “reactive oxygen species” (ROS). These can initiate autocatalytic reactions so that molecules to which they react are themselves converted into free radicals to propagate the chain of damage (Alagumanivasgam *et al.*, 2012). They react quickly with other compounds, trying to capture the electrons needed to gain stability and involved in many pathological conditions. Generally free radicals attack the nearest stable molecules, `stealing' its electrons. When the molecules that has been attacked by a free radical and loses electron, it becomes a free radical itself and begin a chain reaction. Once the process is started, it can cascade, initiating lipid peroxidation which results in destabilization and disintegration of the cell membranes or oxidation of other cellular components like

**Corresponding author: neville@iti.lk*

proteins and DNA, finally resulting in the disruption of cells and cause various human disease as a results of oxidative stress (Awuah and Ramaswamy, 2007). Oxidation caused by free radicals sets reduced capabilities to combat ageing and serious illness, including cancer, kidney damage, atherosclerosis, cardiovascular disease, neural disorders, alzheimer's disease, mild cognitive impairment, parkinson disease, alcohol induced liver disease, ulcerative colitis and aging (Duyn and Pivonka, 2000; Alagumanivasgam *et al.,* 2012; Bazzano *et al.,* 2008; Barrett and Lloyd, 2012; Pyo *et al.,* 2014).To deal with the free radicals, the human body is equipped with an effective defense system which includes various enzymes and high and low molecular weight antioxidants. Antioxidants neutralize free radicals by donating one of their own electrons, ending the electron-stealing reaction. These act as scavengers and play the housekeeper's role by mopping up free radicals before they get a chance to create havoc in a body (Kaur and Kapoor, 2001) and improving the quality of life by preventing or postponing the onset of degenerative disease thus reduce the cost of health cause delivery. Continuous exposure to chemicals and contaminations may increase the amount of free radicals in the body beyond its stability to control and cause irreversible oxidative damage (Plojsak, 2008). The interplay between free radicals and antioxidants is important. Free radicals induce oxidative stress is balanced by the body's endogenous antioxidant systems with the ingestion of exogenous antioxidants (Kumar and Pandey, 2013). If the generation of free radicals exceeds the protective effects of antioxidants, and some co-factors, this can cause oxidative damage which accumulates during the life cycle (Rahman, 2007). Fruits, vegetables and herbs are a major source of dietary antioxidants (Pyo *et al.,* 2014) that increase the plasma antioxidant capacity and thereby scavenging free radicals. Positive effects of the protective substances that originate from food are greater because of the synergic activity among individual antioxidants (Plojsak, 2008). In the preservation of juices, pasteurization (PZ) ensures prevention of microbiological deterioration by thermal destruction, elimination of oxygen and prevention of enzymatic action. Pasteurization causes some effects on organoleptic and/or nutritional properties of juices. Although some of these changes may be desirable, the rather harsh temperature for an extended time period would trigger chemical reactions and loss of nutrients, freshness and sensory characteristics (Awuah and Ramaswamy, 2007). Microfiltration (MF) and ultrasonic (US) are one of two alternate techniques to overcome possible ill effects (O'donnell et al., 2010; Yorn., *et al* 2004). Evaluation and information of antioxidant capacities of fruits, vegetables, herbs juices and effects of different thermal and non-thermal juice processing techniques to antioxidant capacities is more useful for both juice producers and consumers (Sharma and Bhat, 2009; Alagumanivasgam *et al.,* 2012; Selvakumar *et al.,* 2011; Kumar and Abhay, 2013). Determination of active Hydrogen is a meaningful way of estimation of total antioxidant capacity. Every endogenous antioxidant neutralizes one free radical by donating one electron or H-. Hydrogen content in a biological environment expressed as partial pressure of hydrogen (rH or rH2). rH is the absolute indicator of the reductive potential of a substance. Levels of rH are mostly between 0 and 42. Level 42 means dissolved oxygen saturation rate. Level 1 means that the substance is rich with hydrogen and no free oxygen. It is possible to obtain lower or even negative levels of rH, representing greater concentration of active hydrogen. The maximum level is 28, what is above 28 means oxidation, while levels below 28 mean reduction (Plojsk, 2008). The criterion for the reaction capability of a compound is oxidation reduction potentials in mV (ORP) the tendency of a chemical species to acquire electrons and

thereby be reduced. Each species has its own intrinsic reduction potential; the more positive the potential, the greater the species' affinity for electrons and tendency to be reduced. pH of the solution is the criterion of concentration of free positive hydrogen ions in the solution. The use of rH gives a hydrogen proton-unbiased look at the absolute reducing potential of a compound, eliminating the effect of pH in the ORP measurement. It is a true indication of a compounds reduction potential capacity. The shifts in rH can be used to quantify the reducing ability of the compound. The rH is also the indicator of the probability that the compound will react with the free radical. Because of the interaction of protons at the changes of pH, oxidation reduction potential may be biased by the pH and vice versa.

Methodology

Juice was extracted from selected 30 tropical fresh fruits, vegetables and herbs by manual juice extractor and electric blender and filtered through muslin cloth. Star fruit (*Averrhoa carambola*), Water melon (*Citrullus lanatus*) Curry leaves (*Murraya koenigii*) juice was filtered through, 1.18mm sieve and 300µm sieve. The juice was filtered through a clean muslin cloth. The extracted curry leaf, star fruit, and sugar melon juices were mixed according to the formula developed. Citric acid was added to adjust the final pH to 3.5 and food grade sugar was added to adjust the final ^{O}Brix value of 9^{O}Brx.

An ultrasonic juice processor (SJIA–1500 W, Ningbo YinzhouSjia Lab Equipment Co., Ltd.) with a 15 mm probe was used for sonication. Range of increasing treatment of ultrasound wave's power and time were applied to blended juice samples. Samples were treated at a constant frequency of 20 kHz, temperature which was maintained under 40^{O}C and pulse duration (S) of 1.5 on and 2.5 off.

A blended fresh juice sample was micro filtered at 0.5 MPa. The polyvinylidene fluoride membrane (0.3µm) was inserted to the experimental membrane filtration unit. The feed was swapped from time to time when its temperature reached 40˚C and temperature of the feed brought to ambient temperature (± 30^{O}C). Permeate was filled in to sterilized 200 ml polyethylene terephthalate bottles (PET) bottles and capped.

A blended fresh juice sample was heated until the temperature reached 80˚C. Sodium metabisulphite was added (SLS 729-2010) and mixed well. The heated juice was then filled in to the PET bottles and capped. The sealed bottles were pasteurized for 20 minutes by immersing in a boiling water bath (80 ^{O}C). The bottles were allowed to cool, labeled and stored in the refrigerator (4^{O}C).

The re-modelledNernsto's equation used to determine the rH at 25^{O}C (Plojsak, 2008). The RS 232 ORP meter and 510 pH meter (EUTECH Instruments) were used to measure oxidation reduction potential and the pH level of fresh single juices, blended non-thermally and pasteurized processed juices. The ORP value of juice extract was read in mV at room temperature after stabilize the value shown by ORP meter. Two replicates were done from each fruits juice sample were checked for determine rH level.

Results and Discussion

The level of rH is presented in logarithmic scale in Figure 1. According to the results obtained relatively higher values of active hydrogen were observed in Avocado, Katuanoda, Nelli, Lime, Papaya, Mango and Tomato among the sources evaluated.In second place higher active hydrogen were observed in Orange, Pomegranate, Mangusteen, Wood apple, Beet root and Water melon as depicted in the Figure 1.

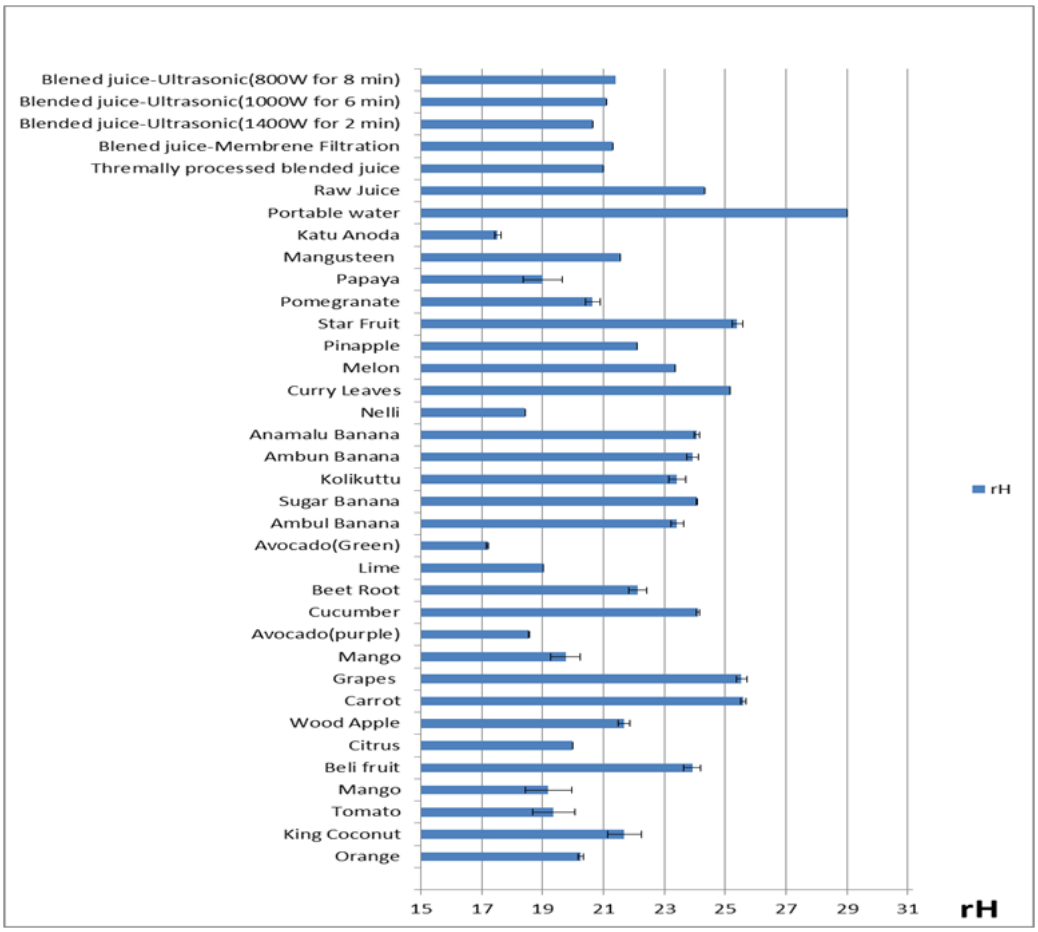


Figure 1. Schematic view of the portion of active hydrogen in the chosen juices.

The results indicate significant difference ($p<0.05$) of active hydrogen among different raw juices. Further, significant differences were observed among blended thermally processed (pasteurized) juices and non- thermally processed (ultrasonically and micro filtrated) juices. In general, the amount of active Hydrogen is increased with the processing compared to unprocessed juices. Blended juice with ultrasonic treatment of 1400W for 2 minutes showed the highest active hydrogen compared to pasteurized and micro filtrated juices. Results indicated positive results of high active hydrogen capacity in ultrasonically treated blended juices compared to pasteurized blended juices. Little reduction of active hydrogen showed in micro filtrated sample compared to

pasteurized blended juices due to share of active hydrogen both in permeate and retentive samples.
Further studies are being carrying out to test the effect of UV sterilization and non-thermal plasma on active hydrogen antioxidant potential. Consumption of fruits, vegetables, herbs can be promoted by increasing the availability of single or blended juices. In the processing of juices, optimum techniques need to be employed to maintain the antioxidant capacities of these juices to obtain optimum health benefits.

Conclusions

The higher values of active hydrogen were observed in Avocado, Katuanoda, Nelli, Lime, Papaya, Mango and Tomato. There is much active Hydrogen in Orange, Pomegranate, Mangusteen, Wood apple, Beet root and Water melon. Active hydrogen differs between different raw juices thus between pasteurized juices and ultrasonically and micro filtrated juices. The amount of active Hydrogen is increased with the blending and processing.

Acknowledgement

Funds provided by the project number TG/13/67 of "Establishment of non-thermal processing technologies for blended fruits, vegetable, and herbal juices is acknowledged.

References

Alagumanivasgam, G., Pasupathy, R., Kottaimuthu, A. and Manvalan, R. 2012. A review on In-vitro Antioxidant methods.International Journal of Pharmaceutical and Chemical Sciences. ISSN: 2277-500,. Vol. 1(2) Apr-Jun 2012, pp. 662-674.

Awuah, G. and Ramaswamy, H. 2007. Economides, A., Thermal processing and quality: Principles and overview. Chemical Engineering and Processing: Process Intensification, 46 (6), pp 584-602.

Barrett, D. M. and Lloyd, B. 2012. Advanced preservation methods and nutrient retention in fruits and vegetables. Journal of the Science of Food and Agriculture, 92 (1), pp 7-22.

Bazzano, L. A., Li, T. Y., Joshipura , K. J. and Hu, F. B. 2008. Intake of Fruit, Vegetables, and Fruit Juices and Risk of Diabetes in Women, Diabetes Care, 2008

Duyn, M. A. S. V., and Pivonka, E., 2000. Overview of the Health Benefits of Fruit and Vegetable Consumption for the Dietetics Professional: Selected Literature. Journal of the American Dietetic Association, 100 (12), pp 1511-1521.

Kaur, C., and Kapoor, H. C. 2001. Antioxidants in fruits and vegetables-the millennium's health, International Journal of Food Science & Technology, 36(7), pp. 703-725.

Kumar, S. and Pandey, A. K. 2013. Chemistry and Biological Activities of Flavonoids: An Overview. The Scientific World Journal Volume 2013 (2013).

O'donnell, C., Tiwari, Bourke, B. P. and Cullen, P. 2010. Effect of ultrasonic processing on food enzymes of industrial importance. Trends in Food Science & Technology, 21 (7), pp 358-367,

Pyo, Y. H., Jin, Y. J. and Hwang, J.Y, 2014. Comparison of the effects of blending and juicing on the phytochemicals contents and antioxidant capacity of typical Korean keranel fruit Juice. PrevNutr Food Sci.2014 Jun, 19(2), pp108-114.

Plojsak, B. 2008. The amount of active hydrogen in selected juices and teas on the Slovenian market. International Journal of Sanitary Engineering Research Vol.2 No. 1/2008, pp 58-64.

Rahman, 2007. Studies on free radicals, antioxidants, and co-factors. Clinical Interventions in Aging 2007 Jun, 2(2), pp 219-230

Selvakumar K., Madhan R., Srinivasan G., Baskar V. 2011. Antioxidant Assays in Pharmacological Research. Asian J.Pharm. Tech. 2011, Vol. 1, Issue 4, pp 99-1030

Sharma, O. P. and Bhat, K. T. 2009. DPPH antioxidant assay revisited. Food Chemistry, Volume 113, Issue 4, pp 1202-1205.

Yorn, K. S., Hong, J. S., Bae, D. H., Kim, S.J. and. Kim, S. D. 2004. Effective clarifying proces of reconstituted apple juice using membrane filtration with filter-aid pre-treatments. Journal of Membrane Science, 228(2), 179-186, 2004

.

THE BEHAVIOR OF SRI LANKAN FOOD PROCESSING COMPANIES TOWARDS VOLUNTARY STANDARD CERTIFICATIONS

Karunasiri J.P.R.N., Mendis B.E.P.* and De Alwis M.[1]
Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Sri Lanka

Summary

The importance of voluntary standard certifications related to food quality, food safety, environmental sustainability and green production has grown in recent years, contributing to higher growth rates in international trade, especially in food and agriculture products. The present research was carried out to study the approach undertaken by Sri Lankan food manufactures to cater to consumer demands by adopting voluntary standard certifications. The findings revealed that voluntary standard adoption do possess a significant association ($p<0.05$) mainly with the international and the both market type, where South Asia does not engage significantly as European Countries, USA/NAFTA, Australia and Middle East and British Retail Consortium (BRC) global standard, ISO 22000, Fair Trade, Halal, organic standards (European Union and USDA) show significant association with market type ($p<0.05$). The majority of the processing plants adhered voluntary standards; in order to, improve product acceptability (91.6%), to increase the market access (83.3%) and as it is a mandatory requirement in target market (75%).

Keywords: Voluntary certifications, Scale of operation, Food processing, Supply category

Introduction

Standardization and the certification process being vital and essential to any sort of industry or manufacturing sector, play a significant role with regard to the food and beverage sector where the quality and safety become the demanding aspects. The scope and the objective of standards differ according to type of firms and companies adopting them, ranging from food quality, safety up to the social, environmental issues along the production chain to the marketing continuum. The motives of complying with particular standards need to be carefully assessed to see whether significant relationship exist in adopting such standard certifications.

The current research study was undertaken with the intention of determining the behavior of food processing firms towards voluntary food standard based on supply category, exporting region, scale of manufacturing and sector of production. The analysis regarding the behavior of the adoption of the voluntary standard certifications is also important for the stakeholders in the food processing industry to predict out their

[1] *Sri Lanka Food Processors Association, Anderson Road, Colombo 05, Sri Lanka*
**Corresponding author: ereshamendis@yahoo.com*

future performance, to work collaboratively with the target customer and also for the customers to be aligned with the manufactures on a reliable basis.
Following Henson (2004), standards can be considered as a continuum characterized according to the degree to which food manufactures have freedom of choice. Voluntary standards are now well established in many developed countries and are gradually extending their global reach to middle income and some low-income countries (Henson and Reardon, 2005).

Methodology

The food and beverage establishments in Sri Lanka were considered as the study population where more than 52% are micro scale establishments. One hundred and fifty food manufacturing companies catering to local, international and both market types were selected for the study using convenient sampling technique. Also, the major exporting regions were identified based on the Export Development Board statistics published in 2016. Thus, five major regions were identified as the export market regions namely, European Union, Middle East, South Asia, NAFTA, Australia. The scale of production was decided based on the classification of small and medium sized enterprises by Ministry of Industry and Commerce applied over both the manufacturing and the service sector and three major categories were identified with regard to the scale of production as large scale, medium scale and small scale.
Pre-tested self-administrated questionnaire was used to collect data from the respondents through an email survey, web link and telephone survey. The published and unpublished reports, newsletters, office record, internet, journal articles and other previous studies were also incorporated as sources of secondary data.
Data analysis was done by using quantitative and qualitative statistics. SPSS 20 was used for the data analysis (IBM, USA).

Results and Discussion

The findings revealed that British Retail Consortium (BRC) global standard, ISO 22000, Fair Trade, Halal, organic standards (European Union and United States Department of Agriculture (USDA) organic standards) have significant association with the market type ($p<0.05$). The results show that the voluntary standard adoption do possess a significant association ($p<0.05$) mainly with the international and the both market type, where South Asia does not engage significantly as European Countries, USA/NAFTA, Australia and Middle East.
According to the results, 55.6% of the BRC adopters cater to both markets while 38.9% prevail in the international market. The analysis furthermore depicts out that BRC standard has a significant association with European Union, USA/NAFTA and Australia where the adoption level is 77.8%, 77.8% and 61.1%. The cross-tabulation analysis shows that the ISO 2200 adoption level have a significant association with the exporting regions such as European Union (57.5%), Middle East (42.5%), USA/NAFTA (49.7%) and Australia (41.1%). Accordingly, 65.8% ISO 22000 earned

manufactures cater their products to both markets; local and international where nearly 20% adopters occur only within the local market.

Halal, voluntary standard targeted on niche market shows significant association with the market type especially with regard to the Middle East (77.9%) and the USA/NAFTA (60%) countries where there is a growing population of Muslims. Concurrently, 75% of the food manufactures export to USA earn the organic standards, where it is 63.0% for Australia 85% for European Union and 52% for the Middle east countries. The sustainable standards and the Fair-trade standards that aligned mostly with the plantation sector, do have a significant association with the market type and exporting regions; and the Fair-trade standard adopters have earned 81% of market share in European Union, 87.5% market share in USA/NAFTA, 62% of market share in both Middle East and Australia.

ISO family standards, pioneered by ISO 9001, ISO 22000 were the leading approaches undertaken by the food and beverage establishments where the contribution varies according to the product category.

The beverage sector represents both the alcoholic products such as wine, arrack, beer etc. and the non-alcoholic sector products mainly with the caffeine products. With regard to the alcoholic product-based firms 85% of the alcoholic product offering firms incorporate company specific policies in their manufacturing procedures. The organic agricultural standards show a significant association of 40.7% with the non-alcoholic beverage sector firms ($p<0.05$) ($\chi 2$, (1),0.010), merely a collaborative approach from 37% USDA organic standards, 29.6% European Union standards and 33.3% Control Union standard certifications. The chi square analysis shows out that the Halal, GMP certification have significant association with the dairy sector firms, ($p<0.05$).

BRC adoption with regard to the scale of production earns a significant association with the kernel-based product categories, where medium scale operators adopt 44.4% BRC significantly compared to large and small-scale operators. Both the HACCP and ISO 22000 standards have significant association over the market catering to both local and international customers where the adoption levels are 88.2% and 94.1% respectively. The large-scale kernel-based product processing firms do have a significant association ($p<0.05$) where it is about 60% while, those cater to both markets; local and international only shows the significance association ($p<0.05$) of 64.7% with the adoption of Halal and Kosher in the manufacturing firms.

The majority of processing plants adhered voluntary standards; in order to increase the market access (83.3%), to improve product acceptability (91.6%), to assess food chains more holistically (66.6%) and as it is a mandatory requirement in the target market (75%). Major barriers to adoption of voluntary standard certification by the small-scale operators catering local market were, resource limitation, poor access to market, lack of proper governance.

Conclusions

It can be concluded that the market type, scale of operation, exporting regions and the product categories affect the adoption of voluntary certification standards and they should be considered on policy formulation and implementing standard certifications.

References

Alli, I., 2004 Food Quality Assurance: Principles and Practices. doi:10.1007/s13398-014-0173-7.2.

Anbumozhi, V. and Kanda, Y., 2005 'Greening the production and supply chains in Asia: Is there a role for voluntary initiatives', IGES Kansai Research Centre KRC, (6), pp. 1–20.

Annual survey of Industries .2015, Department of Census and statistics

Chiputwa, B., Spielman, D. J. and Qaim, M., 2015 'Food standards, certification, and poverty among coffee farmers in Uganda', World Development, 66, pp. 400–412. doi: 10.1016/j.worlddev.2014.09.006.

Clarke, R., 2010 'Private Food Safety Standards : Their Role in Food Safety Regulation and their Impact', 33rd Session of the Codex Alimentarius Commission, pp. 1–36.

Dankers, C., 2003 'Environmental and Social Standards, Certification and Labelling for Cash Crops', p. 120.

Dey, M.M., Rab, M.A., Jahan, K.M., Nisapa, A., Kumar, A. and Ahmed, M., 2005. Food safety standards and regulatory measures: Implications for selected fish exporting Asian countries. Aquaculture economics & management, 9(1-2), pp.217-236.

Export Development Board., 2014. Performance of the Export. Export Development Board, pp.2-10.

Fulponi, L., 2006 'Private voluntary standards in the food system: The perspective of major food retailers in OECD countries', Food Policy, 31(1), pp. 1–13. doi: 10.1016/j.foodpol.2005.06.006.

Gamage, A.S., 2003. Small and medium enterprise development in Sri Lanka: a review. Meijo University, Faculty of Business Management, Nagoya, Japan.

Henson, S. and Reardon, T., 2005 'Private agri-food standards: Implications for food policy and the agri-food system', Food Policy, pp. 241–253. doi: 10.1016/j.foodpol.2005.05.002.

Holleran, E., Bredahl, M. E. and Zaibet, L., 1999 'Private incentives for adopting food safety and quality assurance', Food Policy, 24(6), pp. 669–683. doi: 10.1016/S0306-9192(99)00071-8.

Oosterveer, P., Adjei, B.E., Vellema, S. and Slingerland, M., 2014. Global sustainability standards and food security: Exploring unintended effects of voluntary certification in palm oil. Global Food Security, 3(3), pp.220-226.

Raynolds, L. T. and Ngcwangu, S. U., 2010 'Fair Trade Rooibos tea: Connecting South African producers and American consumer markets', Geoforum. Elsevier Ltd, 41(1), pp. 74–83. doi: 10.1016/j.geoforum.2009.02.004.

Rossignoli, C. M. and Moruzzo, R., 2014) 'Retail Power and Private Standards in the Agri-Food Chain', Agroecology and Sustainable Food Systems. Taylor & Francis, 38(9), pp. 1108–1124. doi: 10.1080/21683565.2014.925530.

Sawyer, E. N., Kerr, W. A. and Hobbs, J. E., 2009 'International marketing of organic foods: Consumers, standards, and harmonization', Journal of International Food and Agribusiness Marketing, 21(1), pp. 44–66. doi: 10.1080/08974430802480644.

Segerson, K., 1998 'Mandatory vs. Voluntary Approaches To Food Safety', (36).
Sharma, L. L., Teret, S. P. and Brownell, K. D., 2010 'The food industry and self-regulation: Standards to promote success and to avoid public health failures', American Journal of Public Health, 100(2), pp. 240–246. doi: 10.2105/AJPH.2009.160960

EVALUATION & COMPARISON OF REFRESHING READY TO SERVE (RTS) BEVERAGE OF COCONUT HAUSTORIUM WITHOUT CHEMICAL PRESERVATIVES

Senarath S.A.C.T. and Perera O.D.A.N.*
Department of Food Science & Technology, Faculty of Livestock, Fisheries & Nutrition, Wayamba University of Sri Lanka

Summary

Coconut haustorium is a healthy food source which contains nutrients and phytochemicals with health-promoting properties. Germinated coconuts are underutilized presently and it can be considered as the only waste in coconut industry, though it is a rich source of nutrients. Therefore, the aim of this study was to develop a refreshing ready to serve (RTS) beverage using coconut haustorium and to compare the product with a commercially available packaged coconut water samples. The most acceptable beverage formula was; 18% extracted haustorium with sugar, citric acid and 0.2% pectin which comply with SLS 729:2010. Both beverage samples were analyzed for physicochemical and microbiological parameters; pH (4.04), total soluble solids (oBrix) (7.5) and titratable acidity was (%0.16) in coconut haustorium RTS beverage. Microbial analysis revealed no any presence of colonies in the formulated beverage. The shelf life of the product is to be further analyzed. The results of the proximate composition revealed that the moisture (93.14 ± 0.09) and ash (0.19± 0.04) of haustorium RTS beverage are significantly different from coconut water (*p* value< 0.05) while protein (0.95± 0.1), fiber (5.39 ± 0.28) & fat (2.94 ± 0.00) are not significantly different. The total phenolic content of the developed beverage (4.5mg (Gallic Acid Equivalent GAE) per 100mL) was higher than the coconut water. Coconut haustorium can be considered as a commercially viable, cheaper food source which has the potential of developing more nutritious beverages which can be introduced as a new product as well as an alternative for commercial coconut water.

Keywords: Coconut haustorium, RTS beverage, Coconut water

Introduction

Coconut (*Cocos nucifera*) is one of the oldest known tropical crops, referred as the 'Tree of Life', "Tree of Heaven" which is a primary source of food, drink and fluid re-hydration. Coconut and coconut based products sector plays a vital role in the foreign exchange earnings by product diversification in the international market. Coconut palm contains two distinct endosperms; liquid form called nut water and solid form called kernel. During the germination process, coconut embryo grows into two directions as the plumule and an absorbent spongy growth called a haustorium (Manivannan *et al.*,

**Corresponding author: aannpp2003@gmail.com*

2018). Haustorium is characteristic within the "Palmae" family (Balachandran and Arumughan, 1995) and it contains two major parts as oil-rich, outer, yellow part and the carbohydrate-rich, inner, white part. Coconut haustorium is a healthy food which is a good source of basic nutrients and important phytoconstituents with disease prevention and health-promoting properties. Though it is a rich source of nutrients, germinated coconuts are presently underutilized and it can be considered as the only waste in coconut industry. Demand for coconut water beverage is being increased in all over the world due to its natural refreshing attributes. Moreover, it creates a huge market as coconut is a change from fruit and carbonated beverage in the market. Therefore, a study was carried out to develop a ready to serve (RTS) beverage using coconut haustorium and to comparatively assess its nutritional and physiochemical properties with a commercially available counterpart.

Methodology

Medium sized coconut (*Cocos nucifera*) haustorium from TXT variety, sugar, citric acid pectin, and water were used as raw materials of the beverage. Trials were conducted by mixing various proportions of the above ingredients to develop a formula which complies with the SLS 729: 2010 standards while having proper acceptance and preference. Sensory analysis was performed on a 7-point Hedonic scale by a panel of thirty semi-trained panelists, to select the most preferred formula which contained a different percentage of pulp, sugar, and ratios of citric to malic acid respectively. The finalized formula for preparation of beverage which complies with SLS 729: 2010 is consisting of 18% extracted haustorium pulp, sugar syrup, citric acid and pectin and water. Then the mixture was homogenized with the help of "D-500 homogenizer at 20000 rpm speed and filtered through muslin cloth. Thus prepared beverage was pasteurized at 85°C in 5min and filled in previously sterilized glass bottles (200 ml) leaving 2.5 cm headspace and sealed airtight by crown corking. Then it was cooled to room temperature and stored at 4 ± 1°C. The developed beverage and the packaged coconut water were subjected to physicochemical analysis (pH, ^{O}Brix, Titratable acidity) microbiological analysis (Yeast & Mould Count, Total Plate Count) and proximate analysis (AOAC 2000). Determination of Total Phenolic Content in both beverages were done using Folin-Ciocalteu (FC) assay as described by Singleton, Orthofer, and Lamuela-Raventos (1999).

Results and Discussion

Sensory evaluation was conducted with untrained panelists to identify the most acceptable proportions of pulp, sugar level and the acid ratios of the haustorium beverage respectively. Eighteen percent pulp incorporated beverage has the highest mean score of sensory attributes while tested samples with different pulp percentages (12%, 14%, 16% & 18%) are significantly different from each other by taste, colour, consistency and overall acceptability. During the second step of sensory evaluation, different sugar levels were tested. It revealed, there was a significant difference (p-value < 0.05) among each level of sugar by taste, overall acceptability and consistency.

Best overall acceptability has shown in the sample that added low amount of sugar syrup (600 ^{O}Brix). Different ratios of citric to malic acid added haustorium beverage samples were tested and it revealed that there were no significant differences among each sample. The beverage containing specific citric to malic acid ratio has been chosen as the final formula as it maintains the pH of the beverage below 4.60 as *Clostridium botulinum* cannot grow and produce toxin at or below pH 4.6.

When comparing the physiochemical properties of these two types of beverages (Table 1) coconut haustorium RTS beverage has a higher value of pH, titratable acidity and ^{O}Brix compared to the coconut water sample. Haustorium beverage's pH is good enough to escape *Clostridium botulinum* as it is below 4.6.

Table 1-Physicochemical properties of beverages.

Beverage Type	Coconut haustorium RTS beverage Mean ± SD[a]	Packed coconut water sample Mean ± SD[b]
pH	4.04	4.95
Brix	7.5	4.00
Titratable acidity (% citric acid)	0.16	0.14 ± 0.04

Microbial analysis (Yeast and Mould count) revealed no any presence of colonies in the formulated haustorium beverage while total plate count revealed 16 Colony Forming Units (CFU) per 1 ml of beverage. The findings of microbial studies on packaged coconut water showed no total plate counts and yeast and mould count since it was subjected to UHT (Ultra- High Temperature) treatment.

The nutritional and proximate composition of the developed Coconut haustorium RTS beverage and the packed coconut water sample were analyzed (Table 2).

Table 2 - Proximate composition of Coconut haustorium RTS beverage (dry basis).

Parameters	Mean ± SD[a] Coconut haustorium RTS beverage	Mean ± SD[b] Coconut water
Moisture	93.14 ± 0.09	96.58 ± 0.12
Ash	0.19 ± 0.04	0.42 ± 0.05
Protein	0.95 ± 0.10	0.54 ± 0.11
Fat	2.94	0.20 ± 0.09
Fiber	5.39 ± 0.28	0.43 ± 0.07

* except moisture other values are in dry weight basis of 100ml of the beverage.

[a] Results are represented as mean ± standard deviation of three independent experiments.(coconut water beverage types N=3)

According to the above results, developed coconut haustorium beverage has a higher amount of protein, fat, and fibre than the packed coconut water 100ml. Moisture content and ash content of the two beverages are significantly different (p-value < 0.05) while protein, fat and fibre content of these two beverages are not significantly different from each other.

Total phenolic content (TPC) in the haustorium beverage was estimated using Folin–Ciocalteu's phenol reagent. And it was 4.5 ± 0.05 mg (Gallic Acid Equivalent GAE) per 100ml of the beverage. Manivannan revealed that the coconut haustorium contained about 146 mg TPC per 100g of dried weight (Manivannan et al., 2018) when analyzed without the outer portion. Total phenolic content (TPC) in the packed coconut water was 3.03 ± 0.06 mg (Gallic Acid Equivalent GAE) per 100ml of the beverage. Packaged coconut water phenolic content is less than the developed beverage.

Conclusions

Coconut haustorium can be considered as a commercially viable since it can be used as the raw material which can be effectively make profit as it has no cost due to a waste in the industry. It has the potential of developing more nutritious beverages which can be introduced as a new product as well as an alternative for commercial coconut water.

References

Balachandran, C. and Arumughan, C. (1995) 'Biochemical and Cytochemical Transformations in Germinating Coconut (Cocos nucifera Linn .)', 1(1), pp. 1385–1391.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. (1995) 'Use of a free radical method to evaluate antioxidant activity', LWT - Food Science and Technology, 28(1), pp. 25–30. doi: 10.1016/S0023-6438(95)80008-5.

Manivannan, A. et al. (2018) 'Biochemical and nutritional characterization of coconut (Cocos nucifera L.) haustorium', Food Chemistry. Elsevier Ltd, 238, pp. 153–159. doi: 10.1016/j.foodchem.2016.10.127.

VALIDATION OF AN IN-HOUSE METHOD DEVELOPED FOR THE DETECTION OF COLIFORMS IN MILK POWDER BASED ON ISO 4831:2006 STANDARD METHOD

Weragala W.N.K. and Arampath P.C.*
Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya, Sri Lanka

Summary

Detection and enumeration of coliforms in a milk powder sample based on ISO 4831:2006 standard method is substantially time consuming and minimum time requirement for obtaining a negative result is 72 hours. An in-house method has been developed in place of ISO 4831:2006 method to obtain negative result within 48 hours. The research objective was to validate the developed in-house method taking ISO 4831:2006 as the reference method according to the guidelines given in ISO 16140:2003, method for validation of alternative methods in the field of microbiology. Artificially contaminated samples (25) and non-contaminated samples (15) were subjected to coliform detection using both test methods. The relative accuracy, relative specificity and relative sensitivity values of the samples were 95, 100 and 90.48 %, respectively. There was no significant difference between the two methods based on discordant result analysis ($\alpha > 0.05$). In conclusion the in-house developed method can be considered as a validated method for the detection of coliforms.

Keywords: Coliform detection, ISO 4831:2006 method, In-house alternative method, Method validation

Introduction

Powdered milk is one of the commonly consumed dairy food commodities. Use of fecal contaminated water in manufacturing operations introduces pathogenic microorganisms to the final product. Unique microbiological isolation techniques are required for the detection of these pathogens due to their low numbers. Thus samples are analyzed for indicator organisms whose presence indirectly indicates the risk of pathogens (Bartram et al., 1996). Fecal coliforms, which is a sub group of coliforms that inhabit in intestinal tract of homoeothermic animals, are considered indicator organisms.

ISO 4831:2006 Microbiology of food and animal feeding stuffs, a horizontal method for the detection and enumeration of coliforms based on most probable number technique is a standard introduced by the International organization for standardization for detection and enumeration of coliforms. ISO 4831:2006 method is time consuming, as it takes at least 72 hours to give a negative result. For a food industry this can incur losses due to delay in release of products. So an in-house method capable of yielding

**Corresponding author: pcarampath@gmail.com*

negative results within 48 hours has been developed. The objective of this study was to validate the developed in-house method based on the ISO 16140:2003 standard; Microbiology of food and animal feeding stuff, a protocol used for validation of alternative methods.

Methodology

Twenty-five artificially contaminated milk powder samples and 15 coliform negative samples were prepared and analyzed by both ISO and in-house methods to detect coliform.

In ISO 4831:2006 method, LSTB (Lauryl Sulfate Tryptose Broth) tube with no inverted Durham tube was inoculated with the sample to be tested and aerobically incubated at 30±1 ℃ for 24±2 hours. After 24±2 hours a tube of confirmation medium (LBBGB-Brilliant Green Lactose Bile Broth) was inoculated with a loopful from LSTB broth and incubated at 30 ℃ for 24 ± 2 hours. If there was no gas formation after 24 hours, incubation continued for another 24 ± 2 hours (confirmation test). If gas production was observed in LBBGB tube, presence of coliform was confirmed.

In In-house method, LSTB tube with inverted Durham tube was inoculated by the sample to be tested and aerobically incubated at 37 ℃ ± 1 ℃ for 24 ± 2 hours and another 24 ± 2 hours if there was no gas formation. After 48 ± 2 hours samples with no gas production were considered coliform negative and no confirmation test was done. For samples with gas production confirmation test was done. If there was a gas production in LBBGB tube presence of coliform was confirmed. Blank control broths were also prepared in all tests.

Relative accuracy (RAC), relative specificity (RSP) and relative sensitivity (RSE) were calculated according to the equations given in ISO 16140:2003 standard. Discordant results were examined using the McNemar's Chi square test.

Results and Discussion

An in-house method which was developed by doing certain modifications to the ISO 4831:2006 MPN procedure can be used in place of the original procedure to reduce the time duration required for giving a negative result in coliform detection test. A comparison between the ISO 4831:2006 MPN method and the developed in- house method is given in Table 1.

In ISO 4831:2006 method since the Durham tubes are not included, confirmation test should be done for all the broths. Although the ISO method states to observe for the opacity of the medium, it is impractical for milk powder sample as milk powder itself is of turbid in nature. In in-house method, broths with gas production can be determined at the end of presumptive test since the inverted Durham tubes are included, thus the confirmation test is done only for the presumptive positive samples. So in-house method is less laborious, saves culture media leading to reduction of the cost, saves the analyst's time and requires lesser incubation space.

Table 1. A comparison between the ISO 4831:2006 MPN method and in-house method

Criteria	ISO 4831:2006 MPN method	In-house method
Incubation temperature	30 ℃	37 ℃
Inverted Durham tubes	Not included	Included
Presumptive stage time duration	24 ± 2 hours	48 ± 2 hours
Confirmation test	Done for all samples	Done only for presumptive positives samples

Ability of the in-house method to produce a negative result within 48 hours allows the industry to release the products early to the market. For an industry one day delay in release of products may cost millions. Loss of profits, consumer dissatisfaction, inability of meeting consumer demand and business targets and requirement of more storage area to retain products are some of the associated adverse consequences.
ISO 16140:2003 alternative method validation protocol comprises of two phases, method comparison study (study performed by the organizing laboratory) and inter-laboratory study (study of the method's performance using common samples in several laboratories). Only the method comparison study phase was conducted during this study. Paired results of the reference method and in-house method for coliform detection are given in Table 2.

Table 2. Paired results of the reference method and in-house method for coliform detection.

Method		Reference method	
	Responses	Positive	Negative
Alternative method	Positive	19 (PA)	0 (ND)
	Negative	2 (PD)	19 (NA)

PA-Positive agreement, NA-Negative agreement, PD-Positive deviation, ND-Negative deviation

The values obtained for three selected criteria, relative accuracy (RAC), relative specificity (RSP) and relative sensitivity (RSE) were 95 %, 100 % and 90.48 % respectively which were considered high and satisfactory values. Relative accuracy is the degree of correspondence between the responses of two methods on identical samples. Relative specificity is the ability of the alternative method to not detect the analyte when it is not detected by the reference method. Relative sensitivity refers to the ability of the alternative method to detect the analyte when it is detected by the reference method.
All 40 samples were analyzed by colony count technique (ISO 4832) simultaneously, for verification of the results. According to the discordant results (positive deviations and negative deviations) analysis by McNemar's Chi-square test, no significant difference was exist between two methods at $\propto$=0.05 level of significance.

Therefore, according to the results of the McNemar test and RAC, RSP and RSE values, the in-house method for coliform detection can be considered as a validated method. It is proposed to conduct a collaborative inter-laboratory study before generally concluding the alternative method as a validated method.

Table 3. 95 % confidence interval for RAC, RSP and RSE criteria

Criteria	Value %	95 % confidence interval
Relative accuracy(RAC)	95	88.2 % - 100 %
Relative specificity(RSP)	100	-
Relative sensitivity (RSE)	90.48	77.65 % - 100 %

Conclusions

According to the study in-house method can be considered a validated method for detection of coliforms in milk powder with satisfactory relative accuracy, relative specificity, and relative sensitivity values of 95%, 100% and 90.48% respectively.

References

Bartram, J. and Ballance, R. (1996). Water quality monitoring: a practical guide to the design and implementation of freshwater quality studies and monitoring programmes. CRC Press.

ISO 4831:2006 - Microbiology of food and animal feeding stuffs - Horizontal method for the detection and enumeration of coliforms - Most probable number technique

ISO 4832:2006 - Microbiology of food and animal feeding stuffs - Horizontal method for the enumeration of coliforms - Colony-count technique

ISO 16140:2003; Microbiology of food and animal feeding stuff- Protocol for validation of alternative

INSTITUTE OF
FOOD SCIENCE & TECHNOLOGY
SRI LANKA