FoodTechno 2019 *Innovation to Application*

organized by

IFSTSL
INSTITUTE OF FOOD SCIENCE & TECHNOLOGY SRI LANKA

What is FoodTechno ?

FoodTechno is the annual research session organized by the Institute of Food Science & Technology Sri Lanka (IFSTSL)

Objectives of FoodTechno

The IFSTSL, works jointly with the Sri Lanka Food Processors Association (SLFPA) as a twin body providing technical inputs for the food industry to upgrade food sector in Sri Lanka. The main objective of this research sessionis to provide a common platform for the researchers from Universities and other research institutes in Sri Lanka to develop adialogue with the food industry aiming to transfer the research knowledge they gathered from scientific research. It is expected that this scientific research findings can be applied into industry practices aiming improvements in the food industry in Sri Lanka. The annual session brings in much needed opportunity for the industrialists to pick innovations to bedeveloped into commercially viable industrial products or processes.

Target Audience

Food industrialists involved in food processing and product development, research and development, quality assurance and any area involving innovations.

4th

Annual ResearchSession of IFSTSL

3rd

August 2019 at BMICH in parallel with ProFood/ProPack Exhibition

What IFSTSL Expects from the Food Industry?

IFSTSL expects fullest cooperation from the food industry by participating in this event to ensure the objectives of this session are achieved.

FORTIFICATION OF DRINKING YOGHURT WITH BETA CAROTENE BY INCORPORATING *Daucus carota* PULP AND *Citrus sinensis* JUICE

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Summary

Beta carotene is a natural antioxidant omnipresent in most of the fruits and vegetables. The current research was carried out to enrich a dairy product; drinking yoghurt with beta carotene which is known as an excellent source of vitamin A. *Daucus carota* pulp was incorporated as the main beta carotene source and *Citrus sinensis* juice was used to enhance the sensory properties of the product. The drinking yoghurt formula was developed as 80% of yoghurt base, 10% of *Daucus carota* pulp and 10% of *Citrus sinensis* juice.The shelf life of developed product was determined as 35 days considering the absence of coliform and yeast & mold counts, variation of pH, beta carotene and antioxidant capacity respectively from 4.41±0.01 to 4.20±0.01, from 4.97±0.01 to 4.39±0.01 µg/g and from 55.24±0.01 to 38.36±0.05% and at 95% confidence level, no significant difference was there between sensory attributes throughout the shelf life according to the analysis done using Kruskal-Wallis non-parametric one-way ANOVA and Mann Whitney tests of Minitab 17 version. These results collectively suggest that the developed product can be identified as a beta carotene enriched dairy product which may encourage further studies on skin fairness effect of the developed product.

Keywords: Beta carotene, yoghurt, *Citrun sinensis, Daucuc carota,* Fortification

Introduction

Milk is a highly nutritious food which contains substances provide both energy and the building materials necessary for growth. Water, fat, protein and lactose are the four quantitatively dominant components of milk while the minor components are minerals, enzymes, vitamins, and dissolved gases. It satisfies consumer demand for high quality innovative dairy products. Fermentation is a value added process of milk which adds more taste, better texture and enhanced shelf life for the product. Yoghurt is also a fermented product preferred by worldwide consumers. However, there are two main types of yoghurt, set and stirred, based on the method of production and on the physical structure of the coagulum. With the innovations of dairy industry, nutritional substances such as probiotic cultures, minerals, natural and artificial fruit and vegetable flavors are incorporated to yoghurts. Drinking yoghurt which is under the category of stirred yoghurt, is now a highly consumable product in both national

and international markets. With the developments of the dairy industry, more innovative types of drinking yoghurt are coming to the market and are in research level based on the fortification of essential nutrients for the human body. Such developments will enhance the nutritional value and also sensory properties of the plain yoghurt drink. The study was carried out in the University of Sri Jayewardenepura to develop a drinking yoghurt enriched with beta carotene by incorporating *Daucus carota (carrot)* pulp and *Citrus sinensis*(orange) juice. Carrot is mostly used vegetable in human nutrition. Carrot juice increases the total antioxidant status and decreases lipid peroxidation in adults (Potter *et al.*, 2011). It is rich in beta carotene, ascorbic acid, tocopherol and classified as vitaminized food (Bello and Wudil, 2012). Therefore, the carrot was used as the main beta carotene source and orange was added to enhance the sensory properties of the product and also as a beta carotene source. Orange is a rich source of vitamin C, flavonoids, phenolic compounds and pectins. The main flavonoids found in citrus species are hesperidine, narirutin, naringin and eriocitrin (Milind and Dev, 2012). Beta carotene pigments are naturally occurring antioxidants in plant materials. According to recent studies, a diet high in carotenoids may reduce the risk of heart attack and assist in cancer prevention (Steinmetz, 1996). Fortification is one of the best methods to deliver the benefits of natural antioxidants for humans (Gad *et al*., 2017).

Methodology

The Raw carrot (*Daucus carota)* was peeled, washed, grated and blended with water (4:1) to prepare the carrot pulp. Orange (*Citrus sinensis*) was washed, peeled and the juice was extracted. Orange juice and carrot pulp were mixed at 1:1 ratio and pasteurized at 105^{o}C for 1 minute for the preparation of pulp to be mixed with yoghurt. According to the usual method, the yoghurt base was prepared using standardized milk containing 3.5% fat and 8.5% MSNF. The yoghurt base was stirred while mixing with pasteurized carrot orange mix at 4:1 ratio. Prepared drinking yoghurt was filled into bottles and stored at 4^{o}C. A preliminary experiment was conducted to select the best formulation of carrot pulp (5%,10%,15%) and yoghurt base (85%, 80%,75%) with constant percentage (10%) of orange juice. The sensory properties of three samples were evaluated using a trained sensory panel on a nine-point hedonic scale. The sample containing 10% carrot pulp, 10% orange juice and 80% yoghurt base was selected as the best after analyzing the data gathered from the sensory trial with respect to appearance, colour, taste, mouth feel, and overall acceptability using Kruskal-Wallis non-parametric one-way ANOVA and Mann Whitney test. Shelf–life of the product stored at 4^{o}C was analyzed for 35 days. For the evaluation of shelf life, the variation of the product pH value, Coliform and Yeast & Mold counts, beta carotene content, antioxidant activity and sensory parameters with shelf life were studied. Ultra violet visible spectrophotometry and 2,2-diphenyl-1-

picrylhydrazyl radical scavenging activity were used to measure beta carotene content and antioxidant activity respectively. The collected data were analyzed statistically using Kruskal-Wallis non-parametric one-way ANOVA and Mann Whitney tests of Minitab 17 version.

Results and Discussion

Sensory analysis

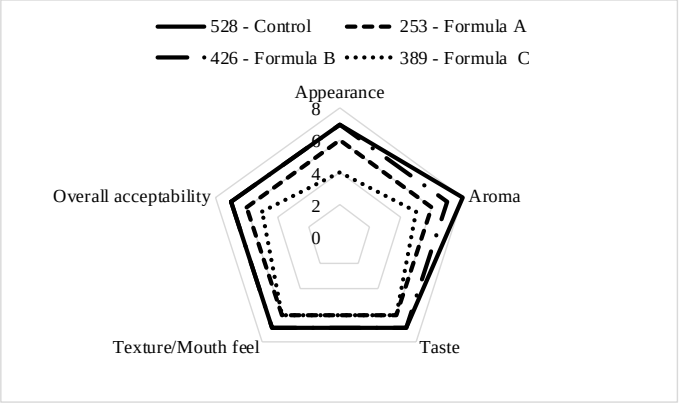

Figure 1: Graphical representation of selecting the best formula with respect to sensory attributes

This web diagram (Figure 1) shows the variation of mean values of each sensory attribute of the four drinking yoghurt samples (528 – Control, 253 – Formula A, 426 – Formula B, 389 – Formula C) according to the data collected from sensory evaluation. The mean values of each sensory attribute of Formula B are similar to the control sample except aroma. Other formulae are having lower values for sensory parameters than formula B and control. Therefore, formula B was selected as the best and subjected to product development and further analysis.

Table 1: H_{cal} values for each sensory attribute

Attribute	H_{cal} value
Appearance	32.65
Aroma	19.38
Taste	26.76
Texture/Mouthfeel	32.28
Overall Acceptability	35.77

Null hypothesis	H_0: All medians are equal (there is no significant difference between two samples under the tested attributes)
Alternative hypothesis	H_1: At least one median is different (there is a significant difference between two samples under the tested attributes)

The degree of freedom of the test samples; 3 – 1 = 2

The H_{cal} values of each sensory attributes are greater than the relevant chi-square value (5.991). Therefore, according to the Kruskal Wallis test, under 0.05 level of significance, H_0 can be rejected. Which concludes that there is a significant difference in each sensory attributes of four samples.

Variation of pH Value with Storage at 4o C

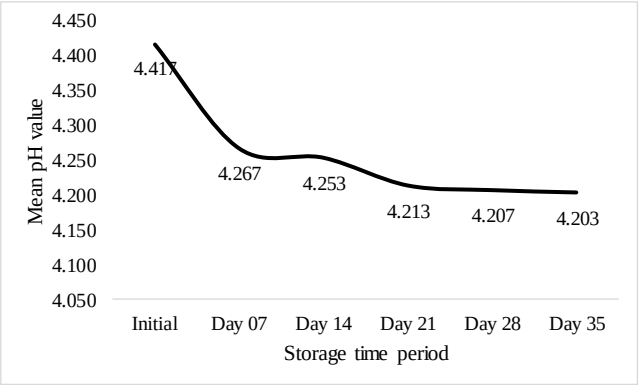

Figure 2: Variation of pH Value with Storage

Figure 2 indicates the graphical representation of the variation of pH value with storage at 4°C. The initial pH value of developed drinking yoghurt was 4.41 and reduced up to 4.20 within 35 days.

Variation of beta Carotene Content with Storage at 4°C

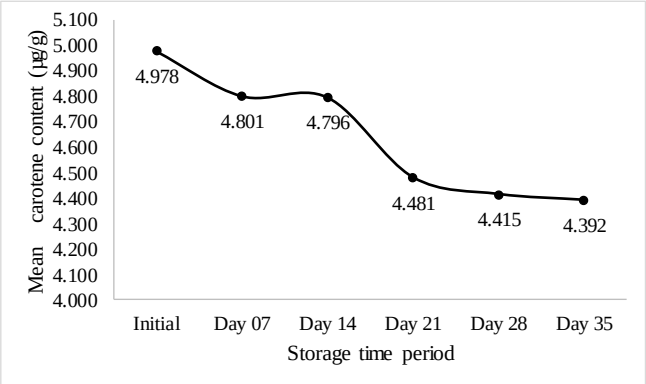

Figure 3: Variation of Beta Carotene Content with Storage

The initial beta carotene content of developed drinking yoghurt was 4.97 µg/g. After 35 days of storage at 4°C, the beta carotene content was reduced to 4.39 µg/g which is shown in figure 3.

Variation of Antioxidant activity with Storage at 4°C

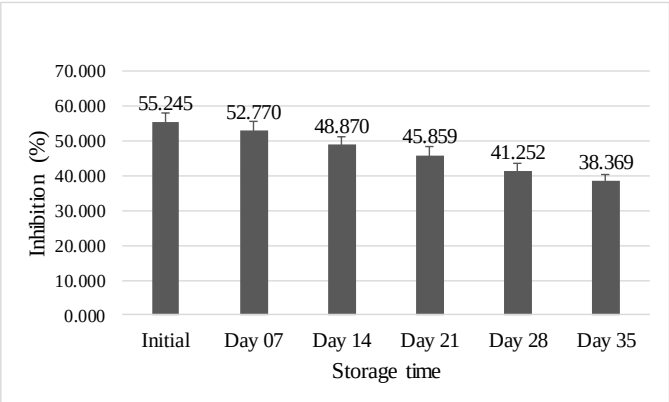

Figure 4: Variation of Antioxidant activity with Storage

The antioxidant activity of developed drinking yoghurt was reduced from 55.2% to 38.3% within 35 days of storage time at 4°C as shown in figure 4

Microbial Analysis

The time period of storage (at 4°C)	Coliform Count		Yeast and Mold count (CFU)	
	Developed drinking yoghurt sample	Control	Developed drinking yoghurt sample	Control
Initial	Negative	Negative	0	0
Day 07	Negative	Negative	0	0
Day 14	Negative	Negative	0	0
Day 21	Negative	Negative	0	0
Day 28	Negative	Negative	0	0
Day 35	Negative	Negative	0	0

Table 2: Coliform, Yeast & Mold counts of the Developed Drinking Yoghurt Samples

Microbial results are indicated in table 3. Coliform counts were negative for 35 days in both developed sample and the control sample. Yeast and mold were also absent from the storage time period.

Conclusion

The drinking yoghurt; developed according to the formula of 80% of yoghurt base, 10% of carrot pulp and 10% of orange juice is a rich source of beta carotene which is 11.63 times higher than a plain yoghurt with 35 days of shelf life.

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ANTIMICROBIAL ACTIVITY OF TWO PROBIOTIC *Enterococcus faecalis* STRAINS ISOLATED FROM SRI LANKAN DAIRIES: A POSSIBLE ALTERNATIVE SOURCE OF ANTIMICROBIAL AGENTS AGAINST INVADING MICROORGANISMS

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Summary

Lactic acid bacteria (LAB) are the most widely used bacteria as starter cultures for the industrial processing of fermented dairy, meat, vegetable and cereal products. Several strains of Lactic Acid Bacteria (LAB) produce bacteriocins that exhibit antibacterial/antifungal activity. The objective of this study was to assess antimicrobial activity of two probiotic *Enterococcus faecalis* strains (*E. faecalis* SLDL-211 and *E. faecalis* SLDL-203) isolated from buffalo milk. These two isolates well survived under simulated gastro intestinal conditions (pH, bile, temperature, simulated gastric enzymes and inhibitory substances) and exhibited satisfactory adhesion and aggregation abilities. They were also proved to be safe for live consumption by *in-vitro* safety testes: DNase and Gelatinase production, haemolytic activities and antibiotic susceptibility patterns. Isolates possessed satisfactory level of milk technological properties and well survived during refrigerated storage. Both test strains showed good antimicrobial activities against 6 human pathogens, *Bacillus cereus*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Salmonella enterica*, *Escherichia coli* and *Listeria monocytogenes.* However, either test strains or the reference strains were unable to inhibit the growth of *Candida albicans*. Therefore, there is a possibility of using these two strains as probiotics to control pathogenic bacteria and for producing bacteriocins as food bio preservatives to control food spoilage.

***Keywords*:** Probiotic, Lactic acid bacteria, Antibacterial activity, Pathogenic bacteria, Well diffusion assay

Introduction

In recent years, due to indiscriminate use of antibiotics for disease treatments and growth promotion of livestock, antibiotic resistance has become a growing therapeutic problem. This has led to increased interest in the application of probiotics and their antimicrobial metabolites as alternative antimicrobial strategies for treatment and prevention of infections. Discovery of new antibiotics is an exclusively important objective among researchers worldwide as the existing compounds are in

danger of losing their efficacy. Therefore, discovery of novel microbial strains may provide an alternative source of antimicrobial agents with potentially new mechanisms of actions. Hence, antimicrobial activity against pathogens is a desirable property of a potential probiotic strain. LAB are generally recognized as safe (GRAS microorganisms) and play an important role in food and feed fermentation and preservation. Antibacterial activity is an important characteristic of probiotic LABs hence, is a main criterion for selection of starter and probiotic cultures for the development of

Methodology

Two *Enterococcus faecalis* strains (*E. faecalis* SLDL-211 and *E. faecalis* SLDL-203) isolated, characterized and identified from buffalo milk (Breed: Murrah, District Anuradhapura) of Sri Lanka were selected for this study. Two known probiotics, *L. casei* subsp*. casei* (ATCCC 393) and *L. delbrueckii* subsp. *lactis* (ATTCC 15808) were used as reference strains. All microbiological media were obtained from Oxoid, UK and chemicals from Sigma, St. Louis, USA.

In a preliminary (unpublished data) study, the strains were isolated, purified, biochemically characterized as per the methods explained in Bergey's Manual of Determinative Bacteriology. Selected LABs were tested for their probiotic attributes (Aswathy *et al*., 2008), confirmed suitability for live consumption by *in-vitro* safety assessment tests (Gupta *et al*., 2007) and also genotypically identified by 16S rDNA sequence analysis. Cultures were stored at −20 °C and −80 °C in appropriate broth media supplemented with glycerol (40% v/v). Before the assay, the strains were pre-cultivated twice in MRS (de Man–Rogosa–Sharpe) broth at 37 °C for 24 h. Seven bacterial strains that are pathogenic to humans were selected as test pathogens to investigate the antagonistic activity of the LAB strains and were obtained from the American Type Culture Collection (USA): *Bacillus cereus* ATCC® 1177, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC® 29212, *Salmonella enterica* ATCC® 14028, *Escherichia coli* ATCC® 35218*, Listeria monocytogenes* ATCC® 11994*, Candida albicans* ATCC® 1023. Agar-well diffusion method (Schillinger and Lücke, 1989) was used with cell-free supernatant (CFS) of test strains to determine the inhibitory effect. Exponential cultures of the test pathogens were diluted to a suitable turbidity (McFarland standard 0.5) and used to inoculate melted and cooled Mueller–Hinton Agar to a final concentration of $\sim 10^6$–10^7 CFU/ml by spreading the cell suspension with a sterile cotton swab. Wells, 7 mm in diameter, were punched in the agar plates and 100 µL of culture supernatant were added to each well. After incubating overnight at 37 °C, the antimicrobial activity was expressed as the diameter of the inhibition zones (mm) around the wells. Inhibition zones of more than 20 mm, 10 to 20 mm, and less than 10 mm were considered as strong, intermediate, and low inhibitions, respectively. The test was performed twice, each in triplicate.

Results and Discussion

Strains *E. faecalis* SLDL-211 and *E. faecalis* SLDL-203 exhibited good survival potential under physiological and simulated chemical conditions existing in the human gastro-intestinal tract and storage conditions. Temperatures of 10 °C, 37 °C and 45 °C , 1.5, 3.0, 9.0 pH, 0.1%, 0.3%, 0.5% ox bile, simulated gastric enzymes, pepsin and pancreatin under 2 different pH levels (pH 2 and 8) and inhibitory substances NaCl (2%,4%,6.5%) and phenol (0.2%, 0.4%, 0.6%). Survival ability of two test strains were equally better compared to the reference strains as shown in Figure 1 therefore, could be considered as potential probiotics for further investigations. Identification of the selected LABs was by PCR amplification of selected regions of 16S rRNA gene with universal primers (forward primer, 27F-AgAgTTTgATCMTGGCTCAg and reverse primer, 1492R-TACggYTACCTTgTT ACgACTT) and amplified products were subjected to DNA sequencing at Macrogen-South Korea. Resulted sequences were analyzed using online Basic Local Alignment Search Tool (BLAST). The sequences were deposited at National Centre for Biotechnology Information (NCBI) and the obtained accession numbers are shown in Table 1. The two strains were safe for live consumption (negative for DNase, gelatinase and haemolytic tests) therefore, were further analyzed for their antimicrobial activities in this study.

Table 1: Identification of the LAB isolates using 16S rRNA gene sequences

Test stain	Source	NCBI Accession Number received	The nearest matched species from GenBank®	Similarity (%)
Enterococcus faecalis strain SLDL-211	Buffalo milk	MH779826	*E. faecalis*	99%
Enterococcus faecalis strain SLDL-203	Buffalo milk	MH645806	*E. faecalis*	99%

Similarity values were determined using the basic local alignment search tool (BLAST) of the GenBank. Sequences with ≥97% similarity to the previously published sequences were used as the criteria to indicate species identity.

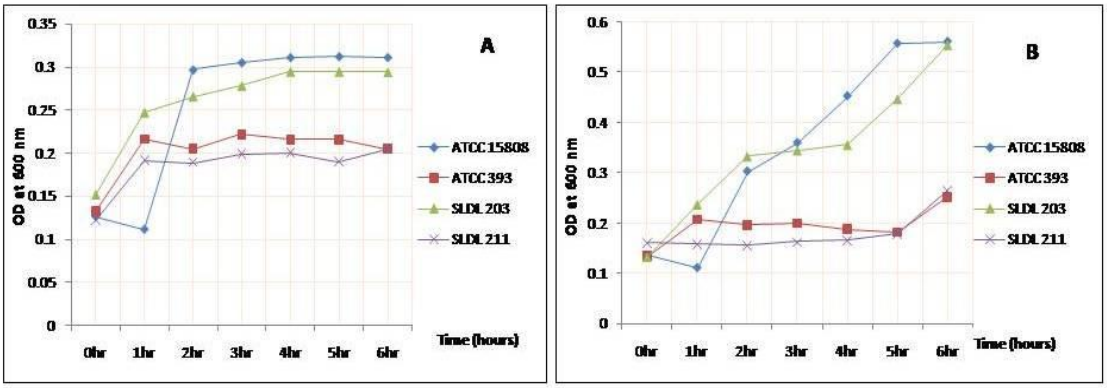


Figure1. Survival pattern of *Enterococcus faecalis* and Reference strains in the presence of pepsin (A) pH 2 and (B) pH 8.0

None of the isolates (test probiotics and reference cultures) were able to inhibit the growth of pathogenic yeast, *C. albicans* ATCC® 1023 used in the study. *C. albicans* strains showed strong resistance to antimicrobial substances and similar results were obtained in a study done by Stephen and Douglas, 1995. Clearance zones produced by *E. faecalis* SLDL-211 and *E. faecalis* SLDL-203 on plates inoculated with *S. aureus* ATCC® 2592 and *B. cereus* ATCC® 117 are shown in Figure 2, (A) and (B). Both test strains produced clearance zones around the wells made on plates inoculated with all other pathogenic bacteria except *C. albicans therefore*, could consider as potential candidates with antibacterial activity. *E. faecalis* SLDL-211 and *E. faecalis* SLDL-203 created inhibition zones of more than 20 mm in plates inoculated with *S. aureus* ATCC® 2592, S*. enterica* ATCC® 14028 as well as *E. coli* ATCC® 35218 hence could consider as strong inhibitors. They were intermediate inhibitors (created inhibition zone of 10 to 20 mm) of *E. faecalis* ATCC® 29212, *B. cereus* ATCC® 117 and *L. monocytogenes* ATCC 11994. Antimicrobial Activity of bacteriocins produced by *Enterococcus faecalis* KT11 against similar pathogens and antibiotic-resistant bacteria has been shown by Abanoz and Kunduhoglu, (2018) in a similar study. A study done by *Atya et al.,* 2015 indicated that the inhibition of *S. aureus* ATCC® 33862 by *E. faecalis* 28 and *E. faecalis* 93 was due to production of lactic acid and bacteriocin, therefore the tested strains in the present study could regard as lactic acid and bacteriocin producers as well.

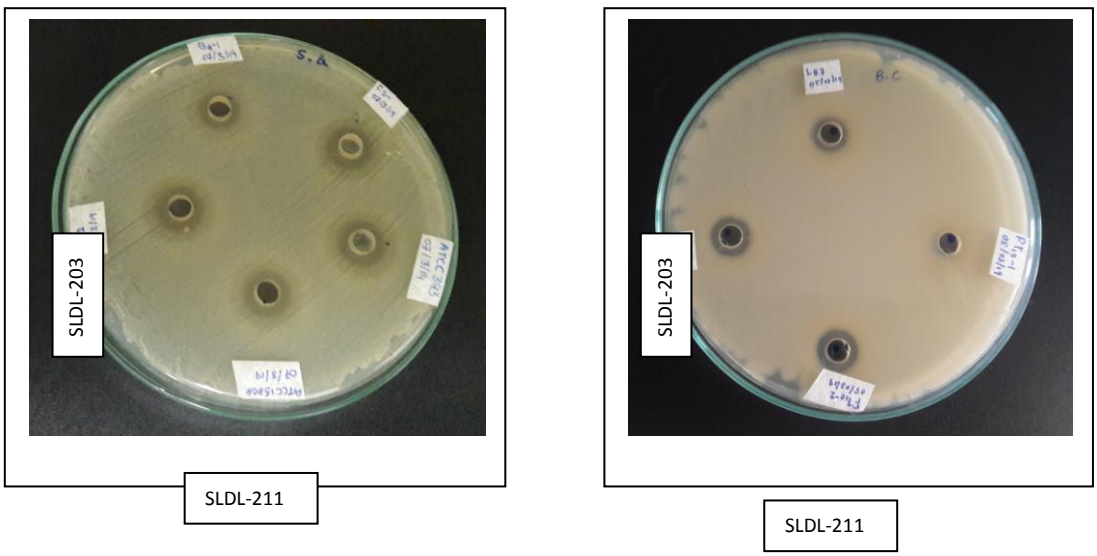
Table: 2 Antagonistic activities of *Lactobacillus* strains against 7 test pathogens

	E. faecalis SLDL-211 Mean ± SD	*E. faecalis* SLDL-203 Mean ± SD	*L. casei subsp. casei* (ATCCC 393)*	*L. delbrueckii subsp. lactis* (ATTCC 15808)* Mean ± SD

	(mm)	(mm)	Mean ± SD (mm)	(mm)
E. faecalis (ATCC 29212)	18.00 ± 1.73 (I)	18.67 ± 1.53 (I)	18.33 0.58 (I)	22.00± 3.46 (S)
B.cereus (ATCC 117)	14.00 ± 1.00 (I)	15.67 ± 0.58 (I)	18.33 ± 0.58 (I)	14.67± 1.53 (I)
S. aureus (ATCC 25923)	24.33 ± 3.06 (S)	26.00 ± 1.00 (S)	25.00 ± 0.00 (S)	25.00 ± 1.73 (S)
S. enterica (ATCC 14028)	26.33 ± 1.15 (S)	26.00 ± 1.73 (S)	28.33 ± 1.15 (S)	23.00 ± 1.73 (S)
E. coli (ATCC 35218)	24.00 ± 3.46 (S)	26.00 ± 1.00 (S)	26.00 ± 1.73 (S)	26.00 ± 1.00 (S)
C. albicans (ATCC 1023)	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
L. monocytogenes (ATCC 11994)	17.67± 0.58 (S)	21.00 ± 1.00 (S)	17.67± 0.58 (I)	21.33 ± 0.58 (S)

Values are means of two independent experiments, each in triplicate.

*Reference strain.



(A) **(B)**

Figure 2: Inhibition zones produced by the 2 test strains against *S. aureus ATCC 2592* (A) and *B. cereus ATCC 117* (B)

Probiotic *E. faecalis* SLDL-211 and *E. faecalis* SLDL-203 strains performed equally better compared to established probiotics and encompass antagonistic activities against human pathogens and could utilize for formulating functional foods to boost human health. The bacteriocins produced by these probiotic strains have to be isolated, identified, characterized and studied further to confirm the minimum inhibitory concentrations (MIC) and to identify the mechanisms against pathogens.

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EXTRACTION OF CRUDE ALGINATE FROM BROWN SEAWEED (*Sargassum* spp.) AND FORMULATION IN TO BEADS AS AN ICE CREAM STABILIZER

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Summary

Sargassum sp. in coastal region of Sri Lanka is currently under-utilized locally. Therefore, purpose of this study was to identify the stabilizing effect of the alginate extract of *Sargassum* sp. to improve the texture and sensory properties of ice-creams. Four types of *Sargassum* sp. were tested to identify the highest alginate yielding type which was compared by different procedures such as, Sulphuric, Hydrochloric and $CaCl_2$ route. From the time-temperature combinations used, 90˚C;1-hour was the optimum at commercial scale compared to 30˚C;24 hours, 30˚C;1 hour and 60˚C;1-hour. The highest yield was obtained from $CaCl_2$ route and the extracted alginates were converted to beads by using $CaCl_2$. Beads were incorporated to ice cream, replacing the commercial stabilizer which contains Guar Gum (E412), Carrageenan (E 407a) and Sorbitan ester of Fatty Acids (E433) and the melting point, texture and other sensory properties were analyzed against the control. *Sargassum* sp. 03 gave the highest yield of alginate and the stabilizing effect of 1.5% inclusion had the first melting drop at 5.57th minute. Without the stabilizers, ice cream showed a quicker melting time with first drop melting at 4.51 minutes which is significantly different (p <0.05). Sensory panelists confirmed the textural improvements of the product.

Keywords: Seaweeds; *Sargassum* spp.; Ice-cream; Stabilizer; Alginate

Introduction

Several studies have been carried out in Sri Lankan coastal areas from which it was revealed that most of the algae species are available in the west coast of Sri Lanka and a very few species in east coast. (Jayasuriya, 1992). As per these studies, the common genera native to Sri Lanka is *Sargassum* (Pheophyceae), with having high level of functional properties. (Durairatnam, 1961). Although they were been used in food diets as well as traditional remedies in Asian countries like China, Japan and Korea (Wijesekara et al., 2010, 2011). Edible sea weeds have been identified as an under exploited plant resource in Sri Lankan context. Currently, in Sri Lanka, these under-utilized species are mostly used to export as in the crude form of it.However, this study was conducted to identify and quantify the alginate polysaccharide present in

Sargassum sp., while finding out the best method to extract the alginates to obtain an optimum yield. Extractions are carried out by solvent extraction method after drying the mass. (Garcia et.al., 2016) Most commonly used solvents for this purpose were water (Cong et al., 2016; Cong, Xiao, Liao, Dong, & Ding, 2014; Shan et al., 2016; Vijayabaskar, Vaseela, & Thirumaran, 2012; J. Wang, Wang, Yun, Zhang, & Zhang, 2012; P. Wang et al., 2012) and ethanol (Foley et al., 2011; Huang et al., 2016). In some instances, slightly acidic solutions (Anastyuk, Shevchenko, Dmitrenok, & Zvyagintseva, 2012; Dinesh et al., 2016; Imbs et al., 2016; Lorbeer et al., 2015; Menshova et al., 2015) have also been used. After the polysaccharide extraction, extracted mass is to be treated further to retain alginates. Alginates are composed of mannuronate and guluronate residue chains alternatingly repeating with 1-4 linkages, which also shows gelation properties in the presence of Ca^{2+} (Garcia et.al., 2016) This property of alginate is utilized in the study to form beads, to form as a carrier of the stabilizing compound.To identify and establish the use of formed alginate beads, ice cream is used in this study as the food matrix. The intention was to study the change in properties of the food matrix, after introducing the bead, against the existing synthetic stabilizers. However, it is known that the chemical structure of the macroalgal polysaccharides varies according to factors affecting the macroalgal biomass (i.e. macroalgae species, environment and geographical location) and the conditions of extraction and purification of the polysaccharides (i.e. temperature, time) (Garcia et.al., 2016). Therefore, the optimum extraction conditions and resultant character improved product will vary from one geography to another, based on the chemical properties.

Methodology

Sample Preparation

Specimens from four different *Sargassum* spp. were detached from its substrate, cleansed and epiphyte removal was done by washing with water at the collection site itself. These samples were then placed in iced containers to facilitate with transportation. After reaching the destination, samples were sun dried for 24 hours and oven dried at 60°C for another 48 hours. Dried samples were grounded until fine powder was obtained and sieved from 350 µm mesh to obtain a fine powder. Acquired powder was place in moisture & air proof containers and stored in 4°C until further use.

Polysaccharide Extraction

Exactly 100.00 g of the dried seaweed powder was mixed with 1 liter of distilled water and allowed to stand in four-time temperature combinations separately (Table 01). After the designated time, the mix was cooled and centrifuged at 2000 rpm for 10

minutes. Supernatant was collected and three volumes of absolute ethanol was added. After standing for 5 minutes, again the mixture was centrifuged, and the supernatant was removed. Resultant was dried in the oven at 40˚C and the weight was measured. The procedure was triplicated to obtain accurate results. The polysaccharide yield is obtained by Eq (1).

$$Polysaccharide\ yield = \frac{Dried\ weight\ of\ the\ extract}{Initial\ weight\ of\ the\ seaweed\ powder}\ X\ 100\% \qquad Eq\ (1)$$

Alginate Extraction

After determining the polysaccharide yield, the species yielding the highest amount of polysaccharide was identified and undergone the alginate extraction. To determine the highest alginate yielding method for the selected species, three routes of extractions were selected.

Hydrochloride Route

Dried seaweed was soaked in 2% Formaldehyde solution at 30˚C for 24 hours. While this step eliminates the pigments and phenolic compounds. Phenolic compounds were removed from the seaweed to reduce discolouration at later stages of extractions. For the resultant colour removed crude, 0.2M HCl was added and was kept for another 24 hours. This step was followed by washing with water and 1-2% Na_2CO_3 for 3 hours in 60˚C. Resulting solution with the crude was centrifuged at 1500 rpm for 15 minutes and the soluble fraction was collected. Soluble fraction was then precipitated with 70% EtOH followed by drying at 65˚C until reaching a constant weight. Precipitation and drying was repeated until no further precipitate were obtained.

Calcium Chloride Route

In analyzing through the $CaCl_2$ route, 2% $CaCl_2$ was used to soak the dried seaweed, before soaking in Formaldehyde and Na_2CO_3 treatment was carried out for 48 hours.

Sulfuric Acid Route

Similarly, in H_2SO_4 route, dried seaweed was soaked in H_2SO_4 for 24 hours before formaldehyde treatment and Na_2CO_3 treatment was carried out for 48 hours. The analysis was triplicated for each of the samples. Crude alginate yield was calculated in all three routes by using the Eq. 1.

Preparation of alginate beads

Extracted Alginate semi solid was dissolved in equal amount of water (50% v/v) and using a dropper, solution drops with equal volume was put into a 2M Calcium Chloride solution. Beads will be formed immediately, but to obtain a rigid outer surface, beads were left in the solution for 10 – 15 minutes. After the time slot, they were removed from the solution and was placed in the refrigerator with 4˚C until further consumption.

Preparation of ice cream

An ice cream recipe of an industrially established local brand was selected and a series of ice creams were prepared with varying amount of stabilizer content of different compounds (Table 02).

All the dry ingredients including the stabilizer was mixed continuously, while adding the liquid and semi solid basic components of ice cream. The only difference is made from the stabilizer amount and type as per table 02. After hand mixing, ingredients were pasteurized at 72˚C for 15 minutes, stirring continuously. Then the mixture was allowed to cool and after 1 hour, it was homogenized using the homogenizer (Goma, SS304). All of the homogenized mixes were allowed to stand in the refrigerated conditions as ageing for 48 hours and the mixes were churned separately with the churner (Krups, GVS141). After the over-run is reached by 60 minutes (for 1 liter of base ice cream mix), ice creams were spooned out and freeze overnight in separate containers. On the following day, they were evaluated for the melting curve and sensory attributes.

Evaluation of melting time

From the freeze ice cream bulk, rectangular cubes (50 mm x 10 mm x 10 mm) were cut while the ice cream was still at the freezing conditions. These cubes should be of equal size and weight from every ice cream made. The cubes were placed in the freezer again and the apparatus was set (Figure 01) to measure the melting weight of the ice cream. Stop watched was switched on immediately after keeping the ice cream cube on the wire mesh and the time taken to drip the first drop of ice cream to the beaker on the scale was recorded. After the first drip, with 1-minute intervals, the melted weight was recorded until the final drop of melted ice cream was recorded. This procedure was repeated for all ice cream variants and the melting curve was plotted for the weight against the time.

Sensory evaluation

Twelve number of trained sensory panelists were selected, and each ice cream variant was tested for its texture being; iciness, sandiness and mouthfeel (Lawless, Heymann, 2010)

Results and Discussion

Polysaccharide yield was highest in the 90°C / 1-hour combination *in Sargassum* sp. 03 (Table 03). Therefore, this combination of time temperature along with the seaweed variety was selected for the extraction of alginates.

In the selected variety, alginate extraction was carried out and the highest yield was obtained from the Calcium Chloride route (Table 04). Therefore, this route was used to extract the alginates from brown seaweeds.

In preparing the beads, Sodium Alginate is allowed to be dropped into a Calcium ion containing solution. In forming the gel, alginate needs to encounter a di valent ion. (Ca^{2+}) In the Sodium based solution after extracting, alginate remains with no cross links in the polymeric form (Figure 03). In the presence of the di valent Ca^{2+}, Ions and polymers become cross linked. Ca^{2+} is able to make cross links (Figure 04) since it can produce two bonds with the polymer, while Na^{+} can make only a single bond. (Waldman et. Al., 1998) To obtain a more rigid structure to the bead, the contact time with the Ca^{2+} ion is increased, since it will facilitate the formation of increased amount of cross links, thereby increasing the bead rigidity. Ca^{2+} solution concentration was selected to be 2M, since the gels will be thermo-reversible at low concentrations (Belitz, HD and Grosch, W. 1999.) In the process of preparing ice cream, this characteristic will be essential since the beads need to dissolve and form linkages in stabilizing the ice creams.

When introducing the produced alginate bead to the ice cream, stabilizing property of the alginate is considered. Melting curve was drawn (Figure 05) for the standard industrial level ice cream, and the intention was to super impose the curve with the newly introduced bead. The most compatible result was obtained for the newly developed bead with 1.5% (w/w) inclusion.

This result was obtained after carrying out the test for alginate beads incorporated ice creams in 0.5% to 1.5% (w/w) concentration series. Therefore, this signifies that the optimum condition of melting is obtained by the 1.5% inclusion. However, when comparing with the commercially available alginate powder, a higher time was obtained to obtain the first drip of melting ice cream. It started dripping at 19.5 (±0.4) minutes while the commercial stabilizer started dripping at 5.5 (±0.5) minutes. When trying to reduce the percentage of inclusion (with preparation of a series from 0.4% to 0.1%), the textural difference existed as identified by the sensory panelists.

Therefore, it was evident that the commercially available alginate powder cannot be used as a stand-alone stabilizer to obtain the existing texture of the commercially available ice cream.

Tested ice creams for melting point was assessed for sensory characteristics with 12 trained panelists in the ice cream industry. Due to the increasing temperature in the oral cavity, ice creams undergo a phase change during consumption. This is referred in the industry as "Ice cream effect" (Hyde & Witherly, 1993). They detailed that the dynamic contrast (changes in ice cream texture in oral cavity from one moment to the other) is creating the palatability of ice cream and other alike products. In a study done by Kokini & Cussler (1983, 1987) that the thickness of melting ice cream in the oral cavity is relating to the Eq 2.

$$\text{Thickness} \propto \mu^{\frac{3}{4}} f^{\frac{1}{4}} V \left[\frac{2(1-\phi)\Delta H_i \rho}{3K\Delta T \pi R^4}\right]^{1/4} \qquad \text{Eq (2)}$$

Where;
μ = liquid phase viscosity
T = temperature difference between the solid phase (frozen ice cream) and the tongue
φ = volume fraction of air in the product (overrun)
Hi= heat of fusion of ice
ρ= density of ice
V = velocity of tongue movements
F = force applied by tongue
R = tongue radius (assuming a circle) in contact with the food
K = thermal conductivity of melted ice cream

In referring to this equation and in relating to the sensory study conducted, μ, Hi and K differs with the variants of ice creams. Other parameters are panelist dependent and the use of a trained panelist is to minimize the differences occurring via the changes in those parameters. By assuming these conditions, a ranking test was carried out for the sensory parameters in 2.7. The Friedman analysis of variance is the most powerful test that can be applied to data sets where all the samples are analyzed and ranked by all the panelists. (Lawless & Heymann, 2010) According to the Friedmann test, it produces a chi-square statistic, based on the total of columns. General formula for calculation is;

$$\chi^2 = \left\{\frac{12}{[K(J)(J+1)]}\left[\sum_{j=1}^{J} T_j^2\right]\right\} - 3K(J+1) \qquad \text{(B.29)} \qquad \text{Eq (3)}$$

The table 05, 06 & 07 summarizes the data obtained through panelists and by applying to Eq 3, Chi-square value obtained will be as below.

For Iciness; X^2 = 1.875 and the df is 7.81. Therefore, the null hypothesis cannot be rejected and henceforth no relationship exists in the categorical variables in the population. But, if we apply the same incident to the two samples of commercially used stabilizer and the alginate beads, for iciness; X^2 = 277.41 and the df is 3.841. Therefore, the null hypothesis is rejected and therefore it is evident that a relationship exists in the existing commercial stabilizer used ice cream and the new alginate bead used ice cream in iciness parameter.

Similarly, for sandiness the two samples of commercially used stabilizer and the alginate beads X^2 = 260.7 and the df is 3.841. Therefore, the null hypothesis is rejected and therefore it is evident that a relationship exists in the existing commercial stabilizer used ice cream and the new alginate bead used ice cream in sandiness parameter as well.

For mouthfeel, when comparing the two samples of commercially used stabilizer and the alginate beads X^2 =223.08 and the df is 3.841. Therefore, the null hypothesis is rejected and therefore it is evident that a relationship exists in the existing commercial stabilizer used ice cream and the new alginate bead used ice cream in sandiness parameter as well. However, when comparing the commercially used stabilizer with the alginate beads and the commercial alginate powder, X^2 = -9.10 and the null hypothesis cannot be rejected and henceforth no relationship exists in the categorical variables in the population. With given these observations, it is evident that in all of the textural parameters tested, formulated alginate bead acts similar to the existing stabilizer in ice creams.

Conclusion

From the coastal brown seaweed species grown in Sri Lanka, *Sargassum* spp. is under-utilized and is majorly found on the southern coast of the country. Out of the four *Sargassum* sp. tested for the study, highest polysaccharide yield was obtained in species 03 with the extraction at 90°C for 1 hour. In this yielding species, the Calcium Chloride route aided in the highest yield of extraction. Using this information of the study, alginate was extracted from *Sargassum* sp. 03 and beads were formulated to be used as a stabilizer in commercial level ice creams. With inclusions at 1.5% of beads, ice cream showed similar melting characteristics to commercially available ice creams. Although the commercially available alginate powder gives more withstanding ability to heat shocks and melting, its overall mouthfeel is not approved by the sensory panelists. Therefore, it can be concluded that the newly developed alginate bead extracted with *Sargassum* sp. 03 can be used as a commercial stabilizer for ice creams.

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EXTRACTION OF CRUDE COCONUT OIL FROM INDUSTRIAL COCONUT WASTE AND DETERMINATION OF ITS PHYSICO-CHEMICAL AND ANTIMICROBIAL PROPERTIES

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Summary

Waste water disposal is a major problem in coconut industry. Objective was to develop a method to extract oil from waste and to check its properties. Crude oil was extracted from 10 L of industrial sludge by density separation and solvent extraction techniques. Physicochemical parameters were determined by standard methods using commercial coconut as control. Best yield obtained with N-hexane (20.14%), whereas in sludge it was 5.00%. According to the chemical analysis, peroxide (mg peroxide kg^{-1}), acid values and moisture content were 2.85±0.09, 1.39±0.01, 1.26±0.09, 1.59±0.01 and 1.36±0.03, 0.097±0.00, 0.099±0.00, 42.2±1.29, and 1.23%, 0.99%, 1.49%, 9.25% for commercial oil, hexane, ether extracted oils and sludge oil respectively while sludge oil showing good antimicrobial properties. This study concludes that the extracted oils contain significant amount of oil with good chemical & antimicrobial properties which can be used in many industrial areas. However further studies needed for its composition analysis.

Keywords: *Law fat, Sludge, Chemical properties, Antimicrobial properties*

Introduction

Coconut oil is very much famous all over the world. Low fat coconut waste and waste water are two main types of wastes found in coconut milk industry. In the recent years of 2010, 2011, 2012 the total production of milk was 7319, 8096, 11000 metric tons respectively (CDA Stat, 2016). However, waste water is used only for production of sludge oil while purified waste water is used for agricultural purposes. The researchers have found many of the applications and benefits of coconut oil in skin care, weight loss, hair care, improving digestion, treating yeast infections, and boosting immunity against a host of infections and diseases (Carpo *et al.,* 2007). Number of researches and investigations have done focusing on the antimicrobial properties of coconut oil. Since waste water contain some amount of oil it is required to find potential value-added products from in order to be profitable in the market. So the objective of this study was to extract crude oil from industrial coconut waste and

to determine its physicochemical and antimicrobial properties with the purpose of using them in many industrial areas.

Methodology

Crude oil extraction from Low fat coconut residue and sludge water

All waste samples were bought to the Protein Chemistry Laboratory in Uva Wellassa University where this research was conducted and stored at room temperature until used. Low fat residue samples (5g) were taken and solvent was added to it in the ratios of 1:1, 1:2, 1:3, and 1:4. Then it was kept in a water bath for four different time periods (2, 4, 6, 12 hours) separately at 40 °C. Finally, solvent was evaporated. Initially 10 L of sludge water was taken and it was put in to an open mouth basin and kept for 8 hours to separate oil and water layers. Then the top layer was separated and it was centrifuged at 3400 rpm for 15 minutes at 4 °C. It was further separated to oil and other impurities.

Chemical property analysis

Moisture content, acid value, Iodine value, peroxide value and fat content was determined based on the AOAC manual 2016.

Determination of antioxidant activity

Determination of antioxidant property of extracted oils were done using Thiobarbituric Acid Reactive Substances (TBARS) Assay with the reference of (Lin *et al.,* 2011) with few modifications.

Determination of Antimicrobial Activity

Agar well diffusion method was used to determine the antibacterial properties of extracted oils (Abbas *et al.,* 2013) where negative and positive controls as distilled water and Augmentin®. All the obtained data were subjected to the one way and two-way analysis of variance using Minitab 2016 statistical software.

Results and discussion

Yield analysis

Proximate analysis of low fat coconut oil was 28.33 ± 0.38%. however, according to the yield analysis 20.14±0.8% (1.007±0.04 g) of oil yield has given for n-hexane by using 1:4 ratio and 4 hour retention time ($p<0.05$) (Table 1). It is well comply with the yields obtained by Suleiman *et al.,* 2013 in his study done using soxhlet extractor which have given a yield of 23.6% at higher temperatures (80 °C). Di-ethyl ether 16.66±0.6% of highest yield have given by 1:4 ratio with 12 hour retention time and it

is significantly higher than others ($p<0.05$). The resulted yield of sludge oil was 5.00±0.2% which is smiliar to the previous finding of 4.41%.

Table 1: Results of yield analysis

Solvent ratio	Time intervals (hrs)	Hexane	Diethyl ether
1:3	2	0.586±0.04	0.297±0.05
1:3	4	0.736± 0.09	0.303±0.06
1:3	6	0.670±0.02	0.490±0.08
1:3	12	0.770±0.10	0.036±0.04
1:4	2	0.550±0.18	0.096±0.09
1:4	4	1.007±0.04	0.044 ±0.04
1:4	6	0.913±0.08	0.017±0.02
1:4	12	0.876±0.35	0.035±0.04

Chemical Property Analysis

Table 2: Results of the chemical analysis

Oil type	Acid value (mg KOH/g)	Peroxide value (mEq/kg)	Iodine value	Moisture Content
Commercial oil	$1.37^{a} \pm 0.04$	$2.85^{a} \pm 0.09$	$8.69^{a} \pm 0.42$	0.01%
N-Hexane oil	$0.11^{a} \pm 0.00$	$1.39^{b} \pm 0.01$	$8.07^{ab} \pm 0.73$	0.01%
Diethyl ether oil	$0.11^{a} \pm 0.00$	$1.27^{b} \pm 0.09$	$6.61^{bc} \pm 0.51$	0.01%
Sludge oil	$42.2^{b} \pm 1.31$	$1.60^{b} \pm 0.01$	$5.28^{c} \pm 0.78$	0.99%
Codex value (2005)	> 10	>5	6.3-10.6	<0.5%

In this study oil extracted using n-hexane and diethyl ether have shown much better acid values of 0.11 and 0.11 respectively (Table 2). It means that these oils are much resistance to hydrolytic rancidity and they will not offer bad odour or aroma for longer periods of time According to the studies done by Okene and Evbuomwan (2014) oil extracted using n-hexane and petrolium ether have shown 0.1 mg KOH/g acid value which exactly comply with the findings of this study. However, with the sludge oil gave a higher acid value of 42.2 mg KOH/g.

According to the results of the peroxide value analysis all the oil types have shown lower peroxide values than the codex requirement (Table 2). According to the literature, the peroxide value of crude oil extracted from solvent extraction technique have shown 0.45 and 0.55 for n-hexane and petroleum ether respectively (Okene and Evbuomwan, 2014). The codex standard value for Iodine value in crude coconut oil is 6.3- 10.6. in this study commercial oil, oil extracted from n-hexane and diethyl ether have shown iodine values in between the codex standard values (Table 2), while sludge oil has shown 5.28 value which is slightly lesser than the standard value (Okene and Evbuomwan, 2014). So that the oils extracted in this study are well complying with the litereature findings.

Antioxident Property

In this study, at end of 16 hour incubation period oil samples have converted pink into white color. When considering the malondialdihyde values of oil samples, all the samples are showing lesser malondialdihyde values than the control sample. It indicates that all the oil samples are showing antioxidant properties while

commercial oil, oil extracted using n-hexane and sludge oil showing zero malondialdihyde value indicating very good antioxidant properties. Oil extracted using diethyl ether have shown 0.07±0.02 mg/L malondialdihyde value indicating some sort of oxidation. But it is still having antioxidant property due to not passing the malondialdihyde value of control.

Antimicrobial Property Analysis

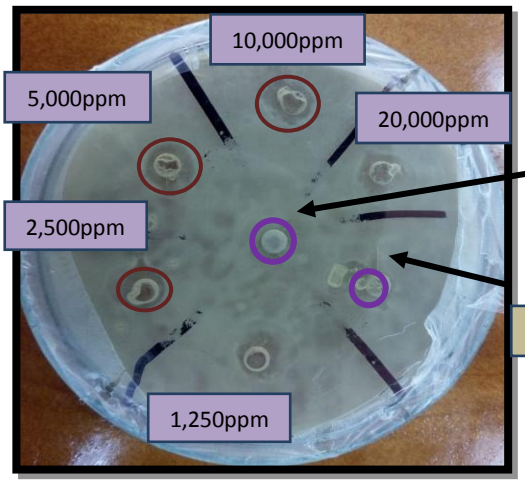

Plate 1: inhibition zone for oil extracted from waste water

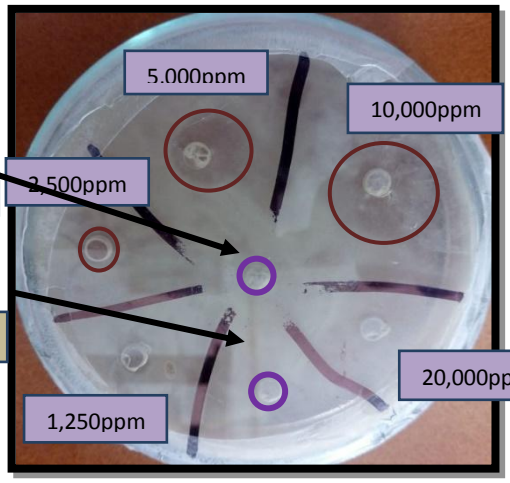

Plate 2: inhibition zone for oil extracted from diethyl ether

Commercial oil and oil extracted using n-hexane have shown lower antimicrobial property against total plate count done using agar well diffusion method. Sulaiman, *et al*, 2012 said that the lauric acid amount present in oil extracted from low fat is around 20%. It might be the reason for the lower inhibition zone obtained from oil extracted from n-hexane. But oil extracted using diethyl ether and sludge oil have shown significant amount of antimicrobial property (Plate 1 & 2). It means that these oils are showing significant amount of antimicrobial property as discussed in the literature. However, further studies should be conducted against several pathogens to check the antimicrobial activity.

Conclusion

Both N-hexane and Di-ethyl ether are good solvents to extract oil from low fat coconut waste. Oil produced from low fat and sludge water provided good physicochemical properties that satisfy codex standard values. Oils extracted from sludge water exhibits good antimicrobial properties.

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COATING DEVELOPED USING LEAF EXTRACTION OF "DAWUL KURUNDU" (*Neolitsea cassia L.)* COMBINED WITH PERFORATED POLYETHYLENE BAG EXTEND STORAGE LIFE OF LIME FRUITS (C*itrus aurantafolia L.*)

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Summary

A study was conducted to evaluate the effect of coating made out of leaf extraction of "Dawul Kurundu" with perforated polyethylene package on extending the storage life of lime *(Citrus aurantifolia L.*). Lime fruits at their optimum maturity were manually harvested from a commercial orchard and brought to the laboratory immediately. The bulk sample was divided into six lots each containing 100 fruits. Three lots were dipped in coating for one minute. Coated three lots were packaged in polyethylene lined plastic crate, perforated polyethylene bag and opened plastic crate. Other three lots were also packaged in polyethylene lined plastic crate, perforated polyethylene bag and opened plastic crate. All samples were stored in ambient conditions (at 32°C ± 2°C and relative humidity 70%). Evaluation of physiochemical attributes were conducted before treatment and thereafter at 2 days interval until the fruits exhibit limit of marketability. Lime that were coated and packaged in perforated polyethylene bag extended the storage life up to 14 days under ambient conditions in contrast to untreated fruits where marketable life ended within a week.

***Keywords*:** Edible coating, Lime fruits, Plant mucilage, Shelf life

Introduction

Lime fruit a non–climacteric, seasonal crop. Lime has an economical value since it has a special place in Sri Lankan cuisine. Price of lime fluctuates drastically. The market price of a one kilogram of lime fruits can be low as LKR 70.00 (0.4US$) in peak time and can be high as LKR 800.00 (4.53US$) during the off-season. Availability of lime limited to couple of months. The major issues in the pathological and physiological breakdown leading to decay and rind disorders and weight loss as a function of respiration and transpiration rates (Ramin and Khoshbakhat, 2008). By regulating the transfer of moisture, gases, aroma, and taste compounds in a food system, "edible coatings" have demonstrated the capability of improving food quality and prolonging

the shelf life of fresh produce (Lin and Zhao, 2007). Edible coatings with proper storage can mimic these problems. Mucilaginous extract of "Dawul Kurundu" (*Neolitsea cassia L.*) leaves contains complex branched polysaccharide (Silva and Kumar, 1986) that shows colloidal properties (Chandrajith et al., 2018). Colloidal properties of the polysaccharide make the extract ideal and economical to use as a coating. Objective of this study is to maintain the internal and external quality of lime for a longer time by building a formula for a coating that contains the leaf extract of "Dawul Kurundu" combined with a packaging to store lime.

Methodology

Preparation of coating: Leaf extraction was obtained by following the method suggested by Kasunmala et al., 2017.In the formula, fraction of 75% "Dawul Kurundu" (*Neolitsea cassia L.***)** leaf extract, 3% Sunflower oil, 2% Lecithin and 20% Glycerin were incorporated and mixed in a mixing machine for 15 minutes and kept in a water bath (~60°C) for 3 minutes.

Experimental procedure: Lime fruits (C. aurantifolia) was obtained from a commercial orchard in Anuradhapura. Fruits were harvested manually at mature green stage (Peel color; L=36.30±1.35, a=-17.54±0.09, b=28.66±0.65, Firmness=188.62±2.30, Titratable acidity (TA)= 8.704±0.21, Total soluble solids (TSS)= 10.1±0.05), packed in plastic basins in 10-15 kg capacity and transported to the laboratory. For the experiment, 600 lime fruits were selected and divided into two lots each containing 300 fruit. One lot was applied the coating prepared as has been mentioned above and the other lot was kept untreated as the control. Both waxed and non-waxed fruits were then separated into three groups each containing 100 fruit and stored in polyethylene lined plastic crate (0.381m x 0.243m x 0.085m), perforated polyethylene bag (gauge 150, size 0.500m x 0.305m, both sides have holes in 0.01m distance, bottom end heat sealed) and a plastic crate without any lining which then stored under ambient conditions (at 32±2°C and relative humidity 70%).

Data collection: Physicochemical parameters on peel color as CIE lab values (color difference meter, Konica. Minolta CR 400), firmness (digital firmness tester: TURONI, 53205), total soluble solids (digital refractometer,3,810, Atago PAL-1), titratable acidity (AOAC, 2005), and physiological weight loss were measured at 2 days of interval. Design of the experiment is two factor factorial and analysis was performed by SPSS16 statistical software at α=0.05.

Scanning Electron Microscopic observations of peel surface: Surface morphologies of the treated and control samples were examined using LEO 1,420 VP model scanning electron microscope (SEM)

Results and Discussion

Results on peel color and fruit firmness are given in the table 1.

Table 1: Peel color (*L,a,b*) and firmness lime fruits subjected to coating vs. control (uncoated) during storage under ambient (at 32°C ± 2°C, relative humidity 70%) condition

Parameter		Time (Days of storage)		
		5	9	13
Peel color	ANW1	67.3±2.1	55.0±15.3	51.1±4.7
"*L* "value	ANW2	51.7±0.8	71.5±10.8	43.1±1.6
	ANW3	50.2±1.3	71.4±1.2	43.6±2.2
	AW1	43.7±1.0	53.4±0.9	65.2±1.5
	AW2	43.0±0.3	49.5±0.9	58.2±1.9
	AW3	42.7±1.1	49.8±0.5	60.3±0.9
	P (Treatment)	0.00	0.00	0.00
	P (Package)	0.00	0.29	0.00
	P (Package* treatment)	0.00	0.07	0.62
"*a*" value	ANW1	-20.7±0.2	-15.3±0.4	-0.2±2.1
	ANW2	-20.2±0.1	-10.9±5.8	-1.3±1.3
	ANW3	-19.7±0.4	-15.6±0.7	-2.2±0.7
	AW1	-19.4±0.5	-20.3±0.5	-20.6±0.3
	AW2	-18.4±0.2	-19.4±0.2	-20.2±0.1
	AW3	-18.6±0.1	-18.3±0.1	-18.6±0.0
	P (Treatment)	0.00	0.00	0.00
	P (Package)	0.00	0.19	0.82
	P (Package* treatment)	0.28	0.14	0.02
"*b*" value	ANW1	35.5±0.6	46.0±1.3	38.3±1.8
	ANW2	34.1±0.6	41.0±0.6	53.4±0.8

	ANW3	33.6±0.5	39.3±0.7	47.1±1.5
	AW1	32.9±0.7	38.2±0.6	43.7±0.4
	AW2	32.3±0.9	35.5±0.5	39.8±0.4
	AW3	31.8±0.7	35.6±0.5	38.5±0.89
	P (Treatment)	0.00	0.00	0.00
	P (Package)	0.01	0.00	0.00
	P (Package*Treatment)	0.50	0.00	0.00
Firmness	ANW1	156.3±5.2	123.0±10.2	86.2±10.2
	ANW2	146.5±3.4	128.6±2.9	89.3±5.7
	ANW3	157.9±10.0	133.0±6.4	92.3±5.6
	AW1	151.6±5.2	140.9±1.5	121.8±3.1
	AW2	151.6±6.4	127.2±8.1	114.4±3.7
	AW3	161.7±1.8	140.5±2.0	128.7±1.5
	P (Treatment)	0.63	0.02	0.00
	P (Package)	0.03	0.08	0.05
	P (Package*Treatment)	0.34	0.06	0.20

Initial (day 1): L=36.30±1.35, a= -17.54±0.09, b=28.66±0.65 and Firmness= 188.62±2.30 N. Each value represents mean ± S.E. of three replicates. ANW1 = ambient storage/no coating/open crate, ANW2 = ambient storage/no coating/polyethylene lined crate, ANW3 = ambient storage/no coating/perforated bag, AW1 = ambient storage/coating/open crate, AW2 = ambient storage/coating/polyethylene lined crate, AW3 = ambient storage/ coating/perforated bag. P (treatment), P (Package) and P (Package*Treatment) are used express probability values obtained for main effect of coating, main effect of package and interaction effect of package and coating respectively.

Peel color measured as *L, a* and *b* values showed significant difference between coated and non-coated samples. Different packages also posed a significant effect on lightness and green color within first 7 days of storage (DOS). Effect of interaction of the two factors i.e. coating and packaging on *L* and *b* values were insignificant after one week. Firmness of coated fruit showed a significant difference after 7 DOS while there was no significant effect with reference to packaging.

Results on physiological loss in weight and juice chemical parameters (TSS and TA) are given in the table 2.

Table 2: Physicochemical parameters of lime fruit subjected to coating vs. control (uncoated) during storage under ambient (at 32°C ± 2°C, relative humidity 70%) condition

Parameter		Time (Days of storage)		
		5	9	13
Percentage weight loss (%)	ANW1	7.8±0.4	6.9±0.1	6.0±0.4
	ANW2	8.1±0.6	6.7±0.3	5.4±0.1
	ANW3	6.8±0.3	5.7±0.2	4.7±0.5
	AW1	7.2±0.4	6.5±0.1	7.5±0.1
	AW2	5.2±0.2	5.4±0.6	4.4±0.4
	AW3	4.5±0.2	3.9±0.6	3.4±1.2
	P (Treatment)	0.00	0.00	0.01
	P (Package)	0.00	0.00	0.00
	P (Package*Treatment)	0.00	0.02	0.48
Total soluble solids (TSS)	ANW1	8.8±0.1	7.7±0.3	7.3±0.3
	ANW2	8.9±0.1	8.0±0.1	7.3±0.1
	ANW3	9.2±0.1	8.1±0.1	7.7±0.3
	AW1	8.7±0.1	8.1±0.1	7.6±0.2
	AW2	9.2±0.3	8.7±0.0	7.9±0.1
	AW3	9.2±0.2	8.6±0.2	8.0±0.1
	P (Treatment)	0.37	0.00	0.00
	P (Package)	0.00	0.00	0.01
	P (Package*Treatment)	0.15	0.27	0.21
Titratable acidity	ANW1	8.0±0.2	7.9±0.3	7.6±0.2
	ANW2	8.3±0.2	7.9±0.0	7.7±0.0
	ANW3	8.7±0.3	8.5±0.2	8.3±0.2

AW1	8.8±0.2	8.8±0.2	8.3±0.2
AW2	8.9±0.2	8.5±0.2	8.4±0.2
AW3	8.9±0.2	8.9±0.2	8.8±0.2
P (Treatment)	0.00	0.00	0.00
P (Package)	0.01	0.00	0.00

Initial (day 1): Total soluble solids (TSS)= 10.1±0.05, and Titratable acidity (TA)= 8.704±0.21%. Each value represents mean ± S.E. of three replicates. ANW1 = ambient storage/no coating/open crate, ANW2 = ambient storage/no coating/polyethylene lined crate, ANW3 = ambient storage/no coating/perforated bag, AW1 = ambient storage/coating/open crate, AW2 = ambient storage/coating/polyethylene lined crate, AW3 = ambient storage/ coating/perforated bag. P (treatment), P (Package) and P (Package*Treatment) are used express probability values obtained for main effect of coating, main effect of package and interaction effect of package and coating respectively.

Total soluble solids demonstrated a significant difference in relation to coating as well as packaging. However, no significant effect was observed from the interaction effect. Titratable acidity showed a significant difference according to packaging and coating, but interaction effect became insignificant after 7 DOS.

Champa *et al*., (2019) have reported that TA showed significant difference between coated and non-coated lime in the first two weeks while TSS showed significant difference after a one week. In the present study also, coated lime showed a significant difference in TSS when compared to non-coated limes. Coating, packaging and their interaction effect were significant with reference to physiological weight loss until 11 DOS and after that the interaction effect became insignificant. Study conducted by applying pure coconut oil on lime has shown a physiological weight loss of 3.7, 5.5, 9.6 % on 6th, 12th and 18th day respectively (Bisen *et al*., 2012).

SEM images of the coated and uncoated lime fruit peel- after 07 DOS under ambient conditions (at 32±2°C and relative humidity 70%) are shown in figure 1(a) and 1(b).

a b.

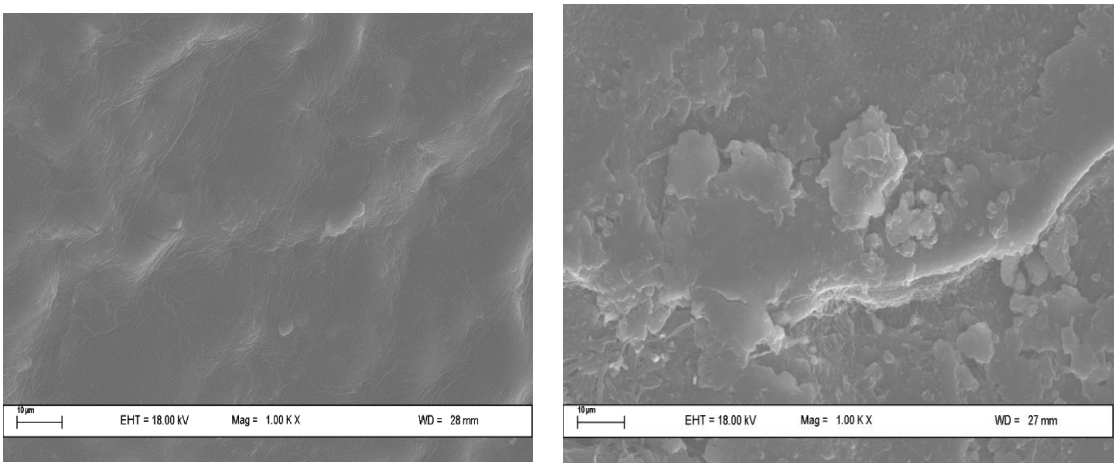

Figure 1: Scanning electron microscopic images of lime coated with developed formula (a) and control (b) after 07 days of storage under ambient conditions (at 32 ± 2°C and relative humidity 70%)

Natural openings could be seen in SEM image of the control sample (Figure a) and those openings are covered in coated sample (Figure 1b). However, bumps and groves could be observed in coated sample (Figure 1a). This might be due to the unevenness of the coating as a result of high viscosity of the formula.

Conclusion

It was revealed that lowest rate of peel yellowing, higher firmness, lowest physiological weight loss, higher acidity and higher soluble solids was maintained by coated lime fruits with the developed formula and packed in perforated polyethylene bag. The shelf life of treated (coated and packed in perforated polyethylene bag) lime was extended up to 2 weeks while control (uncoated and no package) showed a one-week shelf life.

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EFFECT OF COMMONLY USED ARTIFICIAL FRUIT RIPENING METHODS ON RIPENING BEHAVIOUR, QUALITY AND SAFETY OF BANANA

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Summary

Artificial fruit ripening has many commercial advantages. However, improper use of ripening agents may leave harmful residuals on fruit surface. The present investigation was carried out to assess the effect of smoke and ethephon on ripening behavior, quality and safety of banana. The ethephon treatment was done as a fruit spray, dip and expose of fruits to ethylene gas liberated from ethephon while smoking of fruit was done in a smoke chamber. Treated and non- treated fruits were assessed for quality and safety in terms of physico-chemical parameters and residue analysis of Ethephon and Polyaromatic hydrocarbons (PAH) in smoked fruits. The results of PAH analysis revealed that smoke treated fruits contained PAHs (530.5 µg/kg) while control fruits showed no PAH residues. Fruits exposed to ethylene gas liberated from Ethephon were observed to be free from residues while fruits dipped and sprayed in Ethephon were contaminated with residues. Therefore, exposure of bananas to ethylene gas liberated from Ethephon can be recommended as a cost effective, safe fruit ripening technique for vendors to ensure food quality and safety.

***Keywords*:** PAH compounds, ethephon residues, artificial fruit ripening

Introduction

Major postharvest losses in fruits are caused during transportation and handling of ripe fruits. To reduce such losses, ripening could be induced after transportation and prior to distribution by whole sellers to retailers based on demand. For this purpose different artificial ripening agents can be used by traders. However, these are harmful when adulterated with different types of chemicals, such as calcium carbide (CaC_2), acetylene (C_2H_2), ethephon (2-chloroethylphosphonic acid) and other non-recommended pesticides to accelerate ripening of immature fruits (Hakim *et al*., 2012). These chemicals may change nutritional properties of fruits and lead to

serious health hazards like cancer, skin irritation, diarrhea, liver & kidney diseases, gastrointestinal irritation with nausea, vomiting, cardiac disturbances, central nervous system depression and cardiac abnormalities in human (Hossain *et al*., 2015). The most demanded fruit 'Embul' banana in Sri Lanka is often subjected for artificial ripening specially in festival seasons. The ripening techniques suggested even after banning of Calcium carbide, such as use of ethephon is not practiced in a safe manner. Also there are no specific laws and regulations on usage of these substances in Sri Lanka. Hence, the purpose of this study is to select the safest artificial ripening technique to be used by banana traders to ensure the safety of consumption while preserving its marketable quality.

Methodology

Randomly selected hands of Embul banana bunch harvested at green mature stage was used for experiments. six treatments: (T1) control in a jar, (T2) Ethylene gas liberated from Ethephon (25 ml of 1 ml/L Ethephon + 2.5 g NaOH), (T3) Ethephon dip in 1 ml/L Ethephon solution, (T4) Ethephon spray (1 ml/L Ethephon solution), (T5) Smoking (using burnt banana leaves) for 3 minutes and a (T6) control placed outside jars were tested with three replicates per each treatment in Complete Randomized Design (CRD). All ethephon treatments were performed based on recommended concentrations. Sealed 2 L glass jars with fruits were used to trap the gases for 24 hours in smoke and Ethylene gas from ethephon treatments. Initial and final physicochemical properties such as TSS- Total Soluble Solids (°Brix), pH, % Titratable acidity, firmness, % weight loss and color (L, a, b values) were determined.

Polyaromatic Hydrocarbons (PAHs) in smoke was determined by trapping smoke of burnt banana leaves in 100 ml of acetonitrile solution for 30 minutes. For the analysis of PAHs residues in banana fruits, a 5 g portion of peel and flesh from both smoked and control samples were purified with QuEChERS (AOAC, 2007) extraction and clean up kit. Agilent 1260 infinity HPLC system equipped with Florescence detector and PAH analysis column (150 x 4.6 mm, 5 um) was used for quantitative analysis. Ethephon residues were analyzed by QuPPe method (Quick Polar Pesticides Method) by Liquid Chromatography tandem Mass Spectrometry system (LC-MS/MS) using Eksigent ekspert UPLC 100×L HPLC system with an Atlantis HILIC (2.1 mm×100 mm,3 µm) HPLC column and AB SCIEX QTRAP® 4500 system. Treated banana samples of Ethylene gas from Ethephon, Ethephon dip and spray (with recommended dosages) along with a control were used for estimation.

The data obtained from physicochemical parameters were analyzed using ANOVA (Analysis of Variance) and mean separation via Duncan's Multiple Range Test (DMRT) at confidence interval of 95% (SAS 9.0). Results of the organoleptic evaluation were analyzed using Kruskal Wallis Test (SPSS version 20).

Results and Discussion

Physico-chemical and organoleptic quality analysis:

Physicochemical parameters tested showed similar trend in ripening behavior and quality of all treatments except the control as shown in Table 1. Ethephon spray and dip treatments showed faster rate of ripening. Weight loss due to moisture loss in respiration process was observed to be the highest in Ethephon gas treatment indicating accelerated ripening. No significant difference in total soluble solid (°Brix) content was observed in treated fruits ($p>0.05$) though all treated fruits had significantly higher TSS than control. Fruit firmness decreased with ripening and was not significantly different among treated fruits ($p>0.05$). Fruit acidity increased with ripening in Embul variety and was highest in smoke, dip and spray treatments (Table 1). Similar results were reported by Kulkarni *et al*., (2011) .The pH of pulp in all Ethephon treated and smoke treated banana fruits decreased during ripening.

Table 1: Results of physico-chemical analysis of banana subjected to different ripening treatments compared to control after 3 days at ambient temperature (28±1°C)

Treatments	°Brix	Firmness (Kpa)	% Weight Loss	% Titratable acidity	pH
E. gas	24.1 ± 3.3 ^{a}	1.4 ±1.2 ^{b}	5.78 ± 0.1^{a}	0.39 ± 0.0^{c}	4.4 ± 0.0^{b}
E. spray	23.2 ± 1.9^{a}	1.3 ± 0.1^{b}	5.57 ± 0.5 ^{ab}	0.47 ± 0.0^{abc}	4.11 ± 0.2^{d}
E. dip	20.2 ± 2.9^{ab}	1.3 ± 0.5 ^{b}	4.67 ± 0.4^{bc}	0.49 ± 0.0^{ab}	4.13 ± 0.0^{d}
Smoke	21.2 ± 0.6^{a}	1.4 ± 2.7^{b}	4.95 ± 0.2 ^{abc}	0.5 ± 0.1^{a}	4.2 ± 0.0^{cd}
Con-out	15.3 ± 1.5^{b}	6.5 ± 1.1^{a}	4.38 ± 0.8^{dc}	0.42 ± 0.1^{bc}	4.24 ± 0.2^{c}
Control	10.0 ± 4.1^{b}	6.6 ± 0.8^{a}	3.53 ± 0.1^{d}	0.21 ± 0.0^{d}	4.89 ± 0.1^{a}

Note: The data shown are mean ± standard deviation of 3 replicates. Statistically significant values ($p < .05$) within columns are designated by different letters

The organoleptic quality evaluation done using a trained taste panel revealed overall acceptability was high in banana subjected to a treatment of ethylene gas from ethephon and ethephon dip treatments compared to smoke treatment and control fruits.

Residue analysis of Smoke treated fruits:

Smoking is one of the traditional methods used for ripening of banana in Sri Lanka. However, Polyaromatic Hydrocarbons (PAHs) are released during incomplete combustion or pyrolysis (burning) of these organic matter. There are 16 identified PAH compounds (as listed by the United States Environmental Protection Agency – US

EPA) and the smoke released by burning semidried banana leaves produced 14 PAH compounds out of them.

Table 2: PAH residues (µg/kg) detected in smoke treated fruits with ± SD

PAH Compounds	Initial PAH (µg/kg)		Final PAH (after 3 days) (µg/kg)	
	smoked peel	smoked flesh	smoked peel	smoked flesh
Naphthalene	268.34±2.02	ND	ND	ND
Acenapthene	ND	ND	1.65±0.05	ND
Fluorene	123.67±1.85	ND	44.28±5.04	ND
Phenanthrene	50.98±0.18	13.24±0.15	27.7±0.53	13.25±3.37
Anthracene	14.08±0.64	ND	6.24±0.94	ND
pyrene	26.74±5.72	ND	22.71±0.58	ND
Benzo(a) anthracene	5.04±3.57	ND	ND	ND
chrysene	ND	ND	ND	ND
Benzo(k)fluoranthene	ND	ND	ND	ND
Benzo (a) pyrene	3.94±0.12	ND	2.08±0.7	ND
Dibenzo(a,h)anthracene	ND	ND	ND	ND
Dibenzo(g,h,i)Perylene	ND	ND	ND	ND
Fluranthene	34.54±4.62	ND	16.89±0.69	ND
Benzo(b)fluoranthene	3.23±2.28	ND	ND	ND
Indeno(1,2,3-c,d) pyrene	ND	ND	ND	ND

ND – Not Detected

Out of the PAHs, two three ringed PAHs are non-carcinogenic, while several of the four, five and six ring PAHs are carcinogenic. PAH benzo [a] pyrene, have shown various toxicological effects, such as haematotoxicity (effects on the blood), reproductive and developmental toxicity and immune toxicity. Most of these chemicals enter human blood through food and inhalation of gas, Therefore, a long term exposure to smoke alone may cause health hazards on smoke chamber operators in the local market.

Initially, in smoke treated fruits, maximum amount of PAHs were detected from the peel of smoke treated banana (Total 530.56 µg/kg**)** out of which 3.94 µg/kg was benzo (a) pyrene, 5.04 µg/kg benzo [a] anthracene and 3.23 µg/kg Benzo (b) fluoranthene which are carcinogens. Total of 13.24 µg/kg of Phenanthrene was found on the flesh of the treated fruits. However, toxicity data for phenanthrene are limited. No PAH compounds were reported in the control sample. After three days storage, the total PAHs on the smoke treated banana peel had abated to 119.9 µg/kg and 2.075 µg/kg of Benzo (a) pyrene was still remained in the peel without being degenerated. The maximum residue limits (MRL) for PAHs ranges between 2-5 µg/kg (Zelinkova &

Wenzl, 2015).Hence, there is a chance of contamination of the fruit flesh with these residues by penetration.

Residue Analysis of Ethephon:

Ethephon has also been reported to be absorbed by the fruit tissues and when pH exceeds 4.6 it breaks down to release ethylene. The ethylene released from ethephon triggered autocatalytic ethylene production during fruit ripening (Singh, 2016).This indicates that the chemical is directly absorbed into the tissues and gets hydrolyzed leaving some residues. Results on ethephon residues (Initial and after three days) are summarized in Table 3.

Ethephon residues were detected in ethephon spray and dip treated fruits even after three days. Residues found in flesh of spray treatment was 4 times higher than the Maximum residue level of ethephon 0.05 mg/kg for banana according to EU Pesticides database. Control and the fruits treated with ethelyne gas liberated from ethephon were free of ethephon contamination (Table 3). Ethephon is not a food grade chemical. It contains impurities such as 1, 2 Dichloroethane which is moderately acutely toxic in experimental animals. These chemicals may change nutritional properties of fruits and vegetables as well as lead to serious health hazards such as cancer, skin irritation, diarrhea, liver disease, kidney disease, gastrointestinal irritation to human beings (Hakim *et al*., 2012).

Table 3: Ethephon residues of banana just after the treatment of Ethephon and after 3 days at ambient temperature (28±1°C)

Sample	Ethephon content in Initial Samples, mg/kg				Ethephon content in Samples After 3 days, mg/kg			
	Control	E. Dip	E. Spray	E. Gas	Control	E. Dip	E. Spray	E. Gas
Whole fruit with peels	ND	0.4 ± 0.05	0.6 ± 0.01	ND	ND	1.0 ± 0.04	2.0 ± 0.01	ND
Flesh only	ND	ND	0.3 ± 0.01	ND	ND	ND	0.2 ± 0.00	ND

ND- Not Detected

Conclusion

Banana subjected to ethephon dip and spray treatments were contaminated with residues making fruits unsuitable for consumption. Smoke treated fruits were contaminated with PAH compounds which are carcinogenic. Therefore, use of

ethylene gas liberated from ethephon (with and interaction of NaOH) treatment which is free of any chemical contamination can be recommended as the safest artificial ripening technique with preserved marketable quality.

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DEVELOPMENT OF A BIODEGRADABLE CONTAINER USING COCONUT (*Cococus nucifera*) FLOUR AND DETERMINATION OF TECHNO-FUNCTIONAL PROPERTIES

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Summary

Coconut (*Cocos nucifera)* is a dominant crop which is widely used for culinary purposes in household and industrial level. After extracting the coconut milk, the residue is most probably discarded. This study is based on developing a biodegradable container using coconut residue flour. Coconut residue was subjected to blending, squeezing or pressing, drying at 110^{o}C for 2 hours and grinding to make a coconut flour. The obtained yield of coconut flour was 68% and white in color. Commercially available pectin was used as a binding agent mixing in the ratio 10:3 (coconut flour: pectin). The mixture was put into the mold and dried at 110^{o}C for 3 hours. The cup was subjected to following tests and the results indicated, moisture content 14.36% at 91^{o}C, permeability coefficient 5.17 x 10^{-9} cm^2Pa^{-1} s^{-1}. According to the absorption isotherm graph, it showed swellable hydrophilic solid nature until a maximum of site hydration was reached. In relation to the Soil Burial Test, high biodegradation rate was observed within five months period and it completely biodegraded.

Keywords : Absorption isotherm, Biodegradable, Coconut flour, Permeability co-efficient

Introduction

Production of biodegradable containers has become a big trend nowadays to prevent the problem arising due to the plastic crisis. Currently, in Sri Lanka we have a good trend developing in the biodegradable packaging industry. This project focuses on a more practical approach for the development of biodegradable containers in Sri Lanka. Coconut (*Cocos nucifera*) is one of the most available resources in Sri Lanka widely known as the tree of life, due to its significant contribution to human life from all of its meat, water, husk, shell, wood, leaves, spikelet, etc. (Gunethilaka and Abeyrathne, 2008). Every part of the palm are utilized for the benefit of the humans and its fruit particularly provides important constituent of food which is indispensable in every household (Rana *et al.,* 2015). Coconut is one of the main ingredients in Sri Lankan's meal. After extracting the coconut milk from the coconut

meat, the residue is discarded often as a waste and some are only used as an animal feed. But still, there is no good application reported for the residue. Although it is a waste, still it has a high nutritional value such as high dietary fiber content, protein content etc. (Trinidad *et al.,* 2001) Ultimately, development of biodegradable containers from coconut residue by making coconut flour may have a strength to be a valuable application for sustainable development due to its biodegradable nature.

Methodology

The coconut residue was taken and ground gently using a blender by adding little amount of water. After that, the ground sample was taken out and squeezed by hand. Then, it was dried for 2 hours at 110^{o}C in the oven. The dried sample was ground again using a blender into flour. Flour was sifted by the strainer. Sifted flour was packed in a polyethylene bag and stored at the refrigerator. The yield of coconut flour was calculated.

3g of commercially available pectin was taken into the beaker and 50ml of hot water (80^{o}C) was added to it. The mixture was stirred properly until all pectin was dissolved without any clumps. 10g of coconut flour was taken into the container and pectin solution was added to it little by little. While adding pectin, flour and pectin were mixed by hand gently until a non-sticky dough was formed. The dough was put into the mold and dried it at 110^{o}C for 3 hours. The dried biodegradable cup was removed from the mold. Moisture content of the biodegradable cup was measured using moisture meter. And the permeability co-efficiency of biodegradable cup was calculated using Frick's law and Henry's law (Mchugh *et al.,* 1993).

To determine the water sorption properties of biodegradable container three saturation solutions were prepared with $MgCl_2$, KI, KCl. The water sorption data was fitted into the graph of water sorption capacity calculating Equilibrium Moisture Content % (EMC%) and water activity in different RH conditions (Othman *et al.,* 2017).

Biodegradability of the biodegradable container was tested using Soil Burial test. Owing to the test, Compost soil was taken into the five petri dishes. Five biodegradable cups were taken and buried in the compost soil giving same conditions for all samples (30±5^{o}C). Biodegradability of biodegradable cups was observed from time to time.

Results and Discussion

Basic raw material for the production of the biodegradable container was coconut *(Cocus nusifera)* flour made out of coconut residue obtained after extracting coconut milk. It involved several major processing steps; blending, pressing, drying and grinding. Blending and pressing the coconut residue was useful to completely remove remaining coconut milk. Another important thing in pressing is, it is a defatting method and it helps to remove fat content from coconut residue. The most suitable temperature for drying coconut residue was 110^{o}C for 2 hours. If the temperature is increased above this level, brownish-yellow color discoloration and off-odors were obtained from the coconut residue. This may be due to various chemical reactions taking place such as denaturation of proteins and browning reactions. Flour obtained after grinding the dried coconut residue was white in color without any off odors. The calculated coconut flour yield was 68% from coconut residue.

Moisture analysis is very important to control the growth of microorganisms. Presence of high moisture content can affect deterioration by biological as well as chemical changes of the product. It widely affected the shelf life of the product. According to the result of moisture meter, the biodegradable container had 14.36% moisture content when heated to 91^{o}C. Along with the Frick's low and Henry's low, Permiability co-efficient of developed biodegradable container was 5.17 x 10^{-9} $cm^2Pa^{-1}s^1$.

$$\text{Permeability co- efficient (P)} = \frac{Q \times d}{A \times t \times p}$$

P = Permeability co-efficient Q= Quantity of permeant
d= Thickness of material
A= Area of the material
t= Permeability time
p= partial vapor pressure of water

Table 1: Result for permeability co-efficient calculation

Q (cm^3)	d (cm)	A (cm^2)	t (s)	p (Pa)	T (^oC)
2	0.2	54.83	420	3360	26

$$P = \frac{2cm3 \text{ x } 0.2cm}{54.83cm2 \; x \; 420s \; x \; 3360pa}$$

$$P = 5.17 \text{ x } 10^{-9} \text{ cm}^2\text{Pa}^{-1}\text{ s}^{-1}$$

Related to the obtained result, biodegradable cup showed hygroscopic behavior and moisture absorption increased significantly in high RH rather than low RH. When plotting the graph according to the different EMC% and water activity (a_w) obtained from the result, it had swellable hydrophilic solid nature until a maximum of site hydration was ~~is~~ reached.

Table 2: Equilibrium Moisture Content (EMC%) for coconut flour based biodegradable container in different water activities at 25 ± 2^oC

Saturated solution	Sample no	Tare weight of petri dish(g)	Weight of the sample with petri dish (g)		EMC % (dry basis)	Water Activity
			Initial	Constant		
$MgCL_2$	1	12.72`	15.16	15.17	0.409	0.324
KI	2	9.59	12.23	12.24	0.530	0.679
NaCl	3	37.38	40.42	40.44	0.657	0.751
KCl	4	37.81	40.27	40.29	0.813	0.836
KNO_3	5	37.49	40.99	41.03	1.143	0.923

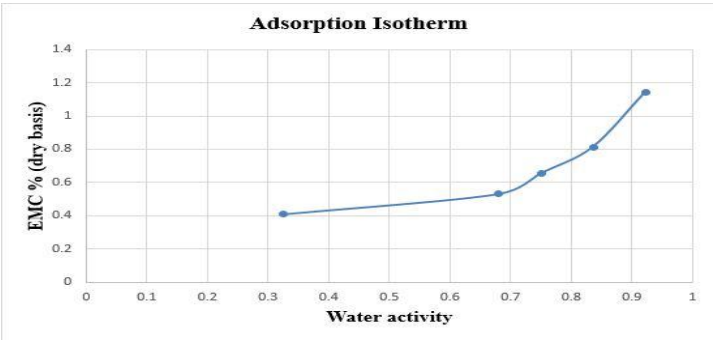

Figure 1: Adsorption isotherm graph of the biodegradable container at 25±2°C for different water activities obtained from the result

Biodegradability test (Soil Burial Test) was continued for five months period providing the same conditions (30±5°C) for all samples produced by using commercial pectin. During this period, high biodegradation rate was observed. On the 8th day of this test, the biodegradable cup showed somewhat similar observations to the initial level. But in 15th day, it showed different characteristics rather than earlier samples. The texture of that sample became poor. In 22nd and 30th days, development of green color mold was observed but it was high in 30th-day sample. The texture of that sample was very poor and tends to break when touching ~~it~~. After 2nd month, about 50% of the container was biodegraded and when observe after five months, it was completely degraded.

Conclusion

Coconut flour can be produced by subjecting coconut residue to blending, squeezing or pressing, drying at 110°C for 2 hours and grinding. It should be stored at a dry place after packing in polyethylene bag. The obtained yield of coconut flour was 68% and white in color. The moisture content of biodegradable cup was 14.36% at 91°C and the permeability coefficient was 5.17 x 10^{-9} cm^2Pa^{-1} s^{-1}. According to the absorption isotherm graph, it showed swellable hydrophilic solid nature until a maximum of site hydration was reached. According to the biodegradability test, it was completely biodegraded within five months period.

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DEVELOPMENT OF HORSE GRAM BASED READY TO SERVE NUTRITIOUS DRINK

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Summary

The horse gram is an underutilized legume containing higher amounts of protein (24.2 ± 0.25%), fibre (6.7 ± 0.20%) and minerals (3.6 ± 0.09%). The main objective of the present study is to formulate Ready To Serve nutritious drink and to evaluate physico-chemical and sensory properties. Raw materials: horse gram, red rice; Kalu Heenati and sesame were processed into blends. Proportionally adding those blends with treacle/bee honey and the stabilizing agent followed by homogenizing, three formulations were done. Samples were bottled and thermally sterilized. The best formula was selected by sensory evaluation using a trained sensory panel using nine - point hedonic scale. The proximate composition and mineral contents of the product were carried out according to the AOAC (2012) methods. Proximate composition of the product showed energy (73.25 kcal), protein (1.69 ± 0.14%), fat (0.30 ± 0.01%), fiber (0.84 ± 0.05%), total minerals (0.20 ± 0.01%) and carbohydrate (15.99%). Product contained minerals; Iron (1.80m g/kg), Zinc (2.20 mg/kg), Calcium (105.0mg/kg) and potassium (785.0 mg/kg) and phosphorus (0.03%). Formulated product is already complying with the SLS 729:2010 requirements (Brix <16) for Ready to Serve drink.

Keywords: Horse gram, Ready to Serve Drink, Nutritious drink, Healthy drink

Introduction

Horse gram (Macrotylomauniflorum) is an underutilized pulse crop grown in a wide range of adverse climatic conditions. Reports on the nutritive value of horse gram indicated as it is an excellent source of protein (up to 25 %), carbohydrates (60%), essential amino acids, energy and low content of lipid (0.58%), iron and molybdenum (Bravo et al., 1999). The importance of horse gram was recognized by traditional medicine as a potential therapeutic agent. The uses of horse gram are limited due to their poor cooking quality and the presence of anti-nutritional factors and unflavored

compounds. Therefore it is important to inactivate anti-nutritional factors before consumption. Horse gram is an excellent source of dietary fiber. As reported it has positive effects on intestine and colon, besides other homoeostatic and therapeutic functions in human nutrition (Ramteke et al. 2016). It is used in the treatment of many health problems especially to treat diarrhea, hemorrhage and hemorrhoids (Ramteke et al., 2016). The regular intake of horse gram helps to flush out the worm infections, it regulates the digestive system and ward offs acidity and flatulence (Zhang et al., 2008) Horse gram also helps in lowering cholesterol levels. Although the importance of raw horse gram and its flour as food is well known in recent days, maximum utilization is lacking due to the presence of poor cooking qualities and anti-nutritional factors like tannin, trypsin inhibitor, phytic acid and bowman-brik inhibitors. These compounds interfere with the bioavailability of nutrients present in horse gram. The purpose of doing this research is the most children and elderly population consume non-nutritious and unhealthy beverages due to the unavailability of nutritious beverages in the local market. The main objective of the present research is to formulate Ready to Serve drink and to determine physico-chemical properties and sensory properties of the product.

Methodology

All the materials were taken from the local market. Preprocessed horse gram, red rice; kalu heeneti and sesame seeds blended with water 1:4, 1:4 and 1:2 ratios. Blenders were mixed 4:4:1 ratio respectively. To get the best combination carried out three formulations such as 1% kithul treacle with 0.5% carrageenan, 1% bee honey with 1% carrageenan and 1% bee honey with 0.5% carrageenan. Then samples were bottled and retorted under 121°C for 15 minutes.

The control sample was prepared by using red rice; kalu heeneti kithul treacle and carrageenan. Sensory analysis of the product was carried out using nine points hedonic scale by giving samples to 10 members of staff, ITI. ANOVA test was used for the analysis

According to the sensory results, the most acceptable formulation was used for further analysis. A comparison was done with the control sample which was prepared using only kaluheeneti, kithul treacle and carrageenan.

Nutritional properties of horse gram drink were carried out according to the standard methods given in the Association of Analytical Chemists (AOAC, 2012). Results were given in Table 1 and 2.

Microbiological analysis was carried out to determine the shelf life of the product by conducting aerobic plate counts (SLS: 516: part I: 1991), yeast and mold count (SLS: part III: 1982) and coliform test (SLS 516: part II: 1991).

Results and Discussion

Three formulations (Formula 1, 2 and3) were prepared using same amounts of blends with different carrageenan percentages (0.5%, 1% and 0.5%) and different sweetening agents (kithul treacle and bee honey) respectively. Among them, two samples (formula 2 and 3) were sweetened using "Bee Honey" with two different percentages of carrageenan. Another sample was sweetened using "Kithul Treacle" and 0.5% of carrageenan.

The best acceptable sample was selected by analyzing sensory data. According to their evaluation, the best formulation (Formula 1) was selected among three formulations.

The sample which contained "Kithul Treacle" was selected as the best formulation. Over the "Kithul Treacle", "Bee Honey" was used because as it contains nutrients than the other sweetening agents. But it is not suitable for the product because waxy nature of "Bee Honey" was affected to the quality of the product after retorting under 121°C for 15 minutes. There was a separation inside the bottle and it was a failure in the product. Because of that reason bee honey products were rejected. Besides the cost of "Bee Honey" is not economically affordable for product formulations.

Due to that reason further analysis was done for the selected product and the control sample.Results of the proximate composition of horse gram based drink are given in Table1.

Table1: Proximate composition of horse gram based drink

Parameters	Horse gram drink	Control sample
Energy (Kcal)	73.25	58.43
Moisture (%)	80.99 ±0.02^{b}	84.98 ± 0.08^{a}
Crude protein (%)	1.69 ± 0.14^{a}	0.54 ± 0.02^{b}
Crude fat (%)	0.30 ± 0.01^{a}	0.08 ± 0.03^{b}
Crude fibre (%)	0.84 ± 0.05^{a}	0.37 ± 0.04^{b}
Ash (%)	0.20 ± 0.01^{a}	0.13 ± 0.01^{b}
CHO (%)	15.99	13.90
Sugar (%)	6.77 ± 0.05^{a}	5.87 ± 0.03^{b}

Values are presented on % wet weight basis.
Means within a column with different letters are significantly different ($p \leq 0.05$).

According to the proximate composition analysis data crude fibre, crude protein and crude fat content of the horse gram based drink was significantly higher than the control sample ($p<0.05$). The reason for the difference is Horse gram contained high protein (24.2±0.25% on dry weight basis) and fibre (6.7 ±0.20%, on dry weight basis) as reported in previously. Fibre - rich foods support for reducing blood cholesterol level and it also good for elders who are suffering from constipation.

Crude fat content was significantly higher in the product compared to the control sample due to the incorporating sesame seed milk into the drink sample. Sesame oil is highly stable and rarely turns rancid in hot climates. It is rich in unsaturated fatty acids where the fatty acids composition is 14% saturated, 39% monounsaturated and 46% polyunsaturated fatty acids (Toma &Tabekhia, 1979). Molecules of sesame seed oil maintain good cholesterol (high density lipoprotein, HDL) and lower bad cholesterol (low density lipoprotein, LDL) (Sirato-Yasumotoet et al., 2001). Ash content was significantly higher in the product sample compared to the control sample. Previous research findings showed that "Horse gram" contains a high amount of minerals compared to the other legumes. It contains Calcium 1571.6 mg/kg, Iron 114.5 mg/kg and Phosphorus 369 mg/kg (Herath et al. 2018).

The physical properties of horse gram drink were presented in Table 2.

Table 2: Physical properties of horse gram drink

Parameters	Horse gram drink	Control sample
Brix value	13	13
pH	5.98 ± 0.02[a]	5.66 ± 0.09[b]
Viscosity	1137.95 ± 21.71[a]	1330.3 ± 0.28[a]

Values are presented on % wet weight basis.
Means within a column with different letters are significantly different ($p\leq0.05$).

Brix value of horse gram based drink ranged in between 14. According to requirements of Ready To Serve drink SLS 729:2010 the Brix value of the RTS drink should be lower than brix 16. Formulated product complies with SLS requirement.

Initial trials were performed to increase the shelf life of the product using the pasteurization process after bottling. Pasteurization was done under 95°C for 20 minutes. The initial aerobic plate count for the product the number of colony counts was> 300. To comply RTS drink it requirement is less than 50 per milliliter (SLS

729:2010). Because of that reason, the pasteurization process was not effective for the extended shelf life and the quality of the product.

Due to the failure in pasteurization the product was undergone with thermal sterilization of the retort process. The initial count for the retorted sample it was zero counts. Microbiology was conducted for two months with two weeks gaps and not detected any colonies furthermore.

Conclusion

The total soluble solids content of the product is 13%. Total plate count, yeast and mold count and total coliform count also not detected during two months with the retorted process. According to the results, the product new beverage comply with the SLS requirements for Ready To Serve drink.

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INNOVATING THE INDUSTRY: DEVELOPMENT OF AN NOVEL CHEMICAL METHOD FOR COCONUT TESTA REMOVAL

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Summary

Coconut testa is removed manually during processing of virgin coconut oil, coconut milk, coconut powder and desiccated coconut and it is a labor-intensive operation. This research was conducted to develop an efficient and convenient method to remove coconut testa using a chemical treatment. Hydrogen peroxide and sodium carbonate were tested with different treatment conditions to remove coconut testa Based on the results, two suitable chemical treatments were selected for the removal of testa from coconut kernels. Steps of the first method include heating of coconut kernel in 20% H_2O_2 at 80^{o}C for 20 minutes and subsequent dipping it in hot water (100^{o}C) for 5 minutes. Steps of the second treatment include heating of coconut kernel in 20% Na_2CO_3 at 80^{o}C for 5 minutes and heating in 20% H_2O_2 at 80^{o}C for another 15 minutes. As the last step of both the treatments, coconut kernels were rubbed and washed using water at room temperature until it removes the odour and the colour of coconut kernels. It was found that method one is much effective in removing the coconut testa.

Keywords: Coconut testa, Ligninolytic enzymes, Solid-state fermentation, coconut testa removal

Introduction

Coconut (Cocos nucifera) is one of the most useful tropical fruits having religious and also traditional values in Asian countries. Coconut testa is the thin brown outer layer present surrounding the fresh coconut kernel. It is manually removed in coconut industries and it is labor intensive, time consuming, contamination prone and inefficient process. Testa mainly contains polyphenols and it has a bitter taste. Polyphenols act as antioxidants, free radical scavengers and peroxidation inhibitors. (Prakruthi et al., 2016). Therefore, if a method of extracting the testa is developed it can be used as an industrial source of antioxidants. There has been no prior scientific evidence found regarding chemical removal of coconut testa. This research was conducted to find a suitable effective and efficient chemical method to remove

coconut testa with the hypothesis that hydrogen peroxide and sodium carbonate will be of good source of removal of coconut testa.

Methodology

This study was conducted in an industrial environment at S.A. Silva & Sons Lanka (PVT) Ltd, (Silvermills), Loluwagoda and at laboratories in the Department of Food Science and Technology, Faculty of Agriculture, University of Peradeniya. Coconuts of medium size (10 – 13 cm) that of TxT variety were used in this study. Coconut (with testa) were dipped in 5% hydrogen peroxide (H_2O_2), until the testa removes. In another experiment 5% H_2O_2 was heated at temperatures 60, 70, 80, 90 and 100^{0}C and coconut were dipped for 40 minutes in order to find the suitable temperature of testa removal. Next a suitable hydrogen peroxide concentration was found by using 5%, 10%, 15%, 20%, 25%, 30%, 35% respectively by heating coconut (with testa) it at 80^{0}C. Using above experiments a suitable hydrogen peroxide and temperature condition was determined and then using that the best suitable time (1-30 min) duration was selected. In another experiment best Na_2CO_3 concentration, time and temperature was determined by using different set of parameters 1-20% concentration, temperatures 70 - 80^{0}C (as it get gets activated at over 70^{0}C temperature), time duration 2 – 20min. After that combined H_2O_2 and Na_2CO_3 experiments were conducted to check the suitability of combined method. In all of the above methods colorimetric analysis and thickness of kernel was done in order to determine the state of testa removal The results were analyzed using SPSS Statistics Version 16 & Minitab 17.

Results and Discussion

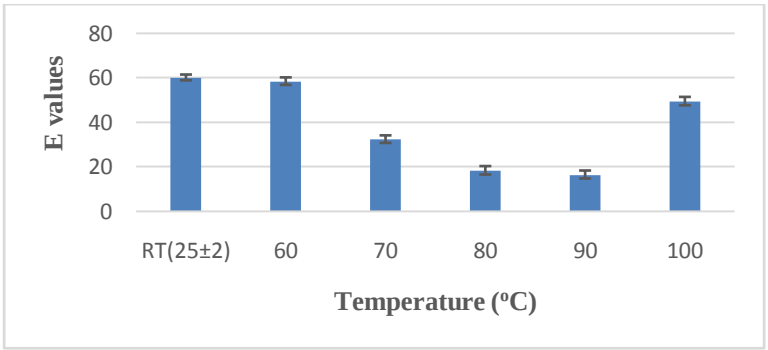

Figure 5- Colorimetric value change with the temperature for Hydrogen Peroxide

Using the above result, it was found that 70, 80 and 90 ^{o}C are much effective in removing the coconut testa. After evaluating the nutritional attributes also 80 ^{o}C was selected as the best temperature with H_2O_2.

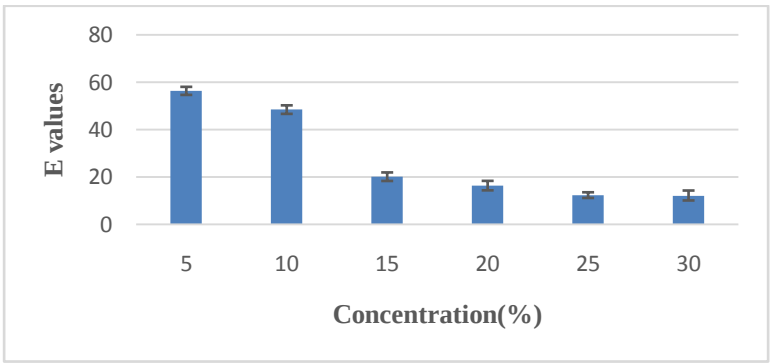

Figure 6- Colorimetric value change with the concentration of Hydrogen Peroxide

It was found out that the concentrations of H_2O_2 above 20% are effective in removing the coconut testa effectively. Also the kernel thickness data showed a significant difference ($P<0.05$). Therefore 20% H_2O_2 concentration along with the 80°C was selected as the optimum.

Concentration (%)	Time (min)			
	10	15	20	30
15	1.71	1.70	1.68	1.67
20	1.70	1.70	1.69	1.65
25	1.45	1.45	1.42	1.40

Figure 7- Thickness (cm) of kernel for combination of Time, and concentration of H2O2.

According to the above results 20% concentration and 20 minutes time duration was selected as the optimum.

Similar experiments showed that there is a significant difference in colorimetric value for the Na_2CO_3 at 80-90°C and 20% concentration. Even though it has shown potential of removing the coconut testa when compared with hydrogen peroxide effectivity of Sodium carbonate is less as only the initial layer (dark brown color) one is been removed. But removal of that upper layer was found to be much effective when sodium carbonate is used. Also the kernel rigidity after the treatments was found to be higher than with the use of hydrogen peroxide. Therefore, a combined treatment (initial dipping is sodium carbonate followed by hydrogen peroxide) set along with hot water dipping was carried out during this study to confirm the results.

Method	Na2CO3 % & Time	H2O2 % & Time	Hot water dipping
8	20%, 5 min	20% 15 min	-

12	-	20%, 20 min	5 min 100°C

Figure 8- Selected combined methodologies

Among other methods above was selected as the optimum based on the colorimetric and kernel thickness data. Hydrogen peroxide has given a significant contribution for the removal of testa while sodium carbonate and hot water has given an equal contribution.

Conclusion

Hydrogen peroxide is a great source for the removal of coconut testa effectively under 20% concentration and also 20 minutes time. Sodium carbonate is relatively lower in effectivity but adds increase in rigidity and quick initial layer removal.

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COMPARATIVE STUDY OF SELECTED ANTIMICROBIAL AND ANTIOXIDANT PROPERTIES OF SWEET ORANGE PEEL AND SOUR ORANGE PEEL

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Summary

Citrus peels which are considered as agro industrial waste are potential source of plant secondary metabolites in the form of essential oils. In the present research, peels of sour orange (*Citrus aurantium*) and sweet orange (*Citrus sinensis*) were used for the comparison of antioxidant and anti-microbial activities. The antimicrobial effects of methanolic extracts of citrus peels were evaluated against the microbial strains of *Escherichia coli, Salmonella typhimurium and Shigella dysenteriae*. Total phenolic content (Folin–Ciocalteu method) and the radical scavenging activity (DPPH assay) of citrus peel extracts were also determined. Both sour and sweet citrus peels showed anti-microbial effects against *S. typhimurium and S. dysenteriae*. Antimicrobial activity of sour orange peel extracts showed relatively high inhibition zones against *S. typhimurium and S. dysenteriae*. Both peels showed no inhibition zones against *E. coli.* The total phenolic content of *C. aurantium* was 78.12±3.54 mg GAE/100 g, in comparison to *C. sinensis* which was 70.60±3.14 mg GAE/100 ml of sample. The highest radical scavenging activity (lower IC50 value) showed by *C. aurantium.* The information obtained from this study highlights the importance of using citrus waste for natural antioxidant production which has high antimicrobial effect.

Keywords: antioxidant, antimicrobial, sour orange, sweet orange

Introduction

Citrus fruits contain a large variety of bioactive compounds in flesh as well as in peel. *Citrus sinensis* which is called sweet orange and *Citrus aurantium* known as sour orange/ bitter orange are most popular orange types. Citrus peels which are considered as agro industrial waste are potential source of plant secondary metabolites in the form of essential oils. The most important applications of citrus peel essential oils are the presence of some bioactive compounds in them which serve as alternatives to synthetic antioxidants, as well as the presence of antimicrobial compounds. In general, fruit skin contains a higher concentration of antioxidant substances than the flesh of the fruit. Citrus peel, which represents roughly one half of

the fruit mass, is a rich source of bioactive compounds including natural antioxidants such as phenolic acids and flavonoids. Flavonoids, especially poly methoxy flavones and flavones (hesperidin, narirutin, neohesperidin and naringin) are identified in citrus peel as well as in flesh. In addition, phytochemicals such as phenolic acids (caffeic, p-coumaric, ferulic, and sinapic acids) have also been identified (Ghafar et al., 2010). Citrus peel extracts have been used as natural antioxidants mainly in foods to prevent the oxidative rancidity of lipid. Polyphenols due to their potent antioxidant properties and their abundance in the diet, have credible effects in the prevention of various oxidative stress associated diseases (Oboh and Ademosun 2012). Therefore, the present study was designed with the main objective of comparing the selected antioxidant properties and antimicrobial effects of peel extracts of *Citrus sinensis* and *Citrus aurantium*.

Methodology

Chemicals and reagents

All the chemicals and reagents used for the study were of analytical grades. Microbial strains required for antimicrobial testing were obtained from Medical Research Institute of Sri Lanka. The used microbial strains include Escherichia coli, Salmonella typhimurium and Shigella dysenteriae.

Preparation of citrus peel powder

Sri Lanka varieties of sweet and sour oranges, randomly collected from Pambahinna area, were peeled carefully and oven dried at 50°C for 20 minutes. The dried peel samples were ground to fine powders and passed through a mesh sieve and packed in polyethylene bags and stored in a refrigerator at 4°C until further use.

Preparation of citrus peel extracts

Two grams of citrus peel powder (sour and sweet) from each type, was added to 70 ml of 95% methanol solution. The mixture was put into a shaker for 8 hours for shaking. Then the samples were taken out and filtered. The supernatant was put into a round bottom flask and the methanol was evaporated by using a rotary evaporator for 6 hours. The extracts prepared were preserved in a refrigerator at 4°C until further use.

Preparation of culture media and determination of anti-microbial effects

Fifteen milligrams of nutrient agar was measured and 250 ml of distilled water was added to it and mixed well. The mixture was autoclaved. Sterilized petri dishes were filled with autoclaved nutrient agar and they were allowed to solidify. After solidification, four wells were made in each petri dish by using a glass rod. Punched white paper was dipped in each microorganisms sample and it was placed in three

wells in one petri dish and other well was kept as the control. Three replicates were prepared for each microbial strain for both sweet and sour orange extracts. Culture plates were properly sealed and incubated overnight at 37^{0}C. The antimicrobial activity was determined by measuring the diameter of the resulting zone of inhibition against the tested organisms (Dhanavade et al., 2011).

Determination of Total Phenolic Content (TPC)

250 µl of 95 % methanol extracts of each sample, was added to a 25 ml volumetric flask filled with 1250 µl distilled water. Folin–Ciocalteu phenol reagent 2000 µl was added to the mixture and shaken vigorously. After 5 min, 1500 µL of 5% Na_2CO_3 solution was added with mixing. Then it was allowed to stand for 30 min before taking measurement, and the absorbance was measured at 765 nm versus the prepared blank. TPC of the sample was expressed as mg/g dry weight of gallic acid equivalent (GAE). A blank solution was prepared by using distilled water. All determinations were performed in triplicates (Oboh and Ademosun 2012).

Determination of antioxidant content by DPPH free radical assay

Five milligram of each peel extract was added to 10 ml of 95% methanol. Dilution series were prepared by changing ratio of sample and methanol. Sample was introduced into test tubes and mixed with 95% methanol. Then 2000 µl DPPH solution was added. The tubes were covered with aluminum foil and allowed to stand for 30 minutes at dark. A blank sample was similarly prepared. Absorbance readings were taken by a UV/ Visible spectrophotometer at 517nm. The same procedure was conducted to the standard solution (methanol) and the standard curve was used to calculate the total antioxidant activity of samples. All determinations were performed in triplicates (Oboh and Ademosun 2012).

Results and Discussion

Antimicrobial activities of sweet and sour orange peel extracts:

Antimicrobial activities of sweet and sour orange peel extracts, against pathogenic *E. coli, S. typhimurium and S. dysenteriae* were compared. The results of antibacterial activity of methanol extracts of citrus varieties are presented in Table 4.1

Table 4.1 Diameter of inhibition areas of citrus peel extracts for microbial strains.

Citrus sample	Microbial strains		
	S. typhimurium	*E. coli*	*S. dysenteriae*
Citrus aurantium	6.00±0.00 mm	ND	7.00±2.12mm

Citrus sinensis	5.50±0.71mm	ND	5.50±0.71mm

Mean±SD (n=3), ND = No activity detected

Data in Table 4.1 revealed that *C. aurantium* peel extract showed higher antibacterial activities than *C. sinensis*. For both peel extracts, no activity was detected against *E.coli*.

Total Phenolic Content of peel extracts:

Table 4.2 Total phenolic content of orange peel extracts.

Citrus sample	GAE mg/100 ml
Citrus sinensis	70.60±3.14[a]
Citrus aurantium	78.12±3.54[b]

Mean±SD (n=3); Values followed by different letters in the same column are significantly different at p<0.05

C. aurantium showed significantly higher total phenolic content ($P < 0.05$) in comparison to *C. sinensis*.

Radical scavenging activity of orange peel extracts:

Table 4.3. IC 50 values of sour and sweet orange peel extracts

Sample concentration (mg/ml)	IC 50 value (mg/ml)	
	Citrus aurantium	*Citrus sinensis*
25	30.88	32.20
50	46.86	47.07
75	66.84	69.37
100	96.25	96.62
150	132.20	133.79

IC 50 value represents the concentration of extract to reduce 50% of free radicals. When consider the same concentration of both samples, sour orange has lower values compared with sweet orange. Since the lower IC50 value indicates better antioxidant activity, it might be concluded that C. *aurantium* had higher radical scavenging activity compared to C. sinensis. The high phenolic content of C. *aurantium* could be the main reason for its high antioxidant activity towards DPPH radicals. Several reports showed a close relationship between total phenolic content and high antioxidant activity (Ghafar et al., 2010).

Conclusion

Citrus peel is a rich source of bioactive and antimicrobial compounds. Both sweet and sour orange peels have anti-microbial activities against the selected pathogenic bacterial strains. However, sour orange peel showed more inhibitory effects and antioxidant properties compared with sweet orange peel.

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CURCUMIN; ETHANOLIC EXTRACTION AND ITS STABILITY IN FOOD APPLICATIONS

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Summary

Curcumin is the main active compound and the coloring agent of turmeric. Color intensity of turmeric powder is very much acceptable and popular in Asian countries. Curcumin was extracted using ethanol as a solvent. Three food products (biscuit filling, pasta and extruded snack) were formulated using three different physical applications in order to investigate the stability of curcumin under different processing conditions (mechanical beating, applied pressure and combination of pressure and temperature). A biscuit filling (cream) was produced with mechanical beating at ambient temperature (30^{o}±1C) without any pressure increase. Pasta was produced as an extruded product with exert of pressure. Extruded snack was produced as an extruded product with increase of both pressure and temperature (170^{0}±1C). Color intensities of the formulated products were compared with turmeric powder and curcumin. The antioxidant activity and curcumin content were analyzed in the biscuit filling, pasta and extruded snack which were subjected to different physical treatments during processing. Out of the three products curcumin content and antioxidant activity were significantly high in the biscuit filling. Curcumin has the highest stability under mechanical beating than with high temperature and pressure.

Keywords: curcumin, antioxidant, ethanoic extraction, color

Introduction

Turmeric is the rhizome of the plant *Curcuma longa* which belongs to the family Zingeberaceae. It is a dominant spice found in sub Asian countries including India and Sri Lanka (Lal, 2012). Curcumin or diferuloylmethane is a polyphenol, extracted from the turmeric rhizome and it is the main coloring agent of turmeric. It is a combination of several phytonutrients such as demethoxycurcumin and bis-demethoxycurcumin, diferuloylmethane and comprised with the most soothing bioactive compound among all the other constituents of turmeric extracts (Priyadarsani, 2014). Curcumin has many therapeutic uses such as antioxidant, anti-platelet, anti-inflammatory,

antibacterial, anti-fungal, anti-cancer and cholesterol lowering properties (Nisar et al., 2015). It has been revealed that curcumin has eight times greater antioxidant power than vitamin E when inhibiting lipid peroxidation (Yadav et al., 2013). Moreover, curcumin recently has been investigated to reduce diabetic mellitus.

Methodology

Turmeric rhizomes were collected from local farmers in Makandura (NWP) area and grounded after sun drying for 3 days. Curcumin, oleoresin and turmeric oil were extracted from turmeric powder. Curcumin and oleoresin were extracted using ethanol as a solvent. Turmeric oil was made by distilling turmeric powder with water. Extracted curcumin was used as a colouring agent in preparation of biscuit cream, pasta and extruded snack. Antioxidant content was analyzed in biscuit cream, pasta and tipi-tip by the DPPH scavenging assay (Chen *et al* 2016) with some modifications. Curcumin content of the extracted turmeric sample and in the three products was calculated through (ASTA method 18.0) according to the following equation; Curcumin% = Absorbance of the extract * 125 / Cell length (cm)* Dry sample weight

Color measurements of turmeric powder and three formulated product's powder were measured using chromo meter (CR-400). Color readings were expressed as Hunter values for L* (brightness), a* (red–green chromaticity) and b* (yellow–blue chromaticity) and value expressed as the average of three measurements.

A GC-MS system(Shimadzu) consisting of a GC-17A with a QP5050 Mass Spectrometer (Kyoto, Japan) equipped with a DB-5 capillary column (60 × 0.25 mm, thickness 0.25 µm; J&W Scientific, Folsom, CA, USA) was used for all chemical quantitative and qualitative analyses. GC-MS analysis was done for turmeric oil.
Curcumin sample was analyzed by HPLC using C_{18} column with UV detection at 425 nm. The mobile phase was acetonitrile and water (50:50 *v/v*) acidified with 2% acetic acid at a flow rate of 1.2 mL min.
Sensory attributes (color, appearance, texture, aroma, flavor, mouth feel and overall acceptability) was evaluated in prepared products using 9 points hedonic scale with 30 untrained panelists.

Results and Discussion

L, a and b values of the turmeric powder were 48.17 ±0.262, +30.67 ±0.613 and +82.23 ±0.262 respectively. The range L, a and b value of curcumin were 64.74 ±0.372, +15.62 ±0.333 and +54.77 ±0.395 respectively. Here b value is more towards to the +60. It is revealed that curcumin has pure yellow color. Evidences have proved that curcumin has pure yellow color than turmeric powder demonstrating that curcumin can be used as natural colorant for food products.
According to the definition, IC50 is the concentration of a drug which inhibits 50% of initial DPPH concentration. Therefore, the lower IC50 value of a sample means less

amount of sample is needed to degrade 50% of DPPH concentration. It means sample that has lower IC50 value considered as a strong antioxidant.

Table 1. Curcumin content and IC50 values of extracted curcumin sample, biscuit cream, pasta and extruded snack.

Sample	Curcumin Content (%)	IC50 (µg/mL)
Extracted curcumin sample	5.32 ± 0.033	20.48 ± 0.653
Biscuit cream	1.18 ± 0.029	108.32 ± 1.535
Pasta	0.27 ± 0.017	253.62 ± 3.142
Extruded snack	0.63 ± 0.022	237.49 ± 3.671

Out of the three products biscuit cream contains highest curcumin content (1.18 ± 0.029%). Due to that it has the lowest IC50 value, highest antioxidant activity. Biscuit cream produced with mechanical beating without any increase of temperature and pressure. Pasta contains the lowest curcumin content hence it has highest IC50 value. So it shows lowest anti-oxidant activity. Pasta and extruded snack both are extruded products under different processing conditions. Pasta has relatively low pressure and not much temperature elevation in the barrel but in extruded snack has high pressure and high temperature (170^0±1C) in the barrel. But after extrusion pasta was kept in an oven at 65^oC for 8 hours. According to the results, higher amount of curcumin has been lost due to the 65^oC temperature for 8 hours than the conditions used for extruded snack (170^0 ±1 C for few minutes).

Curcumin shows higher stability with mechanical beating under the ambient conditions (biscuit cream) than extruded products (pasta and extruded snack). Furthermore, curcumin has low stability in long time, at low temperature conditions than short time, at high temperatures.

Conclusion

Curcumin from the rhizome of turmeric, that extracted using ethanol as a solvent can be used as a colourant in food applications. Curcumin has the highest stability under mechanical beating than with high temperature and high pressure. Curcumin can be incorporated to other food products such as beverages, bakery products and confectioneries which undergo different processing conditions.

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TOTAL QUALITY IMPROVEMENT IN COMMERCIALLY ESTABLISHED PASTEURIZED MILK PLANT UNDER ISO 22000:2005 STANDARD

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Summary

Dairy industry has increased its effort to ensure quality and safety through implementation of proactive strategies because dairy products are identified as high risk food products. A Hazard Analysis was done in the production line from beginning to end (n=17). Accordingly, physical contaminants were observed in raw milk samples. However, no chemical contaminants were detected. Microbiological hazard analysis revealed that total plate count (7.01±0.20 to 7.43±0.27 log cfu/ml) and coliform counts (5.19±0.31 to 5.32±0.37 log cfu/ml) were high in raw milk samples from bowser. All samples collected before pasteurization were positive for coliform (1.13±0.28 to 5.33±0.36 log cfu/ml). Post pasteurization contamination was observed in few batches (0.63±0.20 to 0.86±0.19). Therefore, receiving of raw milk and the storage tanks of pasteurized milk were identified as the critical control points (CCPs). In gap analysis, 34 solutions were identified and highest ranked solutions were screened out. As conclusion, two CCP points were identified and 3 solutions; informing worker's illness, monitoring daily hygiene of workers and covering raw ingredients were ranked high priority from gap analysis.

Keywords: HACCP, Pasteurization, Coliform, Total Plate Count, Critical control points, Gap analysis

Introduction

The most important feature of food quality is its safety. All food laws regulate in order to assure that the food purchased by the consumers meet their safety expectations (Sikora, 2005). Dairy food safety controls the industry in providing safe milk and dairy products. Most of the dairy products are relatively limited shelf life products. Therefore, HACCP analysis of these products is important for public health (Ali and Fischer, 2005). Current dairy industry faces challenges because quality failures are more widespread consequences (Sandrou and Anvanitoyannis, 2000). Dairy products

are potential carriers of veterinary drugs, chemical pollutants and microbial toxins (Karaman *et al*., 2012).

The most effective way to achieve food safety is to focus on prevention of possible hazards and improving the process (Roberto *et al*., 2006). The ISO 22000:2005 deals with issues of safety in food production, which is based on HACCP system (Andrea, 2015). It is a preventive and systematic approach to ensure food safety because it identifies physical, chemical and biological hazards and develop preventive measures throughout food manufacturing process (Ali and Fischer, 2002). The objective of the study was to identify critical control points in the pasteurized milk production process while analyzing the gap needed to be fulfilled to implement ISO 22000:2005 standard.

Methodology

This study was designed to identify the CCPs in pasteurized chocolate milk production process in a commercially established pasteurized milk plant while analyzing the gap needed to be fulfilled to implement the standard. A preliminary study was carried out to familiarize with the production process while identifying the sample collecting points.

Samples were collected from different processing steps including raw milk receiving (P1), whey receiving (P2), skim milk receiving (P3), sugar, skim milk powder, cocoa powder, flash mixing tank (P4), flash mixing tank out (P5), raw milk out (P6), whey out (P7), skim milk out (P8), mixing tank (P9), feeding tank (P10), final product filling (P11) and final product storage (P12). Samples were collected from raw ingredients receiving to the final product.

Collected samples were analyzed for physical, chemical and microbiological hazards. To determine microbiological hazards, total plate count (TPC), Coliform and yeast and mould tests were done. Adulteration tests for chemical hazards were done and visually observed for physical hazards. Numerical data were analyzed through mean value comparison (mean ± standard deviation) by Microsoft Excel 2013 software.

In gap analysis, established prerequisite programs (PRPs) in the factory were studied and requirements for each PRP were identified. Then possible solutions were provided to fulfill the existing gap. Solutions were prioritized according to the order importance based on time gap to be implemented. Collected feedbacks for proposed solutions were ranked based on the Sum of Ranks value in Friedman test using Minitab® 16.1.0 statistical software.

Results and Discussion

Samples from 17 batches were analyzed physically, chemically and microbiologically for determining Critical Control Points (CCPs) and Control Points (CPs). According to analysis, physical contaminants were present in raw milk samples. However, no chemical contaminants were detected. Microbiological analysis revealed that TPC and coliform count were high in raw milk samples from bowser (Table 01).

Table 01: Mean Total Plate Count and Coliform Count of Raw Milk

	Center 1	Center 2	Center 3	Center 4	Center 5
TPC (log cfu/ml)	7.40±0.31	7.43±0.27	7.37±0.31	7.01±0.20	7.40±0.26
Coliform (log cfu/ml)	5.27±0.48	5.18±0.55	5.19±0.31	5.32±0.36	5.23±0.42

Accordingly, mean TPC and Coliform counts were beyond the acceptable levels (TPC 5 log cfu/ml and coliform 1.69 to 2 log cfu/ml) (Figure 01 and 02). Therefore raw milk contain microbiological contaminants. Bacterial agents can contaminate milk at different stages of procurement, processing and distribution (Garedew *et al*., 2012). Contamination of raw milk can occur at farmer level due to less hygienic practices (Lendenbach and Marshal, 2009).

To avoid potentially harmful microorganisms, raw milk should be stored at lowest possible temperature (+4 °C) and used within 72 h. Therefore, receiving of raw milk can be identified as a contamination point. Therefore receiving of raw milk was identified as the first CCP point in the present study.

All the samples collected before pasteurization were tested positive for coliforms. Post pasteurization contamination was observed in few batches while samples collected after pasteurization were tested positive for coliform. Pasteurization of milk is considered as the most effective method of reducing risk of contamination and spreading diseases (Garedew, 2012). However, pasteurization neither destroys nor eliminates toxins, bacterial agglomerates, residues of physical and chemical substances such as metals and antibiotics. Therefore, establishment of at least one CCP before pasteurization is essential (Mavropoulos and Arvanitoyannis, 1999). In pasteurized milk, viable bacterial count should be less than 30,000 cfu/ml (4.48 log cfu/ml) and the coliform bacterial test should be negative (SLS 181, 1983; LuJ *et al*., 2013). But after pasteurization TPC was slightly higher (4.49 log cfu/ml) than the threshold level and coliform count was positive.

Defective pasteurization can lead to survival of pathogenic microorganisms (Anderson *et al*., 2011). Inadequate time temperature combination can be a

contributory factor for survival of coliforms after pasteurization. Though pasteurization temperature is maintained at 80 °C for 15 s in the factory, this may be ineffective in destroying pathogenic microbes due to high level of microflora in raw milk. Therefore, pasteurization can be identified as the second CCP point due to presence of microbiological hazards after pasteurization.

During storage, product temperature must be maintained +4 °C or less to ensure the microbiological safety of the product. Storing pasteurized milk in wrong temperature will affect the safety and shelf life of the product (Ali and Fischer, 2002). Therefore, changes in temperature appear to provide favorable conditions for spoilage microorganisms to grow and multiply thus increasing the plate count. This step identified as a CP.

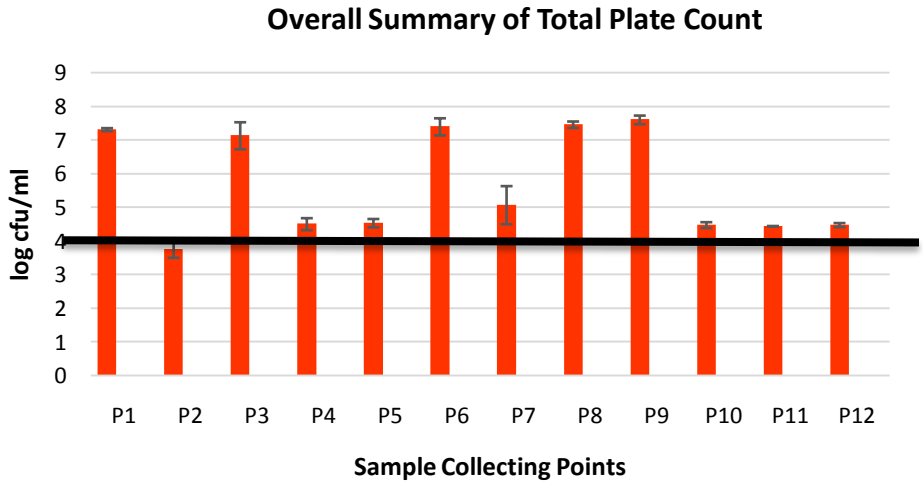

Figure 01: Overall Summary of Total Plate Count in Sample Collecting Points

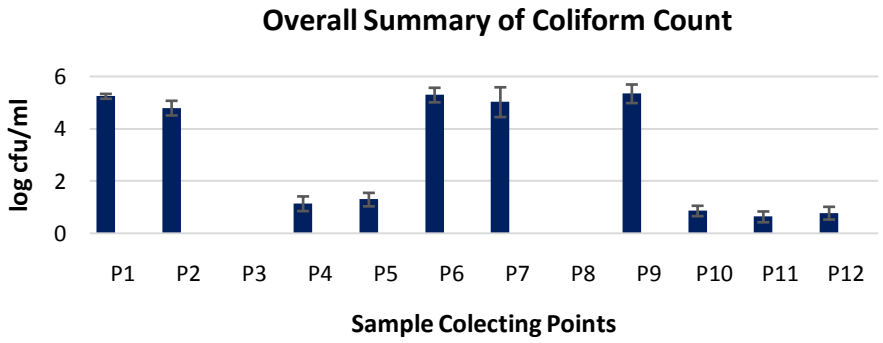

Figure 02: Overall Summary of Coliform Count in Sample Collecting Points

In gap analysis, thirty-four solutions were identified and feedback were collected from the employees in the Quality Assurance Department based on a 5 point Likert scale. Highest ranked solutions (Sum of Ranks 149.5) were informing the workers to report their illnesses, monitoring daily hygiene of workers and covering raw ingredients with wrappers. Lowest ranked solution was implementing a short term training program daily for the temporary employees. Highest ranked solutions need to implement immediately whereas no need of implementing the lowest ranked solution.

Conclusions

Two CCPs were identified as; receiving of raw milk and pasteurization and final product storage as a control point. In gap analysis, highest ranked solutions were informing worker's illness, monitoring daily hygiene of workers and covering raw ingredients. Lowest ranked solution was implementing a short training program for employees. Depending on the results identification and isolation of coliform bacteria can be done. Based on CCPs HACCP plan for pasteurized milk can be developed. Implementing HACCP plan will ensure that the milk produced from plant is safe for consumption.

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FORMULATION OF ICE CREAM INCORPORATED WITH ORANGE-FLESHED SWEET POTATO (*Ipomoea batatas*) PUREE

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Summary

Orange Fleshed sweet potato is a cheap and abundant yet underutilized root crop in Sri Lanka. Even though functional and nutritional benefits of sweet potato were reported by many studies, scanty of value added products are found in the local market. Further, ice cream is a popular dairy product which can be utilized as an excellent carrier to deliver these nutritional and functional benefits of sweet potato. Therefore, the objective of this study is to formulate sweet potato incorporated ice cream along with probiotic culture BB-12. For this purpose, three ice-cream formulations were prepared by replacing cows' milk with sweet potato puree (10 %, 20 % and 30 %). Textural properties of newly formulated ice cream were evaluated with compared to commercially available product by using Brookfield texture analyzer. Consumer preferences for the three ice cream formulations were evaluated using 9 point hedonic scale with 37 applicants and the data were statistically analyzed. The results showed that textural properties of all three formulations were largely deviate from commercially available samples. Ice cream formulation which added 20 % sweet potato puree was the best accepted for color, flavor, odor, texture and overall acceptability. 103.6 % over run was obtained for most preferred ice cream sample. The estimated shelf life of the product was approximately 4-5 weeks.

Keywords: Consumer preference**,** Ice cream, Orange- fleshed sweet potato, Textural properties

Introduction

Ice cream is a dairy product which has a good demand in all round the world. Ice cream acts as a beneficial vehicle that supports considerably for the viability of probiotic strains during production and storage compared with fermented milks (Mohammadi *et al*.,2011). Thus, probiotic ice cream can be formulated with the incorporation of probiotic bacteria either fermented or unfermented mix (Homayouni *et al*.,2008). Most common probiotic bacteria used for the production of fermented and non-fermented ice cream are *Lactobacillus* and *Bifidobacterium* (Mountzouris and

Gibson, 2003). With the incorporation of probiotics in to the ice cream it can be taken as a functional food. Orange-fleshed sweet potato (*Ipomoea batatas*) is a rich source of β-carotene and it act as antioxidant, stabilizes blood sugar, enhance immunity and promote health vision. In order to enhance the probiotic effectiveness of fermented ice cream it is more advantageous to incorporate prebiotic food ingredient. Sweet potato fiber extract has a prebiotic effect (Lestari *et al*., 2013). As Orange-fleshed sweet potato is available in most areas and due to its low price compared to other root crops and generally value added products from sweet potatoes are lacked in the local market it can be incorporated in to probiotic ice cream. As its inherent orange-yellow color, ice cream can be manufactured without adding artificial colorants. Now a days people are not preferred to eat sweet potatoes in boiled form. Thus, such people can be motivated by making value added products like sweet potato ice cream. By considering all these factors it was become significant to develop orange-fleshed sweet potato puree incorporated ice cream.

Methodology

Orange-fleshed sweet potato (OFSP) was purchased from local market and cleaned. Oven is preheated to about 200 ^{0}C. In the sweet potato few holes were poked with a fork and wrapped with Aluminium foil and baked at 200 ^{0}C for about 40 minutes. Then it was allowed for cool and skin was peeled off. The flesh was cut in to small pieces and put in to the blender to obtain a creamy mixture. Then the puree was put in the freezer bag and placed in the refrigerator until it was used.

Whole milk was pasteurized at 85 ^{0}C for 5 minutes and allowed to cool at 42 ^{0}C. DVS (Direct-Vat-Set) probiotic starter culture was added to it and incubated at 42 ^{0}C for 4-6 hours while beating in 1hour time intervals.

The milk and cream(35 % fat) were mixed and brought the temperature to 45 ^{0}C.Then skim milk powder, sugar and water were added and the mixed was pasteurized at 85 ^{0}C for 5 minutes while stirring. Then the mix was allowed to cool to about 10 ^{0}C.Then the prepared OFSP puree was added to it. Then mixture was blended. Then mixture was poured in to a container and allowed to ripen for 4-6 hours at 4 ^{0}C.Then the prepared fermented milk was added to the ice cream mix and freezed in the mini ice cream machine (with ~ 90-100 % overrun).Finally the partially frozen mix was filled in polystyrene cups and stored at -18 ^{0}C for hardening.

Ice cream mix was prepared according to the following formulation:

Cream(35 % fat) 15 % (w/w)
Skim Milk powder 7.4 % (w/w)
Sugar 12 % (w/w)
Stabilizer 0.5 % (w/w)

Water 10 % (w/w)
Fermented Milk 10 % (w/w)

Table 01: Amount of whole milk and sweet potato pulp combine

Sample code	Whole milk	Sweet Potato pulp
416	35 %	10 %
532	25 %	20 %
605	15 %	30 %

Best sample was selected through a 9-point hedonic scale test, evaluated by 37 untrained panelists and statistical data were analyzed by (Friedman statistical test) using Minitab 17 software. Texture Analysis (TA) was performed using the Brookfield CT3 texture analyzer and compared with the commercial ice cream. Shelf life was evaluated by physically observing sensory attributes.

Results and Discussion

According to the sensory evaluation, there was a significant difference ($p < 0.05$) between the three samples for appearance, color, flavor, odor, texture and overall acceptability (Figure 01). For the color, flavor, odor, texture and overall acceptability most selected sample was 532. Only for the appearance, 605 sample was most preferred.

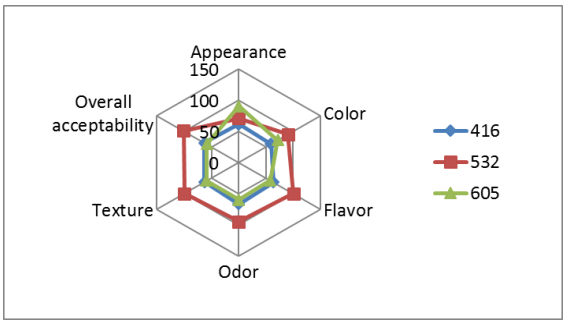

Figure 01: Sensory Evaluation Radar Chart

Table 02: Evaluation of textural parameters of 20% OFSP incorporated ice cream

Sample	Hardness (g)	Adhesiveness (mJ)	Deformation (mm)	Stringiness (mJ)
Market sample	75.803±0.005[b]	0.533±0.005[b]	9.923±0.006[c]	0.213±0.006[b]
10%	70.087±0.006[c]	0.463±0.015[c]	9.987±0.006[b]	0.163±0.006[c]
20%	75.807±0.006[b]	0.533±0.005[b]	9.923±0.006[c]	0.213±0.006[b]
30%	83.873±0.020[a]	1.540±0.010[a]	10.003±0.006[a]	0.417±0.006[a]

The values denoted by different letters in the same column are significantly different ($p<0.05$).

Hardness, adhesiveness, deformation and stringiness was not significantly different ($p<0.05$) between market sample and 20 % sweet potato puree incorporation. But for 10 % and 30 % incorporation those parameters were significantly different ($p<0.05$).

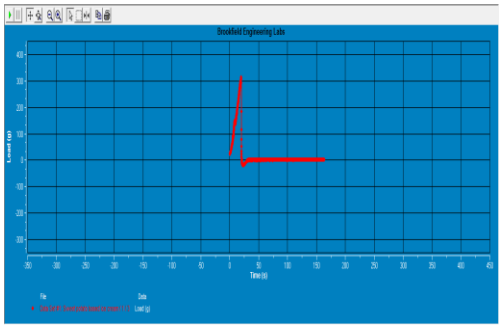

Figure 02: TA for market sample

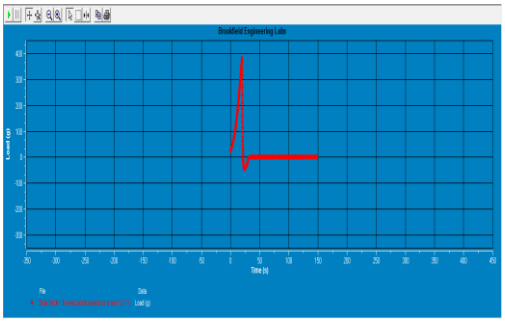

Figure03: TA for 20 % incorporation

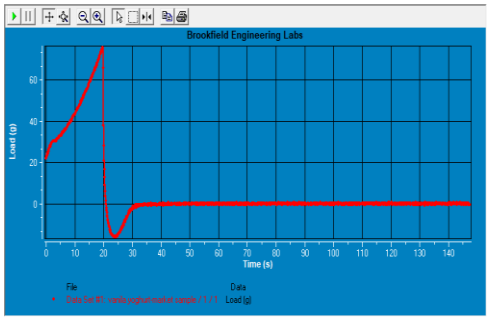

Figure 04: TA for 10 % incorporation

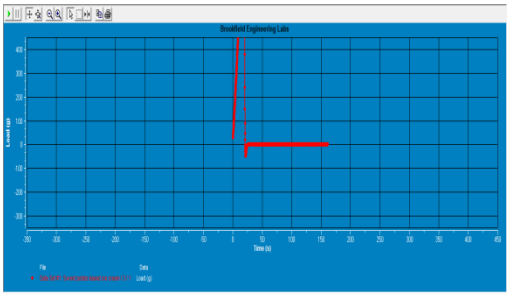

Figure 05: TA for 30 % incorporation

Milk–Solid-Non-Fat Percentage of the best selected sample was 10.9 % and overrun % was 103.6%. Shelf life of the best selected sample was evaluated by physical observations. Sensory attributes of appearance, color, flavor, odor and texture were not changed for 3 weeks of preparation. But after 4 th and 5 th weeks of preparation color and flavor were changed slightly. Although appearance and texture changed slightly after 4 th week, after 5 th week of preparation tiny ice crystal formation on top layer and harder texture was observed.

Table 03: Cost of production

Ingredients	Retail Price Rs.	Weight	Price Rs.
Whole milk	150.00 (1 L)	450 mL	67.50
Orange-fleshed sweet potato	220.00 (1 kg)	100 g	22.00
Cream(35 % fat)	175.00 (200 mL)	150 mL	131.25
Skim Milk powder	450.00 (400 g)	74 g	83.25
Sugar	60.00 (500 g)	120 g	14.40
Stabilizer (cremodan)	100.00 (25 g)	5 g	20.00
Vanilla flavor	75.00(28 mL)	1mL	3.00
ABT 5 culture	*2500.00 (1 packet)	0.5 g	104.00
Water	-	100 mL	-
Empty ice cream cups	72.00(12 cups)	8*25=200 g	6*25=150.00

(*Culture was distributed among 3 students.1 student had to bear Rs. 833.00,Therefore retail price for a packet can be considered as 833.00.This packet was utilized more than 20 trails)

Total cost estimated for 25 cups of ice cream was Rs. 595.40. Therefore unit price of ice cream cup was Rs. 24.00.

Figure 06: Three samples of finished product

Conclusion

It can be concluded that 20 % was the most suitable substitution level among selected levels when considering the sensory and textural properties of final product. This will be a good source to popularize OFSP among the busy population and make profit out of the underutilized food crop.

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DEVELOPMENT OF A NEW FUNCTIONAL COMPOSITE FLOUR BASED PASTA ENRICHED WITH PROBIOTIC: GANEDENBC30 (*Bacillus coagulans* GBI-30, 6086)

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Summary

This study was conducted to develop a composite flour based pasta enriched with a probiotic: *Bacillus coagulans* GBI-30, 6086 (GanedenBC30) and focused to select the appropriate flour blend and to determine the cooking quality of pasta. 0.2% encapsulated version of probiotic was enumerated in raw dough and examined their viability in uncooked, cooked pasta and cooking water. Wheat flour (50%), corn flour (20%), pumpkin seed flour (10%) and semolina flour (20%) were used to make the pasta. Commercially available pasta samples were used as control. Proximate composition (moisture, protein, total Fat, fiber, carbohydrates, ash) and physicochemical properties (pH, acidity, color and texture) were evaluated. The texture was 1.178 N and 0.089 N in uncooked pasta and in cooked pasta respectively. Cooking qualities (cooking loss, weight increase and volume increase), viability of probiotic *Bacillus coagulans* GBI-30, 6086 in pasta samples were determined after storing at 30 ^{o}C for 0, 3, 6, 9, 12, 15 days. Sensory evaluation was conducted with 30 semi-trained panelists using 7 point hedonic scale and color, appearance, odor, flavor, chewing properties, and overall acceptability were evaluated. On the basis of sensory qualities, the highest acceptability was achieved by pasta formulated from wheat (50%), corn (20%), pumpkin seed (10%) and semolina (20%).

Keywords: pasta, composite flour, probiotic, cooking quality, viability

Introduction

Pasta products like macaroni, spaghetti and noodles are largely consumed by the people across the continents (VasanthaKumari and Sangeetha, 2013). It is made from an unleavened dough of durum wheat flour (semolina) mixed with water or eggs, and formed into sheets or various shapes, then cooked by boiling or baking (Laleg *et al*., 2017). Small amounts of optional ingredients, such as salt, celery, garlic, and oils may also be added to enhance flavor and vegetable purees or artificial colorant used to add color to pasta. The applications of new ingredients to the basic product formulation could result in the products with high nutritional value and sensory quality (Bhatt *et al*., 2016). Here, composite flour blend (corn flour: pumpkin seed flour: wheat flour

and semolina flour) was enriched with a probiotic (*Bacillus coagulans* GBI-30, 6086) to enhance the nutritional composition and sensory quality. The probiotic known as GanedenBC30 is a heat stable, gram-positive, spore forming and lactic acid producing strain of *Bacillus coagulans*.GanedenBC30 shows that it is safe to use at levels of greater than 90 billion CFU/day. However,1 billion CFU/day is required for digestive health benefit.1g of encapsulated powder gives 9 billon CFUs and 1 billion CFUs should be in one serving of final product after processing. Therefore, the aim of the study was to develop a composite pasta with a probiotic and to determine the viability of probiotic with different heat application steps.

Methodology

Flours were prepared from raw materials (corn grits and pumpkin seeds) and semolina flour and wheat flour were purchased from a super market and sieved to remove foreign materials such as stones, sand, soil. Each flour blend was mixed with salt and feed into hopper of pasta machine (Dolly - La Monferrina, Italy) with necessary amount of water. Macaroni shaped pasta were made and two separate treatments were applied: (1) Steaming at 100^{o}C before drying and (2) drying without steaming. Both samples were dried at 60-65^{o}C for 8-10 hrs. To select the best sample, tenderness, stickiness, odor, color, flavor and appearance (wetness, slipperiness) were checked in cooked pasta samples.

Table 01: Amount of flour incorporated to formulate 100 g of flour blend

Treatment No	PSF(g)	Semolina flour(g)	Corn flour(g)	Wheat flour(g)
1	10	20	10	60
2	10	20	20	50
3	10	20	30	40
4	10	20	40	30

Probiotic was added to the selected flour blend which was not steamed before drying, mixed with salt and feed into hopper of pasta machine (Dolly - La Monferrina, Italy) with necessary amount of water, extruded in macaroni shape and dried at 60-65^{o}C for 8-10 hrs without steaming. Cooked and/or uncooked (dried) pasta samples were used for analysis of the following parameters (Lale*et al*., 2017). Pasta was cooked at 100^{o}C for 10 minutes.

The proximate composition (moisture, protein, total Fat, fiber, carbohydrates, ash) (AOAC, 2000) and physicochemical properties (water activity, color, texture) were

evaluated. Texture was measured in dried and cooked pasta using a texture analyzer (SHIMADZU compact tabletop testing machine EZT test). Viability of probiotic *Bacillus coagulans* GBI-30, 6086 was determined after storing at 30 ^{o}C for 0, 3, 6, 9, 12, 15 days (Jao *et al.*, 2015). Simple preference test was conducted with 30 semi-trained panelists using 7 point hedonic scale. Color, appearance (wetness, slipperiness), odor, flavor, chewing properties – stickiness, sensation of starch between teeth after each chew and tenderness and overall acceptability were checked. Statistical analysis was carried out using MINITAB-14 version at 0.05 significant levels. The significance of mean differences was determined by Mann-Whitney test.

Results and Discussion

Preliminary trials and sensory test were conducted to select the appropriate flour blend (CF- corn flour, PSF pumpkin seed flour, WF- wheat flour and SF- semolina flour).

Table 02: Amount of flour incorporated to make the 100 g of flour blend

Treatment No(T)	PSF(g)	Semolina flour(g)	Corn flour(g)	Wheat flour(g)
1	10	20	10	60
2	10	20	20	50
3	10	20	30	40
4	10	20	40	30

Then the second trail was done to confirm the flour blend. Trail 02 was included 3 treatments for T1-T2 treatments selected in trail 01(T1, T2 AND T3).10 % of PSF, 20 % SF with CF and WF in three different ratios.

Sensory test results for selection of flour blend

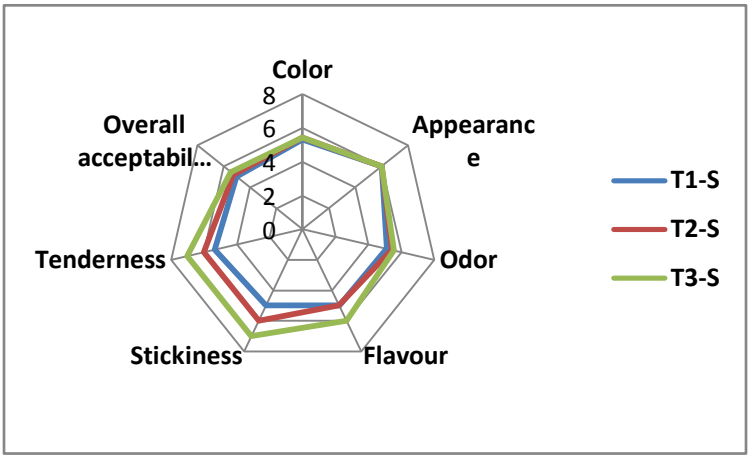

Figure 01. Radar chats for cooked pasta samples (steamed before drying)

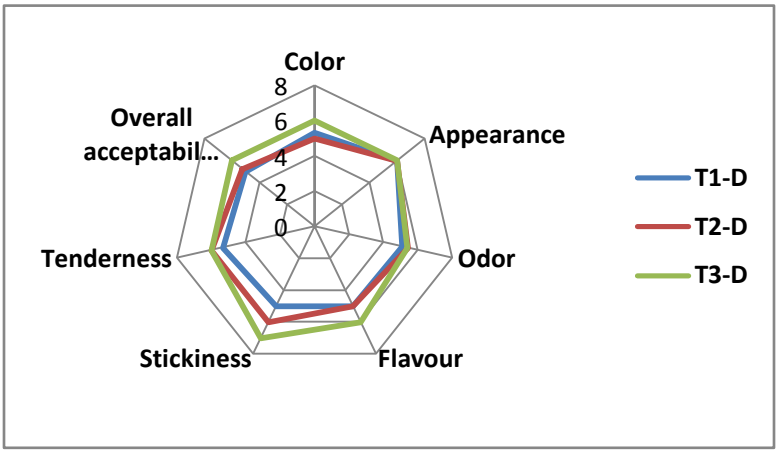

Figure 02. Radar chats for cooked pasta samples (dried without steaming)

Although steaming is a step of pasta processing before drying (precooking step), The appearance of dried form of steamed before drying pasta sample was less acceptable than the appearance of dried form of dried without steaming pasta sample due to not evenly steamed but all were in acceptable range after cooking. Stickiness, flavor and appearance were same in T3 - both treatment applied samples. Color and odor in cooked pasta were slightly changed due to steaming step. Cooking time was reduced but color and odor of dried pasta were also slightly changed due to steaming step. Although the probiotic is an encapsulated version and it is heat stable, dried without steaming step was selected when considering above reasons. Further improvements and analysis were done only for pasta samples dried without steaming.

Texture analysis: Texture was analyzed by Texture analyzer (SHIMADZU compact tabletop testing machine EZT test) in cooked and uncooked probiotic added pasta samples in triplicates. Test method was tensile test method. One end of the sample was fixed in a static grip while the other end of the specimen was pulled at a constant velocity (1mm/sec). The load was continuously monitored during the test. It was conducted until the sample fractures.

Table 03: Texture analysis data for cooked pasta sample

Name parameters	Hardness Calc. at Entire Areas	Comp_Energy 1th Node-Next Node
Unit	N	J
1_1	1.26346	0.00408
1_2	1.10960	0.00296
1_3	1.16237	0.00393
Average	1.17848	0.00366
Standard Deviation	0.07818	0.00061

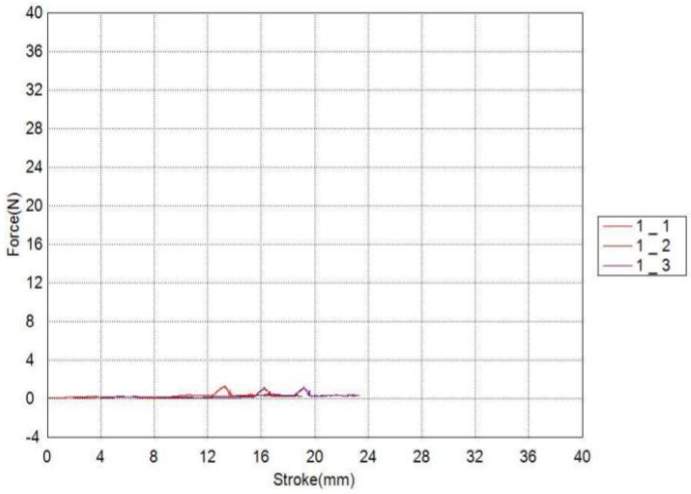

Figure 03: Test data of hardness in cooked pasta sample

Table 04: Texture analysis data for dried form of pasta sample

Name parameters	Hardness Calc. at Entire Areas	Comp_Energy 1th Node-Next Node
Unit	N	J
1_1	12.8755	0.00586
1_2	10.3567	0.00464
1_3	10.4123	0.00401
Average	11.2148	0.00484
Standard Deviation	1.43845	0.00094

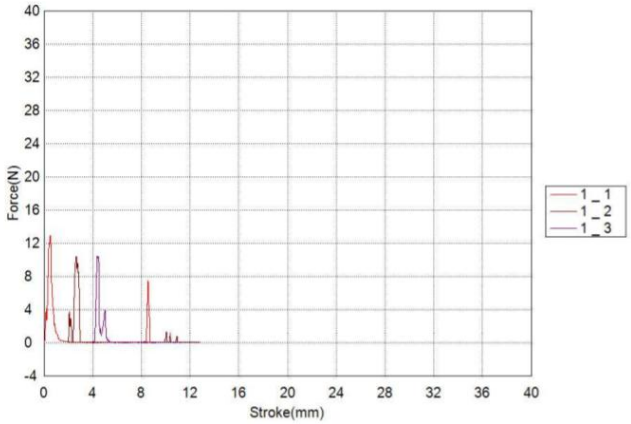

Figure 04: Test data of hardness in dried pasta sample

Cooking qualities:

Cooking time: Aproximately 15.00 g of pasta product was dispersed in 300 mL boiling water. Every minute from 3 minutes after adding pasta, a piece of pasta was held between two glass plates and was compressed. Duplicates were done. Optimum cooking time (min) was established when no white core was observed after compressing, indicating that the starch at the center has gelatinized. White core was not observed after 10 minutes in each sample.

Weight increase : Aproximately 15.00 g of pasta product was dispersed in 300 mL boiling water in triplicates. pasta were cooked withing cooking time(10 minutes). Weight increse(%) was 52.16 ± 3.64.

Weight increase = [(Weight increase of cooked pasta − Weight of uncooked pasta) /Weight of uncooked pasta]×100

Table 04: Measured data for weight increase

Initial sample weight(g)	Final sample weight(g)	Increased weight (g)	Weight increse(%)
15.12	38.66	23.54	55.69
15.49	38.48	22.99	48.42
15.28	38.56	23.28	52.36

Cooking loss : approximately 30.00 g of pasta were boilled in 300 g of distilled water and solids extracted withing 5,8 and 10 minutes from the cooking water were calculated by concentrating the cooking water to dryness in an oven at 100 ^{o}C. Triplicates were done for each.

Cooking loss = [Weight of drained residue in cooking wate /Weight of uncooked pasta] ×100

Table 05: Measured data for cooking lose (n=3)

Time (minutes)	Uncooked sample weight(g)	Beaker weight(g)	Sample+beaker weight(g) after ovening	Cooking lose(%)	Average Cooking lose(%)
5	30.21	172.74	174.15	4.67	4.32 ± 0.35
	30.23	179.53	180.93	4.30	
	30.18	162.38	163.58	3.98	

8	30.02	166.40	168.02	5.34	4.88 ± 0.40
	30.14	182.05	183.45	4.64	
	30.10	174.16	175.56	4.65	
10	30.13	172.71	174.35	5.31	5.31 ± 0.01
	30.20	157.38	158.98	5.30	
	30.16	172.55	174.15	5.31	

Volume increase : 20 pieces of dried and 10 minutes cooked pasta volume were calculated by measuring inner and outer diameter and length using Vernier calliper (6 Inch/15cm and smallest measurement = 0.1mm). Volume was calculated by the formula $V = \pi r^2 h$, and π is about the equivalent of 22/7 or 3.14, r=radius and h= height. Average volume increase was 145.22 ± 0.48 within 10 minutes.

Volume increase = [(Volume increase of cooked pasta − Volume of uncooked pasta)/ Volume of uncooked pasta]×100

Table 06: Measured data for volume increase in 20 pieces of dried and cooked pasta

Pasta sample	Average volume in 20 pieses
Dried	0.502 ± 0.46
Cooked	1.733 ± 0.53

Each value is expressed as mean ± Standard deviation (n=20);

Proximate analysis

Proximate analysis was done for each flour used and dried form of final product (AOAC, 2000).

Table 07: Proximate analysis for each flour

Parameters (%)	Corn flour	Pumpkin seed flour	Wheat flour	Semolina flour
Moisture	11.23	4.06	12.67	11.6
Protein	6.80	34.56	10.55	12.3
Total fat	3.30	36.70	0.94	1.1
Ash	1.02	3.08	0.94	0.8

Fiber	3.50	2.91	0.36	3.9
Carbohydrate	74.78	18.69	74.88	70.9

Table 08: Proximate analysis for final product

Parameters (%)	Amounts
Moisture	4.05 ± 0.01
Protein	13.00 ± 0.45
Total fat	5.66 ± 0.25
Ash	3.45 ± 0.05
Fiber	5.45 ± 0.30
Carbohydrate	72.44 ± 0.13

Each value is expressed as mean ± Standard deviation (n=3);

Water activity: 10.00 g of dried pasta was powdered and put into water activity meter (Novasina ms1, 0.06-0.98 range) and keep 15 minutes to take readings. Triplicates were done. Average water activity was 0.322 ± 0.001.

Microbial analysis:

Sample size was 75.00 g and sample preparation was done by using half of serving size. Aseptically 1.0 mL of the Sample preparation transferred separately into three appropriately labeled sterile 15-mm × 100-mm Petri plates, and then poured 20 mL of the molten MRS agar medium into each plate, Placed the lid on each plate after adding the molten MRS agar medium, then gently swirled the plates to mix the Sample preparation and the agar medium. One blank plate that contains only MRS agar medium was prepare and a second blank plate in which 1.0 mL of peptone diluent has been mixed with MRS agar medium. The plates were allowed to sit at room temperature until the agar medium solidifies, then inverted the plates and incubated them at 40° ± 2° for 48 h. After 48 h of incubation, the colonies on the prepared plates were counted, including both blank plates. Plates containing between 30 and 300 colonies were considered ideal for counting and counted only colonies that appear as follows. Surface colonies should be 1–5 mm in diameter, white to cream in color, convex, with entire margins and smooth surfaces. Colonies inside the MRS agar medium should be 0.5–1 mm in diameter and should appear as cream-colored pinpoints in the MRS agar medium. Results were reported in CFU/g. Average number of colonies per plate were calculate, then multiply the average number of colonies

counted by the reciprocal of the dilution factor to obtain the CFU/g of the sample. 10^{-5}, 10^{-6} and 10^{-7} dilutions for dry pasta and 10^{-4}, 10^{-5}, and 10^{-6} dilutions for cooked pasta were used for plating. 60ml of cooking water was used as the sample size, and then followed the same protocol and 10^{-3}, 10^{-4}, 10^{-5}, and 10^{-6} dilutions were used for plating. Colonies were counted after storing at 30 ^{o}C for 0, 3, 6 days. Viability of probiotic was reduced with the time.

Conclusions: This research was conducted to develop a composite pasta with a probiotic and to determine the viability of probiotic with different heat application steps from processing until consuming. Drying pasta at 60-65^{o}C for 8-10 hrs and cooking at 100^{o}C for 5-10 minutes are the heat application steps involved from processing until consuming. Selected flour blend was wheat flour (50 %): corn flour (20 %): pumpkin seed flour (10 %): semolina flour (20 %) according to sensory results and it can be used for further value additions. Hardness of pasta was reduced after cooking. Pasta quality could be estimated from cooking attributes such as weight increase, cooking loss and volume increase. The results showed that an increase in cooking loses were observed with different cooking time. Water activity of dried pasta should be ≤ 0.5 Water activity of pasta sample was below than 0.5. Viability of probiotic was reduced with the time.

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