DEVELOPMENT OF A SYNBIOTIC BEVERAGE FROM BEET ROOT JUICE USING BENEFICIAL PROBIOTIC *Lactobacillus casei* 431®

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Summary

Consumers are seeking products with greater health benefits. Thus, different products have been designed to meet this demand, especially those with probiotic and prebiotic characteristics. In this study the beet root beverage was formulated by incorporating *Lactobacillus casei* 431® as the probiotic microorganism while testing sensory acceptance at three fermentation periods (2, 4 and 6 h) at 37°C. Viable counts of *L. casei*®, total plate counts, physiochemical characteristics, and sensory characteristics were investigated weekly for six weeks under refrigerated (5±1°C) storage. The beverage fermented for 2 h yielded the highest consumer acceptability. The total probiotic count was greater than 10^7 CFU/mL, meeting the legal requirements of a functional food. *L. casei* 431® also grew well and reached nearly 10^8 CFU/ mL after 2 h of fermentation at 37°C. Titratable acidity was increased significantly ($p \leq 0.05$) during storage. Beet root-based synbiotic fermented beverage could be served as a ready to drink product for 6 weeks under refrigerated storage, meeting the standards (10^8 - 10^{10} CFU/ mL) of a functional drink.

Keywords: Synbiotic; Functional beverage; Sensory characteristics

Introduction

Interest in functional foods has recently increased among consumers due to a greater consciousness of health and nutrition, as well as the need to prevent diseases. The concept of functional foods originated in Japan and defined as being similar in appearance to conventional foods and used as part of a normal diet, but demonstrating nutritional functions beyond those considered basic, physiological benefits or reducing the chronic risk of disease, known as food for specified health use. Hence, food products containing probiotics and prebiotics are considered as functional foods. Most research has focused on evaluating the addition of probiotics and prebiotics, obtaining a product with a better final quality known as synbiotic food.

Prebiotic oligosaccharides are non-digestible (NDO) and low calorific compounds stimulating the growth and development of gastrointestinal microflora described as probiotic bacteria. Dietary carbohydrates that show prebiotic ability include fructans - fructooligosaccharides (FOS) and inulin, galactooligosaccharides (GOS), polydextrose, resistant starch, soyoligosaccharides, xylooligosaccharides, isomaltooligosaccharides,

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and lactulose. Probiotics are defined as selected, viable microbial dietary supplements that, when introduced in sufficient quantities, beneficially affect human organism through their effects in the intestinal tract. Some selected strains of *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Lactococcus* and *Saccharomyces* have been promoted in food products because of their reputed health benefits. Various researchers have discovered the use of different fruits, vegetables and cereals for producing synbiotic beverages in different countries of the world. The beetroot, apart from consumption in its fresh form, is also a valuable vegetable used in the food industry to produce dried and frozen food, non-concentrates and concentrated juices as well as natural colorants (betalains) used as additives in food manufacturing. In many countries there is a growing interest in foods preserved in natural ways. Lactic fermentation is one of the methods of natural preservation and thus production of foods with the highest nutritive value. Thus, based on the above information the present study aimed to evaluate the production of a synbiotic beverage using beet root juice fermented by *Lactobacillus casei* 431®.

Methodology

This research was carried out in the food processing laboratory of the Department of Food Science and Technology, Wayamba University of Sri Lanka. Beet roots were purchased from a local store at Pannala. After being washed, beet roots were peeled, chopped and juice was extracted using a juice extractor (Panasonic, Japan). Coarse particles were separated by centrifugation at 4,000 rpm (model 2-16 K, Germany) for 20 min. Juice was heated up to 50°C and sucrose (40 g/ L) was added. Beet root juice was pasteurized at 90°C for 1 minute. At 43°C, a commercial frozen probiotic culture, *Lactobacillus casei* 431® (CHR-Hanson, Denmark) (0.1g/L) was added. The fermentation of the beet root juice was performed in a thermostat at 37°C for 0 h, 2 h, 4 h and 6 h.

The pH of the beverage samples was measured by using a pH meter (Ohaus, USA). Titratable acidity was determined by titrating 10 mL of sample against 1 N, NaOH in the presence of phenolphthalein as an indicator (Sharma, 2006). The total soluble solid content was measured by using a refractometer (Atago, Japan). Determination of moisture content, ash, protein, fat, total fiber, total sugars, reducing sugars and sucrose were determined (AOAC, 2000). Sensory evaluation was conducted using 31 untrained panelists who were asked to score for colour, consistency, taste, odor, and overall acceptability on 5 point hedonic scales (1 = dislike extremely, 5 = like extremely). Viable cell counts (CFU/mL) of the inoculum were determined by the standard plate method with MRS medium after 48 h of incubation at 30°C. Coliform counts were estimated using MacConkey agar plates incubated at 37°C. Yeast and mold were enumerated by a surface spread plate technique using potato dextrose agar (HiMedia, India) plates in triplicate. To examine effect of cold storage on cell viability samples were taken at weekly intervals, and the viability of probiotic cultures in beet juice was determined. Significant differences between the results were calculated by analysis of variance (ANOVA) with the help of SAS software. Results were expressed as mean ± SD. Values were the average of triplicate experiments. Nonparametric tests were performed to determine the statistical difference of the sensory data, and where appropriate. Differences at $p<0.05$ was considered statistically significant for all analyses.

Results and Discussion

The present study was carried out to investigate the possibility of producing novel synbiotic beverage by incorporating prebiotic microorganism *L. casei*® into beet root juice and fermented for different time intervals (2 h, 4 h and 6 h) at 37 °C. Sensory evaluation results showed that beet root juice fermented for 2h gives the highest ranks for colour, taste, odor, consistency and overall acceptability. Lactobacillus bacteria used in this study *L. casei* 431® is a novel bacterial strain developed by Chrohasen, Denmark. Initial pH of the beet root juice was 5.6 which more close to optimum pH for the *L. casei* 431®. However, in this study *L. casei* 431® reduced the pH of beet juice from an initial value of 5.6 to lower than 5.2 while increasing titrable acidity after 2 h of fermentation due to their ability to produce lactic acid. In fermentation process, the rate of pH decrease is very important. Because the resultant low pH minimize the influence of spoilage bacteria particularly at the beginning of the fermentation when the substrate is rich in sugars. Table 1 illustrates the changes of pH, acidity and viable count of *L. casei* 431® at 2, 4 and 6 h fermentation periods.

Table 1: Changes of titratable acidity, pH and viability of *L. casei* 431® in beet root juice at different time intervals.

Time (h)	pH	Acidity (%)	CFU/mL
0	5.5 ± 0.05^{a}	0.16 ± 0.06^{a}	$2.45\text{x}10^{7}$
2	5.2 ± 0.04^{a}	0.19 ± 0.11^{a}	$2.50\text{x}10^{8}$
4	5.0 ± 0.02^{a}	0.22 ± 0.04^{a}	$3.05\text{x}10^{8}$
6	4.9 ± 0.01^{a}	0.23 ± 0.23^{b}	$3.45\text{x}10^{8}$

The values are mean ±SD of three independent determinations. The means with different superscripts in a row differ significantly ($p\leq 0.05$)

The international standards describe that the probiotic products should be contained minimum of 10^8 viable probiotic bacteria per mL of the product at the time of consumption for health and functional claiming (Samona and Robinson, 1991). However, in this study fermented beet root juice has reached to around 10^8 viable probiotic bacteria per mL of the product at the end of 6th week. Nighswonger *et al*, (1996) revealed that there was a slight fermentative activity by the probiotic organism even at 4°C. Although the lactic acid cultures in the fermented beet juice gradually reduced their cell viability during cold storage at 4°C, the viable cell counts of the lactic acid bacteria in the fermented beet juice still remained at 10^6–10^8 CFU/mL after 4 weeks of storage at 4°C. Some researchers have viewed that several factors may affect the survival of lactic acid bacterial strain, inoculation level, incubation temperature, inhibitors, presence of hydrogen peroxide and oxygen concentration of metabolites, buffering capacity of the media, storage temperature and availability of nutrients. In this study, even after 6 weeks of storage at 5±1°C, *L. casei* was capable of surviving in the fermented beet root juice at low pH. This pH value can still be considered sufficient to positively affect host health. This effect was previously demonstrated for other *Lactobacillus* bacterial strains for which survival under analogous conditions was enhanced by presence of carbohydrates present in vegetable products (Yoon, Woodams and Hang, 2005). The percentages of reducing sugar, sucrose and total sugars were significantly reduced with storage and least was observed at the 6th week of storage

period. Sugar consumption of *L. casei* is correlated with the lactic acid accumulation in the beverage. Therefore, sucrose is added at the time of preparing the beverage for the purpose of safe guarding prebiotics available in the beverage. The probiotic organism has metabolized more reducing and non-reducing sugars due to their high concentrations in the beverage. Therefore, adding sugars is important to this type of beverages where probiotic organism only depend on native prebiotics in the beverage. °Brix value of the beverage has slightly decreased with storage period. Especially, reduction of total sugar content is the main reason for the reduction of the °Brix value because of the fermentation of *L. casei*® within the storage period. Beet Roots contains both insoluble and soluble fiber components in a desirable ratio. Mainly insoluble fibers can be considered as the prebiotics. *L. casei*® is a probiotic which can metabolize above prebiotics and can live by using those prebiotics as substrates. To be a synbiotic beverage, the amount of prebiotics should be maintained until the consumption because there is no introduction of external prebiotics to the product. However addition of sucrose in to the beet root beverage helps to retain desirable prebiotic content in final product. According to the data there was no significant ($p< 0.05$) reduction of content of fiber within the storage period. It seems that prebiotics in the beverage have been preserved until the end of shelf life due to high concentration of sugars in the product. Fiber content is high in this beverage when compared with other ready to serve (RTS) beverages, and with low levels of fat and protein.

Conclusion

Beet roots could serve as a raw material for the production of probiotic incorporated beet juice by lactic acid fermentation with *L. casei*®. The fermented beet juice has a pH value of less than 5.5 and contains a significant number of beneficial lactic acid bacteria (10 CFU/mL). Finally symbiotic beet root beverage had acceptable sensory characteristics for health conscious consumers.

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EFFECT OF PHYSICALLY STRUCTURED WATER PRODUCED BY NANO-TOURMALINE IMPREGNATED CERAMIC BEADS ON IMPROVING EXTRACTABILITY OF CAFFEINE, CURCUMIN AND OIL EMULSIFICATION

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Summary

Physically structured water (PSW) possesses reduced molecular cluster structure. This study researched into identifying the effect of PSW on caffeine and curcumin extractability and oil emulsification. Physically structured water was obtained using a column of nano-tourmaline impregnated ceramic balls was compared with non-structured water (NSW) based on physical properties. Oxidation-reduction potential (ORP), pH and NMR frequency of PSW and NSW were different (P>0.05) in PSW and NSW, and were -113 & +97 mV, 8.64±0.08 & 6.83±0.06 and 55 & 120-Hz respectively. Caffeine was extracted from black tea, green tea, coffee and instant coffee at 24.5±0.2 and 95±2°C for 30 min in triplicate using PSW and NSW (2×2 factorial, CRD) and quantified by spectrophotometry (273 nm). Physically structured water increased (P <0.05) the extractability of caffeine at 95°C by 83.6% (black tea), 79.7% (coffee), 56.6% (green tea) and 3.6% (instant coffee). Caffeine contents from black tea (63.2 mg/L), coffee (60.2 mg/L) and instant coffee (135.0 mg/L) with PSW at 24.5±0.2°C were not different (P >0.05) from the contents extracted at 95±2°C with NSW. Curcumin was extracted from turmeric powder (sieve size, 300 µm) at 24.5±0.2 and 95±2°C for 20 min in triplicate using PSW and NSW (2×2 factorial, CRD), and the absorbencies (420 nm) of the extracts were compared. PSW increased (P <0.05) the extractability of curcumin at 95±2°C by 430% and at 24.5±0.2°C by 282%. Virgin coconut oil (VCO), sunflower and olive oils were sonicated (50 Hz, 5 min) in triplicate with PSW and NSW in a CRD, and the turbidity at 500 nm were measured daily for 6 days. Turbidity of VCO was significantly higher (P <0.05) by 1.1-, 1.2-, 1.2-, 1.2-, 1.2- and 1.5-fold on days 1, 2, 3, 4, 5 and 6 respectively when treated with PSW than with NSW. Similar pattern of turbidity was evident in other oils. Physically structured water is more effective than NSW in extracting caffeine and curcumin and emulsifying oil.

Keywords: Physically structured water; Nano-tourmaline; Curcumin

Introduction

Physically structured water (PSW) is well recognized for its reduced oxidation reduction potential, alkaline pH and reduced molecular cluster structure (Nakamura and

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Kubo, 1992). Naturally PSW can be obtained by a tourmaline treatment (Kubo, 1989), where the water will result in prominent characteristics which facilitate more extractability of compounds, in oil emulsification in the absence of an emulsifier with compared to non-structured water (NSW). Caffeine is less soluble in water and curcumin hardly soluble in water in which both are important constituents in both food and pharmaceutical industry. This highlights the need of use of safe methods for the extraction. The amount of emulsifiers and stabilizers used can be minimized for oil in water emulsions by reducing the cluster structure of water and can obtain stable emulsion. And also due to high emulsification ability this water can be used clean in process (CIP) which again will help to reduce the amount of detergents used.
This paper investigates whether the physically structuring of water is responsible for the increase in extractability of caffeine and curcumin and oil emulsification. The objective of this study was to identify its effect on extractability of caffeine and curcumin and on oil emulsification.

Methodology

Physically structured water was obtained using a column of nano-tourmaline impregnated ceramic beads and was compared with non-structured water (NSW) based on physical properties which are ORP, pH and NMR frequency.
Caffeine was extracted from black tea, green tea, coffee and instant coffee at 24.5±0.2 and 95±2°C for 30 min in triplicate using PSW and NSW (2×2factorial, CRD) and quantified by spectrophotometry (273 nm). Absorbance values were converted into caffeine concentrations by using the standard curve for caffeine. Both caffeine contents and absorbance values obtained for each treatment were analyzed by ANOVA in MINITAB® (2003) statistical software. Treatment means belong to the same category (black tea, green tea, coffee and Instant coffee) were compared at $p<0.05$.
Curcumin was extracted from turmeric powder (sieve size, 300 µm) at 24.5±0.2 and 95±2°C for 20 min in triplicate using PSW and NSW (2×2 factorial, CRD), and the absorbences (420 nm) of the extracts were compared. The absorbences obtained from this experiment were analyzed by ANOVA in MINITAB® (2003) statistical software. Treatment means were compared at $p<0.05$.
Virgin coconut oil (VCO), sunflower and olive oils were sonicated (50 Hz, 5min) in triplicate with PSW and NSW in a CRD, and the turbidity at 500nm were measured daily for 6 days. The data obtained for each day which belongs to the same category was analyzed by ANOVA in MINITAB® (2003) statistical. Treatment means were compared at $P<0.05$.

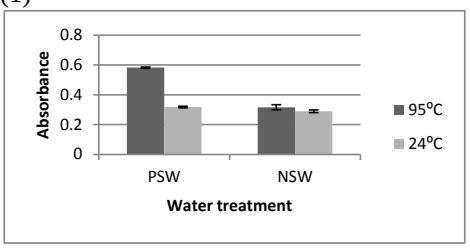
Results and Discussion

Oxidation Reduction Potential of PSW was lower than the NSW ($P<0.05$). And the value is getting lowered with the passing cycles through the column. The mean ORP value for the NSW was +97±2 mV. The ORP value for the PSW was 113±3 mV and this value further reduced to -169±2 mV and then to -231±3 mV in 2nd and 3rd pass respectively.

The pH of PSW was higher (P< 0.05) than NSW. The mean pH value for the PSW was 8.64±0.08 and 6.83±0.06 was obtained as the pH value for the NSW. There was no difference in pH in all the PSW samples up to 6 days of experiment.

Physically structured water increased (P <0.05) the extractability of caffeine at 95±2°C by 83.6% in black tea, 79.7% in coffee, 56.6% in green tea and 3.6% in instant coffee and at 24.5±0.2°C the extractability was 9.7%, 5.2%, 9% and 2.4% respectively (Figures 1,2,3 and 4). Caffeine contents from black tea (63.2 mg/L), coffee (60.2 mg/L) and instant coffee (135.0 mg/L) with PSW at 24.5±0.2°C were not significantly different (P >0.05) from the contents extracted at 95±2°C with NSW which indicates the importance of PSW effect on extractability even in the absence of high temperature.

(1)



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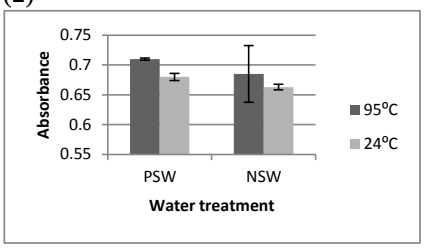
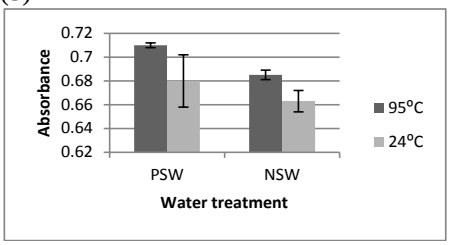


Figure 1: Comparison of caffeine in black tea extracts with PSW and NSW
Figure 2: Comparison of caffeine in instant coffee extracts with PSW and NSW

(3)



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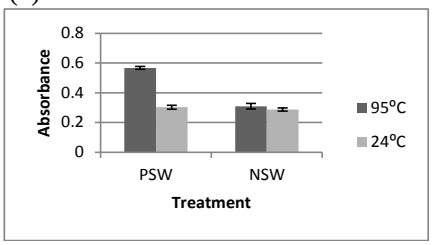

Figure 3: Comparison of caffeine in green tea extracts with PSW and NSW
Figure 4: Comparison of caffeine in coffee extracts with PSW and NSW

Extraction of curcumin is increased with the increasing temperature. Physically structured water has an effect on extraction of curcumin compared to NSW (P <0.05) where PSW has increased (P <0.05) the extractability of curcumin at 95±2°C by 430% and at 24.5±0.2°C by 282% (Figure 5). The most significant extraction was reported in the PSW which had the heat treatment (95°C).

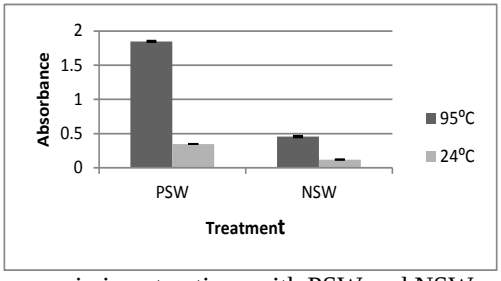

Figure 5: Comparison of curcumin in extractions with PSW and NSW

Physically structured water showed a significant difference in emulsifying and emulsion stability compared to NSW ($p<0.05$) in virgin coconut oil, sunflower oil and olive oil. Turbidity of VCO was higher ($P <0.05$) by 1.1-, 1.2-, 1.2-, 1.2-, 1.2- and 1.5-fold on days 1, 2, 3, 4, 5 and 6 respectively when treated with PSW than with NSW. The turbidity of sunflower oil was significantly higher by 1.3-, 1.2-, 1.3-, 1.3-,1.4- and 1.4- fold on days 1,2,3,4,5, and 6 respectively and 1.2-,1.2-,1.2-,1.2-,1.2-,and 1.2- fold for olive oil respectively when treated with PSW than with NSW.

Conclusions

Physically structured water had higher value for pH compared to NSW ($P< 0.05$) and lower value for ORP. The Negative ORP value further reduces with the passing cycles. The reduced NMR frequency value had shown the reduced cluster size of water molecules in PSW. The caffeine concentration in the tea and coffee samples was influenced by the water treatment and the temperature of extraction. It was observed that thee increase of caffeine concentration directly proportional to the temperature of extraction. Physically structured water showed positive effect on extraction of caffeine in black tea, green tea and coffee ($P< 0.05$), but extraction of caffeine from instant coffee did not show any significant difference ($P> 0.05$). The most significant extraction was reported in the PSW which had the heat treatment (95°C) for all the tea and coffee samples. Extraction of curcumin is increased with the increasing temperature. Physically structured water has an effect on extraction of curcumin compared to NSW ($P <0.05$). The most significant extraction was reported in the PSW which had the heat treatment (95°C). The change in the stability of oil/water emulsion with water treatment was investigated in which showed an effect in emulsification and emulsion stability with PSW compared to NSW ($P< 0.05$).

The application of PSW in the food industry is still in the initial exploration phase. Industry seeks for the extraction of beneficial constituents such as caffeine and curcumin by water rather than using solvents and also there is a demand for the invention of novel techniques to achieve stable emulsions. Physically structured water facilitates in resulting stable emulsions even without emulsifiers for a particular period of time. Further studies need to be carried out to incorporate this phenomenon in to a food, on the area of extraction of other beneficial components from food material, *etc*.

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DEVELOPMENT OF HOT SMOKED FISH PRODUCT BY HERRINGS (*Amblygaster sirm*)

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Summary

Smoking is a historically important method of preparing food because of its preservative qualities. Herrings (*Amblygaster sirum*) considered as an fatty fish which has a great potential to develop novel fish products in all over the world due to its higher content of omega-3 fatty acids and vitamin D. The objective of this research was to identify the most suitable storage condition and to select the best packaging method for prolong the shelf life of hot smoked fish processed in our country. The study was carried out using three packaging methods (none, normal - polypropylene with 100 µm and vacuumed) and different storage conditions (ambient and 4±1°C). Proximate composition of raw and smoked herrings was determined and changes in the quality of smoked fish were evaluated daily, through a systematic study of pysico-chemical, microbiological and sensory analyses during 30 days of storage. For this purpose physico-chemical indexes such as, total volatile basic nitrogen (TVB-N), pH, moisture content, water activity, peroxide value and aerobic plate count as a microbiological index and sensory analyses were carried out daily during storage period. It was found that vacuum-packaging makes it possible to prolong the shelf life of smoked fish, kept in cold stores (4±1^{o}C). The vacuumed packaged, smoked samples stored at ambient and 4±1°C temperatures, spoiled on 6th and the 14th days, respectively. With the hope of extending the shelf life of hot smoked herrings, they were treated with 1.25% sodium metabisulphite as an antioxidant and antimicrobial agent, prior to smoking and were evaluated as previously within 04 days interval for 02 months. According to the analyses, the smoked samples with vacuum packaged had shelf-life for 18 days at ambient temperatures and 06 weeks for 4±1°C conditions. Sodium metabisulphite proved to be more efficient in controlling microbial quality and extending shelf life of hot smoked herrings. Under both situations there were significant ($p<0.05$) differences between the initial and final values of the proximate and chemical constituents of both storage conditions.

Keywords: Hot smokeing; Vacuum-packing; Refrigerated storage; Shelf-life

Introduction

The preservation of fish has been an integral part of every seafaring culture. Smoking is a historically important method of preparing food because of its preservative qualities

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(Doe *et al.*, 1998). There are two general methods of smoking fish, namely hot-smoking and cold-smoking using the traditional method or the mechanical method. Any fish species with high in fat are recommended because they absorb smoke faster and have better texture. Certain compounds given off by burning wood have a preservative or antimicrobial effect on the food, and add flavour (Pigott and Tuckker, 1990). In this study the objective was to develop a smoked fish product using Herrings for the Sri Lankan market, by determining the most suitable packaging material, sensory and microbial quality and to increase the shelf life of the product using preservatives.

Methodology

Fresh Herrings were washed with cold water and then they were headed, gutted and splitted. Then they were dipped in 25% strength of brine solution for 15 min and rinsed again. Prior to smoking, the fish was pre-dried under 20-26°C and about 65% RH condition. To determine the preservative effect on smoked fish, after salting process, the samples were dipped in 1%-5% sodium metabisulphite solutions to select the best treatment. A 1.25% sodium metabisulphite solution (12.5 kg/L) was selected and dipped for 1 min and then rinsed slightly. Prepared samples were placed on a perforated tray, and they were transferred to cooking chamber where hot smoking was done using cinnamon wood under 70-80°C for 3 ½ hours. They were allowed to cool to room temperature, before packaging them. Then they were packaged in three ways (non-packaged, polypropylene with 400 gauge packaged and vacuum packaged) and kept in different storage conditions (ambient and refrigerated).

Moisture content was determined by AOAC 985.14 oven drying method (AOAC, 1995). Results were expressed as g water/100 g muscle. Ash was determined by the AOAC (1980) method 7.009. Lipid content was determined using AOAC (2002) method 960.39 and protein content was determined by AOAC (1980) method 2.507. Mohr method was used to determine salt content (NaCl) in fish muscle as described in Kerskin (1973). The method of Lucke and Geidel was used to determine TVB-N content as described by Inal (1992) and peroxide values of oils were measured by titration of liberated iodine with standardized sodium thiosulphate solution according to the AOAC official method 965.33 (AOAC 1990).

Thirty untrained panelists were selected from the NARA. Panelists were given six samples of smoked fish which were stored under ambient and refrigerated conditions and preference was tested using 09 points Hedonic scale.

Data was analyzed using MINITAB software, version 11. Friedman test was used to analyze the data and the statistical significance was defined as $p<0.05$.

Results and Discussion

The differences in moisture, protein, fat, ash, pH, TVB-N and salt content between fresh and smoked herrings were significant ($P < 0.05$). Moreover, samples stored under ambient temperature showed a statistically significant ($P < 0.05$) increment and the samples stored under refrigerated temperature were shown non-significant ($P > 0.05$) increment. All the non-packaged samples stored under ambient and refrigerated temperature were shown statistically significant ($P < 0.05$) decline and the pH

increment of non-packaged and polypropylene packaged and stored under both storage conditions were significant ($P < 0.05$). Under ambient temperature, non-packaged and polypropylene packaged samples were shown significant ($p < 0.05$) increment and the decline in the non-packaged sample stored under refrigerated temperature was significant ($P < 0.05$). Under ambient temperature, all the three samples were shown significant ($p < 0.05$) increment and the samples stored under refrigerated temperature were shown non-significant ($P > 0.05$) increment. Under ambient temperature all the three samples were shown significant ($p < 0.05$) increment and the samples stored under refrigerated temperature were shown non-significant ($P > 0.05$) increment. Smoked samples at zero days were received higher scores (9), therefore, statistically there was no difference.

At the 2nd day, the mean score value of non-packaged samples were very low and therefore, there were significant differences among ($P<0.05$) the three mean values. Further, it was reached to an unacceptable range.

Table 1: Proximate Composition (w/w %) of Raw and Smoked Herrings

Parameter	**Raw fish**	**Smoked fish**
Moisture	79.40 ± 0.298 [a]	60.52(%) ± 0.04 [b]
Protein	18.67 ± 0.01 [a]	24.883 (%) ± 0.045 [b]
Fat	10.317 ± 0.808 [a]	16.580 (%) ± 0.348 [b]
Ash	01.553 ± 0.090 [a]	03.953 (%) ± 0.07 [b]
Salt	0.973 ± 0.159 [a]	03.75 (%) ± 0.243 [b]
Water Activity (a_w)	0.953 ± 0.005 [a]	0.936 ± 0.0005 [a]
pH	05.38 ± 0.02 [a]	05.70 ± 0.01 [b]
TVB-N (mg/100g)	19.39 ± 0.015 [a]	20.813 ± 0.015 [b]
Peroxide Value (ml/kg)	17.95 ± 0.002 [a]	18.3 ± 0.012 [a]
TPC (cfu/g)	1*103 [a]	6*102 [a]

Results are mean values of three replicates ±SD, a-b - Values in the same line followed by different letter are significantly different ($p <0.05$)

According to the sensory analysis data on the 7th day, polythene packaged samples were reached to an unacceptable level and there was a significant difference ($P<0.05$) between two mean values. Still on the 13th day of storage vacuum packaged samples were not reached to the rejection level. On the 2nd day, the mean value of the non-packaged refrigerated sample was very low and there was a significant difference between the samples ($P<0.05$). On the 10th day when the two smoked products compared, there was a significant ($P<0.05$) difference between two mean values. It was clear that the sensory scores of vacuum packaged samples stored under refrigerator were reduced with time very slowly. Throughout this period it was not reached to an unacceptable range. On the 20th day of storage time, still it was at acceptable level and statistically there was no significant difference ($P>0.05$) between the final and the stored product.

Table 2: Proximate composition (w/w %) of raw and smoked herrings with preservatives (sodium metabisulphite)

Parameter	Raw fish	Smoked fish
Moisture	73.40 ± 0.298[a]	60.52(%) ± 0.04 [b]
Protein	18.30 ± 0.01 [a]	24.883 (%) ± 0.045 [b]
Fat	9.63 ± 0.808 [a]	16.580 (%) ± 0.348[b]
Ash	01.452 ± 0.090 [a]	03.953 (%) ± 0.07 [b]
Salt	0.865 ± 0.159[a]	03.75 (%) ± 0.243 [b]
pH	5.83 ± 0.02 [a]	05.70 ± 0.01 [a]
TVB-N (mg/100g)	18.56 ± 1.28 [a]	19.813 ± 0.711 [a]
TBC (cfu/g)	885 [a]	6*10[2] [a]

Results are mean values of three replicates ±SD

a-b - Values in the same line followed by different letter are significantly different ($p < 0.05$)

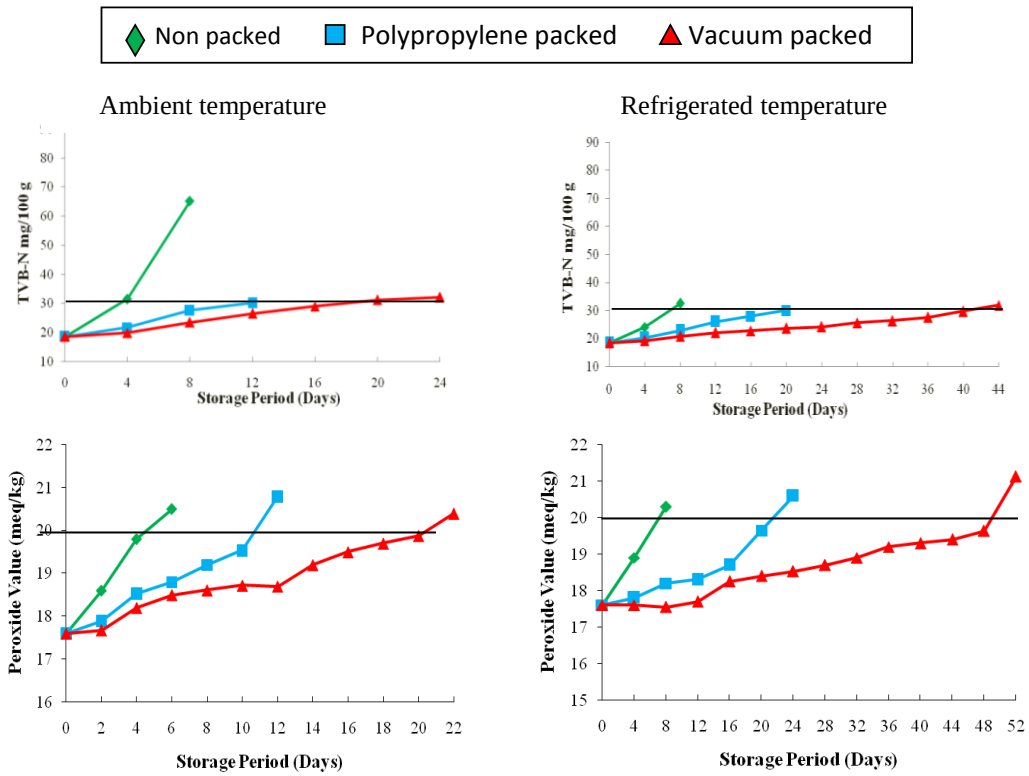

Figure 1. Changes in TVB-N and peroxide value during storage period at ambient and refrigerated temperatures with Preservatives (sodium metabisulphite)

According to the sensory analysis results, no significant difference ($P>0.05$) among three mean values at the storage of 0 days could be observed. But with the time significant difference ($P<0.05$) could be observed among all the sample means. Only up to four days of storage, a non-significant difference ($P>0.05$) was observed between ambient and refrigerated temperature stored non packaged samples. But with the time all the days, all these samples showed a significant difference ($P<0.05$). Under

refrigerated conditions, vacuum packaged samples were remained as the final product by maintaining higher mean values of preference.
Under this study only herrings were used for analysis. The potential of using other fish species should be evaluated. To study how the packaging material affect the preservation, different types of packaging materials with different gauge sizes should be used. Furthermore the effects of brines with different concentrations and different smoke sources instead of cinnamon wood dust should be evaluated.
Usage of vacuum packaging to preserve smoked fish is very effective and modified atmosphere packaging ay also be possible to use for the same purpose, therefore, this should be studied further. Efficiency of other smoking kilns and methods, usage of natural smoking condensates should also be evaluated. Since this is a preliminary study on smoking of fish, this developed product should be compared with the imported smoked fish in order to find out whether this is on in par with imported stuff. Moisture content should be maintained less than 10% in stored smoked fish to reduce the growth of bacteria and moulds. The salt concentration could be raised to a level above 30% as this inhibits growth of insect larvae. For inhibiting some spoilage bacteria smoked fish can be oven-dried and refrigerated or frosted.

Conclusions

The best storage temperature for the smoked product is 4°C and the best packaging method is vacuum packaging which prolongs the shelf life of hot smoked herrings.
The vacuum packaged smoked samples with sodium metabisulphite had higher shelf-life than the vacuum packaged smoked samples without sodium metabisulphite stored at ambient and refrigerated temperatures. Sodium metabisulphite proved to be more efficient in controlling microbial quality and extending shelf life of hot smoked herrings. It is suitable to introduce to small scale fish product manufactures in Sri Lanka.

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FORMULATION OF A NOVEL HIGH ENERGY BREAKFAST BAR FOR ADULTS

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Summary

Previous studies on the breakfast patterns of adults including undergraduates and working crowds have revealed that breakfast is one of the main and most skipped meals due to their busy lifestyles. However, the population is willing to accept a ready-to-eat snack type bar for breakfast considering convenience. Thus the snack bar was formulated using rice flakes as the base ingredient and sweetened condensed milk (SCM), raisins, dates, cornflakes, preserved papaya, roasted green gram, butter, desiccated coconut, sugar, and semolina. A portion of 80 g of the formulated bar has an energy value which fulfills 80 % of breakfast energy requirement of an average woman with sedentary life and 120 g of the bar fulfills the same for an average man with a moderately active life. An 80 g bar costs about Rs. 56.00. Nutritional information per 100 g of a bar was found to contain, 471 kcal of energy, 78 g of carbohydrates, 1.56 g of proteins, 1.78 g of fiber and 6.71 g of fat. Hence, the formulated bar can be recommended as a breakfast snack for adults with a busy lifestyle.

Keywords: Breakfast; Snack bar; Ready-to-eat food; Rice-flakes

Introduction

Breakfast may be defined as the first meal of the day, eaten before or at the start of daily activities, within 2 h of waking, typically no later than 10:00 a.m. and of a calorie level between 20 % and 35 % of total daily energy needs (Giovannini *et al*., 2008). After an overnight fasting period during sleep, the glycogen stores in the body may be used up, therefore a healthy, nutritious breakfast is vital to replace the used glycogen (Thorleifsdottir *et al*., 2002). Surveys show that breakfast is often being skipped by many people. People do not have enough time to devote for meal planning and preparation. They have switched to fast foods and snacks. But most of these types of food available are not very nutritious and may increase the risk of diseases. However, if a nutritious snack food is introduced then the barrier between skipping breakfast and consuming unhealthy snacks can be corrected. There is an increased demand for ready to eat meals. Also with the threat of increasing diet related diseases people tend to seek for nutritious and healthy foods. Therefore, a convenient ready to eat healthy meal is a huge necessity and an ideal product for the modern market. Hence the present study was conducted to develop a ready-to-eat snack bar as a breakfast meal.

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Methodology

The development of the ready to eat snack bar was carried out in few phases. Firstly the ingredients were selected to represent a balanced diet. The selected ingredients were then calculated and formulated under different weights in order to meet the required energy level. Trial sessions were performed with golden syrup and SCM separately to select the best bound product. The final selected ingredients and amounts of ingredients were 150 g of rice flakes, 200 g of SCM, 50 g of raisins 40 g of dates, 20 g of cornflakes, 20 g of preserved papaya, 20 g of green gram, 20 g of butter, 20 g of desiccated coconut, 25 g of sugar and 20 g of semolina. The product was formulated by heating butter, sugar and SCM, then the selected ingredients were mixed with heated mixture and the mixture was put on a baking tray and it was baked for 20-30 min at 150°C. The above ingredients were mixed into three different samples by changing the soaking levels of rice flakes: as without soaked, 50 % soaked and 100 % soaked. These three samples were then subjected to sensory evaluation by a panel of 30 untrained panelists. Thereby the most preferred product was selected using statistical analysis by using minitab-17 software. Kruskalwallis test was used to analyze the data. The selected product was then subjected to analysis of physicochemical properties such as moisture using oven dry method, ash using dry ash method, fat using soxhlet method, protein using Kjeldhal mehod and fibre using chemical digestion method and then calculated the amount of carbohydrates using the reduction method. To assess the microbial quality, a total plate count was performed using nutrient agar medium and spread plate method. Shelf life was tested for two weeks in refrigerated conditions.

Results and Discussion

The selected ingredients and amounts of ingredients for the ready to eat snack bar was 150 g of rice flakes, 200 g of SCM, 50 g of raisins 40 g of dates, 20 g of cornflakes, 20 g of preserved papaya, 20 g of green gram, 20 g of butter, 20 g of desiccated coconut, 25 g of sugar and 20 g of semolina. The sensory evaluation of the snack bar selected the bar with un-soaked rice flakes as the best product. Among the sensory attributes tested it was found that there were no significant difference among the samples for appearance ($p=0.239$) and texture ($p=0.333$) whereas there were significant difference among samples for fragmentation ($p=0.015$), flavor ($p= 0.003$), grittiness ($p= 0.000$), after taste ($p=0.021$) and overall acceptability ($p= 0.001$) at 5% level of significance. The high energy snack bar of 80 g contains 377 kcal of energy. The nutrient profile of a 100 g portion contained, energy: 471 kcal, carbohydrates: 78 g, proteins: 1.56±0.05 g, fiber: 1.78 g, fat: 6.71±0.03 g and moisture: 12.66±0.21. Bacterial Count was 5.59×10^4 CFU/mL.

The formulated high energy ready to eat snack bar of 80 g portion size fulfills about 80% of energy required for breakfast of an average sedentary woman and about 62% of breakfast energy requirement of an average sedentary man. For a moderately active man 120 g of the bar along with a cup of tea can fulfill the breakfast requirement. Therefore bars can be formed as large size 80 g and small size 40 g. However the protein and fiber content of the bar is not at significant levels. According to our previous study on breakfast patterns of undergraduates and working crowd, it was

evident that most women tend to skip breakfast, therefore this product targets women with a busy morning. However majority of them consume a cup of tea or milk in the morning. Therefore with that, the breakfast energy requirement of an average sedentary woman can be fulfilled by this snack bar. It cannot only be used as a breakfast snack but can also be used at other times as a high energy snack bar especially for active kids. A portion of 80 g was selected considering the portion size, energy value and cost. This cost around Rs. 56.00. The shelf life of the product was two weeks in refrigerated conditions without any packaging materials.

Conclusion

The formulated high energy snack bar consists of 377 kcal of energy which compensates to 20% of daily required energy of a sedentary female of average weight 50 kg. The best formulation of high energy snack bar consisted of 78.11% Carbohydrates, 1.56% Crude protein, 6.71% Crude fat, 12.66% Moisture, 1.78% crude fiber and 0.96% ash. The high energy snack bar with a cup of milk will provide a complete breakfast for sedentary females. Average weight of a snack bar is 80 g which cost Rs. 56.50. As per recommendations the energy value should be determined using an experimental method and the shelf life of the product should be analyzed by periodic microbial analyses with a good packaging method under ambient temperatures as well. Selection of an appropriate packaging material should be performed, and to study on additional ingredients that can increase the protein and fiber content of the bar further studies need to be carried out.

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COMPARATIVE STUDY OF INCORPERATION OF MORINGA (*Moringa oleifera*) LEAF POWDER INTO SOY MILK AS A SUBSTITUTE FOR COW'S MILK

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Summary

This study was carried out to develop a nondairy beverage using *Moringa olifera* leaf powder and soy milk as a substitute for cow's milk. The iron, antioxidant and crude fiber contents were significantly ($P<0.05$) higher in soy milk based beverage than those in cow milk based beverage whereas crude fat and total solid contents were significantly ($P<0.05$) lower in soy milk based beverage. In contrast, there was no significant ($P<0.05$) difference in crude protein and calcium content between two beverages. The pH and titratable acidity showed continuous reduction with the storage time, whereas the total plat count exceeded maximum permissible level set by SLSI on 6th day of storage (50 CFU/mL). This study revealed that the moringa fortified soy milk beverage is a good alternative to cow's milk as a nutritious supplement.

Keywords: *Moringa olifera*; Soy milk; Non-dairy beverage; Cow's milk

Introduction

Development of new non-dairy products for those who do not prefer dairy products and are incapable of consuming (lactose intolerant, allergy, heart diseases) dairy products has become essential. The nutrition profile of soy milk proves it as one of the best alternatives for the cow milk. Besides, Moringa (*Moringa oleifera*) is widely cultivated in Sri Lanka and it has been identified as the most nutrient-rich plant in the world (Tahir et al., 2010). Moringa leaves have both nutritional and therapeutic values. More importantly, the micro-nutrient content is high in moringa leaves.

Multinutrient-fortified foods and beverages are worthwhile in the prevention of micronutrient deficiencies, especially in developing countries. Soy beverages fortified with calcium, vitamins A and D which are similar to the nutrient profile of cow's milk are clearly identified as a milk alternatives in the 2010 Dietary Guidelines for Americans policy document.

Combination of soybean and *Moringa oleifera* is considered as a cheap and sustainable product approach in fighting against micro nutrient malnutrition in developing countries (Rweyemamu, 2006). Moreover, soy beverages provide more iron, magnesium, copper

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and manganese than cow's milk (Hajirostamloo, 2009). Hence, moringa leaf powder fortified soy milk beverage is important as an alternative for cow's milk. Therefore, the present study was conducted to develop a dairy milk substitute by soy milk fortified with Moringa leaf powder.

Methodology

Firstly fresh leaves were washed from distilled water and blanched and allowed for air drying under shade for a day. It was then dried at 55°C, ground using stainless steel grinder and stored in room temperature.

Non fumigated soy beans (variety PB1) were brought from local soybean farmer Thalawa, Sri Lanka and soy milk was extracted using three methods including traditional Chinese method, Illinois method and method developed by Bollegala (2011). Sensory evaluations were carried out to determine the best method of soy milk extraction, the maximum level of moringa leaf powder (MOL) that could be incorporated (3%, 5% and 7%) and the level of sugar to be incorporated (8%, 9% and 10%). According to the results of the sensory evaluations, the moringa incorporated soy milk beverage was prepared.

The proximate composition was determined using AOAC, 2005 procedures. Calcium and iron concentrations were measured using atomic absorption flame emission spectrophotometer. The antioxidant capacities of two beverages were analyzed by DPPH radical scavenging activity and FRAP methods. Further, total phenolic content was also measured.

The pH, titratable acidity and total plate count, yeast and mould count and total coliform count of the soy milk based beverage were assessed daily during 7 days of storage at 4°C.

Responses on the products' sensory attributes were analyzed using Freidman test using SPSS-32 software. All the parametric data were analyzed by using two independent sample t -test and using SPSS-32 software.

Results and Discussion

The highest consumer preference for colour, aroma, flavor and overall acceptability were observed in soy milk extracted from new method developed by Bollegala (2011). Further, 5% Moringa leaf powder incorporation showed highest median values for flavor and overall acceptability whereas, the highest consumer preference for sweetness was observed in 10% sugar incorporation. Therefore, 5% level of MOL and 10% sugar level were selected for the preparation of MOL incorporated soy milk based beverage.

Table 1 shows the proximate composition, total solids, calcium and iron concentrations of soy milk and cow's milk based beverages. Soy milk based beverage showed significantly ($P<0.05$) higher iron and crude fiber contents and significantly ($P<0.05$) lower crude fat and total solid content than cow's milk based beverage. There were no significant differences in calcium and crude protein contents between two beverages.

Table 1: Proximate composition, total solids, calcium and iron concentrations of soy milk and cow milk based beverages (g/dried 100 g).

Beverage	Moisture	Crude Protein	Crude Fiber	Total Solids	Iron	Calcium
Soy milk based beverage	81.44±0.15[a]	3.35 ±0.06[a]	0.86 ±0.25[a]	10.20 ±0.16[a]	0.25 ±0.02[a]	0.97 ±0.06[a]
Cow milk based beverage	87.73±0.55[b]	3.25 ±0.06[a]	0.10±0.12[b]	15.30 ±0.11[b]	0.01±0.04[b]	1.03±0.02[a]

Values are expressed as means ± SD. Mean values within a column with different superscript letters were significantly different ($p<0.05$).

Table 2: Total phenolic content (TPC) and antioxidant activity from DPPH and FRAP assays of soy milk and cow milk based beverages.

Beverage(100mL)	TPC (mg of gallic acid equivalents/100 g)	DPPH (mmol/L)	FRAP (mmol/L)
Soy milk based beverage	126.79[a] ±7.25	2.63± 0.22[a]	1.08±0.14[a]
Cow milk based beverage	60.85[b] ±6.61	1.89±0.12[b]	0.63±0.57[b]

Values are expressed as means ± SD. Mean values within a column with different superscript letters were significantly different ($p<0.05$).

According to results obtained from total phenolic content determination, ferric reducing antioxidant power (FRAP) assay, α-diphenyl-β-picrylhydrazyl (DPPH) free radical scavenging method, soy milk based beverage had a significantly ($p<0.05$) higher antioxidant activity than that of cow milk based beverage (Table 2).

The pH and titratable acidity showed a continuous reduction with the storage time. Total plate count exceeded maximum permissible level set by Sri Lanka Standards Institute on the 6th day of storage (50 CFU/mL).

The soy milk and moringa may contribute to the higher iron level in soy milk based beverages since moringa leaves contain high amount of iron content, twenty five times the iron level of spinach (Tahir *et al*., 2010). Moreover, this result is in agreement with Hajirostamloo, 2009 that soy beverages provide more iron than cow's milk. Soy also consists of higher levels of essential fatty acids, soluble fiber and photo-chemicals (Rweyemamu, 2006) and moringa is a rich source of antioxidant compounds (Leone *et al*., 2015). According to the results published by others and the results obtained in this study, soy milk based beverage shows increased fiber and antioxidant activity. Thus, moringa leaf powder incorporated soy milk beverage had a healthier nutritional profile compared to cow's milk beverage

Titratable acidity and pH of the beverage is a measure of the sourness of the product and it also reflects on the stability of the product regarding deterioration during storage.

Though there was a continuous reduction of pH and titratable acidity, slight fluctuations were observed and it could be due to some buffering effects of the beverage proteins.

Conclusion

This study revealed that moringa leaf powder incorporated soy milk beverage had a healthier nutritional profile compared to cow's milk beverage. Thus, moringa incorporated soy milk beverage is a good alternative to cow's milk as a nutritious supplement.

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DEVELOPMENT OF A METHOD TO REMOVE BEANY FLAVOUR IN READY-TO-SERVE SOYA DRINK

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Summary

Soya bean (*Glycine max*) is nutritionally superior to most legumes. It can be made palatable, easily digestible and nutritious by processing into various products, the most popular of which is soya based beverage. Despite the many advantages of bean, the use of soyabean as a food material has been limited because of "beany flavour" or "grassy flavour" generated during processing. The beany flavour of pulses is the result of the action of an enzyme called lipoxygenase. The enzyme is involved in the oxidation of lipids or fat, which result in the off-flavor. Alternations in processing methods namely sprouting, normal soaking, alkaline soaking, pressure cooking and microwave cooking were used to eliminate the lipoxygenase enzyme activity. Effectiveness of each method was determined using thiobarbituric acid (TBA) test and sensory evaluation tests. Variation of nutritional components (crude protein and fat) was concerned within different alterations of processing. Results were analysed using SAS and Minitab statistical data analysis programmes. Thiobarbituric acid test and sensory evaluation tests confirmed that pressure cooking of soya beans at 120°C for 2 min prior to grinding, has a significant ($P \leq 0.05$) effect on reducing beany flavour. Soaking soya bean in 0.25% (w/v) sodium bicarbonate solution (pH = 8.3) for 6 h at room temperature gave significant ($P \leq 0.05$) results on better mouth feel. Combination of processing steps namely, pressure cooking and alkaline soaking was the best method to produce soya drink free from beany flavour while keeping the nutritional quality.

Keywords: Soya bean (*Glycine max*); Beany flavour; Lipoxygenase; Pressure cooking; Sensory evaluation; Thiobarbituric acid (TBA)

Introduction

The beany flavour of soya bean is the result of the action of an enzyme called lipoxygenase. As the name implies, the enzyme is involved in the oxidation of lipids or fat, which result in the beany flavour. There are three forms of the enzyme commonly referred to as lipoxygenase 1, 2 and 3. The reaction takes place at the double bonds of unsaturated fatty acids and can be accelerated by singlet oxygen, free radicals, light and enzymes containing a transition metal prosthetic group such as lipoxygenase. As many researches shown, it is difficult to completely eliminate once it is activated. This study was conducted to inactivate lipoxygenase enzymes altering the processing conditions of soy bean.

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Methodology

Five experiments were conducted using different processing steps. The different processing steps were one day sprouting (T1), three days sprouting (T2), alkaline soaking (T3), normal soaking (T4), microwave cooking (T5) and pressure cooking (T6). Whole dry soya bean (62.5 g) was maintained constant as the initial weight for every treatment.

One day sprouting, three days sprouting, alkaline soaking, normal soaking, microwave cooking and pressure cooking steps were used separately. At the end of each treatment, beans were blended with of warm water (50-60^{o}C) using electric blender at high speed. Macerated slurry was squeezed out using two layers of cheese cloth. Extracted liquid was measured, filled into glass bottles and kept in ambient conditions or stored under refrigerated conditions (4^{o}C). Thiobarbituric acid test was performed after 24 h of processing, for the sample which was stored under the ambient conditions. A ranking test was conducted using 30 untrained panelists and they were instructed to rank the samples (stored under refrigerated conditions) according to the intensity of beany flavor.

Every bean sample was washed and soaked in fresh water with soya bean to water ratio of 1:5 (w/v) at room temperature for 6 h and drained. Soaked beans were put into boiling water pan (bean: water ratio was 1:10). Each bean sample was blanched for several time periods as 5, 10, 15, 20 and 25 min. At the end of each blanching treatment, blanched water was discarded and beans were washed with water at 50-60^{o}C. The further processing steps (grinding, filtering, bottling and storing) were same as to the experiment conducted to choose the best processing method. Extracted juice sample which was stored under ambient conditions was subjected to TBARS test after 24 hours of extraction. A ranking sensory test was conducted using refrigerated samples.
Processing steps which were practiced in the experiment to choose the best processing method, were repeated here but at the end of each treatment soya beans were blanched in boiling water for 20 min. Afterwards further processing steps were carried out as mentioned those in experiment conducted to choose the best processing method. Thiobarbituric acid test and sensory test were also practiced as noted under experiment conducted to choose the best processing method. Kjeldhal method was followed to test the available protein content in each preparation and the result was analyzed using SAS statistical package.

Pressure cooking and microwave cooking of soya bean were done according to the procedure mentioned under first experiment conducted to choose the best processing method. At the end of each cooking method, the beans were put into a solution which contained 0.25% (w/v) of sodium bicarbonate. Pre-cooked beans were soaked in that alkaline solution at room temperature for 6 h. Another two samples (62.5 g each) of soya bean were blanched in boiling water for 20 min after completion of pre-cooking and alkaline soaking steps. Another treatment was done using only alkaline soaking and blanching steps. Extraction, bottling and storing conditions were same as to those of experiment conducted to choose the best processing method. Thiobarbituric acid test and sensory test were performed using the extracted liquid.

Two drink samples were prepared to determine the consumer preference using selected methods in accordance to the previous experiments and those two samples were compared with an imported soya drink product.
Cleaned whole soya bean (62.5 g) was pressure cooked and alkaline soaked. Then the sample was soaked for 6 h in 0.25% (w/v) sodium bicarbonate solution at room temperature. Pre-cooked and alkaline soaked beans were ground and extracted as explained early. Extraction (1000 mL) was taken into a pan and other ingredients (50 g cane sugar, 2 g salt, 0.6 g potassium sorbate, 0.15 g carrageenan) were added and mixed well. Then the mixture was heated at 63 °C for 30 min. Glass bottles were sterilized and hot filled followed by capping. Then they were cooled down rapidly to 7 °C or below in an ice-water and pasteurized drink was then stored in a refrigerator (4 °C).
Crude protein content was determined following Kjeldhal method (A.O.A.C., 2000). The hand refractometer (Aatago at 0-30%) was used to detect the total soluble solids in the plain soya drink sample. The pH of plain soya drink sample was measured using electronic pH meter (Model IM-40S, TOA Electronics Ltd., Japan). Laboratory viscometer (Tokimec, Model BL, Tokimec inc., Tokyo, Japan) was used to measure the viscosity of the product. Plain soya drink sample was subjected to aerobic plate count test after seven days of preparation. The test was performed in accordance to the Sri Lanka Standard Microbiological Test Methods (first revision) 516: Part 1: 1991.

Results and Discussion

According to the estimated median values, processing method, T3 showed highest rank value while others were indicating different lower rank values.
Even though in TBA test, T5 and T6 processing methods have been shown low malonaldehyde content (low TBA value), consumer preference was not much satisfactory on the drink samples resulted through those methods. The reason for comparatively low TBA values for T5 and T6 methods may be due to the use of heat treatments before grinding beans. At high temperature producing by microwave cooking and pressure cooking might inactivated the lipoxygenase enzymes in soya beans. According to the panelists' comments, even though there was lower beany flavour in drink samples produced through T5 and T6 methods there was an objectionable cooked flavour with those samples. That cooked flavour has been affected for the poor consumer acceptability.
Although there was no significant difference among T1, T2, T3 and T4 processing methods lesser mean TBA value has been obtained by method T3. At alkaline medium (pH = 8.3) the activity of lipoxygenase may be retarded. As well as sodium ions in sodium bicarbonate solution have an impact on eliminating isoflavone effect of soya bean which gives undesirable flavour. Higher TBA values were related to processing methods which involved sprouting and normal soaking steps because of the higher interaction with water thus increased lipoxygenase activity.

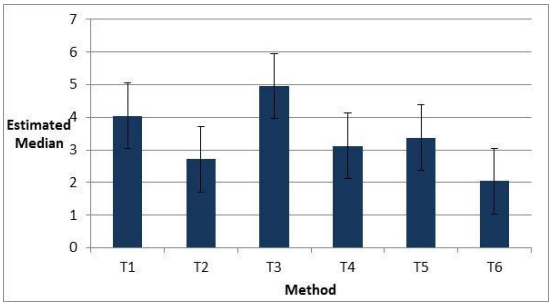

Figure 1. Sensory test result for the beany flavour of soya milk in the first experiment
Untrained panelists (30) were given six samples from different treatments as, 01 day sprouting (T1), 03 days sprouting (T2), alkaline soaking (T3), normal soaking (T4), microwave cooking (T5) and pressure cooking (T6). Processing steps were as described in experiment 01 under the materials and methods.

With the increase of blanching time lipoxygenase inactivation may also be increased. At 5, 10 and 15 min time durations enzyme inactivation was significant ($P \leq 0.05$). However, after 15 min, there was no significant difference between TBA values. Therefore we can assume that at 15 min blanching time almost all the lipoxygenase were destroyed. The least beany flavour was observed for the drink sample prepared through method T4. Long term blanching may also be affected for the producing other undesirable flavours and therefore the panelist was unable to distinguish the intensity of beany flavour clearly. It may be the reason for less consumer acceptability for the drink sample prepared following method T5. A 20 min blanching duration of soya bean in boiling water was selected for further experiments.
In experiment conducted to choose the best processing method, grinding of soya bean just after pressure cooking did not give a satisfactory sensory result. However, alkali soaking of soya bean after the pressure cooking step exhibited a significantly lower beany flavor. There was no significant ($P \leq 0.05$) difference between processing method combinations which involved blanching step and the combination which does not involve the blanching step. It means at pressure cooking step, all most all the lipoxygenase enzymes were destroyed and when soaking the beans were absorbing more water. Then organoleptic properties of bean were well developed. Since there was no significant effect on using blanching step, it is useless to practice combination T3 and can be saved the time and energy. By adding sodium bicarbonate, it changed the optimum pH condition in which the lipoxygenase enzyme can be activated in higher level. As well as sodium ion depromote the activity of isoflavones in soya beans.
Pressure cooking is a moist heat method and microwave cooking is a dry heat method. Soya drink produced, using combination T2 and T4 were not acceptable. The reason may be the less palatable cooked flavor which was caused by the dry heating conditions of microwave cooking. Bean constituents are over cooked and may result toasty flavour during microwave cooking.

Conclusions

One day or three days sprouting has no effect on reducing beany flavour of soya drink. Soaking of soya bean in 0.25% (w/v) sodium bicarbonate solution at room temperature can result soya drink with less beany flavour and good mouth feel compared to normal

soaking method. Soaking and sprouting steps can induce the yield of soya milk extracted. Microwave cooking or pressure cooking can retard the lipoxygenase activity of soya bean yet result an objectionable cooked flavour. Blanching of soya bean for 20 min in boiling water can reduce the beany flavour of extracted soya drink. Use of pressure cooking (2 min at 120°C) as a pretreatment, and subsequent alkali soaking (0.25% sodium bicarbonate solution at room temperature) is the most effective processing method combination to produce soya drink free from beany flavour.
The variation of lipoxygenase activity with the variety and the maturity should also be taken into account. Further studies should be oriented towards the colour improvement of the final product. Chemical or physical bleaching process might be more effective for this purpose. Changing pH of final product with the time of storage should be controlled. An acidity regulator or a buffering reagent could be used to fulfill that requirement.

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EFFECT OF COOKING TIME AND COOKING TEMPERATURE ON ANTIOXIDANT ACTIVITY AND ANTIMICROBIAL ACTIVITY OF CINNAMON, GARLIC, GINGER AND TURMERIC

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Summary

Controlled thermal treatment of spices had shown positive effects on both antioxidant and antimicrobial activities, but substantially lost after prolonged heat treatment during cooking. The effect exerted from the heat during cooking of this study was statistically significant ($P<0.05$) in relation to exert both antioxidant and antimicrobial activities of selected spices. At a given time-temperature combination, malonaldehyde (MDA) concentration of selected spices were ranged as ginger<turmeric<garlic<cinnamon and generally increased with prolonged heat treatment exhibiting reduced antioxidant activity. Garlic had shown a very clear increment in antioxidant activity and antimicrobial activity with increased temperature, but substantially reduced with further increment. According to the diameter of inhibition zones (DIZ), all four selected spices showed inhibition against *Listeria monocytogenes*, which was smaller than *Escherichia coli* or *Salmonella typhi* for a selected spice-time-temperature treatment. The DIZ of a particular spice-time-temperature reached maximum after 12 h of incubation, which gradually reduced and finally disappeared after prolonged incubation. Antioxidant and antimicrobial activities of spices are affected both positively and negatively due to heat changes during processing. Therefore understanding the effect of processing conditions on these activities will provide sound estimation of actual capabilities of these spices.

Keywords: Antioxidant activity; Antimicrobial activity; Malanaldehyde; Inhibition zone

Introduction

Spices are well known for their numerous benefits including improving sensory attributes and also as preservatives, preventing oxidation, microbial spoilage and pathogenesis. Among other phytochemicals, phenolic compounds are recognized being for antioxidant and antimicrobial activity of spices. However, spices are generally consumed along with other staple or major foods which are processed prior to consumption. Processing conditions such as temperature and time can have either positive or negative effect on antioxidant and antimicrobial activity of active compounds in spices. The objective of this study was to identify the effect of cooking time and temperature on antioxidant and antimicrobial activity of cinnamon, garlic, ginger and turmeric. Four hypotheses were tested under this study to determine whether there is a significant effect of cooking time on antioxidant and antimicrobial activity of

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selected spices and whether there is a significant effect of cooking temperature on antioxidant and antimicrobial activity of selected spices.

Methodology

Dried cinnamon quills, fresh garlic, ginger and turmeric were purchased from a local market at Peradeniya. Above spices were cleaned, peeled, sliced, sun dried and ground to obtain spice powder (7-9 % moisture content). For each spice, nine time-temperature combinations with three temperatures (40, 70 and 98°C) and three time durations (10, 20 and 30 min) were provided. Heat treated samples were reacted with 10 mL of 0.01 % linoleic acid for 24 h at room temperature followed by 2- thiobarbituric acid reactive substances (TBARS) assay. The TBARS concentrations were calculated by mg MDA /kg of spice using standard curve (532 nm) obtained for 1,1,3,3 tetramethoxy propane. Agar disc diffusion assay was performed for 15 % (w/v) water extracts of heat treated spices with 75 µL per each disc. Bacterial inoculums (75 µL) corresponding to 2.0 McFarland standards of *Escherichia coli*, *Salmonella typhi* and *Listeria monocytogenes* were used per plate. Antimicrobial activity was determined in terms of DIZ. The DIZ in mm was taken after 12, 16, 20 and 24 h of incubation. Data were statistically analyzed in three factor factorial Completely Randomized Design (CRD) using SAS 9.1.3 Statistical Package.

Results and Discussion

MDA concentration of selected spices were statistically significant ($P<0.05$) which ranges in the order of ginger < turmeric < garlic < cinnamon for a given temperature (Figure 1). Therefore, the antioxidant activity of selected spices ranges as ginger > turmeric > garlic > cinnamon. Effect of temperature and time was significantly different ($P<0.05$) for all spices.

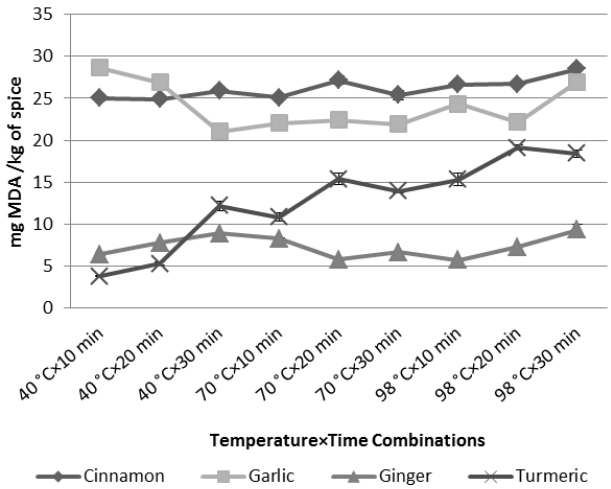

Figure1. MDA Concentrations of treatments with cinnamon, garlic, ginger and turmeric

For cinnamon and turmeric effects of all the temperatures and time durations selected were statistically significant. For garlic and ginger effect of 70°C temperature was significantly different with 40°C and 98°C, however 40°C and 98°C were not. Lowest MDA concentration was observed for 70°C, which increased with increased temperature.

Presence or absence of antimicrobial activity is summarized in Table 1 which shows susceptibility of *L. monocytogenes* is distinctively higher than that of *E. coli* or *S. typhi* where *L. monocytogenes* was susceptible for all four selected spices, while *E. coli* and *S. typhi* were susceptible only to garlic.

Table1. Antimicrobial activity of cinnamon, garlic, ginger and turmeric on *E. coli*, *S. typhi* and *L. monocytogenes*

Spice	*E. coli*	*S. typhi*	*L. monocytogenes*
Cinnamon	-	-	+
Garlic	V	V	+
Ginger	-	-	V
Turmeric	-	-	+

(+) Presence, (-) Absence and (V) Variable antimicrobial activity

Antimicrobial activities vary depending on spice, time-temperature combination and targeted microorganism.

Spices have shown increased antimicrobial activity with the increasing cooking temperature, but the activity was substantially disappeared with prolonged heat treatment. This effect could be clearly observed with activity of garlic over *E. coli* (Figure 2).

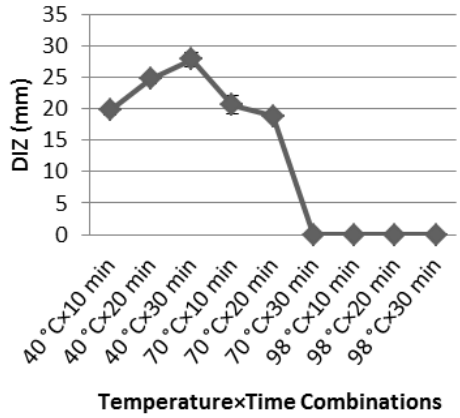

Figure 2. Antimicrobial activity of garlic over *E. coli* after 12 h of incubation

Effect of cooking time on antimicrobial activity was not significant ($p > 0.05$). However effects of spice, temperature and temperature-time combination were statistically significant ($p < 0.05$). Furthermore, for any of the selected spices cooked at 70 °C for

30 min and all the combinations cooked at 98°C, antimicrobial activity was not significantly ($p>0.05$) different.
Diameter of inhibitory zone resulted from a selected spice-time-temperature treatment was maximum after 12 h of incubation, which gradually reduced and finally disappeared after 24 h. This was observed for the antimicrobial activity of garlic over *E. coli*, *S. typhi* and *L. monocytogenes* and activities of cinnamon, ginger and turmeric over *L. monocytogenes*.

Processing conditions impart both negative and positive effects on antioxidant activity on spices by changing structural integrity of the plant material (Reis et al., 2013). Antioxidant compounds can be either transformed in to more active compounds or inactivated due to thermal processing (Horvathova *et al.*, 2007). It is generally known that functionality of phenolic substances can be substantially loss during thermal processing resulting decreased antioxidant and antimicrobial activities (Nwaichi *et al*., 2013). The loss can be varying due to the differences in cooking time *etc*. *E. coli* and *S. typhi* are Gram negative bacteria, comparatively resistant to spice extracts compared with *L. monocytogenes* which is a Gram positive bacteria. Different plant extracts showed varying degree of antimicrobial activities due to absence of outer membrane and periplasmic space in Gram-positives (Mukhtar *et al*., 2012), (Shan *et al*., 2007). Hydrophilic surface of outer membrane of Gram-negative bacteria acts as a barrier to the penetration of antibiotic molecules and associates with enzymes in the periplasmic space, capable of breaking down the molecules introduced from outside. Antibacterial substances can easily destroy bacterial cell wall and cytoplasmic membrane and result in a leakage of the cytoplasm, depletion of proton motive force, change fatty acid and phospholipid constituents, impair enzymatic mechanisms for energy production and metabolism etc. (Shan *et al*., 2007). Antimicrobial activity shown by garlic suggest that garlic has antimicrobial compounds acting upon all three selected microorganisms while cinnamon, ginger and turmeric are unable to exert such effects. It was proven that garlic show better activity in aqueous extracts while cinnamon and turmeric show better activity in organic extracts. The antimicrobial activity of aqueous extracts could be due to anionic components (thiocyanates, nitrates, chlorides and sulphates) in addition to many other compounds naturally present in spices (Mukhtar *et al*., 2012).
According to this study the activity of selected spices under experimental condition is optimum at 12 h of incubation with gradual decreasing followed by complete disappearance after 24 h of incubation time. It could be suggested that complete expense of active compounds allows new generation of microorganisms to grow successfully resulting gradual fading and complete disappearances of DIZs.

Conclusions

In concluding, thermal treatment has shown positive effects on both antioxidant and antimicrobial activities up to some extent, but substantially lost after prolonged heat treatment in which the effect is statistically significant ($P<0.05$) in relation to both antioxidant and antimicrobial activities.

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DEVELOPMENT OF HEALTH BENEFICIAL INSTANT NOODLES BY INCORPORATING EDIBLE FIBER FROM NATURAL FOOD SOURCES

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Summary

This study was conducted to develop an instant noodle having high dietary fiber content by using wheat flour as the base. Dietary fiber was obtained from "kohila" (*Lasia spinosa*) and banana blossom (*Musa* sp.) in flour form. The wheat flour in the traditional noodle formulation was replaced with 5, 10, 15, 20, 25, and 30% of each fiber flour separately. Flour products incorporated with 10 % kohila and 15 % banana blossom flour had higher acceptability scores in sensory analysis and were selected as suitable formulations. However, there were no significant ($p>0.05$) differences between 10 % of "kohila" fiber incorporated noodles and 15 % banana blossom flour incorporated noodles based on sensory parameters tested. Microbiological qualities of the samples were highly acceptable as total plate counts (TPC) were within the acceptable range. The results showed that instant noodles can be incorporated with 10% "kohila" and 15 % banana blossom flour to improve the fiber value without affecting the sensory properties.

Keywords: Instant noodle, Dietary fiber, Kohila, Banana blossom

Introduction

The importance of noodles in the Asian diet is significant. Currently, an average of 20-40% of the total wheat flour consumption in many countries occurs in the form of noodles. Parallal to this, the instant noodle market is rising rapidly in Asian countries because of the convenience, easy to cook and has a relatively long shelf-life. As there is an increasing awareness that health may be modified through diet, it has been a challenge for food scientists in finding more nutritious and healthy substitutes or alternatives to wheat flour for noodle products.

"kohila" (*Lasia spinosa*) is popular due to its fiber content and medical value and has been used in ayurvedic medicine to treat many diseases. The "kohila" rhizome is a rich source of dietary fiber with 40-75% of total dietary fiber on dry weight basis, (7.2-7.5% on fresh weight basis) constituting 35-60% and 4-18% of insoluble and soluble fiber respectively (Shefana and Ekanayake, 2009). Banana blossom is also an excellent source of crude fiber which is incorporated into the human diet and is highly valued for its fiber content. In Sri Lanka, rice flour and "kurakan" flour incorporated instant noodles are available in market other than the wheat flour noodles. Therefore, there is a

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potential exists to incorporate fiber flour with wheat flour to prepare fiber rich noodle having health promoting properties.

The aim of this study was to develop instant noodles incorporating "kohila" and banana bloosom fiber separately to improve the fiber content without affecting their physical and organoleptic properties. The result of this work is therefore expected to bring about significant economical and health benefits to the food industry.

Materials and Methods

"Kohila" (*Lasia spinosa*) and banana blossoms were purchased from Gannoruwa vegetable sales center managed by the Department of Agriculture. Selection of good raw material, cleaning, peeling and size reduction by knife were done and sliced to 3-4 mm pieces. They were dipped in 1% (w/v) citric acid solution for 10 min and blanched at 90 °C for 4 min. Then, banana blossom and "kohila" were dried at 55 °C for 14 h and 18 h respectively (Wickramarachchi and Ranamukhaarachchi, 2005). Dried materials were ground using electronic high speed grinder (3000 rpm) and sieved using 500 μm and 250 μm sieve and extracted into powder. They were stored at room temperature for further use.

The moisture, protein, fat, ash, crude fiber contents of the fiber extracts and the final products were determined by AOAC methods (AOAC, 2000).

The wheat flour in the traditional formulation was replaced with 5, 10, 15, 20, 25, and 30% of each fiber flour separately. The flour blends were mixed with common salt (1 g/100 g flour), tap water (40 mL/100 g of total weight) and vegetable oil (1 g/100 g flour) (Hou and Kruk, 1998). The dough pieces were rested for 20-40 min. Then dough was pressed to get thin sheet and slitting was done by cutting. The noodle was cooked at 100 °C for 30 min and was dried at 60 °C for 6 h. Finally, the noodles were cooled to room temperature and packaged in low density polyethylene packaging material.

Water (250 mL) was brought to boil and 25 g of 2-4 cm noodles were added. The cooking temperature was maintained at 98-100°C throughout the cooking process. Noodles were removed at the end of every 30 seconds and squeezed between clear glass slides. This procedure was then repeated until the white core disappeared. This point is considered the optimum cooking time according to the literature (Sozer *et al*., 2007).

Swelling index of cooked noodles was measured using a formula; swelling index =M1-Mo /Mo, where M1 represents the mass of the fresh noodle (g) and Mo represents the mass of cooked noodles (g).

Breakability study was conducted by Universal Instron texture machine (Model 44665). The peak breaking point of single strand of 3 cm length was read in kilonewton (kN).

Sensory evaluation was conducted involving thirty untrained panelists. Ranking test and nine point hedonic tests were conducted to find out best formulation of noodles, the level of acceptability of noodles respectively.

The tabulated data were analysed by non-parametric analysis, Friedman test (Minitab 15.1). The significant level of $p<0.05$ was used throughout the study.

To determine the microbiological quality of products total plate count procedure (SLS 516: Part I, 1991) was performed with final products.

Results and Discussion

Dietary fiber content of kohila flour and banana blossom flour was 26.56 % and 16.23 %, respectively (Table 1).

Table 1. Chemical composition of kohila and banana blossom

Chemical composition	Fiber source	
% Dry basis	Kohila	Banana blossom
Moisture	6.19	6.58
Crude protein	10.84	25.94
Crude fat	0.58	4.33
Crude dietary fiber	26.56	16.23
Ash	7.99	9.32

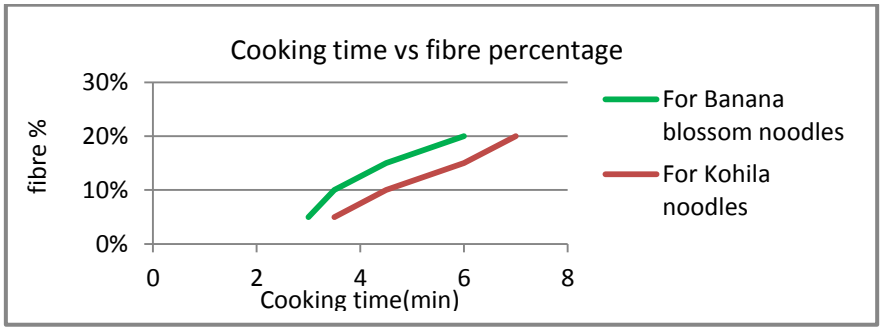

Figure 1. Change of the cooking time with percentage of fiber

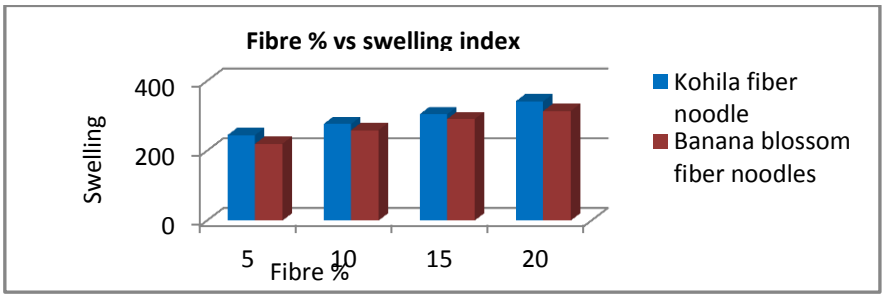

Figure 2. Change of the Swelling index with percentage of fiber

During cooking, noodle become increasingly swollen and less textured. This is normally quantified as "swelling index". Swelling index or water absorption capacity is an important factor in considering consumer acceptability.

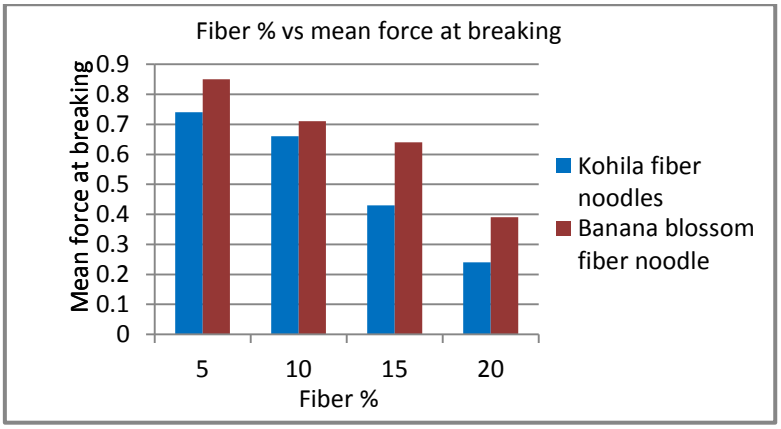

Figure 3. Change of the breakability with percentage of fiber

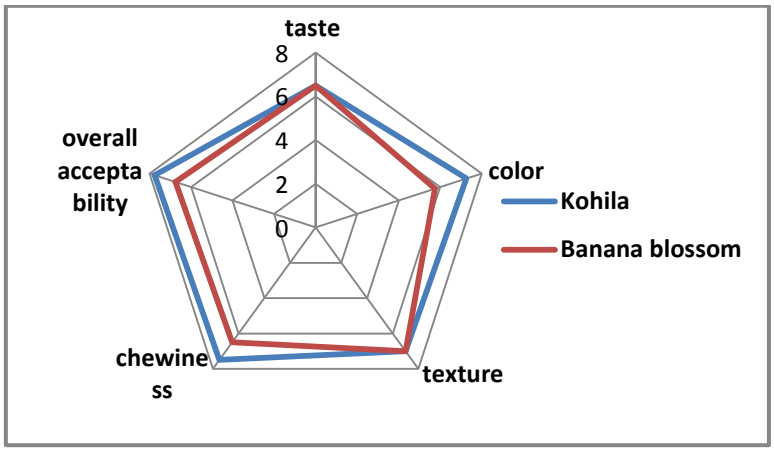

Figure 4. Comparison of "kohila" flour and Banana blossom flour incorporated noodles through sensory parameters

In this study, crude fiber was determined instead of dietary fiber. Crude fiber is a part of dietary fiber. It includes only cellulose and lignin. Dietary fibre content of "kohila" flour and banana blossom flour was found to be 26.56 % and 16.23 %, respectively. In another study it has been reported that the rhizome is the rich source of dietary fiber with 40-55% of total dietary fiber on dry weight basis (Shefana and Ekanayake, 2009). According to the results of this study, crude fibre content of kohila was greater than those of banana blossom flour. The moisture content of "kohila" flour is higher than the Banana blossom flour. It was revealed that the optimum cooking times of all noodle samples ranged from 3 to 7 min. The cooking time of the banana blossom incorporated noodles was less compared to the "kohila" incorporated noodles. It has been resulted due to different composition of flour. "Kohila" incorporated noodles showed high swelling index and swelling index increased with fiber percentage. This may be due to increase water holding capacity. The previous study showed that physical properties of the fiber including water holding capacity, oil holding capacity, viscosity or gel formation significantly affect the product processing and quality (Collar *et al*., 2007). Mean force of breaking point for noodles was decreased

with increasing fibre percentage. This may be due to dilution of wheat gluten with increasing fiber level.

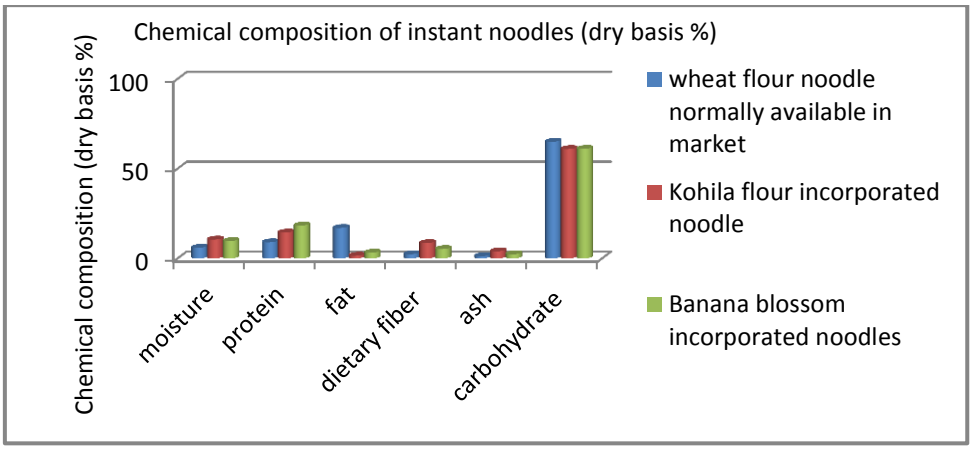

Figure 5. Comparison of chemical composition between developed and wheat flour noodles available in the market

According to the sensory evaluation, preference for "kohila" noodles decreased with increasing fiber percentage. But there was no significant difference between 5 % and 10 %. Preference level decreased with increasing fiber level in banana blossom noodles as well. But there was no significant difference existed among 5 %, 10 % and 15 % dietary fiber inclusions. According to the tested parameters, there was no significant difference between "kohila" incorporated noodles and banana blossom incorporated noodles ($p>0.05$). From the study, 10 % "kohila" incorporated noodles and 15 % banana blossom incorporated noodles were selected as final products.

Crude fiber content of selected "kohila" noodle and banana blossom noodle showed 8.57 and 5.27 % respectively which are higher than that of commercially available wheat flour noodles (figure 5). Total plate count of selected "kohila" noodle and banana blossom noodle were below the maximum acceptable level.

Conclusion

This study revealed that noodle prepared with 10% of "kohila" flour and 15 % of banana blossom flour formulations has acceptable sensory scores than those of noodle containing other percentage of fibre flour. According to the sensory analysis, there was no significant difference between 10% "kohila" flour incorporated noodles and 15 % of banana blossom incorporated noodles. Fiber incorporated noodles are better with regard to their nutrition content and other chemical composition than commercially available wheat flour noodles.

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ENHANCING TEXTURE STABILITY AND GEL FORMATION OF FISH SURIMI PRODUCED FROM SWORDFISH (*Xiphias gladius*) BY REPLACING EXISTENT CRYOPROTECTANTS WITH OVOMUCOID

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Summary

Surimi is concentrated myofibrillar protein obtained from mechanically deboned fish. The objective of this study was to find the suitability of ovomucoid as a cryoprotectant for the production of surimi from swordfish by replacing existing cryoprotectants. Different levels of ovomucoid were incorporated to check the quality of surimi. Improvement in gel strength was noted when 0.75% (w/w) ovomucoid was added to surimi. It effectively prevented the protein denaturation and retarded the lipid oxidation of surimi during frozen storage at -20^{o}C for 21 days. Therefore, ovomucoid can be used successfully as an alternative cryoprotectant in surimi production due to their low sweetness, low caloric value and richness in protein value.

Keywords: Cryoprotectant; Surimi; Ovomucoid; Swordfish

Introduction

Surimi is concentrated myofibrillar protein obtained from mechanically deboned fish (Park and Lin, 2005). Surimi serves as a potential raw material for a variety of products (Park and Morrissey, 2000). Surimi is considered a low-cholesterol, low fat, and low-sodium ingredient that lead to its increasing market demand (Moosavi-Nasab *et al*., 2005). Freshness of fish is considered as the crucial factor determining the surimi quality (Benjakul *et al*., 2002, MacDonald and Lanier, 1991). Frozen storage is an essential step in surimi manufacture, however, myofibrillar proteins have been reported to undergo denaturation under frozen conditions, leading to loss in protein functionality, especially gel-forming ability, water-retention properties and protein solubility. Cryoprotectants minimize protein denaturation during freezing. Among them sucrose is reported as the most effective cryoprotectant (Ryu *et al*., 1994). But problems associated with the sucrose is, it gives a sweet taste. At present, very little is known about the potential of using different cryoprotectants for protecting denaturation of myofibrillar proteins under various storage conditions. Ovomucoid may be an answer.
Egg white is the most commonly available food grade protein denaturation inhibitor due to the presence of ovomucoid. Usually egg white protein is used to enhance the gelling ability of surimi (Ali, 2012). Approximately egg white contains 11 % of ovomucoid (Kovacs-Nolan *et al*., 2005). Ovomucoid also reported to act as a trypsin inhibitor, inhibit the growth of tumours and has antimicrobial properties (Abeyrathne *et*

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al., 2013). Incorporation of ovomucoid into surimi by replacing sugar can reduce sweet taste as well as can increase the protein value of surimi while acting as a trypsin inhibitor. This study was carried out to find the suitability of ovomucoid as a cryoprotectant for surimi to replace the existing cryoprotectants.

Methodology

Fresh swordfish loins (*Xiphias gladius*) were taken from Lihini Sea Food (Pvt) Sri Lanka, and transported into the meat laboratory in the Uva Wellassa University, Sri Lanka and were stored in a polystyrene box under refrigerated conditions. Ovomucoid powder was received from Iowa State University, USA, which had been extracted according to the method developed by Abeyrathne's group (2014). Swordfish fillets were minced for the preparation of both sugar and ovomucoid incorporated surimi. Proximate composition was determined according to AOAC methods (AOAC, 2000). The pH was tested by using the procedure followed by Dhanapala *et al*, (2012) for Surimi with some modifications. Lipid oxidation was measured using TBARS level with some modifications to the method mentioned by Abeyrathne *et al*, (2013). Gel strength was analyzed by following cutting strength test, folding test and teeth cutting test. Colour measurement was done to both cooked and frozen surimi gel using chroma meter. Biochemical, gel cutting strength, proximate composition and sensory parameters were analyzed to study quality changes and shelf life of these products in frozen storage at -20°C. Preliminary investigations were conducted separately to determine the suitable levels of sugar in both cooked and frozen surimi and used different levels of ovomucoid to find out the best ovomucoid percentage for ovomucoid incorporated surimi.

Results and Discussion

Raw surimi yield from sword fish fillets was 51%. There were significant ($P<0.05$) differences in the moisture content and crude protein of sugar and ovomucoid incorporated surimi in both cooked and frozen produced (Table 1). Higher moisture and protein percentages were obtained from ovomucoid incorporated surimi samples than sugar incorporated surimi (both frozen and cooked). Addition of 0.75% egg ovomucoid exhibited gel enhancing effect by increase in gel cutting strength 12.8±1.3 (N) in frozen and 8.2±0.85 (N) in cooked surimi (Fig. 1).

Table 1. Proximate analysis results of surimi products

	Cooked Surimi		Frozen Surimi	
Percentage (%)	**Control**	**Ovomucoid**	**Control**	**Ovomucoid**
Moisture	72.95±0.60^{a}	74.15±1.25^{b}	72.15±0.80^{a}	75.28±0.61^{b}
Crude Protein	17.27±0.11^{a}	18.04±0.95^{a}	15.88±0.04^{a}	16.95±0.41^{b}
Crude fat	0.67±0.06^{a}	0.36±0.02^{a}	0.78±0.05^{a}	0.72±0.04^{a}
Ash	0.64±0.02^{a}	1.20±0.02^{a}	0.77±0.03^{a}	1.44±1.05^{a}

*Mean ± SD (n = 3). a-b Different letters within a raw are significantly different ($P < 0.05$).

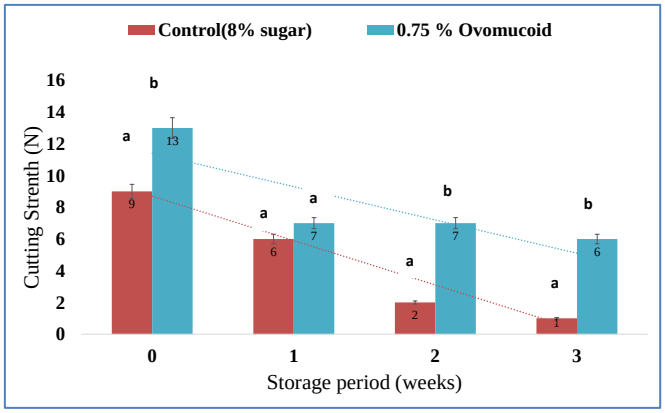

Figure 1. Cutting Strength variation of surimi during frozen storage

pH was increased from 6.25±0.03 to 6.48±0.04 in control surimi and 6.23±0.02 to 6.43±0.06 in ovomucoid added surimi during 3 weeks storage period ($P<0.05$). There were no differences in lipid oxidation of 0.75 % egg ovomucoid added frozen surimi during the storage period ($P>0.05$) and showed lower oxidation rate than control.

Both cooked and frozen control group had significantly higher lightness (L*) than ovomucoid incorporated frozen and cooked surimi sample ($P<0.05$). Redness (a*) and yellowness (b*) were not significantly different for cooked and frozen surimi in both control and ovomucoid incorporated surimi ($P>0.05$). Both cooked and frozen surimi were negative for *Salmonella* test. There were no signs of growth of *Salmonella* in frozen surimi during the three weeks storage period.

Abdelaal *et al*, (2014) have reported that the surimi yield was 55% which made from common carp fillets but our results showed lower yield. This may be due to different methods and techniques used to dewatering. Excess dewatering results lower yield. Proximate analysis revealed that ovomucoid incorporated surimi contains higher amount of protein than the standard (Table 1). When compared the proximate composition of raw sword fish both cooked and frozen surimi showed less value for protein and fat contents. This may due to washing steps, where water-soluble proteins and minerals are removed. When comparing ovomucoid incorporated surimi with control, cooked ovomucoid surimi had higher protein value by 0.77%.

Highest cutting strength was obtained with ovomucoid added surimi than control. Ovomucoid is a proteinase inhibitor, which has inhibitory activity against serine proteinase (Nakamura and Doi, 2000). Egg white at various concentrations (0-3%) was used by Eakpetch *et al*, (2008) in Pacific white shrimp (*Litopenaeus vannamei*) meat. All sample of *L. vannamei* showed increased breaking force of gel and decreasing gel forming ability with increasing frozen storage. When compared the ovomucoid incorporated surimi with control, the rate of lipid oxidation was lower in the ovomucoid added surimi. This may be due to antioxidant activities of ovomucoid (Moon and Song, 2001). Lipid oxidation occurred during frozen storage might cause the denaturation of proteins. When proteins get exposed to an oxidizing environment, those are very susceptible to chemical reactions, such as amino acid destruction, peptide scission and formation of protein lipid complexes (Saeed and Howell, 2002; Xiong, 1997).

Whiteness is one of most important factor in quality of surimi. Ochiai, (2001) has suggested that the high-quality surimi with higher whiteness can be obtained when dark muscle is removed as much as possible. In this study, sugar incorporated surimi showed higher whiteness (W) and lightness (L*). It may be due to the sugar content which added to the control surimi group. When compare cooked surimi with frozen surimi, cooked surimi exhibited more whiteness than frozen one. This may due to non-enzymatic browning due to heating. However, Kim *et al*, (1996) has reported that the colour of surimi can be improved by increasing the washing cycle and washing time.

Conclusion

Sword fish was found to be suitable for surimi production and addition of 0.75% (w/w) ovomucoid showed an increasing gel strength during frozen storage at -20^{o}C for 21 days.

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IMPROVEMENT OF TEXTURE AND FLAVOUR OF A DEEP-FRIED DRIED FISH PRODUCT BY MODIFYING SALTING AND FRYING CONDITIONS

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Summary

Fish and fishery products are important sources of good quality dietary protein and are popular among many Sri Lankans. Development of value-added products from fish employing simple preservation techniques is a successful means of meeting the existing demand throughout the year. The objective of this study was to improve the texture and the flavour of a deep-fried dried fish "keeramin" (*Amblygaster clupeoides*) product developed from salt-dried fish. Salt-dried "keeramin" fish supplied from the market for the production of this product was hard in texture, high in salt content and comparatively less in flavor. Therefore before frying, desalting was required to remove excess salt in the dried fish. In this study fresh "keeramin" fish was dry-salted with 1%, 2%, 3%, 4%, and 5% (w/w) salt for 1 h and 2 h separately to find the best salt level after drying and to avoid desalting. All fish samples were oven dried providing with the same conditions and salt contents were analyzed. Fish dried with 1% (w/w) salt for 2 h had the same level of salt (0.16%) compared with the fish obtained from the suppliers after desalting. Above samples were fried separately with olive oil, soybean oil and vegetable oil at 70°C for 2, 3 and 4 (±10 s) min and the texture and flavour were assessed using a 5-point hedonic scale sensory study with thirty untrained panelists. Fish salted with 1% (w/w) salt for 2 h and fried with olive oil for 3 min was significantly ($p<0.05$) preferred by the panelists. Hardness of the market available fried fish were determined using an instron machine and the force required to break desalted and fried fish (86.83±3.94 N) was significantly ($p<0.05$) higher than that of the treated fish (43.60±3.56N). It can be concluded that fish develop an irreversible hard textured tissue when salted with excessive high salt levels and that can't be removed even after adequate desalting. Moreover frying temperature and the type of oil altered the flavor characteristics of the product. "keeramin" fish salted with 1% (w/w) salt for 2 h reduced the production cost by Rs. 162.75/kg of the final product by avoiding desalting process and, frying with olive oil for 3 min developed the best flavour.

Keywords: *Amblygaster clupeoides*; Salting; Desalting; Hardness

Introduction

Fish preservation is a very important aspect of the fisheries sector, due to its high perishability. When the fish are caught in numbers, greater than amount of consumption, their preservation becomes a necessity for their future use. It is done in

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such a manner that the fish remain fresh for long time, with a minimum loss of flavor, taste, odour, nutritive value and the digestibility of their flesh. Salting as a method of preserving fish has been used for centuries and in many part of the world such as Asia, Europe and Latin America. Salting process can combines with other preservation methods, such as drying or smoking to extend the shelf life and improve the sensory qualities (Bellagha *et al.*, 2007). Salted dried fish is produced as a result of both salting and drying processes. Many fish species are preserved as salted dried fish. Desalting (Rehydration) is a process which is practiced to remove excess amount of salt in salted dried fish. It can be practiced by soaking the salted dried fish in water for several hours. Longer period of desalting will develop a poor texture and poor sensory qualities on final product. "keeramin"/Smoothbelly/Sardinells (*Amblygaster clupeoides*) is one of the small salted dried fish species mostly found in the market and also more popular among consumers due to it is more favorable sensory qualities.

Present study was aimed at standardization of this "keeramin" product by improving the sensory qualities, specially the texture and the flavor. Through this improvement company can increase their production and product will be popular among consumers.

Methodology

Frozen fish were taken and allowed it for thawing. They were headed, gutted, de-scaled, washed and drained well. Two sets of samples were prepared for dry salting. During salting of fish, salt granules were applied in between the layers of fish in the proportion of 1:99 (1%) salt to fish, 2:98 (2%) salt to fish, 3:97 (3%) salt to fish, 4:96 (4%) salt to fish and 5:95 (5%) salt to fish ratio, allowing the developing brine solution to flow off. One set of samples was kept for one hour and the other set of samples was kept for two hours at ambient temperature (28-30°C). There were three replicates for each treatment. Samples were oven dried at 60°C for about 24 h until moisture content reached up to 7%.

The salt content of the samples were determined as chloride, where the ions are precipitated by silver nitrate and the excess silver ions are determined by titration with potassium thiocyanate (AOAC, 1998). All analysis was performed in duplicate.

Even sizes of fried fish were selected from both desalted and the non-desalted samples. Instron machine (SYSTEM ID, 4465 H2239, England) was operated by giving 2kN apply load and 150 mm/min speed. Three replicates from each sample were measured and reading was taken as kN.

The best conditions for frying were selected by conducting a sensory test. Two sensory tests were conducted to select the best oil type and the frying time and evaluated using a 5-point hedonic scale involving 30 untrained panelists. The evaluated sensory attributes were appearance, taste, texture, aroma and overall acceptability. Final sensory evaluation was carried out to select the best process condition from the above selected conditions. The evaluated sensory attributes were taste, texture and overall acceptability.

Sensory data and salt series were statistically analyzed. Salt series was analyzed according to two factor factorial design and pooled t test and mean separation was done according to least significance difference ($p<0.05$) test (LSD). All these are analyzed using SAS Institute Inc., 2000 software program. Sensory data was analyzed according to non parametric procedure- Friedman test and the statistical significance was defined

as p< 0.05. Data obtained from 5- point hedonic test was analyzed using MINITAB software, version 16.

Results and Discussion

By analyzing the final NaCl in all the samples of salt series, it was found that there were significant differences among salt concentrations and also between samples salted for 1 h and 2 h (p>0.05). Initial salt concentration and time kept were affected to the salt penetration in to the flesh. From pooled t test analysis it was found that the sample treated with 1% final sodium chloride and kept for 2 h salt is similar to the sodium chloride % in desalted market available sample (p>0.05). It was proved that the time selected was adequate for salt penetration in to the flesh of fish.

The hardness of the fried desalted fish was measured by instron machine. It was clearly shown that, after desalting the texture of the fried fish obtained from the market was harder (86.83±3.94 N) than the non- desalted fried fish (43.60±3.56 N). It was given as a peak load and expressed as a force. Reading was given for a unit area. Therefore, same size (same surface area) fish were selected.

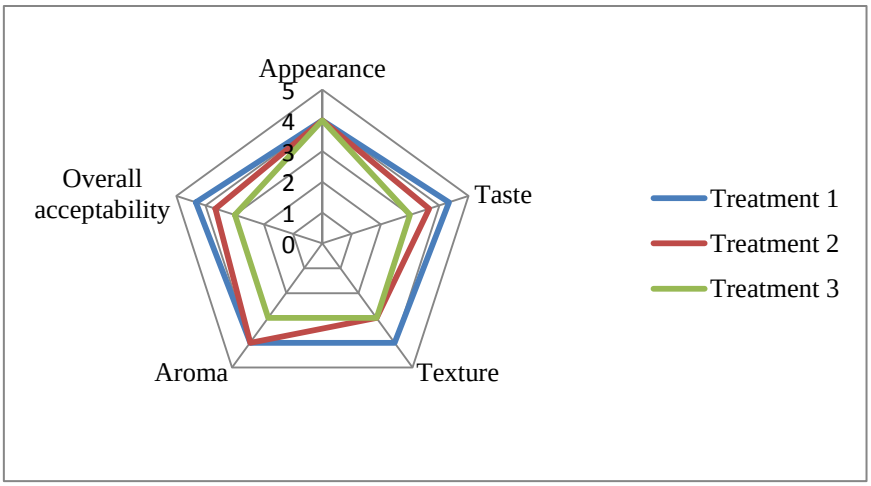

Figure 1. Changes in sensory attributes with different frying oils

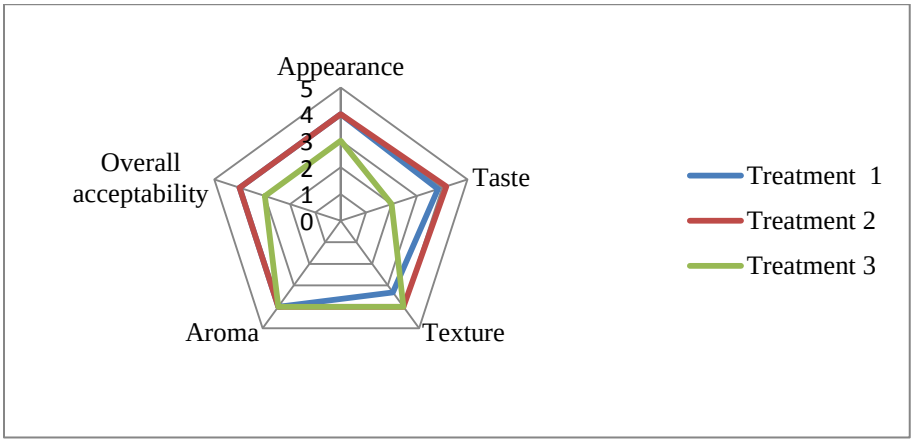

Figure 2. Changes in sensory attributes with different frying time

After carrying out this research, the production line was changed due to cutting down the desalting step. It will be a big change in company's production and the profit increment in the product. It can be proved by doing a cost analysis with desalting step and without the desalting step.

When the desalting step is included in the process line, fish should be dried more hours in the dryer to remove the absorbed excess water. It will take about 16 h and want high energy to run the dryer. Therefore, cost for kerosene is high with the desalting step. By cutting down this step, can reduce the energy and also time taken to processing the product.

As suggestions currently they add only fried curry leaves to the product other than the dried fish. Therefore, new ingredients can be added to the product such as fried big onion to further improve the flavor of the product. And also change of the currently used spice mixture to improve the flavor is recommended. Company can make dried salted fish in the factory premises according to the conditions studied in this study, rather than buying from outsiders. Then they can produce it according to their quality standards. Olive oil can be used in frying dried fish, and have to increase the price per jar according to cost of production due to its high price. Olive oil can be used as an ingredient to the product rather than taken to frying due to its high cost.

Conclusions

Salting of "keeramin" fish using 1% salt and allowing it to stand for 2 h for curing, was successful to obtain NaCl level which is similar to the NaCl level after desalting the market available dried fish. Desalting step can be removed from the processing line without affecting the final quality of the product successfully. Hardness of the fried desalted fish obtained from the market was higher (86.83± 3.94 N) than the non-

desalted fish (43.60 ± 3.56 N). Use of olive oil as the oil type and the 3min±10s as frying time were contributed to improve the sensory attributes.

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DEVELOPMENT OF A READY TO USE RICE BASED OYSTER MUSHROOM (*Pleurotus ostreatus*) BURGER

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Summary

A demand exists among current consumers for healthy, nutritious and ready to eat foods which are free of chemical preservatives. Therefore, food industry is facing a great challenge to cater this emerging demand and strive towards novel product development to meet the requirements. Oyster mushroom is a popular nutritious food among vegetarians as it provides good source of vitamins, proteins and fiber. Despite the nutritive value of oyster mushroom, due to high perishability, nature permits shorter shelf life. This study was carried out to develop a ready to cook oyster mushroom (*Pleurotus ostreatus*) burger which has not yet being exploited for a potential marketable product. A combination of mushroom and filling material levels at a ratio of 10 % to 90% was used to develop burgers. Sensory properties assessed using five hedonic scales with 15 untrained panelists were used to select the best level of filling material and mushroom. The selected best levels of mushroom and filling materials were further evaluated against commercial vegetarian and chicken burger using sensory, storage properties and microbial properties. Based on the preliminary studies 90% mushroom and 10% filling material (rice flour, corn, and wheat) were selected. Rice based mushroom burger had significantly higher value (4.50) for overall acceptability compared to vegetarian burger. Based on the physical properties, the results showed that the pH, aw, color were not significantly ($p < 0.05$) changed with the storage time. Proximate analysis showed that protein content of rice flour based mushroom burger was 41.33%. Microbial analysis revealed, the product was safe for consumption at -18 ℃ for one month. In conclusion, rice based mushroom burger better in overall quality than commercial burger and it can be substitutable with 10 % filling material with90 % of mushroom to produce a good quality vegetarian burger at low cost.

Keywords: Burger; Rice; Corn; Wheat; Sensory evaluation; Storage studies

Introduction

Burgers are one of the most popular ready to eat foods which are mainly made from beef, chicken, fish, turkey and cereal fiber or bean fiber as fillers. Moreover, vegetarian burgers are also available in the international market made with several vegetables. However, in Sri Lanka, market available vegetarian burgers are very few. Therefore, it exists a timely requirement to develop a high quality vegetarian burger at a low cost for

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the local consumers. In order to reduce the cost of production, there is increasing interest in the use of various plant derived proteinous sources and non-meat proteinaceous sources as edible fillers. Mushroom proteins are comparable to animal muscle protein in nutritive value. In addition, mushroom contains high calcium, phosphorous and iron than the amounts contained in beef, pork and chicken meat (Kannayian and Ramasamy, 1980). Mushroom has the highest content of Vitamin B1 and B2 and rich in niacin than any other vegetable. In addition, the consumption of oyster mushroom could reduce cholesterol levels and has been attributed to anti-cancer properties (Kannayian and Ramasamy, 1980). However, Oyster mushrooms are highly perishable in nature, due to the presence of large quantity of water. Moreover, mushroom growers in Sri Lanka face economical loss in market due to consumer rejection of 2-3 days old oyster mushrooms and excess production during raining period. Therefore, it is important to develop mushroom burger using mushroom as a major ingredient considering its nutritive and medicinal values. On the other hand to select the best edible filling material in product development, nutritive value and physical properties such as good binding ability and high viscosity of flour are considered. Therefore, there is an interest in use of rice, corn, wheat like non meat proteinaceous sources as edible filling materials. The main objective of this study was to develop a vegetarian burger using oyster mushroom with the optimization of best ratio of edible filling materials and assessment of its quality attributes.

Methodology

Fresh fully grown Oyster mushrooms (*Pleurotus ostreatus*) with cap diameter 9-11 cm were purchased from the grower Mellone Foods International in Gampaha District, Sri Lanka. Fresh oyster mushrooms (360 g) were blanched in 0.1% ascorbic acid for 3 min and squeezed. The squeezed mushrooms were steam blanched for 3 min then squeezed and ground for 3 min using an electrical grinder (Jaipan, India) until it became a paste. The spices (chili 2g, cumin 0.8 g, ginger powder 2 g, red onion 25 g, garlic 5 g) were mixed with the mushroom paste and fried (30 mL vegetable oil) the mixture until become golden brown for10 min. The fried mushroom paste, 60, 70, 80, and 90% was incorporated with edible filling materials (rice, corn, wheat) at 10, 20, 30, ratios. Also 3.5% soy flour, 2.5g salt, 0.60 g black pepper, 0.30 g cloves and 0.50 g cinnamon and bell pepper pieces (20 g) were added to the mixture. All were mixed together for 5 min to prepare a dough. The final dough was kept for 20 min at 30°C for dough leavening and flavor development. The burger patty (75 g) was formed using a mold and baked in an oven (Genlab-Inc75SS-D16, UK) at 85ºC for 45 min and 65ºC for 45 min. The oven cooked patties were packaged in a polyethylene and kept in a freezer (EVERmed-class3, Italy) at -18ºC until use.

Sensory evaluation of cooked burger was done to select the best combination of oyster mushroom and edible filling material. The selected levels of mushroom and filling materials (rice, corn and wheat) were further evaluated against commercial vegetarian burger using five point Hedonic scale.

The selected final product was subjected to proximate analysis (AOAC, 1995). The colour of the samples were assessed based on L, a and b values by using a chromometer (CR400- Konika Minolta, Japan).

For microbial analysis, 10 g of mushroom burger sample was mixed with 90 mL 0.85% saline solution. A 100 µL of the dilution was spread on to plate count agar (Basingstoke, UK). Plates were incubated at 37ºC for 48 h and colonies were counted.
The results were analyzed using Minitab version 16 with ANOVA for significance. Multiple mean comparison were carried out by Kruskal-wallis test at $P<0.05$.

Results and Discussion

In preliminary studies, results obtained from sensory evaluation of different ratios of mushroom (60, 70, 80, 90): rice, corn or wheat (10, 20, 30, 40) cooked burgers were analyzed. Rice flour can be substituted with mushroom up to 10% level without changing its sensory quality. Similarly, corn and wheat showed same trend of acceptance in sensory attributes. Therefore, for further analysis 10% of rice flour, corn flour, and wheat flour individually combined with 90% of mushroom was used for further evaluations of mushroom burger.
Sensory analyses for the final product are shown in Table 1. Sensory attributes of mushroom burger were significantly ($P<0.05$) different compared to commercial vegetarian burger. Colour, taste, mouth feeling and overall acceptability were significantly different and highest median values were obtained for rice based mushroom burger. Since rice variety (AT405) used helped to increase the binding ability of soy protein concentrates and isolates have some specialized gelling properties which may increase fat emulsification in filling materials (Pearson and Gillett, 1996).

Table 1. Sensory evaluation of developed burger with vegetable burger

Treatments	Mean values of sensory characters						
	Texture	Appearance	Colour	Odour	Taste	Mouth feeling	Overall acceptability
Mush90:Corn10	4.000	4.000	3.500	3.000	3.500	4.000	3.500
Mush90: Rice10	4.000	4.000	4.000	4.000	3.500	3.500	4.000
Mush90:Wheat10	3.000	2.000	4.000	4.500	3.000	2.000	3.000
Vegetable burger	4.000	3.000	2.000	4.000	4.000	3.000	2.000
Probability	0.232	0.516	0.000	0.022	0.000	0.000	0.655

Initially pH of burgers gradually decreased and from 3rd week pH slightly increased (data not presented). This may be due to the formation of lactic acid, at chilled storage and proteins are degraded into a variety of sulfur containing compounds and non nitrogenous components such as ammonia (Forrest *et al.*, 1975) under anaerobic conditions. In addition, the protein denaturation in mushroom increases the pH. Lightness or darkness (L value), redness (a* value) and yellowness (b* value) had no significant ($P<0.05$) effect with storage time. "b" decreased with the increase of time. This may be due to the rancidity of fats in meat result in colour instability (Forrest *et al*., 1975). The protein oxidation of chill storage of burger patties could have affected light reflection and contributed to the discoloration.
The proximate analysis results showed the dry basis of protein content of rice flour based mushroom burger was 41.33% whereas dry based oyster mushroom was 20%-35% (Tripathi, 2005). Moisture and ash content of rice based mushroom burger was 47% and

6.9% respectively. Microbiological load of one month stored mushroom burgers did show very low counts and were within the acceptable limit. The acceptable maximum population of plate count is 500 cfu/g for consumption (SLS, 1997). Hence products were safe for consumption even after 1 month.

This study revealed the possibility of incorporation of oyster mushroom with rice flour as edible fillers. Rice based mushroom burger better in overall quality than commercial burger and it can be substitutable with 10 % filling material with 90 % of mushroom to produce a good quality vegetarian burger at less cost.

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