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Review Article **Open Access**

A Review of *Azolla* spp. as a Potential Resource for Sustainable Agriculture in Sri Lanka: A New Effort for the Green Agriculture among Sri Lankan Farmers

Indika Subhashini Wijeysingha[1] and Sajeewani Rajika Amarasinghe[2*]

Abstract

The present economic crisis in Sri Lanka has limited the usage of inorganic fertilizers in agriculture sector. Furthermore, animal husbandry, the other branch of agriculture has problems due to high prices of animal feed and unavailability. The concept of using *Azolla* spp. as a multifaceted resource for sustainable agriculture, which facilitates the environment-friendly green concept, is receiving great attention in the present crisis. *Azolla* is a small floating, fast-growing aquatic fern distributed globally. Because of its growth habitat, high biomass production and nitrogen-fixing ability, it has acquired substantial value in the agriculture sector, especially as a nitrogen supplier to plants. Besides its usage as a fertilizer, *Azolla* can be used as a nutrient provision for animals, human food, phyto-remediating agent, weed controller, mosquito controller, medicinal plant, and a substrate for biogas production. It is a feed rich in protein, which can be used to feed farm animals, poultry, and fish. As well, it is found to be a more affordable additional feed supplement for the animals. The nitrogen-fixing ability of *Azolla* with the aid of a symbiotic cyanobacterium *Anabaena azollae* has led to the exploitation of *Azolla* as an effective nitrogen fertilizer and a protein supplier. Also, it helps to conserve water, sequestrate carbon, and appropriate for integrated farming systems. *Azolla* is grown in many countries for the aforementioned uses and its cultivation aligns with the goals of sustainable and environmentally conscious agriculture. *Azolla* can be easily grown in containers, soil pits or cement tanks that hold water. The required conditions should be supplied, including optimum light intensity, relative humidity, pH, salinity macro and micronutrients, etc. This review addresses the morphology of *Azolla*, its symbiotic relationship, and the potential usages for sustainable agriculture as a new effort for the green agriculture among Sri Lankan farmers.

Keywords: *Azolla* spp., Biofertilizer, Green Manure, Nitrogen Fixation, Sustainable Agriculture

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INTRODUCTION

As a consequence of the green revolution, farmers tend to apply a huge amount of synthetic fertilizers to fulfill food and nutrition requirements. Farmers in Sri Lanka also have predominantly used synthetic fertilizers for decades expecting rapid prospects in agriculture. However, the synthetic fertilizer application boom has caused many environmental problems and diminished human health in Sri Lanka. At the same time, the recent economic crisis in Sri Lanka has created a serious barrier for importing agricultural inputs. Hence, limited resources such as fertilizer, animal feed and other agrochemicals have created low agricultural production and failed to sustain farmers in the agriculture sector. Therefore, the calamity of the agriculture sector in Sri Lanka cannot be avoided, hence using alternative resources is one of the strategies to overcome the present economic turmoil.

Fertilizer application is one of the management practices that should operate sustainably. Fertilizer usage affects soil nutrient availability, the yield of crops, agriculture production and the environment [1]. Synthetic fertilizer usage for agricultural purposes may be uneconomical, inefficient, and unsustainable, because of nitrogen losses as ammonia or nitrous oxide and methane production from flooded lands [2]. Therefore, there should be sustainable methods for fertilizer application. The green concept in Agriculture arises as a viable solution in this context. At this point, bio-based fertilizers play a major role in sustainable agriculture, while providing nutrients and mitigating negative impacts on the environment. Therefore, it is time to evaluate the prospectus of these fertilizers for sustainable agriculture in Sri Lanka.

Green development in agriculture relates to the concept of sustainability and it is a holistic approach that maintains the balance between the environment and socio-economic impacts. The concept of sustainability was advanced in Neo-Malthusianism discourses during the 1960s and 1970s. The aim of this concept is to accentuate the well-being of the natural ecosystems [3]. Sustainable agriculture is simply, meeting the present needs without depleting the resources on the earth, which aids to meet future needs. Therefore, sustainable agriculture can be defined as a commitment to fulfill food and fiber needs and also to uplift the living standards of the farmers and society, now and in the future [4].

Organic substances such as green manure can be employed as alternative nutrient sources instead of commercial synthetic fertilizers [6]. Green manuring is the addition of green plant tissues into the soil [5], which is inexpensive and environmentally friendly. Green manures that have high nutrient availability and low C/N ratios make significant impacts as fertilizers [1]. Green manure from legumes is a feasible source of nitrogen fertilizer. There are several critical factors for the selection of green manure crops, such as fast-growing ability, succulent nature, adaptability to a broad range of environmental conditions and nitrogen fixation [7]. The average nitrogen accumulation in green manures can substitute the nitrogen availability of synthetic fertilizer at average application rates [1]. The addition of green manure contributes 30 to 100 kg N ha^{-1} and in many instances, it is about 50 to 60 kg N ha^{-1} [5].

Besides nitrogen, green manuring enhances the availability of phosphorus via several mechanisms of reduction, chelation and satisfactory alterations in pH. Also, it helps the mobilization of a wide variety of elements, including sulfur, phosphorus, silicon, zinc, manganese and copper, etc. as a consequence of enhanced microbial activities and diminished redox potential. Furthermore, it decreases the bulk density, while increasing the stability of water-stable aggregates, pore spaces, water intake and water retention [5]. Moreover, green manure application helps to maintain or build organic matter in the soil-plant environment, thus improving the soil structure, water holding capacity, size of pores and efficiency of inorganic fertilizer usage [8]. Among the various green manures, *Azolla* has arisen as a feasible solution in many countries, especially in Taiwan, China, Philippines, India and Indonesia. However, the usage of this plant in the agriculture sector is not much popular among Sri Lankan farmers. Therefore, this article aims to discuss the potential usage of *Azolla* in sustainable agriculture, as a green fertilizer.

Distribution of *Azolla* Spp.

The term *Azolla* is derived from the Greek words *azo* (to dry) and *ollymi* (to kill), suggesting the meaning of death from drought [9]. *Azolla* domestication was in the 11th century and first reported in Vietnam [10] and now it is distributed worldwide. *Azolla* belongs to the genus of aquatic ferns, which primarily exists in tropical and warm temperate zones [11]. It is a free-floating, dichotomously branched, aquatic fern that is naturally existing in wet soils, ditches and marshy ponds [12]. Several *Azolla* species have been domesticated throughout the world, including *A. pinnata*, *Azolla caroliniana*, and *A. microphylla* which are found in the Indian subcontinent [13].

Water is an essential component for the survival of *Azolla* spp. and it floats on the surface of water to survive [10]. *Azolla pinnata* can grow under nitrogen-deficit conditions because it has the ability to intake atmospheric nitrogen and grow without any nitrogen sources [14]. The nitrogen requirement of *Azolla* has accomplished itself through nitrogen fixation, but macro and micronutrients are required for its development [2]. The major limiting nutrient for *Azolla* growth is phosphorus [15]. Besides nitrogen and phosphorus, other macronutrients such as potassium, calcium and magnesium are also required for its growth [10]. In addition, it necessitates micronutrients such as molybdenum, manganese, zinc, copper, iron and cobalt. Also, light intensity affects its optimum growth and the optimal light intensity for *Azolla* growth is 15-18 klux, but high light intensities inhibit photosynthesis and growth of *Azolla*. Furthermore, its productivity depends on the relative humidity, nutrients, pH, salinity, wind and growing season. Near neutral pH and high amount of phosphorus in water body facilitates the growth of *Azolla* spp. [10].

Azolla caroliniana is mostly cultivated in high humidity (80-90%) and low light intensity (50%) conditions. Further, it is reported from environments with high nitrogenase activity [16]. Winds and waves cause vulnerable to disruption in water surfaces for the growth of *Azolla* spp. because of the free-floating nature of the plant and agitation of water, which can break the fronds leading to low absorption efficiencies of nitrogen [10]. Vegetative reproduction of *Azolla* spp. occurs by division of the abscission layer at the base of each branch, while sexual reproduction is uncommon and is influenced by environmental factors such as several stress conditions [11, 17].

Figure 1: *Azolla pinnata* Grown as a Mat on the Water Surface

Morphological Characters of *Azolla* Spp.

Azolla fronds are floating parts on the surface of water and are triangular or polygonal in shape. These fronds range from 1-2.5 cm in length in small species such as *A. pinnata* and 15 cm or more in large species such as *A. nilotica*. It has a main stem that is branched into secondary stems, which bear small leaves that have an alternate leaf arrangement. The unbranched, adventitious roots fall down into the water that arise from nodes on the ventral surfaces of the stems. Furthermore, each *Azolla* leaf comprises with two lobes. The aerial dorsal lobe is chlorophyllous and the partially submerged ventral lobe is cupshaped, colourless and provide buoyancy for the plant [18-20]. Figure 1 shows the *Azolla pinnata* grown in an artificial pond.

Symbiotic Relationship of *Azolla* Spp.

No plant individually can fix nitrogen and use it directly for plant growth unless from a symbiotic association [21]. *Azolla* often makes symbiotic associations with blue-green algae or cyanobacteria, *Anabaena azollae,* which aids to fix and assimilate atmospheric nitrogen [22]. The cyanobacteria live and reproduce in the leaf cavities; however, the association remains extracellular [21]. *Azolla-Anabaena* symbiotic relationship has a capability to fix atmospheric nitrogen rapidly, thus enabling the cultivation of rice under tropical conditions. Also, it has led to the exploitation of *Azolla* as a biofertilizer. The enhanced nitrogen fixation is linked with the production of high biomass of *Azolla* [23]. As shown in Figure 2, *A. azollae* which live within the dorsal lobe of the leaf cavity or 'hairs' of *Azolla*, converts atmospheric nitrogen into usable forms of nitrogen with the aid of nitrogenase enzyme.

The Figure 3 illustrates the microscopic view of *A. azollae*, which live in the *Azolla* leaf cavities. Within the leaf cavities, *A. azollae* are protected and under a micro-aerophilic (low-oxygen) environment, the optimal functioning of the nitrogenase enzyme produced by *A. azollae* occurs. The leaf cavities of *Azolla* maintain a balance between aerobic and anaerobic conditions, where *A. azollae* perform photosynthesis during daylight, producing oxygen, while nitrogen fixation occurs in the absence of light or in shaded conditions, reducing the oxygen concentration. On the other hand, the *Azolla* plants are benefited by the fixed nitrogen to enhance its growth and development. According to the figure 2, the atmospheric N_2 is converted into ammonium through this process.

Beneficial Aspects of *Azolla*

Among many other benefits of *Azolla*, the potentiality of using it as a biofertilizer is popularizing in the present scenario. *Azolla* can be used as an effective biofertilizer, and as a source of nitrogen due to its nitrogen-fixing ability. Biological nitrogen fixation is an environmental friendly mechanism that fulfills nitrogen requirements [20]. Different beneficial aspects of *Azolla* are discussed below.

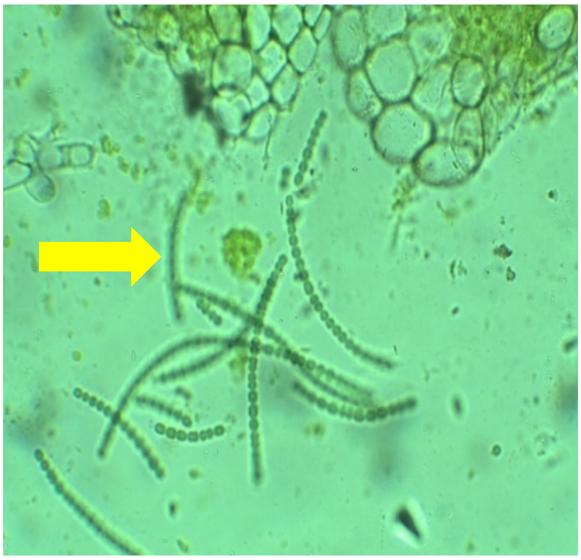

Figure 3: Microscopic View of *Anabaena azollae*

Azolla as a Nitrogen Fertilizer

Azolla is a good biofertilizer and green manure that is distributed globally [23]. Several studies have reported that *Azolla* enhances crop yield [24]. *Azolla* as an effective biofertilizer, reduces the negative impacts of synthetic fertilizers on long-term soil fertility, while enhancing the soil fertility [12] due to increment of total nitrogen, organic carbon and phosphorus availability [17, 25]. The average nitrogen fixing rate can range between 1.0 to 2.6 kg N ha^{-1} day^{-1} [16]. Nitrogen is a vital element for proteins, nucleic acids and other organic nitrogenous composites [21].

Azolla-Anabaena symbiosis provides nutrition requirements for various plant species including rice, wheat and taro etc. [20]. It can be an effective biofertilizer for many crop species other than paddy, especially for crops that grow in a flooded soil ecosystem such as Colocasia esculenta (taro) [25]. azolla application as a mulch for bananas is also practiced [25].

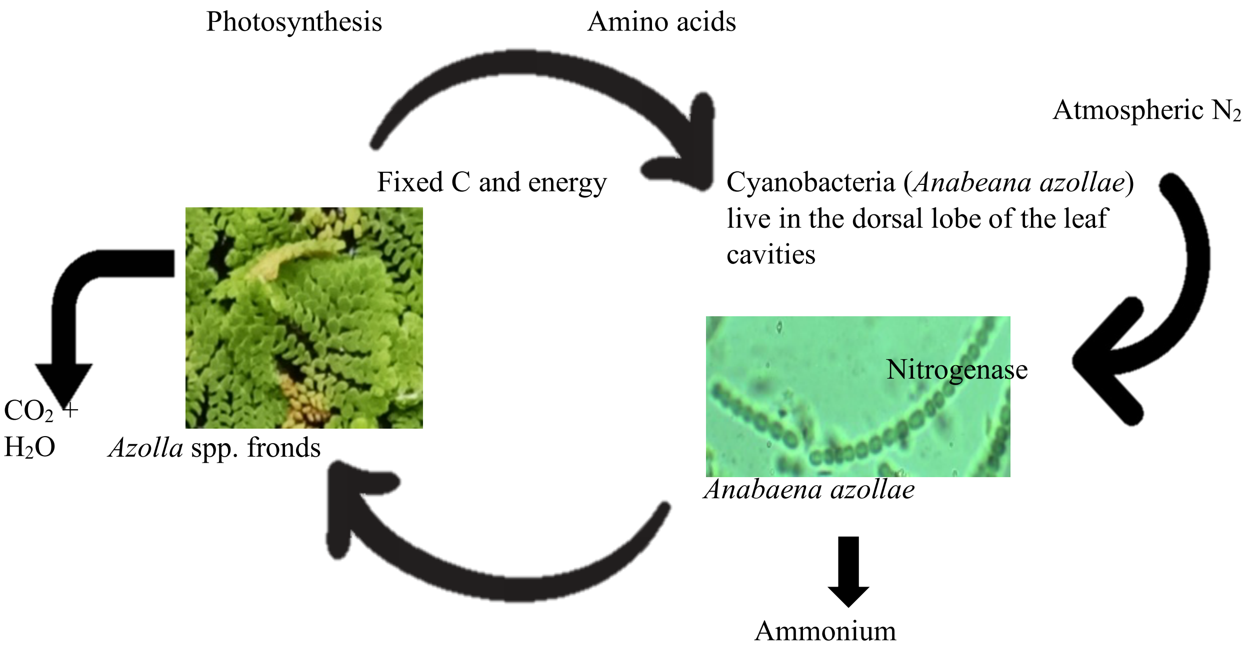

Figure 2: The Process of the *Anabaena azollae* Symbiotic Relationship with *Azolla* spp.

Nitrogen is a vital element for proteins, nucleic acids and other organic nitrogenous composites [21]. *Azolla-Anabaena* symbiosis provides nutrition requirements for various plant species including rice, wheat and taro etc. [20]. It can be an effective biofertilizer for many crop species other than paddy, predominantly for crops that grow in a flooded soil ecosystem such as *Colocasia esculenta* (taro) [25]. *Azolla* application as a mulch for bananas is also practiced [25].

It can be considered as a possible source of organic manure and nitrogen in paddy cultivation because it comprises of 0.2-0.4% nitrogen on a wet basis and 4-5% on a dry basis [26]. *Azolla* as the basal application of 10-12 tones ha^{-1} improves nitrogen in soil by 50-60 kg ha^{-1} and decreases the nitrogen fertilizer necessity by 30-35 kg [16]. Also, *Azolla* has proven to produce twice as a dual crop in rice at 500 kg ha^{-1}, while improving the soil nitrogen by 50 kg ha^{-1} and cutting the nitrogen requirement by 20-30 kg ha^{-1}. The usage of *Azolla* enhances rice productivity by 20 to 30% [16]. *Azolla* association with rice and fish-integrated farming in China, has enhanced the rice yield by 20% and fish production by 30% [22]. Vegetative reproduction is prominent in *Azolla* thus the biomass should be maintained throughout the year [23] to use it as an effective fertilizer. *Azolla* improves the nutrient availability of soil through a biological activity that aids to construct the microflora for mineralization [27].

There are three basic procedures for applying *Azolla* to crops. First, *Azolla* grows as a single crop in the fallow period and then mixes with soil before planting the targeted plant species. Secondly, growing *Azolla* as an intercrop can be practiced. Thirdly, *Azolla* cultures maintained in swamps, ponds, or flooded fields can be harvested and applied for target crops. These applications can be done either by incorporating harvested *Azolla* into the soil before planting or by using it as a mulch. A combination of the above procedures also can be practiced [19]. The most common method of *Azolla* application is as a green manure application collected from ponds and ditches or growing as a dual crop with the paddy [23]. *Azolla* cultivation in rice fields either as a monocrop or as an intercrop is practiced in India, China, Philippines and Indonesia etc. [13]. It is very effective in biomass accumulation and nitrogen-fixing ability which required low-skill and low-cost.

Research and Development regarding Azolla Spp. as a Green Manure for Different Crop Varieties

Table 1 describes different research studies conducted for the usage of *Azolla* spp. for different crop varieties.

Azolla as a Nutrient Supplement for Animals

Azolla has the potential to be used as a feeding ingredient for cattle, goats, pigs, rabbits, chickens, fish and ducks, etc. [13]. *Azolla* meal consists of 21.4% crude protein, 12.7% crude fiber, 2.7%, ether extract, 16.2% ash and 47.0% carbohydrate in dry form [36]. *Azolla* has a substantial amount of proteins, essential amino acids, growth promoter mediators, vitamins (vitamin A, vitamin b12, Beta Carotene) and minerals (Ca, P, K, Fe, Cu, Mg). Furthermore, *Azolla* has a low carbohydrate and oil content. Therefore, it is an economical and effective feeding substitute for livestock [22].

A. pinnata can be considered as an alternative feed ingredient for the poultry industry [37]. High protein and less lignin content can result in easy digestion of *Azolla* [22], while the gross energy value of *Azolla* is 2039 kcal kg^{-1} [36]. *Azolla* as a feed ingredient, increases the weight of broilers and improves the production of eggs in layers. Also, it provides a solution for the cost of production due to high feed costs [22]. *Azolla* is a suitable source of protein that can be used up to 5% in broiler ration, with no deleterious effects on the palatability of diets. These results are based on an experiment conducted using 120 Vencobb commercial broilers. Also, the amount of *Azolla* in the ration does not have any effect on the nutrient digestibility of crude protein, crude fat and crude fiber.

Furthermore, broilers have an ability to readily digest the crude fiber in *Azolla,* but not that in rice bran [37]. Hence digestibleness cannot be considered as a limiting factor for *Azolla* meals [38]. The combination of 1.5-2 kg of *Azolla* with regular feed has been reported to increase milk production by 15-20% in dairy animal trials in Tamil Nadu and Kerala [22]. Further, *Azolla* can be used as a fish meal for a variety of commercial fish species, Nile tilapia (*Oreochromis niloticus*), Mozambique tilapia (*Tilapia mossambica*), Redbelly tilapia (*Tilapia zillii*), Rohu (*Labeo rohita*), South Asia carp (*Catla catla*), orange fin labeo (*Labeo calbasu*), Fringed-lipped peninsula carp (*Labeo fimbriatus*), Grass carp (*Ctenopharyngodon Idella*) and Java barb (*Barbonymus gonionotus*) and many works of literature has reported that adding *Azolla* spp. up to a certain level enhances the growth, feed consumption and survival rate of *Oreochromis niloticus* [39].

Azolla as a Potential Source of Human Food

Azolla has been suggested to be fit for human consumption [25]. Its protein content is somewhat similar to soybean and it contains 10-15% minerals and 7-10% essential amino acids on a dry weight basis. Moreover, it has vitamins and carotenoids [13]. It is used as a salad in Western countries because of its high protein amount [12]. Several experiments have been carried out on the use of *Azolla* as soups and meatballs; which requires further development [40]. Also, there is potential to use *Azolla* as a diet for space stations, space travel and habitation on the Mars and Moon [13]. Studies on consumption potential of *Azolla* app. as a human food would be advantageous as a nutrients and protein supplying substitute in future.

Azolla as a Phytoremediation Agent

Phytoremediation is a green technology that uses either naturally occurring specially selected or genetically engineered plants to decontaminate polluted environmental systems [41]. Industrial activities including paper production, electroplating, printing, soft drinks, oil, pesticide, paints, and pharmaceuticals production can produce a high volume of wastewater [14]. *Azolla* has decent phytoremediation potential, due to its

Table 1: Research on the usage of *Azolla* for Different Crop Varieties

Azolla Species	Crop	Application	Results	Reference
A. pinnata	Squash	Wheat straw and five fertilizer treatments were (control, chemical fertilizer; N 112 kg/ha, P_2O_5 75 kg/ha, and K_2O 75 kg/ha, compost; 7.5 t/ha, vermicompost; 7.5 t/ha and dry *Azolla*; 3.75 t/ha) added to the soil.	The soil treated with *Azolla pinnata* and covered with wheat straw showed the greatest significant levels of accessible N, P and K.	[28]
A. caroliniana	Rice	A pot experiment was carried out using soil, biofertilizer and commercial N, P and K chemical fertilizer. The biofertilizer was prepared by mixing soil with *A. caroliniana.*	*A. caroliniana* has been recommended as a replacement for chemical nitrogenous fertilizer.	[29]
A. pinnata	Rice	Fresh *A. pinnata* has been applied at doses of 0-, 10- and 20-ton ha^{-1} and *A. pinnata* as compost powder at 2.5 ton ha^{-1} and 5 ton ha^{-1}.	Integration of fresh *A. pinnata* at 20 tons ha^{-1} and its compost powder at 5 tons ha^{-1} has improved the accessible P of soil, plant P content and number of tillers.	[30]
A. pinnata	Tomato	Different concentrations of *Azolla* extract (5%, 10%, 20%, 30%, 40%, and 50%) have been applied as a foliar application.	The 20% concentration has shown the highest germination percentage, shoot and root length, fresh and dry weights and highest effect on all vegetative growth parameters.	[31]
A. pinnata	Chinese kale	Application of *Azolla* bokashi fertilizer, consisting of three levels (control, 1.14 kg/plot, 2.28 kg/plot) and liquid organic fertilizer of goat manure (consisting of control, 100 ml/liter of water, 200 ml/liter of water, 300 ml/liter of water) has been investigated.	The administration of *Azolla* bokashi has shown the highest plant height and the chlorophyll quantity at the dose of 2.28 kg/plot.	[32]

A. pinnata	Red spinach	Application of five treatments (control, urea at 50 kg ha-1, poultry manure at 5 t ha-1, *Azolla* at the urea N rate of 23 kg N ha-1, and *Azolla* at the manure N rate of 108 kg N ha-1) in alluvial and peat soils has been investigated.	*Azolla pinnata* added as the manure N rate can be used as an alternative biofertilizer, especially for peat soil.	[33]
A. pinnata and *A. filiculoides*	Rice	Fresh *Azolla* has been used as a basal incorporation in soil and as a dual crop with rice separately, together with and without chemical nitrogen fertilizer in pots kept under net house conditions. (In basal treatments, it has been incorporated in soil before transplanting and equal shares of *A. pinnata* and *A. filiculoides* have been mixed for achieving a more stable plant growth)	Noticeable improvement in height of the plants, number of effective tillers, dry mass and nitrogen content of rice plants have been reported due to the use of *Azolla* and nitrogen fertilizers alone and other mixtures. The use of *Azolla* spp. has also enhanced organic matter and potassium contents of the soil.	[34]
Azolla spp.	Rice	*Azolla* has been used as a green manure and dual crop, while comparing with a commercial chemical fertilizer.	The results have shown that the yield, the number of tillers, plant height, profit to farmers, and the benefit to cost ratio of *Azolla* were higher than the chemical fertilizer.	[35]

high volume of wastewater [14]. *Azolla* has phytoremediation potential, due to its capability to concentrate heavy metals such as copper, cadmium, chromium, nickel, lead and nutrients directly from polluted water or sewage water [13, 16]. Phytoremediation technologies that use chemical chelates such as EDTA, DTPA might be unsustainable because these cause negative impacts on the soil and aquatic environment [2]. Therefore, utilization of *Azolla microphylla*, *A. pinnata* and *A. filiculoides* is more environmentally friendly and cost-effective [14, 41].

Azolla for Biofuel Production

Azolla is one of the rapid-growing plants on the earth, thus it can be considered as a potential source for bioenergy production. The chemical constituents of *Azolla* resemble combinations of terrestrial bioenergy crops and microalgae or cyanobacteria. In this context, *Azolla* is an attractive universal feedstock that is available at low cost, low energy demanding and near zero maintenance system for the production of biofuels [42]. The oil extraction amount from *Azolla* against another feedstock is acceptable as biodiesel [43]. *Azolla* or a combination of *Azolla* with rice straw can be fermented under anaerobic conditions thus results methane gas that can be utilized as a biofuel [20]. The ethanol production of *A. filiculoides* is about 11700 liters $ha^{-1}year^{-1}$ and it is close to the ethanol production from corn stover (13310 liters ha^{-1} $year^{-1}$), greater than from miscanthus (2300 liters ha^{-1} $year^{-1}$) and willow (300 liters ha^{-1} $year^{-1}$), while being lower than from sugarcane (25000 liters ha^{-1} $year^{-1}$) [42].

Azolla for Weed Control and Mosquito Control

Azolla cover can diminish the light intensity by around 90% and reduce photosynthesis in water. This can reduce the dissolved oxygen concentration of water by more than 50%. Besides, it alters the quality of light penetrating the water column. These effects can reduce the germination of light-sensitive seeds of different weeds [20]. Further, Azolla-incorporated rice fields have reported less weed levels, because of the *Azolla* dense cover on the surface of water. There are two possible mechanisms for weed suppression by *Azolla*; the most effectual method by the light-starvation of young seedlings of weeds [19] and by causing physical resistance to the emergence of seedlings of weeds due to dense *Azolla* mat, which does not affect the growth of rice [44].

Azolla forms a thick mat on the surface of water, which prevents mosquito breeding and adult emergence [12, 20]. According to previous studies, the breeding of *Anoplieles* spp. has been completely suppressed by the dense *Azolla* mats in water bodies [45]. *Azolla* is also recognized as the ‘mosquito fern’ due to its capability to reduce mosquito breeding.

Methods of Establishing Artificial Ponds to Grow A*zolla*

Azolla can be grown in artificial ponds or pits while providing optimum growing conditions. Artificial ponds can be created using concrete structures, rectangular or cylindrical in shape. Also, *Azolla* can be successfully grown in a pit of 7 to 10 inches deep, 3 feet wide, and 7-10 feet long. The pit should be placed in a shaded place [46-47] and should be covered using a plastic sheet to prevent leaching. In addition, 10-15 kg of soil, super-phosphate or animal dung (4-5 days old) should be added as a source of phosphorus. After, leaving the pit for 2-3 days to settle the soil, *Azolla* can be introduced. The addition of 2-3 kg of *Azolla* produces 1-3 kg of biomass daily after two weeks. Water (20-30%) should be changed every five days and super-phosphate or animal dung should be added. Also, the pit should be cleaned after 6 months to avoid the production of odours [47].

Limitations of Cultivating and using *Azolla*

Although Azolla has a prolific growth, the application rate of *Azolla* is 0.5 - 1.0 ton ha^{-1}, which limits the promotion of *Azolla* as a biofertilizer [48]. *Azolla* reproduction occurs mainly vegetatively, and this causes the necessity of maintaining *Azolla* biomass throughout the year. Therefore, farmers should carry a huge amount of fresh biomass to the agriculture fields at each time of application. It involves the transportation of

Azolla long distances and perishing the cultures. This reason leads to the poor adoption of *Azolla* by farmers [23]. *Azolla* is more prone to perish and cannot be stored for a long period [48]. Thus, quick application to the fields should be done to avoid deteriorating. Also, the tropical monsoon in Sri Lanka also destroys the *Azolla* cultivations, since heavy rainfall during this period may wash away the *Azolla* plants out of the pits.

Potential Strategies to Promote *Azolla* usage in Agricultural Field in Sri Lanka

Azolla is grown in various countries, including Sri Lanka, because of its vast majority of usage. However, the awareness on the cultivation and variety of benefits are lacking among farmers. Thus, local farmers should be given the necessary information about the technical know-how of growing *Azolla* and its usage in both crop cultivation and animal husbandry. Giving awareness and emphasizing its role as a sustainable and cost-effective source of feed for livestock and a natural biofertilizer for crop fields are some of the possible solutions to promote usage of *Azolla*. Cultivation of *Azolla* is an easy task because it can be grown in containers, either on a small or large scale. The most convenient size for the soil pits is 2m×1m×0.3m [49]. According to the Department of Animal Production and Health, Sri Lanka, the cost of production of 1 kg of *Azolla* can vary between Rs. 1.00-2.00 from one *Azolla* pit with the dimensions of 2 m×1 m×0.3 m within one production cycle of 8 months [49]. Availability of *Azolla* planting materials should be ensured, while conducting training programmes about the growing of *Azolla* [49]. Other than that, establishing demonstration farms, promoting research and development on *Azolla* cultivation and usages, providing financial incentives to farmers, integration into livestock systems, and information dissemination through technology are some other strategies to promote *Azolla* usage among farmer communities in Sri Lanka.

CONCLUSIONS

Against the background of economic challenges faced by Sri Lanka and the increasing concerns about synthetic fertilizers, *Azolla* emerges as a promising biofertilizer with multifaceted benefits to the agriculture sector. Its symbiotic relationship with *Anabaena azollae* makes it an efficient source of biological nitrogen, addressing the drawbacks associated with synthetic fertilizer alternatives. Beyond its role as a biofertilizer, versatility of *Azolla* extends to nutrient supplementation for animals, human consumption, phytoremediation, biofuel production, weed control, and even mosquito repellent properties. Numerous studies emphasize the efficacy of *Azolla*, positioning it as a viable and environmentally friendly alternative to synthetic fertilizers which pose environmental risks. With its rapid growth and sustainable harvesting, *Azolla* offers a reliable and sustainable solution for agricultural needs, during the economic crisis in Sri Lanka. Embracing *Azolla* as an agricultural resource not only addresses immediate concerns, but also aligns with the principles of environmental sustainability as green agriculture, marking it as a vital and sustainable asset for farmer communities in challenging times.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

AUTHORS' CONTRIBUTIONS

ISW: Conceptualized the study and wrote the manuscript. SRA: Conceptualized the study, wrote the manuscript and reviewed the manuscript. All authors read and approved the manuscript.

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APPLIED BIO-SYSTEMS TECHNOLOGY

Research Article Open Access

Formulation and Characterization of a Novel Carrot-based Sandwich Spread

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Abstract

Background: A necessity for developing vegetable-based sandwich spreads has arisen due to the growing number of vegans and the high cost of non-vegan spreads. Thus, the current study aimed to formulate and characterize a carrot-based sandwich spread, in terms of sensory and physicochemical properties.

Methods: Carrot-based sandwich spreads were developed, incorporating a spice mixture, white sauce, garlic paste, and mayonnaise, according to the results of sensory analysis. The variation of the color attributes and pH with time was investigated using a handheld colorimeter and benchtop pH meter, respectively. Shelf-life of the spreads was evaluated using the plate count method. Proximate analysis was carried out using AOAC methods. Sensory data were analysed using the Friedman test, while parametric data were analyzed using Analysis of Variance (ANOVA).

Results: Carrot-based sandwich spreads incorporated with spices, garlic paste, and white sauce exhibited the highest scores for the sensory attributes evaluated in this study. As expected, the physicochemical properties, especially colour, showed significant differences among the different formulations ($P<0.05$). The addition of chemical preservatives – potassium sorbate or sodium benzoate - showed no effect on the pH or colour of the best sandwich spreads and their temporal variation. Further, the shelf-life of the spreads increased to approximately 7 days under refrigerating conditions due to the addition of chemical preservatives.

Conclusions: The addition of spices, garlic paste, and white sauce resulted in the most preferred carrot-based sandwich spread. Additional steps need to be taken for colour preservation and increasing the microbial safety for extending the shelf-life.

Keywords: Carrot, Physicochemical Properties, Proximate Composition, Sandwich Spread, Sensory Properties

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INTRODUCTION

Ready-to-serve convenience foods have secured high demand globally mainly due to the socio-economic changes that have taken place over the last decades. Among the numerous convenience foods in the market, sandwich spreads stand out, since sandwiches are the main food that constitutes the diet of millions of people worldwide. In fact, An *et al.* (2016) reported that "approximately 53.2% of US adults consumed sandwiches on any given day" during the period 2003 – 2012 [1]. Further, the authors revealed that sandwich consumption provided about a quarter of the daily calorie intake and one-third of the fat intake [1-2]. The most popular sandwich spreads are jams, jellies, marmalades, butter, margarine, and cheese spreads that are of either high sugar or fat content [2]. In order to develop healthier spreads, research on meat-based and vegetable-based sandwich spreads has been carried out to a reasonable extent [3-5]. Reports on vegetable-based spreads such as carrot-based spreads are, however, scanty.

Carrot (*Daucus carota* L.) is a root vegetable belonging to the family Apiaceae. Apart from being a rich source of carbohydrates, carrot is a good source of minerals including Fe, Ca, Mg, and P. Further, this vegetable possesses numerous bioactive compounds including β-carotene with provitamin A activity, ascorbic acid, tocopherol, and anthocyanins [6]. Due to these active ingredients, carrot imparts numerous health benefits such as cancer inhibition, muscular degeneration prevention, decreased cataract formation, and cardiovascular disease prevention [6]. Further, the fiber content of raw carrots is approximately 2.8 g in 100 g [7-8]. Consumption of fiber leads to possible health effects such as the prevention or risk reduction of certain types of cancers, prevention of constipation, regulation of blood glucose levels, and shielding against heart disease [9]. Hence, carrot not only function as the base of sandwich spreads, but also may impart nutritive and functional properties to the spreads.

The role of food additives is multifaceted. Among the numerous types of food additives, chemical preservatives have become indispensable in the food industry [10]. The chemical preservatives used in this study were potassium sorbate (PS) and sodium benzoate (SB), which possess antifungal and antibacterial activities. Instances where PS has increased the shelf-life of foods are many. For example, 0.1% of PS has extended the shelf-life of chocolate cake of pH 6 – 7 inoculated with the fungal species *Penicillium citrinum* for 45 days [11]. Further, cooked ground fish inoculated with *Staphylococcus aureus* has shown an increased lag time of bacterial growth with the addition of PS [12]. Moreover, combinations of PS and other preservatives have improved the microbial safety and other quality attributes of foods [13]. SB, like PS, has increased the shelf-life of numerous foods including bread, cake, carbonated beverages, and mayonnaise [14]. Moreover, blends of sodium benzoate with other preservatives have exhibited synergistic antimicrobial potentials [15].

The aim of this study was to develop and evaluate the time-dependent variation of the quality of carrot-based sandwich spreads. Thus, carrot-based sandwich spreads were developed following sensory analysis. The physicochemical properties and microbial quality of the spreads were evaluated for 14 days thereby determining the shelf-life of the spreads. Also, the proximate composition of the spreads was analyzed. Directions for further improvements are finally given.

METHODOLOGY

Materials

Carrot (*Daucus carota* var. New Kuroda), corn flour, gelatin, mustard, salt, pepper, turmeric, sugar, butter, wheat flour, fresh milk, garlic, chilli flakes, coconut oil, eggs, lime juice, vinegar, curry leaves, potassium sorbate and sodium benzoate were purchased from a local retail shop. Fresh milk was pasteurized before use. Curry leaves were dried at 50 – 60 °C for

4 – 5 h in a commercial dehydrator before use.

Experiment 1: Selection of the Best Sandwich Spread Base

A sandwich base was prepared using carrot. Fixed quantities of carrot pulp (60 g) and water (100 mL) were used. For the carrot-based sandwich spread, the three different gelatin-corn flour masses used were 3 g – 4 g (C1), 3.5 g – 3.5 g (C2) and 4 g – 3 g (C3). The mixture was then cooked at 60 °C for 10 min. The resulting carrot spreads were then filled into sterilized glass jars and cooled before storing in the refrigerator.

Experiment 2: Selection of the Best Combination of Spices, Sugar, and Salt

Three combinations of mustard (M), pepper (P), sugar (SR), and salt (ST) were added to the best sandwich base selected from Experiment 1. For the carrot-based spread, the combinations (M-P) used were 0.3 g – 0.3 g (C4), 0.5 g – 0.3 g (C5) and 0.3 g – 0.5 g (C6). The quantities of sugar and salt added were 0.3 g and 2 g, respectively. The best carrot-based sandwich spread was selected via sensory analysis.

Experiment 3: Selection of the Best White Sauce Level

Three different quantities of white sauce were added to the best sandwich spreads selected from Experiment 2. The white source was formulated using butter (30 g), wheat flour (30 g), fresh milk (240 mL), salt (1.8 g) and pepper (0.5 g). The quantities of white sauce added to the carrot-based sandwich spread (60 g of carrot pulp) were 15 g (C7), 20 g (C8) and 25 g (C9). The best sandwich spread was selected via sensory analysis.

Experiment 4: Selection of the Best Garlic Paste Level

Under Experiment 4, three different quantities of garlic paste were added to the best sample identified from Experiment 3. The garlic paste was prepared using garlic paste (70 g), sugar (3 g), salt (3 g), curry leaves powder (5 g), chilli flakes (5 g), and vegetable oil (30 mL). The quantities of garlic paste added to the carrot-based sandwich spread were 1 g (C10), 3 g (C11) and 5g (C12). The best sandwich spread was selected via a sensory analysis.

Experiment 5: Selection of the Best Mayonnaise Level

The best garlic paste level selected from Experiment 4 was added to the best sandwich spreads from Experiment 2 to which three different quantities of mayonnaise were added. Mayonnaise was prepared using egg yolk (1), egg white (2), vinegar (1.5 tbsp), vegetable oil (250 mL), lime juice (1 tsp), salt (¼ tsp), pepper (½ tsp), mustard (¼ tsp), sugar (½ tsp) (tbsp: tablespoon; tsp: teaspoon). The quantities of mayonnaise added to the carrot-based sandwich spread were 10 g (C13), 15 g (C14) and 20 g (C15). The best sandwich spread was selected via a sensory analysis.

Experiment 6: Selection of the Best Sandwich Spread

The carrot-based sandwich spreads used for the determination of the best sandwich spread were the spreads selected from Experiments 2-5 and the control (CC) which was boiled carrot slices. The best carrot-based sandwich spread was selected using sensory analysis.

Sensory Evaluation

The best sandwich spreads selected from the above experiments (Experiments 2 to 5) were subjected to a sensory analysis to identify the best sandwich spread, while boiled carrot slices were used as the control. The sensory attributes considered were appearance, color, density, mouthfeel, texture, taste, aroma, flavor, and overall quality. Purchasing intention was also considered. A semi-trained panel of 30 panelists was used in the sensory evaluation. A five-point hedonic scale ranging from 5: like extremely to 1: dislike extremely was used during the assessment.

Determination of pH

The pH value of the sandwich spreads was tested using a benchtop pH meter (BP3001, Trans Instruments). The best carrot-based spreads selected from Experiments 2-5 were tested for pH. Further, the pH of the final product that contained no artificial preservatives, and the final product with added potassium sorbate (PS) and added sodium benzoate (SB) were tested for 14 days with 7-day intervals. Let the carrot-based spread with added PS be C-PS, and that with added SB be C-SB.

Determination of Colour

The colour of the sandwich spreads was tested using a handheld colorimeter (PCE-CSM4, PCE Instruments). The best carrot-based spreads selected from Experiments 2-5 were tested for colour. In addition, the colour of the final products, C-PS and C-SB, was measured for 14 days with 7-day intervals.

Microbial Count

The best sandwich spreads selected from Experiment 6 were subjected to a microbial analysis, with and without chemical preservatives. Microbial analyses were carried out twice at 7-day intervals, using the tenfold serial dilution method according to the procedure described by Maturin and Peeler in the Bacteriological Analytical Manual Online (BAM) [16]. Three replicates from each treatment were used for the analysis. The growth medium used for the yeast and mold count was Potato Dextrose Agar and that used for the bacterial count was Nutrient Agar. The plates were incubated at 37 °C in the incubator. The colony count was taken from a Quebec Dark Field Colony Counter.

Proximate Analysis

Proximate analysis was carried out for the best products selected from Experiment 6. The percentages of moisture, ash, fat, and protein were analyzed using the AOAC (2010) methods [17]. Briefly, the moisture and ash contents were determined using gravimetric methods, the fat content was determined using the Soxhlet method, and the protein content was determined using the Kjeldahl method. Meanwhile, the reducing sugar content was determined using a titrimetric method [18]. The carbohydrate content was calculated as described by Diddana *et al*. (2021) [19].

Statistical Analysis

Data from sensory evaluations were analyzed using the Friedman Test, while quantitative data (physicochemical data) were analyzed using the Analysis of Variance (ANOVA). The Minitab (version 20) was used for the statistical analysis.

RESULTS AND DISCUSSION

Experiment 1: Selection of the Best Sandwich Spread Base

There was a significant difference ($P<0.05$) among the three carrot-based sandwich spreads for all sensory attributes except appearance and aroma. C1 (carrot paste 60 g, gelatin 3 g, corn flour 4 g) showed the highest mean ranks for all the sensory attributes. Therefore, C1 was selected for further analysis. These results suggested that the best gelatin and corn flour quantities are 3 g and 4 g, respectively for the preparation of a sandwich spread using carrot as the base (60 g). The mean scores for the sensory attributes are shown in Table 1.

Experiment 2: Selection of the Best Combination of Spices, Sugar, and Salt

All sensory attributes of the three carrot-based sandwich spreads were not significantly different ($P<0.05$) at 95% confidence level. The mean scores for the sensory attributes considered in this experiment are shown in Table 2. C5 (M – 0.5 g, P – 0.3 g, SR – 0.3 g, ST 2 g) showed the highest mean rank for the overall quality in addition to texture, mouthfeel, and flavor. Therefore, C5 was selected for further analysis. These results indicate that mustard (0.5 g) and pepper (0.3 g) are ideal in sandwich spreads with carrot as the base (60 g of pulp).

Experiment 3: Selection of the Best White Sauce Level

There was a significant difference ($P>0.05$) among the three carrot-based sandwich spreads for flavor and overall quality. Table 3

Table 1: Mean Scores for the Sensory Attributes of Sandwich Spreads (Experiment 1)

Sensory Attribute	Sandwich Spread			P
	C1	C2	C3	
Spreadability	4.367 ± 0.122	3.500 ± 0.171	3.800 ± 0.139	0.000
Texture	4.100 ± 0.100	3.700 ± 0.128	3.967 ± 0.131	0.026
Appearance	4.333 ± 0.088	4.133 ± 0.124	4.033 ± 0.112	0.059
Colour	4.533 ± 0.104	4.233 ± 0.164	4.167 ± 0.157	0.020
Aroma	3.533 ± 0.150	3.300 ± 0.119	3.333 ± 0.130	0.166
Density	4.100 ± 0.121	3.733 ± 0.143	3.500 ± 0.115	0.007
Overall quality	4.367 ± 0.102	3.800 ± 0.121	3.600 ± 0.123	0.001

Note: *Values are expressed as mean ± SE. Gelatin-corn flour masses: 3 g – 4 g (C1), 3.5 g – 3.5 g (C2) and 4 g – 3 g (C3) in 100 mL*

Table 2: Mean Scores for the Sensory Attributes of Sandwich Spreads (Experiment 2)

Sensory Attribute	Sandwich Spread			P
	C4	C5	C6	
Texture	3.667 ± 0.154	3.733 ± 0.197	3.700 ± 0.160	0.909
Appearance	3.867 ± 0.142	3.833 ± 0.167	3.767 ± 0.171	0.808
Colour	4.033 ± 0.140	3.967 ± 0.162	3.900 ± 0.154	0.662
Aroma	3.500 ± 0.157	3.300 ± 0.187	3.433 ± 0.164	0.640
Density	3.567 ± 0.149	3.500 ± 0.164	3.500 ± 0.178	0.732
Mouthfeel	3.600 ± 0.156	3.667 ± 0.200	3.433 ± 0.141	0.912
Flavour	3.567 ± 0.124	3.600 ± 0.189	3.600 ± 0.141	0.065
Overall quality	3.567 ± 0.164	3.733 ± 0.197	3.500 ± 0.150	0.868

Note: *Values are expressed as mean ± SE. Mustard and pepper masses for 60 g of pulp: 0.3 g – 0.3 g (C4), 0.5 g – 0.3 g (C5) and 0.3 g – 0.5 g (C6).*

Table 3: Mean Scores for the Sensory Attributes of Sandwich Spreads (Experiment 3)

Sensory Attribute	Sandwich Spread			P
	C7	C8	C9	
Texture	3.600 ± 0.177	3.867 ± 0.164	3.667 ± 0.111	0.382
Appearance	3.467 ± 0.184	3.833 ± 0.160	3.500 ± 0.171	0.147
Colour	3.667 ± 0.205	3.867 ± 0.196	3.867 ± 0.202	0.528
Aroma	3.467 ± 0.171	3.567 ± 0.164	3.867 ± 0.171	0.133
Density	3.567 ±0.171	3.933 ± 0.151	3.733 ± 0.159	0.064
Mouthfeel	3.400 ± 0.177	3.767 ± 0.164	3.800 ± 0.151	0.073
Taste	3.433 ± 0.157	3.833 ± 0.160	3.700 ± 0.119	0.168
Flavour	3.367 ± 0.206	3.767 ± 0.164	3.867 ± 0.164	0.043
Overall quality	3.300 ± 0.167	3.767 ± 0.177	3.967 ± 0.131	0.054

Note: *Values are expressed as mean ± SE. White source masses for 60 g of pulp: 15 g (C7), 20 g (C8) and 25 g (C9).*

shows the mean scores for the sensory attributes considered in this experiment. The C9 (white sauce 25 g) treatment showed the highest mean rank for flavour, and overall acceptability. Thus, C9 was selected for further analysis. These results show that incorporating 25 g of white sauce for 60 g of carrot pulp presents favourable sensory attributes to sandwich spreads.

Experiment 4: Selection of the Best Garlic Paste Level

There was a significant difference (P>0.05) among the three carrot-based sandwich spreads for all attributes. The mean scores for the sensory attributes considered in this study are shown in Table 4. Since C11 (garlic paste 3 g) showed the highest mean score for all the attributes, it was selected for further analysis.

Experiment 5: Selection of the Best Mayonnaise Level

Significant differences (P<0.05) were observed among the three carrot-based sandwich spreads for all the sensory attributes (Table 5). C14 (mayonnaise 15 g), which showed the highest mean ranks for all the sensory attributes was selected for the final sensory evaluation. These results indicate that the addition of 15 g of mayonnaise tend to elevate the flavour of sandwich spreads.

Table 4: Mean Scores for the Sensory Attributes of Sandwich Spreads (Experiment 4)

Sensory Attribute	Sandwich Spread			P
	C10	C11	C12	
Texture	3.467 ± 0.142	4.333 ± 0.138	3.400 ± 0.123	0.000
Appearance	3.400 ± 0.149	4.667 ± 0.088	3.433 ± 0.141	0.000
Colour	3.200 ± 0.162	4.333 ± 0.121	3.500 ± 0.115	0.000
Aroma	3.400 ± 0.113	4.300 ± 0.128	3.200 ± 0.176	0.000
Density	3.200 ± 0.139	4.167 ± 0.136	3.033 ± 0.148	0.000
Mouthfeel	3.267 ± 0.151	4.133 ± 0.124	3.400 ± 0.149	0.000
Taste	3.333 ± 0.111	4.333 ± 0.111	3.767 ± 0.141	0.000
Flavour	3.200 ± 0.121	4.367 ± 0.102	3.600 ± 0.132	0.000
Overall quality	3.267 ± 0.117	4.633 ± 0.090	3.367 ± 0.131	0.000

Note: *Values are expressed as mean ± SE. Garlic paste masses for 60 g of pulp: 1 g (C10), 3 g (C11) and 5 g (C12).*

Table 5: Mean Scores for the Sensory Attributes of Sandwich Spreads (Experiment 5)

Sensory Attribute	Sandwich Spread			P
	C13	C14	C15	
Texture	3.733 ± 0.135	4.333 ± 0.133	3.600 ± 0.141	0.001
Appearance	3.733 ± 0.143	4.333 ± 0.111	3.800 ± 0.162	0.002
Colour	4.033 ± 0.140	4.400 ± 0.103	3.800 ±0.155	0.021
Aroma	3.833 ± 0.145	4.167 ± 0.136	3.633 ± 0.155	0.059
Density	3.733 ± 0.179	4.100 ± 0.121	3.667 ± 0.146	0.011
Mouthfeel	3.667 ± 0.200	4.100 ± 0.121	3.633 ± 0.148	0.032
Taste	3.633 ± 0.155	4.300 ± 0.109	3.833 ± 0.160	0.023
Flavour	3.700 ± 0.180	4.367 ± 0.122	3.700 ± 0.174	0.005
Overall quality	3.500 ± 0.142	4.400 ± 0.091	3.500 ± 0.171	0.000

Note: *Values are expressed as mean ± SE. Mayonnaise masses for 60 g of pulp: 10 g (C13), 15 g (C14) and 20 g (C15).*

Experiment 6: Selection of the Best Carrot-Based Sandwich Spread

The final sensory evaluation was carried out using the control sample (CC) and the four best samples selected from Experiments 2-5 (i.e. C5, C9, C11, and C14). According to the Friedman analysis, there were significant differences (P<0.05) in sensory attributes among the five formulations (Figure 1). The sandwich spread produced under the C11 treatment showed the highest mean scores for all the sensory attributes considered in this study, namely appearance (4.300 ± 0.137), colour (4.367 ± 0.131), density (4.367 ± 0.140), mouthfeel (4.500 ± 0.104), texture (4.333 ± 0.138), taste (4.533 ± 0.124), aroma (4.233 ± 0.124), flavour (4.567 ± 0.092), and overall quality (4.633 ± 0.102); hence it was selected as the best carrot-based product. As expected, the mean rank of purchasing intention for C11 (4.733 ± 0.095) was significantly higher (P<0.05) than that for other carrot-based products (< 3.500).

pH and Colour of the Selected Sandwich Spreads

The mean pH values of the different sandwich spreads developed in this study are indicated in Table 6. The pH values of the different carrot-based sandwich spreads were significantly different from each other (P<0.05). However, the pH of the sandwich spreads was within the range of pH 5.5 - 5.8. These results indicate that the incorporation of white sauce, mayonnaise, or garlic paste alters the pH of the carrot-based sandwich spreads only within a narrow range.

The colour (lightness, redness, and yellowness) of the sandwich spreads is given in Table 1. As expected, the incorporation of white sauce resulted in a significant increase in the lightness of carrot-based sandwich spreads (P<0.05). According to sensory analysis, the colour of the white sauce-added sandwich spread with higher lightness was preferred over that of the mayonnaise-added sandwich spread. The redness and yellowness of the carrot-based sandwich spread decreased with the addition of white sauce (C11). In contrast, the redness and yellowness of the carrot-based sandwich remained the same with the incorporation of mayonnaise, as shown in Table 6. Nevertheless, C11 treatment, which contained spices, white sauce, and garlic paste, in addition to the base, was the most preferred sandwich spread concerning the colour.

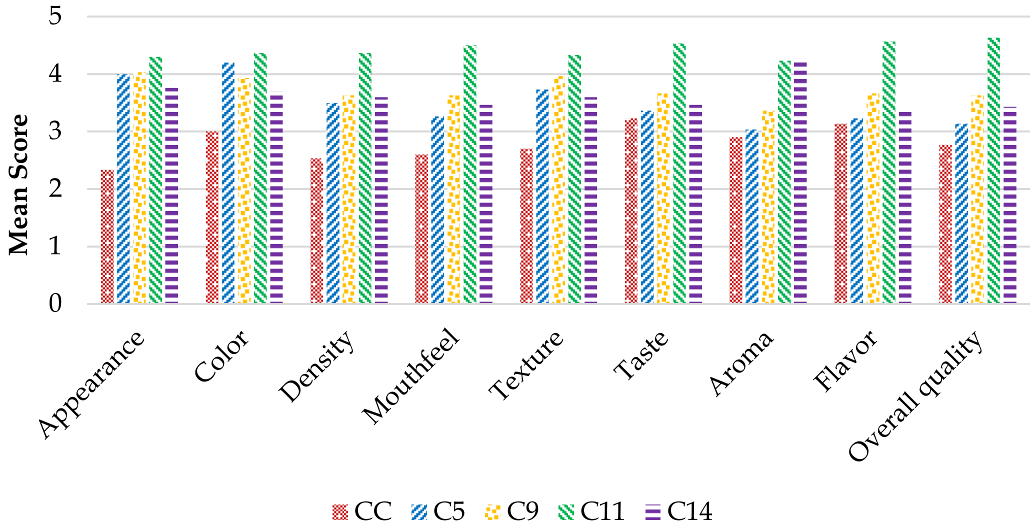

Figure 1: Mean Scores of Carrot-based Sandwich Spreads for Sensory Attributes

Note: *CC: boiled carrot slices, C5: sandwich spread with spices, C9: sandwich spread with spices and white sauce, C11: sandwich spread with spices, white sauce, and garlic paste, C14: sandwich spread with spices, mayonnaise, and garlic paste. Each sensory attribute of the five carrot spreads showed a significant difference (P<0.05).*

Table 6: Variation of pH and Colour Attributes among Sandwich Spreads

Sandwich Spread	pH	Colour Coordinates		
		L*(Lightness)	a*(Redness)	b*(Yellowness)
C5	$5.64^{b} \pm 0.06$	$20.42^{b} \pm 1.99$	$16.45^{a} \pm 2.00$	$105.92^{a} \pm 2.50$
C8	$5.83^{a} \pm 0.02$	$41.76^{a} \pm 3.74$	$7.28^{b} \pm 0.99$	$88.91^{b} \pm 1.57$
C11	$5.47^{c} \pm 0.01$	$40.99^{a} \pm 2.51$	$2.17^{b} \pm 0.99$	$49.07^{c} \pm 2.12$
C14	$5.47^{c} \pm 0.01$	$36.34^{a} \pm 3.00$	$15.57^{a} \pm 3.46$	$111.81^{a} \pm 7.64$

Note: *Values are expressed as mean ± SE. Means with the same letter superscripts within each column of each block are not significantly different at the 0.05 level. C: carrot-based sandwich spread, C5: with spices, C8: with spices and white sauce, C11: with spices, white sauce, and garlic paste, C14: with spices, mayonnaise, and garlic paste.*

Temporal Variation of pH and Colour of the Best Sandwich Spreads with or Without Preservatives

Variation of pH

Table 2 shows the variation of pH and colour of sandwich spreads stored in refrigerating conditions with time. The pH of the carrot-based sandwich spreads, i.e. the best carrot-based sandwich spread with no preservatives (C11), C11 incorporated with potassium sorbate (C11-PS) and C11 incorporated with sodium benzoate (C11-SB), decreased significantly with time ($P<0.05$). However, this variation occurred within a narrow range (pH 6.17 to 6.38). The regression equations revealed that the decrease of pH with time occurred gradually with different slopes. The regression equations for the variation of the pH of the three treatments with time (in days) are as follows.

C11 : $pH = 6.3583 - 0.0145\,Time$ (Adjusted R^2 = 85.26%)

C11-PS : $pH = 6.3606 - 0.0064\,Time$ (Adjusted R^2 = 95.72%)

C11-SB : $pH = 6.3639 - 0.0079\,Time$ (Adjusted R^2 = 94.38%)

As expected, there was no significant difference ($P>0.05$) in the pH of the potassium sorbate and sodium benzoate added sandwich spreads. However, those pH values were lower than that of C11. The mean pH values of the treatments over the 14-day period were close and were as follows: C11: pH 6.18 ± 0.00, C11-PS: pH 6.27 ± 0.01, and C11-SB: pH 6.26 ± 0.01.

Variation of Colour with Time

The variation of colour of the most preferred carrot-based spreads, with or without preservatives, is shown in Table 7. The lightness of the three carrot-based sandwich spreads was not significantly different ($P=0.972$). However, the lightness decreased significantly in the first seven days, after which it increased to a value higher than the original value ($P<0.001$). This experiment revealed that the incorporation of the preservatives (i.e. PS and SB) has no effect on the lightness of the sandwich spreads and the variation of the lightness of the sandwich spreads with time.

The redness of the three carrot-based sandwich spreads was not significantly different ($P>0.05$) and increased significantly with time ($P<0.001$). The results revealed that the incorporation of the preservatives (i.e. PS and SB) had no effect on the redness of the sandwich spreads and the variation of the redness of the sandwich spreads with time. Similar to the lightness and redness, the yellowness of the three carrot-based sandwich spreads also did not differ significantly ($P<0.05$). The yellowness increased in the first week, after which it decreased partially ($P<0.001$). In fact, the yellowness decreased in the first week after which it increased sharply to a value higher than the original one. These results reveal that the incorporation of the preservatives (i.e. PS and SB) has no effect on the yellowness of the sandwich spreads and the variation of the yellowness with time.

Table 7: Variation of pH and Colour of the Best Sandwich Spreads with Time

Sandwich Spread	Time (Days)	pH	Colour Coordinates		
			L* (Lightness)	a* (Redness)	b* (Yellowness)
C11	0	$6.38^{a} \pm 0.01$	$40.92^{b} \pm 2.58$	$3.80^{b} \pm 1.49$	$48.60^{c} \pm 2.06$
	7	$6.21^{b} \pm 0.01$	$30.62^{b} \pm 1.98$	$8.37^{b} \pm 1.59$	$94.99^{a} \pm 2.23$
	14	$6.17^{c} \pm 0.00$	$52.66^{a} \pm 7.11$	$22.23^{a} \pm 3.75$	$73.06^{b} \pm 5.27$
C11-PS	0	$6.36^{a} \pm 0.01$	$47.25^{a} \pm 4.13$	$3.37^{b} \pm 1.88$	$46.37^{c} \pm 4.08$
	7	$6.31^{b} \pm 0.00$	$29.17^{b} \pm 4.51$	$8.08^{b} \pm 1.65$	$100.58^{a} \pm 4.98$
	14	6.27c ± 0.01	$50.22^{a} \pm 4.37$	$31.56^{a} \pm 3.70$	$85.82^{b} \pm 5.00$
C11-SB	0	$6.36^{a} \pm 0.01$	$41.00^{ab} \pm 8.00$	$4.26^{c} \pm 2.06$	$43.86^{c} \pm 6.71$
	7	$6.30^{b} \pm 0.00$	$30.69^{b} \pm 2.02$	$12.16^{b} \pm 3.27$	$100.43^{a} \pm 2.49$
	14	$6.25^{c} \pm 0.01$	$51.57^{a} \pm 2.96$	$21.50^{a} \pm 3.46$	$71.36^{b} \pm 7.00$

Note: *Values are expressed as mean ± SE. The means with the same letter superscripts within each column of each block are not significantly different at the 0.05 level. C11: Carrot-based sandwich spread with spices, white sauce, and garlic paste, PS: Potassium sorbate, SB: Sodium benzoate*

Concisely, the addition of food preservatives – potassium sorbate or sodium benzoate – at a concentration of 0.1% (w/w) had no effect on the color and on the time-dependent color variation of carrot-based sandwich spreads.

Shelf-life of Sandwich Spreads: Microbial Analysis

The sandwich spreads developed in this study were not heat treated to minimize the deterioration of heat-sensitive compounds in carrot or beetroot. Instead, permitted levels (i.e. 0.1% w/w) of potassium sorbate or sodium benzoate were used. Microbial analysis was conducted based on the total plate count and yeast and mold count of the sandwich spreads stored at refrigerating temperature for 14 days.

There was a microbial colony growth within the first week in C11, which had no preservatives. Meanwhile, no microbial colony growth was observed in preservative-added samples (C11-PS, C11-SB) after 7 days. However, bacteria, and yeast and mold overgrowth were observed by day 14. These results indicated the development of a lag phase of microbial growth due to the incorporation of the preservatives PS and SB. The spread with no preservatives had a relatively shorter shelf life than the preservative-added ones. The shelf life of the spreads with no preservatives may be only a few days, while that of the spreads with preservatives may be approximately 7 days. These results highlight the need for heat processing of the sandwich spreads despite the damage it might cause to heat-sensitive compounds.

Proximate Composition

The proximate composition of the best carrot-based sandwich spread (C11) is given in Table 8.

Table 8: Proximate Composition of the Best Sandwich Spread

Food Constituent	Quantity (w/w %)
	Carrot-based Sandwich Spread (C11)
Moisture	87.8
Ash	1.6
Fat	1.9
Protein	1.6
Reducing sugars	1.8
Total carbohydrate	7.1

Note: *C11: The best carrot-based sandwich*

Favourably, the carrot-based sandwich spread developed in this study contained significantly lower fat and sugar contents than other sandwich spreads such as butter, cream cheese, or jam [20-22]. Hence, the carrot-based sandwich spread developed in this study can be identified as a healthier spread.

CONCLUSIONS

This study highlights an opportunity to increase the sensory attributes of carrot-based sandwich spreads by incorporating spices, garlic paste, white sauce, or mayonnaise. The incorporation of spices, garlic paste, and white sauce in carrot-based sandwich spreads enhanced the sensory properties of the spreads, which substantially led to higher purchasing intention of the product. Although it was expected that the addition of chemical preservatives, i.e. potassium sorbate or sodium benzoate, may lead to a substantial increase in the shelf-life, the addition of preservatives was ineffective in maintaining the physicochemical properties of the sandwich spreads.

However, the shelf-life of the sandwich spreads increased as a result of the addition of chemical preservatives to the sandwich spreads according to the results of microbial analysis. Specifically, a lag phase in the growth of microbes, which may be enhanced by incorporating blends of antimicrobial agents, was observed due to the addition of potassium sorbate and sodium benzoate. As the sugar and fat contents of the carrot-based sandwich spreads are significantly lower than those of commonly used sandwich spreads, the carrot-based sandwich spread developed in this study can be called a ‘healthy spread’.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS’ CONTRIBUTIONS

IW: Investigation, Data curation, Analysis of data. GP: Conceptualization, Analysis of data, Writing the manuscript, and Supervision. MA: Investigation, Data curation, Analysis of data.

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APPLIED BIO-SYSTEMS TECHNOLOGY

Research Article Open Access

Development of a Banana Peel Powder Incorporated Ice Cream and Ice Cone Product

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Abstract

Background: Banana peel powder remains as one of the most nutritive waste products that can be utilized as a functional additive. Therefore, the current study aimed to develop a banana peel powder incorporated ice cream and ice cone product with a unique flavour profile, while enhancing the nutritional content.

Methods: Ice cream and ice cone were formulated by incorporating varying levels of banana peel powder. The samples that showed the best sensory attributes were selected. The Kruskal–Wallis test, was used to analyse the significance of differences among the sensory attributes. Proximate analysis was conducted for the best performing ice cream and ice cone products. The microbiological analysis including total plate count and yeast and mould count was performed at 24, 48, 72 hours for ice cream by comparing it against a commercial ice cream product.

Results: The ice cream produced with 15 gL^{-1} of banana peel powder (Treatment 2) and ice cone produced with 67 gkg^{-1} banana peel powder (Treatment 3) showed the highest mean scores for overall acceptability. The proximate composition of the best ice cone product reported 8.3±4.2% (w/w) of crude fibre, while the ice cream (Treatment 2) accounted for a fibre content of 0.65±0.01% (w/w). The ice cream developed in this study (Treatment 2) showed a better microbial safety as revealed by the lower total plate count and yeast and mould counts.

Conclusions: The banana peel powder-incorporated ice cream (T2) and ice cone (T3) developed in this study show greater fibre content, microbial safety, and shelf life than commercial products. Thus, the products developed in this study may serve as better alternatives for ice cream and ice cone available in the market.

Keywords: Banana Peel Powder, Ice Cream, Ice Cone, Proximate Analysis, Sensory Attributes

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INTRODUCTION

Food is one of the basic necessities of life. It is the main nutrient source that supplies essential nutrients for our growth, development, and maintenance of healthy life. Limited availability and accessibility to nutritious food have led to elevated risks of noncommunicable diseases among the population [1, 2]. With this growing interest on the avoidance of noncommunicable diseases and ensuring health and wellness, consumers are focusing more on vitamins, minerals, bioactive substances, and fibre in food items, in addition to taste. Therefore, the customer demand for functional food has increased rapidly around the world recently. Functional foods are developed by removing, or adding one or more functional components or modifying the bioavailability of the components of a food. These are enriched with various physiologically active compounds capable of providing various health benefits beyond the conventional nutritional properties to achieve a balanced diet for optimal wellness [3, 4]. Currently, the global consumer demand for functional foods is increasing by 7-10% per year [5].

The utilization of agricultural by-products for food production has gained an increasing attention across the world [4]. Numerous studies have warranted the ability of incorporating agricultural by-products to elevate the nutritional value and sustainability of food [2, 5]. Indirectly, this approach is capable of contributing to the ensuring of food security. Therefore, utilization of food waste in the formulation of functional foods has become a novel trend. This can further reduce waste, save resources, and establish a more efficient food system by converting these leftovers into useful resources [6].

Banana (*Musa balbisiana*) is one of the most widely consumed tropical and subtropical crops, which remains as a significant fruit at the global level [7]. The overall consumption of banana has shown a notably increasing trend within the last two decades, where the global banana production has reached up to around 117 million tonnes in 2019 [8]. Banana peel is a significant waste created during the processing of fresh bananas. After the fruit has been peeled, these peels are usually thrown away as waste. The weight of a banana peel is around 30 to 40 g/100 g of the total weight, accounting for around 40% of the fresh weight, and is regarded as an industrial/agricultural by-product [7]. At present, many developing countries including Sri Lanka are generating notable amounts of banana peels as waste products [9].

Banana peel is rich in dietary fibre, minerals, and bioactive substances, all having positive effects on human health. Dietary fibre is an essential component of the human diet. The inadequate intake of fibre could lead into numerous health issues such as constipation, haemorrhoids, diverticulitis, overweight and obesity, heart diseases, diabetes, and bowel cancer [7]. Banana peels are having around 12% (w/w) of fibre content (dry weight basis), which will indeed be an excellent ingredient in the development of such high-fibre functional foods [8, 10]. Minerals are micronutrients, essential for human health due to their significance in cellular and physiological functions, including strengthening teeth and bones, growth, maintenance and repair of all tissue cells in the human body, enzyme secretion, regulating nerve functions, and optimizing the immune system [11, 12]. Banana peel contains notable amounts of minerals such as potassium, calcium, sodium, iron, manganese, and zinc.

Apart from fibre and minerals, banana peel is rich in bioactive agents including phenolic compounds (flavanols, hydroxycinnamic acids, catecholamine) that induce health benefits such as cardiovascular disease prevention, cancer prevention, diabetes prevention, and obesity prevention. Further, carotenoids such as lycopene, alpha-carotene, and beta-carotene are present in banana peels that prevent liver problems (hepatocellular carcinoma, cirrhosis, chronic hepatitis, hepatic steatosis, and acute hepatitis), aging-related disorders, susceptibility to atherosclerosis, cataracts, cancer, and oxidative damage to cells [8-9, 11]. Therefore, banana peel has been identified as an ideal functional ingredient readily available in many tropical and sub-tropical countries [13-14].

Conversion of banana peel into powder is one of the widely used approaches to formulate an easily usable ingredient from this valuable byproduct. The banana peel powder can be easily incorporated into food products at standardized compositions to derive various functional foods at the household and industrial levels [8-10]. With this background, numerous studies have attempted to develop a variety of banana peel powder incorporated functional foods ranging from biscuits, cookies, chapatti, chicken sausage, ground chicken and fish patties, Egyptian flatbreads, pasta and noodles, etc. [8-16]. Banana peel powder has the potential to be added as an ingredient in ice cream and ice cone recipes, since it would give the classic frozen sweet touch. Ice cream can be incorporated with banana flavour, while elevating the nutritional value. Therefore, the current study aimed to develop a novel ice cream product along with an ice cone incorporated with banana peel powder, as a functional food.

METHODOLOGY

Materials

Fresh cow milk and butter of acceptable organoleptic and microbial quality were purchased from a reputed local supplier (Kothmale Holdings PLC, Sri Lanka). Sugar (Orient Impex (Pvt) Ltd.), milk powder (Palwatte Dairy Industries Ltd.), corn flour (Motha Confectionary Works (Pvt) Ltd.), gelatine (Motha Confectionary Works (Pvt) Ltd.), vanilla essence (Delmage Forsyt Mills Ltd.) and eggs were purchased from reputed a local food store (COOP City, Pannala, Sri Lanka). Food grade calcium chloride and lecithin were also obtained from a reputed food ingredient store (Pettah Essence Suppliers, Colombo, Sri Lanka). Banana was obtained from an organic banana cultivation.

Preparation of Banana Peel Powder

Organically produced ripe Cavendish banana variety was selected for the banana peel powder production. The banana peels were taken and the damaged particles, the tips and the neck were removed by cutting. Then, the banana peels were cut into small pieces and washed thoroughly with salt water. Subsequently, the pieces were washed twice with lukewarm water. Then, the banana peels were boiled in hot water at 100 °C temperature for about 2 to 3 minutes. After that, the water was removed by a strainer and the peels were vacuum dried at 32 °C. Then the dried peels were ground using a grinder until they became a dusty type powder.

Preparation of Banana Peel Powder Incorporated Ice Cream

The ice cream mixture was developed with fresh milk (1 L), sugar (300 g), milk powder (10 table spoons [tbsp.]), corn flour (4 tbsp), gelatine (4 tsp), vanilla (2 tsp), and varying amounts of banana peel powder. Initially, milk powder was dissolved in water (250 ml). The corn flour was dissolved in water, while gelatine was dissolved in lukewarm water. Thereafter, the fresh milk was heated for a few minutes, and milk powder solution was added to the heated milk along with sugar. The resulting mixture was constantly stirred at low temperature to dissolve all the ingredients completely.

The dissolved gelatine and corn flour were added to that mixture. Thereafter, the resulting mixture was filtered and allowed to cool at room temperature. After cooling, the mixture was transferred into a clean bowl and beaten at high speed for 15 minutes to acquire a foamy mixture using a hand beater. Subsequently, the mixture was placed in a freezer for 2 h. This step was repeated three times and finally, banana peel powder was mixed into the mixture in different quantities to form three treatments, as shown in Table 1.

The resulting mixtures were beaten for 5 minutes, followed by freezing for a period of 12 h.

Table 1: The Banana Peel Powder Content of Different Treatments

Treatment	For Ice Cream (g/L)	For Ice Cone (g/kg)
Treatment 1	30.0	200.0
Treatment 2	15.0	133.0
Treatment 3	42.0	67.0

Preparation of Ice Cone

Three different ice cone mixtures were developed by adding rice flour (5 g), wheat flour (15 g), butter (10 ml), egg-white (20 ml), sugar powder (20 g), calcium chloride (0.15 g), and lecithin (0.25 g), along with varying quantities of banana peel powder, as shown in Table 1. Initially, rice flour, wheat flour, banana peel, and sugar powder were mixed well. Thereafter, butter, egg-white, calcium chloride and lecithin were added into it and were mixed using a hand mixer, until a mixture with a thick texture formed. Subsequently, the mixture was transferred into a clean pan and heated up until a crusted thin layer was formed, which could be rolled up into a cone.

Sensory Analysis

The developed ice cream and ice cone samples were subjected to a sensory evaluation by a semi-trained panel of thirty-five panellists (twenty undergraduates and fifteen academic and non-academic staff members), separately. The samples were graded on a five-point hedonic scale (ranging from extremely dislike to extremely like) for their appearance, flavour, texture/ consistency, aroma, colour, mouthfeel and overall acceptability [17]. Water was provided for mouth-washing each time a panellist ate a sample of formulated ice cream and ice cones [18].

Proximate Analysis

Proximate analysis was carried out on a dry basis for the best sample selected based on the sensory analyses. The moisture content was determined using an Infrared Moisture Analyzer (Kett FD-660). Crude fibre content was determined using a fibre analyser (RAYPA F-6P), adhering to the guidelines of the Weende method [19]. For the fat extraction, around 2 g of ground ice cone sample was measured and stored in an air tight container. The crude fat content was determined using a fat analyser (RAYPA SX-6) according to the Soxhlet extraction method, while the crude protein content was determined using the Kjeldhal method [20]. Further, the total carbohydrate content was determined according to a standard formula as recommended by Schakel *et al.* [21]. All analyses were performed in triplicate.

Microbiological Analysis for Ice Cream

Microbial analysis was conducted for ice cream using the Nutrient Agar media (NA) and Potato Dextrose Agar media (PDA) for bacteria, yeast and mould, respectively. Initially, nutrient agar, potato dextrose agar, peptone water, and distilled water were prepared and autoclaved at 121 °C for 20 minutes at 15 psi. Subsequently, 1 g of the sample was dissolved in 10 ml of peptone water and distilled water separately to prepare inoculation samples to inoculate for yeast and mould count and total plate count, respectively. Thereafter, a dilution series was prepared until 10^{-8} concentration. Then, 1 ml of the 10^{-1} to 10^{-8} concentration series of the sample were plated with nutrient agar and potato dextrose agar, separately. Distilled water was used as the control treatment. Prepared plates were incubated in an incubator at 37 °C and the formation of colonies were recorded at 24 h, 48 h and 72 h intervals, since incubation [22].

Shelf-Life Analysis for Ice Cone

The shelf-life analysis of ice cone was conducted according to the moisture content of the ice cone product. Moisture content was estimated using an infrared moisture analyser (Kett FD-660). About 5 g of crushed ice cone sample was used for the analysis. The result was taken from the analyser directly. The moisture content of a commercial ice cone product was compared against the banana peel powder incorporated ice cone product selected according to the sensory evaluation.

Statistical Analysis
All analyses were done in triplicate except the sensory analysis. The sensory evaluation data were analysed using the Kruskal–Wallis test at 95% confidence level. Descriptive statistics was used to present the data on physicochemical properties. All the statistical analyses were conducted using SPSS (Version 23) software.

RESULTS AND DISCUSSION

Sensory Evaluation
Based on the sensory evaluation, Treatment 3 (T3) was recognized as the best treatment for ice cone, which showed the highest mean scores for all the sensory attributes (Figure 1). Based on the statistics of the Kruskal–Wallis test, all the sensory attributes of ice cone denoted significant differences at 95% confidence level ($P<0.05$). The banana peel powder (67 g/kg) incorporated ice cone (Treatment 3) showed the highest mean overall acceptability of 4.2±0.4. Meanwhile, only appearance and overall acceptability attributes of the ice cream exhibited significant differences, among the treatments ($P<0.05$). In the case of the Ice cream, the T2 combination was identified as the best treatment (Figure 2).

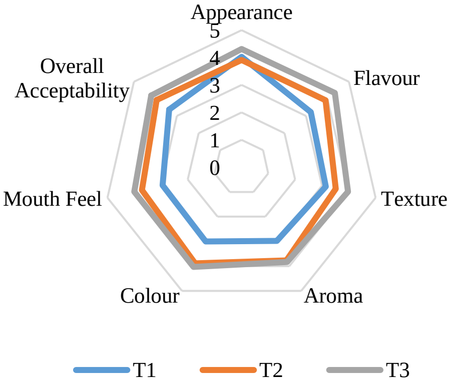

Figure 1: Mean Scores for Sensory Attributes of the Ice Cone
Note: *T1: Ice cone produced under Treatment 1; T2: Ice cone produced under Treatment 2; T3: Ice cone produced under Treatment 3*

Proximate Analysis of Ice Cone
According to the proximate analysis of ice cone, carbohydrates accounted for 77.5 ± 0.3% (w/w), emerging as the prominent nutrient of the ice cone product (Table 2). Because of the high usage of flour and sugar for the product, the carbohydrate amount accounts for a high weight percentage of the ice cone. Carbohydrate is a source of energy and can contribute to the overall calorie content of the dessert [17].

Starches in particular play a significant role in giving the ice cone the proper texture and shape. The stability and shelf life of the ice cone can also be improved by carbohydrates. Further, the browning and visual appeal of the ice cone product are influenced by carbohydrates, since carbohydrates undergo Maillard browning processes while baking or frying, giving the finished product an attractive golden-brown colour [16]. Moreover, caramelization may have contributed to the appealing colour and flavour of ice cone [15]. Therefore, carbohydrates play a vital role in the quality of the ice cone product.

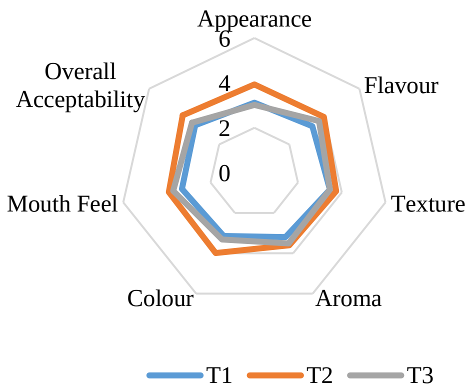

Figure 2: Mean Scores for Sensory Attributes of the Ice Cream
Note: *T1: Ice cream produced under Treatment 1; T2: Ice cream produced under Treatment 2; T3: Ice cream produced under Treatment 3*

The crude protein accounted for 9.3±0.1% (w/w), followed by crude fibre (8.3±4.2% w/w) and crude fat (7.3±0.3% w/w). The main contributors of fat and protein into ice cones are the recipe ingredients like butter and egg. The banana peel powder is the main reason for the high fibre content of the ice cone product [9]. Meanwhile, the total ash content accounted for a lower amount (1.7±0.1% w/w), compared to other food constituents of ice cone (Table 2).

Table 2: Results of Proximate Analysis of Ice Cone

Food Constituent	Available Percentage (% w/w) Mean ± Standard Error
Carbohydrate	77.5 ± 0.3
Crude Protein	9.3 ± 0.1
Crude Fibre	8.3 ± 4.2
Crude Fat	7.3 ± 0.3
Total Ash	1.7 ± 0.1

Proximate Analysis for Ice Cream

Similar to the case of ice cone, the major food constituent of ice cream was carbohydrates, which accounted for 92.0±0.4% (w/w). In addition, the ice cream contained a notable level of protein (5.5±0.01% w/w), followed by crude fat (1.1±0.03% w/w). Previous studies have evidenced that banana peel powder is a good source of amino acids, and thus will contribute to good health [23]. The fibre content was 0.7±0.01% (w/w), and thus it may be beneficial for the gut health too (Table 3).

Table 3: Results of Proximate Analysis of Ice Cream

Food Constituent	Available Percentage (% w/w) Mean ± Standard Error
Carbohydrate	92.0 ± 0.4
Crude Protein	5.5 ± 0.01
Crude Fibre	0.7 ± 0.01
Crude Fat	1.1 ± 0.03
Total Ash	0.7 ± 0.002

Microbial Analysis of Ice Cream

The microbial analysis of the best ice cream product developed in this study (Treatment 2) was conducted and compared against a commercial ice cream product. The total plate count and yeast and mould count obtained for the ice cream produced (Treatment 2) were lower than those obtained for the commercial ice cream product (Figure 3). Therefore, the ice cream produced under Treatment 2 is highly microbially safe to consume.

Previous studies have highlighted antibacterial properties of banana peel powder, especially against various gram-positive and gram-negative bacteria, such as *Bacillus subtilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* [24]. The presence of active ingredients such as ß-sitosterol, malic acid, succinic acid, palmitic acid, 12-hydroxystrearic acid, glycoside, d-malic and 12-hydroxystrearic acid in banana peel has been recognized as the reason for this antibacterial characteristic [25].

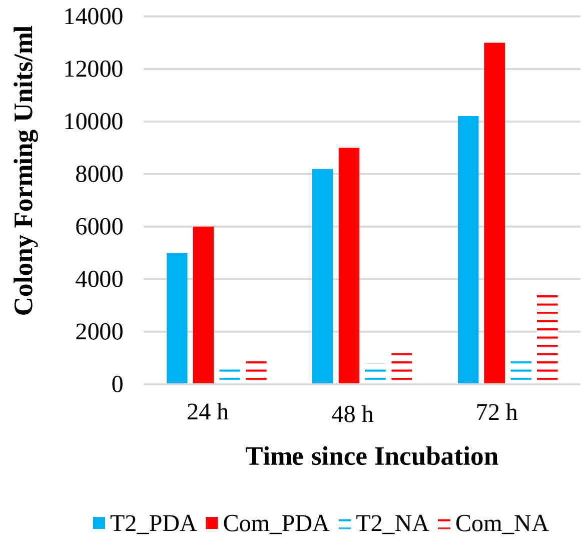

Figure 3: Results of Microbial Analysis of Ice Cream

Note: *Com_PDA: Commercial Ice Cream in PDA, Com_NA: Commercial Ice Cream in NA; T2_PDA: Ice Cream (Treatment 2) in PDA, T2_NA: Ice Cream (Treatment 2) in NA*

Shelf-Life Analysis of Ice Cone

Based on the moisture analysis, the ice cone produced (Treatment 3) showed lower moisture levels even after 6 days (8.9±0.9%), compared to the commercial product, which reported a moisture content of 11.4±1.7% at

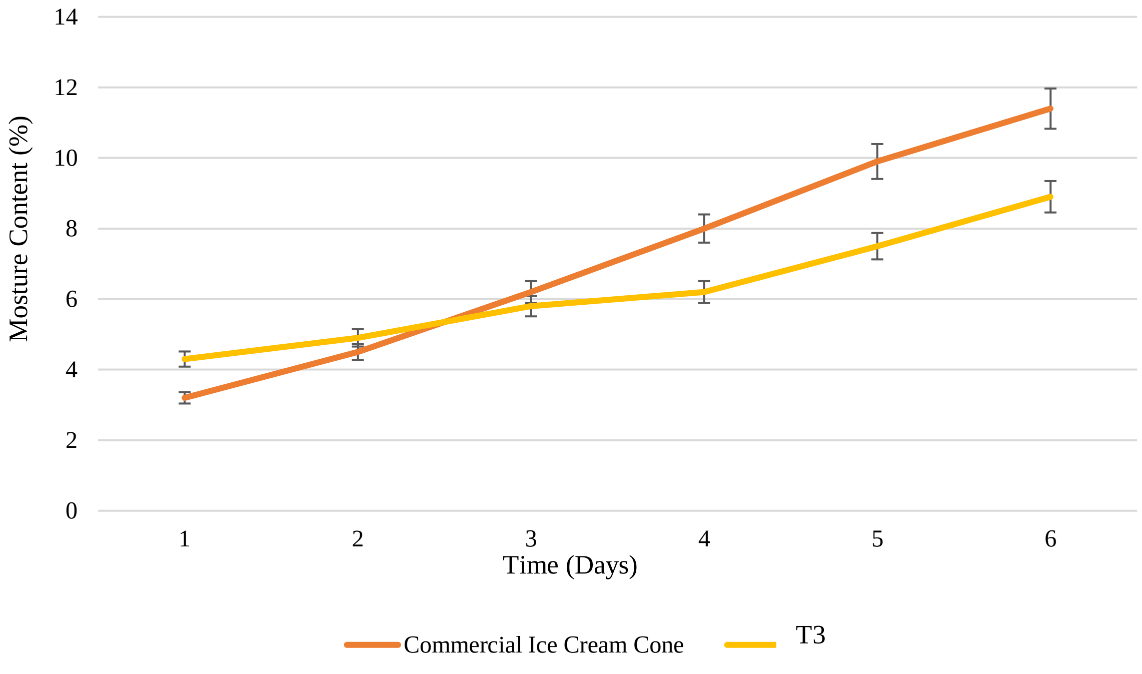

Figure 4: Moisture Analysis of Ice Cone
Note: *T3: Ice cone produced under Treatment 3*

the end of 6 days (Figure 4). Therefore, findings of this study suggest that the ice cone produced (Treatment 3) has a satisfactory shelf life compared to commercial products.

CONCLUSIONS

The findings of this study emphasize that the incorporation of banana peel powder can elevate the taste and overall acceptability of ice cone and ice cream, which are valuable sources of essential nutrients. Based on the sensory evaluation, ice cone (Treatment 3) and ice cream (Treatment 2) scored the highest mean values for overall acceptability. Hence, ice cream prepared by incorporating 15 g/L banana peel powder and ice cone prepared by incorporating 67 g/kg may show higher consumer acceptance. The greater microbial safety of the chosen ice cream and the lower moisture content of the chosen ice cone than the commercial products indicate that the best products according to this study may show greater or comparable shelf-life than the commercial ones. Further studies are recommended to improve the overall quality of these products.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTIONS

HE: Wrote the manuscript and conducted the experiments. IJ, PJ and MW: Conducted the experiments. LU: Conceptualized the study, supervised and wrote the manuscript. GP: Conceptualized the study and reviewed the manuscript. All authors read and approved the manuscript.

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