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## FORMULATION AND STANDARDIZATION OF VANILLA FLAVORED PROBIOTIC ICE CREAM WITH WHEATGRASS POWDER

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### Abstract:

Probiotic Ice cream is a dairy product with benefits of live good bacteria especially *Bacillus Coagulans* SNZ which is a spore forming bacteria that are well known for their therapeutic action in the gut and colon, here in this recipe Wheatgrass is freshly sprouted first leaves of Common Wheat plant which can be used as a food, drink or dietary supplement, prebiotic fibre and nutraceutical. The wheat grass has various nutrients such as protein, vitamins A, E, C, B12, and Some minerals like phosphorous, magnesium, calcium, iron and potassium. The development process involved optimizing the formulation to achieve a harmonious balance of Flavors, textures, and nutritional content to cater to health-conscious consumers. The present aim of this paper is to observe the probiotic strains during production and during storage of 0,1,5,10,14 days and the results appeared as the initial stages of ice cream contains higher counts of *Bacillus coagulans* and the viability of probiotic microorganisms continued to decrease during the storage. The bacteria will undergo various stresses like pH, acidity and antagonistic impact of co-cultures. By the addition of probiotic culture and wheat grass powder will show an impact on its flavor, texture and other sensory characteristics. According to FSSAI regulations the recommended minimum count of bacteria should be at least  $10^6$  cfu/gm in all probiotic products.

**Key words:** Vanilla, Wheatgrass, Probiotics, Protein, Prebiotics, Fiber, Vitamins.

## Introduction:

The sudden appeal in the consumers on functional foods is increased due to increased awareness of the consumers on what they are consuming, so this leads to development of various fortified foods and functional foods by mixing with nutraceuticals and these are the foods with ingredients that can prevent and treat diseases [Scheinbach,1998] <sup>(1)</sup>. Here in this recipe the wheatgrass powder has properties of nutraceutical and have various organic components such as vitamins, minerals, antioxidants, and other bioactive compounds are found and have been linked to various health benefits [Bar-sela et al.,2015] <sup>(2)</sup>. This wheatgrass powder is obtained from fresh shoots of wheat plant *Triticum aestivum*, which are harvested within 10 days (about 1 and a half weeks) after planting the seeds. It is nutrient-dense functional food [Kulkarni et al., 2006] <sup>(3)</sup> which provides more advantages and benefits than basic nutrition and it has therapeutical uses. It has been used for various medicinal purposes from back from ancient Egyptian and Mesopotamian civilizations.

Recently the urge and interest in people leads to popularity and usage of wheatgrass powder and juices in day-to-day life. But it also has drawbacks along with benefits such as it can't be taken by lactating and pregnant women and must mention the allergen information on the developed ice cream. According to studies this wheatgrass powder helps to regulate free radical damage to our body which leads to various chronic diseases as it contains flavonoids, phenolic acids, and carotenoids [Mujoriya and Bodla,2011] <sup>(4)</sup>. It also contains various enzymes like protease, amylase, and lipase which help in digestion as well as all nine essential amino acids which act as building blocks of proteins [Ghumman et al.,2017] <sup>(5)</sup>.

In this recipe the probiotic culture or bacteria plays a crucial role among all the ingredients that are used in the production of this ice cream. Here, *Bacillus Coagulans* is used as probiotic culture in the powdered form. It is a spore forming bacteria which are viable or live organisms which helps in maintaining good gut microbiota as well as good gut health in host [Lee,1999] <sup>(6)</sup>. Recently a great view has been focused on importance of probiotic bacteria in treating and preventing some disorders such as *helicobacter pylori* infection, eczema, allergies, irritable bowel syndrome and supports intestinal health as well as immunological health [Tappenden and Deutsch, 2007] <sup>(7)</sup>.

Ice cream is a delicious sweet creamy dairy product which will help in delivering essential nutrients to consumers of all age groups. Various health benefits have been found by probiotic bacteria and more than 90 probiotic products contain more than one type of probiotic bacteria worldwide [Tharmaraj & Shah, 2003] <sup>(8)</sup>. *Bacillus coagulans* (*B. coagulans*) was first isolated from spoiled milk [Kristjansson et al.,1991] <sup>(9)</sup>. In 1933, it was identified as *Lactobacillus sporogenes* by Horowitz-Wlassowa and Nowotelnow. Afterwards, it was classified as *B. coagulans* [Karri et al.,2016] <sup>(10)</sup>. These are gram positive, facultative anaerobic bacteria, non-pathogenic, lactic acid producing, spore forming bacteria [Asan Ozusaglam et al.,2010] <sup>(11)</sup>.

Other probiotic species such as *Lactobacillus* spp., *Bifidobacterium* spp., show vast probiotic activities but they can't survive in harsh environments and their survival rate is very low [Keller et al., 2019, Ruiz et al., 2011] <sup>(12,13)</sup>. So, researchers have been attracted to spore forming, non-pathogenic probiotic bacteria that can survive harsh environment for better benefits, *Bacillus coagulans* will comes under this category.

## Materials and Methods

**Materials:**

**Milk:** Fresh full cream milk was obtained from Dodla dairy milk.

**Additives:** Some of the additives that are used – Skim milk powder, Emulsifier & Stabilizer, Flavor- Vanilla, Liquid glucose, Organic sugar.

**Wheatgrass powder:** Soaking wheatgrass seeds overnight and fill a tray with evened soil and create surface for the seeds. And let them spray with water and cover with a polythene cover and let them keep in dark place until they sprouted. After that shift the sprouts to sunny area and make them grow up to 10 days (about 1 and a half weeks). The well grown wheatgrass will be harvested, dried under sun and turned into powder.

**Probiotic strains:** Bacillus Coagulans SNZ which is available in powder form.

**Methods:****Procedure for ice cream manufacturing –**

Three ice cream mixes were prepared each of 500ml. All mixes were standardized to contain 10% fat, 12% solids not fat, 0.2% stabilizers & emulsifiers, 8% organic sugar, 8% liquid glucose and 0.2% vanilla. Each sample will be prepared by mixing all these ingredients according to the ratios and homogenized together [Arbuckle 1986] <sup>(14)</sup> and then heated at HTST for about 80°C for 30 seconds [Rothwell,1976] <sup>(15)</sup>. Later it is cooled to 4°C and kept for ageing overnight at the same temperature. The powdered bacteria of 0.10gm will be taken and fermented in skimmed milk, fortified by the addition of 1% D-glucose, 1% tryptone [Hagen and Narvhus,1999] <sup>(16)</sup> and kept for about 12hours at 45°C. The optimal growth temperature for vegetative cell of Bacillus coagulans ranges from 35-50°C [Viktoria Trulsson,2020] <sup>(17)</sup>. 10ml of Fermented milk and various proportions (5gm:8gm:10gm) of wheatgrass powder is added to three ice cream mix samples prior to freezing. The ice cream was packed in an ice cream tub of 500ml, covered with lid and hardened at -26°C for 24 h before analysis.

**Formulations:**

Ingredients	Sample 1	Sample 2	Sample 3
Ice cream mix	500ml	500ml	500ml
Probiotic culture – <i>B.coagulans</i> (fermented milk)	10ml	10ml	10ml
Wheatgrass powder	10mg	8mg	5mg

**Physico - Chemical Analysis of Probiotic Ice cream:**

The titratable acidity of mixes was determined according to Richardson [1986] <sup>(18)</sup>. Acidity of mixes was determined in duplicate by titration with NaOH 0.1N, using phenolphthalein as an indicator.

$$\text{Titratable acidity} = \text{amount of NaOH used} \times 0.064.$$

The pH of ice cream is determined by pH Meter.

Ice cream overrun was determined on samples stored at -26°C for 5 days by the following equation:

$$\text{Over run \%} = \frac{(\text{weight of unit volume of mix} - \text{weight of unit volume of ice cream})}{\text{Weight of unit volume of ice cream}} \times 100$$

**Fat:** The test was done by Gerber centrifuge method.

**Apparatus:** Butyrometer, Butyrometer stand, Ice cream, Sulphuric acid, Amyl alcohol, Gerber

centrifuge, 10.75 ml capacity milk pipette.

**Procedure:**

10gm of ice cream sample will be measured on weighing machine and pipetted out by 10.75 ml capacity milk pipette and transferred into butyrometer. Later the butyrometer will be filled with 10ml of sulphuric acid followed by 5ml of amyl alcohol and make up with some water. Stopper the butyrometer with the help of lock stopper using regulating pin/guiding pin.

The butyrometer will be shaken thoroughly until the sample is well mixed with solutions and turned into mahogany red color is obtained; these butyrometers are placed in Gerber Centrifuge for about 5 minutes and centrifuge them. Take out the butyrometer in an upright position with the stopper end downwards.

Let them stand for some time and note the reading. Reading should be taken from bottom of the fat column to lower border of meniscus on the scale.

The microbial count of *Bacillus coagulans* were estimated at 1, 5, 10, 14 days by doing serial dilutions and plating techniques by using Glucose Yeast Extract Agar prepared by following the formula described by [Evans and Niven et al.,1951 and Rogosa et al.,1951] <sup>(19, 20)</sup>.

**Ash:** Drying the sample at 100°C and churning over an electric heater. It was then ash in muffle furnace at 550°C for 5 hrs. By AOAC (2005).

**Moisture content:**

Moisture content was determined adopting AOAC (2005) <sup>(21)</sup> method.

**Protein:** Protein content was determined using AOAC (2005) <sup>(21)</sup> method. Percentage of nitrogen and protein calculated by the following equation:

**Total carbohydrates:** Total carbohydrate content of the samples was determined as total carbohydrate by difference that is by subtracting the measured protein, fat, ash and moisture from 100 phenol sulphuric acid method as given by AOAC (2005) <sup>(21)</sup>.

**Results and Discussions:**

**Sensory evaluation:**

The sensory evaluation is a process to know about the taste, flavor, appearance, consistency and overall acceptability of the three samples with comparison of control

**Table 2:** Sensory Analysis

Sensory Attributes	Control	Sample 1	Sample 2	Sample 3
<b>Color</b>	9	6	7.5	<b>8</b>
<b>Flavor</b>	8	6	8	<b>8</b>
<b>Consistency</b>	9	8	7	<b>8</b>
<b>Taste</b>	8	6	7	<b>8</b>
<b>Appearance</b>	9	7	7	<b>7.5</b>
<b>Overall acceptability</b>	<b>9</b>	<b>6</b>	<b>7</b>	<b>8</b>

The sensory evaluations of prepared probiotic ice cream are performed by a panel of 5 judges using a 9-point hedonic scale to know the overall acceptability of the product. The overall acceptability of vanilla flavored probiotic ice cream with wheat grass powder is found to be good at the end of 14 days (2 week) of storage period. The score of prepared ice cream during the period is 8. According to the sensory evaluation sample 3 has high overall acceptance and further proximate analysis was done to this sample to determine the nutritional content of produced ice cream

**Proximate Analysis of Ice cream:**

**Table 3:** Proximate Analysis

The pH of ice cream due to its capacity [Narvhus, 1999] certain degree, suppress the flavor.

Nutrition	Control	Sample-3
Moisture	63%	60%
Ash	0.93	0.98
pH	6.2	6.30-6.42
SNF	8.27%	12%
Fat	7%	10%
Protein	2%	3.5%
Carbohydrates	21.5%	24%
Titrable acidity	0.18- 0.20	0.23-0.25
Over run	68.24	59.72

cream is high buffering [Hagen & (22). To a this would probiotic [Hekmat and

McMahon 1992]<sup>(23)</sup> found the preferred pH of probiotic ice cream, based on overall acceptance to be 5.5. The pH can be reduced by adding different amounts of fermented milk to the ice cream mix. [Christiansen et al. 1996]<sup>(24)</sup>.

### Microbial Analysis of Ice cream:

The microbial analysis of Bacillus Coagulans SNZ was done for about 2 weeks on 0, 1, 5, 10, 14 days of storage and noted down the colony count of this probiotic bacterium.

**Table 4: Microbial Analysis**

Days of Storage	0	1	5	10	14
Sample	9.582Aa± 0.019	9.570Aa± 0.029	9.428Aa± 0.039	9.371Aa± 0.41	9.362Aa± 0.047

### Conclusion:

The ice cream which is developed is good to eat and has better nutritional and therapeutic properties and fulfills the demand of probiotic products to the consumers. Ice cream is an excellent source of protein, fat and calories which will be taken by all age groups. Due to this reason, I've chosen this to deliver the richness and medicinal benefits of Wheatgrass powder to all age groups. Apart from pregnant and lactating women all age groups can take this ice cream as a source of nutrients which have both probiotic benefits as well as therapeutic properties of wheatgrass powder in it. The probiotic culture that is used in this product can survive heat conditions and can cure various gut disorders such as irritable bowel syndrome, provides good immunity and maintains gut health. The wheatgrass powder has almost all essential amino acids which are building blocks of protein in our body. Wheatgrass powder has a very strong flavor and aroma due to this reason the sample 3 was liked very much by the judges.

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