Research Article

# DESIGN, DEVELOPMENT OF PROBIOTIC GUMMIES AND DETERMINING THE STABILITY OF GUMMIES

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**ABSTRACT:**

Probiotics have been said to assist healthy immunological and digestive systems. *B.coagulans* has become a focus of research due to its high temperature stable, highly viable, economical and Versatile in nature. The ultimate objective of the present research work is to incorporate *B.coagulans* strain into the gummies, which will benefit the human health. Gummies are formulated by Trio method. Optimization studies for ingredients was carried out to determine the concentration which is ideal for the preparation. Two different formulations were prepared with the help of Optimization data using sucrose, gelatin and pectin as the base for the preparation of gummies. All the prepared probiotic gummies were subjected to various evaluation parameters like pre-formulation studies, microbial analysis, nutritional facts and Stability data. The results of microbial analysis shows that viability of probiotic in gummy is not less than 500 million spores /gummy, total yeast and mold count is < 10 Cfu /gummy and Pathogen are absent in the formulations. Nutritional facts determines the content like Carbohydrate and Total Sugar content varied from 75.1% to 82.76% w/w and 65.4% to 79.91% w/w. Fat and protein in gummies with *B.coagulans* varied from 0.03 % to 0.78 % and 9.9 % to < 1%, respectively. The energy value of gummies varied from 338 kcal/100 g to 340 kcal/100gm. Short-term stability Studies indicate that there are no significant changes in physical characteristics, viable count, traces of yeast & mold and pathogens after 180 days of storage at 25±2º C with 60±5% RH.

**Key words:** *B.coagulans*, Viability, Nutritional facts, Stability studies

## INTRODUCTION

Probiotics are live microorganisms that are meant to improve one's health whether taken orally or topically. They can be discovered in yoghurt and other fermented foods, nutritional supplements and cosmetics. Numerous bacteria could be present in probiotics. The most prevalent bacteria come from the families *Lactobacillus, Bifidobacterium and Weizmannia coagulans.* Yeasts like, *Saccharomyces boulardii* and other microorganisms can both be employed as probiotics [1].

Probiotics helps in Optimal digestion, Vitamin Production, Supports Immunity, Decrease Cholesterol level and weight loss therapy [2]. *Bacillus coagulans (Weizmannia coagulans)* was initially discovered and reported in 1915 by B.W. Hammer at the Iowa Agricultural Experiment Station as the root of a coagulation epidemic in evaporated milk packaged by an Iowa condensary. . *Bacillus coagulans* is a spore-forming, motile, gram-positive, and catalase-positive and measures roughly 0.9 µm by 3.0 µm to 5.0 µm When the growth cycle enters the stagnant phase, it could appear Gram negative. The range of temperatures permitted is 30-55 °C (86-131 °F), with 50 °C (122 °F) being the ideal temperature for growth. [3]. *Bacillus coagulans* or *Lactobacillus sporogeneses* is a kind of probiotic bacteria and it has tolerance to high temperatures. Because of this, it does not require refrigeration and is shelf-stable. *B.coagulans* balance the micro-organism in the gut, controls the blood pressure, relieves depression, anxiety, insomnia and improves sleeping pattern. [4]

Manufacturers consider *B.coagulans* because it is economical, highly temperature stable, never arrive dead, and versatile in nature. Traditional supplements are ones that are swallowed whole in the form of a capsule or tablets. Probiotic Gummies can be chewed rather than consumed whole. Most of gummies taste good, which makes it simpler to remember to take them every day. Gummy supplements are manufactured using substances like gelatin, corn syrup, pectin fiber, sugar, flavors and coloring in addition to probiotics. Probiotic gummies provide the same overall health advantages as probiotic capsules, primarily support for digestive health, immune system function and Gut health. Advantages of Gummies when

compared with Tablets and Capsule is serving size is small, taste better, safeguard is better and the pace of work is efficient. [5]

## RESEARCH INTENTION

The World Health Organization defines probiotics as "live bacteria that, when administered in sufficient proportions, impart a health benefit on the host." Many people in India have bad dietary habits, which have a variety of negative repercussions on our bodies. In India, the majority of people eat tainted food, which disturbs the body's digestive system. Invasive infections are a significant cause of increased morbidity and mortality in this population. [6] *B.coagulans* as probiotic in humans and animals has a long history and is well documented. Because *B.coagulans* can form spores, it can withstand high temperatures and the harsh conditions of the human stomach, which allows it to provide probiotic advantage Due to this Probiotic strains like *Bacillus coagulans* has nutritional value, research from all over the world has been focused on developing methods and products that treat irritable bowel syndrome, boost immunity, and aid in digesting. [7]

By 2023, it is anticipated that the probiotic market would grow to 15 USD billion. A market study revealed that probiotic supplements took advantage of emerging trends to promote probiotics [8]. The market for dietary supplements is anticipated to experience the greatest CAGR of 15.0% between 2015 and 2021. During the review period, the Asia - Pacific region is anticipated to have the quickest market growth. The rising consumer demand for probiotic components in food is what fuels this increase [9]. In terms of *B. coagulans* stability throughout the preparation and storage of functional foods, there is a dearth of scientific evidence. Determining *B. coagulans* survivability during the production process and storage conditions of functional food in the form of gummies is therefore necessary [10]. *Bacillus coagulans* has often been used in adults in doses of 1 – 2 billion (Cfu's) by oral route daily for 4 – 12 weeks [11]. The study goal was to ascertain how *B. coagulans* viability would be affected by the manufacture and storing processes for probiotic gummy preserves.

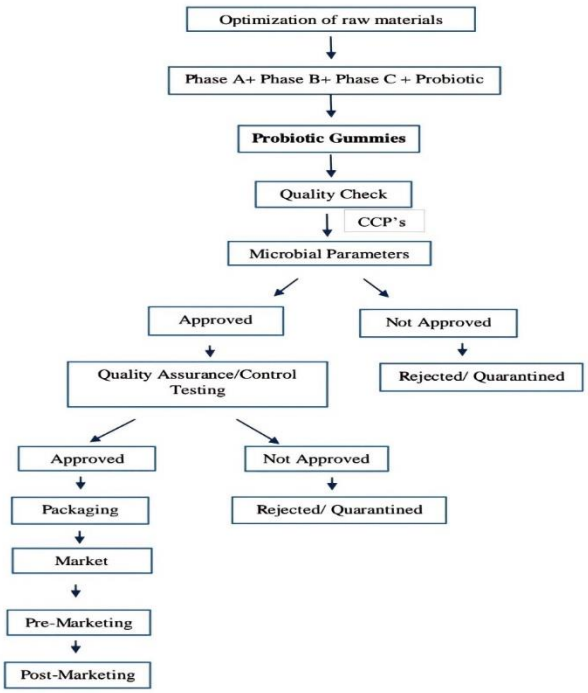



**Fig.1**. Work Plan for Design & Development of Probiotic Gummies

## MATERIALS AND METHODS

*Bacillus coagulans* samples used in the study were manufactured by Unique Biotech (Bangalore, India) Pure *B. coagulans* spores were spray–dried and standardized with food grade maltodextrin to achieve the desired concentration. Optimization Studies of Ingredient's were carried out to determine the Firmness, Strength and Hardness required to design the probiotic gummies. Two different formulations were designed using different gelling agents like Gelatin (Baker's Colors & Flavors) and Pectin (Maple Biotech Pvt.Ltd. Pune) were used as gelling agents, which give the soft texture to the gummies for the formulation (F1 & F2). B. *coagulans*, a probiotic, is incorporated into gummies. (100 billion Cfu/g), wherein Sucrose (Madhur Sugar, Shree Renuka Sugar Limited, Mumbai)contributes 40% of the weight of the gummies, Corn Syrup ( Karo, Light Corn Syrup Superme Traders, Mumbai), acts as a humectant and gives sweetness to the gummies, Citric Acid ( Blue Bird Food India Pvt.Ltd, Mumbai) acts as a preservative; and Flavoring & Coloring Agent (Bush, International Flavors & Fragrance India Pvt.Ltd, Chennai) give the gummies an attractive look and a pleasant taste while consuming them. Each Probiotic gummy weighs about 2.0g ±0.25g and contains NLT 500 million spores/gummy. Physical & Chemical Parameters of Gummies were determined. (Fig.2)

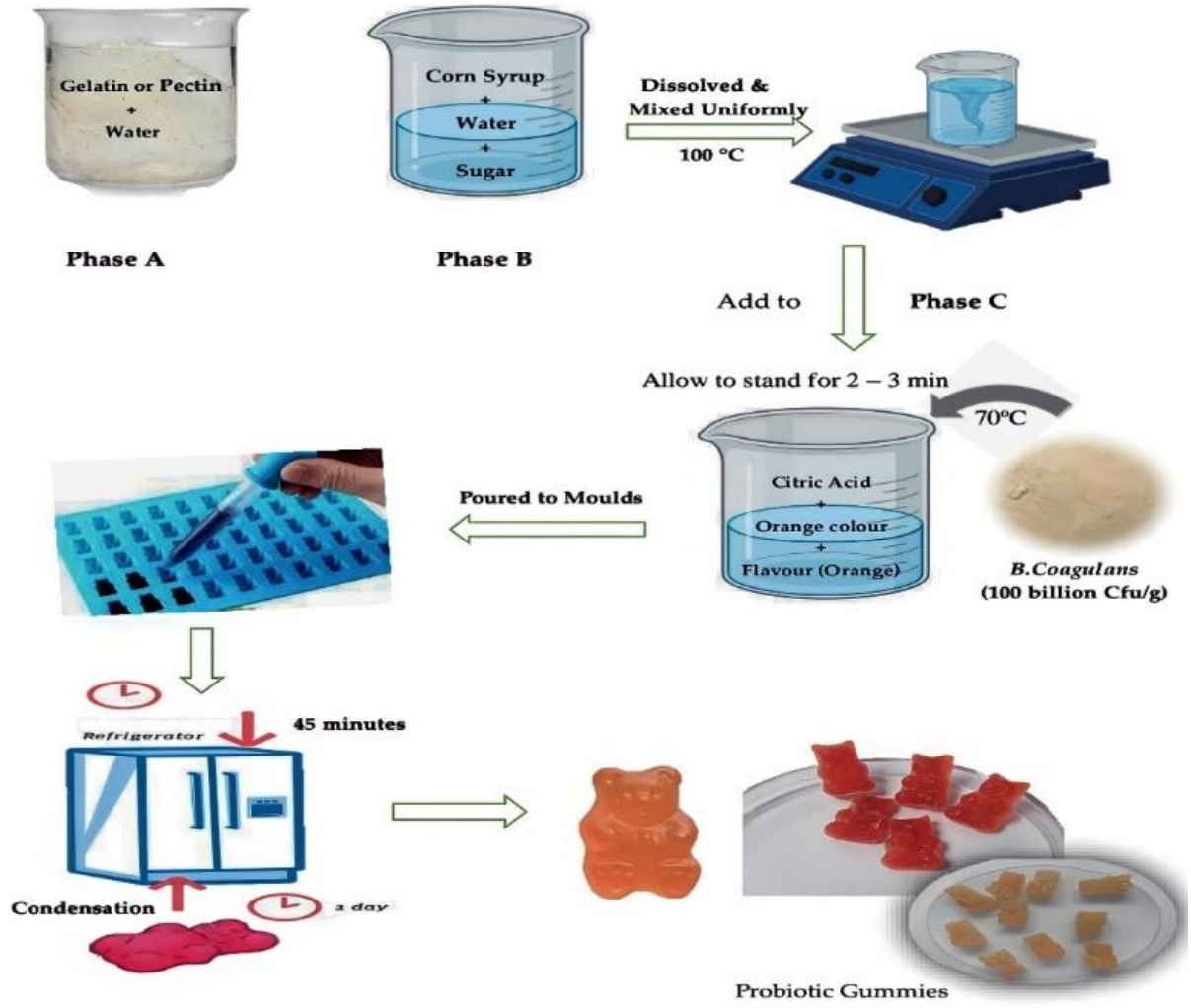



**Fig.2.**Schematic Representation for Preparation of Probiotic gummies

After every time interval for the stability studies, 1.0 g of sample (Probiotic Gummies containing *B. coagulans*) was thoroughly mixed in sterile saline (0.9% NaCl, w/v) and then incubated in water bath for 30 min at 75 °C, followed by immediate cooling to below 45 °C. This suspension was further serially diluted in sterile saline and the viable count was enumerated by plating on glucose yeast extract agar (HiMedia, Mumbai, India) by pour plate method [12]. The plates were incubated at 37 °C for 48–72 h. Total Yeast & Mold count was determined each analysis was performed in triplicate. Average mean of spore viable counts are expressed in $\log^{10}$ Cfu.

The Proximal composition of Gummy supplements with *B.coagulans* was analyzed by the following official method of the Association of Official Analytical Chemists (AOAC) [13].The stability studies were carried out for the Probiotic Gummies F1 and F2 formulations at 25 ± 2°C, 60 % ± 5% RH for six months. [14]

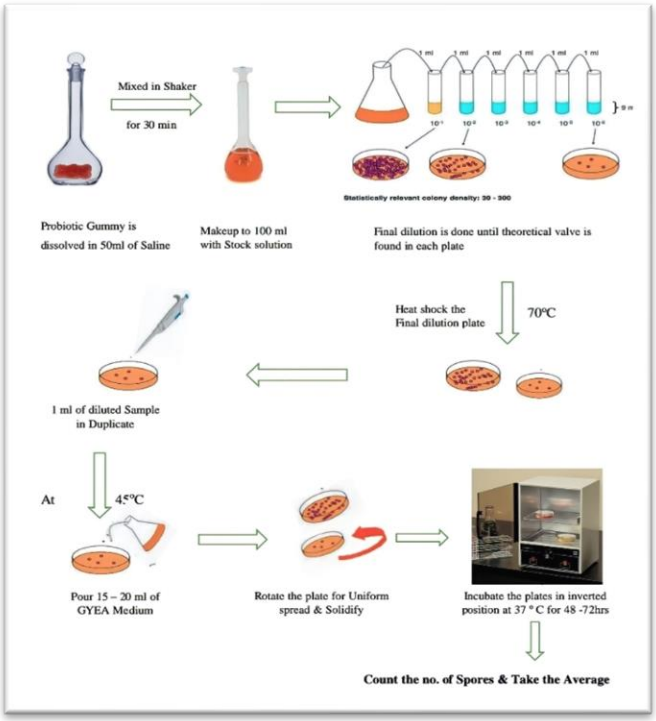



**Fig.3.** Schematic Representation of TVSC Plate for Probiotic gummies

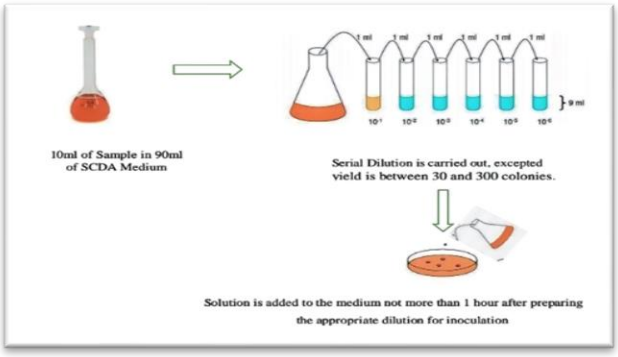
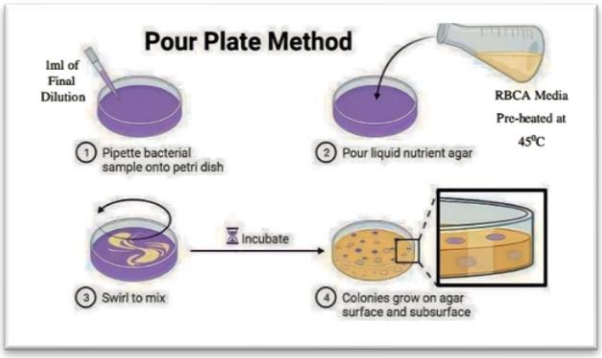



**Fig.4 (A).** Preparation of TYMC Plates for Probiotic Gummies




**(B).** Preparation of TYMC Plates by Pour plate Method for Probiotic Gummies

## RESULTS & DISCUSSIONS

Two different formulations were designed using different gelling agents with the help of optimization studies of the ingredients. *B. coagulans*, a probiotic, is incorporated into gummies. (100 billion Cfu/g), wherein Sugar (Sucrose) contributes 40% of the weight of the gummies, Gelatin and Pectin were used as gelling agents, (F1 & F2). Corn syrup gives sweetness to the gummies Citric acid acts as a preservative; and coloring & flavoring agents give the gummies an attractive look and a pleasant taste while consuming them. Each Probiotic gummy weighs about 2.0g ±0.25g and contains NLT 500 million spores/gummy (Fig.5)

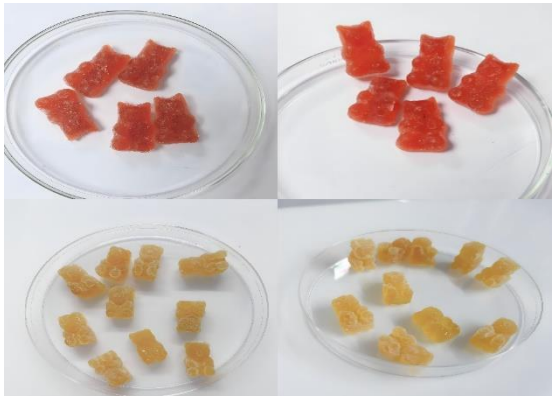


**Fig.5.** Probiotic gummies (F1 & F2)

### Evaluation Parameters for Probiotic Gummies

### a. Determination of Physical & Chemical Parameters

Physical & Chemical Parameters for probiotic gummies were evaluated. Odour and colour of Probiotic Gummies was evaluated. It was found that Gummies has Orange colored, Pleasant Orange Odour and Orange flavored and also the gummies are water soluble in nature. (Table.1)

**Table.1.** Organoleptic Properties of Probiotic Gummies

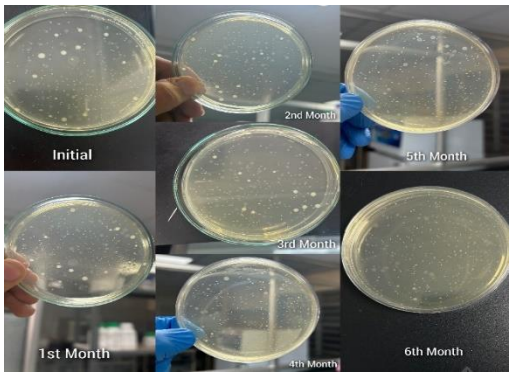
| **Product Name:** Probiotic Gummies containing *Bacillus coagulans* -100 Billion cfu/g | | | |
|---|---|---|---|
| **Sl.no** | **Parameters** | **Specification** | **Results** |
| **A** | Colour | Orange Colour | Complies |
| | Odour | Pleasant Orange Odour | Complies |
| | Taste | Orange Flavour | Complies |
| **B** | Water Solubility Test | NLT 50% | 80% |

### b. Determination of Microbial Parameters

### i. Total Viable Spore Count

TVSC is calculated with GYEA medium and the results are noted down with the help of microbial plate counts of $10^6$ and $10^7$ and it's expressed in Cfu /gummy using the formula. (Fig.6)

Viable Spore per gummy = Average number of colonies formed X dilution factor X 100



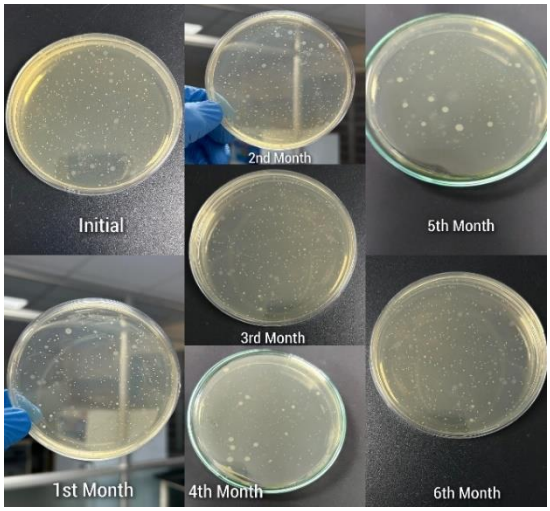



**Fig.6.** TYMC Plate count of Probiotic Gummies (F1 & F2)

**Table.2.** TVSC Calculation for Probiotic Gummies (F1 & F2)

| Time (month) | $Log_{10}$ Cfu /gummy | | Viability (%) | |
|---|---|---|---|---|
| | F1 | F2 | F1 | F2 |
| **0** | 8.7323 | 8.7160 | 100 | 100 |
| **1** | 8.7160 | 8.7160 | 99.8 | 100 |
| **2** | 8.7160 | 8.7075 | 99.8 | 99.9 |
| **3** | 8.7242 | 8.7075 | 99.9 | 99.9 |
| **4** | 8.7242 | 8.6989 | 99.9 | 99.8 |
| **5** | 8.7075 | 8.7075 | 99.7 | 99.9 |
| **6** | 8.7160 | 8.6989 | 99.8 | 99.7 |

## ii. Total Yeast & Mold Count

TYMC is calculated with GYEA medium and the results are noted down with the help of microbial plate counts of $10^1$ and $10^2$ and it's expressed in Cfu /gummy using the formula.

**Total Yeast and Mold Count (TYMC) =** Average number of colonies x Dilution factor (Cfu/g)

**Table.3.** TYMC Calculation for Probiotic Gummies (F1)

| Time (month) | Dilution | | No. of Colonies | | Cfu /gummy |
|---|---|---|---|---|---|
| **0** | $10^{-1}$ | $10^{-2}$ | 00 | 00 | < 10 |
| **1** | $10^{-1}$ | $10^{-2}$ | 00 | 00 | < 10 |
| **2** | $10^{-1}$ | $10^{-2}$ | 00 | 00 | < 10 |
| **3** | $10^{-1}$ | $10^{-2}$ | 00 | 00 | < 10 |
| **4** | $10^{-1}$ | $10^{-2}$ | 00 | 00 | < 10 |
| **5** | $10^{-1}$ | $10^{-2}$ | 00 | 00 | < 10 |
| **6** | $10^{-1}$ | $10^{-2}$ | 00 | 00 | < 10 |

**Table.4.** TYMC Calculation for Probiotic Gummies (F2)

| Time(month) | Dilution | | No. of Colonies | | Cfu /gummy |
|---|---|---|---|---|---|
| **0** | $10^{-1}$ | $10^{-2}$ | 00 | 00 | < 10 |
| **1** | $10^{-1}$ | $10^{-2}$ | 00 | 00 | < 10 |
| **2** | $10^{-1}$ | $10^{-2}$ | 00 | 00 | < 10 |
| **3** | $10^{-1}$ | $10^{-2}$ | 00 | 00 | < 10 |
| **4** | $10^{-1}$ | $10^{-2}$ | 00 | 00 | < 10 |
| **5** | $10^{-1}$ | $10^{-2}$ | 00 | 00 | < 10 |
| **6** | $10^{-1}$ | $10^{-2}$ | 00 | 00 | < 10 |

## c. Nutritional Fact of Probiotic Gummies

The Proximal composition of Gummy supplements with *B.coagulans* was analyzed by the following official method of the Association of Official Analytical Chemists (AOAC).The results of the Nutritional Facts of Probiotic Gummies (Table.5)

The presence of moisture in the gummies was analyzed by the moisture-vacuum oven method and the results varied by 14.6% to 16.28% within the formulations (F1 and F2). Similarly, the Carbohydrate and Total Sugar content in the formulations F1 and F2 varied b/w 75.1% to 82.76% w/w and 65.4% to 79.91% w/w. The content of fat & protein in the probiotic gummies with *B.coagulans* varied from 0.03 % to 0.78 % and 9.9 % to < 1% respectively The energy valve of gummies varied from 338 kcal/100gm to 340 kcal/100gm.The data of the nutritional study suggested that

*B.coagulans* was found to be stable in the gummies with different nutritional profiles.

**Table.5.** Nutritional Study Data of Probiotic Gummies

| Sl.no | Tests | Unit | Results | |
|---|---|---|---|---|
| | | | F1 | F2 |
| 1.1 | Moisture - Vacuum oven method | % w/w | 14.6 | 16.28 |
| 1.2 | Fat | % w/w | 0.03 | 0.78 |
| 1.3 | Protein | % w/w | 9.9 | < 1 |
| 1.4 | Carbohydrates | % w/w | 75.1 | 82.76 |
| 1.5 | Energy | Kcal | 340 | 338.1 |
| 1.6 | Total Sugar | % w/w | 65.4 | 79.91 |

## d. Stability Studies on Probiotic Gummies

The stability studies were carried out for the Probiotic Gummies F1 and F2 formulations at 25 ± 2°C, 60 % ± 5% RH for six months. Initially, the viability of gummies of F1 & F2 was found to be 100%, respectively, in both formulations. During the study period, the probiotic content in the formulations F1 & F2 was found to be 99.8% and 100% at the end of 1st month, respectively. The Probiotic content in the formulations F1& F2 was found to be 99.8% & 99.9%, respectively, at the end of the 2nd month. During the study period, the Probiotic content in the formulations F1& F2 was found to be 99.9% respectively, at the end of the 3rd month. At the end of the study, the Probiotic content in F1 & F2 was found to be 99.8% and 98.8%, respectively, in both formulations. On average, the content of probiotics in gummies in the formulations F1 & F2 was found to be 100% initially; at the 6th month, the probiotic content was found to be 99.8% stable in the formulated gummies. There were no significant differences found in the probiotic content during the stability study. This indicates that Probiotic Gummies are stable under storage conditions. Gummies were stored in an airtight container and there were no significant changes in colour or appearance found during the stability study period

**Table.6.** Criteria required to carry out Stability Studies for Probiotic Gummies

| | |
|---|---|
| **Product :** Probiotic Gummy<br><br>**Strain:** *Bacillus coagulans*<br><br>(F1 & F2)<br><br>**Batch no:** PZ /PG-BC/501<br><br>PZ/PG-BC/502<br><br>**Mfg. Date:** 20/12/2022 | **Sample Pack:**<br><br>Kept in self -sealing double polythene bag enclosed.<br><br>**Storage:** 25 ± 2°C, 60 % ± 5% RH<br><br>**Quantity:** About 16 g per analysis or 8 gummies |
| **Test Performed**<br><br>1.Colour<br><br>2.Flavour<br><br>3.Assay:<br><br>A. Total Viable Spore Count<br><br>B. Total Yeast & Mold Count<br><br>C. Viability % | **Frequency of Testing**<br><br>At intervals of<br><br>1,2,3,4,5& 6months |

**Table.7.** Stability Data of Probiotic Gummies (F1 & F2)

| Period of Testing | Colour | Flavour | Assay | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | A | | B | | C | |
| | | | F1 | F2 | F1 | F2 | F1 | F2 |
| Initial | Orange | Orange | 540 | 520 | <10 | <10 | 100 | 100 |
| 1st Month | Orange | Orange | 525 | 515 | <10 | <10 | 99.8 | 100 |
| 2nd Month | Orange | Orange | 520 | 515 | <10 | <10 | 99.8 | 99.9 |
| 3rd Month | Orange | Orange | 525 | 510 | <10 | <10 | 99.9 | 99.9 |
| 4th Month | Orange | Orange | 525 | 505 | <10 | <10 | 99.9 | 99.8 |
| 5th Month | Orange | Orange | 510 | 510 | <10 | <10 | 99.7 | 99.9 |
| 6th Month | Orange | Orange | 515 | 505 | <10 | <10 | 99.8 | 99.8 |
| LIMITS | Orange | Orange | NLT 500 Million Spore /gummy | | NMT 100 cfu/g | | NLT 98 % | |

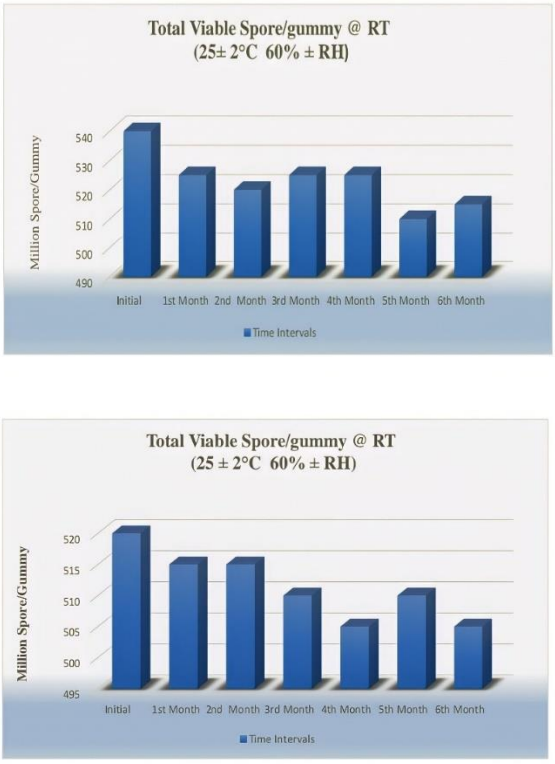



**Fig.7.** Viability Count of Probiotic Gummies (F1 & F2)

## CONCLUSION

Probiotic Gummies were successfully prepared by using *B.coagulans* as a Probiotic with the help of different gelling agents like Gelatin and Pectin. Two different formulations were prepared with the help of Optimization data using sucrose, gelatin and pectin as the base for the preparation of gummies. All the prepared probiotic gummies were subjected to various evaluation parameters like pre-formulation studies, microbial analysis, nutritional facts and Stability data. Microbial parameters, including Total Viable Spore Count (TVSC), Total Yeast & Mold count (TYMC) and Pathogen testing, were carried out and they comply within the limits. Nutritional content in the formulation was analysed and the results were determined and depicted for various constituents like Carbohydrate and Total Sugar content in the formulations, which varied from 75.1% to 82.76% and 65.4% to 79.91% w/w. Fat and protein in the probiotic gummies with *B.coagulans* varied from 0.03 % to 0.78 % and 9.9 % to < 1%, respectively. The energy value of gummies varied from 338 kcal /100 g to 340 kcal/100gm. Short-term stability Studies of the formulations indicate that there are no significant changes in physical characteristics, viable count, traces of yeast & molds and pathogens after 180 days of storage at 25±2º C with 60±5% RH.

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## Conflict of interests

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