# Analysis of Microbial Quality and Sensory characteristics of Probiotic drink powder containing Whey protein and *Piper betle* (Betel leaves) extract

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## Abstract

Today concepts in nutrition are expanding emphasizing on the use of foods to improve health and reduce the risk of diseases. The present study was carried out to evolve a protocol for the development of probiotic drink powder containing whey protein (WP) and betel leaves extract (BLE). BLE was spray dried at inlet and outlet temperature of 110.5-110.9$^0$C and 60-75$^0$C respectively at an aspirator rate of 47%. *Bacillus coagulans* was revived and spray dried at inlet and outlet temperature of 110.2-110.5$^0$C and 55-75$^0$C respectively at an aspirator rate of 48%. Spray dried BLE, *B. coagulans* and 70% WP were combined (5%, 1%, 78% respectively) to develop probiotic drink powder. After baseline sensory evaluation, cardamom powder and fennel seed powder were added to enhance the flavor. Two variations of the powder were prepared, one without sugar (Probiotic drink powder: PDP) and the other containing sugar (Probiotic drink powder containing sugar: PDPS). Samples were packaged and stored in triple laminated bag for 30 days at room temperature (27-32$^0$C) and were analyzed for total viable count at day/s 1, 8, 16 and 30 and sensory characteristics at day/s 1, 8, 16, 30. Viable count was higher for PDP (2.3x10$^8$cfu/g) which reduced by 1 log cycle whereas, for PDPS, it was 0.4x10$^8$cfu/g which remained almost constant to 0.2x10$^8$cfu/g after 30 days of storage. The acceptability scores for aroma, appearance, flavour, taste for PDPS was higher than PDP ($p>0.05$). The study showed that the probiotic organism i.e., *Bacillus coagulans* survived the spray drying procedure and hence it can be used to make a healthy powder with biological benefits which can be made commercially available. The sensory scores showed that PDPS was better acceptable. This could be because of the presence of sugar in the sample. Based on the results of the current study, it can be concluded that the probiotic drink powder has a good potential as a health drink that may help prevent many lifestyle realted diseases.

**Keywords:** Betel leaves extract, Whey protein, Probiotic, Spray drying, Sensory evaluation

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## 1. Introduction

The primary role of diet is to provide sufficient nutrients to meet metabolic requirements while giving the person a feeling of satisfaction and well-being. Recent reviews,

however, support the fact that, beyond meeting nutritional needs, diet may modulate various physiological functions and may play detrimental or beneficial roles in some diseases [1].

The increasing awareness about nutrition, health and quality food consciousness of consumers and the keen competition in the market, compel the food industry to fortify food while preserving and enhancing the nutritional quality of foodstuffs, in order to sell their products profitably [1]. Hence, in recent years, much attention has been paid to create a variety of milk proteins with differing functional properties. The food manufacturing industry has come to realize that milk proteins in general and whey proteins in particular have potential to improve the quality of food products.

Whey is a reliable source for a number of high quality and biologically-active proteins, carbohydrates and minerals. Whey has many nutritional benefits like weight regulation, proliferation and differentiation of cultured osteoblasts thus preventing bone diseases, it helps to stimulate immune system, it has antioxidant activity thus preventing the state of inflammation, it is also found to have anti carcinogenic and anti hypertensive properties, thus helping in prevention of chronic disease [2].

In food industry, recent advances have been probiotic drinks. In addition to their role in fermentation processes, some probiotic lactic acid bacteria like *Bacillus coagulans* have been studied as dietary sources of live microorganisms destined to promote a positive impact in the host by improving the properties of the indigenous beneficial micro biota [3]. Documented benefits of the ingestion of probiotics include: reduction of serum cholesterol, alleviation of lactose intolerance, reduction of cancer risk, resistance to enteric pathogens, among others [4].

Nowadays, there is an increasing interest in the natural remedies [5]. Betel (Piper betle L.) is cultivated in Sri Lanka, India, Indonesia, Philippine Islands and East Africa [6, 7]. Two major propenylphenols: Hydroxyychavicol (HC) and Eugenol (EU), found in betel leaves have contributed to majority of the bioactivities in betel leaves [6]. As a masticatory, it is aromatic, digestive, stimulant and carminative [3]. The betel leaves extract is used as traditional medicine to treat throat inflammation, alleviating coughs and indigestion, and as breath freshener and antiseptic for wounds. Currently there is an abundant scientific finding that has been reported on the bioactivity and beneficial effects of betel leaves including antioxidant, anti carcinogenic, anti inflammatory, anti microbial, anti diabetic properties [3]. According to Rathee *et al*, *Bangla* variety is found to possess the best antioxidant activity [8].

There is paucity of data regarding the development of such a product hence an attempt was made to combine and develop a spray dried powder which can be reconstituted to form probiotic beverage containing whey protein and betel leaves extract and to analyze its microbial quality and carry out sensory evaluation to study its acceptability.

## 2. Experimental Section:

### 2.1 Materials

*Bangla* variety of betel leaves were procured from a wholesale market in Mumbai. Whey protein concentrate (WPC) 70 was purchased from Mahaan Proteins Limited, New Delhi. 1 ampoule freeze dried probiotic culture (Bacillus coagulans 322: DSM-1) was procured from National Collection of Dairy Cultures, Dairy Microbiology Division, National Dairy Research Institute, Karnal- 132 001.

### 2.2 Preparation and Spray Drying of Betel leaves extract

1.1 kg of *Bangla* variety of betel leaves were treated in air convection drier at 60$^{o}$C. The dried leaves was ground in a mixer. 95 g of the powder was obtained which was extracted using distilled water at a ratio of 30:2.5 (ml:g) at 60$^{0}$ C for 1 hour. The mixture was filtered and a feed solution was prepared using an encapsulating material, maltodextrin in the ratio of betel leaves extract to 5% w/v maltodextrin solution of 1:1.

According to Tee *et al* (2012), maltodextrin protects HC (hydroxy chavicol) against adverse conditions like heat and reduces the hygroscopicity of powder [9]. The final volume of the feed solution was 2400 ml. The above solution was then spray dried at the inlet drying temperature of 110.5-110.9$^{0}$ C, outlet temperature of 60-75$^{0}$ C and aspirator rate of 47% (LU 22 Mini Spray Drier, Labultima). Total dried betel leaves powder obtained by spray drying was found to be 32.6 g. Spray drying was performed at Chemistry Laboratory, Kelkar Education Trust's V.G. Vaze College of Arts, Science and Commerce, Mumbai. The spray dried betel leaves extract powder (BLP) was then packed in triple laminated bags.

### 2.3 Revival and spray drying of probiotic culture

Freeze dried *Bacillus coagulans* was revived. The culture was grown using pre sterilized medium (121$^{o}$C, 30 mins), per 100 ml of nutrient broth ([1 g peptone, 0.3 g beef extract, 0.5 ml NaCl, 100 ml distilled water] pH 7). Upon cooling to 38$^{o}$ C, the medium was inoculated with *Bacillus coagulans* and fermentation was carried out on an incubator shaker. Inoculum was built up by inoculating the culture in smaller volumes of broth thus increasing culture volume. The inoculation was done in small volumes of broth and at each step the purity of culture was tested by gram staining and by isolating on agar plate using spread plate technique. Many such cycles were repeated to build up the required cell number.

After complete sporulation, 100 ml *Bacillus coagulans* broth was centrifuged and viable count was analysed by performing serial dilutions of the sample using sterile saline as diluents. 10 fold dilutions were performed till the sample showed an OD of 0.02 in colorimeter. The sample which showed an OD of 0.02 was used to perform 10 fold dilutions by pipetting out 0.5 ml of $10^{-3}$, $10^{-4}$, $10^{-5}$, $10^{-6}$, and $10^{-7}$ in a sterile test tube containing 4.5 ml sterile saline. 0.1 ml sample was taken from each test tube and poured into sterile nutrient agar plates. Spreading was done by surface spreading. The plates were incubated at 37$^{o}$C for 48 hours.

600 ml of *B. Coagulans* and saline solution (1:10, v/v) was fortified with pre sterilized calcium lactate at the ratio of 5:1 (v/g) i.e, 120 g, under aseptic conditions, on complete sporulation. According to Yadav et al (2008), calcium lactate shows 73% survival of

spores of *B. Coagulans*, when it is used as an encapsulating agent [10]. The culture was spray dried at an inlet drying temperature of 110.2-110.5$^0$ C, outlet temperature of 55-75$^0$ C and aspirator rate of 48%. Total dried culture powder obtained by spray drying was found to be 220 g. Spray drying was performed at Chemistry Laboratory, Kelkar Education Trust's V.G. Vaze College of Arts, Science and Commerce, Mumbai. The spray dried powder was then packed in triple laminated bags and 10 fold dilutions were performed to check for viability. The viability of spray dried probiotic powder was found to be 1265.7 x $10^8$cfu/g.

### 2.4 Development of Spray dried powder using suitable additives

Betel leaves have a fresh pepperly taste [11] which highly dominates in the beverage. An attempt was made to improve the acceptability scores based on suggestions made by panelist by addition of fennel seed powder (13%) and cardomom powder (3%). Natural flavoring substances are substances obtained from plant or animal raw materials, by physical, microbiological or enzymatic processes. Cardamom and fennel seeds are used for flavoring of beverages and drinks such as coffee and tea [11].

With an objective to boost its commercial viability and improve the acceptability of the beverage, sugar was added to 78% WPC and 5% BLP. After many trials this percentage of WPC and BLP was selected.

The resulting samples including i) PDP (Probiotic drink powder): WPC + BLP + Fennel seed powder + Cardomom powder (Control) ii) PDPS (Probiotic drink powder containing sugar): Control + Sugar (7.5 gm) were packed in triple laminated bags.

### 2.5 Microbial Analysis

**Viable Count (VC) Assessment (10 fold dilution):**

VC was determined to find out number of *B.coagulans* in the given sample. 10 fold dilution was performed and 0.1 ml was poured into sterile nutrient agar plates. Spreading was done by surface spreading. The plates were incubated at 37$^o$C for 48 hours.

### 2.6 Sensory Evaluation

The evaluation was carried out at Research Laboratory, College of Home Science, Nirmala Niketan, Mumbai, to determine the overall acceptability of the variations of spray dried beverage at day/s 1, 8, 16 and the results were compared. The study had 15 semi trained panelists and the powder was reconstituted with 200 ml water and was served in plastic cups labeled with random digits to the panelist for sensory evaluation. The panelists were briefed about the product and the attributes to be tested before starting the evaluation. Reconstituted beverage was evaluated using a 7 point hedonic scale [18]. Sensory scores were analyzed statistically by ANOVA to evaluate the significance at $p < 0.05$.

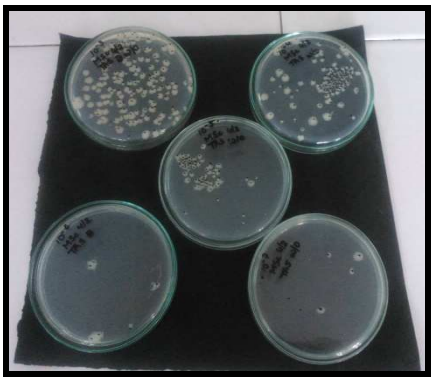
## 3. Results and Discussion

### 3.1 Microbial Analysis

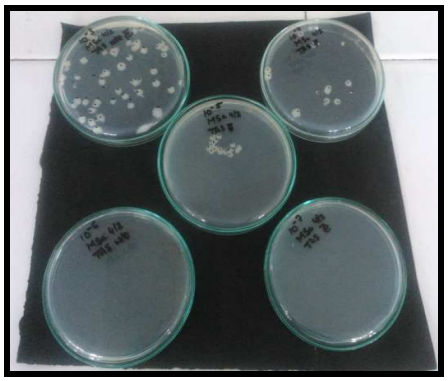
Viable Count (VC) gives a quantitative analysis of the presence of microorganisms in the form of cfu/g [12]. Viability of probiotic bacteria in a product at the point of consumption

is an important consideration for their efficacy, as they have to survive during the processing and shelf life of food and supplements, transit through high acidic conditions of the stomach and enzymes and bile salts in the small intestine [13]. In the present study, VC for the spray dried powder mixtures was estimated for a period of 30 days.

**Figure 1:** Estimation of Viable Count



Day 30: PDP



Day 30: PDPS

**Table 1**: VC of PDP and PDPS

| Storage Period | PDP (x $10^8$ cfu/g) | PDPS (x $10^8$ cfu/g) |
|---|---|---|
| **Day 1** | 2.3 | 0.4 |
| **Day 8** | 1.8 | 0.3 |
| **Day 16** | 0.4 | 0.2 |
| **Day 30** | 0.1 | 0.2 |

Survival of probiotic in a spray-dried form during storage is higher at lower moisture content [14]. The level of survival of probiotic culture remained constant during 2 months of powder storage at 4°C after spray drying. According to Gardiner *et al*., spray drying may be a cost-effective way to produce large quantities of some probiotic cultures [15]. According to Yadav et al (2009), the viable count of *Bacillus coagulans* remained constant to 6.2 x $10^8$ cfu/g for a period of 1 year when calcium lactate was used as a protectant during spray drying of organisms [10]. During spray drying, removal of bacterial cells-bound water leads to damage of surface proteins, cell membrane and cell wall, thereby destablilizing secondary structure and functional integrity of RNA and DNA by tempering different types of weak bonding [16, 17]. Calcium lactate has the property to hold about 23-30% moisture and is found to be protective [10].

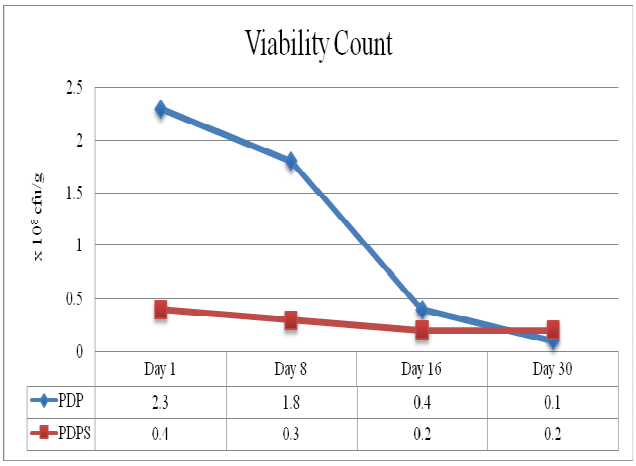



**Figure 2**: VC of spray dried probiotic powder containing whey protein and betel leaves

Figure 2 shows that the cell number in the sample containing sugar remained almost constant till 30 days. In control, there was 10 fold reduction in the number of cells. This proves that sugar stabilizes the bacterial count. So sugar containing product is stable microbiologically.

### 3.2 Sensory Evaluation

At day 1, 8, 16 and 30, sensory evaluation scores showed that PDPS showed higher acceptability in terms of aroma, appearance, flavour, taste than control sample (Figure 3). This could be because of presence of sugar in the sample. However the results were not found to be significant. ($p > 0.05$)

**Figure 3**: Sensory evaluation of reconstituted PDP and PDPS

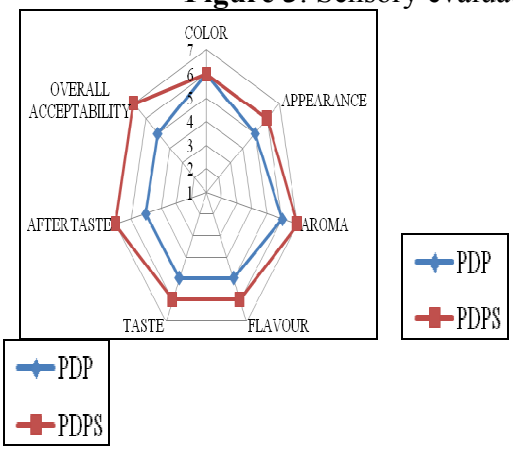



Figure A: Day 1

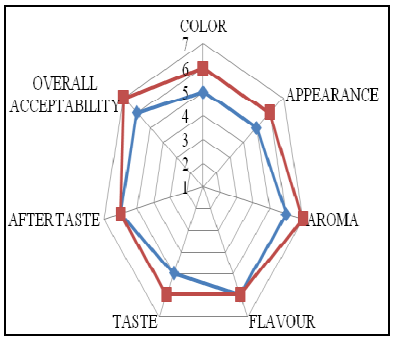



Figure B: Day 8

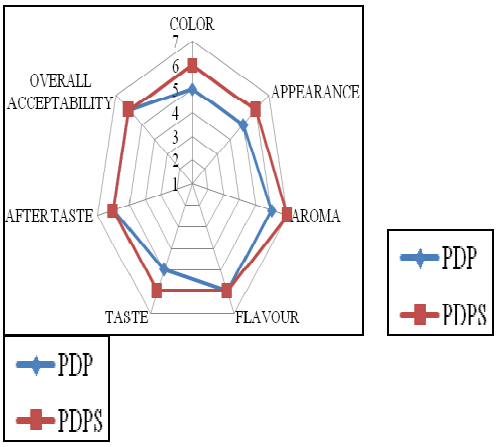



Figure C: Day 16

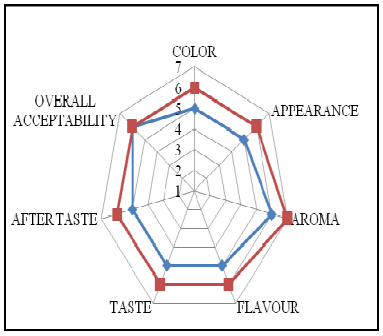



Figure D: Day 30

## 4. Conclusion

Probiotic drink powder containing whey protein and betel leaves was developed with the view to impart nutritional benefits and it can be sold commercially. Based on the results obtained it can be concluded that addition of cardamom, fennel seeds enhanced the acceptability scores. PDPS had higher calories compared to PDP. The control sample showed higher viable counts as against the sample which contained sugar. However, viable count was found to be more constant in case of the sample containing sugar. The study showed that the probiotic organism survived the spray drying procedure hence it can be added to make a healthy powder containing biological benefits. Probiotic products found in the Indian market contains 6.5-7 billion beneficial bacteria (*Lactobacillus casei* strain Shirota). Further studies are required to estimate the antioxidant content, micronutrients and variety of betel leaves can be tested to develop the product.

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