

DEVELOPMENT AND VALIDATION OF SPICE FLAVORED PROBIOTICS

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Abstract

Spices are established sources of natural agents for food flavor and color. In this study, probiotic curd formulations containing varying contents of turmeric and black pepper were prepared and evaluated for the viability of aerobic bacteria during the shelf life of 15 days and consumer acceptability. Based on the results of the viability studies and sensory evaluation composition of a probiotics curd formulation flavored with turmeric and black pepper was arrived at. The formulation with 1.0 and 0.5 % turmeric and black pepper respectively was found to maintain the required levels of probiotic bacteria during the storage for 15 days and was well accepted in the sensory evaluation.

Keywords: Probiotics, Aerobic bacterial plate, count, turmeric, black pepper

Received: 21.01.2022

Reviewed: 25.02.2022

Accepted: 01.03.2022

1. INTRODUCTION

A large amount of beneficial or harmless micro-organisms are naturally found in our mouth, nostrils, stomach, intestine, lungs, urinary tract, vagina, skin, etc. This microflora has major roles in facilitating digestion and absorption of food components and medicines, production of vitamins, defending pathogens, modulation of immunity, etc. They have also been found to produce many bioactive metabolites which can confer benefits to the host when consumed [1]. The conditions such as antibiotic use, travel, diet restrictions, etc can result in the variation of nature and reduction in the content of microflora in the body, which may lead to negative physiological effects. Probiotics are generally used to restore normal microflora in the body and improve health. Probiotics are also being recommended in the treatment of gastrointestinal problems, food allergy [2, 3], antibiotic-related health problems [4], and microbial infections [5–7]. Probiotic supplements are available not only in the forms of foods and drinks but also in the forms of tablets and capsules, sachets of encapsulated and flavored powders, etc. The common probiotic foods include dairy products like yogurt, curd, buttermilk, cheese, and kefir, fermented vegetable products like sauerkraut and kimchi, fermented soybean products like

tempeh, natto, and miso, kombucha (fermented tea), fermented pickles, less cooked soup, etc.

Micro-organisms including different bacterial strains belonging to *Bifidobacterium*, *Lactobacillus*, *Streptococcus*, *Enterococcus*, etc, and the yeast *Saccharomyces boulardii*, [1,8] are being used in probiotics formulations. Among these *Bifidobacterium* and *Lactobacillus* are the most common. *L. casei*, *L. paracasei*, and *L. rhamnosus* are some of the most widely researched and applied probiotic species of *Lactobacilli* [9].

The nutraceutical and functional food sector, the fastest-growing segment of the food industry, is driven by the growing consumer demand for foods with health benefits and the continuous efforts of the industry to formulate foods with unique food ingredients and enhanced health benefits. Recent reports on the detrimental health effects of synthetic food ingredients lead to the increase in consumer demand for foods with coloring and flavoring agents and preservatives of natural origin. Spices and medicinal herbs being natural sources of functional flavoring agents are also being explored for their potential to flavor and color probiotics and impart medicinal effects to the consumers [10]. This study was aimed at the formulation and evaluation of probiotics curd flavored with black pepper (*Piper nigrum*) and turmeric (*Curcuma longa*).

2. MATERIALS AND METHODS

Chemicals and Reagents. Plate count agar (PCA), potassium dihydrogen phosphate, (Himedia, Mumbai, India), sodium hydroxide, acetone, curcumin, piperine (Merck, Mumbai, India).

Spices. Dried black pepper berries and turmeric fingers were purchased from the local markets in Chennai, India. Black pepper berries and turmeric fingers were ground to fine powders in a domestic mixer grinder, passed through a mesh size of US 40. The powders were and sterilized in an autoclave at 100°C, 10 psi before adding to the formulations.

Flavor characteristics of turmeric and pepper. Volatile oil contents in the pepper and turmeric powders were determined by the AOAC 962.17 (1965). Piperin content in black pepper (AOAC 987.07-1990), and curcumin content in turmeric (ASTA 18.0- 2004) were also determined.

Probiotic and raw curd. Probiotic curd of a well-known brand was collected from a local supermarket and coded as Pro-Curd for the study.

The presence of *Lactobacillus acidophilus* and *Bifidobacterium animalis* with a minimum count of 10^6 was claimed on the label of Pro-Curd. Boiled milk (3 L) was inoculated with raw curd (50 mL) and allowed to ferment for 24h at room temperature and named as Raw-Curd.

Preparation of Formulations. Varying amounts (0.5, 1.0, and 1.5 g) of turmeric and/or black pepper powders were mixed with 100 mL Pro-curd or Raw curd to obtain formulations with 0.5, 1.0, and 1.5 % (w/v) spices. The formulations thus prepared stored in airtight food-grade plastic containers at 4 °C for the shelf life studies. The containers once opened for the studies were discarded.

Palatability studies. The sensory evaluation of freshly prepared formulations in terms of color, appearance, texture, flavor, and overall acceptance was assessed by a panel of volunteers. The formulations used for the palatability studies were made either sweet (5g added sugar) or salt (1g table salt).

Total Aerobic Bacterial Plate Count. Total Aerobic bacterial count in the formulations of both Pro-curd and Raw-curd were evaluated at 0, 2, 4, 6, 8, 10, 12, and 15th day of storage as per FDA BAM method (2001). 50g of the formulation was blended for 2 min. with 450 mL of Butterfield's phosphate buffered dilution water.

The mixture was further diluted in phosphate buffer serially up to 10^{-10} . The serial dilutions thus obtained were plated on PCA and incubated at 35°C for 48h. The plates with viable colonies were enumerated.

Statistical Analysis. Results were reported as mean \pm standard deviation of three trials. The significant difference between mean was determined by ANOVA followed by Tukey's pairwise comparison test at a level of $p < 0.05$.

3. RESULTS AND DISCUSSION

Raw and commercially available probiotic curd flavored with turmeric and black pepper at varying concentrations were evaluated for the viability of aerobic bacteria for 15 days and the results are summarized in Figure 1.

The initial aerobic bacterial counts of the Raw-curd and Pro-curd used for the experiments were in the orders of 10^8 and 10^{10} respectively. Raw-curd mixed with 0.5% turmeric (0.5 % Raw-TUR) did not show significant variation in the aerobic bacterial count for 15 days.

At 1% concentration (1.0 % Raw-TUR), a significant reduction in the aerobic count was observed after the 2nd day followed by a slight increase on the 6th day, and thereafter, no significant variation was observed for 15 days.

Table 1. Sensory evaluation of spice-flavored formulations of raw curd (Mean \pm n =3)

Formulation (w/v)		Colour	Appearance	Texture	Flavor	Overall acceptance	Score
Turmeric 0.5 %	Sugared	7.2 \pm 1.1	7.6 \pm 1.0	8.0 \pm 1.1	4.0 \pm 0.6	4.9 \pm 1.0	31.7 \pm 0.8
	Salted	7.1 \pm 1.0	7.6 \pm 1.2	7.9 \pm 0.8	7.2 \pm 0.3	7.2 \pm 0.8	37.0 \pm 0.6
Turmeric 1 %	Sugared	8.2 \pm 0.2	8.2 \pm 0.3	7.6 \pm 1.2	5.2 \pm 0.6	4.8 \pm 0.7	34.0 \pm 0.7
	Salted	7.8 \pm 0.7	8.0 \pm 0.7	7.6 \pm 0.4	8.0 \pm 0.9	7.9 \pm 1.0	39.3 \pm 0.6
Turmeric 1.5 %	Sugared	8.2 \pm 1.0	8.2 \pm 0.8	7.6 \pm 0.7	4.1 \pm 0.5	5.2 \pm 0.9	33.3 \pm 0.7
	Salted	8.0 \pm 0.9	8.0 \pm 0.5	7.2 \pm 0.7	7.5 \pm 0.9	7.2 \pm 0.8	37.9 \pm 0.7
Black pepper 0.5 %	Sugared	7.0 \pm 0.9	7.0 \pm 0.8	6.8 \pm 0.6	4.1 \pm 0.1	4.5 \pm 0.1	29.4 \pm 0.5
	Salted	7.0 \pm 0.7	6.9 \pm 0.6	6.9 \pm 0.7	7.9 \pm 1.1	8.2 \pm 0.9	36.9 \pm 0.8
Black pepper 1 %	Sugared	8.2 \pm 0.9	7.6 \pm 0.9	7.2 \pm 0.7	3.9 \pm 0.0	4.6 \pm 0.2	31.5 \pm 0.4
	Salted	8.2 \pm 1.1	7.6 \pm 0.9	7.2 \pm 0.7	7.6 \pm 0.8	8.0 \pm 0.7	38.6 \pm 0.8
Black pepper 1.5 %	Sugared	8.2 \pm 0.9	7.2 \pm 0.7	7.4 \pm 0.4	3.9 \pm 0.6	4.1 \pm 0.6	30.8 \pm 0.7
	Salted	8.1 \pm 0.9	7.0 \pm 0.7	7.5 \pm 0.5	6.2 \pm 0.7	5.9 \pm 0.6	34.7 \pm 0.8
Turmeric 1 % + Black pepper 0.5 %	Salted	7.9 \pm 0.7	7.6 \pm 0.5	7.8 \pm 0.4	8.1 \pm 0.7	7.9 \pm 0.2	39.3 \pm 0.7

Turmeric powder at 1.5% seemed to exert significant inhibition to the growth and development of bacteria in 1.5 % Raw-TUR, during the storage, at the 15th day the TPC was found as reduced to 10⁴. Black pepper at higher concentrations also reduced the aerobic bacterial count in raw curd formulations during storage. In 0.5% Raw-BP, the count reduced to 10⁸ to 10⁷ after 6 days and remained stable up to the 15th day. In the case of 1.0 % Raw-BP, the count significantly reduced from 10⁸ to 10⁵ between the 6th and 12th day of storage followed by an increase to 10⁶ on the 15th day. At 1.5% black pepper concentration, the aerobic count seemed to reduce continuously from 10⁸ to 10⁴ within 15 days. The variations in the viability of bacteria in Pro-curd in presence of turmeric and black pepper were found to follow trends similar to those of Raw-curd.

The aerobic bacterial counts in 0.5 % Pro-TUR, 1.0 % Pro-TUR, and 0.5% Pro-BP were seemed to be maintained for 15 days of storage without much reduction from the initial value of 10¹⁰. In the case of 1.5 % Pro-TUR, 1.0% Pro-BP, and 1.5 % Pro-BP, the bacterial counts were found to be decreased gradually during storage to 10⁵, 10⁷, and 10⁵ respectively. The results showed that the formulations with turmeric and black pepper at concentrations 1.0 and 0.5 % respectively were able to maintain

the aerobic bacterial count without much reduction from the initial values both in Raw-curd and Pro-Curd formulations. The fragrance of spices directly depends on the content of volatile oils. The black pepper and turmeric used for the formulations respectively contained 3.6 and 4.2 % (v/w) of volatiles. The content of piperine, the compound responsible for the characteristic flavor and pungency of black pepper was 6.5 % (w/w).

The turmeric powder used in the formulations contained 4.2 % (w/w) curcuminoids. Curcuminoids which impart orange-red color to turmeric has been shown to possess many health benefits [11]. The formulations were subjected to sensory evaluation by a panel of 10 volunteers to get an indication of palatability and acceptability of formulations and the results of Raw-curd formulations are summarized in Table 1. Similar results were obtained for Pro-curd, hence the data is not included in the table. Salted formulations were more preferred by the volunteers than sweet formulations. Salted formulations of raw curd containing 1% of both turmeric and black pepper were found to have more acceptance among volunteers followed by their 0.5% formulations.

The assessment aerobic bacterial count showed that the formulations with 1 and 0.5% of turmeric and black pepper maintained the

bacterial count without much variation. Considering the results of sensory evaluation and aerobic bacterial viability studies, a raw curd formulation containing both turmeric (1%) and black pepper (0.5%) with salt was prepared and evaluated for the aerobic count and palatability. The formulation was found to reduce the aerobic count from 10^9 to 10^7 up to 10 days and then increased up to 10^8 and maintained for 25 days (Data not shown). This formulation was also found to be well accepted by the volunteers.

On consumption, the healthy and viable microbes in the probiotic formulations are expected to multiply and colonize in the body to deliver the desired health effects. Hence it is important to ensure that the flavoring and coloring agents and other food additives would not hamper the viability of microbes in the

probiotics. Most of the spices possess considerably higher antimicrobial properties [12] which might stand as a retarding factor for the use of spices as flavoring agents in probiotics. However, recent studies have revealed that spices in lower proportions can be used to impart the required color and flavor to probiotics without affecting the growth and development of the biota [10]. The present study also showed that turmeric and black pepper could be used as flavoring agents to the curd at lower concentrations of 1 and 0.5 % respectively. Both turmeric and black pepper have been demonstrated to have wide spectra of health benefits [11,13,14]. The probiotics formulations containing these spices could be evolved as a medium for delivering their bioactive components to the consumers.

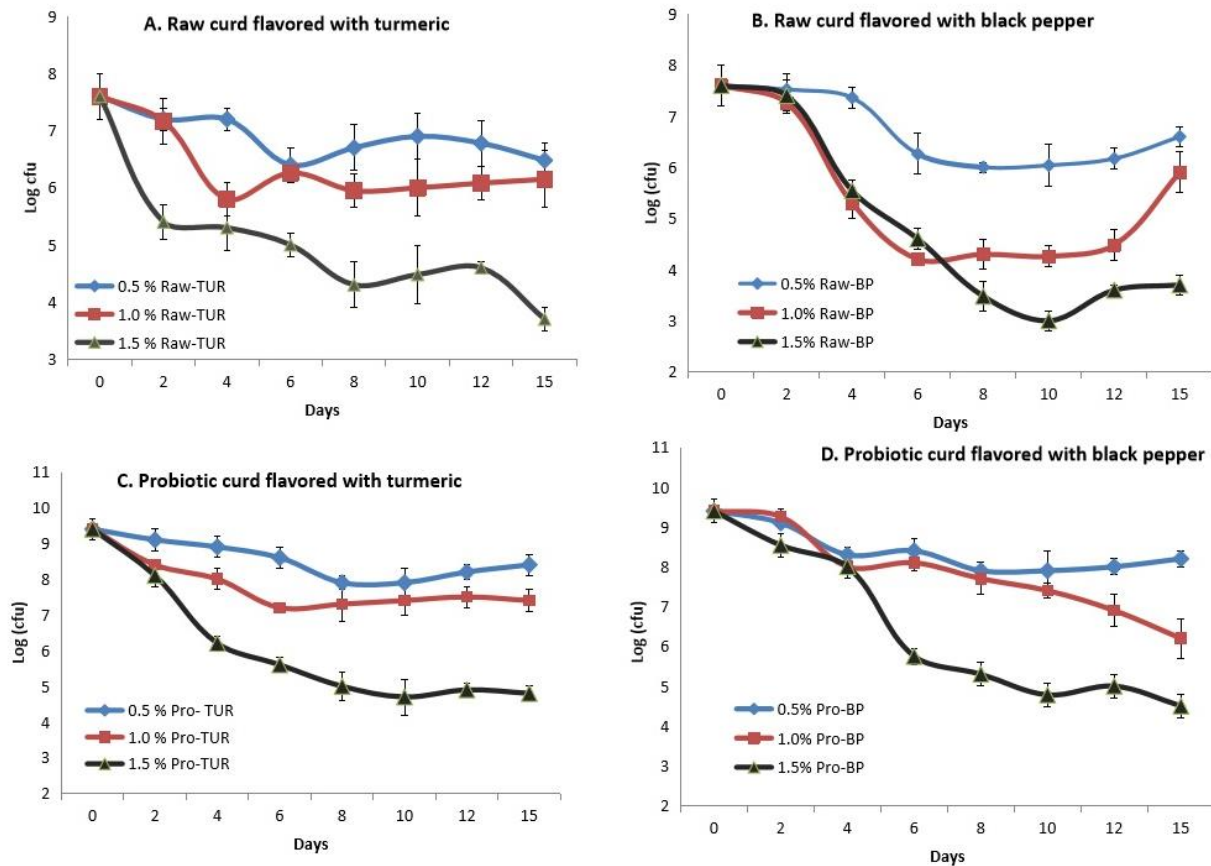


Figure 1. Variations in total aerobic bacterial count in curd flavored with turmeric and black pepper (Mean \pm SD n =3)

The antioxidant nature of these spices might be helpful to improve the oxidative stability of probiotic formulations. There is no fixed definition for the adequate amount of probiotic organisms in the formulations.

A minimum dose of 10^9 colony forming units (CFU) per mL of probiotic organisms is stipulated in the regulations of Canada and Italy. It would be better to add an overage dose of probiotics to the formulations containing turmeric and black pepper to compensate for the possible loss of viability due to the inherent antibacterial nature of these spices.

4. CONCLUSIONS

This study reports the development and assessment of probiotics curd flavored with turmeric and black pepper. The formulation containing 1.0 and 0.5 % turmeric and black pepper respectively was found to maintain the required levels of probiotic bacteria during the period study and was well accepted in the sensory evaluation. A better understanding of the interactions between spice components and probiotic organisms is the key element in developing spice-flavored probiotic food products. Efforts to utilize spices as natural flavoring and coloring agents for probiotics are expected to open up a new area of foods with health benefits.

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