Food ScienTech Journal Vol. 5 (1) 2023 ISSN : 2685-4279 DOI:10.33512/fsj.v5i1.16269

Formulation and Validation of Turmeric and Black Pepper Flavored Probiotics

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Submitted: 16.07.2022; Revised: 26.01.2023; Accepted: 25.04.2023

ABSTRACT

The beneficial or harmless microorganisms naturally found in the digestive tract have major roles in modulating the physiological functions of the host. Probiotics are live microbial feed supplement that helps to improve the intestinal microbial balance and thus imparts beneficial health effects. 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, acceptable compositions of a probiotics curd formulation flavored with turmeric and black pepper were 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: aerobic bacterial plate count, black pepper, probiotics, turmeric

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 against pathogens, modulation of immunity, etc. They have also been found to produce many bioactive metabolites which can confer benefits to the host when consumed (Pandey et al., 2015). 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 recommended in the treatment of gastrointestinal problems, food allergies (Castellazzi et al., 2013; Tuohy et al., 2003), antibiotic-related health problems (Boyanova and Mitov, 2012), and microbial infections (Isolauri et al., 2002; Reid et al., 2003, Ried et al., 2001). Probiotic supplements are available not only in the form of foods and drinks but also in the form 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. Microorganisms including

different bacterial strains belonging to *Bifidobacterium*, *Lactobacillus*, *Streptococcus*, *Enterococcus*, etc., and the yeast *Saccharomyces boulardii* (Czerucka et al., 2007; Pandey et al., 2015), 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 (Saarela et al., 2000).

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 an 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 consumers (Illupapalayam et al., 2014). Black pepper (*Piper nigrum*) and turmeric (*Curcuma longa*) have been reported to possess a wide spectrum of health benefits including antimicrobial, antioxidant, anti-inflammatory, anticarcinogenic, gastro-modulatory, and cardioprotective effects (Ahmad et al., 2012; Jiang, 2019; Lekshmi et al., 2014b, 2012). The presence of spices in the probiotics not only improves consumer acceptability by acting as a natural source of flavor and color but also provides some added health benefits. The presence of spices in the probiotics not only improves consumer acceptability by acting as a natural source of flavor and color but also provides some added health benefits. The antioxidant properties of herbs and spices are of particular interest as they can act as alternatives to synthetic antioxidants that are being used to improve the oxidative stability of probiotics.

Probiotic formulations should be able to provide healthy and viable microbes in a quantity sufficient to multiply and colonize the body to deliver the desired health effects. Hence it is important to ensure that the flavoring and coloring agents and other food additives will not hamper the conditions essential for the viability of microbes in the probiotics. Spices such as turmeric and black pepper contain antimicrobial components that can retard the growth and viability of microbes in probiotic formulations. Hence it is important to establish the composition of these spices sufficient to impart flavor to the probiotics without affecting the viability of microbes. In this study, probiotic curd formulations with varying compositions of black pepper, and turmeric were assessed for consumer acceptance and viability of microbes on storage to arrive at formulations with acceptable flavor and healthy microbial contents.

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 and passed through a mesh size of US 40 and stored at a cool and dry place. The powders were sterilized in an autoclave at 100^{o}C, 10 psi for 15 min. The samples were cooled to room temperature then opened and added to the formulations under aseptic conditions.

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 106 CFU/mL 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 Raw-Curd.

Preparation of Formulations

Pro-curd and Raw-curd samples were evaluated for the total aerobic bacterial count, and the samples with bacterial counts of more than 10^6 colony forming units (CFU)/mL were selected for the formulations. The formulations of Raw-Curd with turmeric were prepared by homogenizing (50 rpm, 5 Min.) 0.5, 1.0, or 1.5 g of turmeric powder with 100 mL Raw-curd and were respectively named 0.5% Raw-TUR, 1.0 % Raw-TUR and 1.5% Raw-TUR. Formulations of Raw-Curd with black pepper (0.5% Raw-BP, 1.0 % Raw-BP, and 1.5% Raw-BP) and Pro-Curd with turmeric (0.5% Pro-TUR, 1.0 % Pro-TUR, and 1.5% Pro-TUR) and Pro-Curd with black pepper (0.5% Pro-BP, 1.0 % Pro-BP and 1.5% Pro-BP) were also prepared in the same manner. Formulations of Raw-Curd or Pro-Curd with 0.5 g turmeric and 0.5 g black pepper, 1 g turmeric and 0.5 g black pepper, 0.5 g turmeric, and 1.0 g black pepper or 1.0 g turmeric and 1.0 g black pepper were also prepared. The formulations thus prepared are stored in airtight food-grade plastic containers at 4^{o}C for shelf-life studies. The containers once opened for the studies were discarded.

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 days of storage as per the 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^{o}C for 48h. The plates with viable colonies were enumerated in terms of CFU/mL.

Palatability studies

Two sets of fresh formulations were prepared as described above. The first set of formulations was made as sweet by adding 5g of sugar and the second set was added with 1g of table salt. Both sweet and salty formulations were subjected to sensory evaluation in terms of color, appearance, texture, flavor, and overall acceptance by a panel of 10 volunteers.

Statistical Analysis

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

RESULTS AND DISCUSSION

Raw and commercially available probiotic curd flavoured 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 7 to 10 log

CFU/mL 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. 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 to be reduced to 3.4 log CFU/mL. 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 the order of 4.3 log CFU/mL after 6 days and remained stable up to the 15th day. In the case of 1.0 % Raw-BP, the count significantly reduced from 7.6 to 4.3 log CFU/mL between the 6th and 12th day of storage followed by an increase to 6.3 log CFU on the 15th day. At 1.5% black pepper concentration, the aerobic count seemed to reduce continuously from 7.6 to 3.3 log CFU/mL 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 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 10^4, 10^6, and 10^4 CFU/mL 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 have been shown to possess many health benefits (Lekshmi et al., 2014a).

The formulations were subjected to sensory evaluation by a panel of 10 volunteers to get an indication of the palatability and acceptability of formulations. The results of the sensory evaluation 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 preferred by the volunteers over 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 of the 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 aerobic count and palatability. The formulation was found to reduce the aerobic count from 10^9 to 10^7 CFU/mL for up to 10 days and then increased up to 10^8 CFU/mL 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 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 spices possess considerably higher antimicrobial properties (Keskin and Toroglu, 2011) 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 (Illupapalayam et al., 2014). 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 (Ahmad et al., 2012; Amalraj et al., 2017; Lekshmi et al., 2014b). The probiotics formulations containing these spices could be evolved as a medium for delivering their bioactive components to the consumers. 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 CFU/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.

CONCLUSION

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 a new area of foods with health benefits.

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Table 1. Sensory evaluation of spice-flavored formulations of raw curd

Formulation (w/v)		Colour (Max Score:10)	Appearance (Max Score:10)	Texture (Max Score:10)	Flavor (Max Score:10)	Overall acceptance (Max Score:10)	Score (Max Score:50)
Turmeric 0.5 %	Sugared	7.2 ± 1.1	7.6 ± 1.0	8.0 ± 1.1	4.0 ± 0.6	4.9 ± 1.0	31.7 ± 0.8[a]
	Salted	7.1± 1.0	7.6 ± 1.2	7.9 ± 0.8	7.2 ± 0.3	7.2 ± 0.8	37.0 ± 0.6[b]
Turmeric 1 %	Sugared	8.2 ± 0.2	8.2 ± 0.3	7.6 ± 1.2	5.2 ± 0.6	4.8 ± 0.7	34.0 ± 0.7[c]
	Salted	7.8 ± 0.7	8.0 ± 0.7	7.6± 0.4	8.0 ± 0.9	7.9 ± 1.0	39.3 ± 0.6[d]
Turmeric 1.5 %	Sugared	8.2 ± 1.0	8.2 ± 0.8	7.6 ± 0.7	4.1 ± 0.5	5.2 ± 0.9	33.3 ± 0.7[c]
	Salted	8.0 ± 0.9	8.0 ± 0.5	7.2 ± 0.7	7.5± 0.9	7.2 ± 0.8	37.9 ± 0.7[b,d]
Black pepper 0.5 %	Sugared	7.0 ± 0.9	7.0± 0.8	6.8± 0.6	4.1± 0.1	4.5± 0.1	29.4 ± 0.5[e]
	Salted	7.0 ± 0.7	6.9 ± 0.9	6.9± 0.7	7.9± 1.1	8.2± 0.9	36.9 ± 0.8[b]
Black pepper 1 %	Sugared	8.2± 0.9	7.6± 0.9	7.2± 0.7	3.9± 0.0	4.6± 0.2	31.5 ± 0.4[a]
	Salted	8.2± 1.1	7.6± 0.9	7.2± 0.7	7.6± 0.8	8.0 ± 0.7	38.6 ± 0.8[d]
Black pepper 1.5 %	Sugared	8.2± 0.9	7.2± 0.7	7.4± 0.4	3.9± 0.6	4.1± 0.6	30.8 ± 0.7[a,e]
	Salted	8.1± 0.9	7.0± 0.7	7.5± 0.5	6.2± 0.7	5.9± 0.6	34.7 ± 0.8[c]
Turmeric 1 % + Black pepper 0.5 %	Salted	7.9± 0.7	7.6± 0.5	7.8± 0.4	8.1± 0.7	7.9± 0.2	39.3 ± 0.7[d]

Mean ± n =3, Different superscripts indicate the values are significantly different ($p < 0.05$)

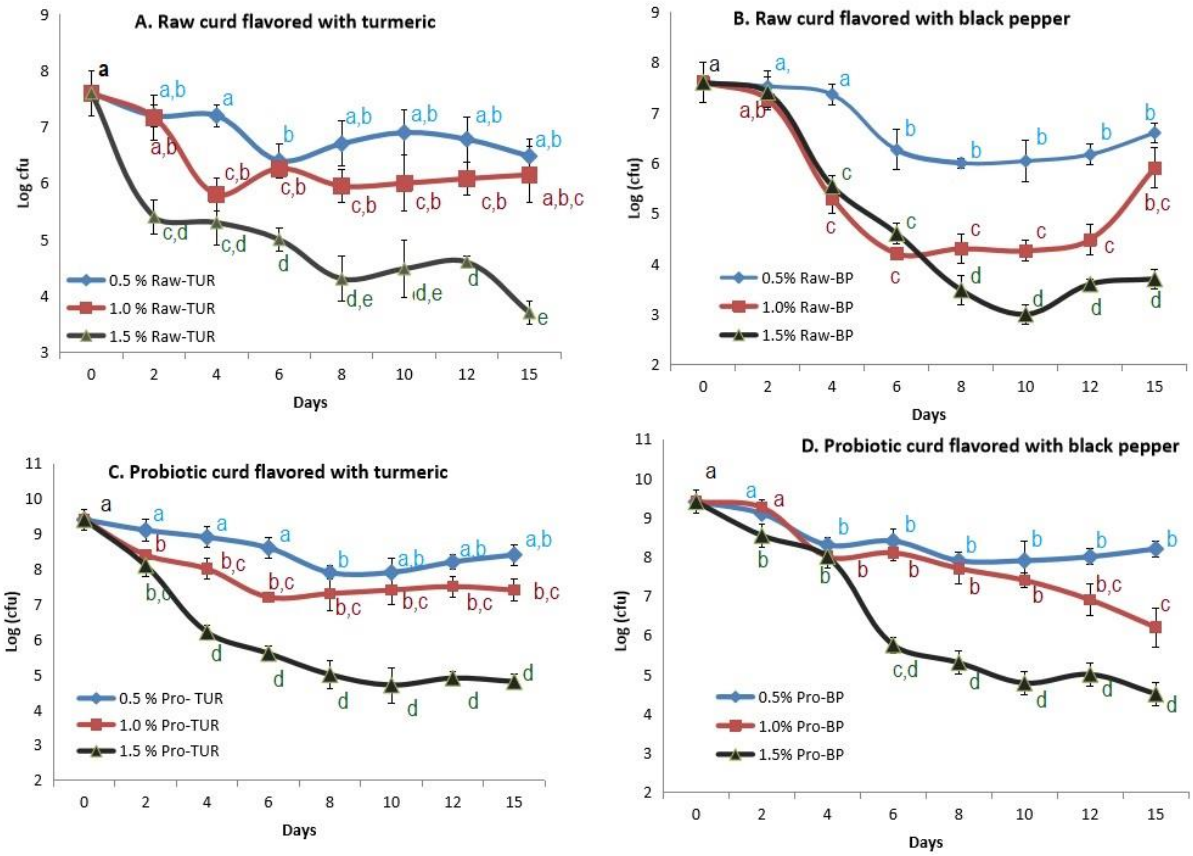

Figure 1. Variations in total aerobic bacterial count in curd flavored with turmeric and black pepper (Mean ± SD, n =3. Different notations indicate the values are significantly different ($p < 0.05$).