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Original Article

Effect of white oyster mushroom and basil powder on physicochemical and sensory characteristics of probiotic yogurt

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Abstract

Mushrooms are a rich source of proteins and carbohydrates, while basil is rich in dietary fibers containing non-digestible oligosaccharides acting as prebiotics. The present study was conducted to understand the impact of adding white oyster mushroom powder and basil powder in the probiotic yogurt. Different concentrations of basil powder (0.5%, 1%, 1.5%, and 2%), along with the 0.5% of white oyster mushroom powder and 5% sugar, was used to make the yogurt. Physicochemical properties such as moisture, fat, titratable acidity, pH, total ash, protein and total solid; and sensory attributes were analyzed on the fermented product. There was decrease in the pH, moisture and fat content while increase in the titratable acidity, ash, protein and total solid in the probiotic curd fortified with basil and white oyster mushroom. The sensory analysis proved basil powder as a flavoring agent besides acting as prebiotic in yogurt. Results obtained showed that the addition of white oyster mushroom and basil powder considerably improved the properties of probiotic yogurt.

Keywords: white oyster mushroom, basil, *Lactobacillus acidophilus*, probiotics, potential prebiotics, yogurt

1. Introduction

The convergence of hectic lifestyle and increase in health awareness among people has raised the demand for functional foods containing probiotics. In India, yogurt / curd/ *Dahi* has an important place among the traditional microbial food supplements. It is the probiotic food that improves the intestinal microbial balance by establishing a desirable microbiome environment in consumers' gastrointestinal tract, thus producing beneficial health effects. The Expert Panel of FAO (Food and Agricultural Organization of the United Nations)/WHO (World Health Organization) first defined probiotics as the "live microorganisms *which* when administered in adequate amounts confer a health benefit on the host" (FAO/WHO, 2002). However, in 2013, the definition was modified to "Live microorganisms *that*, when administered in adequate amounts, confer a health benefit on the host" (Hill *et al*., 2014). Thus, probiotics provide health benefits by ameliorating and/or restoring the gut microflora, alleviating the lactose intolerance, reduction in the harmful metabolites produced in the gut and many more due to the interaction of various immune signaling mechanisms within the host and gut microbiome (de Vrese & Schrezenmeir, 2008; National Health Service [NHS], 2018; Sanders *et al*., 2013).

Probiotics contain a variety of microorganisms ranging from bacteria to yeast. However, only specific genera such as *Bacillus*, *Bifidobacterium*, *Enterococcus*, *Lactobacillus*, *Leuconostoc*, *Saccharomyces*, *Streptococcus*, *Escherichia coli* etc. (Fijan, 2014), have shown the health benefits, hence probiotics. Among the probiotics, *Bifidobacterium* and *Lactobacillus* are widely used strains of bacteria (Guarner *et al*., 2005; Reid, Jass, Sebulsky, & McCormick, 2003).

The probiotics need some agents that can stimulate their growth in the intestine; such agents are termed prebiotics. The International Scientific Association for Probiotics and Prebiotics (ISAPP) in 2016 defined prebiotics as "a substrate that is selectively utilized by host microorganisms conferring a health benefit" (Gibson *et al*., 2017). The primary constituents of prebiotics are fibers, natural sugars, or oligosaccharides, which are found in artichoke, garlic, onion, mushrooms, herbs, and others.

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(Nowak, Nowacka-Jechalke, Juda, & Malm, 2018). Although prebiotics are generally composed of carbohydrate, few studies have shown that polyunsaturated fatty acids and polyphenols, when gets reduced to their respective conjugated fatty acids, also show prebiotic potential (Gibson *et al*., 2017). Researches have suggested that basil seed gum provides a rich source of dietary fibers containing non-digestible oligosaccharides, thus acting as prebiotics (Wongputtisin & Khanongnuch, 2015). Studies on basil have reported that they have higher prebiotic activity, showing abundant growth of Bifidobacterium and Lactobacillus when compared to other known prebiotics and fructo-oligosaccharide (FOS) such as ginger, black pepper (Narendra Babu et al, 2018). Use of basil mucilage have reported to increase viability of lactic acid bacteria, acidity and reduction in pH. Further study on the effect of incubation time of the yoghurt could reduce fermentation time of yoghurt production (Noiduang, Ittakornpan, & Marukatat, 2013).

Mushrooms are rich in bioactive polysaccharide in the form of short chain sugars such as glucose, galactose, fructose and N-acetylglucosamine, thus providing a good source of prebiotic (Patel & Goya, 2011). Oyster mushroom contains protein (29%), carbohydrate, dietary fibers (13%), vitamins, mineral, and essential amino acids like valine, leucine, and glutamine (Deepalakshmi & Mirunalini, 2014; Reis, Barros, Martins & Ferreira, 2012). Their polysaccharide fractions are also resistant to gastric acidity. They effectively reach the intestine unharmed and stimulate the probiotic bacteria, thus showing the potential of mushroom as prebiotics (Nowak *et al*., 2018). Sawangwan, Wansanit, Pattani, and Noysang (2018) investigated the prebiotic properties of edible mushrooms**:** *Auricularia auricula-judae*, *Pleurotus citrinopileatus Lentinus edodoes*, *Pleurotus djamor*, *Pleurotus ostreatus* (Jacq.Fr.) Kummer and found that *P. ostreatus* had the highest carbohydrate content and reducing sugar thus indicating the prebiotic yield. In fact, few studies have shown that use of even 0.5% oyster mushroom (*P. ostreatus*) in the preparation of yogurt and dairy products, also shows promising result (Salama, Mohamed, & Elsisi, 2009; Tupamahu & Budiarso, 2017).

Fermented products like yogurt have a short shelf life due to the low viability of probiotics (Mosiyani, Rezvan, & Mohammad, 2017; Shoji *et al*., 2013). Probiotic cultures must be viable in the product (>10^6 cfu mL^{-1}) throughout the shelf-life, to exert a beneficial impact on consumer health (Costa *et al*., 2019; Hashemi & Hosseini, 2021; Sangami & Sri, 2017; Savedboworn *et al*., 2017). Prebiotics help stimulates the growth, activity and viability of probiotics as they are tolerant to gastrointestinal tract that comprises of salivary amylase, gastric juice and bile extract, thereby, maintaining properties to activate the beneficial microbes for host health (Chowdhury *et al*., 2015; Nowak *et al*., 2018). Thereby, new advances are being made in making functional food by addition of prebiotics to probiotics.

The combination of two prebiotics and their effect on the probiotic lactobacillus species haven't been yet explored much. Therefore, the present study was undertaken with an aim to understand the effect of white oyster mushroom (*Pleurotus ostreatus*) and basil (*Ocimum sanctum* L.) powder on the physicochemical and sensory characteristics of the probiotic yogurt.

2. Materials and Methods

2.1. Materials

Pasteurized full cream milk (Paras Dairy Pvt. Ltd, India), sugar and powdered basil (Figure 1) were purchased from the local market of Lucknow, Uttar Pradesh, India. Probiotic *Lactobacillus acidophilus* ATCC SD5221 strain was used as preculture/inoculum. It was isolated from the commercially available probiotic yogurt "Nestlé Actiplus Dahi" (Nestlé India Ltd., India). The sun-dried white oyster mushroom was also brought from the local market and then grinded to powder (Figure 2).

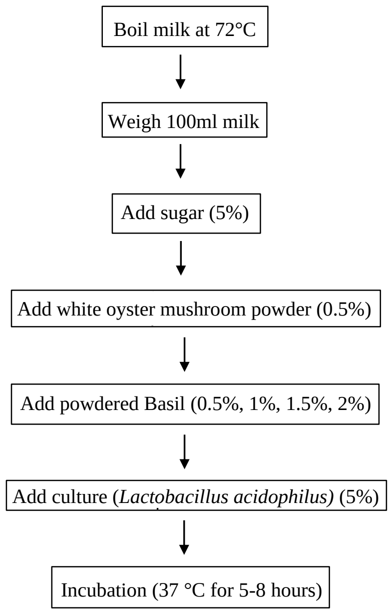

Figure 1. Flow chart for the preparation of probiotic yogurt added with white oyster mushroom powder and basil powder

Figure 2. Basil (*Ocimum sanctum* L.) powder

2.2. Preparation of starter culture

Strains of *Lactobacillus acidophilus* ATCC SD5221 were used starter culture as well as probiotic culture. The content of the package (25 g) was poured into 1 L of sterilized milk, mixed, and stored at 4 °C for inoculation.

2.3. Preparation of yogurt

The probiotic yogurt was prepared according to guidelines of National Dairy Research Institute (Kanawjia, 2006), with slight modifications. Milk was heated at 72 ºC, cooled down to 45 ºC, and divided into five equal portions of 100 ml. To each portion, added sugar (5% or 5gm), white oyster mushroom powder (0.5%) and basil powder at different concentrations (0.5%, 1%, 1.5% and 2%). 5% starter culture/inoculum was added followed by incubation at 37ºC for 8 hours. For further analysis, the yogurt was stored at 4±2 ºC. The preparation of the probiotic yogurt is summarized in Figure 3. The experiment was performed in triplicates. Table 1 illustrates different combinations used as treatments for the experiment.

2.4. Physicochemical characteristics

All samples were homogenized before testing the physicochemical characters. The pH value was measured using a digital pH meter (model PICO, Labindia Analytical Instruments Pvt. Ltd., India). The titratable acidity (expressed as lactic acid %) was evaluated using the titration method (Association of Official Analytical Chemists [AOAC], 2005). The moisture content of the yogurt was measured by drying the sample at 105ºC for 3 hours in hot air oven till the color changed to brown (Manual of Dairy chemistry, 1972). The fat content of the sample was determined using the Soxhlet extraction method using hexane as solvent (AOAC, 2005). Total ash % was determined by keeping the sample at 550 ºC for five hours to get off white/brownish color ash (AOAC, 2005). The protein % was calculated using the Kjeldahl method and converted to total protein by multiplying with the conversion factor 6.25. Total solid was evaluated using the Gravimetric method (AOAC, 2005).

2.5. Viability test

The viability of *Lactobacillus acidophilus* was tested for one week at the interval of two days (1st, 3rd, 5th, and 7th day) by measuring the Total Viable Count (TVC). After preparing homogenates, serial dilution was done using 9 mL of peptone water and taking 1 mL of inoculum from appropriate dilutions. Pour plate technique was used for the enumeration of *L. acidophilus* using de Mann Rogosa and Sharpe (MRS) agar (Himedia, India); the plates were incubated at 43 °C for 72 hours (Tharmaraj & Shah, 2003).

2.6. Sensory analysis

Sensory analysis of the probiotic yogurt was done by the panel of 50 members (untrained; 32 males and 18 females; aged 21 to 40 year; free of lactose intolerance) consisting of students and staffs at the Regional Food Research and Analysis Centre (R-FRAC) Lucknow. The hedonic scale of 9-points (where 9-like extremely and 1-dislike extremely) was used for the evaluation. The sensory/organoleptic parameters considered for analysis were color & appearance, texture, flavor, taste, and overall acceptability. Each sample was given a 3-digit code and served randomly for unbiased analysis. The panelists were served water to clean their palate prior to tasting each sample (Bodyfelt, Tobias, & Trout, 1988). The analysis was done on the 1st day of product formation.

Figure 3. Powdered white oyster mushroom (*Pleurotus ostreatus*)

2.7. Statistical analysis

Experiments were conducted in triplicates, and the results obtained were expressed in mean ± standard deviation. The data were analyzed statistically using a one-way analysis of variance (ANOVA). Post-hoc Tukey's test analysis of means was employed to determine significant differences among treatments at the significance of p-value (< 0.05) using Microsoft Excel (2016 MSO; Version 2103).

3. Results and Discussion

3.1 Physicochemical characteristics

The result obtained showed a significant difference between the control and the probiotic yogurt fortified with basil and white oyster mushroom (Table 2). The pH count was lower in the fortified probiotic yogurt. The addition of basil and white oyster mushroom considerably increased the acidity in the fortified yogurt. The pH and acidity count varied from 5.70 to 5.58 ($p<0.05$) and 0.77% to 0.81% ($p<0.05$) respectively. This might be due to the increased growth of *L. acidophilus*. The activity of lactic acid bacteria increased the acidic count of the product. During fermentation, lactose present in milk is broken down due to the enzymatic activity

Table 1. Different combinations used as treatments for the experiment

Treatments	Milk (%w/v)	Sugar (%w/w)	White oyster mushroom (%w/w)	Basil powder (%w/w)	Starter culture/inoculum (%w/v)
Control (T0)	100	5	-	-	5
T1	100	5	0.5	0.5	5
T 2	100	5	0.5	1.0	5
T 3	100	5	0.5	1.5	5
T 4	100	5	0.5	2.0	5

Table 2. Physicochemical Properties of the probiotic curd samples

Physicochemical properties	Treatments				
	T0	T1	T2	T3	T4
pH	$5.75^{b} \pm 0.01$	$5.70^{b} \pm 0.01$	$5.68^{a} \pm 0.01$	$5.63^{a} \pm 0.01$	$5.58^{b} \pm 0.01$
Titratable acidity (Lactic acid %)	$0.75^{a} \pm 0.01$	$0.77^{a} \pm 0.01$	$0.79^{b} \pm 0.02$	$0.80^{b} \pm 0.01$	$0.81^{a} \pm 0.02$
Moisture (%)	$76.01^{a} \pm 0.02$	$78.51^{c} \pm 0.01$	$78.56^{b} \pm 0.01$	$78.61^{b} \pm 0.02$	$78.69^{a} \pm 0.01$
Fat (%)	$3.48^{a} \pm 0.01$	$3.41^{b} \pm 0.01$	$3.39^{b} \pm 0.01$	$3.37^{ab} \pm 0.01$	$3.36^{b} \pm 0.01$
Ash (%)	$0.67^{b} \pm 0.0$	$0.72^{b} \pm 0.01$	$0.73^{c} \pm 0.01$	$0.75^{a} \pm 0.01$	$0.76^{a} \pm 0.01$
Protein (%)	$3.20^{b} \pm 0.01$	3.38 ± 0.01	3.40 ± 0.01	3.41 ± 0.01	$3.42^{a} \pm 0.01$
Total solid (%)	$17.80^{a} \pm 0.02$	$19.55^{a} \pm 0.02$	$21.22^{c} \pm 0.02$	$23.41^{b} \pm 0.01$	$25.80^{b} \pm 0.02$

(T0= Control, T1 = 0.5% basil, T2=1% basil, T3= 1.5% basil, T4= 2% basil). Data presented as Mean± Standard Deviation. Equal letters in the same line demonstrate significant difference between the formulations ($p < 0.05$).

of lactic acid bacteria **(**LAB**)** present in the yogurt and gets converted to lactic acid, thus increasing the acidity as reported by Bosnea, Kopsahelis, Kokkali, Terpou, and Kanellaki (2017). The average acidity, during one week was found to be highest in treatment samples as compared to control (T0).

The moisture content varied from 78.51% to 78.60% (Table 2), while the fat content showed a significant reduction in treatments than the control. The analysis of total ash % and protein % showed a significant increase ($p<0.05$) in the fortified probiotic yogurt as compared to control. The treated samples showed the total ash in the range of 0.72 to 0.76% (Table 2). The protein-% were insignificant between treated samples (T1-T4) ($p>0.05$); however significant difference could be seen between the control (T0) and treatments (T1-T4). The increased ash and protein content in the yogurt is possibly due to the addition of 0.5% oyster mushroom, as also indicated by Salama *et al.* (2009). This is because mushrooms are rich in carbohydrates (40-43%) and protein (25-29%) besides dietary fibers (Deepalakshmi & Mirunalini, 2014). Their addition increased the nutritional value (carbohydrate, protein, dietary fibers, and others) of the yogurt that also reflected in the result. The total solid % in the fortified yogurt increased gradually from control-T0 (17.80) to treatment sample (T4) containing 2% basil (25.80); and was in accordance with the study of Yadav and Shukla (2014).

3.2 Viability count of *Lactobacillus acidophilus*:

The viability of *Lactobacillus acidophilus* increased significantly during one week (Figure 4). The highest total viable count was 9.85 log CFU/g (T4) while the lowest (9.40 log CFU/g) was seen in T0, but the results obtained were higher than the minimum viable count (10^6 CFU/gm) required for a product to be considered as probiotic (Costa *et al*., 2019). The count was significantly highest in the sample, having 0.5% white oyster mushroom powder and 2% basil powder. The findings were concordant with Allaithy (2014), who reported a similar increment in the no. of *Lactobacillus plantarum* when the different concentration of white oyster mushroom powder (0.5% and 1%) was used. The increased viability of Lactic Acid Bacteria (LAB) is due to the enzymatic activity of bacteria. LAB produces an enzyme α-galactosidase, that breakdown carbohydrate into galactose and sucrose. Sucrose further degrades to glucose and fructose. This glucose becomes an additional nutrient source for the growth and viability of Lactobacillus (Irving *et al*., 2014; Tupamahu & Budiarso, 2017).

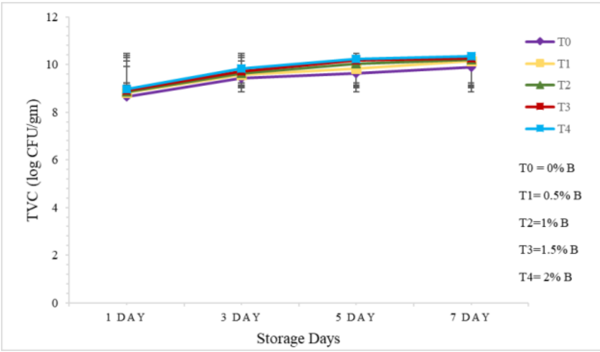

Figure 4. Total viable count (TVC) of the probiotic curd samples at 4 ºC

3.3 Sensory characteristics

The evaluation of sensory/organoleptic parameters, namely, color and appearance, texture, flavor, taste, and overall acceptability (Table 3). On an average, the highest score of overall acceptability was for control. Although, the use of 1% basil powder was favored in terms of color and appearance. The brownish-cream appearance of the treated probiotic yogurt (Figure 5) shows the potential of basil and mushroom as a natural food coloring agent. The texture analysis showed the preference of control over fortified probiotic yogurt. Basil having strong aroma due to essential oils, overpowered the aroma/flavor of white oyster mushroom, thus providing a pleasant flavor. Similar results were seen in taste parameter where probiotic yogurt fortified with 1% basil powder was preferred. However, basil powder having 2% concentration was not accepted due to the bitterness. The highest overall acceptability score was for the control sample, and the lowest was for the sample containing 2% basil powder. No significant difference ($p>0.05$) was found between control and T2 (sample having 1% basil). Contrary, Mosiyani *et al.* (2017) found that the use of 8% and 10% basil extract decreased the taste, aroma, color and overall acceptance. This might be due to the use of a high concentration of basil extract.

Table 3. Sensory evaluation of probiotic curd samples

Sensory properties	Sensory Scores				
	T0	T1	T2	T3	T4
Color &Appearance	$8.00^{a} \pm 0.20$	$8.50^{b} \pm 0.42$	$8.75^{ab} \pm 0.43$	$7.75^{a} \pm 0.32$	$7.50^{a} \pm 0.50$
Texture	$8.50^{a} \pm 0.52$	$7.10^{a} \pm 0.29$	$7.13^{b} \pm 0.43$	$6.75^{a} \pm 0.43$	$6.50^{ab} \pm 0.32$
Flavor	$8.75^{a} \pm 0.41$	$7.75^{a} \pm 0.74$	8.50 ± 0.24	$7.50^{b} \pm 0.32$	$6.85^{b} \pm 0.68$
Taste	$8.75^{a} \pm 0.32$	$8.00^{b} \pm 0.38$	$8.50^{c} \pm 0.32$	$7.13^{a} \pm 0.78$	$6.50^{ab} \pm 0.38$
Overall acceptability	8.75 ± 0.50	$7.50^{a} \pm 0.28$	8.37 ± 0.40	$7.36^{b} \pm 0.51$	$7.00^{a} \pm 0.42$

(T0= Control, T1 = 0.5% basil, T2=1% basil, T3= 1.5% basil, T4= 2% basil) Equal letters in the same line demonstrate significant difference between the formulations ($p < 0.05$).

Figure 5. Appearance of the probiotic curd samples. Note: T0= Control, T1 = 0.5% basil, T2=1% basil, T3= 1.5% basil, T4= 2% basil

Therefore, it can be stated that among the treated samples, 1% basil powder fortification in the probiotic yogurt was highly favored, which also did not differ significantly from control (T0). Scores of color and appearance, taste and flavor also suggest the acceptance of basil and mushroom as a flavoring and natural coloring agents besides acting as prebiotics.

4. Conclusions

The present study showed that white oyster mushroom and basil considerably increased the quality of the probiotic yogurt. Based on the viability count and total acidity, yogurt with 2% basil powder was found optimal for the fermentation and use of 1% basil powder was favored based on the sensory/organoleptic evaluation. The increment in the viability of *Lactobacillus acidophilus* was also observed, which could be attributed to the basil and white oyster mushroom powder, thus showing their prebiotic potential used in combination and their acceptance as a fermented food product. However, the functional properties of the powders (white oyster mushroom and basil), and their modifications for the development of other food/fermented products and how does it impact the rheological characteristics of the fermented product, is yet to be studied for their application in various food industries. The molecular and functional characteristics of the combination of various prebiotics and their role in the gut microbiome are still to be explored.

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