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Enhancing Probiotic Labneh with Broccoli Florets: Impact on Nutritional Quality, Antioxidant Activity, and Microbial Viability

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Abstract

The purpose of this study is to develop functional probiotic Labneh cheeses supplemented with broccoli florets. Probiotic Labneh cheese was produced using broccoli florets paste at four different levels (0, 5, 10 and 15%), with Lactobacillus casei NRRL B-1922 as a probiotic strain, to evaluate its physicochemical, phenols, antioxidant activity, minerals, vitamins, textural, microbiological and sensory characteristics during storage for 15 days. The results indicated that Labneh cheese with added broccoli paste exhibited significantly (p 5 0.05) higher level of moisture, acidity, soluble nitrogen, phenols, antioxidant activity, minerals and B vitamins, and lower protein, fat, ash and pH values when compared to control Labneh cheese. Textural analysis of Labneh cheese indicated that Labneh with higher levels of broccoli (15%) exhibited a harder texture than others. Higher viable counts of Lactobacillus casei and Streptococcus thermophilus were detected in Labneh with broccoli paste, and the counts (107 cfu/g) were higher than the number should be present to achieve their health benefits. The most acceptable Labneh cheeses were those supplemented with 5 and 10% broccoli paste. Originality/value – This study revealed broccoli florets could enhance the growth of Lactobacillus casei and Streptococcus thermophilus in the Labneh matrix, which resulted in a wider spectrum of health benefits of Labneh cheese to the consumers.

Keywords: Labneh, Probiotic, Lactobacillus, Broccoli, Antioxidant activity.

INTRODUCTION

Probiotic is a relatively new word meaning 'for life', which is used to name microorganisms that are associated with the beneficial effects for humans and animals. These microorganisms contribute to intestinal microbial balance and play a role in maintaining health. The probiotic microorganisms consist mostly of the strains of the genera Lactobacillus and Bifidobacterium, but strains of Bacillus,

Pediococcus and some yeasts have also been found as suitable candidates. Together they play an important role in the protection of the organism against harmful microorganisms and also strengthen the host's immune system.Regular consumption of food containing probiotic microorganisms is recommended to establish a positive balance of the population of useful or beneficial microbes in the intestinal flora. Foods should contain a sufficient number of probiotics (above 106–107 cfu/g) to be called a probiotic food with functional characteristics and numerous health benefits (Behera and Panda, 2020). Probiotic foods can reduce the risk of cancer diseases, lower cholesterol level, enhance immunity and contribute to maintaining the balance of bacteria in the human gut, which help consumers enjoy life and do well in their routine activities (Kilic, 2018; Elkot,2020). In addition, probiotics can enhance the lactose digestion by their digestive enzymes, minimize the side-effects of antibiotics by improving the quick recovery of the human intestine after the treatment with antibiotics, protect against the intestinal infections by organic acids and their antibacterial activities (Kilic, 2018).

The genera of Lactobacillus casei was reported to enhance the immune system, cell function, promote the healthy bacteria in human intestine and modify the activities of harmful bacteria, which make such probiotic Probiotic Labneh cheese Probiotics are defined as ‘live microorganisms that, when administered in adequate amounts, confer a health benefit on the host’ (Food and Agriculture Organization of United Nations; World Health Organization – FAO/WHO, 2001).Probiotic food is defined as a processed product which contains viable probiotic microorganisms in a suitable matrix and in sufficient concentration (Saxelin,Korpel Mäyrä-Mäkinen, 2003).

The number of available products and the consumer's familiarity with the ‘probiotic’ concept has been increasing, and, as a consequence, the research into these products has also been increasing. More than 600 food products launched by the dairy industry in 2006 used the term probiotic (Sveje, 2007). The best-known examples of probiotic foods are fermented milks and yogurts, which are generally consumed within days or weeks of manufacture (Nagpal et al., 2007).

Cheese is one of the most versatile food products available nowadays, appealing to many palates and suitable for all age groups. Its versatility offers opportunities for many marketing strategies (Wilkinson, Meehan, Stanton, & Cowan, 2001), as a probiotic food carrier. However, the development of probiotic cheeses implies obligatory knowledge of all their processing steps, as well as on their level of influence – positive or negative – on the survival of these microorganisms

throughout their shelf life. In this context, this review covers the main hurdle technologies involved in the manufacture and stability of probiotic cheese.

MATERIALS AND METHODS

Isolation of bacteria from curd sample

The medium which was selected for the lactic acid bacteria was de Man, Rogosa, and Sharpe (MRS) agar medium. A loopful of curd sample was diluted in serial dilution method and performed a spread plate method, under aseptic conditions. After performing the spread plate method on all the Petri plates, they were incubated at 37°C for 24 to 48 hrs. After the incubation, colonies were streaked on the MRS agar Petri plate for the formation of isolated colonies.

Identification of bacteria

Lactobacilli confirmation can be done microscopically by performing gram staining. The colour, shape and motility was also observed. Biochemical tests were also performed for the identification of probiotic bacteria.

Determination of bile salt tolerance

Bile salt tolerance of the isolated bacteria was examined by inoculating the freshly cultured isolates at 1% (%) in MS both containing Bile salt at different (0,0.1,0.3.0.5 and 1% (w). The medium was then incubated at 37°C for 24 hours in anaerobic condition. The growth of the isolates were monitored as 0, 3, 5 and 24 h by measuring the absorbance of the culture broth at 620 nm using a spectrophotometer.

pH acidity

(Sample preparation) weight 10g of probiotic cheese sample, add 10ml of distilled water in the diluted sample. Filter the diluted sample in watsmann filter paper to remove solid particles. Collect the filtered sample in a beaker. After filter the sample perform titration , for titration 10ml of filtered sample taken in the conical flask ,added 2 to 3 drops of phenolphthalein indicator to the sample. Fill the burette with 0.1N (NaOH) solution. The NaOH was titrated against with the phenolphthalein the colour was changed.

Protein estimation (kjeldhal method)

Weight 0.5 to 1g of the sample and transfer into a kjeldhal flask , and add 10ml of distilled water in the diluted sample. Add 10-15ml of concentration sulfuric acid [H2SO4]. Add a pinch of catalyst example

of the copper sulphate [cuso4] + k2so4. Heat the flask in a digestion unit until the solution turns clear [2to3 hrs], which means all the nitrogen is converted to ammonia sulphate, and cool the sample. [distillation] releasing ammonia gas; add an excess amount of NAOH solution and connect the flask to the distillation apparatus. The ammonia [NH3] formed is released and absorbed in a boric acid solution containing a mixed indicator .For titration the boric acid solution with absorbed ammonia is titrated using Hcl (or) H2So4 solution until the indicator change the colour from green to pink .

Phenol analysis (Folin-Ciocalteu method)

1 to 2 g of cheese sample was dissolved in 10 to 20ml of ethanol or methanol. Shake the sample for 10 mins in a metabolic shaker. Filter the sample in watsmann filter paper. For reaction 0.5ml of sample extract was taken in a test tube and added 2.5ml of folin-ciocalteu reagent and let it react for 5 minutes in the dark. Add 2ml of sodium carbonate solution and mix well, leave it for room temperature at 30mins. Measure the absorbance at 670nm using spectrophotometer.compare with a standard curve of gallic acid to determine the phenol content.

Antimicrobial Test (Agar well diffusion method)

The Muller Hinton agar is prepared and poured into sterile petri dishes and allows it to solidify. Then swab the two different bacterial inoculum uniformly over the agar surface using a sterile cotton swab. After inoculation sterile the wells were made in the agar plate. Add 100µl of cheese extract was added into the well. Allow the extract to diffuse at room temperature for 30 minutes, and incubate the plates at 37 degree celcius for 24 hours. After incubation check the zone of inhibition and and measure the diameter of the inhibition zone of millimeters.

Pathogenic test

Prepare a nutrient agar and pour it into sterile petri plates and perform serial dilution using the cheese samples and make three plates for thermophilic, psychrophilic and mesophilic . for bacteria the required dilution was taken and performed a pour plate method 0.1ml of diluted sample was taken and pour it into an agar plate and stored the plate in a mesophilic (room temperature), thermophilic (high temperature) and psychrophilic (refrigerated condition) incubate the plate for 24 hours and after incubation analyse the growth of the microorganism.

RESULTS AND DISCUSSION

Isolation of bacteria from curd sample

MRS medium plates were observed to have isolated bacterial samples from curd .The bacterial sample was further tested for confirmation of lactobacillus species. The bacterial isolates were observed to be round, flat,slightly mucoid, creamy in colour, gram staining was performed and observed to be gram positive bacteria and observed to be rod shaped. A biochemical test was also performed.

Identification of bacteria

Lactobacillus spp,was identified microscopically. They are psychrophilic, non -spore forming, rod shaped, non-motile, gram positive, and facultative anaerobic.

Antimicrobial Test (Agar well diffusion method)

In this test the probiotic cheese was used in the test. The clear zone was formed in a well and its highly resistant strain was taken in a process and 5mm of zone formation was observed.

Pathogenic test

In this probiotic cheese was inoculated in a plate and kept at a different temperature. The growth was observed in mesophilic and thermophilic temperature, the growth was not observed in psychrophilic temperature.

Conclusion

This study assessed the development of broccoli and buffalo milk-based labneh cheese represents a significant advancement in functional food innovation. This combination not only enhances the nutritional profile of labneh but also introduces a new flavor dimension to dairy products. Buffalo milk, known for its rich protein and fat content, provides a creamy texture and robust taste, making it a suitable base for labneh production. Incorporating broccoli into labneh cheese brings added value by increasing its fiber, vitamin, and antioxidant content.When combined with buffalo milk labneh, it results in a product that not only satisfies taste preferences but also meets the growing consumer demand for nutritious and functional foods. This product could cater to a wide range of dietary preferences, including those who prioritize high-protein diets, probiotic intake, and nutrient-dense foods. In conclusion, the integration of broccoli into buffalo milk-based labneh cheese offers multiple advantages, from improved nutritional composition to unique sensory attributes. This innovation underscores the

potential for expanding the dairy industry by incorporating plant-based nutrients, thus contributing to a more diverse and health-oriented food market.This product highlights the potential for incorporating vegetables into dairy-based foods, paving the way for more diverse and health-promoting food choices in the market.

Conflict of interests

The authors declare that there is no competing interest.

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Table 1. Biochemical Test

S.no	Basic characteristics	Properties (*Lactobacillus spp*)
1	Indole test	Negative
2	Methyl red (MR)	Negative
3	Voges-proskauer (VP)	Negative
4	Citrate utilization test	Negative
5	Catalase	Negative

Table 2. Determination of bile salt tolerance

Time In Hour	0.10 %	0.30 %	0.50%	1%
0	0.218	0.218	0.219	0.21
3	0.211	0.261	0.203	0.126
5	0.374	0.354	0.116	0.152
24	0.923	0.571	0.096	0.086

Table 3. Ph acidity

S.No	Weight Of The Cheese Sample (g)	Initial Ph	Final Ph	Volume Of Naoh Used (ml)	Normality Of Naoh	Acidity (ml)
1	10	4.5	7	15	0.1	36.5

2	10	4.6	7	14.5	0.1	36.5
3	10	4.4	7	15.2	0.1	36.5

Table 4. Protein estimation (kjeldhal method)

S.No	Weight Of Cheese Sample (g)	Weight Of H2so4 Used (ml)	Volume Of Naoh Used (ml)	Normality Of Naoh (N)	Protein Content (%)
1	1	25	15.2	0.1	49
2	1	25	14.8	0.1	49
3	1	25	15	0.1	49

Table 5. Phenol analysis (Folin-Ciocalteu method)

Test Tube	Volume Of Cheese Samples	OD At 670nm
B	-	0
S1	0.2	0.13
S2	0.4	0.15
S3	0.6	0.16
S4	0.8	0.2
S5	1	0.26
T	g	0.29