LWT - Food Science and Technology 91 (2018) 339–344

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Development of non-dairy fermented probiotic drink based on germinated and ungerminated cereals and legume

Mayuri Chavan[a], Yogesh Gat[b], Mugdha Harmalkar[a], Roji Waghmare[a,*]

[a] Food Science and Technology, School of Biotechnology and Bioinformatics, D. Y. Patil University, Navi Mumbai, India
[b] Department of Food Technology and Nutrition, Lovely Professional University, Jalandhar, India

ARTICLE INFO

Keywords:
Grains
Non-dairy milk
Lactobacillus acidophilus
Probiotic drink

ABSTRACT

In the present study probiotic drinks were developed using germinated and ungerminated seeds of barley, finger millet and moth bean. All three grains were washed, soaked, germinated, dried; both germinated and ungerminated grains were roasted, grinded and mixed in the ratio of 2.5:1.5:1 (barley: finger millet: moth bean) with sugar and cardamom. The drink mixtures were added to distilled water and milks like soy, almond and coconut in different concentrations (0 g, 2 g, 4 g, 6 g and 8 g). Drink mixtures were inoculated with *Lactobacilli acidophilus*, a probiotic bacterium. Physicochemical analysis like pH, acidity, bacterial enumeration and antioxidant activity were carried out, along with the evaluation of organoleptic properties. Fermentation with *L. acidophilus* starter culture improved the overall acceptability and functional properties of beverage during fermentation. Changes in the pH and acidity, bacterial count, DPPH assay and polyphenol content were increased as the concentration of drink mixture increased in three different milk and distilled water. In sensory evaluation it was found that the coconut milk based probiotic drink scored highest than the water, soymilk, almond milk probiotic drink. The overall acceptance score of 4 g drink mixture in probiotic drink was highest among all the drink mixture concentration.

1. Introduction

Probiotic drinks can be made from variety of raw materials such as cereals, millets, legumes, fruits and vegetables (Mradula & Somesh, 2016; Rajyalakshmi et al., 2016; Vasudha & Mishra, 2013). Barley malt is a good source of starch, sucrose, vitamins, essential minerals and phytochemicals (Dabina-Bicka, Karklina, & Kruma, 2011). Cereal based probiotic products develop antimicrobial activity against common microbial pathogen (Sharma, Trivedi, & Gat, 2017). Germinated cereals and grains are more nutritious than raw grains and cereals. Germinated cereals are rich in digestible energy, bioavailable vitamins, minerals, amino acids, proteins, and phytochemicals (Aboulfazli, Shori, & Baba, 2016). Barely, ragi and moth bean help in management of various diseases (heart diseases, diabetes, hyperlipidemia and obesity).

Functional beverages constitute one of the most developed segments and are very appreciated for their nutritional characteristics in the market (Aliakbarian et al., 2015; Silva, Bezerra, Santos, & Correia, 2015). Non-dairy milk is good alternative for dairy-based drinks because dairy milk contains whey and casein protein which can cause allergy. The advantage of non-dairy milk such as soy milk, almond milk and coconut milk is due to the absence of cholesterol and lactose sugar (Neus & Maite, 2015). Non-dairy probiotic drinks are available in market which can be consumed by people having lactose intolerance, who cannot drink dairy-based probiotic drinks (Mradula & Somesh, 2016; Vasudha & Mishra, 2013). This milk is a rich source of highly valuable proteins, iron, unsaturated fatty acids, dietary fibers, vitamin B and isoflavones, which are vital part of our diet (He & Hekmat, 2014).

Probiotics are live microorganisms which are introduced into host for the health benefits they provide (Millette, Luquet, Ruiz, & Lacroix, 2008). Probiotic bacteria are used in the form of nutritional supplements and functional foods having advantage of easily digestibility. It also provides more soluble calcium, reduces flatulence, destroys undesirable pathogens and also improves taste and texture of probiotic fermented food (Sharma et al., 2017). There are various microbial strains which are used as probiotics. The most common bacteria used as probiotics on commercial basis are *Bifidobacteria* and lactic acid bacteria (LAB) such as *lactobacilli*, *lactococci* and *streptococci* (Isolauri, Kirjavainen, & Salminen, 2002). *L. acidophilus* is a lactic acid bacteria which is rod shaped and gram positive in nature. Lactic acid fermentation helps to increase nutritional quality of food as well as maintains the environment and shelf-life of the lactic acid bacteria (Mridula & Sharma, 2014).

* Corresponding author. Food Science and Technology, School of Biotechnology & Bioinformatics, D. Y. Patil University, Sector 15, CBD Belapur, Navi Mumbai 400614, Maharashtra, India.
E-mail addresses: roji.waghmare@dypatil.edu, rosewaghmare@gmail.com (R. Waghmare).

https://doi.org/10.1016/j.lwt.2018.01.070
Received 25 October 2017; Received in revised form 23 January 2018; Accepted 24 January 2018

Various authors have carried out studies on non-dairy probiotic drinks. Mridula and Sharma (2014) prepared non-dairy probiotic drink using sprouted cereal, legumes and soymilk. Antioxidant activity and polyphenol content in fermented soy milk supplemented with WPC-70 by probiotic Lactobacilli have also been previously studied (Subrota, Shilpa, Brij, Vandna, & Surajit, 2013). The mixture of geminated barley, ragi and moth bean can thus be suitable for the growth of *L. acidophilus* as such mixtures may supply required amounts of carbohydrates, starch and protein for growth of bacteria (Maselli & Hekmat, 2016).

The objectives of the present work were (1) to develop cereal, grain and legume based powder and increase its nutritional value, (2) to develop and evaluate non-dairy probiotic beverages using soymilk, almond milk and coconut milk and (3) to compare the physicochemical properties of probiotic drink having germinated and ungerminated drink mixtures.

2. Materials and methods

2.1. Materials used

Barley (*Hordeumvulgare* L.), ragi (*Eleusinecoracana*), moth bean (*Vignaaconitifolia*), soyabean, almond and coconut used in this study were procured from APMC market, Vashi, Navi Mumbai. Pure culture of probiotic bacteria *L. acidophilus* was purchased from Jevan Pharmaceuticals, Navi Mumbai. All chemicals were supplied by Himedia, Mumbai, India.

2.2. Germination and milk preparation

All the grains were cleaned, washed and soaked in water in the ratio of 1:2 (seed to water) separately for 8 h at 30 °C. After 8 h of soaking, water was drained and soaked grains were allowed to germinate in a controlled germinator (30 °C and 95% relative humidity). Barely was germinated for 48 h, ragi was germinated for 36 h while moth bean was germinated for 24 h. The germinated grains were then dried in hot air oven at 55 ± 5 °C to 8% moisture content. Dried germinated and ungerminated grains were roasted for 5 min at 130 °C and ground in an electric grinder. Ground powder was sieved through 0.0075μ sieves.

For milk preparation, 250 g of soybean, 350 g of almond and 350 g of coconut were soaked in water in the ratio of 1:6, 1:4.2 and 1:4 respectively for 12 h at room temperature. After soaking, it were grinded it in an electric grinder and extracted milk was filtered through muslin cloth. The milk was boiled for 5 min and cooled.

2.3. Preparation of culture

Probiotic culture was prepared by using lyophilized culture of *Lactobacillus acidophilus*. Probiotic bacteria were streaked on MRS agar plate and for its preservation sub-culturing of bacteria was done after every 2 weeks. For preparation of inoculums, bacteria were inoculated in MRS broth and incubated for 48 h. After 48 h, broth was centrifuged at 8000 rpm and cell pellets were washed with saline water for 2–3 times until colourless pellet were observed. To obtain required cell count, optical density of inoculum was checked by spectrophotometer at absorbance 660 nm.

2.4. Preparation of probiotic drink

All the germinated and ungerminated ground powder were mixed in the ratio 2.5:1.5:1 (barley: finger millet: moth bean). This ratio of drink mixtures were added in 100 ml of milk and distilled water for the preparation of germinated distilled water probiotic drink (GDWPD), ungerminated distilled water probiotic drink (UGDWPD), germinated soymilk probiotic drink (GSPD), ungerminated soymilk probiotic drink (UGSPD), germinated almond milk probiotic drink (GAPD), ungerminated almond milk probiotic drink (UGAPD), germinated coconut milk probiotic drink (GCPD) and ungerminated coconut milk probiotic drink (UGCPD) in different weights of 0 g, 2 g, 4 g, 6 g and 8 g. Hence, the concentration of drink mixture in probiotic drink was 0.02%, 0.04%, 0.06% and 0.08%. Further, 4 g of sugar and 0.07 g cardamom were added to the drink mixture. The probiotic culture at a level of 1ml/100 ml having cell count 10^4 cell/ml was added to the liquid portion containing the formulation mixture. Then the drinks were allowed to incubate for 6 h at 37 °C. All the samples were prepared in triplicates in a batch of 300 ml.

2.5. Acidity

Acidity in terms of lactic acid was determined using titrimetric method (Ough, Amerine, & Sparks, 1969). Titratable acidity was measured by titration against 0.1 N sodium hydroxide solution and using 1% ethanol solution of phenolphthalein as an indicator.

2.6. pH

pH of the non-dairy milk was measured using a pH meter (Equiptronics, India). The measurements were carried out in triplicates.

2.7. Trolox equivalent antioxidant capacity (TEAC)

For antioxidant activity, probiotic drinks were diluted in 1:100 ratio of probiotic drink: water. TEAC free radical scavenging activity was calculated on the basis of the method described by Gat and Ananthanarayan (2015). All the experiments were performed in triplicates.

2.8. Total phenolic content

Determination of polyphenols was determined using Folin–Ciocalteureagent (Gat & Ananthanarayan, 2015). Absorbance of the clear supernatant solution was measured at 765 nm using allic acid as a standard. Results were expressed as milligram gallic acid equivalent per 100 g dry weight.

2.9. Sensory characteristics

The sensory characteristics of probiotic drink samples were evaluated three times in three different sessions by a group of 15 panelists, who had been trained following the standards procedure before conducting the sensory evaluation (Kilcast, 2010; Waghmare & Annapure, 2015). 100 ml of probiotic drink sample in glass containers, duly coated with code was given to panelist for evaluating the sensory attributes like appearance, flavor, consistency, taste and overall acceptability using nine point hedonic scale. Hedonic scale was in the following sequence: like 9- like extremely, 8- like very much, 7- like moderately, 6- like slightly, 5- neither like nor dis like, 4- dislike slightly, 3- dislike moderately, 2- dislike very much, 1- dislike extremely (Chen, Zhu, Zhang, Niu, & Du, 2010).

2.10. Enumeration of probiotic count

Enumeration of viable cells was performed by estimating colony forming unit number on De Man, Rogosa and Sharpe (MRS) agar plates (pH- 6.4 ± 2) after incubation at 37 °C for 6 h. Probiotic count was done by using streak plate method after incubation at 37 °C for 48 h. Mixture of barley, ragi and moth bean is suitable for the growth of probiotic bacteria hence as there is increasing in concentration of drink mixture there is increasing in count of probiotic bacteria (Maselli & Hekmat, 2016).