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RESEARCH ARTICLE

Development of Herbal Bio-yoghurt Powder using Freeze Drying Technology

Heema R[1], Murugan B[2], Rita Narayanan[3], Pugazhenthi TR[4]

[1]College of Food and Dairy Technology, Tamil Nadu Veterinary and Animal Sciences University, Koduveli, Chennai - 600 052.

[2]Department of Food Safety and Quality Assurance, College of Food and Dairy Technology, Tamil Nadu Veterinary and Animal Sciences University, Koduveli, Chennai - 600 052.

[3]Department of Food Processing Technology, College of Food and Dairy Technology, Tamil Nadu Veterinary and Animal Sciences University, Koduveli, Chennai - 600 052.

[4]Department of Livestock Products Technology (Dairy Science), Madras Veterinary College, Tamil Nadu Veterinary and Animal Sciences University, Vepery, Chennai - 600 007.

Corresponding author mail id: heemaravidrapandian@gmail.com

ABSTRACT

A study was undertaken to produce freeze-dried herbal bio-yoghurt with *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Streptococcus thermophilus,* and an additional probiotic strain *Lactobacillus rhamnosus GG*. Aqueous extract of licorice (*Glycyrrhiza glabra*) at different concentration levels (0.5, 1.0, 1.5, 2.0, 2.5%) was incorporated into the yoghurt, to study the effect of licorice as a prebiotic in promoting the growth of yoghurt starters and *Lactobacillus rhamnosus GG.* The incorporation of licorice extract at different levels positively affected the total viable count of the bio-yoghurt. Antioxidant properties of the herbal bio-yoghurt were analysed and it ranged between 39.26±0.02 and 68.13±0.04% when compared to control yoghurt (22.08±0.03%). The optimum level of incorporation of licorice extract was standardized based on the physico-chemical, functional, and viability of microbes. Among the five different levels of incorporation of herbal extract, 2% level was ideal based on the sensory and physico-chemical parameters for the preparation of herbal bio-yoghurt. On freeze-drying the herbal bio-yoghurt with trehalose and lactose as cryoprotectants, trehalose showed more promising results in protecting the viability of microbes during freeze-drying when compared to lactose, and there was no significant difference between two cryoprotectants and control concerning acidity, pH, moisture and ash of the freeze-dried herbal bio-yoghurt.

Keywords: *Licorice; Bio-yoghurt;Freeze drying; Cryoprotectant; Yoghurt Powder*

INTRODUCTION

In recent years, the demand for highly nutritious and -promoting functional dairy products is on the rise, which acts as a driving force for developing novel food products. In humans, the gut microbiota plays a major role in maintaining various metabolic reactions and helps to protect human health from any pathogenic organisms by increasing the immune response. Any imbalance in the gut environment will lead to disorders or discomfort such as faecal impaction, constipation, unhealthy weight gain, bloating, etc. To overcome these defects, various food products are being formulated. Among them, milk and milk products act as a perfect carrier for delivering nutrients to the host. Fermented dairy products are gaining popularity among the consumers due to the presence of potential health promoting microorganisms in the product and one such product is yoghurt. Yoghurt is a well-known fermented dairy product produced by using two starter cultures, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Streptococcus thermophilus*. It is believed that yoghurt is one of the appropriate vehicles to carry scientifically proven probiotic bacteria and prebiotic ingredients such as fructooligosaccharides, inulin, lactulose, and galactooligosaccharides. A symbiotic relationship exists between these two bacteria which results in the modulation of various metabolites. The term "bio-yoghurt" or 'yoghurt-like product' is defined as product made by alternative yoghurt culture or adding probiotic culture in addition to yoghurt culture (i.e, a specific Lactobacillus probiotic strain is added to *Lactobacillus delbrueckii* subsp*. bulgaricus* and *Streptococcus thermophilus*.)

The herb 'Licorice' belongs to the Fabaceae family and is gaining attention in the food industry as a natural sweetening, flavoring agent and other clinical formulations (Hamad *et al*., 2020). Licorice acts as a natural antioxidant due to the presence of bio-active components, viz. glycyrrhizin, glabridin, phenols, saponins, flavanones, isoglycyrrhizin, isoflavonoids, 18β-glycyrrhetinic acid, licochalcone A, liquiritigenin. These bio-active components exhibited antioxidant activity to overcome oxidative stress caused by free radicals (Rosa *et al*., 2016).

It has been found that spray drying of food products with viable microorganisms affects cell viability resulting in decreased microbial load. Alternatively, freeze-drying is a widely accepted drying process for food products with heat-sensitive ingredients. This technique has always been recognized as the best drying process for preserving foods, which is based on dehydration, by the sublimation process of frozen food products (Bielecka and Majkowska, 2000).

The main objective of the study is to develop an herbal bio-yoghurt powder supplemented with aqueous extract of licorice herb along with an additional probiotic strain *Lactobacillus rhamnosus* GG apart from the yoghurt starter culture and analyze the sensory and physico-chemical properties of the bio-yoghurt and bio-yoghurt powder.

MATERIAL AND METHODS

Starter culture and raw materials:

Fresh skim milk containing about 0.5% fat was procured from the dairy plant at the College of Food and Dairy Technology, Chennai, Tamil Nadu. The yoghurt starters viz. *Lactobacillus delbrueckii* subsp*. bulgaricus* and *Streptococcus thermoplilus* and a probiotic strain *Lactobacillus rhamnosus* GG in freeze-

dried form were procured from NCDC, NDRI, Karnal. The licorice herb powder was purchased from a local shop.

Culture preparation and maintenance:

The freeze-dried cultures of *Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus thermophilus,* and *Lactobacillus rhamnosus* were revived as per the standard protocol and were sub-cultured in skim milk before use.

Preparation of aqueous herbal extract:

Aqueous extract of Licorice root was prepared as per the method described by Hasneen *et al.* (2020) with minor modifications. Briefly, licorice root powder (1:10 w/v) was dissolved and extracted with hot distilled water (60 °C) for 15 min. The suspension was then filtered twice, first through cheese-cloth and then through filter paper (Whatman No. 1). The clear aqueous extract was immediately used.

Preparation of herbal bio-yoghurt:

Fresh skim milk having 0.5% fat, 0.14% acidity, and 9.25% total solids was used for the preparation of bio-yoghurt. Bio-yoghurts were prepared with starter cultures *Lactobacillus delbrueckii* subsp. *bulgaricus, Streptococcus thermophilus* and probiotic *Lactobacillus rhamnosus GG* as constant and varying the level of incorporation of licorice extract at 0.5, 1.0, 1.5, 2.0 and 2.5%. The control sample consisted of one without the herbal extract. To the fresh skim milk, 4% of skim milk powder was added and tempered to 85 °C for 15 min, cooled to 42 °C with the addition of 1% of each of all the three starter cultures and 0.5, 1.0, 1.5, 2.0 and 2.5 % of licorice extract. All the treatments were analyzed for the microbial count when fresh and after 7 and 14 days of refrigerated storage.

Functional property of herbal bio-yoghurt:

The antioxidant activity of herbal bio-yoghurt with different concentrations of licorice extract (0.5, 1.0, 1.5, 2.0, 2.5%) was assessed by DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) radical scavenging activity as per the method described by Behrad *et al*. (2009). In brief, 250 µL aliquot of the sample solution (supernatant) was mixed with 3 mL of 60 µM DPPH in 100 mL of ethanol solution and vortexed. The reaction mixture was left in the dark for 20 min, after which the absorbance was measured at 517 nm. DPPH with ethanol was used as blank. The scavenging activity was calculated as % scavenging effect using the following equation:

$$\text{Antioxidant activity (\%)} = \frac{A_0 - A_1 \times}{A} 100$$

Where,

A_0 is the absorbance of the control at 20 minute

A_1 is the absorbance of the sample at 20 minute

Freeze drying

Freeze drying was carried out using mini freeze dryer (LARK) as per the method described by Venir *et al.* (2007) with minor modifications. Bio-yoghurt samples of 1 mm thickness were frozen in a deep freezer at -18°C for overnight and freeze-dried at -40°C and pressure at 0.006 atm for 6-7 hrs. The herbal

bio-yoghurt powder thus obtained was subjected to sensory, physico-chemical, and microbiological analysis.

Sensory evaluation of herbal bio-yoghurt with different levels of incorporation of licorice extract

The sensorial analysis was carried out for control yoghurt without the addition of aqueous extract of licorice and treatment yoghurt containing 0.5, 1, 1.5, 2 and 2.5% of licorice extract incorporated yoghurt to determine the ideal level of licorice extract incorporation. The bio-yoghurt prepared with different levels of incorporation of licorice extract were analyzed for their sensory qualities such as color and appearance, flavour, body & texture and overall acceptance using 9 point hedonic scale.

Statistical analysis was performed using one way analysis of variance (ANOVA). Statistical analysis was conducted with Vetstat software program.

RESULTS AND DISCUSSION

Physico-chemical characterization for herbal bio-yoghurt:

The effect of incorporation of licorice aqueous extract at different concentrations *viz*. 0.5 (T_1), 1.0 (T_2), 1.5 (T_3), 2.0 (T_4) and 2.5 (T_5) % of the physico-chemical characteristics of bio-yoghurt is presented in Figure 4. There was no significant difference in the pH among Treatments 1, 2, 3, and 4 but significant differences existed between Control, T_5 and the rest of the group. The lowest pH was observed in Treatment 5 (T_5). The results are in line with the findings of Shan *et al*. (2011) in cheese enriched with herb extracts. It was concluded that high levels of phenolic compounds contributed to the lower pH in cheese and other dairy products like yoghurt. As far as acidity is concerned, T5 showed the highest acidity at 0.825 % of lactic acid. An increase in acidity was mainly due to an increase in the number of lactic acid bacteria, which converted lactose into lactic acid (Abdalla and Ahmed, 2010). While no significant difference was observed for fat among different treatments and control, T_5 showed the highest total solids content. The addition of herb extract resulted in an increase in ash content for all the treatments. Keke *et al.* (2009) mentioned that there was an increase in the ash content of cheeses treated with *Sorghum vulgaris* and *Pimenta racemosa*. The control sample scored the highest moisture (in %) with 84.78, whereas T5 outscored other treatments and control in ash content (in %) at 0.75±0.01.

Sensory analysis of herbal bio-yoghurt:

The sensorial analysis of control and treatment yoghurt with 0.5, 1, 1.5, 2 and 2.5% of herb extract addition was depicted in Figure 5. From the analysis, it has been concluded that 2% addition of licorice aqueous extract was awarded the highest overall acceptability further addition of herb extract resulted in sourness because of an increase in the acidity level. The result was in accordance with the observation of Srivastava et al. (2015) that a 2% addition of beet root and ginger extract resulted in the highest sensory score.

Anti oxidant activity of herbal bio-yoghurt:

The antioxidant activity of herbal bio-yoghurt is presented in Figure 6. The result showed that increasing the level of incorporation of the herbal extract resulted in a progressive increase in antioxidant activity and there was a significant increase in antioxidant activity between treatments and control. The

highest level of antioxidant activity was observed in the 2.5% herb extract incorporated bio-yoghurt sample (68.13 ± 0.04%). The findings are in agreement with that of Rosa *et al*. (2016), who found that the addition of licorice root extract boosted antioxidant activity as the concentration of root extract increased due to the DPPH hydrogen donating ability in addition to the correlation with increasing of extracted plant phenolic compounds. According to Thompson *et al.* (2007) and Velioglu *et al.* (1998), the increase in antioxidant activities of herb extract supplemented yoghurt was mostly due to the phytochemical constituents present in the herb and also due to the microbial metabolism. Consequently, the consumption of herbal bio-yoghurt with live bacteria has potential health benefits with antioxidant activity.

Microbiological characteristics for herbal bio-yoghurt

The effect of different levels of incorporation of licorice extract on the total viable count of herbal bio-yoghurt is presented in figure 7. It can be inferred from the results that the addition of herbal extract has a definite effect on improving the viable count of yoghurt and probiotic bacteria. Increasing the level of addition of licorice extract progressively increased the viable count. The viable count (in CFU X 10^9/g) of the control sample and the herbal bio-yoghurt with 2.5% supplemented herbal extract at the end of 14 days of storage were 2.06±0.03 and 27.01±0.04, respectively, thereby confirming the prebiotic property of the herb. The prebiotic effect could be due to the presence of higher levels of fructans in the licorice. The findings are in agreement with the results of Tsirulnichenko and Kretova (2020), who investigated the effect of licorice extract on the growth of probiotic organisms and concluded that a 1% addition of licorice extract was found to be more favorable for microbial growth than 10% addition under in vitro conditions.

Based on the sensory and physico-chemical parameters, a 2.0% level of incorporation of licorice extract was considered ideal for preparing herbal bio-yoghurt, and treatment 4 (T_4) was used for further studies, i.e. in the preparation of herbal bio-yoghurt powder by freeze drying process.

The effect of different levels of incorporation of cryoprotectants at a 5% level on the total viable count of herbal bio-yoghurt powder is presented in Figure 8. Out of the two cryoprotectants, trehalose was found to be a better cryoprotective agent ($p \leq 0.01$) with a viable count (CFU X 10^9/g) of 16.20 ± 0.18 when compared to lactose with 14.11 ± 0.17. The present findings are in agreement with the work carried out by Giulio *et al.* (2005), who concluded that trehalose, compared to other sugars, would enhance the performance of starter cultures and readily protected the viability of microbes during freeze-drying process.

The effect of incorporation of different cryoprotectants on the physico-chemical properties of freeze-dried herbal bio-yoghurt is presented in Figure 9. The results showed no significant difference between the two cryoprotectants and control with respect to acidity, pH, moisture, and ash. The pH value of herbal bio-yoghurt decreased from 4.62±0.01 (Figure 9) before freeze drying to 4.51 ± 0.01 after sublimation in trehalose and lactose incorporated samples. This desired value of acidity indicated that the lactic acid content in yoghurt powder was present after freeze drying. The freeze drying technique was chosen to remove the water content in yogurt, which had viable lactic acid bacteria that were heat sensitive. Based on the test result of moisture content in control and treatment with trehalose and lactose, the moisture content value obtained was quite low and within the standard prescribed for yoghurt powder (< 5%).

CONCLUSION

Supplementation of licorice extract as a prebiotic increased the viability of yoghurt starter culture and probiotic when compared to the control yoghurt. Moreover, a constant increase in antioxidant property was observed as the level of incorporation of licorice extract increased. Among the five different levels of incorporation of herbal extract, a 2% level was found to be ideal based on the sensory and physico-chemical parameters for the preparation of herbal bio-yoghurt. When freeze drying the herbal bio-yoghurt with trehalose and lactose as cryoprotectants, trehalose showed more promising results in protecting the viability of cells during freeze-drying when compared to lactose and there was no significant difference between two cryoprotectants and control concerning acidity, pH, moisture and ash of the freeze dried herbal bio-yoghurt. The prepared freeze dried herbal bio-yoghurt may be used for making pellets with a suitable binding agent that will serve as a ready source of starter and probiotic bacteria for subsequent bio-yoghurt preparation at the household level.

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There was no conflict of interest in the publication of this content

Author contributions

Research grant-TANUVAS,

Idea conceptualization- Heema. R and Murugan. B,

Experiments- Heema. R,

Guidance – Murugan. B, Rita Narayanan, Pugazhenthi T. R,

Writing original draft –Heema. R, Murugan. B, Rita Narayanan, Pugazhenthi T. R,

Writing- reviewing &editing – Murugan. B, Heema. R, Rita Narayanan, Pugazhenthi T. R

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Figures

Figure 1. Aqueous extraction of licorice herb

Figure 2. Freeze dryer

Figure 3. Herbal bio-yoghurt powder

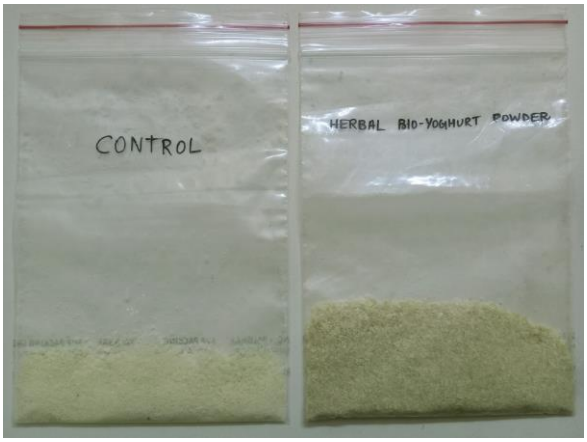


Figure4. Effect of incorporation of different levels of licorice extract on the physico-chemical properties of herbal bio-yoghurt

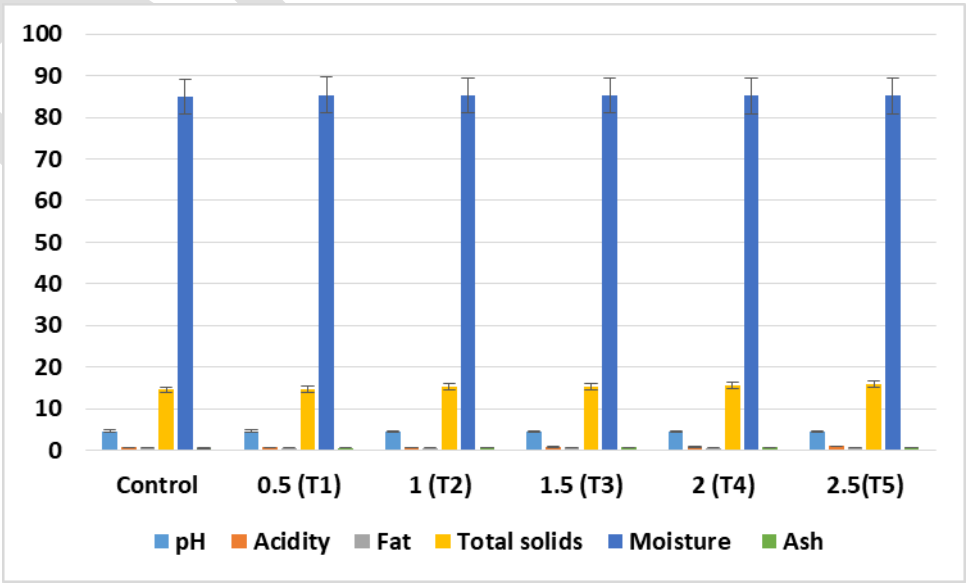


Figure5. Sensory analysis on herbal bio-yoghurt with different level of incorporation of aqueous extract of licorice

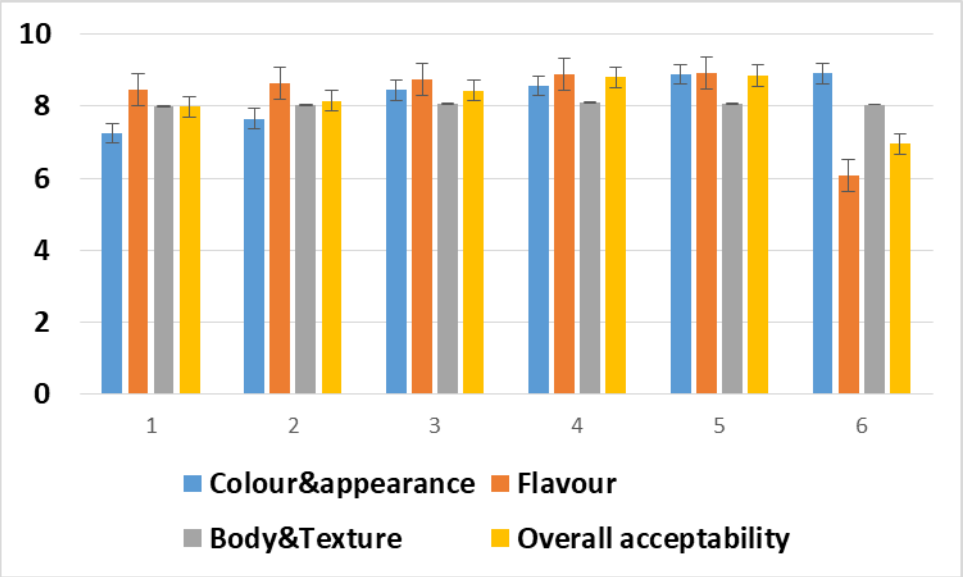


Figure 6 Effect of incorporation of different levels of licorice extract on the antioxidant activity (%) of herbal bio-yoghurt

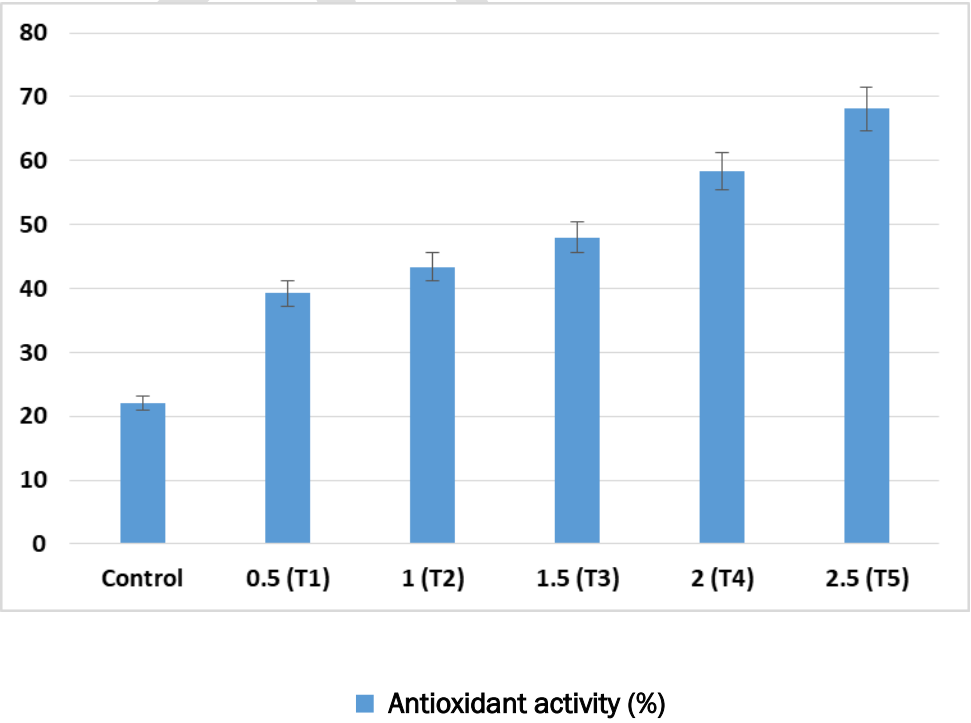


Figure 7. Effect of incorporation of different levels of licorice extract on the total viable count of herbal bio-yoghurt

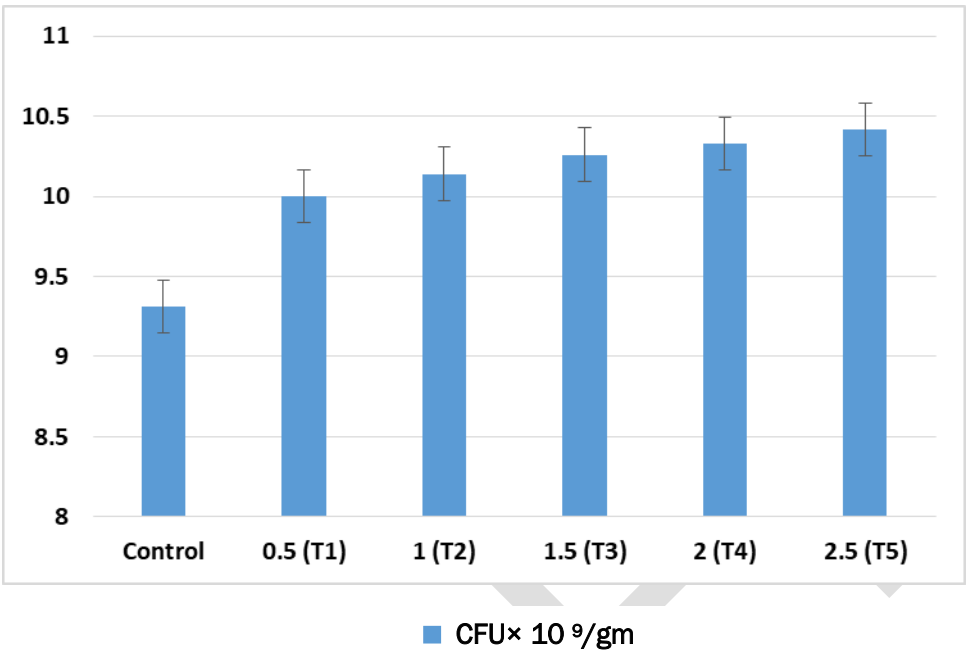


Figure 8. Effect of incorporation of cryoprotectants on the total viable count of herbal bio-yoghurt powder

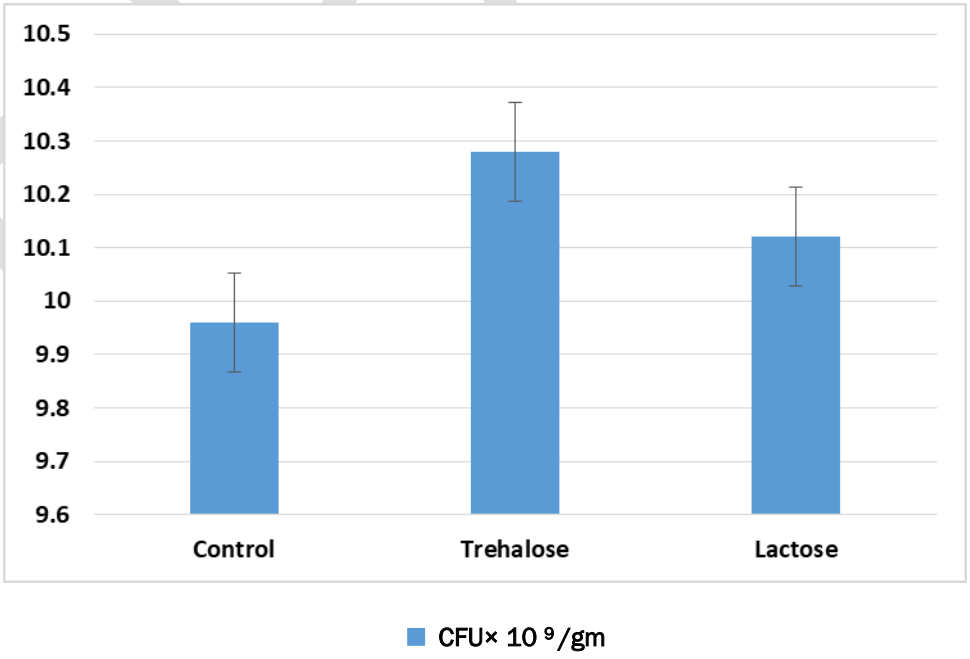
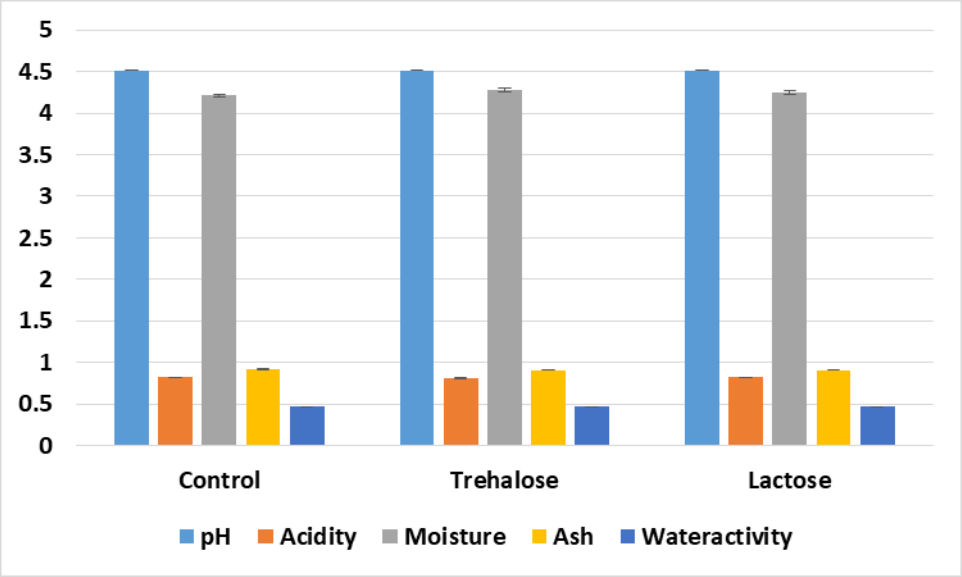


Figure 9. Effect of incorporation of different cryoprotectants on the physico chemical properties of freeze dried herbal bio-yoghurt


5
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Control
Trehalose
Lactose
pH
Acidity
Moisture
Ash
Wateractivity