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Research Article
Bacterial Strains as Vitamins Supplements to Prepare Functional Dairy Beverages

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Abstract

Objective: The capability of production of vitamins by some strains of bacteria is the target of this research to prepared a new style of dairy beverage. **Methodology:** Three different strains of bacteria (*L. acidophilus*, *L. rhamnosus* and *Bifidobacterium bifidum*) or their mixture were evaluated for their ability for production of different vitamins in a dairy beverage. An experiment was designed to prepare papaya-whey-beverage (40:60 w/v) inoculated with 1% of each of the above strains or their mixture to serve four treatments. Estimation of 11 vitamins in the fresh beverages were conducted and the sensory properties of the resultant beverages were also evaluated. **Results:** Obtained data revealed that all the estimated vitamins were increased by fortification of probiotic bacteria; however their contents showed intra-values differences. Results showed that *L. rhamnosus* had the highly ability in production of vitamin E, D, B2 and B12, however, *L. acidophilus* succeeded in production of vitamin A, K, nicotinic and thiamin. On the other side, *Bifidobacterium bifidum* had the highest ability for production of folic acid, it produced 315.5 μg/100 mL sample rather than all other strains. Using of 1% of mixture of strains lead to decrease the values of all estimated vitamins except vitamin C. No clear differences were observed in the organoleptic properties within all samples. **Conclusion:** It could be concluded that using of some probiotics bacteria can be able to produce natural vitamins in dairy beverages to improve health concept.

Key words: Probiotic bacteria, dairy beverages, vitamins contents, *Bifidobacterium bifidum*

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Although, most vitamins are present in a variety of foods, human vitamin deficiencies still presents in various countries. A new trend to produce vitamins by microorganisms was recommended. Probiotics mean special strains of bacteria that do specific benefit roles, however using of probiotic bacteria in dairy products is considered as one of the effective additives and recent trend in food technology. One of these roles is producing nutritional and bioactive compounds such as synthesize vitamins[1]. Probiotic bacteria, mostly belonging to the genera Lactobacillus and Bifidobacterium have been studied for their capability to produce vitamins. The ability of vitamins productions is between varied[2-7].

One another side, fruits are excellent sources of phytochemicals that are essential for human health, they considered rich sources of nutritional and bioactive compounds that confer immense health benefits[8,9]. Among all fruits, papaya is a good source of bioactive compounds[10]. Regarding to the anti-nutrients constituents, peel and pulp of ripe papaya fruits contain low amounts of anti-nutrients like tannin, phytate and oxalate creating incompatibility problems as reported by Srividya and Ramachandran[11].

So, the present study was planned to evaluate some bacterial strains for their capability for vitamins production in new style dairy beverage prepared from papaya pulp and sweet whey-to produce functional probiotic dairy beverage.

MATERIALS AND METHODS

Main materials: Papaya fruits (*Carica papaya* L.) were obtained from Horticultural Research Institute, Agriculture Research Center, Giza, Egypt. However fresh sweet whey was obtained from Faculty of Agricultural, Cairo University, Egypt.

The bacterial cultures are *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Bifidobacterium bifidum* separated or mixed by (1:1:1) in the form of freeze-dried culture. They obtained from Dairy Department, Microbiology Laboratory, National Research Centre, Egypt. They prepared as the mother culture by adding 1% of lyophilized cell culture into sterilized reconstituted skim milk powder (12%) and incubated at 42°C for 4-6 h.

Experiments

Preparation of papaya pulp: Papaya fruits were carefully washed, cut into pieces and blended, then filtered through a fine cloth. The mixed pulp was heated at 90°C for 3 min to inactivate pectin enzymes, after that they cooled rapidly to 15°C and filled into glass bottles. The fruit pulp were stored at -18°C until used[12].

Preparation of probiotic papaya-whey beverage: Homogenized Papaya pulp and sweet whey were mixed in proportion 40:60 (w/v) as advised by Mohamed *et al.*[12]. Carboxyl methylcellulose (0.2%), citric acid (0.1%) and potassium sorbets (0.1%) were supplemented. The total soluble solids content was adjusted to 18.0% in all blends using sucrose. The blends were poured in 100 mL five glass bottles. They closed and pasteurized at 65°C for 30 min. The first bottle was serve as control (c), whereas the other four bottles (T1, T2, T3 and T4) were individually fortified (under sterilized conditions) with 1% *Lactobacillus acidophilus*, *Lactobacillus rhamnosus* and *Bifidobacterium bifidum* separated or mixed (1:1:1), respectively. The inoculated samples were incubated at 42°C until pH 4.7, then cooled to 5±2°C. The resulting beverages were examined for bacterial counts fresh and after 1 month of storage at 5±2°C. The gross chemical composition of fresh beverages was as follow: TS were 18.0%, lactose content was 2.81 mg/100 g, while titratable acidity was 0.30%.

Methods

Sensory evaluation: Twenty expert judges were selected from staff member of Dairy Department., National Research Center, Egypt, to evaluate flavor (60 points), appearance (30 points) and color (10 points) of the dairy beverages samples according to Deka *et al.*[13].

Determination of bacterial counts: Lactobacilli counts were determined using MRS agar (Oxoid) according to De Man *et al.*[14] and *Bifidobacterium bifidum* was determined according to Blanchette *et al.*[15] using modified MRS agar (Oxoid) supplemented with 0.05% L. cysteine-HCL (Merck, Germany). Plates were incubated at 37°C for 48 h under anaerobic conditions (BBL Gas Pak, Becton Dickinson, Cockeysville MA, USA).

Determination of vitamins contents: Vitamin B-group content is estimated as mentioned by Sharaf *et al.*[7] using HPLC technique, while vitamin E was determined according to the methods described by Pyka and Sliwiok[16]. Vitamin A was determined according to the methods described by Noll[17].

RESULTS AND DISCUSSION

Organoleptic properties of papaya whey beverages: Organoleptic evaluation of the fresh resultant beverages showed no clear differences in all examined items (flavor, appearance and color) in all samples and all examined parameters were accebtable.

Bacterial count of the probiotic in beverages: Figure 1 showed the number of bacterial counts in fresh and stored papaya-whey-beverages. It could be concluded that the log count of probiotic strains were slightly decreased from the initial time (after incubation) to ted end of storage period (after one month) except *L. rhamnosus* which was slightly increase. This result may be due to the sensitivity of these strains to acid developed during the month of storage. These results were agree with the results of De Souza Oliveira *et al.*[18] and El-Batawy *et al.*[19].

Vitamins content: One of the multiples benefits that probiotics have is the capacity to synthesize vitamins. We have kenned for sometime that commensal bacteria engender vitamins, concretely B vitamins and play a major role in meeting our desiderata for these essential nutrients.

Figure 2-6 revealed the capability of the bacterial strains in syntheses of hydrophilic and hydrophobic vitamins. The present results revealed that *L. rhamnosus* had the highly ability in production of vitamin E, D, B2 and B12 where their values were: 18.042, 463.5, 1880 and 827.7 µg/100 mL sample, respectively compared to 2.315, 33.387, 5.378 and 338.1 µg/100 mL for control sample. On the other hand, *L. acidophilus* produced the highly amounts of vitamin A, K, nicotinic and thiamin where their values were 1551.4, 97357, 9829 and 23694 µg/100 mL sample respectively in comparison to 0.0281, 3331, 50.85 and 61.60 µg/100 mL for control samples. However, *Bifidobacterium bifidum* had the highest ability for production of Folic acid. It produced 315.5 µg/100 mL sample rather than all other strains. Using of 1% of mixture of the three-mentioned strained lead to decrease the values of all estimated vitamins except vitamin C. This may be due to the competition between others. Jones *et al.*[20] reported that bacteria in the gut engender vitamin K and recent evidence produce vitamin D is as well. While, Eck and Friel[1] mentioned that in vitro and studies in humans have documented the capacity of some probiotic strains to synthesize vitamin K, folic acid, vitamin B2

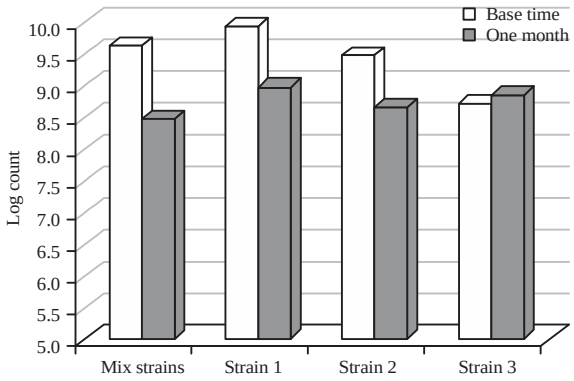

Fig. 1: Log count of probiotic strains during storage period in papaya-whey beverages

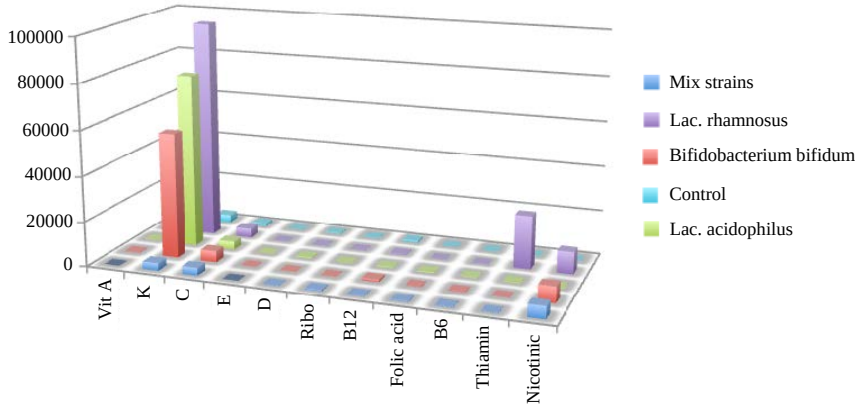

Fig. 2: Different vitamin contents produced by different species of probiotic bacteria in papaya whey beverages

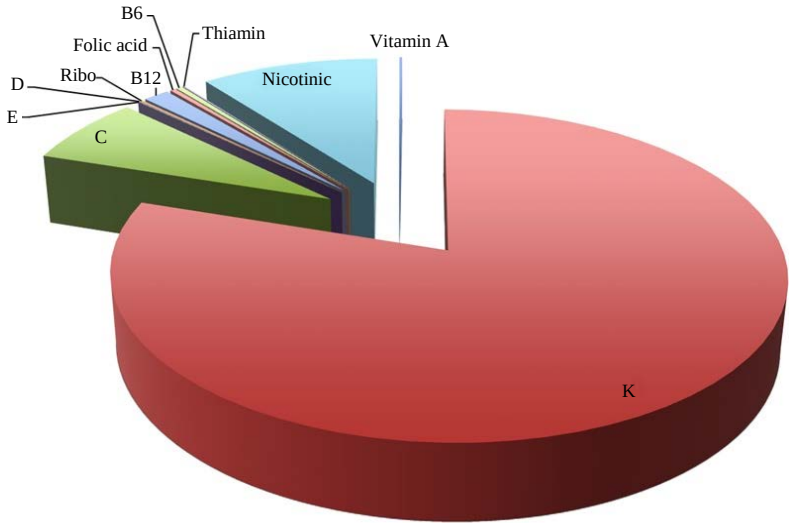

Fig. 3: Capability of *L.acidophilus* strain for production of different vitamins in papaya-whey beverage (T1)

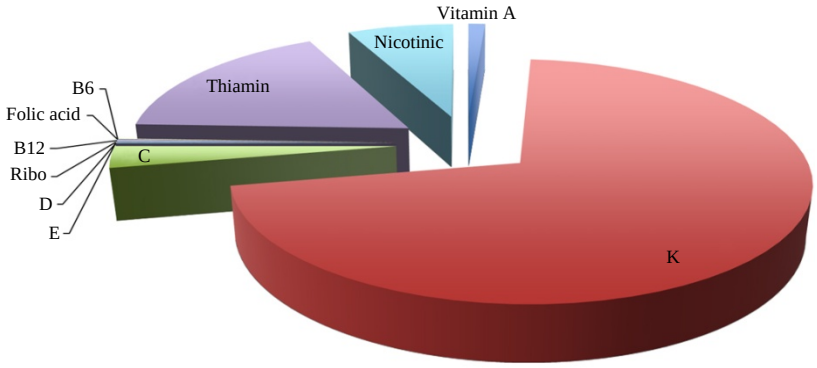

Fig. 4: Capability of *L. rhamnosus* strain for production of different vitamins in papaya-whey beverage (T2)

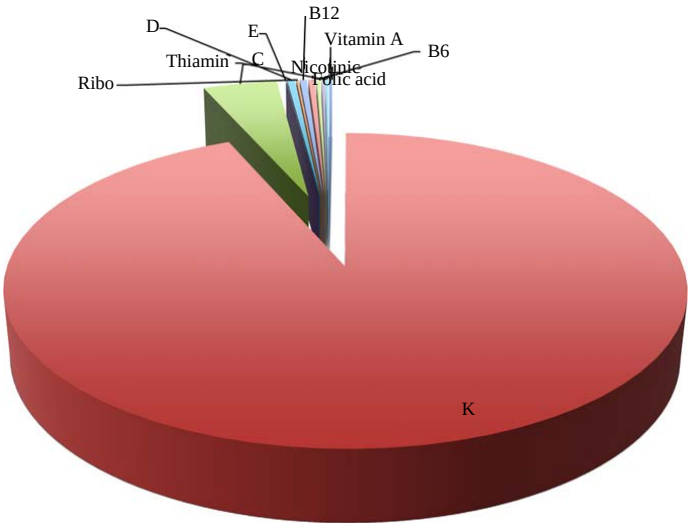

Fig. 5: Capability of *Bifidobacterium bifidum* strains for production of different vitamins in papaya-whey beverage (T3)

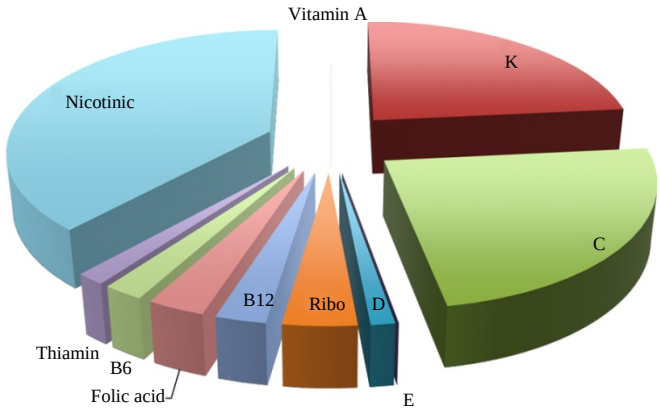

Fig. 6: Capability of mixed strains (1:1:1) for production of different vitamins in papaya-whey beverages (T4)

and B12. In 2006, Thijssen *et al.*[21] explained that there are two different forms of vitamin K and K1 (Phylloquinone) present in all photosynthetic plants and menaquinons and vitamin K2 which is primarily of bacterial origin.

Le Blanc[3] reported that B-group vitamins can be successfully produced by lactic acid bacteria, while Rossi *et al.*[4] and Eck and Friel[1] reported that several folate-producing strains have been selected within the genus Bifidobacterium.

CONCLUSION

It be concluded that, some lactic acid bacteria can be successufully used to produce vitamins in papaya-whey bevarege to produced fanctial product and improve health.

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