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VIABILITY OF LACTIC CULTURES BEFORE AND AFTER FREEZE DRYING IN POMEGRANATE JUICE

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ABSTRACT
The purpose of this research was to study the viability of lactic cultures in pomegranate juice before and after freeze drying.Two dietary adjuncts of a combination of 50% pomegranate juice and 50% skim milk were fermented separately with 1% culture of Lactobacillus plantarum and Lactobacillus casei and freeze-dried. The viability of the freeze-dried strains was evaluated after 24 h on *MRS* agar. Results indicated a 2 log reduction in the viability of lactic cultures in fermented combination of pomegranate juice and skim milk and also in fermented skim milk (control) after freeze-drying . The viable count of *L.casei* after freeze drying was reported as $8.04 \pm 0.1470 \log_{10}$cfu/ml and $8.030 \pm 0.1483 \log_{10}$cfu/ml in control and pomegrante juice and skim milk mixed sample respectively . The viable count of *L. plantarum* after freeze drying was reported as $8.04 \pm 0.1470 \log_{10}$cfu/ml and $8.02 \pm 0.0151 \log_{10}$cfu/ml in control and pomegrante juice and skim milk mixed sample respectively . Survival of the lactic cultures were studied after freeze drying at intervals of 7 days, 15 days, 30 days and 90 days of storage under refrigeration conditions. A higher percent of survival was noted upto 30 days in both the lactic organisms in skim milk (control) and fermented combination of pomegranate juice with skim milk . However there was a reduction in viability of both the cultures on the 90th day of enumeration.

INTRODUCTION

Fruit juices are a rich source of calcium and vitamin and could serve as a suitable media for cultivating starter cultures. Considerable amount of total soluble solids, total sugars, reducing sugars, anthocyanins, phenolics, ascorbic acid, proteins and antioxidants are present in fruit juices. Pomegranate (Punica granatum) is one of the important table fruit and is known to have considerable health-promoting properties such as antimicrobial, antiviral, antioxidant and anti-mutagenic effects (Negi *et al*., 2003). Development of foods that promote health and wellbeing is one of the key research priorities of the food industry (Klaenhammer and Kullen ,1999)

It has been suggested that fruit juices could serve as suitable media for cultivating probiotic bacteria (Mattila-Sandholm *et al*., 2002). Fermented fruit beverages are known for their health beneficial effects and nutritional properties. *L.casei* and *L.plantarum* related species have been utilized widely in food processing. *L.casei* is a common inhabitant of the human intestinal tract and also found naturally in fermented vegetables, milk and meat. *L.plantarum* also colonizes naturally in plant surfaces and is also able to successfully ferment fruit juices and vegetable juices (tomato, beetroot) with a high viability (Santo *et al*., 2011).

Freeze drying is considered as a suitable method for stabilizing microorganisms that are greatly sensitive to high temperature (Goderska, 2012; Fonseca *et al*., 2015). However, freezing and subsequent sublimation of frozen water could be attributed to cellular injuries including damage to cell membrane and DNA (Tripathi and Giri, 2014). Additionally, the changes in the physical state of membrane lipids during storage may result in severe loss of bacterial viability during storage (Fonseca *et al*., 2015).A number of cryoprotective agents such as proteins, sugars and carbohydrates have been used to minimize the bacterial inactivation after freeze drying and subsequent storage (Carvalho *et al*., 2004). Freeze drying has been a method of choice for the long term preservation of bioactive materials. This dehydration method causes little shrinkage and results in a completely soluble product that is easily rehydrated. Moreover, lyophilization is frequently used to preserve lactic acid bacterial starter cultures involved in dairy and food fermentations (Lodato *et al*., 1999). Cell immobilization in various carriers, including composite carrier matrix systems has recently attracted interest targeting to protect cultures from different types of environmental stress.

Methods of production of lactic starter juice powders should be such that adequate numbers of viable bacteria are maintained in the dried powder following manufacture, and throughout the shelf-life of powder. Both freeze-drying and spray-drying can be used for manufacture of lactic starter fruit juice powders on a large scale (Wang *et al*., 2004). Keeping this in view the aim of this research was to investigate the growth rate and substrate metabolism by the lactic cultures of *Lactobacillus plantarum* and *Lactobacillus casei* in a medium containing 50% of pomegrante juice and 50% skim milk after fermentation and to evaluate their viability after freeze drying.

MATERIALS AND METHODS

Pomegranate (Punica granatum- Arakta variety) skim milk

powder with 5 per cent moisture and 95 per cent solubility were purchased locally. Lactobacillus casei (NCDC- 142) and *Lactobacillus plantarum* (NCDC- 21) were purchased from the National Dairy Research Institute, Karnal, Haryana. Sterilized skim milk acted as control. The protocol for freeze drying the cultures in omegranate substrate was done as per the modified procedure adopted by Ambuj and Athmaselvi (2016). Fifty percent of pomegranate juice was filter sterilized and mixed with 50 per cent sterilized skim milk for the study . The samples were inoculated separately with L.casei and L.plantarum and incubated overnight . The overnight cultures were then dispensed in sterile cryogenic vials and freeze dried.

Enumeration of viable cultures after freeze drying

After freeze-drying, the freeze-dried powders were re-hydrated in skim milk powder and the cell suspensions were allowed to stand for 10 min at room temperature, and subsequently plated on MRS agar. The number of viable cells were determined before and after freeze-drying after incubation at 24 h at 37ºC. The viability of the freeze-dried cells were also determined at regular intervals for a period of 90 days.

The data obtained were analyzed statistically as per the standard procedure of Snedecor and Cochran (1980).

RESULTS AND DISCUSSION

Viability of *L.casei* before and after freeze drying in various medium

Results in table 1 shows that there is a highly significant difference in the viable count of L.casei before and after freeze drying. There is almost a 2 log reduction in the viable count after freeze drying. The viable count of L.casei after freeze drying was reported as 8.04 ± 0.1470log10cfu/ml and 8.030 ± 0.1483log10cfu/ml in control and pomegrante juice and skim milk mixed sample respectively.This was in agrrement with the results of Jofre *et al*. (2015) on freeze dried samples of Lactobacillus casei/paracasei CTC1677 and L. casei/paracasei CTC1678 . They reported survivability of ($\geq$ 94%) and best performance on freeze drying Lactobacillus rhamnosus CTC1679, Lactobacillus casei/paracasei CTC1677 and L. casei/paracasei CTC1678 in skim milk alone or in supplementation with trehalose or lactose. The results are also concordant with the findings of Ambuj and Athmaselvi (2016) on pomegranate juice samples containing L.rhamnosus treated at "40º C which had more regenerative power.

Table 1: Total viable count#($\log_{10}$ cfu/ml) of *L. casei* before and after freeze drying in various medium @

	Lactobacillus casei#	
	Control	Combination of skim milk and pomegranate (50% each)
Before freeze drying	10.03 ± 0.0141	10.02 ± 0.0192
After freeze drying	8.04 ± 0.1470	8.030 ± 0.1483
t test	**	**

@Average of six trials;#$\log_{10}$ cfu/ml;**Statistically highly significant ($P \leq 0.01$)

Table 2: Total viable count#($\log_{10}$ cfu/ml) of Lactobacillus plantarum before and after freeze drying in various medium @

	Lactobacillus plantarum#	
	Control	Combination of skim milk and pomegranate (50% each)
Before freeze drying	10.03 ± 0.0141	10.01 ± 0.022
After freeze drying	8.04 ± 0.1470	8.02 ± 0.0151
t test	**	**

@Average of six trials;#$\log_{10}$ cfu/ml;**Statistically highly significant ($P \leq 0.01$)

Viability of Lactobacillus plantarum before and after freeze drying in various medium

Results in table 2 shows that there is a highly significant difference in the viable count of L. plantarum before and after freeze drying. There is almost a 2 log reduction in the viable count after freeze drying. The viable count of L. plantarum after freeze drying was reported as 8.04 ± 0.1470log10cfu/ml and 8.02 ± 0.0151log10cfu/ml in control and pomegrante juice and skim milk mixed sample respectively. This differed from the study of Sofia Carvalho *et al*. (2002) who found that no significant differences in the viability of Lactobacillus plantarum cells during freeze-drying in the presence of inositol, sorbitol, fructose, trehalose, monosodium glutamate and propyl gallate. This decrease of cell count is in line with the results on freeze drying of. L.plantarum by Sawaminee et al (2012) who reported in their study , a decrease in cell viability of approximately 40% during freeze drying leading to an initial cell concentration in the instant dried powders to 6×107 CFU/m *L. lactobacilli* .

Viability of Freeze dried Lactobacillus casei during storage at refrigeration temperature.

Small case shows significant difference within treatments

Results in table3 shows that there is a highly significant difference in the viable count of *L.casei* during storage. There was no difference in viability of cells till 30 days of storage. However there was a significant difference in their viability on the 90 th day.

No difference in the viability of cells between the treatments was noticed indicating that substitution of pomegranate juice for skim milk can also be considered to improve the functional property of dairy adjuncts.

The findings corroborated with the work of Caroliny *et al*

Table 3: Total viable count# ($\log_{10}$ cfu/ml) of Lactobacillus casei during storage at refrigeration temperature @

Treatment	Viability during storage in days				
	7th day	15th day	30th day	90th day	F value
Control	8.033b $\pm$0.180	7.983b$\pm$0.184	7.913b$\pm$0.215	6.408a$\pm$0.3380	** 31.07
Skim milk + Pomegranate juice	8.025 b $\pm$0.180	7.983 b $\pm$0.178	7.773 b $\pm$0.062	6.460 a $\pm$0.358	** 24.04
t Test P Value	0.9692NS	0.0573NS	0.6724 NS	0.9059NS	

@Average of six trials;#$\log_{10}$ cfu/ml;**Statistically highly significant (P 0.01);NS – non significant (P > 0.05)

Table 4:Total viable count#(log_{10} cfu/ml) of Lactobacillus plantarum during storage at refrigeration temperature @

Treatment	Viability during storage in days				F value
	7th day	15th day	30th day	90th day	
Control	8.043b ±0.177	7.983 b ±0.18	7.873 b ±0.222	6.4600 a ±0.388	** 34.04
Skim milk + Pomegranate juice	8.008 b ±0.185	7.967 b ±0.184	7.740 b ±0.062	6.511 a ±0.375	** 23.02
t Test P Value	0.8719 NS	0.9445 NS	0.4982 NS	0.9063 NS	

@Average of six trials; #log_{10} cfu/ml; **Statistically highly significant ($P \leq 0.01$); NS – non significant ($P > 0.05$)

(2020) who repoarted a small decrease (<1 log CFU/g) in viable counts of L. casei L-26 after 15 days of storage under refrigeration when the strain was freeze-dried with acerola, cashew juice and guava juice. The viable counts of L. casei L-26 freeze-dried without or with substrates decreased from 15 days of refrigerated storage onward. They reported a higher viable counts of L. casei L-26 freeze-dried with acerola (5.3 ± 0.3 log CFU/g) when compared to other juices after 90 days of storage under refrigeration.

Viability of Freeze dried Lactobacillus plantarum during storage at refrigeration temperature.

Small case shows significant difference within treatments

Results in table4 shows that there is a highly significant difference in the viable count of L.plantarum during storage. There is no difference in viability of cells till 30 days of storage. However there is a significant difference in their viability on the 90th day.

Trial showed no difference in the viability of cells between the treatments thus indicating that substitution of pomegranate juice for skim milk can also be considered to improve the functional property of dairy adjuncts. This corroborates with the results reported by Sawamineenualkaekul *et al*. (2012) that the survival of freeze dried Lactobacillus plantarum cells mixed with several freeze dried instant fruit powders (strawberry, pomegranate, blackcurrant and cranberry) is possible during storage with pomegranate showing (~ 0.9 log decrease) after 12 months

This was also in concordance to the results reported bylaconelli *et al*. (2015) that a minimum concentration of viable lactic starter bacteria of 106 -107 CFU/ml was obtained during freeze drying . In order to preserve lactic starters for long-term viability and functionality, dehydration process which involved the transition of microorganisms from a liquid to a solid medium is recommended for production of dried powder of probiotics.

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