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RESEARCH PAPER

Quality Aspects and Storage Evaluation of Freeze Dried Probiotic Pineapple *Lassi* Powder

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ABSTRACT

Freeze dried probiotic pineapple *lassi* powder was developed using Bifidobacterium bifidum (NCDC 235). Quality aspects were determined in terms of physico-chemical parameters, vitamin composition and probiotic viability. Storage changes were monitored at 25±20 °C and -18±20 °C for one year. The reconstituted *lassi* has carbohydrate content of 22.2 per cent, thiamin 2 mg which meets 2/3rd of suggested micronutrient intake goal for those working either in cold or at high altitudes. The niacin content in the product is 2.27 mg, riboflavin 0.375 mg and pyridoxine 67.5 mg. An increasing trend in ΔE values 0 to 6.06 was observed at 25±2 °C while no significant difference ($p<0.05$) was observed at -18±20 °C. pH decreased from 4.71 to 4.1 at 25±2°C and to 4.3 at -18±2°C after 12 months. Browning in terms of absorbance increased significantly ($p<0.05$) from 0.015 to 0.061 at 25±2°C. *Bifidobacterium bifidum* count decreased from 9.55 $\log_{10}$ cfu.ml^{-1} to 8.87 $\log_{10}$ cfu.ml^{-1} and 8.95 $\log_{10}$ cfu.ml^{-1} after 12 months at 25±2 °C and -18±2 °C respectively. Probiotic viability did not change significantly throughout the storage period at both the temperatures. This instant powder can also be utilised as a health drink mix with reduced bulk suitable for combat conditions and in high altitudes to overcome logistic problems.

Keywords: Probiotic; Freeze drying; Pineapple *Lassi*; B-Vitamins; High altitudes

NOMENCLATURE

NCDC	National Centre for Dairy Culture
CFU	Colony Forming Unit
UHT	Ultra high temperature
ΔEc	Colour change
WPC	Whey protein concentrate
OAA	Over all acceptability

1. INTRODUCTION

Exposure to high altitudes is associated with decreased oxygen pressure and can result in oxidative stress. Requirements for carbohydrates and vitamins are high in cold and at high altitudes for the production of sufficient energy for thermo genesis and for physical exertion. Carbohydrates help in replacing depleted muscle glycogen stores, and they require less oxygen for metabolism. All of the water-soluble vitamins, with the exception of folic acid and vitamin C, are intimately involved in the oxidation and conversion of food to energy at multiple steps leading up to and in the functioning of the Krebs cycle[14]. The suggested micronutrient intake of thiamine for those working either in cold or at high altitudes is set at 3mg/day, niacin 20mg/day, riboflavin 2.5 mg/day and panthothenic acid 10mg/day and there are no suggested recommendations for pyridoxine at high altitudes[23]. Fermented foods provide plenty of vital nutrients and bioactive components that affect a number of functions of human body in a positive way. Fermented milks can be made more functional by incorporating probiotic strains and furthermore, if they are capable of synthesising essential bio molecules such as vitamins, enzymes, exopolysaccharides, bacteriocins or bioactive peptides serve in to the functions and technological properties of the products. Fruit flavoured *lassi* drinks are popular in India which offer distinct health advantages due to the combination of dairy protein, calcium and probiotics with fruit based vitamins, minerals, dietary fibers and antioxidants[38]. Pineapple is a tropical fruit having exceptional juiciness, vibrant tropical flavour and immense health benefits. It contains considerable amount of calcium, potassium, vitamin C, carbohydrates, crude fibre, water and different minerals that is good for the digestive system and helps in maintaining ideal weight and balanced nutrition. It has minimal fat and sodium[29]. Blending of *lassi* (Indian Yoghurt drink) with pineapple juice not only increases its nutrient content including minerals and vitamins but also adds to its health promoting value.

Probiotic bacteria, mostly belonging to the genera *Lactobacillus* and *Bifidobacterium*, confer a number of health benefits to the host, including vitamin production[20]. *Bifidobacterium* are commonly found in GT tract of mammals including humans. They are known for their capacity to restore the balance of gut microbiota and to deliver other health benefits to consumers[23]. *Bifidobacterium* species are generally believed to produce B-vitamins including folate, cobalamine, pyridoxine, riboflavin and thiamine[10]. Because of their probiotic property they are frequently incorporated as functional ingredients in food products. It is generally accepted that probiotic products should have a minimum concentration

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of 10^6 CFU/ml or gram and that a total of some 10^8 to 10^9 probiotic microorganisms should be consumed daily for the probiotic effect to be transferred to the consumer. Furthermore, the strains must be able to grow and retain viability during manufacture and storage[30].

Freeze-drying is the best method of water removal with final products of highest quality compared to other methods of food drying[15]. It is based on sublimation of a frozen product. It offers the highest quality of dried product next to fresh product with maximum retention of colour, good solubility, light weight and long shelf life. It is frequently used to preserve lactic acid bacterial starter cultures involved in dairy and food fermentations[18]. It is one among the methods which allow maintaining viability of microbial biomass[30] It has been used to preserve microbial mixtures in UHT milk without reducing the cell viability and probiotic properties Rathnayaka[27].

The shelf life of fruit *lassi* is very short i.e. 1 day at ambient temperature (25 °C – 35 °C) and about 2-3 days at 7 °C. Improvements in the shelf life of fruit *lassi* can be brought about by its dehydration and conversion into a shelf stable powder. Several authors have studied the retention of starter organisms, vitamins antioxidants, occurrence of colour and oxidative changes in food powders during storage[16,34]. *Dahi* powder has been produced using freeze drying technology[11]. Freeze-dried yoghurt powder can be stored for up to 1–2 years at 4 °C[17]. The anticipated storage changes in food powders which limit their acceptability and shelf life colour changes, non enzymatic browning, oxidative changes, lump formation due to moisture increase, decrease in probiotic viability[6].

Considering the additional requirements of B vitamins in high altitudes, a probiotic product containing *Bifidobacterium bifidum* was envisaged. This paper focuses on the quality aspects and storage changes of freeze dried (FD) probiotic pineapple *lassi* powder in terms of physico- chemical parameters, vitamin composition and probiotic viability.

2. MATERIALS AND METHODS

A mature giant variety of pineapple (*Ananas cosmosus*) was procured from local market, Mysuru, India. The standard cultures NCDC-167 (Mixed mesophilic dahi culture), NCDC-010 (*Lactobacillus delbrueckii*) and NCDC-235 (*Bifdobacterium bifidum*) were procured from NDRI, Karnal, Haryana. Process flow chart for FD probiotic pineapple lassi is as shown in Fig. 1.

2.1 Freeze Drying

Freeze drying of probiotic pineapple *lassi* was carried out in a freeze drier (Martin Christ, Germany). The samples were loaded on trays and frozen to - 40 °C and final temperature during freeze drying was 60 °C. A vacuum of 0.1 mm of Hg was maintained. The freezing temperature was - 40 °C and secondary heating was carried up to 60 °C. The freeze drying was completed in 16 h and the flakes were powdered in Raymond Mill and packed in paper/Al-Foil/polyethylene (45 GSM, 20 microns foil, 37.45 microns LDPE) pouches (PFP) under controlled humidity conditions (18-20 per cent RH). It took 16 h - 17 h for drying to a moisture content of 1-2 per cent.

2.2 Proximate Composition and Quality Attributes

Moisture in the FD probiotic pineapple *lassi* powder was determined by using vacuum drying to a constant weight as per[25]. 5 g of powder in a dried, pre weighed aluminium dish was kept in vacuum oven at 70 °C for 6 h - 7 h and moisture content was calculated as per cent. Fat content was determined using soxhlet method[2]. Protein was estimated by Kjeldhal's method described as per[1]. Carbohydrate was calculated by difference and total energy of the sample was calculated as kcal/100 g using the Atwater factors of 4, 9, and 4 for protein, fat and carbohydrate, respectively Chancey[8].

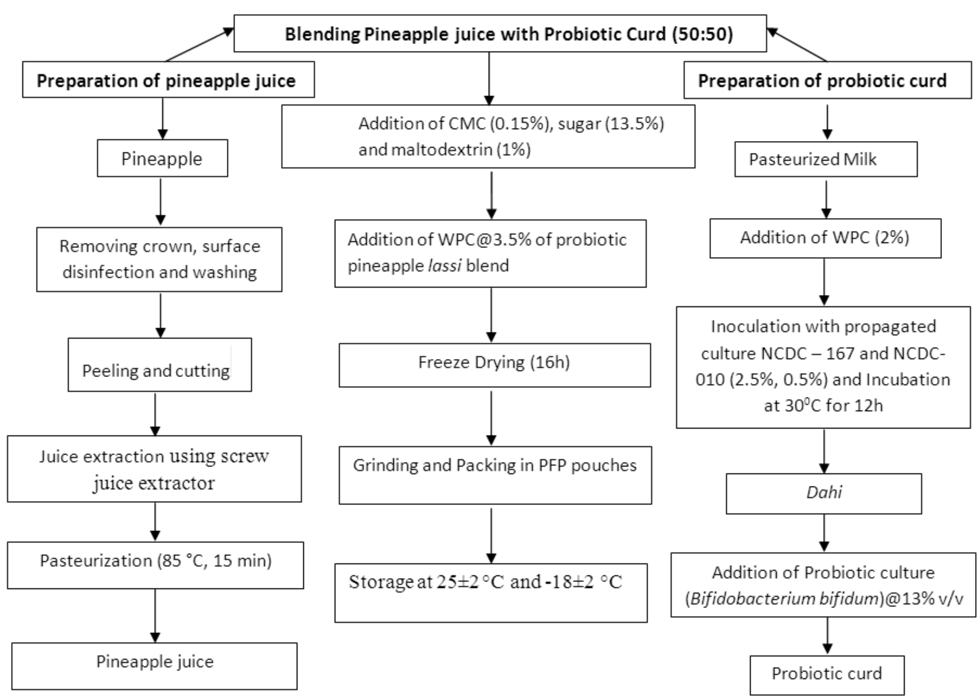

Figure 1. Process flow chart for FD probiotic pineapple *lassi*.

2.3 Vitamin Analysis

B- vitamins Thiamine, Riboflavin, Niacin and pyridoxine in the sample were analysed on HPLC (Waters Model 1525, India) using Atlantis dC_{18} 5 µm, 4.6 mm x 150 mm column using a gradient elution of 0.1 per cent tri flouro acetic acid (TFA) and acetonitrile, starting with 100 ml 0.1 per cent TFA and 100 ml acetonitrile over 25 min. UV detector at 260 nm was used for detection.

2.4 Bulk Density

The bulk density of the powder was measured by weighing 2 g of sample and allowing it to fall freely into 50 ml graduated cylinder. The cylinder was tapped by hand five times and bulk density was calculated by dividing mass of the powder by volume occupied in the cylinder and expressed as g/ml.

2.5 Hygroscopicity

Hygroscopicity of the powder was determined by a method described by[5] with some modification. 1 g sample placed at 25 °C in a container with NaCl saturated solution (72.29 per cent RH). After 1 week samples were weighed and hygroscopicity was expressed as g of absorbed moisture per 100 g dry solids.

2.6 Solubility

Solubility was determined using the procedure[9]. 1 g of powder (dry basis) was dispersed in 100 ml distilled water by blending at high speed (13000 rpm) for 5 min using an Oosthuizen blender (Oster, Mexico). The dispersed *lassi* powder was then centrifuged at 3000 rpm for 5 min. A 25 ml of aliquot of supernatant was carefully pipetted and transferred to a pre weighed aluminium dish and then oven dried at 105 °C for 5 h. Drying was continued and weighed every hour for 2 h. The solubility of the powder in (per cent) was determined by taking the weight difference.

2.7 Storage and Evaluation

The FD probiotic pineapple *lassi* powder was packed in paper-aluminium foil-polyethylene (PFP) laminate pouches and stored at two different temperatures 25±2 °C and -18±2°C. Initially as well as periodically at an interval of 2 months, the samples were analysed for different parameters.

2.8 Colour Change

The colour was measured as *L, a, b* values using Hunter colorimeter[32] (Hunter Lab, Reston, VA, United States of America). The colour change (ΔEc) of samples during storage was estimated using the Eqn 1.

$$\Delta Ec = \sqrt{(Li - Lr)^2 + (ai - ar)^2 + (bi - br)^2} \quad (1)$$

where *Li, ai, bi* are initial colour values of reconstituted fresh *lassi* and *Lr, ar, br* are colour values of reconstituted *lassi* during storage.

2.9 pH, Acidity and Non Enzymatic Browning

pH of the sample was determined using microprocessor controlled digital pH meter with a probe (Systronics, India). Acidity was determined as per cent lactic acid by titration against N/10 NaOH using phenolphthalein indicator[24]. Browning of the sample was measured by spectrophotometric method[26]. 2 g of sample was mixed in 50 ml of 66 per cent alcohol. The solution is then filtered through Whatman No.541 filter paper. The absorbance was measured at 420 nm.

2.10 Viability of Probiotic Bacteria

Viable cells of *Bifidobacteria* in FD probiotic pineapple *lassi* were enumerated using MRS agar containing 1 per cent L- cysteine hydrochloride (w/v) by pour plate method. Plates were incubated at 37 °C for 24 h in an anaerobic jar.

2.11 Sensory Wvaluation

The samples were subjected to sensory evaluation on 9 pt hedonic scale by a semi-trained panel of 11 judges and over all acceptability (OAA) was determined.

2.12 Statistical analysis

All experiments were repeated three times and data sets were subjected to analysis of variance (ANOVA) using the general linear models. Significant differences between the samples mean were determined at the ($p < 0.05$) levels by ANOVA.

3. RESULTS AND DISCUSSION

3.1 Proximate and Vitamin Composition

Moisture content in FD probiotic pineapple *lassi* powder was 2 per cent. Similar results have been reported for FD products[34] where the moisture content in FD grape juice beverage mix was between 1-2 per cent. Moisture content of 2.3 per cent in FD guava concentrate[20] and 2 per cent in FD muskmelon *lassi*[33] has been reported. The fat content in FD pineapple *lassi* powder was 10 per cent, protein 12 per cent, carbohydrate 74 per cent, ash 1.57 per cent, and crude fibre 2.23 per cent. The energy value of the FD probiotic pineapple *lassi* was 434 kcal/100g. The product is rich in carbohydrates and is suitable for high altitudes where requirement of carbohydrates is more. The optimal diet at high altitudes contains at least 400 g of carbohydrate -believed to best food for high altitudes[12]. The reconstitution level is standardised as 30 g of freeze dried powder in 100 ml water based on its consistency and overall acceptability. One serving of reconstituted product (100 ml) has thiamin content of 2mg which meets 2/3rd of suggested micronutrient intake goal of thiamine for those working either in cold or at high altitudes. The niacin content is 2.27 mg, riboflavin 0.375 mg and pyridoxine 67.5 mg per 100 ml. The proximate composition and vitamin content is as shown in Table 1.

3.2 Physico - Chemical Attributes

The bulk density of the probiotic pineapple *lassi* powder is 0.717 g/ml. A similar value has been reported in FD guava concentrate and in FD muskmelon *lassi*[20,33]. The hygroscopicity of FD probiotic pineapple *lassi* powder was 16.28 per cent (g of absorbed moisture/100 g dry solids). The hygroscopicity value obtained for spray dried mango powder is 16.5 per cent and FD mango powder is 18 per cent[5]. Both maltodextrin and WPC added @ of 1 per cent and 3.5 per cent respectively might have contributed towards reduced hygroscopicity of the freeze dried product. The hygroscopicity of spray-dried acai

Table 1. Proximate composition of FD probiotic pineapple *lassi* powder

Parameter	Value
Moisture (%)	2.0±0.02
Protein (%)	12±0.01
Fat (%)	10±0.01
Ash (%)	1.57±0.01
Crude Fibre (%)	2.23±0.02
Carbohydrate (%)	74±0.1
Energy Value K cal/100g	434± 1
Vitamin – B /100 ml Serving (30 g)	
Thiamine (mg)	2±0.02
Riboflavin (mg)	0.375±0.002
Niacin (mg)	2.27±0.03
Pyridoxine (mg)	67.5±0.1

mean±SD (n=6)

powder gets lower as the concentration of maltodextrin was increased[35]. Solubility is the most reliable criterion to evaluate the behaviour of powder in aqueous solution[8]. Solubility of FD probiotic pineapple *lassi* powder was 86 per cent. A similar result has been obtained for FD mango powder with solubility of 89.7 per cent[5].

3.3 Storage and Evaluation

FD probiotic pineapple *lassi* powder was packed in PFP pouches and stored at 25±2 °C and -18±2 °C. The changes in colour values, pH, acidity, browning, over all acceptability (OAA) and viability of probiotic organisms were monitored initially and during storage at an interval of 2 months for a period of 1 year. The storage changes were quantified and discussed in following sections.

3.4 Colour values

The *L**, *a**, and *b** values of reconstituted FD probiotic pineapple *lassi* during storage were determined. The colour change (ΔE) was evaluated initially and during storage, at an interval of two months for a period of 1 year (Fig. 2). An increasing trend in ΔE values (0 to 6.06) was observed in the sample kept at 25 °C ± 2 °C. No significant difference ($p<0.05$) was observed in ΔE values (0 to 1.25) during storage at -18 °C ± 2 °C. The lightness (L*) was increased from 78.99 to 79.75 and yellowness (b*) was decreased from 30.98 to 25.06 during storage at ambient conditions (25±2 °C). An increase in the redness values from 0 to 1.07 was also observed which might be due to Maillard reaction caused by the chemical reactions between sugars and proteins[21]. The lightness (L*) was increased from 78.99 to 79.47 No significant difference was observed in b * value (yellowness) in the probiotic pineapple *lassi* stored at -18±2 °C. A similar observation has been observed in FD mango powder[5]. The dominant colour in pineapple *lassi* is yellow and hence can be best represented by Hunter color b* (yellowness) to distinguish the color difference as affected by storage temperature.

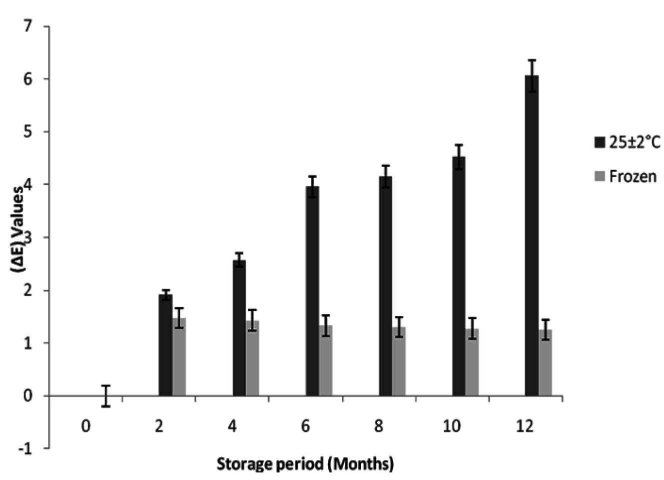

Figure 2. Change in (ΔE) value of reconstituted FD probiotic pineapple *lassi* at different temperatures.

3.5 pH, Acidity and Non Enzymatic Browning

Stored samples were reconstituted and were compared for pH at periodical intervals (Fig. 3). pH decreased from 4.71 to 4.1 after 12 months at 25 °C ± 2 °C and at -18 °C ± 2 °C, a frozen condition, the decrease in pH was less compared to ambient temperature. Similar observation has been reported in Aloe supplemented probiotic *lassi* stored

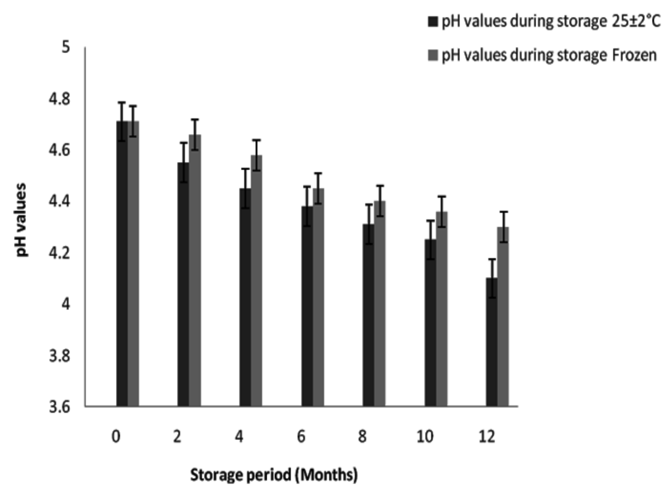

Figure 3. Changes in pH value of reconstituted FD probiotic pineapple *lassi* at different temperatures.

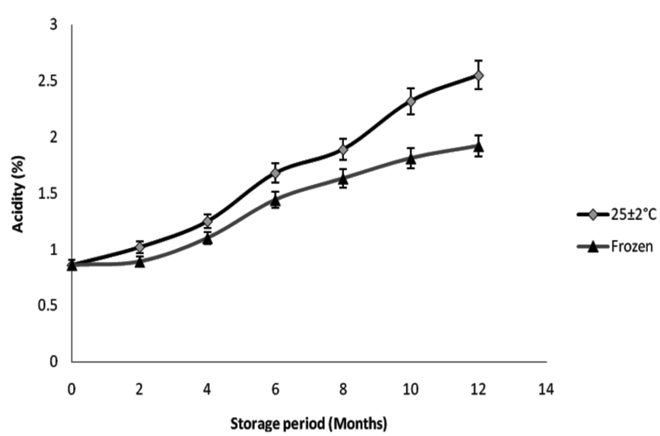

Figure 4. Change in acidity value of reconstituted FD probiotic pineapple *lassi* at different temperatures.

at 5±1 °C[12]. An increase in acidity was observed in the FD probiotic *lassi* powder stored at both the temperatures however it was less at -18±2 °C compared to sample stored at 25±2 °C (Fig. 4). The initial acidity in the reconstituted sample was 0.86 per cent and increased to 2.55 per cent and 1.92 per cent at 25±2 °C and -18±2 °C, respectively, during storage up to 12 months. Though the value is on higher side, sensorially the product was acceptable. There was significant difference ($p<0.05$) in the browning values determined using optical density at 420 nm. In the samples stored at 25±2 °C, the initial reading was 0.015 and final reading was 0.061 at the end of 12 months storage. However it was limited to 0.03 after 12 months storage at -18±2 °C (Fig. 5).

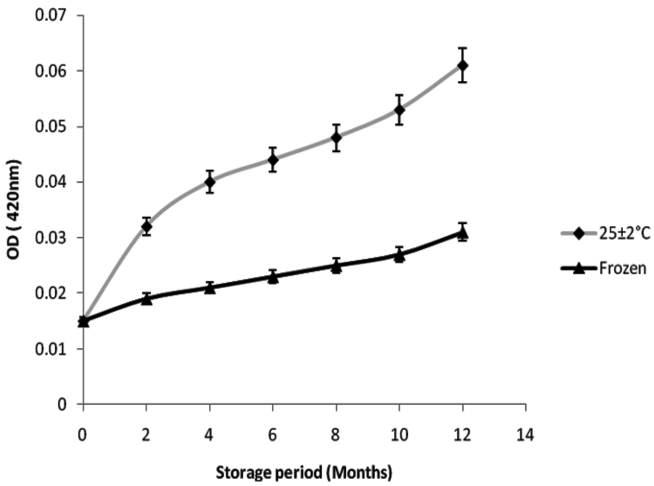

Figure 5. Change in browning value of FD probiotic pineapple *lassi* powder at different temperatures.

3.6 Probiotic Viability

The changes in the counts of probiotic organism (*Bifidobacterium bifidum*) in FD probiotic pineapple *lassi* powder were monitored periodically during storage at 25±2 °C and -18±2 °C up to 12 months (Table 2). As shown, initial count of *Bifidobacterium bifidum* was 9.55 $\log_{10}$ cfu.ml^{-1}. After 12 months of storage at 25±2 °C the viable count decreased to 8.87 $\log_{10}$ cfu.ml^{-1}. At -18±2 °C the viable count was 8.95 $\log_{10}$ cfu.ml^{-1} after 12 months. The viability of the probiotic organism did not change significantly throughout the storage period at both the temperatures. Viability loss of *bifidobacteria* was gradual and steady during the storage of probiotic yogurt during storage at 4 °C for 21 days[29].

Table 2. Viability of probiotic organism ($\log_{10}$ cfu ml^{-1}) during storage

Storage period in months	25±2 °C	-18±2 °C
0	9.55	9.55
2	8.56	9.83
4	8.70	8.65
6	8.72	8.82
8	8.69	8.71
10	8.17	8.50
12	8.87	8.95

mean±SD (n=6)

3.7 Sensory Evaluation

Significant difference ($p<0.05$) was found in OAA score of the sample stored at 25±2 °C OAA score decreased from 8.8 to 7.4 after 12 months of storage. However, in the sample which was stored at -18±2 °C the OAA score was 8 when evaluated on 9 point hedonic scale. This is represented by the graph as shown in Fig. 6.

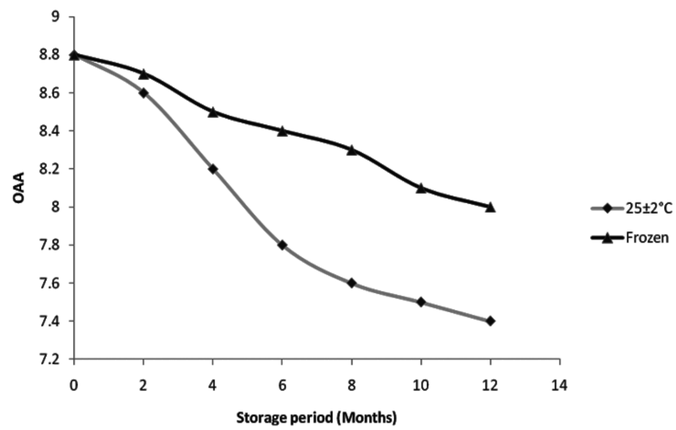

Figure 6. Change in OAA score of reconstituted FD probiotic pineapple *lassi* at different temperatures.

3.8 Shelf Life of Probiotic Pineapple *Lassi* Powder

Freeze dried probiotic pineapple *lassi* powder can be stored up to 12 months at both the 25±2 °C and -18±2 °C. However the quality and sensory attributes were found better in the sample which was stored at -18±2 °C. FD yoghurt powder can be stored for 1-2 years at 4 °C[14]. This shows that the powder has potential application at high altitude condition. The powder remained free flowing and retained good flavour at frozen storage condition. In terms of probiotic viability also the product was acceptable up to 12 months at frozen condition.

4. CONCLUSION

It is expected that the food industry will exploit novel and efficient vitamin producing strains to produce fermented food products. Such products are expected to provide economic benefits to food manufacturers since increased natural vitamin concentrations would be an important value added trait without increasing production costs. The developed FD probiotic pineapple *lassi* powder is fermented special product which combines the health benefits of pineapple and curd with probiotic strains for vitamin-B production. The product has improved sensory scores and nutritional quality than plain *lassi* powder. This can also be utilised as ready -to -reconstitute health drink mix with reduced bulk suitable for combat conditions and in high altitudes to overcome logistic problems.

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