DEVELOPMENT OF INSTANT *LASSI*

BY

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CERTIFICATE–I

This is to certify that the dissertation entitled **"Development of Instant *Lassi*"** submitted for the degree of Master of Science in the subject of Food Science and Technology to Chaudhary Charan Singh Haryana Agricultural University, Hisar, is a bonafide research work carried out by **Kritika Rawat** Admn. No. **2016FST32M** under my supervision and guidance and that no part of this thesis has been submitted for any other degree.

The assistance and help received during the course of investigation have been fully acknowledged.

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CERTIFICATE-II

This is to certify that the thesis entitled **"Development of Instant *Lassi*"** submitted by **Kritika Rawat** Admn. No. **2016FST32M** to Chaudhary Charan Singh Haryana Agricultural University, Hisar in partial fulfilment of the requirements for the degree of Master of Science in the subject of Food Science and Technology has been approved by the Student's Advisory Committee after an oral examination on the same.

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It is certified that the Thesis submitted by **Ms. Kritika Rawat**, Admn. No. **2016FST31M,** a M.Sc. student of Centre of Food Science and Technology has been checked and found as per specifications of the format circulated by Dean, PGS vide his Memo No. PGS/A- 1/09/6926-90 dated 26/8/2009.

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ABBREVIATIONS

%	-	Percentage
C.D	-	Critical difference
e.g	-	*example gratia*; for example
et al.	-	*et alibei*; and other
MRS	-	Mann Rogasa and Sharpe
CCRD	-	Central Composite Rotatable Design
RSM	-	Response Surface Methodology
Sp.gr.	-	Specific gravity
MTCC	-	Microbial Type Culture Collection
SAS	-	Statistical Analysis System
O.D.	-	Optical density
TS	-	Total Solids
SNF	-	Solid Not Fat
RFT	-	Refrigerated Temperature
RT	-	Room Temperature
SRP	-	Survival of Probiotics
ANOVA	-	Analysis of Variance
2FI	-	Two Factor Interaction
R	-	Regression
df	-	Degree of Freedom
v/v	-	Volume by Volume
g	-	Gram
mg	-	Milligram
ml	-	Milliliter
NaOH	-	Sodium Hydroxide

CHAPTER-I

INTRODUCTION

Milk obtained from cow or buffalo or combination of both is considered to have nutrients containing all essential amino acids, carbohydrates, fat, vitamins, minerals and with 99% energy value. The shelf life of milk is considered to be up to 2 to 3 days only when kept at refrigerated condition, so to increase the shelf life with retainment of all nutritional characteristics, it can be converted into another form or simply be processed to form different products naturally. The simplest and ancient way of processing milk for human consumption is fermentation. Fermentation is an oldest bioactive technology used in food processing and is very common traditional practice done in home since time immemorial. It is generally completed by certain desirable microorganisms, commonly known as probiotics. According to FAO/WHO "Probiotics are live or viable microorganisms, which when consumed in adequate quantities confer health benefits to the host", mainly lactic acid bacteria. Milk contains a sugar called lactose, which on fermentation, converts into lactic acid. Fermentation improves digestibility, flavour and aroma of food, and put forth health promoting benefits through biological enrichment of food substrate with protein, essential amino acids, fatty acids and vitamins. It may also assist in the destruction and detoxification of certain undesirable compounds, which may be present in raw food (Sathe and Mandal, 2016). Many studies have shown that probiotics can stimulate the immune system, decrease serum cholesterol, alleviate lactose intolerance, decrease diarrhoeal incidence, avoid allergy, control infections and protect against cancer (O'Bryan *et al*., 2013).

Varieties of fermented milk products are common in the global market depending on the origin or geographical indication of countries. One of the popular and traditionally consumed fermented products of Indian market is *lassi*, which is usually consumed as a summer drink with thirst quenching properties especially by the people of North India. It is ready-to-serve traditional fermented milk beverage. It is a blend of dahi/curd, water, sweetened and sometimes even spiced or fruits are added (Sudheendra *et al*., 2018). The starter culture is generally added to transform the substrates biochemically and organoleptically in to edible products, which are generally appetizing, harmless and nourishing (Campbell-Platt 1987). The composition of *lassi* is as follows: water (96.2%), total solids (3.8%), fat (0.8%), solid not fat (3.0%), protein (1.3%), lactose (1.2%), ash (0.4%) and lactic acid (0.44%). The microorganisms have health benefits, improve digestibility of the product and produce vitamins which are useful for our body, thus, amplify the nutritional characteristics and add on nutrients or increase the amount or percentage of a particular nutrient. It has its own therapeutic and nutritional value and is used for the treatment of

diarrhoea, dysentery, chronic specific and non-specific colitis, jaundice and piles (Padghan *et al*., 2015). The product is very much popular in summer and contains all nutritional properties, but with a drawback that its availability is less and shelf life of *lassi* is only 2 to 3 days under refrigerated condition and up to few hours when kept at room temperature due to increase in acidity and decrease in pH.

To enhance the availability throughout the year and to increase the shelf life of the product by conversion of liquid to the powder form, it will be better option to the consumers and they can relish this drink any time, any place and the whole year. Powder requires less packaging, storage and distribution cost because of reduction in bulk water and the refrigeration is also not needed (Schuck *et al*., 2016). Liquid-based probiotic products require refrigeration during storage and transport, thereby increasing its cost, whereas, drying the probiotic formulation provide a suitable growth suspended state for its long term storage. When rehydrated for consumption, the cells start dividing again as normal cells and also benefit the host (Bohbot and Cardot, 2012). Traditionally drying was mainly done by Sun drying but with a disadvantage that it causes maximum losses to the product by affecting its colour and nutritional properties. As the time passed much advancement came in the drying technologies and that are tray drying, drum drying, microwave drying, freeze drying and spray drying. The most appropriate technology which is in boom in the present scenario is spray drying. Spray drying is a technology, which involves drying of the product, may be food or non-food item, in less time with minimal nutritional losses in the product. Spray drying process involves the evaporation of solvent. The rapid drying in spray dryers is advantageous for preserving heat-sensitive components in food materials. The low residence time in the drying chamber minimizes contact with the hot air and consequently avoids damaging the material at high temperatures. This method is profitable because it is a continuous process, owns faster drying rate and large throughput as compared with other drying methods (Seth *et al*., 2017a). Spray drying produces very fine powder (10-50µm) or large size particles (2-3 mm). The degrees to which the microorganisms survive depend on the inlet and outlet temperatures of spray dryer. Spray drying is considered better as takes less time and is economical to use and bring down the moisture content between 4-5%. There are a very few studies on spray drying of *lassi*. The present study is first of its kind to prepare spray dried *lassi* powder and to evaluate the shelf life of the spray dried *lassi* powder. Therefore, the present study was planned with the following objectives:

1. To standardize the preparation of instant *lassi*
2. To evaluate the shelf life of the developed product

CHAPTER-II

REVIEW OF LITERATURE

The literature pertaining to the study entitled "Development of Instant *Lassi*" has been reviewed in this chapter under the following headings:

2.1 Fermentation

2.2 Probiotics

2.3 Fermented milk products and their starter culture

2.4 *Lassi*

2.5 Spray drying technology

2.6 Survival of microorganisms during spray drying

2.7 Spray dried powders

2.8 Antimicrobial activity

2.9 Antibiotic sensitivity

2.1 Fermentation

Since the beginning of human civilization, fermented foods play an important part in human food and integral component of human diet. Fermented foods are made through controlled microbial growth and enzymatic activities. Basically fermentation is one of the oldest techniques that are available to us for producing and preserving foods. Fermentation involves metabolic activity in the absence of oxygen, which results in enzymatic activity producing organic acids, gases and alcohol. Other than increasing important nutritional value, they also have benefits of enhancing food flavour, increased digestibility and pharmacological values. Fermentations also described on the basis of food substrates, which include dairy, cereals, starchy roots, meats, fish, vegetables, grapes and other fruits. Various manufacturing techniques; raw materials and microorganisms are used in making fermented foods. Four main types of fermentation processes that are mainly use world-wide: alcoholic, lactic acid, acetic acid and alkali fermentation (Soni and Sandhu, 1990 and Menezes *et al*., 2018).

Alcohol fermentation is mainly responsible for results in the production of ethanol, and yeasts which are prevailing organisms (e.g. wine and beer). Lactic acid fermentation is mainly carried with the help of lactic acid bacteria (e.g. fermented milk, dahi and cereals). Acetobacter species are second group of bacteria plays an important role in food fermentations which produces acetic acid. As it convert alcohol to acetic acid in the presence of surplus oxygen. Alkali fermentation often takes place during the fermentation of fish and seeds, popularly used as condiment (McKay and Baldwin, 1990). Lactic acid bacteria have been employed to ferment food for at least 4,000 years and have resulted in different cultured

milk products with improved preservation accompanied with characteristic flavour and texture, non-identical from the original food (Saloff-Coste, 1994; Sarkar 2008 and Amadoro *et al*., 2018). In modern fermentation technologies, specific kind of lactic acid bacteria used in controlled conditions to result in a fermented product with high nutritional and therapeutic qualities (Campagnollo *et al*., 2018). Many indigenous fermented foods and traditional beverages are still prepared in a conventional way as a house art. They are produced in various states of Indian homes, villages and small-scale industries. However, in the last 20 years, the number of books and articles dealing with indigenous fermented beverages and foods found around the whole world have rapidly increased (Blandino and Asseri 2003 and Rawat *et al*., 2018).

2.2 Probiotics

Probiotic is derived from a Greek word meaning "for life". Probiotics are defined as "live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" (Fuller, 1989). According to (FAO/WHO, 2002) "probiotics are defined as live microorganisms which when administered in adequate amounts confer a health benefit in the host." A product is said to be probiotic when obtained by fermenting milk by accomplishment of appropriate starter microorganisms that should be present in the product and should be viable, active and abundant to the date of minimum durability of the manufactured product Codex Alimentarius Commission (2003). In addition, probiotics should not have adverse effects on the taste or aroma of the product and should not enhance acidification during the shelf life of the product. Russian scientist Elie Metchnikoff is well thought out to be the inventor of probiotics (Nagpal *et al*., 2012). First scientists who anticipated the use of a celiac *Lactobacillus acidophilus* to produce *Acidophilus-Milch,* or reform yogurt was Henneberg (Heller, 2001) in Kiel.

The microorganisms used in probiotic arrangements should be generally recognized as safe (GRAS). Many strains are discovered and are used as probiotics Some stains used as probiocs are*: Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus crispatus, Lactobacillus delbrueckii subsp. Bulgaricus, Lactobacillus fermentum, Lactobacillus gasseri, Lactobacillus johnsonii, Lactobacillus paracasei, Lactobacillus plantarum, Lactobacillus reuteri, Lactobacillus rhamnous, Lactobacillus helveticus, Lactobacillus lactis, Bifidobacterium bifidum, Bifidobacterium breve, Bifidobacterium infantis, Bifidobacterium longum, Bifidobacterium lactis, Bifidobacterium adolescentis, Bifidobacterium essensis, Bifidobacterium laterosporus, Escherichia coli Nissle, Saccharomyces boulardii, Streptococcus thermophilus, Enterococcus francium, Propionibacterium, Pediococcus and Leuconostoc* (Metchnikoff, 1908; Shortt, 1999; Senok *et al*., 2005; Tabasco *et al*., 2007; Tamang *et al*., 2016; Shangpliang *et al*., 2018 and Bury *et al*., 2018). Several evidences are

been reported numerous clinical applications of probiotics in the treatment and inhibition of diseases of the gastrointestinal, respiratory and urogenital tracts (Gardiner *et al*., 2002; Falagas *et al*., 2006 and Oh *et al*., 2018). Probiotics have potential health benefits mainly: lowering risk and therapy support for gastrointestinal diseases, improving immune systems' response , producing and improving the bioavailability of nutrients, reducing lactose intolerance (Levri *et al*., 2005 and Corgneau *et al*., 2017), maintaining good balance and composition of intestinal flora, natural resistance of body to diseases of the colonic tract (De Vrese and Schrezenmeir, 2008); cancer reduction (Allsopp and Rowland, 2009); serum cholesterol reduction (Andresen and Baumgart, 2006), helping to increase the body's ability to resist the invasion of pathogens and maintain the host's well-being (D'Aimmo *et al*., 2007). Specifically dairy products are used as a superlative vehicle for carrying probiotic microorganism to the human GI tract (Zoumpopoulou *et al*., 2008), managing food allergies (Castellazzi *et al*., 2013 and Hsiao *et al*., 2017)

2.3 Fermented milk products and their starter culture

Starter culture defined as large numbers of cells minimum of one microorganism to be added to a raw material to yield a fermented product by enhancing its fermentation process. LAB are the workhorses of most food fermentation processes, and have been extensively studied for their metabolic and genetic properties (Pophaly *et al*., 2018). The assemblages of lactic acid bacteria (LAB) plays an important role in processes, and have an extended and nontoxic application consumption in the production of fermented foods and beverages (Caplice and Fitzgerald, 1999; Gilliland 2018 and Perez-Diaz *et al*., 2017). Functional starter cultures are those which retain at least one intrinsic property and contribute to nutritional, technological, organoleptic, and health advantages to the consumer (Leroy and Vuyst, 2004). Numerous amalgamations of lactic acid starter and probiotic cultures allow the production of fermented dairy products with target technological characteristics, and potential nutritional and health benefits (Vinderola *et al*., 2002). However, microbial interactions should be beneficial should not generate toxic substances after manufacture and cold storage of fermented dairy products (Bellengier *et al*., 1997 and Vinderola *et al*., 2002). At present dairy fermented products are in great demand and are listed under various categories: a) cultured milk, b) dry cultured milk products, c) whey based cultured dairy products d) thermophilic fermented milk e) fermented dairy beverage f) yogurt beverages g) cultured buttermilk h) acidophilus milk i) sour cream and others (Shiby and Mishra 2013). There are endless health promoting benefits of fermented milk products. As per studies conducted *Streptococcus thermophiles* and *Lactobacillus acidophilus* or *L. rhamnosus* bacteria do not exhibit the hypocholesterolemic effect (Agerholm-Larsen *et al*., 2000). It has been proven that a long-term consumption of fermented milk foods rises the serum HDL cholesterol, and

advances the ratio LDL/HDL (Kiessling *et al*., 2002). Dairy fermented products also contribute significant quantities of other indispensable nutrients to the diet which include calcium, phosphorus, magnesium, zinc, potassium, vitamin A, vitamin B12, riboflavin (Ebringer *et al*., 2008 and Lan *et al*., 2018).

2.4 *Lassi*

Lassi is a ready-to-serve very prevalent traditional fermented milk beverage consumed frequently as a summer beverage having its origin in India, especially in northern India and occupies protruding place in the Indian diet (Mathur, 1991). *Lassi* can also be described as a fermented beverage achieved after the growth of specific culture usually *Lactobacillus* and *Streptococcus* or commonly known as dahi (Padghan *et al*., 2017). It is prepared by blending of dahi, water, sugar or salt and sometimes even addition of spices or fruits. It has creamy texture, sweetish rich aroma and mild acidic flavour, and has shelf life of a day or two at room temperature (Mittal, 2003). It can be similar to sweet yoghurt (Tamime and Robinson, 2007). It is consumed chilled and is refreshing, thirst quenching and therapeutic beverage. The initial composition of the milk decides the composition of *lassi*. The estimated configuration of *lassi* is as follows. Milk fat (1.5-3.8%), milk total solids (9.0%) sugar (13-20%), sodium dihydrogen phosphate (0.5%), low methoxy pectin (0.5%) and lactic acid (0.7%) (Aneja *et al*., 2002). In the southern part of India, *Lassi* is favoured as a salty drink and commonly mentioned as "buttermilk", as it is the by-product attained after churning of *dahi.* Several researchers have reported different raw materials and treatments for manufacture of *lassi* like buttermilk and soybean (Deka *et al*., 1984), probiotic and symbiotic *lassi* (Zohra *et al*., 2017). Reconstituted milk *lassi* prepared by (Sudheendra *et al*., 2018). Hussain *et al*., (2015) prepared *lassi* supplemented with *Aloe barbadensis* Miller juice. *Lassi* prepared by addition of different artificial sweetener and evaluated the shelf life, physicochemical, sensory and microbial properties (George *et al*., 2012). Hingmire *et al*., (2009) prepared instant *lassi*, Kumar and Kumar, (2016) developed antioxidant rich fruit fortified probiotic *lassi* using *Lactobacillus rhamnosus* culture, *lassi* prepared by using finger millet (Pardhi *et al*., 2014), Bhoir *et al*., (2012) prepared *lassi* from cow milk by lactobacillus culture and Mane *et al*., (2014) developed instant probiotics *lassi* mix prepared by using whole milk powder, sucrose, and *lactobacillus* culture. Shuwu *et al*., (2011) prepared value added *lassi* using honey. Kamdi, (2017) prepared low fat *lassi* by addition of curry leaves.

2.5 Spray drying technology

Liquid-based probiotic products require refrigeration during storage and transport thereby increasing its cost. Whereas, drying the probiotic formulation provide a suitable growth suspended state for its long-term storage. When rehydrated after consumption, the cells start dividing again as normal cells and also benefit the host (Bohbot and Cardot, 2012).

At present to increase the shelf life of the product drying is considered to be the best option. Many drying techniques are common now days which are: freeze drying, drum drying, conventional drying and spray drying. One of the common techniques of reducing moisture content of food materials causing minimal structural and functional changes in the product is spray drying (Rodriguez *et al*., 2005). In 1865 first spray drying techniques was used for the process of drying eggs. The first items produced from the process of spray drying were milk and washing powder. It has a wide application in chemical, pharmaceutical and food industries. The process of spray drying consists in conversion of a product from liquid state to a solid state in the form of powder, with the help of inside hot chamber through the process of dispersion, where product's droplets come in contact with hot air (Rodriguez *et al*., 2005 and Samantha *et al*., 2015). Spray drying process results from rupture and disintegration on liquid point of product, which divides it into trillions of small individual particles, by creating spray or mist of spray of droplets. It has ability to obtain a product in fine powder form, and also takes short drying time. The mechanism of spray-drying is based on the process of moisture elimination by heating the atmosphere at certain controlled temperature. It involves three major basic phases which includes atomization, droplet-to-particle conversion and particles collection, and also have other steps according to different authors. Other than that it involves five major basic steps which are as follows:

(i) Concentration: Product is put into the concentrated prior to going into the spray dryer.

(ii) Atomization: this stage is responsible for creating optimum condition of evaporation to get a dried product having desired characteristics.

(iii) Droplet-air contact: closed controlled chamber helps, atomized liquid comes into contact with hot gas which result in evaporation of 95%+ small droplets of liquid present in product in just few seconds.

(iv) Droplet drying: This process happens in two stages in which moisture evaporation takes places; 1. in first stage, sufficient moisture in the drop to substitute the liquid evaporated of the surface of particles and relatively at constant rate of evaporation takes place (Keey and Pham, 1976). At seconds stage dried shell form at the surface, because of insufficient moisture which unable to maintain saturated conditions. Basically evaporation depends on the diffusion of moisture through droplet shell, which ultimately results in increasing thickness.

(v) Separation: for final separation stage, electrostatic precipitators, cyclones and bag filters is used. Wet scrubbers help in this state to purify and cool the air, which by the end released to atmosphere.

Advantages of spray drying process is that it can be standardized according to our products or after product required. Feed rates can vary from few pounds per hour to over 100

tons per hour. This technique is advantageous because it is a continuous process, possesses faster drying rate and large throughput (Schuck *et al*., 2016). Operation is continuous and changeable to full automatic control (Gharsallaoui *et al.,* 2007). It also can be used with heat-resistant and heat sensitive products. But there are some limitations of spray drying process as it includes limited versatility in producing complex morphological products. Spray drying leads to minimal loss in the food products (D. Santos *et al*., 2018).

2.6 Survival of microorganisms during spray drying

Recently numerous researches have observed the opportunity of using spray drying technology for the preservation of probiotic bacteria, with the advantage of comparatively economical and high throughput processes compared to other drying process (Morgan *et al*., 2006). Spray drying is well-thought-out a good long-term preservation method for starter cultures and probiotic cultures (Riveros *et al*., 2009). To date, most studies have concentrated on the drying of *Lactobacillus*, *Lactococcus* and *Bifidobacteria* species. The drying of probiotic bacteria depend on many factors: a) resistance of a bacterial strains an important criteria to enhance the spray-dried powders' probiotic viability. b) Drying temperature that is inlet temperature of spray dryer with drying time, which influence the probiotic viability due to heat sensitive nature of bacterial cells. c) Heat adaptation, by exposure of bacteria to sub-lethal osmotic stress may confer tolerance to heat and spray-drying (Huang *et al*., 2017). Seth *et al.,* (2017a) optimized the spray drying conditions in developing a sweetened yoghurt powder using response surface methodology. The best optimal condition was inlet air temperature 148 °C, feed rate 0.54 L/h, and atomization pressure 898 kPa, the response variables including the moisture content, bacteria survival ratio, acetaldehyde retention, and overall sensory score were predicted and validated as 3.69% (db), 2.37×10^{-3}, 76.39%, and 8.0, respectively. 100% viability of *Lactobacillus paracasei* was attainable after one week storage at 4 °C and 15 °C (Desmond *et al*., 2002). Two *Propionibacteria acidipropionici* strains were spray dried with at 140°C inlet temperature and 60°C outlet temperature and observed high degree of viability with approximate 100% survival and 10_{10} CFU g-1 cell counts in powder (Schuck *et al*., 2013). Huang *et al*., (2016a) observed *Propionibacteria freudenreichii* ITG P20 (70%) survived better than *Lactobacillus casei* BL23 (40%), under drying conditions (180ºC T inlet, 73ºC T outlet versus 140ºC T inlet, 63ºC T outlet). *Streptococcus* was observed to be more impervious than *Lactobacillus* in spray drying and *S. thermophilus* showed better survival than *Lb. delbrueckii* ssp. *bulgaricus* in spray-dried yoghurt (Bielecka and Majkowska, 2000). *S. thermophilus* CCRC14085 had better survival rate than *Lb. acidophilus* CCRC 14079 in spray dried fermented soymilk (Wang *et al*., 2004). The survival of both *Lb. casei* BL23 and *P. freudenreichii* ITG P20 improved significantly after spray-drying when provide harsh hyperosmotic conditions during their multiplication in

concentrated sweet whey (Huang *et al*., 2016a and Huang *et al*., 2016b). When *Lb. rhamnosus* cells were harvested in the lag, early exponential and stationary phases their viability increased from 2%, 14% and 50% (Corcoran *et al*., 2006). The viability of encapsulated cells of *L. rhamnosus* ATCC 7469 decreased from 10.60 to 10.97 log cfu/g in the first week to 8.72–10.78 log cfu/g in the last week of storage at 25°C (Moayyedi *et al*., 2018). Moumita *et al*., (2018) prepared synbiotic microcapsules using *Pleurotus florida* extract as prebiotics and *Enterococcus faecium* as probiotic, through lyophilization and spray drying methods. There were 4 log unit reductions in lyophilized formulation as compared to 3 log unit reduction in spray dried form, after 15 weeks of storage and founded spray dried form of symbiotic fortification was suggestively more stable than lyophilized form. Survivability of probiotics in dry food matrix is inevitable to overcome the disadvantages of liquid-based cold stored formulations. Lohiya and Avari, (2016) studied on spray drying *Lactobacilli acidophilus* by using three protecting agents (microcrystalline cellulose, Lactose and skimmed milk) and were used in 5%w/v, 10%w/v and 15%w/v concentration and reported maximum viablitity (82%) obtained with 15%w/v SM with moisture content of 3.3 percent.

2.7 Spray dried powders

The products with short shelf life or are perishable can be spray dried as to extend the shelf life of the product. Predominantly milk and milk products are spray dried to change its form and enhance the shelf life and bio- availability of the product. Many spray dried dairy products are prepared: such as high milk protein powder (Mistry and Hassan, 1992), whey protein powder (Hall and Iglesias, 1997), wholemilk powder (Holsinger *et al*., 2000) and high fat powder (McCluskey *et al*., 1997), yogurt powder (Koc *et al*., 2014), dahi powder (Kothakota *et al*., 2014), Kim *et al*., (2002) developed sodium caseinate, anhydrous milk fat and four industrial spray-dried dairy powders (skim milk powder, whole milk powder , cream powder and whey protein concentrate. Reddy *et al*., (2014) developed goat milk powders, Jinapong *et al*., (2008) developed soymilk powders, and whey powder, whey protein concentrate, whey protein hydrolase, and whey protein isolate (Lavari *et al*., 2014 and Tamm, 2016). The inclusive benefit of dairy powders are effortlessly transported, stored, and incorporated into products such as baked goods, confectionary, and ready-to-use therapeutic foods (Seth *et al*., 2017b).

Thomas *et al*., (2004) Reported the protein of bovine whole milk and whey was (3.4%) and (0.8%) before drying and after drying it increased to (26%) in whole milk and (13%) in whey powder. During storage of milk powders it was observed that browning increases due to increase in free radical formation, lactose hydrolization which increases water activity due to which browning and caking occur in milk powders and ultimately effect the reconstitution properties of powders. (Norwood *et al*., 2017; Thomsen *et al*., 2005; Tatiana 2018; Le *et al*.,

2011 and Huppertz and Gazi, 2016). Koc *et al*., (2010) prepared yogurt powder and observed the titratable acidity and pH of fresh yogurt was (0.98% and 4.37), but after drying there was increase in titratable acidity and decrease in pH (1.04% and 4.34) Kumar and Mishra, (2004) researched on Storage stability of mango soy fortified yoghurt powder in high density polypropylene (HDPP) and aluminum laminated (ALP) and concluded ALP was better than HDPP during storage in terms of quality product.

After drying Dispersibility increased as the concentration of soy milk increased (62.3 to 92.1) (Jinapong *et al*., 2008). Dispersibility decreased with increase in inlet air temperature of spray dryer, and was observed to be 57.4% (Reddy *et al*., 2014). Kothakota *et al*., (2014) recorded increase in Dispersibility on increasing the maltodextrin concentration of dahi powder (96.2 to 98.4%). Dispersibility of sweetened yogurt powder showed a variation in the range of (70.62 – 88.74%) (Seth *et al*., 2017a).

Wettability decreased with concentration of soy milk increased (308.0 to 72.4) (Jinapong *et al*., 2008). Kothakota *et al*., (2014) recorded increase in wettability was observed on increasing the maltodextrin concentration of dahi powder 178 to 300 sec. (Jha *et al*., 2002) estimated wettability in instant kher mix was (2 min). Seth *et al*., (2017b) wettability of sweetened yogurt powder varied from (132 to 378 s). Reddy *et al*., (2014) observed increase in wettability in seconds as the concentration of goat milk powder increases (418 to 594)

Kothakota *et al*., (2014) observed decrease in moisture content by increase in concentration of maltodextrin in dahi powder (8.2 to 3.2). If there is an increase in outlet temperature of spray dryer from (65.5°C to 85°C) there was a decrease in moisture content 4.59 to 1.48 (Izadi *et al*., 2014). Seth *et al*., (2017b) observed the moisture content of the spray dried sweetened yogurt powder varied from (1.38 to 5.26%). Koc *et al*., (2010) observed a range of moisture content from (3.98 to 6.88%) in yogurt powder while changing the inlet temperature of spray dryer between 150 and 180°C. Jha *et al*., (2002) estimated 1.91% moisture in instant kheer mix. Davis *et al*., (2017) observed 2.1% moisture content of goat milk powder.

Thomas *et al*., (2004) found solubility decreases with increase in Maillard reaction and temperature. Koc *et al*., (2010) however, found the solubility of yogurt powder in the range of (65.0-72.5%). Kothakota *et al*., (2014) reported the solubility index of spray dried dahi powder without addition of maltodextrin was 22% and then with increasing concentration of maltodextrin content in dahi powder which increases the solubility index up to 48%. It was reported that a reasonably good-quality shelf-stable powder can be developed from sweetened yoghurt having adequate bacteria count and acetaldehyde content by spray drying (Seth *et al*., 2017a).

2.8 Antimicrobial activity

Antimicrobial activity refers to the process of elimination or inhibiting the disease causing microbes. It can be anti-bacterial, anti-fungal or antiviral. Common antimicrobial microorganism, which are defined are probiotics that have a beneficial effect on human health. They are most commonly used in foods, especially in dairy products and also in pharmaceutical. Probiotics widely used in diary technology for making dairy products such as yogurt, as it an important generating source of probiotics (Tamang *et al*., 2016). *S. thermophilus* are second commonly most used bacterium in dairy industry (Xavier *et al*., 2016). It has been observed that *L. acidophilus* is active against gram negative bacteria which include *E. coli, P. aeruginosa, Klebsiella, Salmonella, Shigella* and Gram-positive which includes: *B. cereus, B. subtilis, C. perfringens, S. aureus* and *L. monocytogenes* (Dinev *et al*., 2017). Four different species of *Lactobacillus* {*L. bulgaricus* (PTCC 1332), *Lactobacillus casei* (PTCC 1608), *L. plantarum* (PTCC 1058) and *L. Fermentum* (PTCC 1638)} were experimented to examine the inhibitory activity against four bacterial enteric pathogens (*E. coli, S. aureus, Shigella dysenteriae* and *Salmonella paratyphi* which were separately inoculated in MRS medium (de Man, Rogosa and Sharpe medium) for 50 hours at 37 °C and pH 7 (Hamid *et al*., 2017). Results showed that enteropathogens growth was refrained in the presence of all Lactobacillus and inhibition zone was between 12 and 32 millimetres. So these four Lactobacillus strains had potential antimicrobial compounds against human enteric pathogens (Hamid *et al*., 2017). Kamal *et al*., (2018) studied the antimicrobial activity of *L. rhamnosus* against four common foodborne pathogens, both in vitro and in a food model (yoghurt). By using an agar well diffusion assay, acidified *L. rhamnosus* cell free supernatant was shown to notably inhibit all pathogens tested: *E. coli* O157:H7, *S. aureus, Y. enterocolitica* and *S. typhimurium.* Neutralised supernatant proved to be an antimicrobial effect most pathogens. Also with the addition of *L. rhamnosus* to pathogen yogurt, result in complete elimination and least reduction of pathogens were achieved. Lekha *et al*., (2016) studied antimicrobial activity of *L. bulgaricus, L. casei* and *L. bulgaricus* with *S. thermophilus*. It was reported that mix culture of *L.bulgaricus* and *S. thermophilus* had highest antimicrobial activity against *S. aureus. L. bulgaricus.* Similar efficiency of antimicrobial activity was found in *L.casei.*

2.9 Antibiotic sensitivity

Antibiotic susceptibility is a type of resistance at which a microorganism is able to endure in contact to an antibiotic (Cocconcelli *et al*., 2003 and Toomey *et al*., 2009). Sixty three *L. rhamnosus* strains isolated from Parmigiano Reggiano cheese and *L. rhamnosus* DSM 20021 and antibiotic susceptibility was check against 41 antibiotics. All isolated stains showed resistance to six antibiotics (cefixime, vancomycin, neomycin, enoxacin, pefloxacin and sulphamethoxazole) and DSM 20021T was resistant to nine antibiotics (the above six

plus cephalexin, bacitracin and lincomycin) (Coppola *et al*., 2005). Five lactic acid bacteria species (*L. acidophilus, L. helveticus, L. delbrueckii, L. delbrueckii* subsp. *lactis* and *S. thermophiles*) were tested against 27 antibiotics and observed the strains were resistant to trimethoprim/sulfonamides and fluoroquinolones (Karapetkov *et al*., 2011). Eom *et al*., (2017) checked antimicrobial activity against in yogurt and observed high rate of erythromycin and tetracycline resistance in strains. Georgieva *et al*., (2015) researched on 23 stains of *Lactobacillus* and *Bifidobacteria* as a useful starter culture and reported L. *rhamnosus* was resistant to streptomycin, *L. acidophilus* and *L. brevis* were resistant to kanamycin and clindamycin and *L. casei* was resistant to clindamycin and chloramphenicol. It was also observed that all the strains were resistant to Vancomycin.

CHAPTER-III

MATERIALS AND METHODS

The research work entitled, "Development of Instant *Lassi*" was carried out in Centre of Food Science and Technology CCSHAU, Hisar during the year 2017-2018. The research work was undertaken as per details given below:

3.1 PROCUREMENT OF MATERIALS

3.1.1 Microorganisms

Pure culture of *Streptococcus salivarius* ssp. *thermophilus* (ATCC 19258), *Lactobacillus rhamnosus* (ATCC 7469) *and Lactobacillus acidophilus* (ATCC 4356) were procured from HiMedia company.

3.1.1.1 *Streptococcus salivarius* ssp. *thermophilus* (ATCC 19258)

Streptococcus salivarius ssp. thermophilus was revived by the method prescribed by ATCC. Vial was opened and lyophilized culture pellet was rehydrated using 5 – 6 ml Mann Rogasa and Sharpe (MRS) broth (Table 1) presterlized by autoclaving at 121°C at 15 psi for 15 minutes. One ml of broth from vial was aseptically transferred in laminar air flow to MRS broth and incubated at 37°C for 24 hours. For pure culture (Plate1a) it was streaked on the surface of solidified *Streptococcus thermophilus* Agar (Table 2) and incubated at 37°C. The colony grown after 24 hours were further maintained on slants at refrigerated condition.

3.1.1.2 *Lactobacillus rhamnosus* (ATCC 7469) and *Lactobacillus acidophilus* (ATCC 4356)

Lactobacillus rhamnosus was revived by the method prescribed by ATCC. Vial was opened and lyophilized culture pellet was rehydrated using 5-6 ml MRS broth. One ml of broth from vial was aseptically transferred to MRS broth, kept in anaerobic bottles and incubated at 37°C for 24 hours. The cultured broth was opened under laminar air flow and streaked on the surface of solidified MRS media and incubated at 37°C temperature for the colonies to grow (Plate 1b and 1c). Pure cultures were further maintained on slants at refrigerated condition.

Table 1. Composition of Mann Rogasa and Sharpe Broth (MRS Broth)

Ingredients	Gms / Litre
Proteose peptone	10.000
Beef extract	10.000
Yeast extract	5.000
Dextrose	20.000
Polysorbate 80	1.000
Ammonium citrate	2.000
Sodium acetate	5.000
Magnesium sulphate	0.100
Manganese sulphate	0.050
Dipotassium phosphate	2.000
Final pH (at 25°C)	6.5±0.2

***For preparing MRS Agar:** agar added @ 2%

Table 2. Composition of *Streptococcus thermophilus* Agar

Ingredients	Gms / Litre
Casein enzymic hydrolysate	10.000
Yeast extract	5.000
Sucrose	10.000
Dipotassium phosphate	2.000
Agar	15.000
Final pH (at 25°C)	6.8±0.2

3.1.2 Milk

Double toned milk was purchased from the market, which have predetermined fat (1.5%) and SNF (9.0%).

3.1.3 *Lassi*

Lassi was procured from the local market, Hisar

3.2 Preparation of *lassi*

3.2.1 Starter Culture preparation:

The preparation of starter culture involves following steps:

1. Milk was boiled, divided in pre sterilized conical flasks and cooled at room temperature.
2. After cooling the milk was inoculated with probiotic bacterial cultures *viz. Streptococcus salivarius* ssp. *thermophilus* (ATCC 19258), *Lactobacillus rhamnosus* (ATCC 7469) *and Lactobacillus acidophilus* (ATCC 4356) @ 2% each separately.
3. Kept at 37°C for 12 hours for curd formation.
4. 2% v/v curd prepared from each probiotic bacterial culture were inoculated in pre-boiled milk and incubated at 37°C for 12 hours for curd formation. The curd thus formed was used as starter culture for *lassi* preparation (Plate 2).

3.2.2 *Lassi* preparation

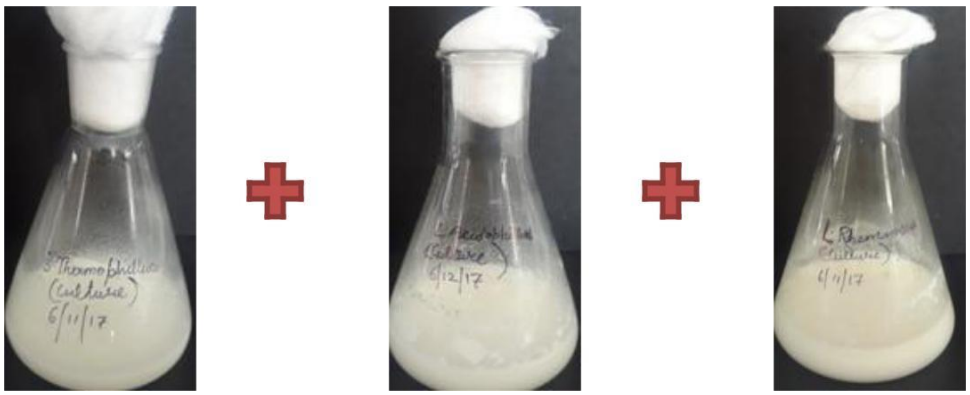
Double toned milk procured from the market was boiled in a stainless steel vessel for 10 to 15 minutes and was then allowed to cool at room temperature. After cooling inoculated with starter culture (3.2.1) at the rate of 2.0% (v/v) and then the vessel was covered with muslin cloth and lid to provide anaerobic environment. The milk was then incubated at 37 °C for 12 hours. After the formation of curd, breaking of curd was done by mechanical stirrer. Stirred curd was blended with water at a ratio of 1:1 to prepare *lassi*. The *lassi* was prepared in three variations as follows:

a.) Plain *lassi* (Fig. 1)

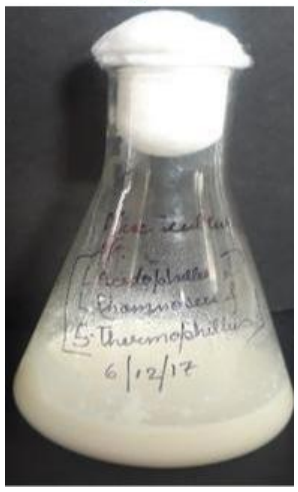
b.) Salted *lassi* by addition of salt @ 0.15 g per 100 ml before spray drying and cumin powder was added at the rate of 0.1 mg per 25 g in spray dried powder (Fig. 2)

c.) Sweetened *lassi* was prepared by addition of sucrose 2 grams per 100ml before spray drying (Fig. 3)

Plate 1. a.) *S. thermophilus* on *Streptococcus thermophilus* Agar b.) *L. rhamnosus* on MRS Agar and c.) *L. acidophilus* on MRS Agar



S. thermophilus *L. acidophilus* *L. rhamnosus*



Starter culture with 2% (v/v)

Plate 2 Starter culture for *Lassi* preparation

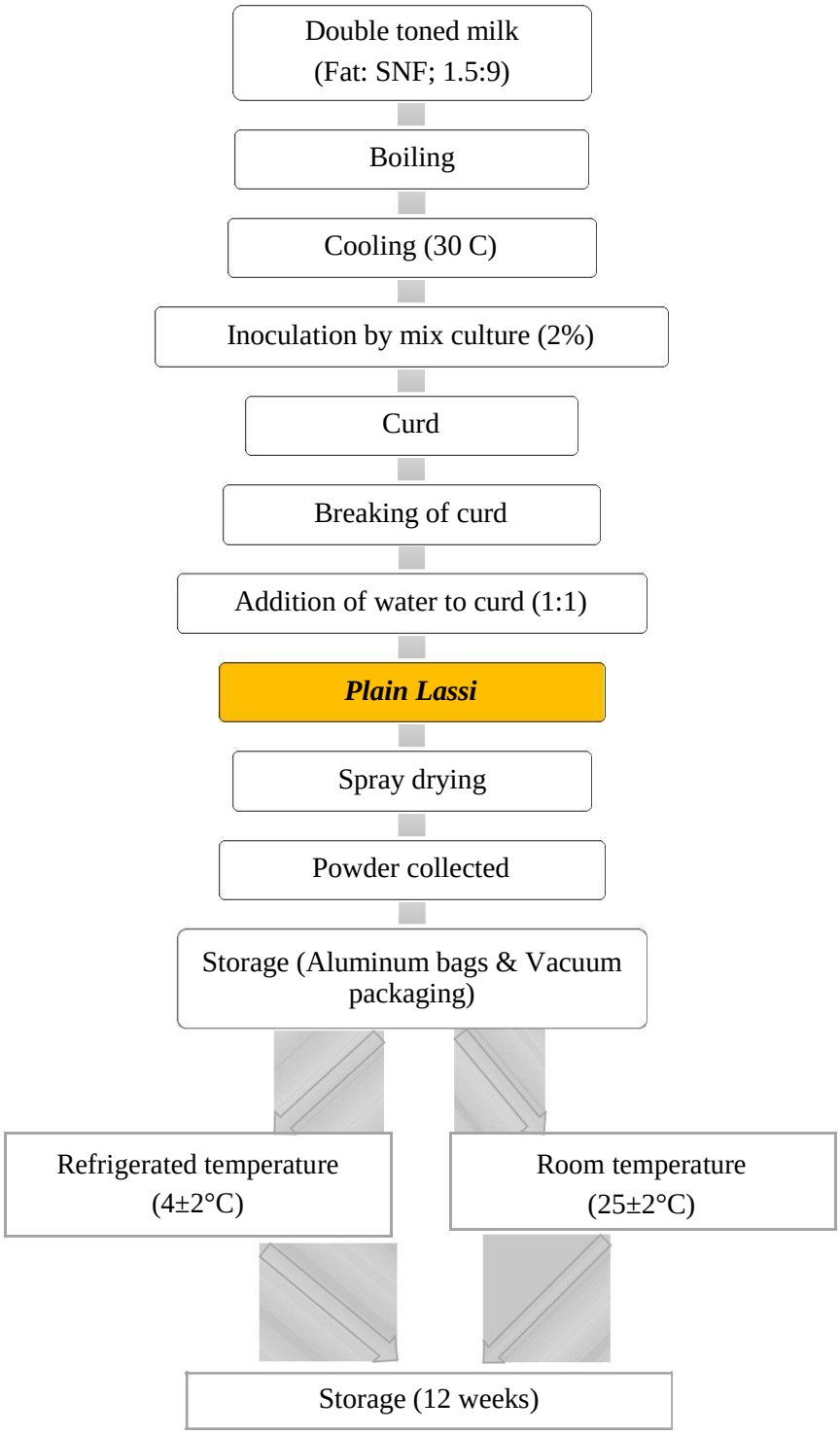


Fig 1. Flow chart for the preparation of plain *Lassi*

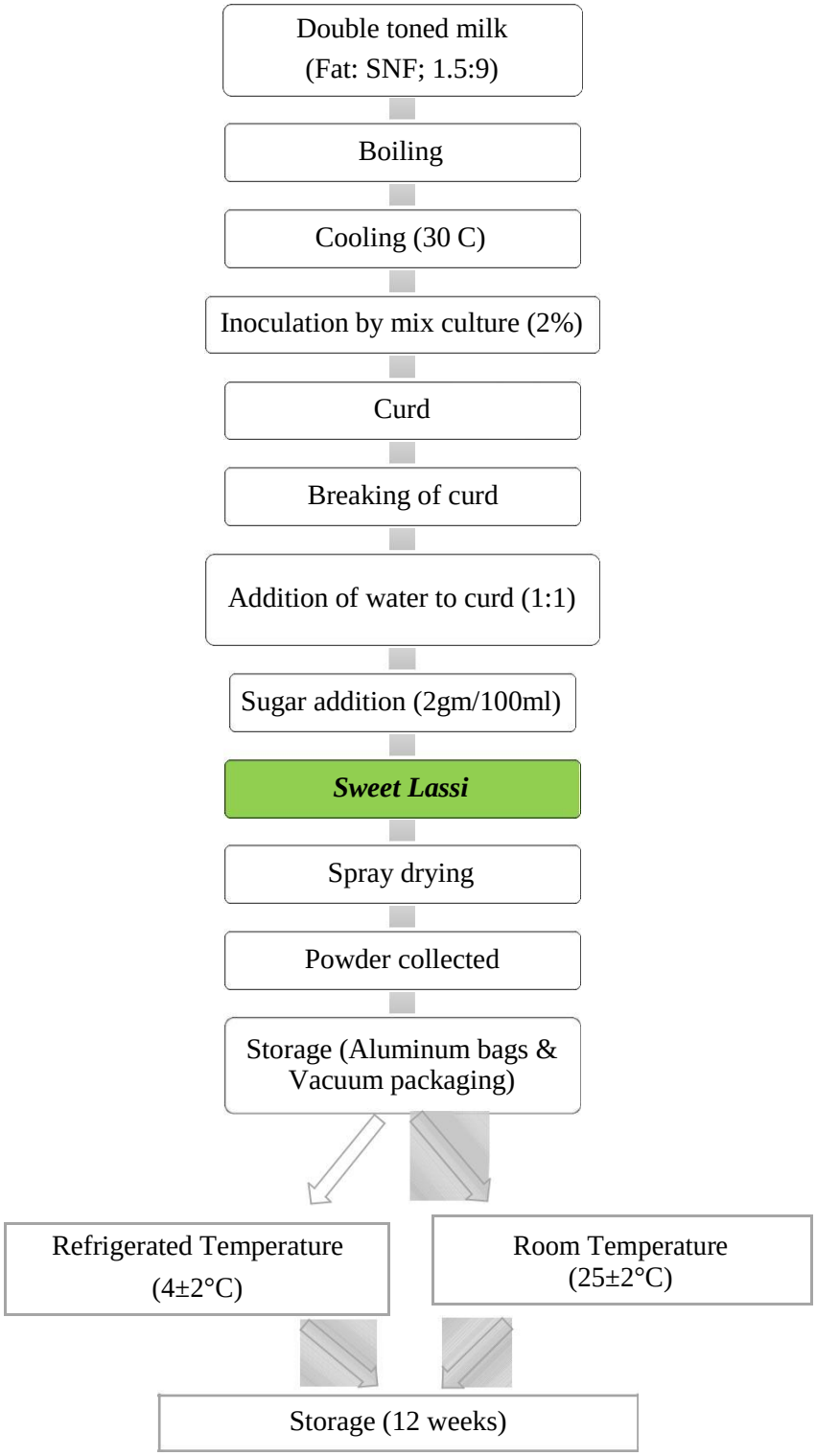


Fig 2. Flow chart for the preparation of sweet *Lassi*

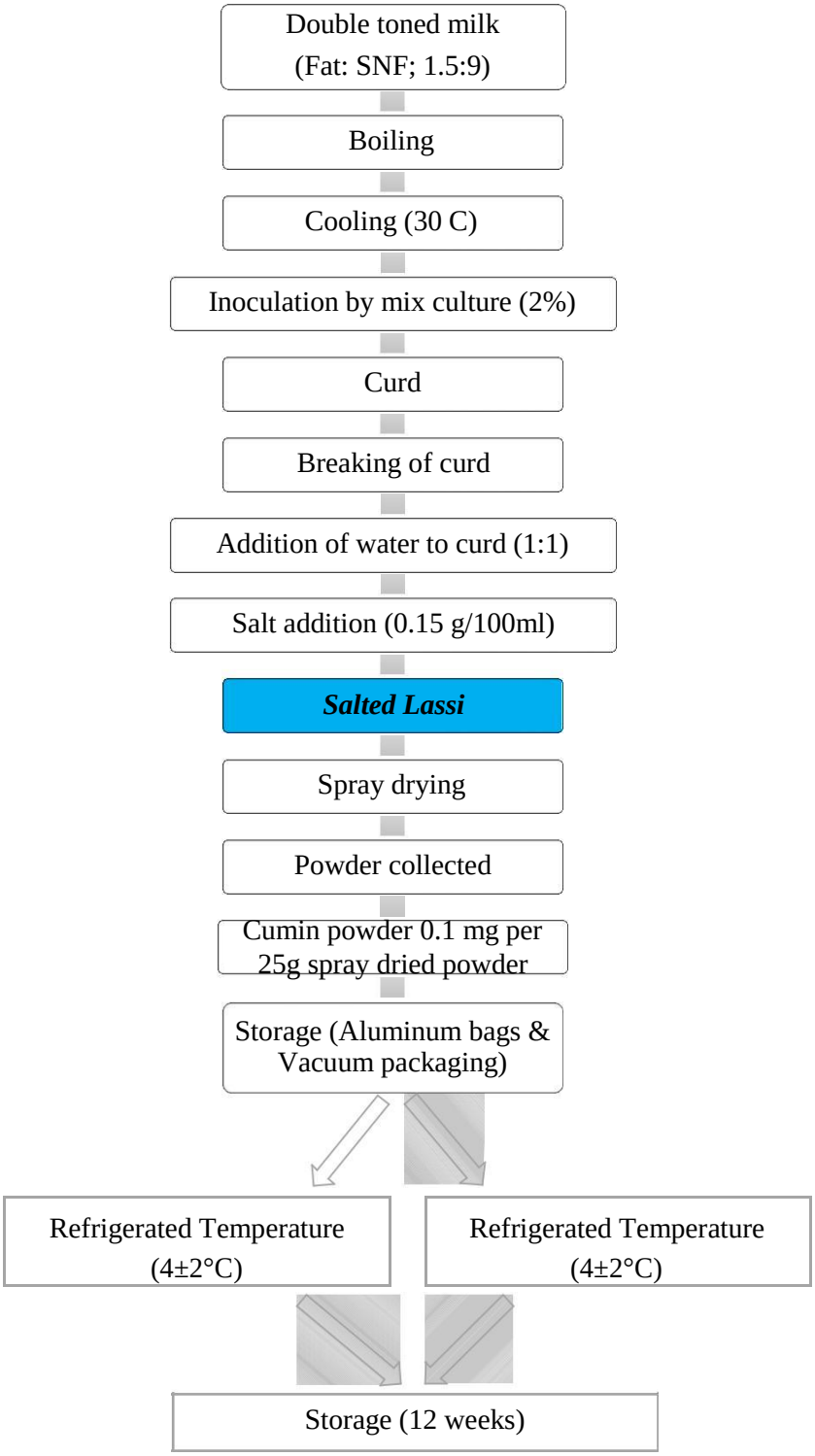


Fig 3. Flow chart for the preparation of salted *Lassi*

3.3 Spray drying of *lassi*

The inlet temperature, outlet temperature and speed of the spray dryer were optimized with the help of Central Composite Rotatable Design (CCRD) of Response Surface Methodology (RSM). Polynomial models were developed using multiple regression technique for lack of the dependent variable using Design Expert 11 using software procured from Stat Ease Inc., USA. CCRD design constituted of 20 runs which were carried out in randomized order. The response was generated with reference to Survival rate of probiotic (SRP) microorganisms. There were total 4 variants of *lassi* (market procured, plain, sweet and salted respectively) were pre heated to 30˚C and spray dried at optimized condition. After every spray drying operation the spray dried powder was collected and remaining was scrapped of the spray dryer by opening the spray dryer followed by cleaning of spray dryer and nozzle by water. The collected spray dried powder was packed in aluminium laminated bags followed by vacuum packaging. And was then stored at room temperature (25±2˚C) and refrigerated temperature (4±2˚C) for further storage studies for weekly intervals.

3.4 Optimizing reconstitution of *lassi*

After spray drying the powder was reconstituted by blending with water by mixing in a blender to obtain *lassi*.

3.5 Physico-chemical analysis

Physico-chemical analysis was performed at different stages of manufacturing of *lassi* powder.

3.5.1 Physico-chemical analysis of milk

3.5.1.1 Total solids

Total solids in milk were determined by gravimetric method (IS 12333 - 1997 / ISO 6731: 1989). Five ml sample was transferred to a beaker and was warmed slowly to 35-40°C on a water bath with careful mixing to incorporate any cream adhering to the sample. Sample was quickly cooled to room temperature. Five ml of milk was dried on a boiling water bath by pouring the milk in pre-dried and pre- weighed aluminum dish without placing the lid on the top followed by vigorous boiling in water bath in such a way that the bottom of the dish is directly heated by the steam. Heating was continued till all the water was removed. Remove the dish from the water bath. Sample was dried in hot air oven at 102±2°C and from the weight of the residue, the total solids content in milk was determined by the following method:

$$\text{Total solids} = \frac{M2-M0}{M1-M0} \times 100$$

Where,

M0 = mass in g of dish + lid

M1 = mass in g of dish + lid and test portion

M2 = mass in g of dish + lid and dried test portion

3.5.1.2 Fat

Fat in milk was estimated by Rose-Gottlieb Method (AOAC 2000). In this method the milk sample was treated with ammonia and ethyl alcohol to dissolve the protein and the latter to help precipitate the proteins. Fat is extracted with diethyl ether and petroleum ether. Mixed ethers are evaporated and the residue weighed.

Reagents

1. Ammonia solution, containing approximately 25% (m/m) of NH_3, sp.gr ($\rho 20$) ≈910 g/l
2. Ethyl alcohol (95%).
3. Diethyl ether, peroxide-free.
4. Petroleum ether, boiling range 40-60°C.
5. Mojonnier fat extraction flask or any other suitable extraction tube (as per IS specification).
6. Cork or stopper of synthetic rubber unaffected by usual fat solvents.

Procedure

Ten gram milk was weighed and transferred to extraction tube and for powders one gram powder and 10 ml of warm water. 1.25 ml of ammonia specific gravity 0.91 was added, mix and shake thoroughly. Add 10 ml ethyl alcohol and mix again. 25 ml of diethyl ether (peroxide free) was added; stopper was placed and shaken vigorously for about a minute. Then 25 ml petroleum ether was added and shaken again vigorously for about half a minute. It was allowed to stand until the upper ethereal layer has separated completely and was clear. The clear ethereal layer was decant off in a suitable vessel. The delivery end was washed of the extraction tube with a little ether. The flask were dried in hot plate to evaporate the ether, followed by final drying I the oven at 102±2℃ for 30 min. Cool in a desiccator and weigh. It was calculated as follows

Fat % (w/w) = Weight of Extracted Fat/ Weight of milk X 100

3.5.1.3 pH

The pH of milk was determined by digital pH meter (Elico India Ltd., using standard buffers pH 4.0 and 7.0)

3.5.1.4 Titratable acidity

Titratable acidity was determined by NaOH method *(IS 1166 – 1973).* In this method 10 g sample was weighed accurately (milk) in a suitable dish or basin. 30 ml of warm water was added. 1 ml of phenolphthalein indicator was added. Shaken well and titrated against 0.1N NaOH solution till faint pink colour appeared and was calculated as:

Titratable acidity as Lactic acid= 9 AN/ W

Where,

A = Volume of standard NaOH required for titration

N = Normality of Standard NaOH solution

W = weight of the sample taken for test

3.5.1.5 Protein

Crude protein was estimated using Micro-Kjeldhal method (*ISO 8968-1/IDF 020-1:2001*).

Reagents

1. Digestion mixture – $CuSO_4$ and K_2SO_4 was mixed in ratio 1: 8
2. H_2SO_4
3. 0.01 N HCl
4. 4 % Boric acid solution
5. 40 % NaOH

Procedure

One gram of milk sample, 20 ml concentrated H_2SO_4 and a pinch of digestion mixture was taken in a digestion flask. The mixture was heated till digested. After digestion the contents were cooled, diluted and volume made up to 100 ml with distilled water. The liberated ammonia was distilled into 10 ml boric acid solution containing a few drops of mixed indicator. The distillate was titrated with N/100 hydrochloric acid to determine the ammonia absorbed in boric acid.

$$\text{Nitrogen (\%)} = \frac{\text{(S-B) x V x 0.00014)}}{\text{V1 x W}} \times 100$$

$$\text{Crude protein (\%)} = \text{Nitrogen (\%)} \times 6.38$$

Where,

W = Weight of sample taken (g)

V = Volume of extract made (ml)

V1 = Volume of aliquot taken for distillation

S = Volume of standard acid (0.01 N HCl) used for titration (ml) of sample

B = Volume of 0.01 N HCl used for blank (ml)

3.5.2 Physico-chemical analysis of *lassi* from local market

3.5.2.1 Total solids

Total solids were determined after neutralization of developed acidity with alkali. The method used for determination of total solids in *lassi* is sodium hydroxide method (IS 12333: 1997). In this procedure NaOH is used for neutralization of developed acidity followed by determination of total solids by evaporation at 100 ± 2°C.

Apparatus

Shallow flat-bottom dishes of aluminum, nickel, 7-8 cm diameter, about 1.5 cm in height and provided with easily removable but closely fitting lids.

Procedure

Heated clean dry empty dish and lid in an oven maintained at 100 ± 2°C for one hour, cooled in a desiccator and weighed. Four to five gram sample of curd was weighed. Added 1-2 drops of phenolphthalein solution to the sample in dish and neutralized with 0.1 N NaOH solution to a faint pink colour. The volume of 0.1N sodium hydroxide required to neutralize the sample was noted. The dish without lid was placed in boiling water bath until the whole water is removed from the sample. The dish was then placed in the oven maintained at 100±2°C for 3 hrs. The dish along with the lid was cooled in a desiccator and weighed. Continued heating and re-weighing was performed at hourly intervals until successive weighing do not vary by more than 0.5 mg, and was calculated as followes:

$$a = \frac{N \times T.V \times 40}{1000 \times 2}$$

$$\text{Total solids percent w/w} = 100\ (W2 - a)/1$$

Where:

N = Normality of NaOH

T. V = Titre value

W**2** = Weight in g of residue left after drying

W1= Weight in g of the prepared sample taken

a = Half of the volume of 0.1 N Sodium hydroxide added

3.5.2.2 SNF

Determined Total solids by the method given under 3.5.2.1 and sugar as described in Lane–Eynon Method.

$$\text{Total milk solids} = \text{Total solids} - \text{Added sugar}$$

3.5.2.3 Fat

Fat content in *Lassi* procured from the local market was estimated by the same method as given under 3.5.1.4.

3.5.2.4 pH

The estimation pH of *Lassi* was determined by method under 3.5.1.5.

3.5.2.5 Titratable Acidity

Titratable acidity was estimated by the method given under 3.5.1.6.

3.5.2.6 Lactic acid

Lactic acid is measured in the term of titratable acidity.

3.5.2.7 Lactose

Lactose was estimated by Lane Eynon Method (IS 4079 – 1967)

Reagents

1. Sodium hydroxide solution: 0.1N
2. Stock solution of invert sugar: To 9.5 g of pure sucrose, 5ml of concentrated hydrochloric acid was added in 1000 ml flask and subsequently 100 ml of water was added. It was allowed to stand for 3 days at 20 to 25℃ and it was made to volume with water
3. Standard solution of invert sugar: 20 ml of stock solution was neutralized using NaOH and volume was made to 1000 ml using distilled water
4. Methylene blue indicator
5. Fehling Solution A
6. Fehling Solution B
7. Zinc acetate solution: Dissolve 21.9 g of crystalline zinc acetate ($Zn(CHCOO)_2$, 2HO) in water and add 3 ml of glacial acetic acid. Make up to 100 ml.
8. Potassium ferrocyanide solution: Dissolve 10.6 g of crystalline potassium ferrocyanide and make up to 100 ml with water.
9. Concentrated Hydrochloric acid solution (Sp.gr. 1.16).
10. Concentrated ammonia solution (Sp.gr. 0.88).
11. Dilute ammonia solution: 10 ml of concentrated ammonia solution diluted to 100 ml with water.
12. Dilute acetic acid solution: Approximately equivalent to the dilute ammonia solution in strength.

Procedure

For the analysis 50 g well mixed sample was taken in 250ml beaker followed by 65ml of hot distilled water at 80°C to 90°C and transferring to 250 ml volumetric flask. Distilled water at 60°C was added until the volume reached 150-160 ml. it was mixed well and allowed to cool. 7ml of dilute ammonia solution was added and was allowed to stand for 15 min followed by addition of dilute acetic acid the solution was mixed and 15 ml zinc acetate solution was added followed by addition of 15 ml Potassium ferrocyanide solution. The solution was mixed and volume was made to 250 ml and was allowed to settle and filtered. 50 ml of the filtrate was taken in beaker and 5 ml concentrated hydrochloric acid solution was added and was kept at water bath at 65- 68°C for 5 min. it was cooled neutralized and volume made to 100ml with distilled water. It was then used for titrating 10ml felling solution (5ml Fehling A and 5 ml Fehling B) using methyl blue as an indicator till the appearance of brick red end point. The amount of sample used to reduce Felling solution was noted. Amount of sugar was calculated as follows:

$$\% \text{ Sugar} = \frac{\text{Titre Value (standard)} \times \text{dilution} \times 2}{\text{Titre Value (sample)}}$$

3.5.3 Physico-chemical analysis of *lassi* (specific culture)

3.5.3.1 Total solids

Total solids in *lassi* of specific culture was estimated by the method given as discribed under 3.5.2.1

3.5.3.2 SNF

SNF content in *lassi* was determined by the method given under 3.5.2.3

3.5.3.3 Lactose

Lactose content was determined by the method 3.5.2.7

3.5.3.4 Fat

Fat content was estimated by the same method as given under 3.5.1.4.

3.5.3.5 pH

The estimation pH of *lassi* was determined by method under 3.5.1.5.

3.5.3.6 Titaratable Acidity

Titratable acidity was estimated by the method given under 3.5.1.6.

3.5.4 Physico-Chemical Analysis of Instant *Lassi* Drink

Instant *lassi* in three variation reconstituted from spray dried *Lassi* powder was analyzed for

3.5.4.1 Total solids

Total solids in reconstituted *lassi* was estimated by method given as under 3.5.2.1

3.5.4.2 Fat

Fat content in reconstituted was estimated by the same method as given under 3.5.1.4.

3.5.4.3 pH

The estimation pH of reconstituted *lassi* was determined by the method under 3.5.1.5.

3.5.4.4 Titratable Acidity

Titratable acidity was estimated by the method given under 3.5.1.6.

3.5.4.5 Browning

The browning in *lassi* powder was estimated by method of Rangana (2014). The increase in absorbance of sample extract at 440nm was taken as a measure of non-enzymatic browning. Ten grams of sample was blended with 10 ml of water and 30 ml of 60% ethyl alcohol and was kept overnight. The solution was filtered. The filtrate was taken and its color was measured at 440 nm using 60% aqueous ethyl alcohol as blank

3.5.5 Physico-chemical analysis of spray dried *lassi* powder

3.5.5.1 Moisture and Total solids

The sample is dried to constant weight at 102±2°C and the loss in weight reported as moisture. The method described here has been followed from IS: SP-18 (part XI) -1981

Procedure

The uncovered dish was placed in hot air oven at 102±2°C for 1 h. the dish was kept in desiccators. Allow it to cool to room temperature and weighed. One gram of the dried milk sample was weighed in the dish and dish was kept uncovered in hot air oven maintained at 102 ± 2°C for 2 h. the lid was covered and transferred to the desiccator, and allowed to cool to room temperature and weighed. Again the dish was kept in the hot air oven at 102±2°C for further 1 h, and was weighed again after cooling in desiccator. The process was repeated until the successive weighing do not differ by more than 0.5 mg. it was calculated as follows:

$$\text{Moisture \% by mass (Z)} = \frac{100\ (M1 - M2)}{M1 - M}$$

Where,

M = mass in g, of the empty dish;

M1 = initial mass in g, of the dish and lid with the material taken for analysis;

M2 = final mass in g, of the dish and lid with the material after drying.

Percent Total Solids = 100 -Z

3.5.5.2 Solubility Index

Solubility index was estimated by with slight modification 14 g of the sample was added to 100 ml distilled water in the mixing jar. The jar was placed in the mixer and was stirred for exactly 90 seconds. Sample was allowed to stand until the foam was separated. The reconstituted *lassi* was immediately filled to the 50 ml mark in Centrifuge tube and was centrifuged for 5 minutes. Immediately transparent liquid to within 5 ml of the surface of the sediment level was siphon off. Twenty five ml of distilled water was added again in centrifuge tube to disperse the sediment, dislodging it and was again centrifuged for 5 min and was calculated was followed:

Solubility index: Report the solubility index as the milliliters of sediment in the tube.

3.5.5.3 Dispersibility

Dispersibility measurement was performed according to the procedure described by Jinapong (2008). Ten ml of distilled water, was poured into a 50 ml beaker. One gram powder was added into the beaker. The stop watch was started and the sample was stirred vigorously with a spoon for 15 s making 25 complete movements back and forth across the whole diameter of the beaker. The reconstituted *Lassi* was poured through a sieve (212 ppm). The sieved *lassi* (1 ml) was transferred to a weighed and dried aluminum pan and dried for 4 h in a hot air oven at 105± 2°C. The dispersibility of the powder was calculated as follows:

$$\text{\% Dispersibility} = \frac{(10+a) \times \text{\% TS}}{a \times 100 - b/\ 100}$$

Where,

a = amount of powder (g) being used,

b = moisture content in the powder

% TS = dry matter in percentage in the reconstituted *lassi* after it has been passed through the sieve.

3.5.5.4 Wettability

The wettability is defined as the time in seconds required for all the particles of an instant dry milk sample to become wetted (to sink below the water surface or assume a 'typical' wet appearance) when placed on the surface of water.

Procedure

Ten gram of well mixed instant dried *lassi* was weighed and 250 ml of deionized water was poured in dry 600 ml glass beaker. The steel plate was placed on top of the beaker, with one edge of the plate closed to the rim of the beaker. The test portion was spreaded evenly over the glass plate. The stop watch was started. After 10 seconds, the glass plate with one hand was withdrawed allowing the powder sample to fall progressively over a period of 2.5 seconds onto the surface of the water. The time in seconds was recorded from the beginning of withdrawal of the glass plate until all the particles become wetted. Measurements are to be carried out in duplicate and calculated as:

Wetting time = T-10

Where,

T = time recorded (in 6.6) in seconds.

10 = time elapsed before withdrawal of the glass plate.

3.5.5.5. Browning

Browning in dried *lassi* powder was estimated as per the method given under 3.5.4.5.

3.5.5.6 SNF

SNF content in *lassi* was determined by Jha *et al.,* (2002)

3.5.5.7 Fat

Fat content in reconstituted was estimated by the same method as given under 3.5.1.4.

3.5.5.8 pH

The estimation pH of reconstituted *lassi* was determined by method under 3.5.1.5.

3.5.5.9 Titratable Acidity

Titratable acidity was estimated by the method given under 3.5.1.6.

3.6 Microbiological analysis

Microbial analysis of spray dried *lassi* powders were done at monthly every monthly interval up to 12 weeks storage.

3.6.1 Yeast and mold count

Yeast and mold count was estimated on Yeast Extract Peptone Dextrose (YEPD) Agar (Table 3). The agar was prepared by addition of desired amount of media in warm distilled water and autoclaved at 121°C at 15 psi for 15 minutes. The agar was then poured in sterile petri plates and was allowed to solidify, and left for 72 hours to check any contamination. One gram powder was weighed and poured in pre sterilized water blanks and serial dilutions were prepared till 10^7. After solidification 0.1ml of each serial dilution was poured on agar and was spreaded by spread plate method. The plates were incubated at 25°C temperature for 72 hours and then colonies were counted by colony counter.

3.6.2 Coliform count

Coliform count was estimated on Eoisine Methylene Blue Agar (EMB) Agar (Table 4) by serial dilution followed by spread plate method. The plates were incubated at 37°C temperature for 24 hours and then colonies were counted by colony counter.

Table 3. Composition of Yeast Extract Peptone Dextrose Agar (YEPD)

Ingredients	**Grams/Litre**
Peptone	20.00
Yeast extract	10.00
Dextrose	20.00
Agar	15.00

Table 4. Composition of Eoisine Methylene Blue Agar (EMB Agar)

Ingredients	**Gms / Litre**
Peptic digest of animal tissue	10.000
Dipotassium phosphate	2.000
Lactose	5.000
Sucrose	5.000
Eosin – Y	0.400
Methylene blue	0.065
Agar	13.500
Final pH (at 25°C)	7.2±0.2

3.6.3 Probiotic count in *lassi* powder

The microbial count of spray dried *lassi* powders were estimated on two media that were ST Agar for S. *thermophiles*, and MRS Agar for *L. rhamnosus and L. acidophilus* by pour plate method. One gram powder was weighed and poured in pre sterilized water blanks and serial dilutions were prepared till 10^7. 0.1ml sample from each serial dilution was poured into plates and then the media was poured and plated were allowed to solidify and were kept under anaerobic condition at 37°C temperature for 24 – 72 hours

3.6.4 Antimicrobial activity

Antimicrobial activity against food pathogens viz. *E. coli* (MTCC 249), *Staphylococcus aureus* (MTCC 133) and *Bacillus subtilis* (MTCC 070) was estimated on Nutrient Agar plates (Table 5). For estimation of antimicrobial activity all the food pathogens grown in Nutrient Broth (Table 5) for 24 hours at 25±2°C. Samples were prepared by incubating the spray dried powder of different *lassi* variants in MRS Broth for 24 hours at 37°C. Pre solidified Nutrient agar plates spreaded with 0.1ml of food pathogen culture. After spreading wells were created on the plates and 0.1ml sample of lassi powder as prepared above was poured in wells and incubated at 37°C for 24 hours. Antimicrobial activity was checked by measuring the zone of inhibition.

Table 5. Composition of Nutrient Broth

Ingredients	Gms / Litre
Peptone	5.000
Sodium chloride	5.000
HM peptone B#	1.500
Yeast extract	1.500
Final pH (at 25°C)	7.4±0.2
*For preparation of Nutrient Agar, agar added @ 2%	

3.6.5 Antibiotic Sensitivity

Antibiotic Sensitivity was estimated against thirty two different antibiotics with the help of ICOSA UNIVERSAL II and ICOSA G-I-PLUS disc on MRS Agar plates. These disc were impregnated with different antibiotic, with their corresponding symbols printed on the ring. 1ml of the spray dried powder sample prepared above (3.6.4) was spreaded on MRS Agar plates and disc was placed on the spreaded sample with the help of forceps. The plates were then incubated at 37°C for 24 - 42 hours. The antibiotic impregnated disc functions as individual antibiotic susceptible discs, generating precise circular zone of inhibition and measured by zone scale.

3.7 Sensory Evaluation

Sensory evaluation of reconstituted spray dried *Lassi* powder with different variation was done by using 9-point hedonic scale at interval of 0, 30, 60 and 90 days of storage period by a panel of 10 semi-trained or non-trained judges. The products were evaluated on the basis of color and appearance, taste, aroma and flavor, body and texture, after taste and overall acceptability. The overall acceptability of instant *lassi* was based on mean scores obtained from all the sensory characteristics.

3.9 Statistical Analysis

The data was statistically analyzed using SAS software.

CHAPTER-IV

RESULTS

The experimental results of the present study "Development of instant *lassi*" are presented in this chapter. The present study was studied with the objectives to standardize the preparation of instant *lassi* and to evaluate the shelf life of the developed product. All the experiments were conducted in triplicates and the results presented are the mean values. For each individual experiment, ANOVA was calculated using SAS (Statistical Analysis System) software. Results are presented in this chapter under following heading.

4.1 Physico-chemical analysis of milk and local market *lassi*

4.2 Physico-chemical analysis of *lassi* before drying

4.3 Optimization of spray drying conditions

4.4 Optimization and physico-chemical analysis of reconstituted *lassi* powder

4.5 Changes in physico-chemical properties during storage at weekly interval

4.6 Organoleptic quality of *lassi* during storage

4.7 Microbial Analysis

4.1 Physico-chemical analysis of milk and local market *lassi*

4.1.1 Milk

Pasteurised double toned milk of Amul was procured from the market, Hisar which have pre-determined fat (1.5%) and SNF (9.0%) level. In physico-chemical analysis of fresh milk pH obtained was (6.7), titratable acidity in milk was observed (0.11%) and protein content was (3.06%) (Table 6)

Table 6: Physicochemical analysis of fresh milk

Parameters	Milk
pH	6.70±0.005
Titratable Acidity	0.11±0.028
Protein	3.06±0.057

*The values are mean ± S.D. of three replicates

4.1.2 Local Market *lassi*

Lassi was procured from the local market, Hisar. Different physico-chemical analysis of local market *lassi* was done and the average values were calculated. Different chemical constituents of local market *lassi* Total Solids (TS), Solid Not Fat (SNF), Fat, Lactose, pH, Titratable acidity content were estimated to (10.17%), (9.4%), (0.77%), (1.2%), (4.33) and (0.633) respectively (Table 7)

Table 7: Physicochemical analysis of fresh local market *lassi*

Parameters	**Local Market *lassi***
Total Solids	10.17 ± 0.28
Solid Not Fat	9.4±0.36
Fat	0.77±0.25
Lactose	1.2±0
pH	4.33±0.057
Titratable Acidity	0.633±0.057

*The values are mean ± S.D. of three replicates

4.2 Physico-chemical analysis of *lassi* before drying

Lassi was prepared with probiotic bacterial cultures *viz.* {*S. thermophiles* (ATCC 19258), *L. rhamnosus* (ATCC 7469) *and L. acidophilus* (ATCC 4356)} in different variations that were plain, sweet and salted. All variations were analyzed for different physico-chemical properties. Estimated pH of plain, sweet and salted *lassi* was (4.70), (4.64) and (4.63) respectively. It was observed that pH of plain is significantly higher than sweet and salted *lassi*. Titratable acidity after addition of salt to *lassi* increased from (0.33%) to (0.41%). As compared to plain (0.33%) there was slight change in acidity of sweet *lassi* (0.37%). There was no significant change in SNF of plain (9.4%) and salted *lassi* (9.50%). Due to the addition of sucrose SNF was high in sweetened *lassi* (10.53%).Fat content in plain, sweet and salted *lassi* was found as (1.6%), (1.67%) and (1.53%). Lactose in different composition of *lassi* was observed as (1.16%, 1.03% and 1.16%) respectively (Table 8).

Table 8: Physico-chemical analysis of *lassi* before drying

Variable	**Treatments**			**C.D. at 5%**
	Plain (Mean ± SD)	**Sweet (Mean ± SD)**	**Salt (Mean ± SD)**	
pH	4.70^{a} ± 0.005	4.64^{b} ± 0.015	4.63^{b} ± 0.035	0.0447
Titratable Acidity	0.33^{a} ± 0.03	0.37^{a} ± 1.74	0.41^{a} ± 0.015	0.04
Fat	1.6^{a} ± 0.1	1.67^{a} ± 0.15	1.53^{a} ± 0.15	0.274
Total Solids	11.5^{ba} ± 0.5	12.13^{a} ± 0.23	11.33^{b} ± 0.29	0.717
Solid Not Fat	9.40^{b} ± 0.1	10.53^{a} ± 0.35	9.50^{b} ± 0.46	0.675
Lactose	1.16^{a} ± 0.057	1.03^{b} ± 0.058	1.16^{a} ± 0.057	0.115

*The values are mean ± S.D. of three replicates

4.3 Optimization of spray drying conditions

The optimized spray drying inlet temperature, outlet temperature and speed was studied with the help of Central Composite Rotatable Design (CCRD) of Response Surface Methodology (RSM). Polynomial models were developed using multiple regression technique for lack of the dependent variable using Design Expert 11 using software procured from Stat Ease Inc., USA. The CCRD design constituted of 20 runs which were carried out in

randomized order. The response was generated with reference to SRP. The best inlet, outlet temperature and speed with maximum probiotic count was optimized as 190°C, 70°C and 300 rpm speed.. The fitted model was 2FI. The Model F-value of 19.28 implies the model is significant. P-values less than 0.0500 indicate model terms are significant. In this case inlet temperature and interaction between inlet and outlet temperature are significant model terms which means inlet and outlet temperature affect the SRP. The R^2 of the model was observed to 0.8990 means the model is significant. Multiple regression equation generated to predict the microbial count as affected by different factors in terms of actual factors is as follows:

Microbial Count = +109.91588 - 0.735627 inlet temperature + 0.167588 outlet temperature - 0.160017 speed + 0.001700 inlet temperature × outlet temperature + 0.001591 inlet temperature × speed - 0.001891outlet temperature × speed

There were total 4 variations in *lassi*: Market procured, plain, sweet and salted, which were spray dried optimized condition at 190°C, 70°C and 300 rpm and stored in aluminium laminated bags followed by vacuum packaging

Table 9: Survival rate of probiotics (SRP) w.r.t inlet temperature, outlet temperature and speed of spray dryer

Inlet temperature (ºC)	Outlet temperature (ºC)	Speed (rpm)	SRP (log10 CFU/ml)
270	100	350	2.1
270	120	350	1.2
180	60	300	9.0 (slurry)
190	**70**	**300**	**9.2 (powder)**
180	60	350	10
200	94	300	6.4
220	110	350	1
270	130	350	0
220	100	350	2.5
270	120	350	1.2
230	90	350	4.2
190	75	350	8.5
240	124	350	1.2
250	120	350	1
200	90	350	2.1
210	93	350	3
190	85	250	6.5
260	130	350	1.5
210	90	300	2
240	105	300	1

Table 10: ANOVA for 2FI model for Response: SRP

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	186.72	6	31.12	19.28	< 0.0001	Significant
A-A	9.46	1	9.46	5.86	0.0309	
B-B	4.78	1	4.78	2.96	0.1090	
C-C	2.50	1	2.50	1.55	0.2354	
AB	13.20	1	13.20	8.18	0.0134	
AC	5.08	1	5.08	3.15	0.0994	
BC	2.94	1	2.94	1.82	0.2002	
Residual	20.98	13	1.61			
Lack of Fit	20.98	12	1.75			
Pure Error	0.0000	1	0.0000			
Cor Total	207.70	19				
Std. Dev.	1.27					
Mean	3.69					
C.V.%	34.43					
R^2	0.8990					
Adjusted R^2	0.8524					
Predicted R^2	0.7449					

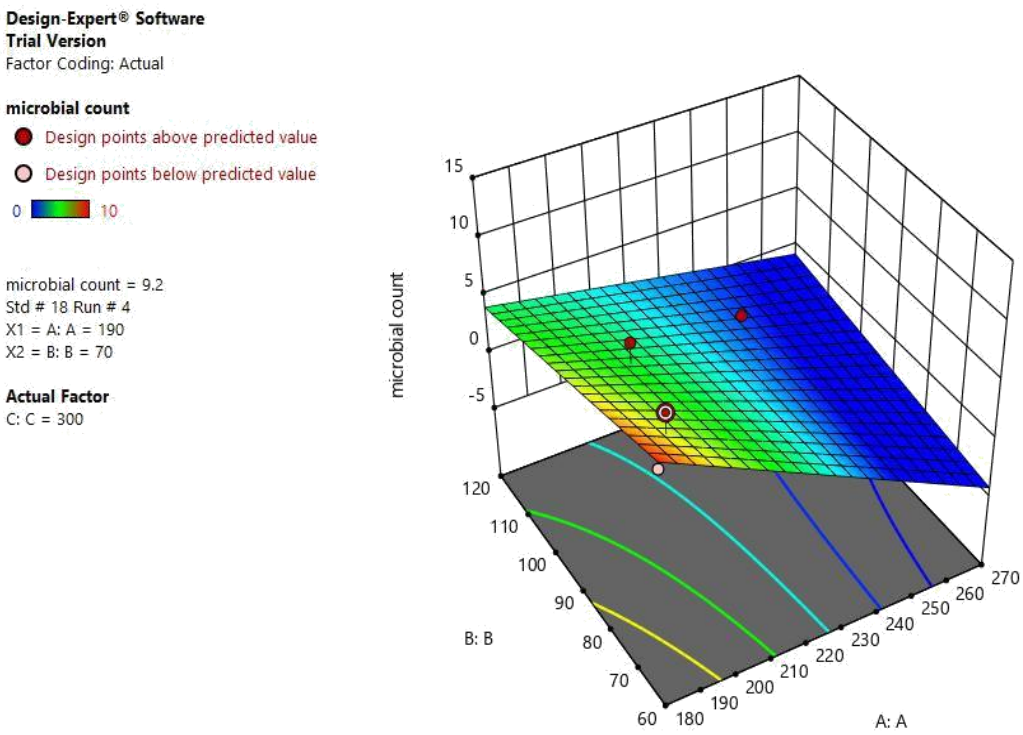


Fig 4. Optimized inlet and outlet temperature w.r.t SRP

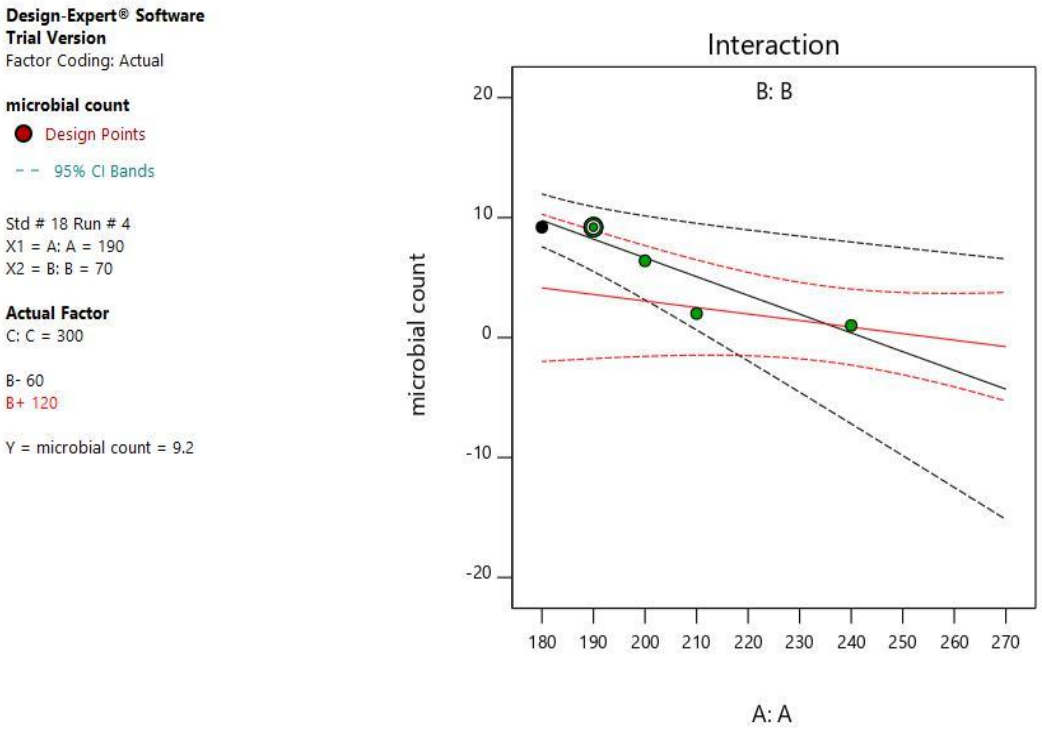


Fig. 5 Effect of inlet and outlet temperature w.r.t SRP

4.4 Optimization and reconstitution of *lassi* powder

After spray drying the powder obtained {plate 3. a), 3. b), 3. c), 3. d)} was reconstituted in various ratios with water by mixing in a blender to obtained *lassi*. On the basis of organoleptic characteristics best was obtained by dissolving 10 grams of *lassi* powder in 100 ml water. Different physico-chemical analyses were done after reconstitution of different spray dried *lassi* powder. pH of plain *lassi* (4.7) was found to be significantly higher than those of sweet and salted spray dried *lassi* powder. There was no significant difference of between sweet (4.64) and salted *lassi* powder (4.67). After reconstitution the titratable acidity of the powder was comparatively higher than the fresh *lassi*. The titratable acidity of plain, sweet and salted powder was (0.52%), (0.57%) and (0.66%). It was observed that titratable acidity of salted *lassi* powder was significantly higher than that of plain and salted. Highest browning was observed in sweet *lassi* that is (0.05), than plain (0.02) and least browning was observed in salted *lassi* powder (0.01). Total solids after reconstitution were (14%), (15.23%) and (15.70%) in plain, sweet and salted respectively.

Table 11: Physico-chemical analysis of reconstituted spray dried *lassi* powder

Variable	Treatments			C.D. at 5%
	Plain *Lassi* Powder (Mean ± SD)	Sweet *Lassi* Powder (Mean ± SD)	Salt *Lassi* Powder (Mean ± SD)	
pH	4.70^{a} ± 0.005	4.64^{b} ± 0.01	4.63^{b} ± 0	0.04
Titratable Acidity	0.52^{b} ± 0.03	0.57^{b} ± 0.02	0.66^{a} ± 0.02	0.05
Browning	0.023^{b} ± 0.005	0.05^{a} ± 0	0.01^{c} ± 0	0.006
Total Solids	14^{b} ± 0.5	15.23^{a} ± 0.25	15.70^{a} ± 0.1	0.65

4.5 Changes in physico-chemical properties of spray dried *lassi* powders during storage at weekly intervals

The spray dried *lassi* powder was store at two different temperature that were Refrigerated temperature (RFT) and Room temperature (RT). Under storage spray dried *lassi* powder was evaluated for physico-chemical parameters. The results of the evaluation on weekly interval are stated as under:

4.5.1 Physico-chemical evaluation of spray dried *lassi* powder of specific cultures

4.5.1.1 Browning

It was observed as in Table 12 after every weekly interval there was change in browning in *lassi* powder variants. When plain *lassi* powder is considered the change in RFT is from zero day to twelve week was (0.023 to 0.088), and in RT was observed (0.023 to 0.054), which was significantly higher from zero to 12^{th} week. Browning at the end of 12^{th} week at RT was significantly higher than that of RFT. Same is the case for sweet and salted *lassi* powder. Browning in sweet *lassi* powder was observed from zero day to twelve week were (0.05 to 0.14) for RFT, and (0.05 to 0.63) for RT which were significantly higher as of plain.

There were significantly less changes in RFT as compare to RT. Similarly in salted *lassi* powder there was increase in browning in RFT (0.01 to 0.054), and (0.01 to 0.44) in RT. The overall comparison of three *lassi* variants there was less change in browning of salted *lassi* powder as compared to others. It was observed that among all *lassi* variants sweet *lassi* powder had maximum browning in both storage conditions that are RFT and RT.

Table 12: Changes in browning (O.D. at 440 nm) of spray dried *lassi* powder during storage

Treatments	Plain *lassi* Powder		Sweet *lassi* Powder		Salted *lassi* Powder	
Storage (weeks)	RFT	RT	RFT	RT	RFT	RT
0	0.023^{i}	0.023^{g}	0.05^{g}	0.05^{d}	0.01^{i}	0.01^{i}
1	0.021^{i}	0.055^{g}	0.055^{fg}	0.056^{d}	0.01^{i}	0.012^{i}
2	0.032^{h}	0.032^{gf}	0.055^{fg}	0.057^{d}	0.012^{h}	0.013^{i}
3	0.034^{h}	0.039^{gf}	0.058^{fg}	0.068^{d}	0.015^{g}	0.045^{h}
4	0.04^{g}	0.05^{gf}	0.061^{ef}	0.070^{d}	0.018^{f}	0.052^{h}
5	0.042^{g}	0.052^{gef}	0.061^{ef}	0.081^{d}	0.019^{f}	0.067^{g}
6	0.058^{f}	0.068^{dgef}	0.071^{e}	0.097^{d}	0.023^{e}	0.074^{g}
7	0.077^{e}	0.084^{def}	0.083^{d}	0.12^{cd}	0.045^{d}	0.085^{f}
8	0.078^{de}	0.098^{de}	0.093^{cd}	0.34^{bc}	0.047^{c}	0.97^{e}
9	0.081^{dc}	0.12^{cd}	0.095^{c}	0.38^{b}	0.05^{b}	0.14^{d}
10	0.082^{bc}	0.15^{c}	0.099	0.45^{ab}	0.051^{b}	0.28^{c}
11	0.085^{ba}	0.35^{b}	0.11^{b}	0.51^{ab}	0.054^{a}	0.39^{b}
12	0.088^{a}	0.54^{a}	0.14^{a}	0.63^{a}	0.054^{a}	0.44^{a}
C.D. at 5%	0.0035	0.055	0.01	0.24	0.001	0.086

Note: Mean bearing different subscript differ significantly ($p<0.05$)

4.5.1.2 pH

It was observed as in Table 13 after every weekly interval there were changes in pH in different *lassi* powder. When plain *lassi* powder is considered the change in refrigerated condition is from zero day to twelve week was (4.71 to 4.11), and in RT was observed (4.71 to 3.13), which was significantly lower from zero to 12^{th} week. pH at the end of 12^{th} week of RT was significantly lower than that of RFT in all variants. In sweet *lassi* powder the observation from zero day to twelve week were (4.63 to 4.12) for refrigerated condition, and (4.63 to 3.76) for RT which were significantly lower as of plain. Significantly less changes in pH observed in refrigerated conditions as compare to room condition. In salted *lassi* powder the same happened there was decrease in pH in RFT from (4.63 to 4.003), and from (4.63 to 2.96) in RT. Similarly when we compare the both conditions RT pH was significantly lower than that of RFT. If the overall comparison among variants there was more change in pH of salted *lassi* powder as compared to others.

3. a) Spray dried plain *lassi* powder

3. b) Spray dried sweet *lassi* powder

3. c) Spray dried salted *lassi* powder

3. d) Spray dried local market *lassi* powder

Plate 3

Table 13: Changes in pH of spray dried *lassi* powder during storage at weekly intervals

Treatments	Plain *lassi* Powder		Sweet *lassi* Powder		Salted *lassi* Powder	
Storage (weeks)	RFT	RT	RFT	RT	RFT	RT
0	4.71^{a}	4.71^{a}	4.63^{a}	4.63^{a}	4.63^{a}	4.63^{a}
1	4.62^{b}	4.50^{ab}	4.62^{a}	4.59^{ab}	4.63^{a}	4.5^{b}
2	4.52^{c}	4.49^{abc}	4.59^{ab}	4.47^{c}	4.57^{ab}	4.40^{c}
3	4.5^{cd}	4.34^{bcd}	4.54^{b}	4.41^{bc}	4.5^{bc}	4.3^{d}
4	4.45^{e}	4.30^{bcd}	4.48^{c}	4.37^{cd}	4.44^{cd}	4.28^{d}
5	4.45^{de}	4.24^{bcde}	4.44^{c}	4.28^{de}	4.41^{d}	4.20^{e}
6	4.40^{e}	4.22^{bcde}	4.34^{d}	4.22^{ef}	4.31^{e}	4.17^{e}
7	4.34^{f}	4.2^{cde}	4.34^{d}	4.18^{efg}	4.28^{ef}	4.11^{f}
8	4.33^{f}	4.14^{de}	4.37^{d}	4.10^{gf}	4.24^{efg}	4.0^{g}
9	4.27^{g}	4.07^{de}	4.27^{e}	4.07^{gh}	4.22^{gh}	3.86^{h}
10	4.23^{gh}	4.0^{ef}	4.24^{e}	3.96^{h}	4.16^{h}	3.53^{i}
11	4.18^{h}	3.72^{f}	4.16^{f}	3.63^{i}	4.13^{i}	3.16^{j}
12	4.11^{i}	3.13^{g}	4.12^{f}	3.76^{j}	4.003	2.96^{k}
C.D. at 5%	0.053	0.302	0.059	0.12	0.085	0.052

Note: Mean bearing different subscript differ significantly ($p<0.05$)

4.5.1.3 Titratable Acidity (TTA)

It was observed as in Table 14 after every weekly interval there was a significant change in TTA in different *lassi* powder. In plain *lassi* powder the changes in RFT from zero day to twelve weeks were (0.52 to 1.62), and in RT (0.52 to 2.03), which was significantly higher from zero to 12^{th} week. TTA at the end of 12^{th} week of RT was significantly higher than that of RFT. In sweet *lassi* powder the observation from zero day to twelve week were (0.57 to1.61) for RFT, and (0.57 to 2.4) for RT which were significantly higher as of plain in RT. There were significantly less changes in RFT as compared to RT. In salted *lassi* powder there was increase in TTA in RFT from (0.65 to1.81), and from (0.65 to 3.00) in RT. At RT TTA was significantly higher than that of RFT. In plain, sweet and salted *lassi* powder there was significantly low change in TTA of sweet and plain *lassi* powder as compared to salted *lassi* powder. And if we compare the condition days and as well as treatments it was observed that salted *lassi* powder had maximum TTA at both conditions that are refrigerated and RT storage.

Table 14: Changes in titratable acidity (%) on spray dried *lassi* powder during storage at weekly intervals

Treatments	Plain *lassi* Powder		Sweet *lassi* Powder		Salted *lassi* Powder	
Storage (weeks)	RFT	RT	RFT	RT	RFT	RT
0	0.52^{k}	0.52^{j}	0.57^{k}	0.57^{h}	0.65^{k}	0.65^{k}
1	0.66^{j}	0.76^{i}	0.67^{j}	0.69^{g}	0.71^{j}	0.79^{j}
2	0.71^{i}	0.80^{i}	0.65^{j}	0.77^{g}	0.78^{i}	0.88^{i}
3	0.80^{h}	0.91^{h}	0.79^{i}	0.93^{f}	0.87^{h}	0.98^{h}
4	0.91^{g}	0.97^{gh}	0.93^{h}	0.98^{ef}	0.98^{g}	1.03^{gh}
5	0.98^{f}	1.025^{fg}	0.98^{g}	1.04^{e}	0.99^{g}	1.05^{fg}
6	1.024^{e}	1.028^{fg}	1.049^{f}	1.05^{e}	1.059^{f}	1.08^{fg}
7	1.036^{e}	1.06^{f}	1.04^{f}	1.077^{e}	1.08^{ef}	1.097^{f}
8	1.05^{e}	1.30^{e}	1.084^{e}	1.20^{d}	1.097^{e}	1.38^{e}
9	1.23^{d}	1.36^{d}	1.3^{d}	1.47^{c}	1.53^{d}	1.38^{d}
10	1.37^{c}	1.50^{c}	1.40^{c}	1.54^{c}	1.48^{c}	1.65^{c}
11	1.51^{b}	1.65^{b}	1.58^{b}	1.77^{b}	1.65^{b}	1.90^{b}
12	1.62^{a}	2.03^{a}	1.61^{a}	2.4^{a}	1.81^{a}	3.00^{a}
C.D. at 5%	0.0536	0.065	0.022	0.11	0.029	0.066

Note: Mean bearing different subscript differ significantly ($p<0.05$)

4.5.1.4 Moisture content

After drying the moisture content of plain, sweet and salted *lassi* powder are (5.03%), (4.07%) and (2.08%) respectively (Table 15). There were no significant changes in moisture among *lassi* variants during storage at RFT and were same at end as on the zero day of evaluation. At RT there was slightly increase in plain sweet and salted *lassi* powder which were (5.03% to 5.16%), (4.07% to 4.16%) and (2.10% to 2.36%) respectively.

Table 15: Changes in moisture content (%) of spray dried *lassi* powder during storage at weekly intervals

Treatments	Plain *lassi* Powder		Sweet *lassi* Powder		Salted *lassi* Powder	
Storage (weeks)	RFT	RT	RFT	RT	**RFT**	**RT**
0	5.03^{a}	5.03^{b}	4.07^{a}	4.07^{b}	2.08^{a}	2.08^{b}
1	5.03^{a}	5.03^{b}	4.07^{a}	4.07^{b}	2.08^{a}	2.08^{b}
2	5.03^{a}	5.03^{b}	4.07^{a}	4.07^{b}	2.08^{a}	2.08^{b}
3	5.03^{a}	5.03^{b}	4.07^{a}	4.07^{b}	2.08^{a}	2.08^{b}
4	5.03^{a}	5.03^{b}	4.07^{a}	4.07^{b}	2.08^{a}	2.08^{b}
5	5.03^{a}	5.16^{a}	4.07^{a}	4.16^{a}	2.08^{a}	2.36^{a}
6	5.03^{a}	5.16^{a}	4.07^{a}	4.16^{a}	2.08^{a}	2.36^{a}
7	5.03^{a}	5.16^{a}	4.07^{a}	4.16^{a}	2.08^{a}	2.36^{a}
8	5.03^{a}	5.16^{a}	4.07^{a}	4.16^{a}	2.08^{a}	2.35^{a}
9	5.03^{a}	5.16^{a}	4.07^{a}	4.16^{a}	2.08^{a}	2.36^{a}
10	5.03^{a}	5.16^{a}	4.07^{a}	4.16^{a}	2.08^{a}	2.34^{a}
11	5.03^{a}	5.16^{a}	4.07^{a}	4.16^{a}	2.08^{a}	2.33^{a}
12	5.03^{a}	5.16^{a}	4.07^{a}	4.16^{a}	2.08^{a}	2.36^{a}
C.D. at 5%	0.096	0.096	0.193	0.19	0.019	0.35

Note: Mean bearing different subscript differ significantly ($p<0.05$)

4.5.1.5 Fat

Initially fat content in all variants was 2.4% under RFT However, there was significantly change in fat content from zero day to twelve week of storage (Table 16) which were as follows (2.4% to 3.37), (2.4% to 3.39%) and (2.4% to 3.44%) of plain, sweet and salted *lassi* powder respectively. If we compare among variants the fat content of salted *lassi* powder was comparatively higher than that of sweet and plain *lassi* powder.

Table 16: Changes in fat (%) of spray dried *lassi* powder during storage at weekly interval

Treatments	Plain *lassi* Powder		Sweet *lassi* Powder		Salted *lassi* Powder	
Storage (weeks)	RFT	RT	RFT	RT	RFT	RT
0	2.4^{a}	2.4^{f}	2.4^{a}	2.4^{g}	2.4^{a}	2.4^{f}
1	2.4^{a}	2.4^{f}	2.4^{a}	2.4^{g}	2.4^{a}	2.4^{f}
2	2.4^{a}	2.60^{ef}	2.4^{a}	2.66^{gf}	2.4^{a}	2.7^{ef}
3	2.4^{a}	2.7^{de}	2.4^{a}	2.80^{ef}	2.4^{a}	2.85^{de}
4	2.4^{a}	2.8^{cde}	2.4^{a}	2.86^{def}	2.4^{a}	2.94^{cde}
5	2.4^{a}	2.9^{cd}	2.4^{a}	2.95^{cdef}	2.4^{a}	2.95^{cde}
6	2.4^{a}	2.99^{bc}	2.4^{a}	2.99^{bcde}	2.4^{a}	3.09^{bcd}
7	2.4^{a}	3.093^{abc}	2.4^{a}	3.12^{abcd}	2.4^{a}	3.16^{abcd}
8	2.4^{a}	3.12^{abc}	2.4^{a}	3.16^{abcd}	2.4^{a}	3.16^{abcd}
9	2.4^{a}	3.19^{ab}	2.4^{a}	3.19^{abc}	2.4^{a}	3.19^{abcd}
10	2.4^{a}	3.29^{a}	2.4^{a}	3.29^{ab}	2.4^{a}	3.29^{abc}
11	2.4^{a}	3.33^{a}	2.4^{a}	3.34^{a}	2.4^{a}	3.34^{ab}
12	2.4^{a}	3.37^{a}	2.4^{a}	3.39^{a}	2.4^{a}	3.44^{a}
C.D. at 5%	0	0.284	0	0.30	0	0.31

Note: Mean bearing different subscript differ significantly ($p<0.05$)

4.5.1.6 Wettability

Initially the wettability of plain *lassi* powder was 60.3s, sweet *lassi* powder was 50s and salted *lassi* powder was 50s respectively. There was no significant change observed in wettability during storage of plain sweet and salted *lassi* powder under RFT and RT as per (Table 17).

Table17: Changes in wettability (seconds) of spray dried *lassi* powder during storage at weekly intervals

Treatments	Plain *lassi* Powder		Sweet *lassi* Powder		Salted *lassi* Powder	
Storage (weeks)	**RFT**	**RT**	**RFT**	**RT**	**RFT**	**RT**
0	60.3^{a}	60.3^{a}	50^{b}	50^{b}	50^{b}	50^{b}
1	60^{a}	60^{a}	50^{b}	50^{b}	50.33^{a}	50.33^{a}
2	50^{b}	50^{b}	50.33^{a}	50.33^{a}	50.33^{a}	50.33^{a}
3	60^{a}	60^{a}	50^{b}	50^{b}	50.33^{a}	50.33^{a}
4	60.33^{a}	60.33^{a}	50^{b}	50^{b}	50^{b}	50^{b}
5	60^{a}	60^{a}	50^{b}	50^{b}	50.33^{b}	50.33^{b}
6	60^{a}	60^{a}	50^{b}	50^{b}	49.66^{b}	49.66^{b}
7	60^{a}	60^{a}	50^{b}	50^{b}	50.33^{b}	50.33^{b}
8	60.3^{a}	60.3^{a}	50^{b}	50^{b}	50^{b}	50^{b}
9	60^{a}	60^{a}	50^{b}	50^{b}	50.33^{b}	50.33^{b}
10	60^{a}	60^{a}	50^{b}	50^{b}	49.66^{b}	49.66^{b}
11	60^{a}	60^{a}	50^{b}	50^{b}	50.33^{b}	50.33^{b}
12	60^{a}	60^{a}	50^{b}	50^{b}	50.33^{b}	50.33^{b}
C.D. at 5%	0.06	0.06	0.05	0.05	0.07	0.07

Note: Mean bearing different subscript differ significantly ($p<0.05$)

4.5.1. 7 Dispersibilty

80% dispersibility for plain *lassi* powder, 85% for sweet *lassi* powder and 88% for salted *lassi* powder in both the temperature conditions. No significant change in dispersibility was observed during storage of powders at RFT and RT. conditions (Table 18).

Table 18: Changes in dispersibity (%) of spray dried *lassi* powder during storage at weekly intervals

Treatments	Plain *lassi* Powder		Sweet *lassi* Powder		Salted *lassi* Powder	
Storage (weeks)	RFT	RT	RFT	RT	RFT	RT
0	80.00 [a]	80.00 [a]	85.00 [a]	85.00 [a]	88.00 [a]	88.00 [a]
1	80.00 [a]	80.00 [a]	85.00 [a]	85.00 [a]	88.00 [a]	88.00 [a]
2	80.00 [a]	80.00 [a]	85.00 [a]	85.00 [a]	88.00 [a]	88.00 [a]
3	80.00 [a]	80.00 [a]	85.00 [a]	85.00 [a]	88.00 [a]	88.00 [a]
4	80.00 [a]	80.00 [a]	85.00 [a]	85.00 [a]	88.00 [a]	88.00 [a]
5	80.00 [a]	80.00 [a]	85.00 [a]	85.00 [a]	88.00 [a]	88.00 [a]
6	80.00 [a]	80.00 [a]	85.00 [a]	85.00 [a]	88.00 [a]	88.00 [a]
7	80.00 [a]	80.00 [a]	85.00 [a]	85.00 [a]	88.00 [a]	88.00 [a]
8	80.00 [a]	80.00 [a]	85.00 [a]	85.00 [a]	88.00 [a]	88.00 [a]
9	80.00 [a]	80.00 [a]	85.00 [a]	85.00 [a]	88.00 [a]	88.00 [a]
10	80.00 [a]	80.00 [a]	85.00 [a]	85.00 [a]	88.00 [a]	88.00 [a]
11	80.00 [a]	80.00 [a]	85.00 [a]	85.00 [a]	88.00 [a]	88.00 [a]
12	80.00 [a]	80.00 [a]	85.00 [a]	85.00 [a]	88.00 [a]	88.00 [a]
C.D. at 5%	0	0	0	0	0	0

Note: Mean bearing different subscript differ significantly ($p<0.05$)

4.5.1.8 Solubility index

Solubility index was observed 0.50 ml for plain *lassi* powder, 0.60 ml for sweet *lassi* powder and 0.60 ml for salted *lassi* powder was observed in both the temperature conditions. No significant change in solubility index was observed during storage of powders at different conditions (Table 19).

4.5.1.9 Total solids

Initially TS of plain *lassi* powder was 94.96%, sweet *lassi* powder was 95.93% and salted *lassi* powder was 95.93% respectively. There were no significant change in total solids of plain, sweet and salted *lassi* powder during RFT storage (Table 20), but a significant difference after fourth week of storage which was (94.96% to 94.86%), (95.93% to 95.93%) and (97.91% to 97.63%) was observed in plain, sweet and salted *lassi* powder of RT storage.

4.5.1.10 Solid not fat

Solid not fat of plain, sweet and salted *lassi* powder was observed to be same during storage at RFT that was (92.56%) for plain, (93.53%) for sweet and (95.51%) for salted *lassi* powder (Table 21). Significant decrease in solid not fat in plain, sweet and salted *lassi* powder that is (92.56% to 91.49%), (93.53% to 92.40%) and (95.51% to 94.19%) respectively at RT. At RT

more than one subscript depict the interaction of the values from each other, which means the value are closely related to another value, but with a little significant difference. If, compare room and RFTs then it was observed that Solid Not Fat decreased gradually in RT and maximum decrease was observed in plain *lassi* powder from zero day to twelve week of storage.

Table 19: Changes in Solubility index (ml) of spray dried *lassi* powder during storage at weekly intervals

Treatments	Plain *lassi* Powder		Sweet *lassi* Powder		Salted *lassi* Powder	
Storage (weeks)	RFT	RT	RFT	RT	RFT	RT
0	0.50^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}
1	0.50^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}
2	0.50^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}
3	0.50^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}
4	0.50^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}
5	0.50^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}
6	0.50^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}
7	0.50^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}
8	0.50^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}
9	0.50^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}
10	0.50^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}
11	0.50^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}
12	0.50^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}	0.60^{a}
C.D. at 5%	0	0	0	0	0	0

Note: Mean bearing different subscript differ significantly ($p<0.05$)

Table 20: Changes in total solids (%) of spray dried *lassi* powder during storage at weekly intervals

Treatments	Plain *lassi* Powder		Sweet *lassi* Powder		Salted *lassi* Powder	
Storage (weeks)	RFT	RT	RFT	RT	RFT	RT
0	94.96^{a}	94.96^{a}	95.93^{a}	95.93^{a}	97.91^{a}	97.91^{a}
1	94.96^{a}	94.96^{a}	95.93^{a}	95.93^{a}	97.91^{a}	97.91^{a}
2	94.96^{a}	94.96^{a}	95.93^{a}	95.93^{a}	97.91^{a}	97.91^{a}
3	94.96^{a}	94.96^{a}	95.93^{a}	95.93^{a}	97.91^{a}	97.91^{a}
4	94.96^{a}	94.96^{a}	95.93^{a}	95.93^{a}	97.91^{a}	97.91^{a}
5	94.96^{a}	94.80^{b}	95.93^{a}	95.76^{b}	97.91^{a}	97.63^{b}
6	94.96^{a}	94.86^{b}	95.93^{a}	95.76^{b}	97.91^{a}	97.63^{b}
7	94.96^{a}	94.86^{b}	95.93^{a}	95.76^{b}	97.91^{a}	97.63^{b}
8	94.96^{a}	94.86^{b}	95.93^{a}	95.76^{b}	97.91^{a}	97.63^{b}
9	94.96^{a}	94.86^{b}	95.93^{a}	95.76^{b}	97.91^{a}	97.63^{b}
10	94.96^{a}	94.86^{b}	95.93^{a}	95.76^{b}	97.91^{a}	97.63^{b}
11	94.96^{a}	94.86^{b}	95.93^{a}	95.76^{b}	97.91^{a}	97.63^{b}
12	94.96^{a}	94.86^{b}	95.93^{a}	95.76^{b}	97.91^{a}	97.63^{b}
C.D. at 5%	0.096	0.096	0.193	0.193	0.019	0.423

Note: Mean bearing different subscript differ significantly ($p<0.05$)

Table 21: Changes in solid not fat (%) of spray dried *lassi* powder during storage at weekly intervals

Treatments	Plain *lassi* Powder		Sweet *lassi* Powder		Salted *lassi* Powder	
Storage (weeks)	RFT	RT	RFT	RT	RFT	RT
0	92.56^{a}	92.56^{a}	93.53^{a}	93.53^{a}	95.51^{a}	95.51^{a}
1	92.56^{a}	92.56^{a}	93.53^{a}	93.53^{a}	95.51^{a}	95.51^{a}
2	92.56^{a}	92.36^{ab}	93.53^{a}	93.26^{ab}	95.51^{a}	95.21^{ab}
3	92.56^{a}	92.26^{abc}	93.53^{a}	93.13^{abc}	95.51^{a}	95.06^{b}
4	92.56^{a}	92.16^{bcd}	93.53^{a}	93.06^{bcd}	95.51^{a}	94.97^{bc}
5	92.56^{a}	91.96^{cde}	93.53^{a}	92.81^{cde}	95.51^{a}	94.67^{cd}
6	92.56^{a}	91.87^{def}	93.53^{a}	92.77^{cde}	95.51^{a}	94.54^{de}
7	92.56^{a}	91.77^{efg}	93.53^{a}	92.64^{de}	95.51^{a}	94.47^{de}
8	92.56^{a}	91.74^{efg}	93.53^{a}	92.60^{e}	95.51^{a}	94.47^{de}
9	92.56^{a}	91.67^{efg}	93.53^{a}	92.57^{e}	95.51^{a}	94.44^{de}
10	92.56^{a}	91.57^{gf}	93.53^{a}	92.47^{e}	95.51^{a}	94.34^{de}
11	92.56^{a}	91.53^{f}	93.53^{a}	92.43^{e}	95.51^{a}	94.29^{e}
12	92.56^{a}	91.49^{f}	93.53^{a}	92.40^{e}	95.51^{a}	94.19^{e}
C.D. at 5%	0.096	0.33	0.193	0.452	0.019	0.37

Note: Mean bearing different subscript differ significantly (p<0.05)

4.5.2 Physico-chemical analysis of spray dried local market *lassi* powder

Local market *lassi* was spray dried at optimized conditions of spray drier, but quality evaluation of reconstituted local market *lassi* powder was not accepted on the basis of organoleptic. Physico-chemical analysis was done till 4^{th} week and after it got spoiled. The gradual increase was observed in browning of the powder from zero day to four weeks (0.316 to 0.47), pH also decreased from zero day to fourth week (4.40 to 4.31), there was an increase in titratable acidity from zero day to fourth week (0.816 to 0.9), no changes in moisture content, total solids, solubility index and solid not fat was observed during storage (Table 22). As well as SRP was not detected after spray drying of local market *lassi*, hence was rejected.

Table 22: Physico-chemical analysis of spray dried local market *lassi* powder during storage at weekly intervals

Variable	Weekly Data					C.D. at 5%
	0	1	2	3	4	
Browning	0.316^{a}	0.35^{b}	0.43^{c}	0.46^{cd}	0.47^{d}	0.0373
pH	4.40^{a}	4.39^{a}	4.31^{b}	4.31^{b}	4.30^{b}	0.0156
Titratable Acidity	0.816^{a}	0.87^{b}	0.88^{b}	0.89^{bc}	0.9^{c}	0.0293
Moisture Content	3.10^{a}	3.10^{a}	3.10^{a}	3.10^{a}	3.10^{a}	0.3179
Wettability	51.66^{a}	52.33^{a}	52.33a	52.0^{a}	52.66^{a}	3.9858
Dispersibility	81.0^{a}	81.0^{a}	81.0^{a}	81.0^{a}	81.0^{a}	1.8193
Fat	2.60^{a}	2.60^{a}	2.60^{a}	2.60^{a}	2.60^{a}	0.021
Solubility Index	0.7^{a}	0.7^{a}	0.7^{a}	0.7^{a}	0.7^{a}	0.1819
Solid Not Fat	94.39^{a}	94.39^{a}	94.39^{a}	94.39^{a}	94.39^{a}	0.021
Total Solids	96.89^{a}	96.89^{a}	96.89^{a}	96.89^{a}	96.89^{a}	0.31

Note: Mean bearing different subscript differ significantly (p<0.05)

4.6 Organoleptic evaluation of spray dried *lassi* powder and local market *lassi* powder during storage

The spray dried *lassi* powder variants under storage was evaluated organoleptically for color and appearance, taste, aroma and flavor, body and texture, after taste and overall acceptability characteristics on a 9-point hedonic scale by a panel of semi-trained judges. The results of the evaluation on monthly interval are stated as under:

4.6.1 Organoleptic evaluation of spray dried *lassi* powder

4.6.1.1 Color and appearance

The color and appearance score on zero day was highest, but on storage it decreased in all variants (Table 23). As compared with RFT and RT there was significant loss in color and appearance of plain, sweet and salted *lassi* powder in RT which was (8.15 to 4.20), (8.25 to 4.20) and (8.95 to 4.30) respectively. If treatments are considered then maximum color and appearance values was observed in salted *lassi* powder on zero day (8.95). After three months in different storage conditions the values of salted powder were still higher than that of plain and sweet *lassi* powder that is (7.8) for RFT and (4.30) for RT

Table 23: Effect of color and appearance on of spray dried *lassi* powder during storage at monthly intervals

Treatments	Plain *lassi* Powder		Sweet *lassi* Powder		Salted *lassi* Powder	
Storage (months)	RFT	RT	RFT	RT	RFT	RT
0	8.15^{a}	8.15^{a}	8.25^{a}	8.25^{a}	8.95^{a}	8.95^{a}
1	7.6^{b}	7.15^{b}	7.40^{b}	6.80^{b}	7.80^{b}	7.20^{b}
2	7.4^{b}	5.5^{c}	6.45^{b}	5.95^{c}	7.45^{b}	6.2^{c}
3	7.65^{b}	4.20^{d}	7.65^{c}	4.20^{d}	7.8^{b}	4.30^{d}
C.D. at 5%	0.462	0.41	0.52	0.26	0.35	0.40

Note: Mean bearing different subscript differ significantly ($p<0.05$)

4.6.1.2 Taste

The taste of plain, sweet and salted *lassi* powder were significantly different from each other (8.5), (7.8) and (8.45) respectively at zero day (Table 24). A significant decrease was observed during storage period as well as in different storage conditions which was found (8.5 to 7.6) in RFT, (8.5 to 4.45) in RT of plain *lassi* powder, (7.85 to 7.25) in RFT, (7.85 to 4.10) in RT of sweet *lassi* powder and (8.45 to 7.60) in RFT, (8.45 to 4.55) in RT of salted *lassi* powder respectively. Significant decrease in taste was observed in variants stored at RT as compare to RFT.

4.6.1.3 Body and Texture

Body and texture of salted *lassi* powder (8.55) was higher as compared to plain (7.95) and sweet (7.9) *lassi* powder respectively at zero day (Table 25). A significant decrease was observed during storage period as well as in different storage conditions which was found (7.95 to 7.35) in RFT, (7.95 to 5.75) in RT of plain *lassi* powder, (7.90 to 7.4) in RFT, (7.90

to 6.10) in RT of sweet *lassi* powder and (8.55 to 7.30) in RFT, (8.45 to 5.80) in RT of salted *lassi* powder respectively. Significant decrease in Body and texture was observed in variants under RT as compare to RFT.

Table 24: Changes in taste of spray dried *lassi* powder during storage at monthly intervals

Treatments	Plain *lassi* Powder		Sweet *lassi* Powder		Salted *lassi* Powder	
Storage (months)	**RFT**	**RT**	**RFT**	**RT**	**RFT**	**RT**
0	8.5^{a}	8.5^{a}	7.85^{a}	7.85^{a}	8.45^{a}	8.45^{a}
1	7.60^{b}	6.75^{b}	7.7^{a}	6.75^{b}	7.90^{ab}	6.95^{b}
2	7.70^{b}	5.50^{c}	7.25^{a}	5.50^{c}	7.50^{b}	5.7^{c}
3	7.6^{b}	4.45^{d}	7.25^{a}	4.10^{d}	7.60^{b}	4.55^{d}
C.D. at 5%	0.499	0.50	0.60	0.54	0.61	0.42

Note: Mean bearing different subscript differ significantly (p<0.05)

Table 25: Changes in body and texture of spray dried *lassi* powder during storage at monthly intervals

Treatments	Plain *lassi* Powder		Sweet *lassi* Powder		Salted *lassi* Powder	
Storage (months)	**RFT**	**RT**	**RFT**	**RT**	**RFT**	**RT**
0	7.95^{a}	7.95^{a}	7.9^{a}	7.9^{a}	8.55^{a}	8.55^{a}
1	7.90^{ab}	7.55^{a}	7.55^{ab}	7.5^{b}	7.90^{b}	7.70^{a}
2	7.40^{bc}	6.9^{b}	7.50^{ab}	6.55^{c}	7.50^{bc}	6.90^{b}
3	7.35^{c}	5.75^{c}	7.4^{c}	6.10^{d}	7.3^{c}	5.80^{c}
C.D. at 5%	0.50	0.41	0.44	0.34	0.44	0.42

Note: Mean bearing different subscript differ significantly (p<0.05)

4.6.1.4 Aroma and Flavour

The aroma and flavour of salted *lassi* powder (8.35) was maximum as compared to plain and sweet *lassi* powder (Table 26). Significant decrease in aroma and flavor at RT was observed than of RFT. It was found maximum in plain, sweet and salted *lassi* variants in zero day (8.35, 8.15 and 8.35) and after 3 months it decreased to (3.55, 3.1 and 3.55). On comparing the treatments maximum aroma and flavor was found in salted (7.5) followed by plain *lassi* powder (7.4) as compared to sweet *lassi* powder (7.35) at RFT.

4.6.1.5 After Taste

After taste significantly decreased from zero to third month in storage of both RFT and RT storage in all variants (Table 27). After taste of salted *lassi* powder was maximum as compared to plain and sweet *lassi* powder in RFT and RT. Significantly higher decrease was observed in RT storage than RFT. On zero day after taste was observed (8.25, 8.15 and 8.55), but at the end of third month it was found (3.9, 3.9 and 3.80) for plain, sweet and salted *lassi* powder stored in RT storage.

Table 26: Changes in aroma and flavour of spray dried *lassi* powder during storage at monthly intervals

Treatments	Plain *lassi* Powder		Sweet *lassi* Powder		Salted *lassi* Powder	
Storage (months)	RFT	RT	RFT	RT	RFT	RT
0	8.35^{a}	8.35^{a}	8.15^{a}	8.15^{a}	8.35^{a}	8.35^{a}
1	7.60^{b}	7.1^{b}	7.40^{b}	7.10^{b}	8.10^{ab}	7.1^{b}
2	7.40^{b}	5.45^{c}	7.4^{b}	6.15^{c}	7.75^{bc}	5.95^{a}
3	7.4^{b}	3.55^{d}	7.35^{b}	3.1^{d}	7.5^{c}	3.55^{a}
C.D. at 5%	0.53	0.42	0.49	0.38	0.44	0.29

Note: Mean bearing different subscript differ significantly (p<0.05)

Table 27: Changes in after taste of spray dried *lassi* powder during storage at monthly intervals

Treatments	Plain *lassi* Powder		Sweet *lassi* Powder		Salted *lassi* Powder	
Storage (months)	RFT	RT	RFT	RT	RFT	RT
0	8.25^{a}	8.25^{a}	8.15^{a}	8.15^{a}	8.55^{a}	8.55^{a}
1	7.80^{b}	6.9^{b}	7.55^{ab}	6.7^{b}	8.10^{a}	6.8^{b}
2	7.60^{b}	6.3^{c}	7.0^{b}	5.60^{c}	7.65^{b}	5.80^{c}
3	7.4^{b}	3.9^{d}	7.15^{b}	3.90^{d}	7.5^{b}	3.80^{d}
C.D. at 5%	0.50	0.29	0.64	0.35	0.44	0.43

Note: Mean bearing different subscript differ significantly (p<0.05)

4.6.1.6 Overall Acceptability

The overall acceptability of salted *lassi* powder was found more as compared to plain and sweet *lassi* powder (Table 28). There was significant decrease in overall acceptability at RT as compared to RFT. The maximum value of plain sweet and salted *lassi* powder during zero day of refrigerated and RT were (8.24, 8.06 and 8.57) respectively which, decreased to (7.46, 7.13 and 7.54) in RFT, (4.37, 4.28 and 4.40) in RT respectively.

Table 28: Changes in overall acceptability of spray dried *lassi* powder during storage at monthly intervals

Treatments	Plain *lassi* Powder		Sweet *lassi* Powder		Salted *lassi* Powder	
Storage (months)	RFT	RT	RFT	RT	RFT	RT
0	8.24^{a}	8.24^{a}	8.06^{a}	8.06^{a}	8.57^{a}	8.57^{a}
1	7.70^{b}	7.09^{b}	7.51^{b}	6.97^{b}	7.96^{a}	7.15^{b}
2	7.50^{b}	6.10^{c}	7.36^{bc}	5.95^{c}	7.57^{a}	6.12^{c}
3	7.46^{b}	4.37^{d}	7.13^{c}	4.28^{d}	7.54^{a}	4.40^{d}
C.D. at 5%	0.28	0.25	0.29	0.15	0.39	0.15

Note: Mean bearing different subscript differ significantly (p<0.05)

4.6.2 Organoleptic evaluation of spray dried Local Market *lassi* powder during storage

The sensory evaluation was done for local market *lassi* powder after drying and was not found acceptable. Overall acceptability score was (3.81) which means disliked very much hence was rejected on sensory basis. As well as SRP was not detected after spray drying of local market *lassi* (Table 29)

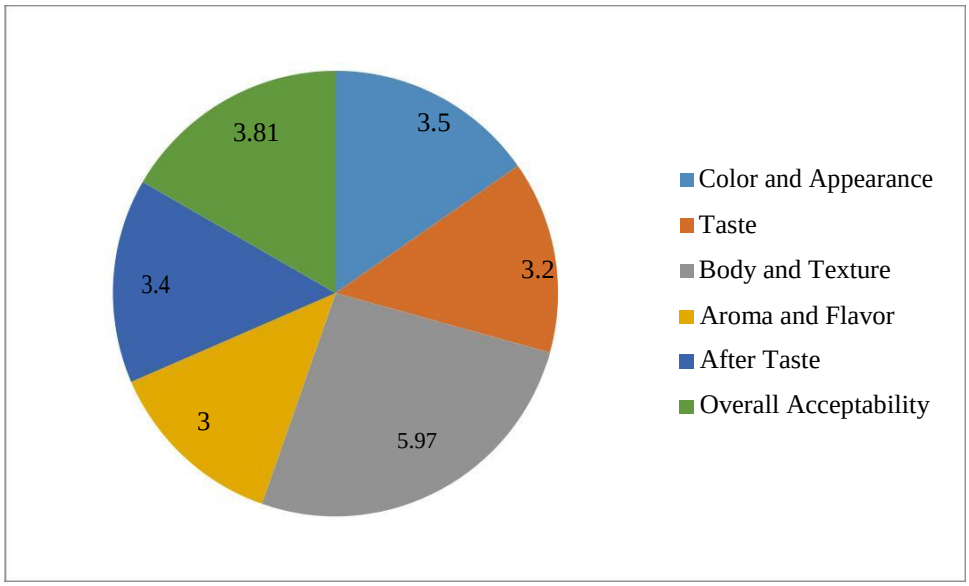


Fig. 6 Organoleptic evaluation of spray dried local market *lassi* powder at zero day

4.7 Microbial count

4.7.1 Yeast and Mold Count

No Yeast and Mold count was observed in spray dried *lassi* powder variants when were plated on YEPD Agar and incubated at 25±2°C for 72 hours and checked at monthly interval till 3 months

4.7.2 Coliform Count

No coliform count Coliform count was observed in spray dried *lassi* powder variants when plated on EMB Agar and incubated at 37±2°C for 24 hours and checked with at monthly interval till 3 months

4.7.3 Probiotic count of *lassi* powder

Total plate count was done after spray drying of *lassi* powder and was found to be 9.2 on zero day and after third month of interval it decreased to 8.5 under RFT and 7.0 under RT storage.

4.7.4 Antimicrobial activity

Antimicrobial activity was recorded separately for RFT and RT storage. Under RFT maximum zone of inhibition was observed for fresh curd sample (17.33) and plain *lassi* powder (17.33) against *S. aureus*. No zone of inhibition observed in salted *lassi* powder against *E.coli* at RFT storage (Fig. 7). Plain and sweet variants show inhibition zone against all three pathogens *B. subtilis, E. coli and S. aureus*

Plate 4. Plain *lassi* powder at RFT and RT after 3 months

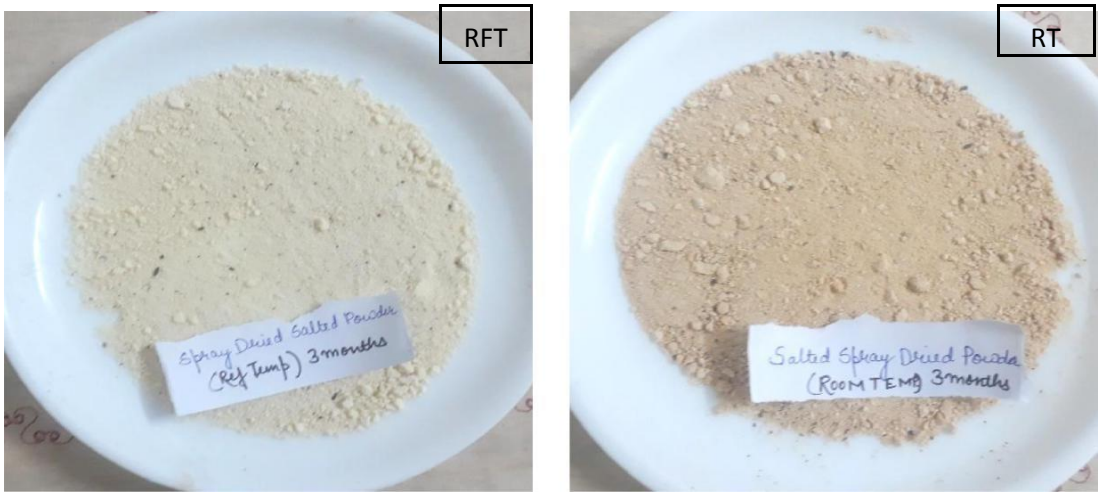


Plate 5. Salted *lassi* powder at RFT and RT after 3 months

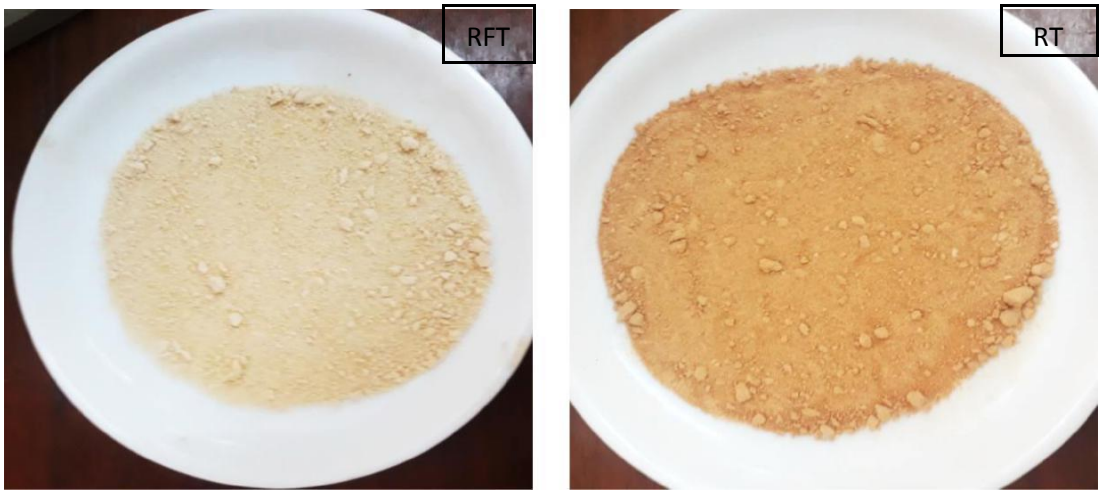


Plate 6. Salted *lassi* powder at RFT and RT after 3 months

At RT storage maximum zone of inhibition (13.33) was observed by plain lassi powder against *B. subtilis* and no inhibition zone was observed in sweet *lassi* powder against *B. subtilis, E. coli and S. aureus* (Fig. 8). In both storage conditions plain *lassi* powder was observed to have maximum zone of inhibition against food pathogens, however, less but non-significant inhibition is showed by RFT when compared with fresh curd sample (Fig. 7), while less but significant inhibition recorded by plain at RT when compared with fresh curd (Fig. 8).

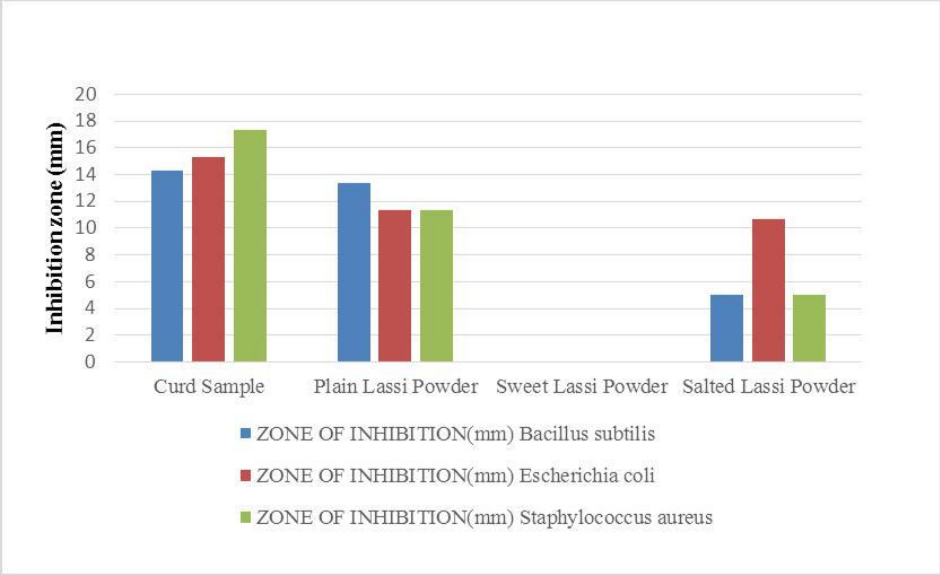


Fig. 7 Antimicrobial activity of *lassi* powders after storage at RFT

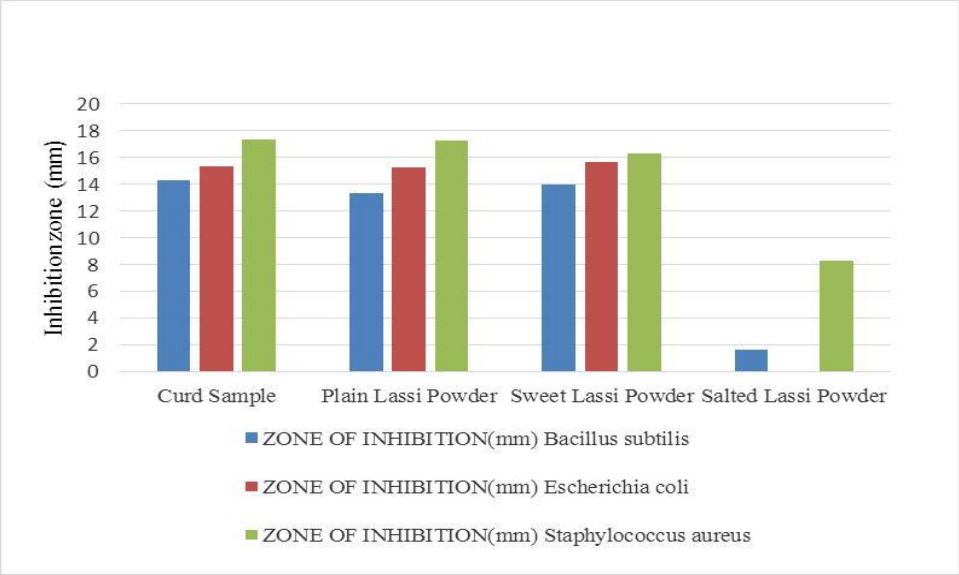


Fig. 8 Antimicrobial activity of *lassi* powders after storage at RT

4.7.5 Antibiotic sensitivity

Antimicrobial sensitivity was observed against 32 antibiotics with the help of ICOSA UNIVERSAL II and ICOSA G-I-PLUS disc. It was observed that plain, sweet and salted *lassi* powder of RFT and RT storage was susceptible against ampicillin, amoxicillin, cefadroxil, cefoperazone, ceftriaxone, chloramphenicol, ciprofloxacin, cloxacillin, co-trimoxazole,

erythromycin, netillin, nitrofurantoin, norfloxacin, penicillin, cephalothin, ofloxacin, azithromycin, methicillin and novobiocin antibiotic, moderately susceptible against ceftazidme, gentamicin and tobramycin tetracycline amoxyclav and oxacillin antibiotics and showed resistance against amikacin, nalidixic acid, vancomycin, clindamycin, linezolid, clarithromycin and teicoplanin antibiotics (Table 29)

Table 29 Antibiotic sensitivity of lassi powders and fresh curd against 32 antibiotics

Antibiotics	**Fresh curd**	**Plain *lassi* powder**		**Sweet *lassi* powder**		**Salted *lassi* powder**	
		RFT	**RT**	**RFT**	**RT**	**RFT**	**RT**
Amikacin	R	R	R	R	R	R	R
Ampicillin	S	S	S	S	S	S	S
Amoxicillin	S	S	S	S	S	S	S
Cefadroxil	S	S	S	S	S	S	S
Cefoperazone	S	S	S	S	S	S	S
Ceftazidme	M	M	M	M	M	M	M
Ceftriaxone	S	S	S	S	S	S	S
Chloramphenicol	S	s	s	s	S	S	S
Ciprofloxacin	S	S	S	S	S	S	S
Cloxacillin	S	S	S	S	S	S	S
Co- trimoxazole	S	S	S	S	S	S	S
Erythromycin	S	S	S	S	S	S	S
Gentamicin	M	M	M	M	M	M	M
Nalidixic acid	R	R	R	R	R	R	R
Netillin	S	S	S	S	S	S	S
Nitrofurantoin	S	S	S	S	S	S	S
Norfloxacin	S	S	S	S	S	S	S
Penicillin	S	S	S	S	S	S	S
Tobramycin	M	M	M	M	M	M	M
Vancomycin	R	R	R	R	R	R	R
Cephalothin	S	S	S	S	S	S	S
Clindamycin	R	R	R	R	R	R	R
Ofloxacin	S	S	S	S	S	S	S
Oxacillin	M	M	M	M	M	M	M
Linezolid	R	R	R	R	R	R	R
Azithromycin	S	S	S	S	S	S	S
Clarithromycin	R	R	R	R	R	R	R
Teicoplanin	R	R	R	R	R	R	R
Methicillin	S	S	S	S	S	S	S
Novobiocin	S	S	S	S	S	S	S
Tetracycline	M	M	M	M	M	M	M
Amoxyclav	M	M	M	M	M	M	M

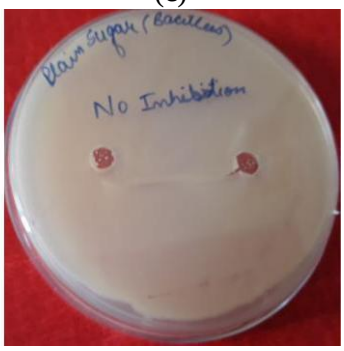
*S= Susceptible, M= Moderately susceptible and R= Resistant

(a)

(b)

(c)

(d)

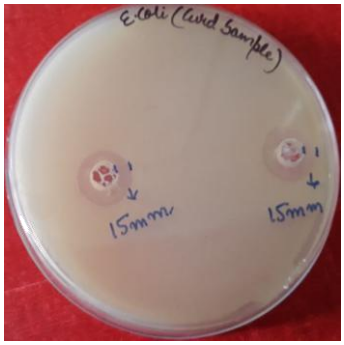


(e)

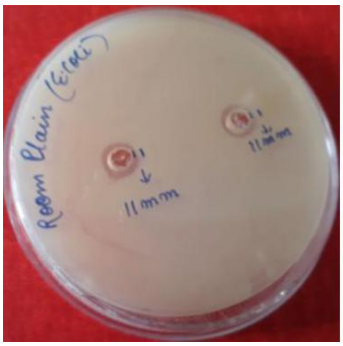
(f)

(g)

Plate 7. Antimicrobial activity of *lassi* powders stored at RFT and RT against *Bacillus*: (a) Fresh curd (b) Plain *lassi* powder (RFT), (c) Plain *lassi* powder (RT), (d) Sweet *lassi* powder (RFT), (e) Sweet *lassi* powder (RT), (f) Salted *lassi* powder (RFT) and (g) Sweet *lassi* powder (RT)



(a)



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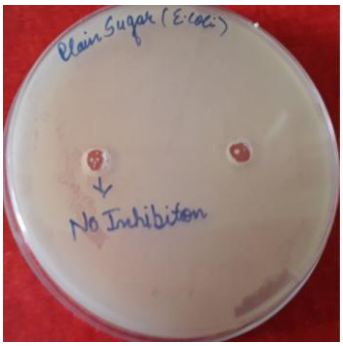

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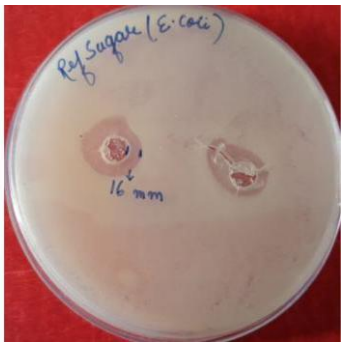

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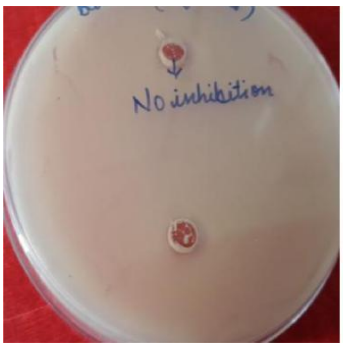
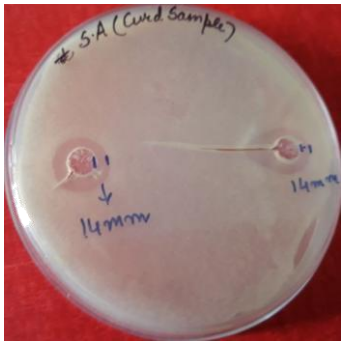


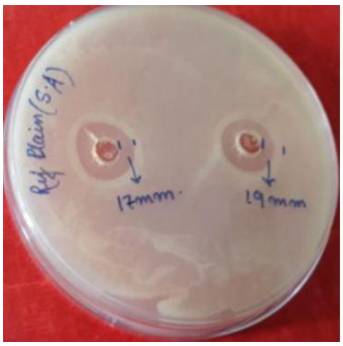
(f)

(g)

Plate 8. **Antimicrobial activity of *lassi* powders stored at RFT and RT against *E. coli*: (a) Fresh curd (b) Plain *lassi* powder (RFT), (c) Plain *lassi* powder (RT), (d) Sweet *lassi* powder (RFT), (e) Sweet *lassi* powder (RT), (f) Salted *lassi* powder (RFT) and (g) Sweet *lassi* powder (RT)**



(a)



(b)

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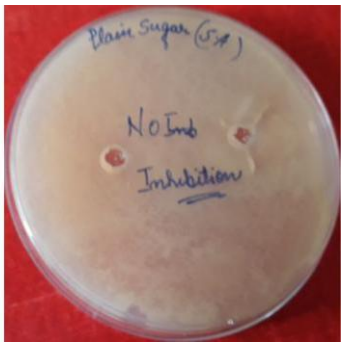

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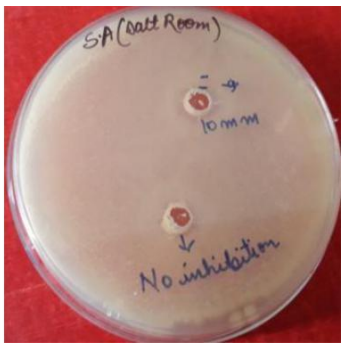

(g)

Plate 9. **Antimicrobial activity of *lassi* powders stored at RFT and RT against *S. aureus*: (a) Fresh curd (b) Plain *lassi* powder (RFT), (c) Plain *lassi* powder (RT), (d) Sweet *lassi* powder (RFT), (e) Sweet *lassi* powder (RT), (f) Salted *lassi* powder (RFT) and (g) Sweet *lassi* powder (RT)**

Plate 10: Antibiotic sensitivity of salted *lassi* powder of RFT against Thirty two antibiotics

CHAPTER-V

DISCUSSION

The present investigation was carried out on the topic entitled with "Development of Instant *lassi*". The results on physicochemical composition of plain, sweet and salted variants prepared with specific probiotic cultures local market *lassi*, optimization of spray drying conditions, physic-chemical property of *lassi* before drying after drying, changes in chemical constituents during storage and organoleptic quality of instant *lassi* powder and microbial activity during storage at room and refrigerated temperature have been discussed under following heading:

5.1 Physico- chemical analysis of Fresh Milk

Fresh milk was analyzed for various physicochemical characteristics and were estimated as pH (6.70), titratable acidity (0.1), and protein (3.06). Similar results were observed by (Mahmood and Usman, 2010 and Reddy *et al*., 2014).

5.2 Physicochemical analysis of Local Market *lassi*

Fresh local market *lassi* was analyzed for various physicochemical characteristics that were Total Solids (10.17%) Solid Not Fat (9.4%), and Lactose (1.2%) respectively. In local market *lassi* titratable acidity (TTA) was recorded as 0.633% and the similar results were also reported by (Kumbhar *et al*., 2009 and Munde, 2015) in their experimental *lassi*. pH in present study was recorded as 4.33, Munde, (2015) reported a range of pH between 3.18 to 4.33 for *Lassi* sold in Kholapur district. Fat in local market *Lassi* was recorded to be (0.77%). (Morin *et al*., *2007*) in buttermilk also reported fat content (1.43), which was much higher than the fat in present study.

5.3 Physicochemical analysis of *Lassi* varients prepared with specific cultures before drying

Various physicochemical analysis were done before drying that were fat (1.6%, 1.67% and 1.53%) and lactose (1.16%, 1.03% and 1.16%) of plain sweet and salted *lassi* respectively. Total solid were estimated (11.5%, 12.13% and 11.33%) in plain sweet and salted *lassi*. Morin *et al*., (2007) also reported the similar findings in regular buttermilk. pH and TTA was observed to be (4.7, 4.64 and 4.63) and (0.33%, 0.37% and 0.41%), for plain sweet and salted *lassi* powder respectively. Sudhakar, (2004) also estimated pH and TTA and observed dissimilar results than the present study which were (4.52 and 0.91%) respectively. Rashmi, (2010) reported TTA in low Fat *lassi* prepared by *Leuconostoc* strains and observed to be higher than the present study (0.60), Sharma (2016) studied on pH and TTA in symbiotic *lassi* supplemented with honey and observed pH and TTA in range of (4.50 to 4.44)

and (0.67 to 0.66%) respectively. George *et al*., (2012) reported (0.87% and 4.56) pH and TTA in sweetened *lassi*.

5.4 Optimization of Spray Drying

The best optimized condition was observed at condition 190°C inlet temperature, 70°C outlet temperature and 300rpm speed with 9.2 Survival probiotic count. Kim, (1990) optimized suitable conditions for inlet air 160°C outlet air 60°C. Bielecka and Majkowska, (2000) reported similar studies and observed outlet temperatures within the range 70 –75°C ensured acceptable survival of yoghurt cultures (*L. delbrueckii* subsp. *bulgaricus* – 13.7–15.8%; *S. thermophilus* – 51.6–54.7%). Koc *et al*., (2010) optimized air inlet temperature of 171°C, air outlet temperature of 60.5°C, and feed temperature of 15°C was the optimum processing condition for spray dried yogurt powder, Triboli *et al*., (2016) optimized inlet air temperature 160 °C and yoghurt feed flow rate 8.7 kg/h for yogurt powder production. To standardize the process for goat milk powder inlet air temperature of 160, 170 and 180°C were chosen best to produce the spray dried powder by (Reddy *et al*., 2014). Seth *et al*., (2017b) also optimized inlet air temperature 148°C, feed rate 0.54 L/h, and atomization pressure 898 kPa and reported moisture content, bacteria survival ratio, acetaldehyde retention and overall sensory as 3.69%, 2.37×10^{-3} 76.39%, and 8.0, respectively.

5.5 Physicochemical analysis of reconstituted *Lassi* powder

Total solids in reconstituted *lassi* powder were higher than the fresh *lassi*. After drying the TTA of plain sweet and salted *lassi* powder were significantly higher than fresh *lassi* (0.52%, 0.57%and 0.66 %). Koc *et al*., (2010) also reported the similar observation in yogurt powder and concluded the lactic acid percent in yogurt powder was higher than fresh yogurt. No browning was observed in fresh product but after drying sweet *lassi* powder had more browning than plain and salted *lassi* powder, this may be due to Maillard reaction in high temperature. Fat content after drying was higher than the fresh lassi (2.6%) similar results were reported by (Jha *et al*., 2002) in instant kheer premix.

5.6 Changes in physicochemical properties of spray dried lassi variants during storage at weekly interval

5.6.1 Browning

There was a significant increase in browning during storage but less change in browning was observed during RFT storage as compare to RT storage. It may be due to Maillard reaction and enzymatic reaction occurring at higher rate at room temperature in the product (Thomas *et al*., 2004). Thomsen *et al*., (2005) conducted research on effect of lactose crystallization, lipid oxidation, and browning reactions in whole milk powder during storage and they reported increase in water content favors lactose crystallization and lipid oxidation which lead to the formation of free radical oxygen availability increases and ultimately increase browning during storage. Powder browning during storage at high temperature

suggested that Maillard reaction is the main phenomenon responsible for powder alteration. (Burgain *et al*., 2016) and Norwood *et al*., 2017) stored whey protein isolate and reported when storage done at high temperature protein lactosylation occur which lead to degradation of Maillard reaction products, results in a higher browning and no significant change in browning observed in low temperature storage 0 to 20°C.

5.6.2 pH

In the present study there was significant decrease in pH with time. More decrease in pH was observed in RT storage as compare to RFT storage. Davis *et al*., (2017) stored goat milk powder and founded significant increase in pH during storage upto24 months but on comparing the two different temperatures (4°C and 22°C) no significant changes were observed in storage condition.

5.6.3 Titratable Acidity (TTA)

In the present study significant increase in TTA in *lassi* powders during storage and more significant changes in *lassi* powders were observed in RT as compare to RFT. After 12 weeks of study the product became unacceptable to drink due to high acidity. It may be due to the decrease in pH of the *lassi* powder. Acidity of reconstituted *dahi* powder was 0.936% Kothakota *et al*., (2014) and Koc *et al*., (2010) reported TTA of fresh yogurt was 0.98 and after drying increased to 1.04, but no reports were found for TTA under storage for lassi powder.

5.6.4 Moisture content

Moisture content of *Lassi* powders in RT storage increased significantly and no significant change in *lassi* powder was observed during RFT storage. Koc *et al*., (2010) studied moisture sorption isotherm and storage stability of spray dried yogurt at 25°C in aluminum laminated bags and observed similar results that there was increase in moisture content during storage and concluded that moisture uptake can be governed by environmental condition, water activity and package permeability. Chavez and Ledeboer, (2007) also concluded the similar studies, penetration of moisture through packaging material increase moisture content.

5.6.5 Fat

In the present study there was no significant increase in fat content of *Lassi* powder stored under RFT. A significant increase in fat was observed at RT storage of *lassi* powders. Increase in fat may be due to auto-oxidation of fat (Stapelfeldt *et al*., 1997). Davis *et al*., (2017) stored goat milk powder and founded an increase in free fatty acid content at 22°C storage which may increase the fat content. Paez *et al*., (2006) studied on changes in free fatty acid composition during storage of whole milk powder and concluded that changes in fat is due to microbial thermo stable lipases and found there was an increase in free fatty acid content during storage which increased the rancidity in product.

5.6.6 Wettability

Initial and final wettability at optimized condition was 60.33s for plain, 50s for sweet and 50s for salted lassi powder. No significant change in wettability was observed during storage of *Lassi* powders in different storage temperatures. Dis similar results by Koc *et al*., (2010) they observed wettability in a range of 307 sec to 756 sec with variation of outlet temperature of spray drier. Wettability of sweetened yogurt powder varied from 132 to 378 sec with the variation in processing condition of spray drier (Seth *et al*., 2017b). The powders were stored at 4°C to 7°C in RFT and 25°C at RT and no change was observed in solubility , hence due to this reason wettability would not had changed.

5.6.7 Solubility

There was no significant change in solubility observed during storage of *Lassi* powders in different storage temperatures. Stapelfeldt *et al*., (1997) studied the effect of heat treatment on milk powder storage and reported no difference was found for storage at 25°C whereas storage at 45°C more browning occurred and the solubility decreased. Thomas *et al*., (2004); Thomsen *et al*., (2005); Burgain *et al*., (2016) and Norwood *et al*., (2017) reported similar results that during high temperature storage there is loss in solubility of the product., but as per present study solubility has no effect as the temperature range were between 7 to 25°C.

5.6.8 Dispersibility

Initial and final dispersibility at optimized condition was 80% for plain, 85% for sweet and 88% for salted lassi powder. Reddy *et al*., (2014) observed dispersibility decrease with increase in temperature of spray drier and was in range of 78 to 89%. Similar results were quoted by (Seth *et al*., 2017b). There was no significant change in dispersibility observed during storage of *lassi* powders in different storage temperatures as the powders were stored at 4°C to 7°C in RFT and 25°C at RT and no change was observed in solubility , hence due to this reason Dispersibility would not had changed

5.6.9 Total solids

The present study total solids content of *lassi* powders showed no significant increase in RFT storage, but observed a decrease in total solids content of *lassi* powders during RT storage. It may be due to increase in moisture content of *Lassi* powders in RT storage.

5.6.10 Solid Not Fat

In the present study SNF content of *lassi* powders showed no significant increase in RFT storage, but observed decrease in RT storage of *lassi* powders. The decrease may be due to increase in fat content of RT storage.

5.7 Changes in organoleptic quality of spray dried *Lassi* powder during storage

In the present study a significant decrease in color and appearance, taste, body and texture, aroma and flavor, after taste and overall acceptability was observed for spray dried

plain, sweet, salted and market *Lassi* powders. The sensory scores were higher for RFT *lassi* powders and was still accepted after three months. RT plain, sweet and salted *lassi* and local market *lassi* powder powders were not accepted on sensory basis after three months, this may be due to the chemical changes occurring in the product kept at high temperature were higher than at low temperature. Stapelfeldt *et al*., (1997) also observed similar results in milk powder and concluded sensory quality is affected by an increase in storage temperature, which give rise to off-flavors produced by autoxidation and Maillard reaction.

After the storage studies up to three months for physicochemical analysis and organoleptic quality salted lassi powder is considered best followed by plain lassi powder of RFT storage. The lassi powder is considered as probiotic due to SRP was 9.2 log10 cfu/ml after spray drying and have 8.5 log10 cfu/ml SRP in RFT storage after three months of have therapeutic property and can be helpful in curing disease.

5.8 Microbial activity

5.8.1 Yeast and mold count

There were no yeast and mold count detected after three months of storage studies at RT and RFT of spray dried lassi powder variants. According to microbial standards for milk and milk products there should be zero yeast and mold count in powder (F. No.1-110(2)/SP (Biological Hazards)/FSSAI/2010).

5.8.2 Coliform count

In present study there was no coliform count detected after 3 months in *lassi* powders stored at different storage temperature. According to microbial standards for milk and milk products there should be zero coliform count in powder (F. No.1-110(2)/SP (Biological Hazards)/FSSAI/2010).

5.8.3 Probiotic count in *lassi* powder

In the present study SRP decreased after three months of storage but the decrease was not much in RFT as compared to Moumita *et al*., (2018) found the similar results by studying the effect of long-term storage on viability of lyophilized and spray-dried synbiotic microcapsules in dry functional food formulations and reported the similar study that 4 log unit reduction in lyophilized formulation as compared to 3 log unit reduction in spray dried form, after 15 weeks of storage and concluded that spray-dried form of synbiotic fortification was more stable than lyophilized form. Agudelo *et al*., (2017) studied on disaccharide incorporation to improve survival during storage of spray dried L. *rhamnosus* and concluded that partial replacement of maltodextrin by sucrose maintained the viable count after 196 days and Cold storage enhanced cell preservation, although after 7 storage months

5.8.4 Antimicrobial Activity

Spray dried *lassi* powder variants showed different antimicrobial activity against the food pathogens that are *B. subtilis*, *E. coli* and *S. aureus*. Plain lassi powder kept at RFT storage (17.33mm) showed higher zone of inhibition against *S. aureus*, but plain lassi powder stored at RT storage showed higher zone of inhibition against *B. subtilis* (13.33mm). Sweet lassi powder showed maximum zone of inhibition against *S. aureus* at RFT storage (16.33mm) and showed no zone of inhibition against the three food pathogen under RT storage. *L. acidophilus* active against gram negative pathogenic *E. coli* (including enteroaggregative *E. coli and E. coli* 0157:H7) (Coconnier *et al*., 1993; Klewicka and Libudzisz, 2004; Ogunbanwo *et al*., 2008; Wang *et al*., 2010; Omemu and Faniran, 2011; Elamathy and Kanchana, 2013; El-Kholy *et al*., 2014; Venkadesan and Sumathi, 2015; Kumar *et al*., 2016; Bertuccini *et al*., 2017; Campana *et al*., 2017 and Abdelhamid *et al*., 2018) *L. acidophilus* exhibits antimicrobial activity against gram positive food pathogenic bacteria that are *B. subtilis* and *S. aureus* (Nigam *et al*., 2012; Emami and Bazargani, 2014; El- Kholy *et al*., 2014; Venkadesan and Sumathi, 2015; Georgieva *et al*., 2015). Das *et al*., (2016) studied on antimicrobial activity of fermented oats (*Avena sativa*) against food borne pathogens. Oats were fermented by *L. acidophilus* culture and and observed a potent antimicrobial activity against *E. coli* and *S. aureus*.

L. rhamnosus showed mild inhibitory activity against diarrheagenic *E. coli* (Davoodabadi *et al*., 2015 and Anand et al., 2017). El-Shenawy, *et al*., (2017) studied on antimicrobial activity of Lactic Acid Bacteria and observed lactic acid bacteria in yogurt shows antimicrobial activity against *E.coli, Bacillus species and S. aureus.*

5.8.5 Antibiotic sensitivity

In the present study it was observed that lassi powder of all variants were resistant against Amikacin, Nalidixic Acid, Vancomycin, Clindamycin, Linezolid, Clarithromycin and Teicoplanin antibiotics. Salminen *et al*., (1998) demonstrated safety of probiotics and observed 30 strains of *L. acidophilus* were resistant against Vancomycin. Charteris *et al*., (1998) gave detailed study on antibiotic susceptibility of 46 *Lactobacillus* strains from human and dairy sources against 44 antibiotics and observed that lactobacillus stains were resistant against nalidixic acid, Amikacin and all strains were resistant to Vancomycin. Gueimonde *et al*., (2013) reported the similar result and concluded vancomycin-resistant phenotype of some lactobacilli is perhaps the best characterized intrinsic resistance in LAB, Wong *et al*., (2015) also coated similar results. Zhou *et al*., (2005) also reported antibiotic susceptibility and observed *L. rhamnosus* strains (HN001, HN067 and GG) were resistant to vancomycin. Coppola *et al*., (2005) isolated *L. rhamnosus* strains from cheese and showed resistance to six

antibiotics (cefixime, vancomycin, neomycin, enoxacin, pefloxacin and sulphamethoxazole plus trimethoprim).

Present study optimized a procedure for manufacturing of instant probiotic lassi powder formation without any additives through which dry and powder ingredients are made to form instant lassi by dissolving in water. This instant *Lassi* powder can be used as a base material for kadhi, rabri, can be used for making idli, dosa and dhokla. The powder is easy to store, handle, transport and can be conveniently used in restaurants, ships, aeroplanes, astronauts and mid-day meal in schools. It may be prescribed to ill persons for good gut microflora and fight against disease. Spray dried *lassi* powder variants showed antimicrobial activity against the food pathogens: *B. subtilis*, *E. coli* and *S. aureus*. In antibiotic susceptibility test it was observed that lassi powder had resistance against amikacin, nalidixic acid, vancomycin, clindamycin, linezolid, clarithromycin and teicoplanin antibiotics. If these antibiotics are given to ill person the infectious microflora will die, but probiotic microflora of the instant *lassi* may be retained in the gut.

CHAPTER-VI

SUMMARY AND CONCLUSION

The present investigation entitled "Development of Instant *Lassi*" are summarized below

- Pure culture of *Streptococcus salivarius* ssp. *thermophilus* (ATCC 19258), *Lactobacillus rhamnosus* (ATCC 7469) *and Lactobacillus acidophilus* (ATCC 4356) were used as a starter culture for *lassi* preparation
- Pasteurised double toned milk of Amul was procured from the market, which have pre-determined fat (1.5%) SNF (9.0%), pH (6.7), titratable acidity (0.11%) and protein content (3.06%)
- *Lassi* was procured from the local market, with TS, SNF, fat, lactose, pH, titratable acidity content were estimated as (10.17%), (9.4%), (0.77%), (1.2%), (4.33) and (0.633) respectively
- Three variations of *lassi* were prepared that were plain, sweet by addition of sugar @ 2gm/100ml and salted by addition of salt 0.15g/100ml with cumin powder 0.1g/ 25g after drying
- Plain, sweet and salted *lassi* were analyzed for different physico-chemical properties before drying. pH of plain, sweet and salted *lassi* were (4.70, 4.64 and 4.63). titratable acidity increased after salt addition. Solid not fat of plain, sweet and salted *lassi* were (9.4%, 9.50% and 10.53%), fat content in plain, sweet and salted *lassi* were (1.6%, 1.67% and 1.53%). Lactose in different composition of *lassi* were observed as (1.16%, 1.03% and 1.16%) respectively.
- Optimization of spray drying inlet temperature, outlet temperature and speed was studied with the help of CCRD of RSM, using Design Expert 11. The best optimized condition were 190°C, 70°C and 300rpm with maximum SRP of 9.2 $\log_{10}$ CFU/ml
- After spray drying the powder was reconstituted by mixing 10 grams of *lassi* powder in 100 ml water. Different physico-chemical analysis were done after reconstitution of different spray dried powders. Titratable acidity of the powder were comparatively higher than before drying. The titratable acidity of plain, sweet and salted *lassi* powder was obtained as following (0.52%), (0.57%) and (0.66%)
- Storage studies were done at RT and RFT up to 12 weeks. Significant increase in browning was observed at RT. While among variants highest browning was observed in sweet *lassi* powder of both RT and RFT that were (0.05 to 0.63) and (0.05 to 0.14) respectively

- Significant decrease in pH was observed in all *lassi* variants. But as compared to refrigerated temperature significant decrease was observed in room temperature, and highest decline in pH was found in salted *lassi* powder in both room and refrigerated condition that were (4.63 to 2.96) and (4.63 to 4.003)
- Significant increase in titratable acidity (0.65 to 3.00) was found in salted *lassi* powder of room temperature
- No significant increase of moisture content was found in *lassi* powders variants during storage in refrigerated condition. Fat content (2.4%) in *lassi* powder was same in refrigerated temperature, whereas in room temperature there was significantly change in fat content from zero day to twelve week of plain, sweet and salted *lassi* powder which were as follows (2.4% to 3.37), (2.4% to 3.39%) and (2.4% to 3.44%) respectively.
- There was no significant change in wettability, dispersibility, solubility index and total solids was observed during storage of powders
- SNF of plain, sweet and salted *lassi* powder was observed same during storage at refrigerated temperature (92.56%) for plain, (93.53%) for sweet and (95.51%) for salted *lassi* powder, but at room temperature there was significant decrease in solid not fat in plain, sweet and salted *lassi* powder (92.56% to 91.49%), (93.53% to 92.40%) and (95.51% to 94.19%) respectively
- Local market *lassi* powder was not accepted on the basis of quality and organoleptic evaluation. Significant increase in browning and titratable acidity was observed from zero day to four weeks (0.316 to 0.47) and (0.816% to 0.9%) respectively. pH also decreased from zero day to fourth week (4.40 to 4.31).
- There was significant decrease in overall acceptability of *lassi* powders in RT as compared to RFT. The maximum value of plain sweet and salted *lassi* powder during zero day of RFT and RT (8.24, 8.06 and 8.57) respectively which decreased to (7.46, 7.13 and 7.54) in refrigerated temperature, (4.37, 4.28 and 4.40) in room temperature respectively.
- No Yeast, Mold and coliform count was detected in spray dried *lassi* powders
- Maximum antimicrobial activity of sweet *lassi* powder in RFT (14 mm, 15.66 mm and 16.33mm) and of plain *lassi* powder in room condition (14 mm, 15.66mm and 16.33mm) against the food pathogens *B. subtilis*, *E. coli* and *S. aureus* was observed.
- Antibiotic sensitivity checked against 32 antibiotics and observed all *lassi* powder variants were resistant against amikacin, nalidixic acid, vancomycin, clindamycin, linezolid, clarithromycin and teicoplanin antibiotics
- After drying of *lassi* variants 9.2 $\log_{10}$ CFU/ml SRP was observed and after three months of storage count reduced to 8.5 $\log_{10}$ CFU/ml in RFT and 7.0 $\log_{10}$ CFU/ml in RT, respectively.

CONCLUSION

The present study was conducted to standardize the preparation of instant *lassi* and first of its kind to evaluate the shelf life of the spray dried *lassi* powder. Three variants of *lassi* (plain, sweet and salted) were prepared with specific probiotic cultures {*S. salivarius* ssp. *thermophillus* (ATCC 19258), *L. rhamnosus* (ATCC 7469) *and L. acidophilus* (ATCC 4356)} and one sample was procured from local market. Spray dryer conditions were optimized with RSM as inlet temperature (190°C), outlet temperature (70°C) and speed (300 rpm) with 9.2 $\log_{10}$ CFU/ml survival rate of probiotics. Instant probiotic lassi can be reconstituted by dissolving 10g/100ml water. The powder was stored for 3 months at refrigerated temperature and room temperature in aluminium laminated pouches followed by vaccum packaging. Increase in acidity, pH, browning and significant decrease in organoleptic properties was observed after one month in the powders stored at room temperature while, the powders stored at refrigerated temperature were acceptable with regard to oganoleptic, physicochemical and microbiological properties up to three months. Plain *lassi* powder stored under refrigerated temperature showed maximum antimicrobial activity against the food pathogens *B. subitilis*, *S. aureus* and *E. coli*. Antibiotic susceptibility test was done against 32 antibiotics for all the *lassi* variants and observed that they were resistant to amikacin, nalidixic acid, vancomycin, clindamycin, linezolid, clarithromycin and teicoplanin antibiotics. After all physicochemical, organoleptic and microbiological analysis plain *lassi* powder was recommended as best and can be provided as probiotic supplement and can be manufactured commercially with lower cost, easy availability, easy to store, handle, transport and convenient to use.

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APPENDIX -I

SENSORY EVALUATION

(HEDONIC RATING SCALE)

Name: Date:

Product: Time:

INSTRUCTIONS

Taste the given samples and check how much you like or dislike the product. The appropriate scale to show your attitude by assigning points that best describe your feelings about sample. An honest expression of yours' will help us. Evaluate base on the following scale

SCORE PREFERENCE

Like Extremely	9
Like Very Much	8
Like Moderately	7
Like Slightly	6
Neither Like nor Dislike	5
Dislike Slightly	4
Dislike Moderately	3
Dislike Very Much	2
Dislike Extremely	1

Code	Colour And Appearence	Taste	Body and Texture	Aroma and Flavour	After Taste	Overall Acceptbility

Signature

ABSTRACT

Title of Thesis	**:Development of Instant *Lassi***
Full Name of Degree Holder	**: Kritika Rawat**
Admission No.	**:** 2016 FST32M
Title of Degree	**: Master of Science**
Name of Discipline	**:**Food Science and Technology
Name and Address of Major Advisor	**:Dr. Anju K. Shehrawat** Assistant Professor Centre of Food science and Technology CCS Haryana Agricultural University Hisar- 125 004 (India)
Degree Awarding University/ Institute	**:**CCS Haryana Agricultural University Hisar- 125 004 (Haryana), India
Year of Award of Degree	**:** 2018
Major Subject	**:**Food Science and Technology
Total Number of Pages in Thesis	**:56 + xi + I**
Total Number of Word in Abstract	**: 317**

Keyword: *lassi*, spray dryer, optimization, instant powder, storage, antimicrobial, antibiotic sensitivity

The present investigation entitled "Development of Instant *Lassi*" was carried out to standardize the preparation of instant *lassi* and first of its kind to evaluate the shelf life of the spray dried *lassi* powder. Three variants of *lassi* (plain, sweet and salted) were prepared with specific probiotic cultures {*S. salivarius* ssp. *thermophillus* (ATCC 19258), *L. rhamnosus* (ATCC 7469) *and L. acidophilus* (ATCC 4356)} and one sample was procured from local market. Spray dryer conditions were optimized with RSM as inlet temperature (190°C), outlet temperature (70°C) and speed (300 rpm) with 9.2 $\log_{10}$ CFU/ml survival rate of probiotics. Instant probiotic lassi was reconstituted by dissolving 10 g/100 ml water. Titratable acidity and fat of lassi variants before drying was observed (0.33%, 0.37% and 0.41%) and (1.6%, 1.67% and 1.53%) respectively, but after drying it was found to be increased titratable acidity (0.52%, 0.57% and 0.66 %) and fat (2.6%). After reconstitution maximum overall acceptability score was of salted lassi powder (8.57). The powder was stored for 3 months at refrigerated temperature and room temperature in aluminium laminated pouches followed by vaccum packaging. Increase in acidity, pH, browning and significant decrease in organoleptic properties was observed after one month in the powders stored at room temperature while, the powders stored at refrigerated temperature were acceptable with regard to oganoleptic, physicochemical and microbiological properties up to three months. Plain *lassi* powder stored under refrigerated temperature showed maximum antimicrobial activity against the food pathogens *B. subitilis*, *S. aureus* and *E. coli*. Antibiotic susceptibility test was done against 32 antibiotics for all the *lassi* variants and observed that they were resistant to amikacin, nalidixic acid, vancomycin, clindamycin, linezolid, clarithromycin and teicoplanin antibiotics. After all physicochemical, organoleptic and microbiological analysis plain *lassi* powder was recommended as best and can be provided as probiotic supplement and can be manufactured commercially with lower cost, easy availability, easy to store, handle, transport and convenient to use.

MAJOR ADVISOR **SGNATURE OF DEGREE HOLDER**

HEAD OF DEPARTMENT
CENTRE OF FOOD SCIENCE AND TECHNOLOGY

CURRICULUM VITAE

Name of Degree Holder	:	Kritika Rawat
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Academic Qualification

Degree	Board / University	Year of passing	Percentage of marks	Subjects
10	C.B.S.E	2009	60.4	English, Hindi, Maths, Science, Social Science
10+2	C.B.S.E	2011	69.6	Physics, Chemistry, Biology, Hindi, Physical Education
B.Sc. (Food Technology)	G.B.P.U.A&T (Pantnagar)	2016	6.87 (CGPA)	Food Science and Technology

Medal/Honours received

- Won first prize in nutritious snacks making at CFST, CCS HAU.
- Won third prize in Declamation at CFST, CCS HAU.
- Won second prize in nutritious drink at CFST, CCS

HAU **List of publications**

Patent filed: Appl. No. 201811016859 Date/Time 2018/05/04 11:18:36

Review article

- Rawat, K., Kumari, A., Kumar, S., Kumar, R. & Gehlot, R. (2018) Traditional Fermented Products of India. *International Journal of Current Microbiology and Applied Sci*ence, **7**(4), 1873-1883.

Book Chapter

- Arora, S., **Rawat, K.,** Sucheta and Bhardwaj, R. 2018. Advancement in food processing and preservation technology. Advances in food processing techniques.

Abstracts

- **Rawat, K.**, Kumari A., Panwar, K., Roperia, M., and Sadiq, M. 2017. Spray dried lassi powder. National conference on "Advances in Food science & technology-current trends & future prospective" AFST-2017. March 25-25, 2017. RP126 Kumari,
- A., Roperia, M., Jangra, R., **Rawat, K.** Kumar, R., and Sadiq, M. 2017. Agriculture to sustain food security with growing population. International conference on Emerging areas of Environmental Sciences and Engineering (EAESE-2017), GJU, Hisar (India) 16-18 Feb, 2017
- Roperia, M., Kumari A., **Rawat, K.** and Sadiq, M. 2017. Endophytic microorganisms and their role in phytoremediation. International conference on Emerging areas of Environmental Sciences and Engineering (EAESE-2017), GJU, Hisar (India) 16-18 Feb, 2017

- Roperia, M., Kumari A., **Kritika** and Sadiq, M. 2016. Nanotechnology: Applications in Food. National conference on Trends in Nanobiotechnology (NCTN-2016). CCS HAU, Hisar. (India). 29-30 Nov, 2016. Pp.SP110
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- Jattan, P., Kumari, A., Rekha, **Rawat, K.** and Bansal, A. Nano-Medicine: A Boon in Healthcare. National conference on Trends in Nanobiotechnology (NCTN-2016). CCS HAU, Hisar. (India). 29-30 Nov, 2016. Pp.SP 149.

Co-curricular activities

Participated in National Summit-2018 at CCS HAU, Hisar ; Participated in Agri startups convention 2018 under Council of Food and Agriculture (ICFA), held at constitution club, New Delhi. Given 30 minutes programme in Pantnagar Janvani radio station, have NSS "B" and "C" certificate. Participated in netritva: The leadership hunt contest held from 16th to 30th January 2014, Organised by Vivekanand Swadhya Mandal, GBPUA & T.

Signature of Student

UNDERTAKING OF THE COPY RIGHT

"I, **KRITIKA RAWAT**, Admn. No. **2016FST32M** undertakes that I give copy right to the CCS HAU, Hisar of my thesis entitled "**Development of Instant *Lassi***".

I also undertake that, patent, if any, arising out of the research work conducted during the programme shall be filed by me only with due permission of the competent authority of CCS HAU, Hisar.

KRITIKA RAWAT